The Role of the Electrochemical Gradient in Determining Potassium Fluxes in Frog Striated Muscle

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INTRODUCTION

Nearly two decades ago, B. Katz reported some important observations on electrical rectification in skeletal muscles immersed in isotonic solutions of K_2SO_4. In these solutions, he found that the membrane resistance was fairly low for passage of inward current but high for passage of outward current. He suggested that this rectification was a property of the potassium conductance system (Katz, 1949).

Since that time a number of investigators have studied this rectification process, measuring electrical parameters and the rates of $^{42}K^+$ movement (Adrian, 1962; Adrian and Freygang, 1962 *a*, 1962 *b*; Hodgkin and Horowicz, 1959; Nakajima, Iwasaki and Obata, 1962; Sjodin, 1965). The generally accepted view is that this rectification is in fact attributable to the potassium conductance system as originally suggested by Katz. Furthermore, for a wide range of potassium equilibrium potentials, V_K, the behavior of this conductance system can be qualitatively described by saying that the potassium permeability is fairly high for inward movement but very low for outward movement. Although important and useful information has been gathered in support of this view, no satisfactory physical explanation for the process has yet emerged.

The present experiments were designed as a study of the unidirectional potassium fluxes for two types of condition. In one type, the potassium equilibrium potential was varied by changing $[K^+]_o$ while the internal potential, V_i, was kept constant; in the other type, the internal potential was varied by changing $[Cl^-]_o$ while the potassium equilibrium potential was kept constant. These methods are based on the observation that, under appropriate conditions, the internal potential can be made to depend mainly on chloride ion concentration while being relatively insensitive to changes in external potassium ion concentration (Hodgkin and Horowicz, 1959).

The observations described in this article were performed on small bundles of muscle fibers isolated from the semitendinosus muscles of the frog *Rana pipiens*. Measurements were made of the internal potential, $^{42}K^+$ influx, and $^{42}K^+$ efflux on preparations initially equilibrated in a Ringer's fluid to which KCl was added so that the final external $[K^+]$ and $[Cl^-]$ were 100 and 217 mmoles/liter respectively. This treatment produces no change in fiber volume (Boyle and Conway, 1941).

Reprinted from THE JOURNAL OF GENERAL PHYSIOLOGY, 1968, Vol. 51, No. 5, Part 2, pp. 193 s–203 s *Printed in U.S.A.*

EFFECTS OF CHANGING EXTERNAL POTASSIUM ION CONCENTRATION

The results from experiments dealing with effects on K^+ efflux produced by lowering $[K^+]_o$ from an initial value of 100 mM are summarized in Fig. 1. The K^+ efflux at reduced $[K^+]_o$ has been expressed in dimensionless terms by dividing by the K^+ efflux found in 100 mM K^+. In these experiments, the average internal potential, as measured by microelectrodes, was not detectably altered at first by the reduction in $[K^+]_o$ from its initial value of −18mv measured with the fibers equilibrated in 100

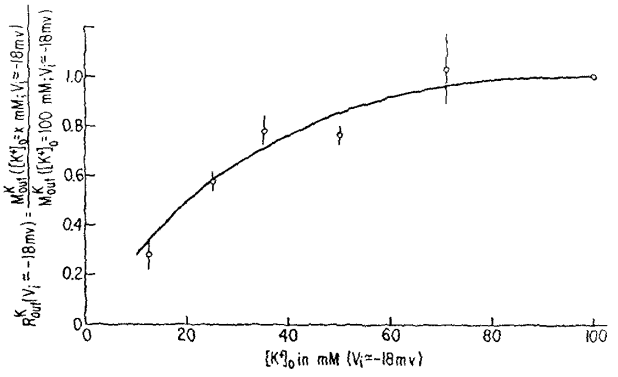

FIGURE 1. The effects of external potassium ion concentration on the efflux of potassium ions from muscle fibers. Fibers had been equilibrated initially in a solution containing 100 mM K^+ and 217 mM Cl^-. The ordinate represents potassium efflux during the first 5 min after reduction of the external K^+ concentration. This efflux has been divided by the K^+ efflux in the equilibrating solution and is plotted as a dimensionless ratio. Each point is the mean of several experiments: the bars show ±2SEM. The curve shown is given by the equation $R^K_{out} = [52200\ mM^2/([K^+]_o^2 + 42200\ mM^2)] \times [(1.6([K^+]_o)/(60\ mM + [K^+]_o)]$.

