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A Model for Anomalous Rectification: Electrochemical-Potential-Dependent Gating of Membrane Channels

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Summary. A model is presented for "anomalous rectification" based upon electrical measurements on the egg cell membrane of the starfish. The objective is to postulate a plausible molecular mechanism which yields an expression for the conductance similar to that deduced empirically by Hagiwara and Takahashi (1974), i.e.,

$$G_K = \frac{B c_K^{1/2}}{1 + \exp\left(\frac{\Delta V - \Delta V_h}{v}\right)},$$

where B, ΔV_h and v are constant, c_K is the external K^+ concentration, and $\Delta V (= V - V_0)$ is the displacement of the membrane potential from its resting value. It is shown that a similar dependence of the conductance on ΔV is expected for a particular class of models in which the K^+ ions are also implicated in "gating". To give a specific example, we consider the case in which the formation of ion-permeable pores requires a voltage-induced orientation of membrane-bound, electrically-charged groups and subsequent complexation of these groups with the external cations. Furthermore, the proportionality between G_K and $c_K^{1/2}$, when the internal K^+ concentration is constant, is accounted for by conventional descriptions of the ionic fluxes using Eyring's rate reaction theory. In terms of the present model, B and ΔV_h are explicit functions of the internal K^+ concentrations and are thus constant only as long as this is unvaried. The particular value of v required to fit the data ($v \simeq 8.4$ mV) is rationalized by the assumption that each of the orientable groups carries three negative elementary charges. In addition, the predictions of the present model are compared with those deduced from an alternative viewpoint, which is related to Armstrong's "blocking particle hypothesis", in that the probability for opening and closing of the pore is assumed to depend on whether the pore is occupied or empty. Differences and similarities between the two models, as well as ways to discriminate between them, are discussed.

A. Introduction

Katz (1949) studied the electrical characteristics of frog twitch muscle fibers in an isotonic K_2SO_4 solution. Despite the fact that in this

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condition the external and internal K^+ concentrations are almost identical, he found that the membrane conductance was much higher for inward than outward current. This phenomenon has been referred to as "inward-going" or "anomalous" rectification, and its electrophysiological properties have been studied in detail (Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962*a–b*; Nakajima, Iwasaki & Obata, 1962; Adrian, 1964, 1969; Horowicz, Gage & Eisenberg, 1968; Adrian, Chandler & Hodgkin, 1970; Almers, 1971, 1972).

Recently, a similar rectification was found in other tissues, namely, in the egg cell membrane of a tunicate (Takahashi, Miyazaki & Kidokoro, 1971; Miyazaki *et al.*, 1974), and also of certain starfish (Hagiwara & Takahashi, 1974; Miyazaki, Ohmori & Sasaki, 1975; Hagiwara, Miyazaki & Rosenthal, 1976). An extensive characterization of the permeability properties of these cells has been carried out, leading to the discovery of some analogies with those of skeletal muscle fibers.

This paper deals with speculations on a possible mechanism for "anomalous rectification" and develops a quantitative description which accounts for the steady-state electrical properties of starfish egg cell membranes when K^+ is the only permeant ion in the bathing medium.

The relevant properties can be summarized as follows (Hagiwara & Takahashi, 1974; Hagiwara *et al.*, 1976):

1) The membrane is impermeable to anions and behaves as an ideal potassium electrode when potassium is the only permeant cation present in the bathing medium.

2) The current-voltage relationship shows a very pronounced inward rectification. When potassium is the sole permeant ion present, the steady-state conductance can be fitted by the expression

$$G_K = \frac{B\rho(c_K)}{1+\exp\left(\frac{\Delta V-\Delta V_h}{v}\right)}, \tag{1}$$

where c_K is the external concentration of potassium and $\rho(c_K)$ is approximately

$$\rho(c_K) \simeq c_K^{1/2}. \tag{2}$$

B, ΔV_h and v are constants for a particular type of cell and ΔV is the displacement of the membrane potential, V, from the zero-current membrane potential, V_0; namely

$$\Delta V = V - V_0. \tag{3}$$

In addition, these channels exhibit the property that in $K^+ - Tl^+$ mixtures both the conductance and zero-current potential go through a minimum when the mole fraction is varied (Hagiwara & Takahashi, 1974; Hagiwara *et al.*, 1977), and the model proposed here can easily be extended to account for this observation.

In the attempt to develop a model that predicts a dependence on ΔV ($= V - V_0$) for the conductance similar to that of Eq. (1), we shall consider two classes of mechanisms, each of which is compatible with a variety of particular physical models. Since a relationship between the conductance and ΔV, as shown in Eq. (1), is just a convenient way of formulating concisely the dependence on two separate parameters, membrane potential and external ion concentration, we shall start from the hypothesis that both these parameters play an essential role in "gating". In both classes of mechanisms we have limited our considerations to the case in which the channel is either empty or occupied by a single ion (i.e., we don't consider the cases in which more than one ion can be present inside the channel at the same time).

In one class of mechanisms, the formation of permeable channels requires a chemical interaction between the external ions and some membrane-bound structures via pathways that are totally different from those of permeation, while the role of the electric field can be by action either on the membrane-bound structures prior to the interaction with the ions, or after, or also on the ions themselves. A typical example of this class of mechanisms would be that in which the formation of pores occurs via orientation of membrane-bound dipoles (by the applied field) and subsequent complexation with the external ions. For the sake of convenience, we shall refer to this class of mechanisms as "electrochemical gating".

In the second class of mechanisms, the ions are also implicated in "gating", although, in contrast to the previous case, the pathways through which they influence gating are the same as those through which they permeate the membrane. More precisely, the channel is viewed as a sequence of activation energy barriers whose heights can vary as a result of kinetic transitions between different conformational states. Thus, a blocked channel is one in which at least one barrier is sufficiently high to prevent significant translocation of ions, and opening of the channel corresponds to a transition whereby that barrier is lowered and permeation restored. The probability for opening and closing are assumed to depend on the state of occupancy of the channel, and thus, if the channel has at least one internal site (i.e., local energy minimum), the presence of an ion at that site will influence "gating". For convenience, we shall refer to this second class of mechanisms as "occupancy gating".

A model for "inward rectification" proposed by Armstrong (1975), in which opening of the pore is caused by direct interaction between the ion in the pore and a postulated blocking particle ("knock-on" mechanism), may be considered as an example of this second class of mechanisms.

We have found that the predictions of the "electro-chemical gating" model can account for Eq. (1) more readily than the "occupancy gating" model, and thus the main part of the paper will derive and examine the predictions for "inward rectification" based on the former model, whereas a treatment of the latter will be given in *Appendix B*. The principal results of both classes of models will be examined and compared in the Discussion section.

Mechanism for Pore Formation in the "Electro-Chemical Gating" Hypothesis

As we have already suggested, there are undoubtedly many particular physical models, all within the domain of the "electrochemical gating" hypothesis, that could be described by similar equations and that would eventually give the same result. Therefore, the physical model analyzed below should be considered one of the many alternatives which has been chosen for its simplicity, but without implying that it faithfully represents physical reality.

One of the reasons for selecting the physical model presented here is that it was suggested by mechanisms which have been postulated to explain the voltage-dependent conductance induced in artificial lipid bilayers by various antibiotics, most notably alamethicin (Baumann & Mueller, 1974; Boheim, 1974; Gordon & Haydon, 1975; Boheim & Kolb, 1978; Kolb & Boheim, 1978). It may be useful to illustrate some similarities between the behavior of the biological systems considered in this paper and that of artificial bilayers in the presence of alamethicin. At a given ion concentration, and in the simple case where potassium is the sole permeant ion, the membrane conductance of starfish egg cells, as well as of muscle, is a strong function of voltage, which increases with hyperpolarization and decreases with depolarization. Qualitatively similar voltage dependences and analogous rectifying properties can be reproduced in bilayers when the antibiotic alamethicin is added to one side of the membrane only. In this case, positive potentials on the alamethicin-containing side induce sharp increases of the conductance, while oppositely directed potentials have little effect. A commonly accepted interpretation of this finding is that the highly polar alamethicin molecules adsorb to the membrane surface adjacent to the antibiotic-containing solution, and, on account of their dipole moment, orient themselves perpendicularly to the surface when a positive potential is applied on the side of alamethicin. Subsequent stepwise aggregation of the oriented molecules or, according to more recent work, orientation of partially pre-formed aggregates and consecutive aggregation of additional monomers (Boheim & Kolb, 1978; Kolb & Boheim, 1978) would eventually lead to the formation of conductive pores of progressively larger sizes. The failure to produce any conductance when a voltage is applied in the opposite direction is generally explained by the assumption that the negative end of the molecule adsorbs more strongly to the membrane solution interface. A similar mechanism is postulated in our model, in which preformed aggregates of interconnected, negatively-charged, and simultaneously orientable subunits are assumed to exist in the membrane (*see* Fig. 1).

Besides being dependent on voltage, the conductance of "anomalously rectifying cells" is also a function of the external concentration of potassium. This can be recognized from inspection of Eq. (1), where the concentration of external potassium appears explicitly in the numerator, and implicitly in the denominator, via ΔV. Whereas it seems plausible that the square root dependence on potassium concentration in Eq. (1) reflects properties of the single channel conductance (*see* Section *B*-3), it is less likely that single channel effects are responsible for the dependence on c_K via ΔV. We shall, therefore, assume that the dependence on ΔV is the result of an effect of potassium concentration on the actual number of permeant channels. As is shown later, this feature can be accounted for if, in addition to voltage-dependent orientation of the macromolecular components, binding with the external cations is also assumed necessary for pore formation (*see* Fig. 1).

