Potassium Channels as Multi-Ion Single-File Pores

BERTIL HILLE and WOLFGANG SCHWARZ

From the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195. Dr. Schwarz's present address is Physiologisches Institut der Universität des Saarlandes, 6650 Homburg/Saar, West Germany.

ABSTRACT A literature review reveals many lines of evidence that both delayed rectifier and inward rectifier potassium channels are multi-ion pores. These include unidirectional flux ratios given by the 2-2.5 power of the electrochemical activity ratio, very steeply voltage-dependent block with monovalent blocking ions, relief of block by permeant ions added to the side opposite from the blocking ion, rectification depending on $E - E_K$, and a minimum in the reversal potential or conductance as external K$^+$ ions are replaced by an equivalent concentration of Tl$^+$ ions. We consider a channel with a linear sequence of energy barriers and binding sites. The channel can be occupied by more than one ion at a time, and ions hop in single file into vacant sites with rate constants that depend on barrier heights, membrane potential, and interionic repulsion. Such multi-ion models reproduce qualitatively the special flux properties of potassium channels when the barriers for hopping out of the pore are larger than for hopping between sites within the pore and when there is repulsion between ions. These conditions also produce multiple maxima in the conductance-ion activity relationship. In agreement with Armstrong's hypothesis (1969. J. Gen. Physiol. 54:553-575), inward rectification may be understood in terms of block by an internal blocking cation. Potassium channels must have at least three sites and often contain at least two ions at a time.

Evidence has accumulated, for the sodium channel and for several types of potassium channels of electrically excitable cells, that ions interact with the channel and with other ions in it while diffusing across the membrane (French and Adelman, 1976). An earlier paper of Hille (1975 b) discussed a model of the sodium channel in which the permeating ion must pass across a sequence of four energy barriers to cross the membrane. Inasmuch as the model assumed that no more than one ion could be in the channel at a time, it was called a one-ion pore. In this paper we consider a similar type of linear, multibarrier model for a multi-ion pore where more than one ion may be in a channel at a time, and the ions are not permitted to pass by each other as they move through the channel. These assumptions lead to phenomena commonly referred to as "single-file diffusion" or the "long pore effect" which have been reported in measurements of the passive movement of ions in potassium channels of nerve, muscle, and other cell membranes. Our goal is to show that the major transport properties of potassium channels may be accounted for by this class of multi-ion channel models. For practical reasons, only a qualitative agreement is demonstrated here. An attempt to make more realistic models would need many more
free parameters and would take orders of magnitude more time to calculate than the simpler systems we have considered. A preliminary account of this work has been given (Hille and Schwarz, 1978).

We consider first the evidence that potassium channels in excitable cells are multi-ion pores and then show that the phenomena can be reproduced qualitatively by models elaborated in the Theory and Results sections. In this paper, potassium channels of the type activating with a brief delay during a depolarization, for example, potassium channels used in the action potential of typical axons (Hodgkin and Huxley, 1952), are called "delayed rectifier" channels, and those that conduct better in the inward direction than outward, for example, in K⁺-depolarized skeletal muscle (Katz, 1949), are called "inward rectifier" channels. These latter are also frequently referred to as "anomalous rectifiers" or "inward-going rectifiers" in the literature.

**Potassium Channels Are Multi-Ion Channels**

In free-diffusion systems or in one-ion pores, the ratio of outward to inward unidirectional particle fluxes (measured by tracer methods) equals the ratio of internal to external electrochemical activities of the diffusing particles. For ions this rule is the Ussing (1949) flux ratio:

\[
\frac{J_S}{J_S} = \frac{[S]_i^{1/n'}}{[S]_e^{1/n'}}
\]

where \( J_S \) and \( J_S \) are the unidirectional efflux and influx of ion \( S \); \([S]_i\) and \([S]_e\) the internal and external activities; \( z \) the valence of \( S \); \( E \) the membrane potential; and \( F \), \( R \), and \( T \) the usual thermodynamic quantities. Hodgkin and Keynes (1955) found that K⁺ ion fluxes in electrically polarized axons of the cuttlefish, *Sepia officinalis*, do not satisfy Eq. 1 unless the right hand side is raised to a power \( n' \) giving the equivalent forms:

\[
\frac{J_K}{J_K} = \left( \frac{[K]_i}{[K]_e} \right)^{n'} \exp \left( \frac{zF[E - E_K]}{RT} \right)
\]

where \( E_K \) is the potassium equilibrium potential defined by the Nernst equation. The experimental value for \( n' \) in *Sepia* axons was roughly 2.5. Potassium flux ratios in depolarized frog skeletal muscle exposed to elevated external potassium concentrations are also reported to satisfy Eq. 2 with an exponent \( n' = 2.0 \) (Horowicz et al., 1968; but for muscle in low potassium see a contrary report of Sjodin, 1965).

A value of \( n' \) larger than 1.0 suggests that the diffusing particle is a multimer of the single ion and can be obtained with single-file pores containing more than one ion at a time (Hodgkin and Keynes, 1955) or with carriers complexing with more than one ion at a time (Horowicz et al., 1968). The high value of \( n' \) in *Sepia* axons was the first evidence for a multi-ion nature of potassium channels or indeed of any ionic channel. In the *Sepia* axon experiments the primary pathway for K⁺ ion flow would have been delayed rectifier channels, whereas in
the frog muscle experiments, flow would have been in inward rectifier potassium channels. Hence, both delayed rectifier and inward rectifier are reported to have multi-ion flux ratios. These difficult experiments are of sufficient importance that they ought to be repeated.

A second, less obvious line of evidence against one-ion pore or one-ion carrier models for potassium channels comes from the voltage dependence of block obtained when a blocking ion is added on one side of the membrane. Consider an internally applied impermeant blocking ion which acts by moving part of the way across the channel to form the blocking complex. If the block is not influenced by other ions, then the fraction of blocked, \( R_B \), to not blocked, \( 1 - R_B \), channels is given by a Boltzmann distribution at equilibrium:

\[
\frac{R_B}{1 - R_B} = \frac{[B]}{K_B} e^{\frac{F}{z' RT}} = e^{\frac{F}{z' E - E_0} RT},
\]

where \([B]\) is the activity of the blocking ion, \(K_B\) the dissociation constant of the blocking complex at \( E = 0 \) mV, \( E_b \) the membrane potential at which half the channels are blocked, and \( z' \) the effective valence of the blocking reaction given by the valence of the blocking ion multiplied by the fraction of the total potential drop through which it moves. For an external blocking ion, there should be a minus sign in front of \( z' \). Taking the logarithm of Eq. 3 and differentiating at constant \([B]\) gives:

\[
z' = \frac{RT}{F} \frac{\partial}{\partial E} \ln \left( \frac{R_B}{1 - R_B} \right).
\]

For a one-ion channel there can be competition between permeant ions and blocking ions so Eq. 3 may not be satisfied with a constant \( z' \) or \( K_B \); nevertheless, for each set of ionic conditions and membrane potential, a value of \( z' \) can be determined using Eq. 4. In a one-ion channel with monovalent blocking ions and monovalent permeant ions, the value of \( z' \) cannot exceed 1.0.

In multi-ion pores or multi-ion carriers where the movement of several ions may occur in the formation of the blocking complex, the effective valence of the blocking reaction may be larger than 1.0, even though the blocking particle itself may be monovalent. Indeed potassium channels are blocked by some monovalent cations in a voltage-dependent manner requiring values of \( z' \) larger than 1.0. The most carefully analyzed examples are the block by external \( Cs^+ \) ions of inward \( K^+ \) currents in the inward rectifier of starfish eggs, where \( z' \) is 1.4-1.5 (Hagiwara et al., 1976) and in the delayed rectifier of squid giant axons where \( z' \) increases with \([Cs]\) to 1.3 at 200 mM Cs (Adelman and French, 1978). In this latter case the block at very negative potentials (−106 and −86 mV) is also second order in \([Cs]\), requiring a coefficient \(([B]/K_B)^2\) in Eq. 3 rather than \([B]/K_B\). Other experiments also suggest high values of \( z' \) for block of delayed rectifiers (Hille, 1975 a) and inward rectifiers (Gay and Stanfield, 1977).

Armstrong (1969, 1975 b; see also Cleeman and Morad, 1978) has suggested that the normal rectification of the inward rectifier could itself be due to movement of a physiological blocking ion coming from the cytoplasmic side of the channel, although other authors have suggested an explanation in terms of
a highly charged carrier molecular (Horowicz et al., 1968; Adrian, 1969). In the starfish egg this normal rectification fits Eq. 3 with an equivalent valence \(z'\) of 2.9-3.6 (Hagiwara and Takahashi, 1974 a; Hagiwara et al., 1977). In frog skeletal muscle the equivalent valence is 2.3 from the curves of Adrian and Freygang (1962), and for the \(I_{K}\) channel of mammalian cardiac Purkinje fibers it is near 1.0 (McAllister et al., 1975). To explain an equivalent valence of 3 by a blocking-pore model would require at least a trivalent blocking ion in a one-ion channel, or a three-ion channel with a monovalent blocking ion, or a tetravalent carrier.