mM K^+. A lowering of $[K^+]_o$ to 71 mM produced no statistically significant change in the K^+ efflux; further lowering of $[K^+]_o$ produced a reduction in the K^+ efflux. For $[K^+]_o$ between 35 and 12.5 mM the values for K^+ efflux fall on a line which appears to extrapolate toward the origin. It is clear that with V_i and internal potassium concentration, $[K^+]_i$, constant, the system responsible for K^+ movements, as assayed by K^+ efflux, was gradually activated by increasing $[K^+]_o$. At sufficiently high values of $[K^+]_o$ this transport system apparently becomes saturated.

Starting from the same initial conditions, K^+ influx was also measured in solutions with lowered $[K^+]_o$. The results of these measurements are summarized in Fig. 2. In this graph the ordinate represents the average ratio of K^+ influx at the reduced $[K^+]_o$ to the K^+ influx in 100 mM K^+ and is plotted as a filled circle. It is evident from inspection of the figure that the K^+ influx seems to increase approximately as the square of $[K^+]_o$. For intermediate concentrations the points do not quite follow the law of squares; on the average, the points fall below this relation.

If the effect of reduced $[K^+]_o$ on K^+ efflux is ascribable to an inactivation of the potassium transport system, then dividing the K^+ influx at a given $[K^+]_o$ by the efflux ratio of Fig. 1 for the same $[K^+]_o$ should give an estimate of the effect of $[K^+]_o$ on influx at a constant level of activation. The open circles in Fig. 2 represent the results

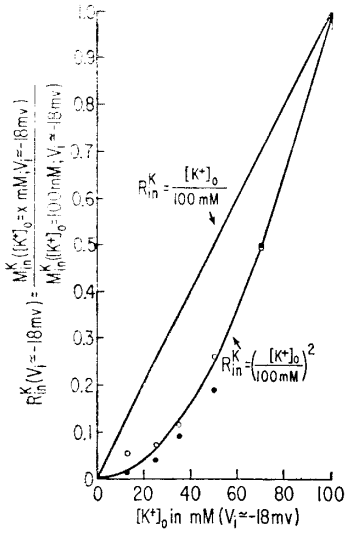

FIGURE 2. The effect of external potassium ion concentration on the influx of potassium ions. Fibers had been equilibrated initially in a solution containing 100 mM K^+ and 217 mM Cl^-. The ordinate (filled circles) gives the K^+ influx during the first 6 min after a reduction of the $[K^+]_o$. This influx has been divided by the K^+ influx in the equilibrating solution and is plotted as a dimensionless ratio. The significance of the open circles is explained in the text.

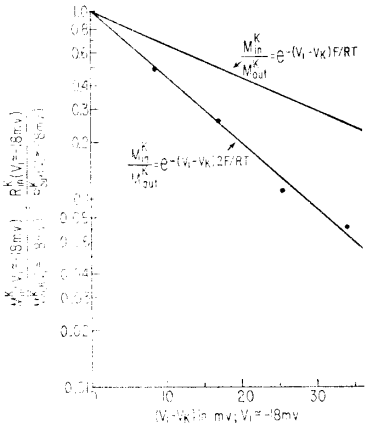

FIGURE 3. The effect of external potassium ion concentration on the ratio of the K^+ influx to the K^+ efflux. The ordinate, which represents this flux ratio, has a logarithmic scale. The abscissa gives the external K^+ concentration as the difference between the internal potential and the potassium equilibrium potential ($V_K = RT/F \ln\{[K^+]_o/[K^+]_i\}$).

of such a calculation. Taken as a group, these values more nearly follow the quadratic relation. The natural conclusion, therefore, is that, at constant V_i and $[K^+]_i$, the potassium influx is largely controlled by a second-order reaction mechanism for K^+.