The following basic features of the model are illustrated in Fig. 1: The internal side of the membrane contains molecules constituted of n interconnected subunits, which orient themselves perpendicularly to the membrane (and simultaneously) under the influence of an electric field, ($n = 3$ in our model). More precisely, one end of each subunit in the molecule is anchored to the internal side of the membrane, while the opposite one, which bears a negative charge, can flip toward the exterior. When the molecule is oriented by the electric field, it spans the whole membrane thickness, so that the negative charges become exposed to the external solutions and may each bind one cation. The oriented molecules are denoted by A_s^*, where the subscript, s, $(0 \leq s \leq n)$ specifies the number

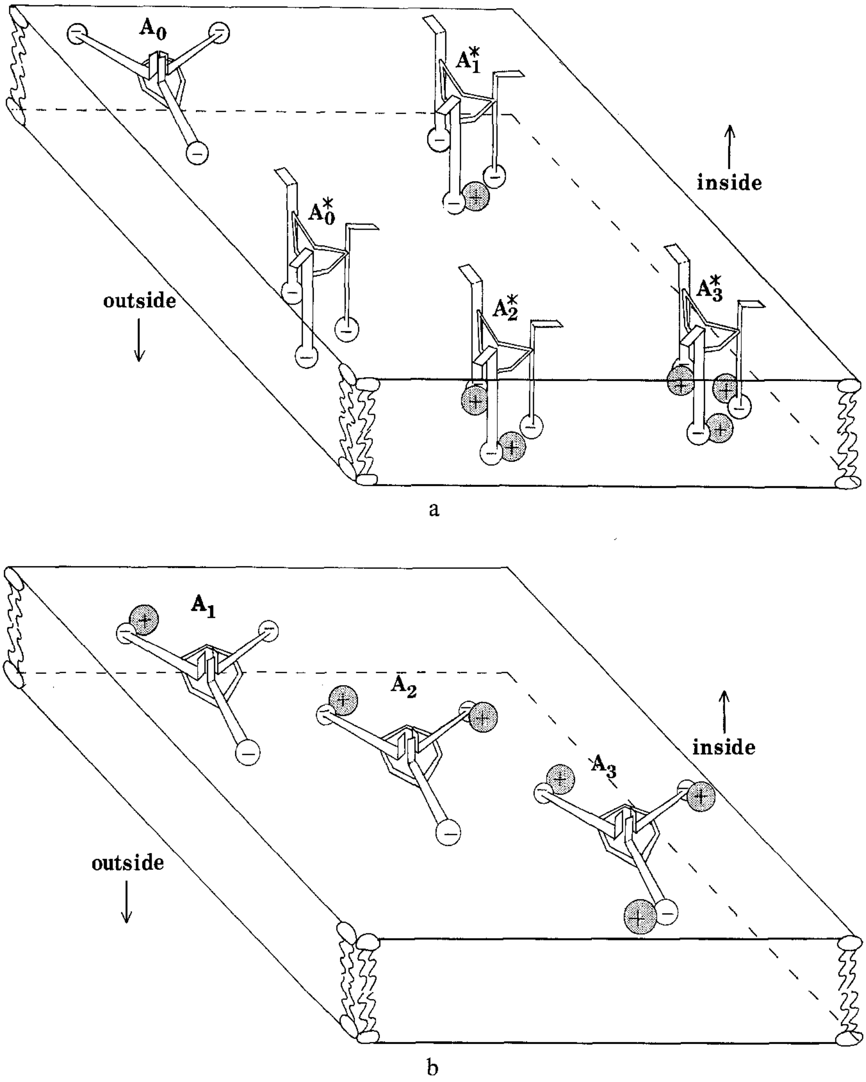

Fig. 1. Schematic representation of a particular case of the "electrochemical gating" class of models. (*a*): It is assumed that orientation of the charged molecules, A_0, located at the inner side of the membrane, is required for the formation of permeable pores and that stepwise complexation of the three charged sites with the external cations is necessary for stabilization of such pores. (*b*) Complexes between the unoriented channel precursors and the internal cations. It is assumed that orientation of these species is negligible compared to that of the uncomplexed ones, A_0^*. Justifications for this assumption are given in the text

of charged subunits with one bound cation, and the asterisk is used consistently to denote those molecules which have been oriented by the electric field.

Whereas it is convenient to schematize the pore-forming molecule as composed of an aggregate of n subunits, the present formalism describes equally well a single unit (e.g., a globular protein) containing n negative groups which traverse the membrane under the influence of an electric field, bind cations, and, in so doing, change the conformation of the molecule from a nonconducting to a conducting channel. Results similar to those deducible from this class of models would also be obtained from a model in which n external cations ("gating ions"), rather than any polar membrane components, move under the action of the electric field through the pore-forming molecule and bind to n "gating" sites within the molecule near the inner membrane surface, thereby inducing a conformational change (from "closed" to "open"). It should be emphasized, however, that according to this picture the pathway for the movement of the "gating" ions is meant to be distinct from the pathway of permeation; which also implies that the "gating" ions are generally not in equilibrium either with the internal solution or with the permeating ions in the channel.

Formally, the major requirement of the model is that the effect on gating of voltage and external K^+ concentration be representable as a function of the following combination of these variables: $c_K^n e^{-n\phi}$, where ϕ denotes the membrane potential in units of RT/F. After we demonstrate that this requirement is satisfied by the specific physical model analyzed below, it will probably become clear to the reader that similar functional dependences are to be expected also for various different physical pictures, including the few mentioned above.

B. Theoretical Results

If n is the total number of subunits in each pore-forming molecule ($n = 3$ in Fig. 1), there are $n+1$ types of pores corresponding to the number of cations bound. Since all of these may be conductive, the specific membrane conductance is

$$G = \sum_{s=0}^{n} \bar{g}_s [A_s^*], \tag{4}$$

where $\bar{g}_s$ is the conductance of an individual pore with s bound cations, denoted by A_s^*, and $[A_s^*]$ is the number of such pores per unit surface. From now on, the A_s^* species ($s=0, 1, 2, \ldots, n$) will be called either "pores," "channels," or "oriented molecules," whereas the same molecules, prior to orientation by the field, will be denoted by A_s ($s=0, 1, 2, \ldots, n$) and will be referred to as "unoriented (pore-forming) molecules," or "channel precursors."

In Section *B*-1, we shall evaluate how $[A_s^*]$ depends upon membrane potential and ion concentration. In Section *B*-2, we shall show that, under certain conditions and assumptions, the density of permeable channels can be expressed as a function of ΔV, namely, of the displacement of the membrane potential from its equilibrium value. Section *B*-3 will present the derivation of an expression for the "average" single pore conductance and of the overall membrane conductance, as defined by Eq. (4).

B-1. Dependence, at Steady State, of the Density of Permeable Pores on Ion Concentration and Membrane Potential

It is shown in *Appendix A* (*see also* Fig. 4) that, in order to deduce rigorously the dependence, at steady state, of the pore density $[A_s^*]$ on the total concentration of pore-forming molecules, $[A]^{\mathrm{Tot}}$, on the rate constants for ion binding to and orientation of these molecules, on the membrane potential, and on the concentration of binding ions, one would have to solve a nonhomogeneous system of $2n+2$ linear equations. For $n \geqq 2$, therefore, the number of parameters would be very large and the algebra impractically cumbersome. We shall circumvent this difficulty by making the following simplifying assumption:

> The only molecular species capable of undergoing orientation (in one direction or the other) and which therefore respond to changes of a transmembrane electric field are those which have no bound cations and therefore carry their full negative charge, ne. The remaining species with at least one bound cation, either oriented or not, are assumed to adopt a physical configuration in which the process of rotation is blocked.

As is shown in *Appendix A*, this assumption reduces the very lengthy steady-state kinetic problem mentioned above to a much simpler "partial equilibrium" problem, and provides probably the simplest route to the derivation of Eq. (1) from the particular physical model that we have chosen to describe. Some implications of this assumption are intuitively plausible, whereas others may sound objectionable and require some justification. Thus, the implication that the molecular species most sensitive to the electric field are those carrying the highest charge is clearly reasonable. On the other hand, the notion that the uncharged or partially charged molecules don't rotate or, in any case, move less easily than the fully charged ones is in conflict with simple electrostatic arguments, whereby the hydrocarbon interior of the membrane should provide a higher barrier to the most charged species than to the neutral and the less charged ones. Rather than speculating arbitrarily on the possible ways to obviate this difficulty (which can certainly be done), we prefer simply to recall that, on the basis of the past experience with permeation through bilayers, the net charge of a molecular species is neither the only, nor necessarily the most important, parameter determining the rate constants for crossing a lipid membrane. It has been found, for example, that the ion-carrier valinomycin crosses the membrane at the same rate in its neutral as in its complexed charged form (Stark *et al.*, 1971), which is probably accountable by the fact that the acquisition of an electric charge with consequent increase of the Born energy barrier is compensated by a conformational change, whereby either the hydrophobic interaction with the membrane is enhanced and/or the stabilizing interaction between water and the polar groups of valinomycin is reduced. It is, therefore, quite reasonable to conceive that analogous compensating effects may be present in the gating molecules of our model: In particular, ion binding might be accompanied by a change of physical state which either hinders the rotation sterically or stabilizes the interaction between polar groups of the molecule and water at the interfaces, thus blocking the process of orientation. Furthermore, a number of physical factors may differ for the ion-binding sites in the unoriented and oriented molecules, such that the equilibrium constant for ion binding may be large for the oriented molecule but small for the unoriented molecule (e.g., the charge density of the binding site might be lower for the unoriented molecule, as schematically illustrated in Figs. 1*a* and *b*, or steric constraints which disfavor ion binding may exist in the unoriented form). In this case, the predominant species of unoriented molecules might actually be the one with no complexed ions, this species, therefore, being the only one to give a significant contribution to the reorientation induced by the field.[1]

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[1] In any case, it should be pointed out that the difficulties intrinsic to the assumption discussed above arise from the particular physical picture for "electrochemical gating" that we have chosen to describe. Had we selected one of the alternative models briefly mentioned previously in the paper, such as the one, for example, in which the channel formation requires the movement of n external "gating" ions up to the n binding sites situated close to the internal surface (although inaccessible from the internal solution), the derivation of Eq. (1) would have been possible without that type of restrictive assumption.