A third line of evidence for multi-ion potassium channels is the effect of external potassium concentration on block or inward rectification. A characteristic and distinguishing property of the inward rectifier is that the conductance change is a function of the potential difference \(E - E_K\) (Katz, 1949), rather than of the membrane potential alone. The voltage dependence of the conductance \(g\) is approximately described by the empirical equation (Hagiwara and Takahashi, 1974 a):

\[
g_{\text{max}} - g = \frac{[B]}{K_b} e^{\frac{z'qE}{RT}} e^{\Delta E_b/RT} = e^{\frac{z'qE - E_K - \Delta E_b}{RT}},
\]

where, for the inward rectifier, \([B]/K_b\) may be regarded simply as coefficient and \(\Delta E_b\) is the potential displacement from \(E_K\) at which half the channels are blocked. Outward currents can flow at potentials just above \(E_K\), but at much more positive potentials, they shut off. In this range of small outward current, \(> 20\ \text{mV}\) positive of \(E_K\), adding external \(K^+\) ions actually increases the net outward flux of \(K^+\) ions (Hodgkin and Horowicz, 1959; Horowicz et al., 1968), causing the current-voltage relations measured in different external potassium concentrations to cross above \(E_K\) (Noble and Tsien, 1968; Adrian, 1969), a phenomenon sometimes referred to as “crossover”. An analogous crossover, is seen with delayed rectifier channels of squid and frog axons that are partially blocked by internally or externally applied blocking cations (Bezanilla and Armstrong, 1972; Hille, 1975 a; Adelman and French, 1978), and raising the potassium concentration on the opposite side relieves the block. The requirement for at least a small amount of external \(K^+\) (or \(Cs^+\)) to get maximal outward currents in delayed rectifier channels of frog axons (Dubois and Bergman, 1977) may be a related phenomenon.

Crossover effects with conductance dependence on \(E - E_K\) cannot be explained by a channel obeying independence or by a one-ion channel, where adding \(K^+\) ions to one side always decreases net current to that side. Armstrong (1969, 1975 a) has postulated that pores may simultaneously contain a \(K^+\) ion and a blocking ion so that \(K^+\) ions coming from one side may repel, knock off, or compete against blocking ions coming from the other. This type of multi-ion model, which is elaborated later in this paper, produces crossover of the current-voltage relations (Hille, 1975 a). Crossover can also be produced in rectifying carrier models (Horowicz et al., 1968; Adrian, 1969) and in rectifying pore models (Cleeman and Morad, 1978), if the number or permeability of carriers or pores is postulated to increase with the external potassium concentration.
Finally, a fourth line of evidence indicates that inward rectifier channels of echinoderm eggs are not one-ion pores or one-ion carriers (Hagiwara and Takahashi, 1974 a; Hagiwara et al., 1977). In bi-ionic conditions, Tl⁺ ions may be considered more permeant than K⁺ ions because with external solutions of TlNO₃ the membrane conductance is higher and the resting potential is more positive than with external solutions of KNO₃ at the same concentration. However, surprisingly, when the Tl⁺ and K⁺ solutions are mixed together, the membrane conductance can become smaller and the reversal potential more negative than with either of the pure salt solutions outside indicating that Tl⁺ is apparently now less permeant than K⁺. Such behavior where conductance or reversal potential go through a minimum as a function of the ratio of cation activities in the bathing solution has been called "anomalous mole-fraction" behavior. The anomaly requires a model in which one permeant ion influences either the channel or another permeant ion while that ion is being transported. Thus, if there is only one transport site in the channel, there must be at least one other externally accessible modulatory site that can alter the properties of the channel. A model with modulatory sites has been used to describe anomalous mole-fraction effects in gramicidin A pores (Sandblom et al., 1977; Eisenman et al., 1977, 1978). As is shown later, multi-ion channels (which have more than one transport site) also show anomalous mole-fraction effects even without a modulatory site and without any special modulatory effect of ions bound at the transport sites.

Potassium Channels Are Pores

Although several of the phenomena discussed can be modeled using pores or carriers, the conventional delayed rectifier potassium channel of axons is already known to be a pore (Armstrong, 1975 a, b). In the squid giant axon, the single-channel conductance was calculated to be 0.9–3 pS from the kinetics of block by quaternary ammonium ions (Armstrong, 1966, 1969, 1975 b) and 12 pS from current fluctuations (Conti et al., 1975), and, in frog nerve, 4 pS from current fluctuations (Begniesch and Stevens, 1975). Even the lowest value means that one open potassium channel can pass a K⁺ ion at least every microsecond, a turnover number too high for a carrier mechanism. The crossover effect and related actions of external K⁺ on internal blocking ions have also been considered as arguments for a pore (Armstrong, 1975 a, b).

For the inward rectifier there is no direct evidence and more work is needed. However, we suggest that it is also a pore on the following two indirect grounds. (a) The permeability properties of the inward rectifier show several striking parallels with those of the delayed rectifier which is a pore, such as steeply voltage-dependent block with Cs⁺ ions, a permeability sequence from potential measurements of Tl⁺ > K⁺ > Rb⁺ > NH₄⁺ (Hagiwara and Takahashi, 1974 a; Hille, 1973), crossover effects with changes in external K⁺, and Hodgkin-Huxley flux ratio exponents near 2. (b) The total membrane conductance due to inward rectifiers in electric eel electroplax is so high that an explanation in terms of carriers might be difficult. In electroplax bathed in 175 mM K⁺ the maximum conductance for inward K⁺ current is on the order of 1.6 S/cm² (Nakamura et
al., 1965), neglecting any folding of the membrane, and 100 mS per microfarad of membrane capacity (Keynes and Martins-Ferreira, 1953). A conductance of 100 mS/cm² is three times higher than the maximum potassium conductance of a squid giant axon and could be accounted for by 250 pores of 4 pS conductance per square micrometer but might require a density of carriers two orders of magnitude or more higher.

For these reasons we suggest that inward rectifier and delayed rectifier potassium channels are both multi-ion pores. Some of the arguments may be weak individually, but together they seem strong. More direct experiments would be useful. In these arguments we have assumed that inward rectifier channels in a variety of tissues are basically similar in construction, although at least minor differences are known.

THEORY

This section outlines one type of model for describing the movements and interactions of ions in a multi-ion pore. For definiteness it is necessary to make a variety of specific assumptions in the model, but many of these must be regarded as both arbitrary and of little consequence to the overall conclusion reached in Results that multi-ion pore models qualitatively reproduce many of the steady-state flux properties of potassium channels.

The elements of the theory are the same as those in several previous papers (Heckmann, 1965 a, b, 1968; Hill and Chen, 1971; Hadiky, 1972; Läuger, 1973; Markin and Chizmadjev, 1974; Hille 1975 a, b; Chizmadjev and Aityan, 1977; Aityan and Kalandadze, 1977; Aityan et al., 1977, Kohler, 1977; Levitt, 1978 b). We use some of the notation of Hille (1975 b). A channel is represented in terms of a free energy profile corresponding to the free energy of an ion at various positions across the membrane in the absence both of an applied electric field and of other ions within the channel. Following absolute reaction rate theory (Glasstone et al., 1941), the rate constant for an elementary chemical step is exponentially related to the height of the energy barrier to be crossed. For example, for an ion crossing from energy well 2 (Fig. 1 A) to the outside, the fixed "chemical part" of the rate constant is: \( b_{21} = Q \exp(-G_{21} + G_2) \), where the frequency factor \( Q \) is taken as an unspecified constant and the reduced free energies \( G \) are defined as the free energies divided by \( RT \) and subscripted as in Fig. 1 A. In the text and figure legends the \( G \)'s are given in this dimensionless form.

In addition to the fixed, chemical component of free energy, there is an electrostatic component from the applied membrane potential and a repulsive electrostatic component from Coulomb forces between ions when the channel is at least partially occupied. The contribution from the membrane potential \( E \) is expressed as the product of the reduced potential \( V \), defined as \( EF/RT \), and the fraction \( d_i \) of the total potential drop acting on the transition from the energy minimum to the energy maximum. Although the "electrical distances" \( d_i \) may differ for different barriers, the same \( d_i \) applies when the \( i^{th} \) barrier is approached from the left or the right. In the numerical calculations, but not in the formulae in the text, \( RT/E \) is set at 25 mV, the lateral binding sites (energy wells) are placed arbitrarily at an electrical distance 20% from the membrane surface, and, with the three-site model, the central site is placed in the center of the membrane. The minima and maxima of the energy profile are assumed to be so sharp that the \( d_i \)'s are invariant with added external or local electrical fields.

Repulsive Coulombic interactions could be expressed in terms of the local potential from the other ions, but we express them equivalently as factors multiplying the rate constants. As a simplification we assume that the energy profiles \( G \) for different ion species are different and uninfluenced by the other ions in the channel except through...
the mutual repulsion terms which are identical for all ions of the same valence. For a
two-site model there are just two such factors, $F_{in}$ and $F_{out}$, describing the effect of an ion
occupying one site on the rate of jumping of another ion into or out of the second site.
For a three-site model there are more repulsion factors because the sites may be occupied
in a variety of ways. A range of values of 2.0–500 for the net effect ($F_{out}/F_{in}$) of one ion on
the equilibrium dissociation constant of another seems easily possible. These extreme
values would be obtained for example if two monovalent ions were moved from infinity
to separations of 40 Å or of 4.5 Å, respectively, in an infinite medium of dielectric
constant 20. Levitt (1978 a) gives more detailed calculations for a pore. In numerical

Calculations (but not in the general formulae), it is necessary to decide how much of net
repulsive effect is due to $F_{out}$ and how much to $F_{in}$. For the lack of definite information,
we arbitrarily made the $F_{out}$ factor for a step where an ion moves away from another ion
equal to the reciprocal of the $F_{in}$ factor for the reverse step, so in the figures the
repulsion factors are given simply as $F$, defined by $F = F_{out} = 1/F_{in}$. This assumption,
equivalent to assuming that the potential from an ion in one well drops linearly between
the neighboring wells, was also made by Aityan et al. (1977) in their model of potassium
channels.