The calculation just discussed in fact represents the ratio of the influx to the efflux of potassium. It is informative to plot this ratio as a function of the difference in electrochemical potential across the membrane, i.e. $V_i - V_K$; this is shown in Fig. 3. For values of $(V_i - V_K)$ less than 35 mv the flux ratio is described by the equation

$$\frac{M_{in}^{K}}{M_{out}^{K}} = \exp\,[-2(V_i - V_K)F/RT]. \qquad (a)$$

This equation follows directly from the equation used to approximate the data in Fig. 2, i.e.,

$$\frac{M_{in}^{K}}{M_{out}^{K}} = \left(\frac{[K^+]_o}{[K^+]_o'}\right)^2 \qquad (b)$$

where $[K^+]_o'$ is the initial external potassium concentration (= 100 mM); V_i is taken as

$$V_i = V_K' = \frac{RT}{F} \ln \frac{[K^+]_o'}{[K^+]_i};$$

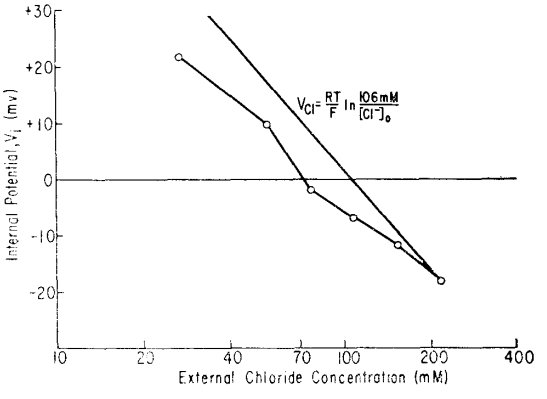

FIGURE 4. The effect of lowered external chloride ion concentration on the internal potential of fibers initially equilibrated in a solution containing 100 mM K^+ and 217 mM Cl^-.

and $[K^+]_i$ is constant. Thus the deviation of the potassium flux ratio from the well known Ussing independence relation in this case seems to result from the dominance of a reaction mechanism involving second-order kinetics for K^+.

EFFECTS OF CHANGING EXTERNAL CHLORIDE ION CONCENTRATION

In view of the above findings, it is of interest to examine the dependence of the flux ratio on $(V_i - V_K)$ when V_K is kept constant and V_i varies.

In Fig. 4 the average transmembrane potential found during the first 5–6 min after a reduction of $[Cl^-]_o$ from its initial value of 217 mM is shown as a function of $[Cl^-]_o$. The potassium ion concentration was kept constant at 100 mM. It is apparent that the internal potential becomes more positive when $[Cl^-]_o$ is reduced. In these experiments the glucuronate anion was used as a substitute for chloride. The values of the internal potential obtained result from the fact that, in the solutions used, the chloride conductance of the membrane is much higher than that for potassium and other ions (Hodgkin and Horowicz, 1959). By employing these solutions, it is possible to study the effects of V_i on the K^+ fluxes.

The averaged results from such experiments are given in Figs. 5 and 6. In Fig. 5 the individual fluxes are plotted (as above) in terms of dimensionless units; both K^+ influx and K^+ efflux fall as the internal potential goes progressively more positive. Since the influx falls more rapidly than the efflux, there is a net flux of potassium out of the cells. This net flux of potassium has a maximum because the efflux continues to fall at values of $(V_i - V_K)$ for which the influx is already quite small. The origin of the equations used to fit the data will be discussed below.

The relation of the flux ratio to $(V_i - V_K)$ is shown in Fig. 6. It is clear that the flux ratio is again described by equation *a* for values of $(V_i - V_K)$ less than 35 mv.