With the aid of the above assumption, it is shown in *Appendix A* that the steady-state densities of oriented, uncomplexed molecules, $[A_0^*]$, of oriented, complexed molecules, $[A_s^*]$, of unoriented, uncomplexed molecules, $[A_0]$, and of unoriented, complexed molecules, $[A_s]$, are interrelated as follows:

$$[A_0^*] = \Gamma e^{zn\phi}[A_0]. \tag{5}$$

$$[A_s^*] = K_s^*[A_0^*]\, c_i^s \quad (s=1, 2, \ldots, n) \tag{6}$$

$$[A_s] = K_s[A_0]\, c_i'^s \quad (s=1, 2, \ldots, n), \tag{7}$$

where c_i and c_i' denote the external and internal concentrations, respectively, of the permeant ion (e.g., K^+), $\Gamma e^{zn\phi}$, K_s^*, and K_s are the equilibrium constants of the following reactions

$$A_0 \overset{\Gamma e^{zn\phi}}{\longleftrightarrow} A_0^* \tag{8}$$

$$A_0^* + s\cdot I \overset{K_s^*}{\longleftrightarrow} A_s^* \tag{9}$$

$$A_0 + s\cdot I' \overset{K_s}{\longleftrightarrow} A_s, \tag{10}$$

and ϕ is the potential in units of RT/F.

Combining the conservation equation,

$$[A]^{\mathrm{Tot}} = \sum_{r=0}^{n}\{[A_r]+[A_r^*]\} = [A_0]+[A_0^*]+\sum_{r=1}^{n} K_r c_i'^r [A_0] + \sum_{r=1}^{n} K_r^* c_i^r [A_0^*] \tag{11}$$

with Eqs. (5) and (6), and recalling that $z=-1$, then yields

$$[A_0^*] = \frac{\Gamma e^{-n\phi}[A]^{\mathrm{Tot}}}{1+\sum_{r=1}^{n} K_r c_i'^r + \Gamma e^{-n\phi}\left[1+\sum_{r=1}^{n} K_r^* c_i^r\right]} \tag{12}$$

and

$$[A_s^*] = \frac{K_s^* \Gamma e^{-n\phi} c_i^s [A]^{\mathrm{Tot}}}{1+\sum_{r=1}^{n} K_r c_i'^r + \Gamma e^{-n\phi}\left[1+\sum_{r=1}^{n} K_r^* c_i^r\right]} \quad (s=1, 2, \ldots, n). \tag{13}$$

Equations (12) and (13) can be rewritten more concisely. Defining

$$\omega = \left[1+\sum_{r=1}^{n} K_r c_i'^r\right]^{-1} \tag{14}$$

which is a function of the internal concentration only, and defining also $K_0^* = 1$, Eqs. (12) and (13) are equivalent to the following expression:

$$[A_s^*] = \frac{\omega \Gamma e^{-n\phi} K_s^* c_i^s [A]^{\text{Tot}}}{1 + \omega \Gamma e^{-n\phi} \left[1 + \sum_{r=1}^{n} K_r^* c_i^r\right]} \qquad (s = 0, 1, \ldots, n). \tag{15}$$

Equation (15) gives the density of all types of oriented molecules in terms of experimentally controllable variables, such as the membrane potential and the external concentration of ions, and shows that the density of oriented molecules of any type vanishes at high depolarizing potentials, whereas it approaches a finite limit at high hyperpolarizing potentials.

Although Eq. (15) would be adequate to fit all the available data for the starfish egg cell membrane conductance, the much simpler empirical expression, Eq. (1), which is sufficient to describe the data for starfish egg cell membranes, will be shown in the next section to correspond to the limiting case in which the fully-complexed pores, A_n^*, predominate over all the other species of oriented molecules.

B-2. Dependence of the Density of Permeable Channels on ΔV

When potassium is the sole permeant ion present, the zero-current potential, V_0, coincides with the potassium equilibrium potential, so that we can write

$$\frac{c_i}{c_i'} = e^{\phi_0}. \tag{16}$$

Substituting Eq. (16) into Eq. (15) and defining terms in a way which will be convenient for a comparison between the theoretical expression of the conductance, Eq. (23), and the empirical expression, Eq. (1), we can write

$$[A_s^*] = \frac{L_s}{L} \frac{[A]^{\text{Tot}}}{1 + \exp\left(\dfrac{\Delta V - \Delta V_h}{v}\right)} \qquad (s = 0, 1, \ldots, n) \tag{17}$$

where

$$\Delta V = \frac{RT}{F} (\phi - \phi_0) \tag{18}$$

$$v = \frac{RT}{nF} \tag{19}$$

$$L_s = \omega \Gamma c_i'^n K_s^* c_i^{(s-n)} \qquad (s=0, 1, \ldots, n) \tag{20}$$

$$L = \sum_{s=0}^{n} L_s \tag{21}$$

and

$$\exp\left[-\frac{\Delta V_h}{v}\right] = L^{-1} = [\omega \Gamma c_i'^n]^{-1} \frac{c_i^n}{\sum_{s=0}^{n} K_s^* c_i^s}. \tag{22}$$

Substituting Eq. (17) into Eq. (4) yields

$$G = [A]^{\mathrm{Tot}} \frac{\sum_{s=0}^{n} \frac{L_s}{L} \bar{g}_s}{1 + \exp\left[\frac{\Delta V - \Delta V_h}{v}\right]}. \tag{23}$$

Comparing now this expression with Eq. (1), and attributing the square root term in Eq. (1) to the properties of the individual channel conductances, $\bar{g}_s$ (*see* next section), it is easy to realize with the help of Eq. (22) that a meaningful similarity between the theoretically derived Eq. (23) and the phenomenological Eq. (1) can be claimed if the inequality

$$K_n^* c_i^n \gg \sum_{s=0}^{n-1} K_s^* c_i^s \tag{24}$$

is satisfied in the range of the examined concentrations. As shown by Eq. (22), it is in fact only in this case that ΔV_h becomes independent of the external ion concentrations and can thus be considered constant. Clearly, if experiments could be carried out at sufficiently low concentrations (e.g., $c_i^n \leqq 1/K_n^*$), a domain should be eventually found in which relationship (24) would not be satisfied and ΔV_h would depend on c_i. From the presently existing data, which are certainly consistent but do show a certain amount of scattering from cell to cell, it is difficult to assess how strictly the constancy of ΔV_h is required for a good fit. However, two important observations pertinent to this point can be readily made: (i) If relationship (24) is not satisfied so that ΔV_h does vary with c_i according to Eq. (22), it is hard to obtain from Eq. (23) a square root dependence on c_i (at equal ΔV's) similar to that found experimentally. (ii) In addition, it is worth pointing out that the discrepancy in Fig. 2 between the data and the theoretical curve at 10 mM cannot be corrected by the assumption that at such a low concentration ΔV_h starts varying with c_i according to Eq. (22). In fact, in order to bring the curve closer to the data, a decrease of ΔV_h from the value corresponding to the upper curves would be required as the external K^+ concentration is decreased; whereas Eq. (22) predicts a change in the opposite direction for decreasing external K^+ concentrations.

Therefore, restricting considerations to the case in which relationship (24) is satisfied, it is straightforward to realize with the help of Eqs. (20) and (21) that

$$L_s \ll L_n \simeq L, \qquad s=0, 1, \ldots, n-1. \tag{25}$$

With this approximation and recalling Eq. (17), we find that

$$[A_s^*] \ll [A_n^*] = \frac{[A]^{\text{Tot}}}{1+\exp\left[\dfrac{\Delta V-\Delta V_h}{v}\right]}, \qquad s=0, 1, \ldots, n-1 \tag{26}$$

where, by virtue of relationship (24), Eq. (22) becomes independent of the external K^+ concentration and simplifies to

$$\exp\left[-\frac{\Delta V_h}{v}\right] \simeq [\omega \Gamma c_i'^{\,n} K_n^*]^{-1}. \tag{27}$$

Equations (26) and (27) show that, when the condition (24) is satisfied, the dependence of the pore density on the membrane voltage and on the external ion concentration can be expressed as a dependence on ΔV alone. Within the limits of validity of the same approximation, the membrane conductance, Eq. (23), reduces to

$$G = [A]^{\text{Tot}} \frac{\bar{g}_n}{1+\exp\left[\dfrac{\Delta V-\Delta V_h}{v}\right]}. \tag{28}$$

Note that the rectification properties embodied in Eq. (28) are due to the feature of the present model that the number of pores vanishes at large depolarizing potentials while approaching the finite value, $[A]^{\text{Tot}}$, at large hyperpolarizing potentials.