In developing the flux equations, we follow the principles outlined by Heckmann
Jumps over energy barriers occur sufficiently infrequently and in a brief enough time for the states of the channel to be described simply by specifying which well is occupied by what ion. Thus, as in Fig. 2 A, the four states of a two-site model bathed on both sides by K⁺ ions are 00, 0K, K0, and KK, where 0 means an empty site and K means a K⁺-occupied site. Permitted transitions among these states, shown by lines in the figure, involve the movement of only one ion from an occupied site or a bathing compartment to a neighboring unoccupied site or bathing compartment. Thus, diffusion here requires vacancies, unlike the strict "knock-on" theory suggested by Hodgkin and Keynes (1955).

Each full, voltage-dependent rate constant $k$ can now be written down in explicit form, for example the rate constant for the KK-0K transition is: $k_{KK-0K} = F_{out}b_2\exp(d_2V) = QF_{out}\exp(-G_{12} + G_a + d_1V)$. (In this paper, unlike the previous one (Hille, 1975 b), the ionic activities $[K]_o$ of the external and $[K]_i$ of the internal bathing compartment are not combined ahead of time with the rate constants of jumping into a channel to make a single coefficient.) We restrict the analysis to the steady state, where the flux $J$ of K⁺ ions may be expressed as the difference in the rates of crossing of any one of the barriers. Thus, for the diagram of Fig. 2 A the net flux across the central energy barrier is: $J = k_{KK-0K}R_{KK} - k_{0K-OK}R_{KK}$, where the $R$'s are steady-state probabilities of the designated states, usually given by long algebraic expressions obtained by using the diagram method of King and Altman (1956) either by hand for simpler diagrams or by a computer program kindly sent to us by Professor K. Heckmann, University of Regensburg, West Germany (Heckmann et al., 1969). A fuller description of the mathematical and numerical methods used is given in the Appendix. Numerical calculations were done in BASIL on an LM² laboratory minicomputer, kindly built for us by Dr. T. H. Kehl, University of Washington, and his staff (Kehl et al., 1975).

A few further simplifications are used in some cases later. We refer to a "symmetrical" channel when the free-energy profile is symmetric about the center of the membrane, and we refer to a "uniform" channel when all energy maxima are equal, all energy minima are equal, and all $d_i$ values are equal. If the energy peaks are equal, they are symbolized $G_p$, and if the energy wells are equal, they are symbolized $G_w$. Sometimes when the wells are equal it is convenient to convert the bathing K⁺ ion activities $[K]$ into reduced activities $\sigma$, defined by: $\sigma = [K]/\exp(G_w)$. The term $\exp(G_w)$ is the equilibrium dissociation constant of the wells in the absence of additional electrical forces, so by
definition, equilibrium occupancy of each site would be 0.5 when \( \sigma \) on both sides of the membrane is 1.0 and there is no repulsion and no applied field. The activities \( [K] \) and reduced activities \( \sigma \) are dimensionless and neither is intended to be equivalent to moles per liter. The fluxes, currents, and conductances are also given in arbitrary units, as we do not specify a value for the frequency factor \( Q \). Therefore, the actual values given for barrier peaks could be changed uniformly by an additive constant with no effect on the results given.

**RESULTS**

*Occupancy and Conductance*

The transport properties of multi-ion pores vary as the activity of permeant ions is increased. At the lowest activity, where the pore is nearly empty, the fluxes increase linearly with ion activity in accord with the "independence relation" (Hodgkin and Huxley, 1952). The flux equations for this low-activity range have been given by Eyring et al. (1949) and Woodbury (1971), and for the two-site model with monovalent ions may be written (Hille, 1975b):

\[
J = Q \frac{[K]\exp(V/2) - [K]\exp(-V/2)}{\exp[G_{34} + (d_1 + d_2)\cdot V] + \exp[G_{23} + (d_1 - d_2)\cdot V] + \exp[G_{12} -(d_2 + d_3)\cdot V]}
\]

In this range of activities the permeability of the channel is determined by the energy maxima alone, while the depth of the energy minima and the repulsion factors play no role. For a uniform two-site channel, Eq. 6 simplifies to:

\[
J = Q \cdot \exp(-G_p) \frac{[K]\exp(V/2) - [K]\exp(-V/2)}{\exp(V/3) + 1 + \exp(-V/3)}.
\]

In a middle activity range, occupancy of the channel becomes significant, the independence relation is definitely not satisfied, and the flux saturates as exit from the pore becomes the rate-limiting step. Steady-state flux equations for one permeant species in a two-site model may be derived from the state diagram in Fig. 2 A and have been given by Heckmann (1965a), Hill and Chen (1971), Hladky (1972), and Markin and Chizmadjev (1974). For a symmetrical two-site channel bathed in equal K+ activities, the conductance around 0 mV is (Hladky, 1972):

\[
g = \frac{F^2}{RT} \frac{[K]Q\exp(-G_{23})}{1 + 2\sigma + \sigma^2 \frac{F_{in}}{F_{out}}} \left(1 + \frac{2\exp(G_{12} - G_{23})}{\sigma \cdot F_{in} + 1}\right).
\]

Conductance and flux show complex dependence on ion activity and depend on energy maxima \( (G_{12} \text{ and } G_{23}) \), on energy minima (which are contained in the \( \sigma \)'s), and on repulsion factors. In a channel that accepts no more than one ion at a time (one-ion channel), the conductance rises asymptotically to a plateau at high activities (Läuger, 1973; Hille 1975a, b); however, in a multi-ion channel, the conductance actually decreases again at the highest activity (Heckmann, 1965b; Hill and Chen, 1971; Hladky, 1972). Transport at high activity may be regarded as limited by the requirement that a vacancy move from one side of
the membrane to the other, and conductance begins to vary inversely with activity when there are almost no vacancies and when the new ones forming at the edge of the membrane are more rapidly filled by an ion in the bathing solution than by an ion crossing from the next site. In this high-activity range the general two-site flux equation becomes (without assuming symmetry):

\[
J = \frac{b_{32}b_{21}}{b_{12}} \frac{F_{\text{out}}}{F_{\text{in}}} \left\{ \frac{\exp[(d_2 + 2d_2)\cdot V]}{[K]_\infty} - \frac{\exp[-(d_2 + 2d_2)\cdot V]}{[K]_i} \right\}. \tag{9}
\]

Note that the ion activities appear only in the denominator of the flux equation. Further, the flux depends on the energy wells and the central energy barrier but not on the lateral barriers, as may be seen by expanding the rate constants as energy terms:

\[
b_{32}b_{21}b_{23}b_{34} = \frac{Q^* \exp(-G_{23} + G_2 + G_3 + G_4)}{b_{12}b_{43}}. \tag{10}
\]

For a symmetrical channel Eq. 9 simplifies to:

\[
J = \frac{b_{32}}{b_{12}} \frac{F_{\text{out}}}{F_{\text{in}}} \left( \frac{e^{V/2}}{\sigma_0} - \frac{e^{-V/2}}{\sigma_1} \right). \tag{11}
\]

The range of possible transport properties for the relatively simple two-site model may be appreciated from numerical solutions of the transport equations corresponding to the diagram in Fig. 2 A. In the log-log plots given in Fig. 3, the K⁺ activity of the symmetrical bathing solutions ([K]₀ = [K]₀ = [K]) increases from a low value to an extremely high value. Four different energy barrier profiles are considered: (1) a high central barrier; (2) equal barriers; (3) a high external barrier at one end; and (4) high barriers at both ends. In each case the well energies G₂ and G₃ are zero, so that an external ion activity of 1.0 begins to give significant occupation of the channel. Fig. 3 C plots the fraction of channels occupied by no, one, and two ions for the case without ionic repulsions. As [K] increases from a low level, most channels are empty; singly occupied channels increase with a slope of 1 on the log-log plot; and doubly occupied channels with a slope of 2. At [K] = 1.0, singly occupied channels reach a maximum. Finally, at higher activities, doubly occupied channels dominate, while empty channels fall with a slope of −2, and singly occupied channels with a slope of −1. These equilibrium occupancies are entirely unaffected by the heights of the energy barriers separating the energy wells. By contrast, the zero-potential conductances (Fig. 3 A) depend both on barrier heights and on the relative positions of the high and low barriers. The four energy profiles used are chosen to give identical conductances at low activities. As the K⁺ activity increases, conductance rises with a slope of 1, then peaks and, at high activity, falls again with a slope of −1, as expected from the limiting flux equations (Eqs. 6 and 9). The peak conductance and the corresponding K⁺ activity are clearly highest when the lateral barriers are the high barriers (case 4), and the peak conductance is lowest when the central barrier is the highest barrier (case 1). The explanation is again that net flux is reduced when a newly formed vacancy is preferentially filled by a particle reentering from the same side, an event facilitated by a low lateral barrier and a high central barrier.
So far we have not considered the effect of repulsion between the occupying ions. Including repulsion can change the occupancy and conductance considerably. The log-log plots on the right hand side of Fig. 3 include repulsion factors of $F_{\text{out}} = 1/F_{\text{in}} = 20$, so that loading the second site in the channel is now 400 times more difficult than before, and therefore there is a broad activity range over which almost all channels are singly occupied (Fig. 3 D). Repulsion also increases the channel conductance (Fig. 3 B) at higher activities, because it increases by a factor $F_{\text{out}}$ the rate of exit from the channel and lowers by a factor $F_{\text{in}}$ the chance that a newly formed vacancy is refilled from the same side. Depending on the barrier pattern and the amount of repulsion, the conductance