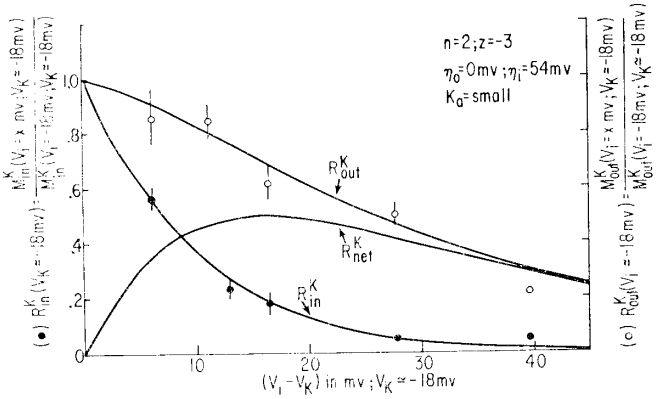

FIGURE 5. The effect of the internal potential on the K^+ efflux (open circles) and K^+ influx (filled circles). The internal potential was varied by lowering $[Cl^-]_o$. Bars show ±2SEM. Equations (16) to (18) of the text were used to draw the curves shown. The values of the parameters used are given in the upper right-hand corner. See text for further details.

In summary, equation *a* for the passive K^+ flux ratio is valid whether the driving force, $V_i - V_K$, on the potassium ions results from a variation of V_i or of V_K.

A MODEL FOR PASSIVE POTASSIUM ION MOVEMENTS IN FROG MUSCLE

Since the data suggest that the fluxes are dominated by a second-order reaction, a carrier model of the type described below becomes an attractive possibility. The detailed behavior of the fluxes measured under the conditions stated above cannot be fitted by a simple model in which the total amount of carrier sites remains constant. Consequently, a number of additional elements need to be incorporated into the model. The elements which have been added are fairly simple. As it now stands, the model generates some relations which are suggestive and can be used to fit the data. In what follows, the assumptions will be grouped under three headings.

A. Geometry and Potential

The first assumption will be that in addition to the membrane there are two juxtamembranous regions which separate the inside of the muscle cell from the outside

(see Fig. 7). For convenience, the region separating the membrane from the outside solution shall be termed the outer j region (oj) and the one separating the membrane from the myoplasm, the inner j region (ij). It shall be further assumed that the electrostatic potentials in these j regions are maintained at levels which differ from those found in the bulk phases adjoining them. The spatial distribution of potential on moving from the bulk phase to the adjoining j region need not be specified. It will be assumed, however, that the rate of movement of potassium via the transport system in the membrane is sufficiently limiting to allow the potassium ions to be main-

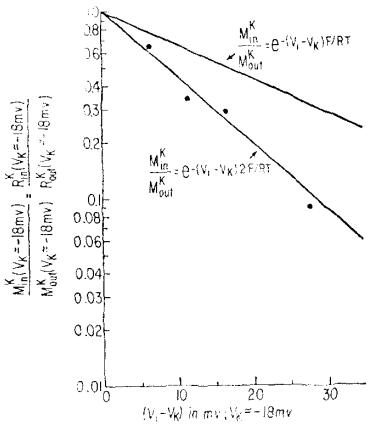

FIGURE 6. The effect of the internal potential on the ratio of the K^+ influx to the K^+ efflux.