B-3. Dependence of the Open Pore Conductance on Ion Concentration and Final Expression for the Membrane Conductance

We can see from the results of the previous section that the conductance per unit area, Eq. (28), is the same function of ΔV as that in the empirical expression, Eq. (1), provided that the conductance of the single channel $\bar{g}_n$ is independent of transmembrane potential and is proportional to $\sqrt{c_i}$.

Although little information about the properties of the single pore is available, we show here that when the internal ion concentration is constant, a dependence of the membrane conductance on the square root of the external concentration, such as that found experimentally [*see* Eqs. (1) and (2)], can be justified on the basis of conventional flux equations. In the absence of effects due to saturation of the channel and in the approximation of constant electric field, the expression for the flux of ions through the A_n^* pore, as deduced either from Eyring's rate theory or the equation of Nernst-Planck, can be written in the form

$$J_i = [A_n^*]\, f(\phi)\, \{c_i - c_i' e^{\phi}\}, \tag{29}$$

where $f(\phi)$ is a function of the voltage and of the structure of the channel, but is independent of the ionic concentrations, c_i and c_i'. Rearranging Eq. (29), adopting the convention that the outward fluxes are positive, and assuming that the monovalent cation i is the only permeant ion, the membrane conductance becomes

$$G = \frac{F^2}{RT}\,[A_n^*]\,\sqrt{c_i c_i'}\, f'(\phi)\, \frac{2\sinh\left[\frac{\phi-\phi_0}{2}\right]}{\phi-\phi_0}, \tag{30}$$

where

$$G = \frac{I}{V-V_0} = \frac{F^2}{RT}\,\frac{J_i}{\phi-\phi_0} \quad \text{and} \quad f'(\phi) = f(\phi)\, e^{\phi/2}. \tag{31}$$

The behavior of Eq. (30) clearly depends on the function, $f'(\phi)$, which is unknown. Nevertheless, valuable inferences can still be made if one considers certain limiting cases, and takes account of the fact that in the usual experimental range of the driving force: ($|\phi-\phi_0| \leqq 2$), the magnitude of the absolute value of $2\sinh\left[\frac{\phi-\phi_0}{2}\right]/(\phi-\phi_0)$ varies only from 1 to 1.17, and thus can be approximated by unity in most practical cases. Using this approximation, Eq. (30) simplifies to

$$G \simeq \frac{F^2}{RT}\,[A_n^*]\,\sqrt{c_i c_i'}\, f'(\phi), \tag{32}$$

which shows that G depends on the square root of c_i for comparable values of $[A_n^*]$, (and therefore of ΔV) only if $f'(\phi)$ is a sufficiently weak function of ϕ or, ideally, a constant.[2] Whatever formalism is used to describe the fluxes, the explicit form of $f'(\phi)$ depends on the structure and the chemical nature of the pore, which in the present system are basically unknown. For example, in a description based on Eyring's rate theory, the number, height, and position of the energy barriers should be known; similarly, the spatial dependence of the diffusion coefficient and of the standard chemical potential should be specified when the Nernst-Planck equation is used. Despite the lack of knowledge of the microscopic properties of the pore, it is worth showing that there are simple cases in which $f'(\phi)$ varies very little with the potential. For example, if Eyring's formalism is used and the pore is schematized as a symmetrical sequence of n sites separated by $n-1$ identical activation energy barriers, it can be shown that $f'(\phi)$ is proportional to

$$\frac{\sinh[\phi/2(n-1)]}{\sinh[\phi/2]}, \tag{33}$$

where ϕ is the normalized potential and $(n-1)$ is the number of energy barriers. The most favorable situation is clearly that in which the pore is described by a single barrier separating the two solutions. In this case, $n-1=1$ and $f'(\phi)$ is rigorously constant. It can be shown that the greater the number of barriers, the less satisfactory the approximation of $f'(\phi)$ with a constant becomes. The least satisfactory situation is that in which the number of sites and barriers is so high that $\phi<2(n-1)$ in the whole range of applied potentials. In this case, Eyring's formalism merges into that of Nernst-Planck, and the function (33) can be approximated by

$$\frac{1}{2(n-1)}\,\frac{\phi}{\sinh[\phi/2]}. \tag{34}$$

Given that the highest absolute value of the membrane potential for which measurements on starfish egg cells were made is of the order of 140 mV ($\phi=5.6$),[3] and recalling that, in the worst case, $f'(\phi)$ is pro-

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[2] Recent "noise analysis" studies of the membrane conductance of certain tunicates strongly suggest that the average single channel conductance is indeed proportional to the square root of the external potassium concentration (Ohmori, 1978).

[3] The most extreme ranges of absolute membrane potential measured are those obtained from the largest hyperpolarization at the lowest value of c_K and the largest depolarization at the highest value of c_K. Experimentally, the lowest value of c_K measured (*see* Fig. 2) is 10 mM for which $V_0=-76$ mV and the most hyperpolarizing applied potential was $\Delta V\simeq -60$ mV. The highest value of c_K was 100 mM for which $V_0=-20$ mV and the most depolarizing applied potential was $\Delta V\simeq +15$ mV. So, for the data in Fig. 2, the actual range of voltages was from -5 to -136 mV. It is also worth noting that the experimental points for 10 mM K^+ at the highest hyperpolarizing potentials lie below the theoretical curve, which is the type of deviation which is expected if $f'(\phi)$ were not rigorously constant over the whole voltage range.

portional to the function (34), we find

$$\frac{f'(5.6)}{f'(0)} = 0.35. \tag{35}$$

Equation (35) shows that in the most unfavorable case, namely when the Nernst-Planck formalism is appropriate, $f'(\phi)$ will vary by less than a factor of 3 for the most extreme excursions of the voltage.

From the above considerations, it seems that the approximation of $f'(\phi)$ with a constant is not too arbitrary. Using this approximation, defining

$$f' = f(\phi) \simeq \text{const.} \quad \text{and} \quad B \equiv \frac{F^2}{RT}[A]^{\text{Tot}}\sqrt{c_i' f_i'}, \tag{36}$$

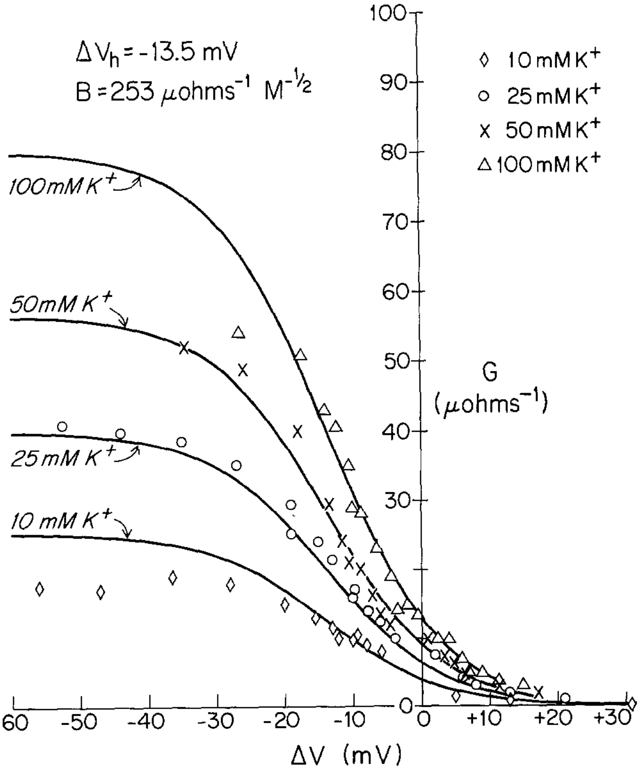

Fig. 2. Fit of the steady-state conductance data for the egg cell membrane of the starfish *Mediastera aequalis* with Eq. (37). The four sets of experimental points refer to different concentrations of external potassium for the same cell (diameter, 900 μm), and the corresponding theoretical curves are calculated from Eq. (37) using the same values for the parameters, v, ΔV_h and B, namely: $v = 8.43$ mV; $\Delta V_h = -13.5$ mV; $B = 253\ \mu\Omega^{-1} \cdot \text{M}^{-1/2}$

and substituting Eqs. (26) and (36) into Eq. (32), we finally find

$$G = B \frac{\sqrt{c_i}}{1 + \exp[(\Delta V - \Delta V_h)/v]}. \tag{37}$$

Equation (37) is formally identical to the empirical expressions given by Eqs. (1) and (2) at the beginning of the paper.

A fitting of steady-state conductance data with Eq. (37) for the egg cell membrane of the starfish, *Mediaster aequalis* is shown in Fig. 2. Note that a reasonable fit of the theoretical curve to the data can be obtained and that this fit yields the value $v = 8.43$ mV, which, according to Eq. (19) is equivalent to $n = 3$. Thus, the present model would suggest that the "gating site" of the inward-going rectification channel in the starfish egg cell membrane has a net charge of -3.