![Figure 3](image-url)
plot (Fig. 3 B) may just show a broad maximum or it may turn upwards and even rise again with a slope near one before reaching its maximum. The elevation of the conductance maximum by repulsion is much more significant, when one or both lateral barriers are the highest ones (cases 3 and 4) so exit is indeed rate-limiting, than when the central barrier is the highest (case 1). Considering again that occupancy of the channel sites (Fig. 3 D) has the same concentration dependence with each of the four barrier patterns used demonstrates clearly that the shape of the conductance-activity curve cannot be predicted from the occupancy-activity curve.

General flux equations for models with more than two sites are cumbersome to write down, but numerical results may be obtained by solving state diagrams like that in Fig. 4 A using a computer. Some log-log plots of conductance vs. activity are shown in Fig. 5 A for a three-site, symmetrical channel with higher lateral barriers, low internal barriers, and no binding ($G_w = 0$). The mean occupancy of the channels is given on a linear scale below (Fig. 5 B). As may also be shown analytically for any number of sites above one, the conductance rises linearly with activity when most channels are empty and falls inversely with activity when most channels are full. Adding repulsion spreads out the activity range over which the filling of channels occurs and can split the single maximum of the conductance into two widely separated maxima. In this example and in later calculations with the three-site model, the repulsion terms are powers of a basic term $F_{out}$ chosen to satisfy the principle of detailed balances (e.g., Hill, 1977) and the arbitrary assumption of a linear potential drop between wells as follows: for the transitions $00K \rightarrow K0K$ and $K0K \rightarrow 0KK$, $F_{out}$; for $0K0 \rightarrow KK0$, $F_{out}^2$; and for $0KK \rightarrow KKK$, $F_{out}^3$. A similar choice of repulsion terms was made by Aityan and Kalandadze (1977). The four curves in Fig. 5 have $F_{out} = \frac{1}{F_{in}} = 1, 2, 4, \text{ and } 8$. Note that eight orders of magnitude of activity are shown. It is evident that depending on what part of this vast activity range is experimentally

![Figure 4. The state diagram for flux and block of three-site channels (Fig. 1 B). (A) Eight occupancy states for channel bathed on both sides by $K^+$ ions. (B) 19 additional occupancy states when a blocking ion is added to the right side. Most of the states are unlabeled. This diagram joins to part A through the dashed lines representing the entry of the first blocking ion into the right side of the channel. In the calculations of Figs. 8, 9, and 10, these dashed transitions are assumed to be slow steps.](image-url)
accessible in a practical case, one could obtain quite a variety of conductance-activity results with multi-ion channels.

There are actually no experiments on excitable cells involving conductance measurements with symmetrically varying $[K]$. When only $[K]_o$ is increased, the conductance of delayed rectifier channels increases, although perhaps less than is predicted by independence (Frankenhaeuser, 1962; Dubois and Bergman, 1977), and the conductance of inward rectifier channels measured at a fixed displacement from $E_K$ increases as $[K]_o^{0.3}$ (Hagiwara and Takahashi, 1974 a; Almers, 1971). In order to show a conductance increase and still have the high occupancy suggested by their multi-ion properties (see later), biological potassium channels may have significant ionic repulsion and at least one high end barrier and are operating on one of the secondary rises at the high-occupancy end of the conductance curve (c.f., Fig. 5). Fig. 6 A is a log-log plot of the calculated current at 0 mV for three-site models bathed on one side by no $K^+$, and on the other by an eight order of magnitude range of $K^+$ activities. Beginning at very low activities, the current rises with a slope of 1.0 (dashed line), then passes through two inflection points and regions of slope $< 1.0$, and finally levels off to a plateau at very high activities. There is no final current decrease at the highest $[K]$, as there is when $K^+$ is added to both sides (Fig. 5). In these models, the rise of $K^+$ inward current is always monotonic on addition

---

**Figure 5.** Conductance and occupancy in a three-site channel as a function of the bathing ion activity and the ionic repulsion factor $F$. Barrier peaks 8/6/6/8 and $G_w = 0$. State diagram of Fig. 4 A. $[K]_o = [K]_i$ and $E = 0.5$ mV.
of external K⁺, whatever the internal K⁺ activity, provided that [K]ᵢ is constant. Cases 2 and 3 in Figs. 6 A demonstrate that the current (and apparent permeability) in an asymmetric channel depends on whether the ions are placed on the side with the high barrier (case 2) or with the low barrier (case 3). Parts of the same two curves up to [K] = 10⁵ have been scaled and redrawn on linear coordinates in Fig. 6 B to show how deviations from independence in an asymmetric channel might look in an experiment. Notice that not only is the behavior at high activities nonlinear, but also (case 3) it can seem not to extrapolate back to the origin because of the very steep rise of current at very low activities.

**Unidirectional Fluxes and Flux Ratio**

The ratios of unidirectional tracer fluxes in a one-ion channel at any bathing ion activity obey the Ussing equation (Eq. 1), while in a multi-ion channel they deviate in the direction suggested by Eq. 2 with a flux-ratio exponent n' larger than one. As the permeant ion activities are increased from zero, the exponent n' rises from a limiting value of 1.0 to a peak and then at very high activities falls back to 1.0 again. The maximum value of n' does not exceed the maximum number of ions permitted in a channel but otherwise bears no unique relation to the occupancy of the channel. Heckmann (1965 a, 1972) has stated the general requirement for achieving the highest values of n' in terms of two conditions: (a) the multi-ion channel should have no more than one vacancy; and (b) the vacancy should be mobile enough to move across the membrane before being destroyed by an entering external ion. We now illustrate these conclusions using the simplest single-file system, the two-site model.
The properties of unidirectional flux ratios may be determined in generality for a two-site, single-file channel by solving the rate equations corresponding to Fig. 2 B which shows the eight permitted occupancy states of a channel bathed only by K⁺ ions on the right and S⁺ ions on the left. Four transitions are one-way transitions, as they involve irreversible exit of K⁺ or S⁺ into the compartment not containing them. If K⁺ and S⁺ are identical particles except for their labels, their fluxes are the desired unidirectional tracer fluxes. Heckmann (1965a, b, 1968) has solved the more general problem of two species of identical particles bathing both sides of a two-site, single-file system, but restricted to a symmetrical channel without electrical forces (nonelectrolyte particles). Using his "correlation factor" we can derive (See Appendix) an expression for the flux-ratio exponent for a two-site symmetrical channel including repulsion but exact only for nearly symmetrical ion activities and small applied electric fields:

$$n' = 1 + \frac{2 \exp(G_{12} - G_{23})}{[F_{in}\sigma + 2][F_{in}\sigma + 1 + 2 \exp(G_{12} - G_{23})]}.$$  \hspace{1cm} (12)

At low concentration $n'$ rises from 1.0 linearly with activity:

$$n' = 1 + \frac{F_{in}\sigma}{2 + \exp(G_{23} - G_{12})}.$$  \hspace{1cm} (13)

At high concentration $n'$ falls to 1.0 inversely with activity:

$$n' = 1 + \frac{2 \exp(G_{12} - G_{23})}{F_{in}\sigma};$$  \hspace{1cm} (14)

and at an activity of

$$F_{in}\sigma = [2 + 4 \exp(G_{12} - G_{23})]^{0.5},$$  \hspace{1cm} (15)

$n'$ reaches its maximum value, given by:

$$n' = 1 + \frac{\exp(G_{12} - G_{23})}{[\exp(G_{12} - G_{23}) + 0.5^{0.5} + 1]^2}.$$  \hspace{1cm} (16)

Limiting expressions corresponding to Eqs. 13 and 14 but without assuming a symmetrical channel are given in the Appendix (Eqs. 10 a and 11 a).