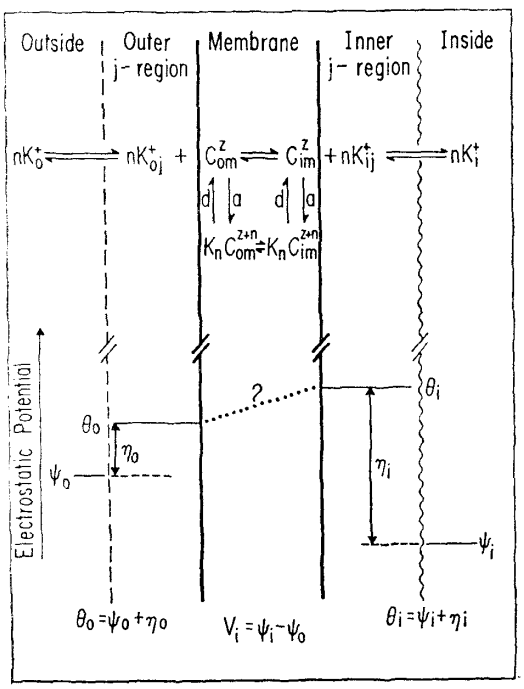

FIGURE 7. Diagram of a model for passive K^+ movements, showing carrier reaction mechanism in membrane and electrostatic potentials in regions separating the myoplasm from the extracellular fluid.

tained at electrochemical equilibrium between a given bulk phase and its adjoining j region even under conditions of net transport. Employing the notation given in Fig. 7, one can write

$$[K^+]_{ij} = [K^+]_i \exp [(\psi_i - \theta_i)F/RT] = [K^+]_i \exp (-\eta_i F/RT) \quad (1)$$

and

$$[K^+]_{oj} = [K^+]_o \exp [(\psi_o - \theta_o)F/RT] = [K^+]_o \exp (-\eta_o F/RT), \quad (2)$$

where

$$\theta_i = \psi_i + \eta_i, \quad \theta_o = \psi_o + \eta_o, \quad \text{and} \quad V_i = \psi_i - \psi_o.$$

B. The Carrier System and Reaction Laws

Potassium ions are assumed to move through the membrane by associating with a mobile carrier system which is confined to the membrane. The carriers, C^z, when not associated with K^+ have a charge of zF coulombs per mole; in this condition they will be called empty. The carrier associates with n potassium ions to form the predominant complex K_nC^{z+n}, which will be called the filled carrier. All other possible complexes are assumed to exist only in negligible concentrations. The rates of movement of the empty and filled carriers across the membrane are much faster than the rates of association and dissociation, so that these species are at electrochemical equilibrium across the membrane for all conditions. Taking the electrostatic potentials at the membrane borders to be continuous with the adjoining j regions, one can write

$$[C^z]_{im} = [C^z]_{om} \exp [-(\theta_i - \theta_o)zF/RT] \quad (3)$$

and

$$[K_nC^{z+n}]_{im} = [K_nC^{z+n}]_{om} \exp [-(\theta_i - \theta_o)(z + n)F/RT]. \quad (4)$$

At the outside border of the membrane, the velocities of association (v_a) and dissociation (v_d) obey mass reaction laws given by

$$v_{ao} = a \cdot [K^+]_{oj}^n \cdot [C^z]_{om} \quad (5)$$

and

$$v_{do} = d \cdot [K_nC^{z+n}]_{om}, \quad (6)$$

and at the inside border of the membrane the reaction laws are

$$v_{ai} = a \cdot [K^+]_{ij}^n \cdot [C^z]_{im} \quad (7)$$

and

$$v_{di} = d \cdot [K_nC^{z+n}]_{im}. \quad (8)$$

The equilibrium state defines a unique association constant for the two borders:

$$v_{ao} = v_{do} \quad \text{implies} \quad K_{ao} = \frac{[\mathrm{K}^+]_{oj}^n \cdot [C^z]_{om}}{[\mathrm{K}_n C^{z+n}]_{om}} = \frac{d}{a} = K_a \tag{9 a}$$

and

$$v_{ai} = v_{di} \quad \text{implies} \quad K_{ai} = \frac{[\mathrm{K}^+]_{ij}^n \cdot [C^z]_{im}}{[\mathrm{K}_n C^{z+n}]_{im}} = \frac{d}{a} = K_a. \tag{9 b}$$