Discussion

The Physical Basis for Rectification and the ΔV Dependence of the Conductance According to the Present Model

The model presented here is based on the concept that rectification is intrinsic to the mechanism of pore *formation*, and is not due to asymmetric profiles of the energy barrier in the open pores. The membrane potential and the concentration of external potassium are both assumed to affect the density of open pores, and the way in which these parameters are combined in the treatment results in an expression for the number of open pores, Eq. (26), that can be written as a function of the displacement of the membrane voltage from the equilibrium potential of potassium. The suggestion that the permeability to potassium may be viewed as a function of $V - V_K$, rather than of the membrane potential alone, has already been made in the past by a number of investigators working on muscle fibers (Hodgkin & Horowicz, 1959; Nakamura, Nakajima & Grundfest, 1965; Horowicz *et al.*, 1968; Adrian, 1969), and a very clear quantitative description of this property of the conductance was given for the rectifying membranes of a starfish egg cell (Hagiwara & Takahashi, 1974).

In terms of the present model, the intuitive explanation for the conductance decrease at positive ΔV's is that positive membrane potentials and/or low external potassium concentrations, both of which make ΔV positive, are unfavorable conditions to the formation of permeable pores, the reason being that a positive increase of the membrane potential has the effect of preventing the molecules from orienting themselves perpendicularly to the surface and a decrease of the external potassium concentration diminishes the degree of ion binding to the oriented molecules. Conversely, the saturation of the number of available pores at high negative ΔV can be rationalized in terms of the fact that at high hyperpolarizing membrane potentials and/or at high external potassium concentrations, both of which make ΔV more negative, most of the available channel-forming molecules are in the "permeable channel" configuration, so that a further increase of either hyperpolarization or external potassium concentration has no effect on their number.

Recently, an internal perfusion technique has been developed for tunicate egg cells (Takahashi & Yoshii, 1978) which could, in principle, be applied to starfish eggs, thereby allowing one to assess the dependence of conductance on internal ion concentration and to determine whether the predictions of the "electrochemical-gating" model apply to that measurement as well. Therefore, it is worth examining explicitly the way in which this model predicts that the conductance will vary as a function of the internal concentration of potassium.

Assuming that the external potassium concentration, c_i, is held constant and that the membrane is clamped to a given potential, ϕ, then introducing Eqs. (14), (16), (26), and (27) into Eq. (32) yields (after simplifying),

$$G = D_1 \frac{\sqrt{c_i'}}{1 + D_2 \left(1 + \sum_{r=1}^{n} K_r c_i'^r\right)} \tag{38}$$

where D_1 and D_2 are constants given by

$$D_1 = \frac{F^2}{RT} [A]^{\text{Tot}} \sqrt{c_i}\, f'(\phi) \tag{39}$$

and

$$D_2 = \frac{e^{n\phi}}{K_n^* c_i^n}. \tag{40}$$

Equation (38) illustrates that in general, the conductance measured at a constant voltage and external K^+ concentration will vary with changes in the internal K^+ concentration. The nature of this variation will depend, however, on the fraction of unoriented pore-forming molecules which have bound one or more internal K^+ ions. When that fraction is small $\left(\text{i.e., } \sum_{r=1}^{n} K_r c_i'^r \ll 1 \text{ in Eq. (38)}\right)$, the ratio $G/\sqrt{c_i'}$ is expected to remain approximately constant, and so is, of course, the density of pores; whereas when a significant fraction of unoriented molecules have bound internal K^+'s $\left(\text{i.e., } \sum_{r=1}^{n} K_r c_i'^r \geqq 1\right)$, the quantity $G/\sqrt{c_i'}$ is expected to decrease with increasing internal K^+. It is thus clear that experimental findings showing either a decrease or constancy of $G/\sqrt{c_i'}$ with increasing internal K^+ would be compatible with the model, whereas an increase of the same quantity would not be so, suggesting that major modifications are necessary.

The Conductance Behavior Expected from the "Occupancy-Gating" Class of Mechanisms

An alternative class of models for the dependence of conductance on ΔV (rather than V) is the one, which we have referred to as "occupancy gating", in which the probability that a channel is in an "open" (conducting) state is determined by the presence of ions *inside* the channel. In Appendix *B* we have derived the expression for the ionic flux predicted by this type of model, assuming that the channel can be occupied by no more than one ion at a time. Briefly, the pore is schematized as consisting of a single site (= free energy well) separated from the aqueous solutions by two activation-energy barriers. The transition from the "open" to the "closed" state is described as a first-order reaction as a result of which the energy barrier between the site and the internal solution rises so high as to prevent further exchange of ions with that solution. The different states of the channel (open/empty, closed/empty, open/occupied, and closed/occupied) as well as the rate constants describing transitions between those states are illustrated in Fig. 3. Note that the heights of the activation-energy barriers and of the energy well can be different for each state of the channel, whereas the *positions* in the membrane of these barriers and wells (i.e., the fraction of the transmembrane potential which they sense) are assumed to be the same for all states.

Utilizing this model, a general expression was derived in Appendix *B* for the ionic flux, and it could be seen that the only case in which the expression for the conductance becomes somewhat similar to Eqs. (1) or

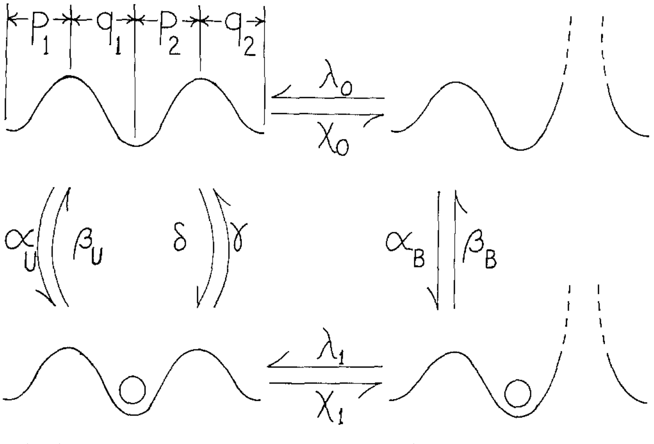

Fig. 3. Schematic diagram of the four states and of the transitions between them for the two-barrier, one-site model of a channel considered in the analysis of the "occupancy gating" mechanism. The crucial feature in the model is that the values of the rate constants for transition between a "closed" to an "open" state (and vice versa) depend on the state of occupancy of the pore

(37) was that in which the peak of the external barrier is displaced almost to the "internal end" of the pore (i.e., in Fig. 3, $p_1 \simeq 1$ and $p_2 \simeq q_1 \simeq q_2 \simeq 0$). In this limit, the conductance can be written in the form [*cf.* Eq. (B23)]

$$G=\frac{F^2}{RT}\,\frac{\left(\Omega_0+\frac{\Omega_1}{c'}\,e^{\Delta\phi}\right)\sqrt{c_i\,c_i'}\left(\frac{2\sinh\frac{\Delta\phi}{2}}{\Delta\phi}\right)}{W_1\left[1+2\,\frac{\sqrt{W_0\,W_2}}{W_1}\cosh(\Delta\phi-\Delta\phi_h^*)\right]}\,e^{-\frac{\phi}{2}} \tag{41}$$

where

$$\Delta\phi_h^*=\ln\sqrt{\frac{W_0\,c_1'^2}{W_2}} \tag{42}$$

and W_0, W_1, W_2, Ω_0 and Ω_1 are constants (assuming $p_1=1$ and $p_2 \simeq q_1 \simeq q_2 \simeq 0$) for a given internal K$^+$ concentration, as it can be seen from Eqs. (B17)–(B21).

Considering that over the present experimental ranges of $\Delta\phi$ (*cf.* Fig. 2)

$$\frac{2\sinh\frac{\Delta\phi}{2}}{\Delta\phi}\simeq 1, \tag{43}$$

Eq. (41) can be made to come closest to the empirical behavior observed for anomalous rectification (*cf.* Eq. (1)) by assuming the validity of the approximations

$$\frac{\Omega_1}{c'} \ll \Omega_0 \tag{44}$$

and

$$\cosh(\Delta\phi - \Delta\phi_h^*) \simeq \tfrac{1}{2}\exp(\Delta\phi - \Delta\phi_h^*). \tag{45}$$

Inserting the limits defined by Eqs. (43)–(45) into Eq. (41) yields

$$G = \frac{z^2 F^2}{RT} \frac{\Omega_0}{W_1} \frac{\sqrt{c_i c_i'}\, e^{-\frac{\phi}{2}}}{1 + \exp(\Delta\phi - \Delta\phi_h)} \tag{46}$$

where

$$e^{-\Delta\phi_h} = \frac{W_2}{W_1 c_i'}. \tag{47}$$

Two discrepancies still exist between Eq. (46) and the behavior observed for anomalous rectification. The first is the factor $e^{-\phi/2}$ which does not appear in Eq. (1); the second is the fact that the exponential in the denominator should contain a factor n times the term $(\Delta\phi - \Delta\phi_h)$, this factor equaling 3 for the present experiments. It is possible that this second deviation could be adjusted by assuming that three ions (rather than one) must simultaneously occupy a binding site within the channel near the inner mouth of the pore. Even if this assumption were made, however, one could not readily get rid of the factor, $e^{-\phi/2}$ in the numerator.[4] At first, it might seem that this factor could be reduced by assuming that the potential energy profile for the "open" channel was different from that of the "closed" channel (e.g., perhaps the highest barrier for ion migration through an open channel could sense one-half of the voltage drop across the membrane, whereas this same barrier could be located at a position near the inner surface of the membrane when the channel is closed). However, such an assumption will not remedy the situation, since allowing for the possibility of shifting the location of the wells and the peaks in the "open to closed" and "closed to open" transitions will influence the voltage-dependence of the rate