Fig. 3 E and F show the concentration dependence of the flux-ratio exponent for the set of barrier profiles already discussed in the previous section calculated with $[K]_o = [K]_i$. The curves of log ($n' - 1$) vs. log [K] are symmetrical, as may be verified analytically from Eq. 12. The contrast in $n'$ for case 1 ($n'_{max} = 1.006$), with a rate-limiting central barrier, and case 4 ($n'_{max} = 1.77$), with rate-limiting external barriers, illustrates the need for high relative mobility of a vacancy within the channel to get a high flux-ratio exponent. In a symmetrical channel, the larger the barrier energy difference $G_{23} - G_{12}$, the larger is $n'$. Making only one of the lateral barriers high (case 3, $n'_{max} = 1.25$) does not increase the maximum $n'$ much over that with all equal barriers (case 2, $n'_{max} = 1.20$). Making one of the lateral barriers low, i.e., taking barrier peaks of 4/8/8 $RT$ units, lowers $n'_{max}$ to 1.014 (curve not shown). Taking a symmetrical channel with a low central barrier and then raising just one of the lateral barriers by an
additional amount has little effect. Thus, profiles of 8/4/8 and 8/4/12 both have
n'_{\text{max}} = 1.77. As all of these models have identical equilibrium occupancy-activity
curves (shown in Fig. 3 C and D), it is clear, as with conductance, that the flux-
ratio exponent cannot be predicted from the equilibrium occupancy of the
channel. Two types of ions with identical occupancies could give quite different
n' values if the barriers to them differed. Adding repulsion to the two-site
model simply shifts the curves of n' by a factor $F_{\text{in}}$ on the activity axis.

Our discussion of n' is deficient in two respects for comparison with measure-
ments on biological potassium channels. Flux-ratio exponents have not been
measured near 0 mV in symmetrical solutions, and the values of 2-2.5 obtained
away from equilibrium are higher than can be accounted for by a two-site
channel. The question of measurements away from equilibrium is easily studied
numerically. Case 4, which gave $n'_{\text{max}} = 1.77$, was recalculated with symmetrical
solutions but at an applied potential of 100 mV. The log-log plot of n' had a
peak with $n'_{\text{max}} = 1.67$ at just slightly higher activities than in the original
calculation. Then case 4 was calculated once again with a 10-fold activity
gradient and no applied potential. The plot now had a peak with $n'_{\text{max}} = 1.73$ at
a point where the geometric mean of the activities was about equal to the
original peak activity. Thus, it seems safe to consider that n' is not very sensitive
to the deviations away from equilibrium used in typical experiments. In the
Appendix we show that n' can be measured at equilibrium by a method that
may at times be easier than a flux-ratio measurement. Calculation of flux ratios
with more than two sites by diagram methods involves expressions too large for
our laboratory computer at present, and we have not tried the other more
practical methods. Certainly the n' values can be made larger, and we anticipate
that repulsion would not simply shift the n' curves in these cases but would
rather split them into steps, something like the conductance curves in Figs. 3 A
and 5, corresponding to regions of double occupancy, triple occupancy, etc.
Biological potassium channels with n' values in the range 2-2.5 for K⁺ ions
might have at least three internal sites with easy internal mobility and a high
barrier at each end, and would have occupancies of at least two and three K⁺
ions.

In multi-ion channels without blocking ions (see later), the unidirectional
tracer fluxes from one compartment increase on addition of ions to that
compartment and decrease on addition of ions to the other compartment
(Heckmann, 1965 b). Fig. 7 A shows unidirectional fluxes for a two-site channel
as $[K]_o$ is increased over six orders of magnitude and $[K]_i$ is held constant at
10³. The calculations were done at two values of the membrane potential. When
$[K]_o$ becomes high enough, unidirectional efflux begins to fall, ultimately with a
maximum slope of $-1$ on the log-log plot. At very low $[K]_o$, the unidirectional
influx rises with a slope of 1, but in the middle activity range, the slope increases
to a maximum of 1.52 and then decreases again, ultimately to 0. Unidirectional
fluxes that depend on a power higher than 1.0 of the “cis” concentration are
characteristic of multi-ion, single-file pores under conditions when the pore is
already partly occupied by ions from the “trans” side (in contrast to Fig. 6 where
there are no “trans” ions). Heckmann (1968, 1972) says that the concentration
dependence can be as steep as $[K]^m$, where m is the number of sites in the pore.
The unidirectional flux curves up to $[K]_o = 10^3$ have been scaled and replotted on linear coordinates in Fig. 7 B, as they might appear in an experiment. The efflux curves are similar to those measured in squid giant axon by Begenisich and DeWeer (1977). The upward curvature of the influx curves reflects again the multi-ion nature of the channel.

This concludes the description of transport properties when there is only one relevant cation species in the bathing solutions. We turn now to a variety of cases with two types of ions.

**Blocking Ions**

The transport of permeant ions can be blocked by other ions stuck in some sites of the channel. These blocking ions might bind to one or more sites, or they might be trapped because they have proceeded part of the way across the membrane and then encounter an impassable barrier from which they cannot retreat because other ions have followed behind. We consider the particular case of a monovalent blocking ion $B^+$ on one side of the membrane which can enter the channel and cross all barriers but the last one. The block is then voltage-dependent, inasmuch as the probability of finding one or more $B^+$ ion in the channel is influenced by the electric forces on $B^+$ ions coming from one side and on $K^+$ ions coming from both sides of the membrane. Fig. 2 C shows a state diagram for the two-site version of this blocking scheme with the blocking...
ion in the inside compartment. Blocking adds five new states. Fig. 4 B shows in an abbreviated form the 17 blocked states obtained in a three-site blocking model. In each case there are dead-end branches of the diagram, terminating in fully occupied channels with a blocking ion stuck at the outer impassable barrier.

Although the diagrams of states are complicated, some simple properties can readily be extracted from limiting cases. Of particular interest are the conditions needed for steep voltage-dependent block and for unblock by permeant ions coming from the other side of the membrane. The distribution coefficient \( R_B/1 - R_B \) between blocked and unblocked states is a useful measure of block. Consider a channel with equal barrier spacings \( d_i \)'s equal), uniform wells for K\(^+\), and uniform wells for B\(^+\), and let the reduced B\(^+\) activity be represented by \( \theta \) and the quantity \( \exp (V) \), by \( \beta \). When the reduced activity \( \sigma \) of K\(^+\) ions is very low, the distribution coefficient for a two-site model is (see Appendix):

\[
\frac{R_B}{1 - R_B} = \frac{\theta \beta^{1/3} + \theta \beta^{2/3} + \frac{F_{in}}{F_{out}} \cdot \theta \beta}{1 - \frac{F_{in}}{F_{out}} \cdot \theta \beta}.
\]

(17)

Depolarization favors block, but because \( \sigma \) is low, the K\(^+\) activities do not affect the block. For higher numbers of barriers, the distribution coefficient is still a series with powers of \( \frac{F_{in}}{F_{out}} \), \( \theta \), and \( \beta \), and the maximum power of \( \beta \) is \( m/2 \), where \( m \) is the number of sites. In a channel where the \( d_i \)'s are not equal, the maximum power of \( \beta \) at low \( \sigma \) can range from 0 to \( m \) as the binding sites are moved from an extreme of all sites internal to the electrical potential drop to all sites external. Any symmetrical set of \( d_i \)'s gives \( m/2 \).

A more interesting result is obtained at the opposite limit, at extremely high \( \sigma \), where the maximum power of \( \beta \) is \( m - 1 \), independent of the \( d_i \)'s, and there is both competition and unblock from the permeant ion. The distribution coefficient for a two-site model with uniform wells and arbitrary \( d_i \)'s is:

\[
\frac{R_B}{1 - R_B} = \frac{\theta_i \beta^{1/3} + \theta_i \beta^{2/3} + \frac{F_{in}}{F_{out}} \cdot \theta \beta}{1 - \frac{F_{in}}{F_{out}} \cdot \theta \beta}.
\]

(18)

and for a four-site model it is:

\[
\frac{R_B}{1 - R_B} = \frac{\theta_i \beta^{1/3} + \theta_i \beta^{2/3} + \frac{F_{in}}{F_{out}} \cdot \theta \beta}{1 + \left( \frac{\theta_i + \sigma_i}{\sigma_o} \right) \beta + \left( \frac{\theta_i + \sigma_i}{\sigma_o} \right)^2 \beta^2 + \left( \frac{\theta_i + \sigma_i}{\sigma_o} \right)^3 \beta^3}.
\]

(19)

The high powers of \( \beta \) indicate a steep voltage dependence of block. When the \( \sigma \)'s are very high, repulsion factors don't appear because they all cancel, and it is not even essential for B\(^+\) to cross any of the membrane field to give voltage-dependent block. The terms of Eq. 19 arise respectively from the formation of the filled blocking complexes KKKB, KKXB, KBXX, and BXXX, where \( X \) is either B\(^+\) or K\(^+\), and each arises from the preceding one by a process involving shifting in the outward direction of the entire pore contents by one site and loss of an external K\(^+\) and gain of an internal K\(^+\) or B\(^+\). Eq. 19 may be written in another more general form to show that the voltage-dependent part of the block is a function of the deviation \( E - E_X \) from the potassium equilibrium potential and depends inversely on the K\(^+\) activity (\( \sigma \)) on the side of the membrane "trans" to
the blocking ion:

$$R_B = \frac{\theta_i}{\sigma_1} \left\{ \frac{1}{1 + \sum_{j=1}^{m-1} \frac{\theta_i}{\sigma_o} \exp(V) + \exp(V - V_b)} \right\}.$$ (20)

Thus, in the range of high $\sigma$, adding external K+ ions relieves block by an internal blocking ion. This unblocking effect in turn can lead to a crossover of the current-voltage relations above $E_K$. The intimate interdependence of block and the driving force on K+ ions arises simply from the law of mass action for occupancy and is quite independent of repulsion effects.