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[4] This factor would appear as a deviation of the data points at low K^+ concentrations and large ΔV's in Fig. 3 *above* the theoretical curve (e.g., at $\Delta V = -60$ mV the data for 10 mM K^+, for which $V_0 = -71$ mV, would be more than three times larger than the value predicted by the (saturating) theoretical curve. If anything, a deviation in the opposite direction is observed).

constants for "blocking" and "unblocking" in such a way that the dependence of gating on ΔV would be lost.[5]

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Appendix A

Derivation of the Explicit Expression for the Density of Pores, Eqs. (12) and (13)

A complete kinetic analysis for the calculation of the density of pores in the membrane would require that all the steps shown in the diagram of Fig. 4 be taken into account. For example, the rate of formation of the pore molecules, A_s^*, in the case in which $0<s<n$ would be given by the sum of the net rates of the three following reactions:

$$A^*_{s-1} + I \xleftrightarrow[k^*_{s,\,s-1}]{k^*_{s-1,\,s}} A^*_s \tag{A1}$$

$$A^*_{s+1} \xleftrightarrow[k^*_{s,\,s+1}]{k^*_{s+1,\,s}} A^*_s + I \tag{A2}$$

and

$$A_s \xleftrightarrow[k_s^B\, e^{-z(n-s)(1-\alpha)\phi}]{k_s^F\, e^{z(n-s)\alpha\phi}} A^*_s. \tag{A3}$$

Equations (A1) and (A2) describe the binding of an external cation to a pore with $s-1$ already complexed subunits, and the dissociation of a

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[5] Such voltage-dependence of "gating" is not always easy to predict intuitively, however. For example, in the limit in which the pore will open only when occupied by a K^+ ion (Armstrong's "knock-on" mechanism), one might expect intuitively that the number of "open" pores would be simply proportional to the external K^+ concentration and to the exponential of the voltage drop between the external solution and the K^+ binding site inside the pore (*cf.* Armstrong, 1975). Such a result would be expected to follow from the assumptions that the rate constant for "opening" is proportional to the number of closed pores with occupied sites, and that the degree of ion-binding by sites of closed pores can be related to voltage and external K^+ concentration by simple equilibrium laws. As a matter of fact, however, if the pores are not permanently "closed", and if there is a flux of K^+ when the pores are "open", the steady-state probability of K^+ occupying the internal sites in closed channels will not be relatable to voltage and external potassium by simple equilibrium laws, but will be described by a more complex function of the voltage, of all the rate constants for transition between "closed" and "open" states, as well as of the external *and internal* K^+ concentrations.

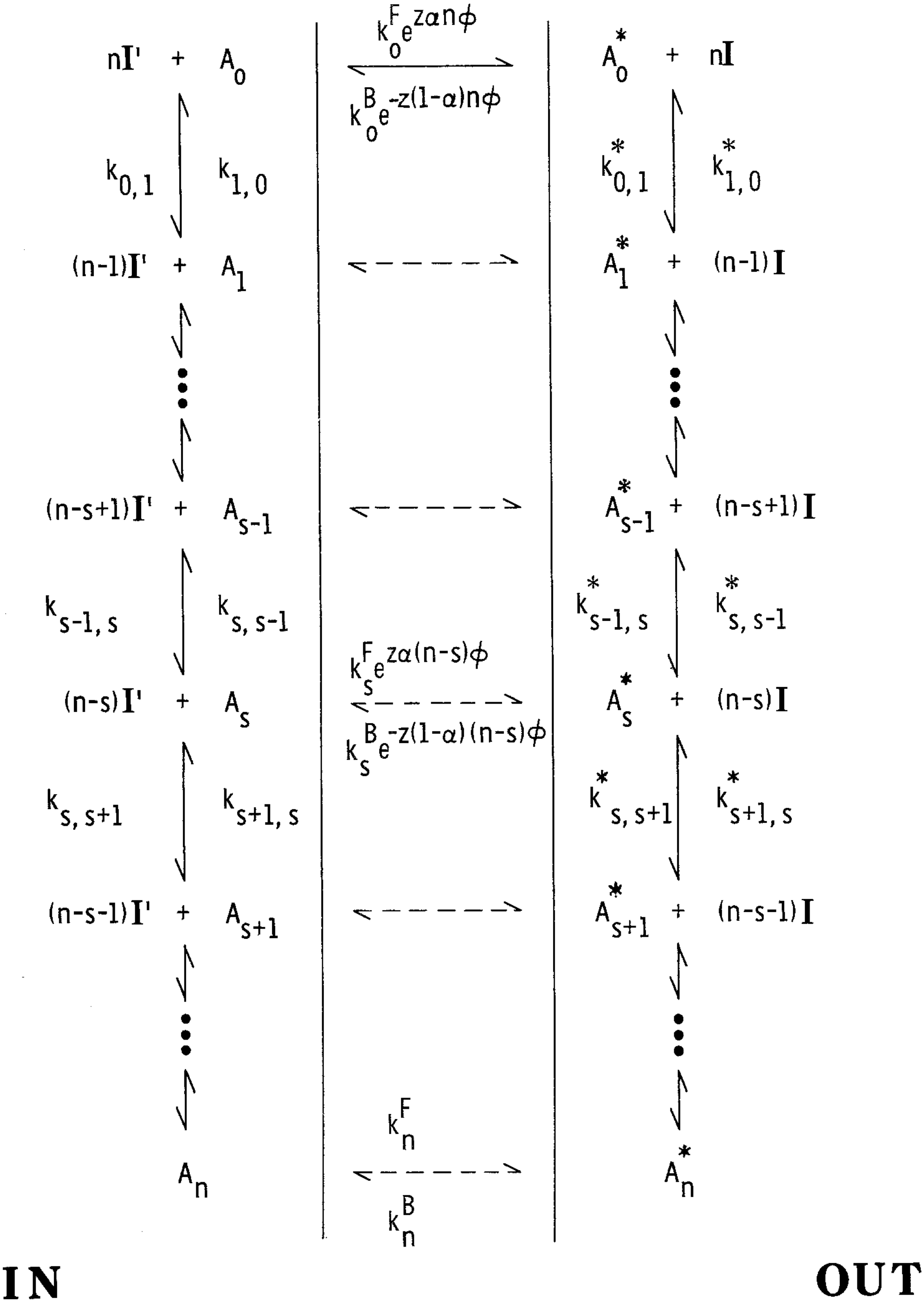

Fig. 4. A diagram of the postulated kinetics in "electrochemical gating". The reactions at the inner side of the membrane (vertical arrows at the left side of the figure) indicate the various stages of complexation between the unoriented channel precursors and the internal cations; those at the outer side of the membrane (vertical arrows on the right side) refer to analogous reactions between the pore molecules and the external cations. The horizontal arrows suggest that, in principle, all types of molecules may rotate. However, all the results in this paper correspond to the limiting case in which the fully charged molecules, A_0 and A_0^* are the only ones to undergo orientation

cation from a pore with $s+1$ complexed subunits, respectively. Clearly, reaction (A1) is not applicable to the case of $s=0$, nor is reaction (A2) to the case of $s=n$. Equation (A3) denotes the orientation of a gating molecule assumed to be describable by an Eyring formalism with a single activation energy barrier. z is the valency of the individual groups in the gating molecules ($z=-1$ in our model), $n-s$ is the number of net negative charges, ϕ is the membrane potential in RT/F units, and α is the fractional distance (distance in units of membrane thickness) of the activation energy peak from the inner side of the membrane. The exponentials, $e^{z(n-s)\alpha\phi}$ and $e^{-z(n-s)(1-\alpha)\phi}$, express the extent to which the height of the energy barrier relative to the two adjacent wells is modified by the membrane potential. Stipulating that the rate constants, $k^*_{-1,0}$, $k^*_{0,-1}$, $k^*_{n,n+1}$ and $k^*_{n+1,n}$ are equal to zero, the net rate of formation of A^*_s for $s=0, 1, \ldots, n$ can be condensed in the following expression

$$\frac{d[A^*_s]}{dt}=k^*_{s-1,s}\,c_i[A^*_{s-1}]-k^*_{s,s-1}[A^*_s]+k^*_{s+1,s}[A^*_{s+1}]-k^*_{s,s+1}\,c_i[A^*_s]$$
$$+k^F_s[A_s]\,e^{z(n-s)\alpha\phi}-k^B_s[A^*_s]\,e^{-z(n-s)(1-\alpha)\phi}, \qquad (s=0, 1, \ldots, n), \qquad \text{(A4)}$$

where c_i denotes the external concentration of cations and the various rate constants are all defined in Eqs. (A1) to (A3). For the unoriented gating molecules, we could write the analogous equations

$$\frac{d[A_s]}{dt}=k_{s-1,s}\,c'_i[A_{s-1}]-k_{s,s-1}[A_s]+k_{s+1,s}[A_{s+1}]-k_{s,s+1}\,c'_i[A_s]$$
$$+k^B_s[A^*_s]\,e^{-z(n-s)(1-\alpha)\phi}-k^F_s[A_s]\,e^{z(n-s)\alpha\phi} \qquad (a=0, 1, \ldots, n) \qquad \text{(A5)}$$

where it is again understood that $k_{-1,0}=k_{0,-1}=k_{n,n+1}=k_{n+1,n}\equiv 0$. Imposing the conditions of steady-state on Eqs. (A4) and (A5) $\left(\frac{d[A^*_s]}{dt}=\frac{d[A_s]}{dt}=0\right)$, we would obtain a system of $2n+2$ homogeneous linear equations, only $2n+1$ of which, however, would be linearly independent. These $2n+1$ relationships, together with the conservation equation