Armstrong (1969, 1975 a, b) has proposed that the normal inward rectification of the inward rectifier arises from an unknown cellular cation or gating particle that acts like a blocking ion. Indeed the major features of inward rectification can be duplicated by the multi-ion blocking model, as may be seen from the current-voltage relations in Fig. 8 calculated for a three-site channel with a K+ occupancy near 2. In the calculations, the inside [K] is held constant and the outside [K] is set at 1, 3, 10, 30, and 100% of the inside value. The current-voltage relations show inward rectification depending on $E - E_K$, with good qualitative resemblance to measurements on vertebrate skeletal muscle (Adrian and Freygang, 1962; Almers, 1971), heart muscle (Noble and Tsien, 1968), and electric organ (Nakamura et al., 1965) and on echinoderm egg (Hagiwara and Takahashi, 1974 a). Quantitatively, however, the steepness of the rectification in the model is too small. Fig. 9 A compares the probability, 1-$R_B$, of unblocked channels in this model (squares) with a smooth curve derived from the simple

![Figure 8](image-url)
empirical equation, Eq. 5, used by Hagiwara and Takahashi (1974 a). The effective valence $z'$ of the smooth curve is only 1.5, much smaller than the value of 2.3 seen with the inward rectifier of frog skeletal muscle (Adrian and Freygang, 1962) or of 2.9-3.6 seen with echinoderm eggs (Hagiwara and Takahashi, 1974 a; Hagiwara et al., 1977). More reasonable values could be obtained by increasing the number of ion sites in the channel to 4 for muscle and 5 for eggs, but the diagram method we use is probably not suitable for these more complex cases. We did investigate the effect of increasing the valence of the blocking particle. Making the particle divalent increases the effective $z'$ dramatically, but then the electrical forces on $B^{++}$ and $K^+$ no longer match and the block shifts much less than the change of $E_K$ when external $[K]$ is increased.

Figure 9. Voltage dependence of block and activity dependence of conductance in the three-site model of Fig. 8 with an internal blocking ion. (A) Fraction of channels not blocked (squares) as a function of voltage in the calculation when $[K]_o = [K]_+ = 10^5$. Smooth curve calculated from Eq. 5 with $z' = 1.5$ and $\Delta E_b = -10$ mV. (B) Chord conductance, $I_K/(E - E_K)$, at a potential 50 mV more negative than $E_K$ (triangles). Smooth curve is a line with slope 0.40 in the log-log plot.

The chord conductance of inward rectifiers measured at a fixed displacement from $E_K$ is reported to increase as the square root of $[K]_o$ (Hagiwara and Takahashi, 1974 a; Almers, 1971). Similarly (Fig. 9 B) the conductance in the model calculation of Fig. 8 increases as $[K]_o^{0.4}$.

The multi-ion model can also be used to imitate the effect of known blocking ions like $Cs^+$ and other small ions on potassium channels. If the blocking model is turned around to consider blocking ions in the external solution which can cross all but the innermost barrier of the channel, then $K^+$ current in the inward direction is blocked. Fig. 10 shows current-voltage relations in a three-site channel bathed in solutions with high equal $K^+$ activities ($K = 10^5$) and with an external blocker activity $[B]_o$ of 0, 8, 40, 200, 1,000, and 5,000. The result closely resembles the effect of external $Cs^+$ ion on the inward rectifier channel of
echinoderm eggs (Hagiwara et al., 1976) and frog skeletal muscle (Gay and Stanfield, 1977). With the parameters used, the model shows slightly steeper voltage dependence \( z' = 1.8 \) than is seen with external Cs\(^+\) blocking inward rectifiers \( z' = 1.4-1.6 \) and a roughly similar dependence on the activity of the blocking ion. Rotation of Fig. 10 by 180° would give the concentration dependence of block of outward currents by internal blocking ions in this model. The analogous experiments with a variety of blocking ions on delayed rectifier channels often show block with an effective valence \( z' \) between 0.2-1.6 (Bezanilla and Armstrong, 1972; Hille, 1975b), but with some small ions the block seems to be decreased again at very positive potentials and the current then increases steeply (French and Wells, 1977). We have not explored multi-

![Figure 10](image)

**Figure 10.** Current-voltage relations in a three-site model with several concentrations of external blocking ion B. The blocking ion cannot cross the innermost potential barrier. \([K]_0 = [K]^+ = 10^5\). For the unlabeled curve \([B]_0 = 0\). Barriers 8/4/4/8. \(F = 4\). Calculations with the state diagrams of Fig. 4 A and B, except that the sequence of ions read from left to right in each state corresponds to the sequence from inside to outside in the channel.

ion channel models in which the blocking ion is actually very slightly permeant (Adrian, 1964; French and Wells, 1977) to imitate these phenomena better. The crossover effects (Bezanilla and Armstrong, 1972; Hille, 1975b; Adelman and French, 1978) seen when added K\(^+\) relieves block by blocking ions applied to the opposite side are, of course, explained by the model.

**Permeability Ratios and Mole-Fraction Effects with Two Permeant Ions**

We have seen that the effects of ion activity on conductance and on flux-ratio exponent could be discussed in terms of simple limits at low and high activity and a complex behavior in the middle. The same holds true for zero-current potentials and permeability ratios with mixed ions. For the bi-ionic case with pure K\(^+\) on the inside and pure ion S\(^+\) on the outside, the state diagram of Fig.
2 B applies. At the low-activity limit the bi-ionic flux ratio becomes
\[
\frac{J_K}{J_S} = \exp(V) \frac{[K]}{[S]}
\]
\[
= \frac{2\exp[G_{34S} + (d_4 + d_2)\cdot V] + \exp[G_{23S} + (d_1 - d_3)\cdot V]}{\exp[G_{34K} + (d_4 + d_2)\cdot V] + \exp[G_{23K} + (d_1 - d_3)\cdot V] + \exp[G_{12K} - (d_2 + d_3)\cdot V] + \exp[G_{12S} - (d_2 + d_3)\cdot V]},
\]
(21)
where the different barriers for K and S ions are distinguished by subscripts. At the high-activity limit the bi-ionic flux ratio depends only on the electrochemical activity ratio and on the difference between the central barriers:
\[
\frac{J_K}{J_S} = \exp(V) \frac{[K]}{[S]} \{\exp(G_{23S} - G_{23K})\}.
\]
(22)
Eqs. 21 and 22 are clearly not identical and show that the bi-ionic permeability ratio \(P_K/P_S\) changes with activity. This ratio is defined by the zero-current potential (where \(J_K/J_S = 1\)),
\[
\exp(-V_{rev}) = \frac{[K]}{[S]} \frac{P_K}{P_S},
\]
(23)
and is given by the terms in braces, \{ \}, in the two expressions. Indeed, \(P_K\) may be larger than \(P_S\) under one set of ionic conditions and smaller under another. At very low activity \(P_K/P_S\) depends on all high barriers, which in a practical case could turn out to be the lateral barriers, whereas at very high activity, only the central barrier counts. This result is quite different from that with a one-ion channel (even with several sites) where Läuger (1973) showed that the expression for \(P_K/P_S\) at all ion activities is the same as that for the low-activity limit.

In the mid-activity range the expressions for bi-ionic flux ratio include terms with the depth of energy wells (Heckmann, 1965 b; Hladky, 1972) so that even in a channel with energy barriers giving identical limiting permeability ratios at very low and very high activity, the permeability ratio may be different at intermediate activity. Some of the possibilities are shown as plots of \(E_{rev}\) vs. the logarithm of activity in Fig. 11. Each line is calculated for a different symmetrical, two-site channel model with high end barriers to both types of ion and with repulsion factors of \(F_{out} = 1/F_{in} = 20\). The channel is bathed on one side by pure \(K^+\) and on the other side by pure \(S^+\) at the same activity, and the activity on both sides increases by six orders of magnitude from a very low value to a very high value. In Fig. 11 A only the relative height of the low, central energy barrier to \(S\) is varied, and therefore \(E_{rev}\) (and hence \(P_K/P_S\)) has similar behavior in the four cases at low and intermediate activities and the major divergences occur on approaching the high-activity limit. In Fig. 11 B only the depth of the wells is varied, and now both limiting \(E_{rev}\) values are invariant and the divergences occur in the intermediate activity range. The crossing of two of the curves in this example shows that, depending on ionic activities, increasing the binding of an ion may increase or decrease the relative permeability for that ion.
At the zero-current potential, the probability that an ion moves completely across the membrane to the right exactly equals the probability that an ion moves completely across the membrane to the left. With multi-ion channels these probabilities depend on competition for sites within the channel and are influenced by mobilities, binding strengths, and also the composition of the bathing solutions. Anything that changes the relative probabilities of the different occupancy states can affect the permeability ratios. Therefore, a potential measurement with mixed salt solutions on one or both sides need not give the same permeability ratios as a bi-ionic potential measurement with pure salts on the two sides, even if the total salt activities are the same in the two measurements, i.e., both the total activities and the mole fraction of the bathing ions can affect the permeability ratio. Fig. 12 A shows zero-current potential and conductance calculations for a two-site model with pure K\(^+\) inside and K\(^+\) + S\(^+\) mixtures on the outside. As the mole-fraction of S\(^+\) cation is increased while keeping the total ion activity constant, both \(E_{\text{rev}}\) and \(g\) pass through a minimum similar to the "anomalous mole-fraction" effect reported with echinoderm eggs bathed in K\(^+\) + Tl\(^+\) mixtures. (Hagiwara et al., 1977).

The anomalous mole-fraction effect arises from correlations among occupancy states but does not require that the K\(^+\) or S\(^+\) ions have special barrier-modifying properties. With different choices of energy profiles there may be no anomalous effect, a minimum or maximum of \(g\) only, a minimum or maximum of \(E_{\text{rev}}\) only, or an extremum of both, as in the case of Fig. 12. Empirically we find that to give an extremum the channel must be of the type that gives high \(n'\) values and must have significant occupancy. Repulsion is necessary for an
extremum of $g$, but not for an extremum of $E_{rev}$. To have a minimum in both simultaneously, as in Fig. 12, requires that the ion with the lower bi-ionic permeability ($K^+$ here) have the stronger binding. The conductance for inward current then decreases in mixtures because a single $K^+$ ion in the channel gets stuck for a long time. As Dr. S. B. Hladky has pointed out to us, entering $S^+$ ions are quickly repelled back over their low barrier whence they came, and only another entering $K^+$ ion has a long enough lifetime in the channel to have a high probability of expelling the original $K^+$ ion in the direction needed to get inward current. Fig. 12 B shows that underlying the extremum of $E_{rev}$ there is actually a relatively simple, monotonic change of the permeability ratio $P_S/P_K$, defined by:

$$\exp(V_{rev}) = \frac{[K]_o + (P_S/P_K)[S]_o}{[K]_i} = \frac{[K]_o + [S]_o + [K]_i}{[K]_i}.$$

Figure 12. Anomalous mole-fraction dependence of potential and conductance in an asymmetrical two-site channel with two permeant cations. The external solution is a mixture of $S^+$ and $K^+$ with constant total activity, $[K]_o + [S]_o = 300$. The internal solution has only $K^+$ with $[K]_i = 300$. (A) Zero-current potential ($E_{rev}$) and chord conductance at 50 mV more negative than $E_{rev}$. Barriers and wells from outside to inside: $K^+$, 8.5/−1/4/−1/10 (thin line); $S^+$, 8.5/2/4/1/4 (thick line). $F = 30$. Calculations from a state diagram like Fig. 2 B but with state KS and five more transitions added. (B) Permeability ratio calculated using Eq. 24 and the $E_{rev}$ values in part A.
An extremum of $E_{rev}$ requires a fairly steep mole-fraction dependence of $P_s/P_K$ or a crossing of unity, but does not require an extremum of $P_s/P_K$ itself.

**DISCUSSION**

*Potassium Channels Are Multi-Ion Pores*

The pivotal observation of Hodgkin and Keynes (1955) has by now been extended to include a constellation of transport properties for potassium channels that seems impossible to explain in terms of a one-ion pore or one-ion carrier model. This includes a unidirectional flux-ratio exponent $>1.0$, an effective valence of blocking reactions $>1.0$, properties dependent on $E-E_K$ rather than on the membrane potential alone, and permeability ratios depending on the ionic composition of the bathing solutions. Each of these properties is readily obtained with multi-ion pores, and to get them all requires a significant probability of multiply-occupied states, some degree of single-file restriction, repulsion between the ions, and lower barriers for movement within the channel than for exit from the channel. The properties of real potassium channels also suggest at least three internal sites. The energy required to place three mutually repelling ions in one channel might seem prohibitive (Levitt, 1978a), but could well be offset by fixed negative charges or dipoles within the channel as well as by counterions attracted to or associating with the ends of the channel (Eisenman et al., 1978) which would be equivalent to making the energy wells for cations in the channel deep. In that case, the energy required to remove all the cations from the channel could seem prohibitive. This concept is similar to the classical fixed-charge membrane except that single-file diffusion with highly correlated ion movements is also essential. The single-file restriction must in part arise simply from ionic repulsion as well as from the steric constraints imposed by the pore and the water molecules in it. Hence, the mechanical pore diameter could still be larger than two K$^+$-ion diameters throughout much of the channel.

Delayed rectifier potassium channels are generally thought to be single-file pores (Armstrong, 1975a, b). Be of the similarities between delayed rectifiers and inward rectifiers and because of the ease of explaining inward rectification by Armstrong's (1969, 1975a, b) hypothesis of a charged blocking particle, we suggest that the inward rectifier channel is a single-file pore as well. During a step hyperpolarization from $E_{rev}$, inward rectification in frog skeletal muscle and echinoderm egg has some time dependence, part of the conductance increase appearing by the time the first measurement can be made, and the remainder developing over a period 5-1000 ms (Almers, 1971; Hagiwara et al., 1976). This time-course, like the steady state, depends on $E-E_K$. We have not investigated the transient properties of multi-ion blocking models in detail, but Kohler (1977) has given transient solutions for one three-site model with internal blocking particles and a few general predictions can be made. In a hyperpolarization that produces unblock, the conductance increase can have fast, slow, and delayed components as the various blocked states (Fig. 4) are cleared out. The time-course will depend on the initial conditions. For a large depolarization that produces block, the conductance decrease should be a single exponential as the first blocking particle enters each channel. Particularly in the middle of the
region of rectification (near $E_{\text{rev}}$), the potassium current should be fluctuating with a detectable variance which could be used to obtain the single channel conductance. Armstrong's hypothesis also suggests that experiments could be done to try to identify the hypothetical blocking particle in myoplasm. Conceivably, however, the particle is covalently attached to the channel rather than being completely freely diffusible (Armstrong, 1975 a).

Several experiments seem worth pursuing on the delayed rectifier of perfused squid giant axons including a study of the concentration dependence of the channel conductance in symmetrical solutions ($[K]^i = [K]^o$), a search for mole-fraction and concentration effects on permeability ratios, and further measurements of flux-ratio exponents $n'$ with several types of permeant cations. Preferably $n'$ should be measured with only one cation present, as any interpretation of permeability with mixtures is formidable (Heckmann, 1965 a, b, 1972). In addition, it would be useful to have a more complete description of the voltage dependence of blocking effects with small cations. These experiments might give a clearer picture of the sites and barriers in delayed rectifier channels. However, any model complex enough to give a good fit may have more free parameters than could be uniquely determined given the constraints of biological experimentation.

Other Channels May Be Multi-Ion Channels
Although potassium channels are by far the best known example, other channels also seem to have properties of multi-ion channels. The available data suggest that the chloride channel of frog skeletal muscle has an $n'$, determined by Eq. 5 $a$, > 1.0 (Hodgkin and Horowicz, 1959). Chloride channels of elasmobranch and frog skeletal muscle show anomalous mole-fraction dependence of conductance but not of zero-current potential in $\text{Cl}^-\text{-SCN}^-$ mixtures (Hagiwara and Takahashi, 1974 b; Hutter and Padsha, 1959). Hence, we believe that some chloride channels are multi-ion channels and that interpretation of their complex properties would be facilitated by using such models (see further references in French and Adelman, 1976). Historically the sodium channel has been regarded as the prime example of a system obeying independence. However, it shows clear deviation from independence (Hille, 1975 a, b) and even a hint of multi-ion properties. The $P_{\text{Na}}/P_{\text{K}}$ ratio depends on the internal K$^+$ concentration (Chandler and Meves, 1965; Cahalan and Beginisich, 1976; Ebert and Goldman, 1976); the internally acting blocking cation strychnine is less effective with high external Na$^+$ than in the absence of external Na$^+$ (Shapiro, 1977); and crossover of outward currents can be observed on changing the external cation.$^1$ The Ca$^{2+}$ permeability of Na channels may increase on removing most permeant monovalent ions (Meves and Vogel, 1973), and conversely the Na$^+$ permeability of calcium channels may increase on removing divalent ions (Kostyuk and Krishtal, 1977).

Some clear examples of multi-ion properties outside of excitable cells are found with the gramicidin A channel (Hladky and Haydon, 1972; Neher, 1975; Eisenman et al., 1977, 1978; Shagina et al., 1978) and hydrated ion-selective glass

$^1$ Hille, B. Unpublished observation.
(Eisenman et al., 1967). Indeed, several authors have already discussed multi-ion models in some detail for gramicidin A (Hladky, 1972; Sandblom et al., 1977; Eisenman et al., 1977, 1978; Levitt, 1978a, b) and that system is likely to be the best described single-file pore for some time. Gramicidin A pores have a flux-ratio exponent higher than one, concentration and mole-fraction-dependent permeability ratios, and a conductance rising in several stages and finally falling again as the permeant ion concentration increases. Although the channel is made of neutral molecules, it can hold more than one cation at a time.