$$[A]^{\mathrm{Tot}}=\sum_{r=0}^{n}\{[A_r]+[A^*_r]\} \qquad \text{(A6)}$$

constitute a nonhomogeneous system of $2n-2$ linear equations, from which $[A_s]$ and $[A^*_s]$ could be finally calculated as explicit functions of all the rate constants, of $[A]^{\mathrm{Tot}}$, of the membrane potential, and of the concentration of the binding ions. Although conceptually trivial, the algebra is already unwieldy for $n=2$. A substantial simplification, however, is brought about by the assumption, discussed in the text, that the only species of gating molecules which are oriented by the electric field are the ones which have no bound cations. Under this approximation, all the rate constants for orientation of the aggregates which have at least one complexed cation, k_s^F and k_s^B, with $(s \neq 0)$ are set equal to zero. With this simplification, it is very easy to verify that the steady-state solution of the system of Eq. (A4) is given by the relationships

$$[A_n^*] = \frac{k_{n-1,n}^*}{k_{n,n-1}^*} c_i [A_{n-1}^*] \tag{A7}$$

$$[A_{n-1}^*] = \frac{k_{n-2,n-1}^*}{k_{n-1,n-2}^*} c_i [A_{n-2}^*] \tag{A8}$$

$$\vdots \qquad\qquad \vdots$$

$$[A_s^*] = \frac{k_{s-1,s}^*}{k_{s,s-1}^*} c_i [A_{s-1}^*] \tag{A9}$$

$$\vdots \qquad\qquad \vdots$$

$$[A_0^*] = \frac{k_0^F}{k_0^B} e^{-n\phi} [A_0]. \tag{A10}$$

By successive elimination of the intermediate concentrations in the above equations and defining

$$\Gamma = \frac{k_0^F}{k_0^B}, \qquad K_s^* = \frac{k_{0,1}^* \, k_{1,2}^* \dots k_{s-1,s}^*}{k_{1,0}^* \, k_{2,1}^* \dots k_{s,s-1}^*} \qquad (s=1, 2, \dots, n), \tag{A11}$$

it is easy to deduce that

$$[A_s^*] = K_s^* \, c_i^s \, \Gamma e^{-n\phi} [A_0] \qquad (s=1, 2, \dots, n). \tag{A12}$$

Using similar considerations for the unoriented molecules, the steady-state solution of the system (A5) is found to be

$$[A_s] = K_s \, c_i'^s [A_0] \qquad (s=1, 2, \dots, n) \tag{A13}$$

where

$$K_s = \frac{k_{0,1} \, k_{1,2} \dots k_{s-1,s}}{k_{1,0} \, k_{2,1} \dots k_{s,s-1}} \qquad (s=1, 2, \dots, n). \tag{A14}$$

Substituting Eqs. (A10), (A12) and (A13) into Eq. (A6) then yields

$$[A]^{\mathrm{Tot}} = [A_0] \left\{ 1 + \sum_{r=1}^{n} K_r \, c_i'^r + \Gamma e^{-n\phi} \left[1 + \sum_{r=1}^{n} K_r^* \, c_i^r \right] \right\}. \tag{A15}$$

Finally, eliminating $[A_0]$ from Eqs. (A10) and (A15), as well as from (A12) and (A15), we obtain Eqs. (12) and (13) of the text.

Appendix B

A Single-Site, Two-Barrier Eyring Model for an Ion-Permeable Pore, Extended to Allow for a Transition between Closed and Open States

In this appendix we will attempt to account for "inward rectification", assuming that the ion-permeable pore is capable of undergoing transitions between conductive (=open or unblocked) and nonconductive (=closed or blocked) states.

Using an Eyring formalism, we represent the pore as a sequence of free energy wells separated by activation energy barriers, and, for simplicity and without compromising the generality of our main conclusions, we shall assume that such a pore can be described by a single internal well and two barriers (*see* Fig. 3). "Blocking" of the pore will be ascribed to a monomolecular process as a result of which the inner barrier, which separates the site in the pore from the internal solution, becomes sufficiently high to stop further ionic movement. Since the transition from an "open" to a "closed" state may occur when the internal site is either empty or occupied, we shall consider two rate constants for "opening", λ_0 and λ_1, as well as two rate constants for closing, χ_0 and χ_1, where the subscripts, 0 and 1, denote that the pore is empty or occupied, respectively. It will be assumed that all the four rate constants, λ_0, χ_0, λ_1 and χ_1 are independent of voltage. When the channel is open, the rate constants for "inward" movement (i.e., from the cell exterior towards the cell interior) across the external and internal barriers will be denoted by α_U and γ, respectively, while those for "outward" movement by β_U and δ. The subscript U in α and β is meant to indicate that the rate constants, α_U and β_U refer to the case in which the pore is "unblocked". If p_1, q_1, p_2 and q_2 denote fractional distances (distances in units of membrane thickness) between the peaks of the activation energy barriers and the neighboring wells (*see* Fig. 3), the dependence of the four rate constants: α_U, γ, β_U and δ on the total membrane potential, ϕ, can be expressed, using the usual Eyring formalism, by

$$\alpha_U=\bar{\alpha}_U e^{-p_1\phi}; \quad \gamma=\bar{\gamma}e^{-p_2\phi}; \quad \beta_U=\bar{\beta}_U e^{q_1\phi}; \quad \delta=\bar{\delta}e^{q_2\phi}, \tag{B1}$$

where ϕ is electric potential in units of RT/F, and the four quantities, $\bar{\alpha}_U$, $\bar{\gamma}$, $\bar{\beta}_U$ and $\bar{\delta}$ are assumed to be independent of voltage as well as of ion concentration.

When the pore is "closed", translocation of ions across the internal solution and the pore is impeded, whereas ion exchange between the site in the pore and the external solution is still possible and will thus be described by the two additional rate constants, α_B and β_B. Assuming that the location of barriers and wells is not affected by whether the pore is open or closed, the dependence of such rate constants on the membrane potential will be

$$\alpha_B = \bar{\alpha}_B\, e^{-p_1 \phi}; \qquad \beta_B = \bar{\beta}_B\, e^{q_1 \phi} \tag{B2}$$

where p_1 and q_1 have the same values as in Eq. (B1).

According to this schematization of the pore, the total number of pores per unit surface, N^{Tot}, can be subdivided into the following four classes:

$N_U(0)$ = unblocked and unoccupied
$N_B(0)$ = blocked and unoccupied
$N_U(1)$ = unblocked and occupied
$N_B(1)$ = blocked and occupied.

If c_i and c_i' denote the external and internal concentrations of ion, respectively, the rates of formation of each of the above-listed states are given by

$$\frac{dN_U(0)}{dt} = \beta_U\, N_U(1) - \alpha_U\, c_i\, N_U(0) + \lambda_0\, N_B(0) - \chi_0\, N_U(0) + \gamma N_U(1) - \delta c_i'\, N_U(0) \tag{B3}$$

$$\frac{dN_B(0)}{dt} = \chi_0\, N_U(0) - \lambda_0\, N_B(0) + \beta_B\, N_B(1) - \alpha_B\, c_i\, N_B(0) \tag{B4}$$

$$\frac{dN_U(1)}{dt} = \lambda_1\, N_B(1) - \chi_1\, N_U(1) + \alpha_U\, c_i\, N_U(0) - \beta_U\, N_U(1) + \delta c_i'\, N_U(0) - \gamma N_U(1) \tag{B5}$$

$$\frac{dN_B(1)}{dt} = \chi_1\, N_U(1) - \lambda_1\, N_B(1) + \alpha_B\, c_i\, N_B(0) - \beta_B\, N_B(1) \tag{B6}$$

where all the rate constants have been defined previously. Adding up the four equations above, one realizes that the sum of the right-hand sides is identically equal to zero, which is consistent with the conservation equation

$$N_U(0) + N_B(0) + N_U(1) + N_B(1) = N^{\mathrm{Tot}}. \tag{B7}$$

The *steady-state* number of pores of each type per unit surface can be calculated by solving a linear system of four equations, constituted by Eq. (B7) and by any group of three of the four equations (B3), (B4), (B5) and (B6), in which the left-hand side has been set identically equal to zero. The transmembrane flux of ions at steady state will finally be given by

$$J=\gamma N_U(1)-\delta c_i' N_U(0). \tag{B8}$$

Constraints on the Rate Constants Imposed by "Microscopic Reversibility"

Since the rate constants introduced in the previous section are independent of ion concentration, so also must be any relationship between them. We shall now consider the case of equal concentrations in the two solutions simply because the derivation of certain important relationships is easier in this situation, although it should be clear that they remain valid in much more general conditions, such as in the presence of ionic and potential gradients.