**Other Multi-Ion Models Are Possible**

The type of model we have used has a small number of fixed barriers and binding sites that represent all interactions of the ion with its environment in crossing the membrane. Movements between sites are represented as elementary transitions given by rate theory with work terms linear in potential and with repulsion terms included in a simple arbitrary manner. We view these assumptions as idealizations of a more complicated many-body problem involving the continuous correlated motions of many water molecules, several ions and counterions, and a macromolecular channel, all of which are in close contact and interacting. Probably the only important general features of the model are that there are several mutually repelling ions in the channel constrained to move more or less in single file and driven by thermal and electrical forces. Thus, we imagine that a similar success might be obtained using models with some knock-on character (Hodgkin and Keynes, 1955), or with a continuum approach that takes ion correlations into account, or with a more flexible or fluctuating view of the barriers to ionic motion. For example, the model of Sandblom et al. (1977) explains mole-fraction effects by supposing that each species of bound ion has a different, specific influence on the diffusion barriers in the channel. This is certainly possible in potassium channels as well, although it is not required to give mole-fraction effects.

An important practical advantage of the type of models introduced by Heckmann (1965a, 1972) and used here is that they are simple enough to calculate and yet they give qualitatively the spectrum of flux properties reported for biological potassium channels. For the present there may not be much justification for attempting much more sophisticated modelling of potassium channels, as one would require much more detailed information than is available on the channel to fix the many resulting parameters.

**APPENDIX**

**Implementation of Diagram Method**

The major task in obtaining the steady-state flux expressions for any of the problems discussed is to determine the steady-state probabilities $R_j$ for the occupancy states of the channel. For example with the three-site system of Fig. 4 A the numerator of the expression for each $R_j$ contains the sum of 288 products of seven rate constants and the denominator contains the sum of the numerators for the eight different states. We used the diagram method of King and Altman (1956; Hill, 1977) which, in the language of graph theory, constructs the terms of $R_j$ from the topology of the appropriate state
diagram (e.g., Figs. 2 and 4) by a one-to-one mapping of each term onto each maximal “directed tree” that can be constructed to state $j$. Maximal trees are all the noncyclic partial diagrams that may be formed from the state diagram by dropping all but $s-1$ of the reaction steps, where $s$ is the number of states. To shorten expressions and to save calculation time, it is always desirable to start with the simplest state diagram that is adequate. This involves eliminating all arrows and states not used in particular problem and then “reducing” the diagram by exact methods that simplify treatment of dead-end inhibitory steps (Cleland, 1963), of parallel steps (Volkenstein and Goldstein, 1966), and of reversible steps preceding an irreversible step (Cleland, 1975; Stein, 1976), and where permissible, by approximate methods that simplify the treatment of transient intermediate states of low occupancy and of diagrams with a clear separation between slow and fast transitions (Hill, 1977; Chizmadjev and Aityan, 1977; Aityan et al., 1977).

In some cases after the diagram had been simplified, the flux equations could be written down by inspection and manipulated algebraically to give the desired quantities. However, for more complex cases (e.g., two-site channels with two permeant species and all problems with three-site channels), the directed trees were generated by the computer algorithm of Heckmann et al. (1968) and stored in symbolic form on the floppy disc of our computer. Numerical evaluations were done later by calculating the products and sums represented by the list of directed trees. Even with the computer this could be a slow process, because for the full three-site model > 16,000 multiplications go into an evaluation of the denominator of the $R_j$'s at one voltage and concentration.

The specific simplifications made in deriving equations in the text are now listed. The very high-activity behavior of the two-site model (Eqs. 9, 18, 22, and 11a) was obtained by omitting the empty state 00 from the relevant state diagram in Fig. 2, writing down the trees and flux equations, and then dropping small terms. Blocking ion effects (Eqs. 18 and 19) are simple dead-end inhibition in this approximation. At very low activities, blocking effects (Eq. 17) are again dead-end inhibition added now to state 00 of a diagram without state KK. However, the low-activity flux ratio needed for Eqs. 6a and 10a requires more complete treatment; the fluxes through states KK, SS, and SK of Fig. 2 B must be retained but can be treated as fluxes through transient states of low occupancy (Hill, 1977). Finally, small terms are dropped to get Eq. 6a. All calculations shown in the figures used exact methods rather than the approximations used to get the equations in the text, except that in solving the three-site model with block (Figs. 8 and 10), the entry and exit of blocking ions (dashed steps in Fig. 4 B) were assumed to be much slower than the movements of $K^+$ ions. This permits all blocked states to be included as simple dead-end inhibition. Conductances in Figs. 3 and 5 were calculated from the fluxes with an applied potential of 0.5 mV, and $n'$ in Fig. 3 was calculated from unidirectional flux ratios (diagram of Fig. 2 A) at 0.5 mV. Zero-current potentials in Figs. 11 and 12 were obtained by six iterations of the Newton-Raphson method on the appropriate full flux equations. The bi-ionic case used the diagram of Fig. 2 B, and the mixed ion case added to this the state KS and five more transitions. Both of these diagrams were first simplified by the exact method of replacing several reversible steps by equivalent irreversible ones.

**Flux Ratio Exponent**

This section describes the relation between the correlation factor (Heckmann, 1965 b) and the flux ratio exponent and gives a method for deriving expressions for $n'$. The correlation factor $f$ is defined by Heckmann as the ratio of the tracer permeability $P^*$ at equilibrium to the permeability $P_{net}$ in the face of a net electrochemical driving force. Consider a membrane with no applied electric field and with an ion distribution very close to equilibrium, and let $a$ and $a + \delta a$ be the activities of an ion or nonelectrolyte.
molecule and let $J$ and $J + \delta J$ be the unidirectional fluxes. Then the net permeability becomes $\delta J / \delta a$, and the equilibrium tracer permeability may be expressed as $J/a$, leading to the correlation factor:

$$f = \frac{P^*}{P_{\text{net}}} = \frac{J}{a} \frac{\delta a}{\delta J},$$

(1a)

From Eq. 2 the unidirectional flux ratio for a deviation from equilibrium is:

$$\frac{J}{J} = \frac{J + \delta J}{J} = 1 + \frac{\delta J}{J} = \left(1 + \frac{\delta a}{a}\right)^{n'} = \left(1 + \frac{\delta a}{a}\right)^{\nu}. \quad (2a)$$

When $\delta a / a$ is very small, i.e., near equilibrium, Eq. 2a may be simplified:

$$\frac{J}{J} = 1 + \frac{\delta J}{J} \approx 1 + n' \frac{\delta a}{a}. \quad (3a)$$

Solving for $n'$ shows that $n'$ is the reciprocal of $f$:

$$n' = \frac{\delta J}{J} \frac{a}{\delta a} = 1 - \frac{P_{\text{net}}}{P^*} \quad (4a)$$

Hodgkin and Keynes (1955, p. 79) give a completely analogous expression which relates $n'$ to the ionic conductance, $g$, and the equilibrium tracer flux, $J$:

$$n' = \frac{g}{J} \cdot \frac{RT}{F^2}, \quad (5a)$$

at the equilibrium potential. Eqs. 4a and 5a ought to be easier to apply to many experimental situations in measuring $n'$ than the original defining relationship Eq. 2.

Expressions for $n'$ may be obtained from flux diagrams either by the correlation-factor route or by the flux-ratio route. Heckmann (1965b) gives the correlation factor for a symmetrical, two-site channel with no electrical forces. In translating his expression into our notation, Eq. 12, we inserted repulsion factors $F_{\text{in}}$ by inspecting all the flux diagrams (Hill, 1977) of Fig. 2 B needed for forming the bi-ionic flux ratio $J_K / J_S$ and noting that all repulsion terms cancel out, except in every tree with a $[K]$ or $[S]$ term where one $F_{\text{in}}$ remains.

We also derived limiting expressions for $n'$ directly, without assuming channel symmetry. For low activities the bi-ionic flux ratio can be simplified to a form containing the limiting bi-ionic permeability ratio defined in Eq. 21:

$$\frac{J_K}{J_S} = \exp(V) \frac{[K] P_K}{[S] P_S} \frac{1 + Y_K [K]}{1 + Y_S [S]}, \quad (6a)$$

where the $Y$'s are voltage-dependent expressions. If $K^+$ and $S^+$ are identical ions of activity $a + \delta a$ and $a$, and if there is no electric field, Eq. 6a reduces to:

$$\frac{J_K}{J_S} = \left(1 + \frac{\delta a}{a}\right) \frac{1 + Y (a + \delta a)}{1 + Ya}. \quad (7a)$$

Then, making approximations that $Ya$ is small and $(\delta a)^2$ is negligible gives:

$$\frac{J_K}{J_S} \approx \left(1 + \frac{\delta a}{a}\right) (1 + Y \delta a) \approx 1 + (1 + Ya) \frac{\delta a}{a}. \quad (8a)$$
Finally, comparison with Eq. 3 \( a \) permits the identification at low activity:
\[
\eta' = 1 + Y_a. \tag{9 a}
\]
Applying this method to a two-site channel at low activity and 0 mV gives:
\[
\eta' = 1 + \frac{F_{in}[K]}{\exp(G_{12}) + \exp(G_{23}) + \exp(G_{34})} \frac{\exp(-G_{34}+G_a)}{\exp(-G_{12}+G_3) + \exp(-G_{12}+G_2)}. \tag{10 a}
\]
A similar method at high activity and 0 mV gives:
\[
\eta' = 1 \frac{1}{F_{in}[K]} \left[ \exp(G_{34} + G_2) + \exp(G_{12} + G_3) \exp(-G_{23}) \right]. \tag{11 a}
\]
Unlike Eqs. 13 and 14, these two expressions apply even to asymmetric barriers.

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