When the permeant ion concentrations are the same in the two solutions ($c_i=c_i'$), and when there is no potential difference across the membrane, the energy levels at the two channel mouths must be the same. Recalling that in Eyring theory the rate constants are exponential functions of the activation energy barriers, this implies that at equilibrium

$$\alpha_U\gamma=\beta_U\delta. \tag{B9}$$

If we then make the additional assumption that the profile of the electric potential within the membrane is flat at equilibrium (i.e., the electric field equals 0), Eq. (B9) becomes, with the help of Eq. (B1),

$$\bar{\alpha}_U\bar{\gamma}=\bar{\beta}_U\bar{\delta}. \tag{B10}$$

Since the flux of ions is zero at equilibrium, recalling that $c_i=c_i'$ and that the electric field is assumed to vanish, we deduce from Eqs. (B8) and (B10)

$$\frac{N_U(1)}{N_U(0)}=\frac{\bar{\delta}c_i}{\bar{\gamma}}=\frac{\bar{\alpha}_U c_i}{\bar{\beta}_U}. \tag{B11}$$

As a consequence of Eqs. (B11) and (B1), Eqs. (B3), (B4) and (B5) show that at equilibrium, for $c_i=c_i'$ and zero electric field inside the membrane, we shall have

$$\frac{N_U(0)}{N_B(0)}=\frac{\lambda_0}{\chi_0}; \quad \frac{N_B(1)}{N_B(0)}=\frac{\bar{\alpha}_B\, c_i}{\bar{\beta}_B}; \quad \frac{N_U(1)}{N_B(1)}=\frac{\lambda_1}{\chi_1}. \tag{B12}$$

Making use of the identity

$$\frac{N_U(1)}{N_B(1)}=\frac{N_U(1)}{N_U(0)}\,\frac{N_U(0)}{N_B(0)}\,\frac{N_B(0)}{N_B(1)} \tag{B13}$$

the relationship (B11) and (B12) give

$$\frac{\lambda_1}{\chi_1}=\frac{\bar{\alpha}_U}{\bar{\beta}_U}\,\frac{\lambda_0}{\chi_0}\,\frac{\bar{\beta}_B}{\bar{\alpha}_B} \quad \text{or} \quad \lambda_1\,\chi_0\,\bar{\alpha}_B\,\bar{\beta}_U=\lambda_0\,\chi_1\,\bar{\alpha}_U\,\bar{\beta}_B. \tag{B14}$$

Equations (B10) and (B14) indicate that out of the ten rate constants introduced only eight are independent.

Steady-State Ionic Flux

Setting the left-hand sides of eqs. (B3), (B4), (B5) and (B6) equal to zero, and solving for J from the system of five Equations, (B7), (B8) and any group of three of the four equations (B3), (B4), (B5) and (B6), one finds

$$J=-2\,\frac{\Omega_0\,c_i\,e^{-\phi}+\Omega_1}{W_0\,c_i^2\,e^{-2\phi}+W_1\,c_i\,e^{-\phi}+W_2}\,e^{[\frac{1}{2}-p_1-p_2]\phi}\sqrt{c_i\,c_i'}\,\sinh\left[\frac{\Delta\phi}{2}\right] \tag{B15}$$

where

$$\Delta\phi=\phi-\phi_0=\phi-\ln\frac{c_i}{c_i'} \tag{B16}$$

$$\Omega_0=N^{\mathrm{Tot}}\,\bar{\alpha}_U\,\bar{\gamma}\lambda_1\,\bar{\alpha}_B\,e^{(1-p_1)\phi} \tag{B17}$$

$$\Omega_1=N^{\mathrm{Tot}}\,\bar{\alpha}_U\,\bar{\gamma}\lambda_0(\bar{\beta}_B\,e^{q_1\phi}+\lambda_1+\chi_1\,\bar{\beta}_B/\bar{\beta}_U) \tag{B18}$$

$$W_0=(\chi_1+\lambda_1)\,\bar{\alpha}_U\,\bar{\alpha}_B\,e^{2(1-p_1)\phi} \tag{B19}$$

$$\begin{aligned}W_1=[&\bar{\alpha}_B\,\bar{\delta}\,e^{q_2\phi}(\chi_1+\lambda_1)\,c_i'+\bar{\alpha}_B(\bar{\beta}_U\,e^{q_1\phi}+\bar{\gamma}e^{-p_2\phi})(\lambda_1+\chi_0)\\&+(\chi_1+\lambda_1)(\lambda_0\,\bar{\alpha}_U+\chi_0\,\bar{\alpha}_B)+\bar{\alpha}_U\,\bar{\beta}_B\,e^{q_1\phi}(\lambda_0+\chi_1)]\,e^{(1-p_1)\phi}\end{aligned} \tag{B20}$$

$$\begin{aligned}W_2=\bar{\delta}\,e^{q_2\phi}&[\chi_1\,\bar{\beta}_B\,e^{q_1\phi}+\lambda_0(\bar{\beta}_B\,e^{q_1\phi}+\chi_1+\lambda_1)]\,c_i'\\&+(\lambda_0+\chi_0)[\chi_1\,\bar{\beta}_B\,e^{q_1\phi}+(\bar{\beta}_U\,e^{q_1\phi}+\bar{\gamma}e^{-p_2\phi})(\bar{\beta}_B\,e^{q_1\phi}+\lambda_1)].\end{aligned} \tag{B21}$$

From these equations it is apparent that the expression for the conductance deducible from Eq. (B15) is in general much more complicated than either Eqs. (1) or (37). The case in which Eqs. (B15) and (37) become as close to each other as possible is the limiting situation in which the peak of the external barrier is so close to the inner boundary that

$$p_1 \simeq 1; \qquad q_1 \simeq q_2 \simeq p_2 \simeq 0. \tag{B22}$$

This is the only case in which the five quantities, Ω_0, Ω_1, W_0, W_1 and W_2 are all independent of potential. Recalling Eqs. (B16) and (B22) and rearranging, Eq. (B15) can be written in the following form

$$J = -2 \frac{\Omega_0 + \frac{\Omega_1}{c_i'} e^{\Delta\phi}}{W_1 \left\{ 1 + 2 \frac{\sqrt{W_0 W_2}}{W_1} \cosh\left[\Delta\phi - \Delta\phi_h^*\right] \right\}} e^{-\phi/2} \sqrt{c_i c_i'} \sinh\left[\frac{\Delta\phi}{2}\right]; \tag{B23}$$

where

$$e^{\Delta\phi_h^*} = \sqrt{\frac{W_0 c_i'^2}{W_2}}. \tag{B24}$$

Equation (41) in the text can now be obtained from Eq. (B23), recalling that

$$G = \frac{I}{\Delta V} = \frac{FJ}{\Delta V} = \frac{F^2}{RT} \frac{I}{\Delta\phi} \tag{B25}$$

where I is the current density.

List of Symbols

Latin Alphabet

1) A_s: unoriented pore-forming molecule with s complexed cations ($s = 0, 1, \ldots, n$).
2) A_s^*: pore with s complexed cations ($s = 0, 1, \ldots, n$).
3) B: defined by Eq. (36).
4) c_i, c_i': external and internal concentration of the permeant cation.
5) D_1, D_2: defined by Eqs. (39) and (40).
6) $f(\phi), f'(\phi)$: *cf.* Eqs. (29), (30), and (31).
7) $\bar{g}_s$: conductance of a single A_s^* pore ($s = 0, 1, \ldots, n$).
8) $k_{s-1,s}$, ($k_{s,s-1}$): rate constants for binding (dissociation of) an ion by (from) an unoriented pore-forming molecule with $s-1(s)$ complexed cations [*see* Eq. (A5)].
9) $k_{s-1,s}^*$, ($k_{s,s-1}^*$): same as above, except that they refer to pore molecules [*see* Eqs. (A4) and (A1)].
10) k_s^F, k_s^B: electric field-independent fractions of the forward and backward rate constants for orientation of pore-forming molecules with s complexed cations [*cf.* Eq. (A3)].

11) K_s: equilibrium constant for the binding of s internal cations by an unoriented pore-forming molecule [*cf.* Eq. (10)].
12) K_s^*: equilibrium constant for the binding of s external cations by an oriented gating molecule [*cf.* Eq. (9)].
13) L_s, L: defined by Eqs. (20) and (21).
14) n: number of subunits in each pore-forming molecule and maximum number of ions bound by such a molecule.
15) p_1, p_2, q_1, q_2: fractional voltage drops between energy-peaks and wells in the pore considered in the "occupancy gating" model. *See* Fig. 3.
16) v: defined by Eq. (19).
17) V: membrane potential, defined as the electric potential difference between the interior and the exterior of the cell.
18) W_0, W_1, W_2: defined by Eqs. (B19), (B20) and (B21).
19) z: valency of the subunits of the gating molecules.

Greek Alphabet

1) α: fractional voltage drop between peak and well of the activation energy barrier for orientation of the gating molecules (*cf.* Eq. (A3)).
2) $\left\{\begin{array}{l}\alpha_U, \alpha_B, \beta_U, \beta_B, \\ \gamma, \delta, \\ \lambda_0, \chi_0, \lambda_1, \chi_1\end{array}\right.$ state-transition rate constants in the "occupancy gating" model (*see Appendix B* and Fig. 3).
3) $\bar{\alpha}_U, \bar{\alpha}_B, \bar{\beta}_U, \bar{\beta}_B, \bar{\gamma}, \bar{\delta}$: particular values of the above first six rate constants in the condition of zero electric field.
4) Γ: k_s^F/k_s^B with $s=0$ (*cf.* 10 in the list of Latin alphabet letters).
5) ΔV_0: $V-V_0$, where V_0 is resting potential.
6) ΔV_h: defined by Eqs. (22) and (27) in the "electro-chemical-gating" model.
7) $\Delta\phi$: $\frac{F}{RT}\Delta V$.
8) $\Delta\phi_h$: defined by Eq. (47).
9) $\Delta\phi_h^*$: defined by Eq. (42).
10) ϕ: $\frac{F}{RT}V$.
11) ω: defined by Eq. (14).
12) Ω_0, Ω_1: defined by Eqs. (B17) and (B18).

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