In general, the total number of carrier molecules actively present in the system, empty and filled, is controlled by a mechanism dependent on the concentrations of potassium ions in the two j regions. The greater the concentration of potassium ions in the j regions, the greater the total number of carrier molecules activated. Hence one can write

$$[\mathrm{K}_n C^{z+n}]_{om} + [\mathrm{K}_n C^{z+n}]_{im} + [C^z]_{im} + [C^z]_{om} = f([\mathrm{K}^+]_{oj}\,;[\mathrm{K}^+]_{ij}) = Q. \tag{10}$$

C. Specifications of Unidirectional and Net Fluxes

When a steady-state rate of movement of potassium is achieved, the net rate of association at one membrane border will equal the net rate of dissociation at the other membrane border; on this assumption one can write

$$M_{\mathrm{net}}^{\mathrm{K}} = v_{ai} - v_{di} = v_{do} - v_{ao}\,. \tag{11}$$

With the above 11 equations, it is possible to derive the net flux vs. internal potential relation for potassium ions, which, using Faraday's constant, converts to the current-voltage relation for these ions. Before stating this relation, it will be convenient to pursue the scheme of assumptions one stage farther in order to develop the equations governing the movement of an isotope of potassium. On the hypothesis that the carrier sites do not discriminate between isotopes of potassium, one can write

$$v_{ao}^* = v_{ao} \cdot S_{oj}^{\mathrm{K}} \tag{1*}$$

$$v_{do}^* = v_{do} \cdot S_{om}^{\mathrm{K}_n C} \tag{2*}$$

$$v_{ai}^* = v_{ai} \cdot S_{ij}^{\mathrm{K}} \tag{3*}$$

$$v_{di}^* = v_{di} \cdot S_{im}^{\mathrm{K}_n C} \tag{4*}$$

where the v^*'s are the velocities of association and dissociation for the isotope and the S's are the specific activities. Since the isotope will distribute itself rapidly in the membrane carrier system, then as in equation 11 one can write

$$M_{\mathrm{net}}^* = v_{ai}^* - v_{di}^* = v_{do}^* - v_{ao}^*\,. \tag{5*}$$

Before proceeding to the operational definitions of the unidirectional fluxes, it will be convenient to obtain an expression for the specific activity of the filled carriers

in terms of the specific activities of the K^+ outside and inside the cell. Substituting equations 1*–4* into equation 5*, one gets

$$v_{ao} \cdot S^{K}_{oj} + v_{ai} \cdot S^{K}_{ij} = v_{do} \cdot S^{K_n C}_{om} + v_{di} \cdot S^{K_n C}_{im}. \qquad (6^*)$$

Since both filled and empty carriers are at equilibrium across the membrane, then

$$S^{K_n C}_{om} = S^{K_n C}_{im}. \qquad (7^*)$$

In addition, the equilibrium of K^+ between the j regions and their adjoining bulk phases insures that

$$S^{K}_{oj} = S^{K}_{o} \qquad (8\,a^*)$$

and

$$S^{K}_{ij} = S^{K}_{i}. \qquad (8\,b^*)$$

Substituting equations 7*, 8 a*, and 8 b* into 6*, one gets

$$S^{K_n C}_{om} = \frac{v_{ao} \cdot S^{K}_{o} + v_{di} S^{K}_{i}}{v_{do} + v_{di}}. \qquad (9^*)$$

With equation 9* in hand, one can proceed to the equations for unidirectional fluxes. Operationally, the definitions for the influx and efflux of potassium are given by

$$M^{K}_{in} = -\frac{M^{*}_{net}}{S^{K}_{o}} \quad \text{when} \quad S^{K}_{i} = 0 \qquad (10^*)$$

and

$$M^{K}_{out} = \frac{M^{*}_{net}}{S^{K}_{i}} \quad \text{when} \quad S^{K}_{o} = 0. \qquad (11^*)$$

Using the asterisked equations, it follows, therefore, that

$$M^{K}_{in} = \frac{v_{ao} \cdot v_{di}}{v_{do} + v_{di}} \qquad (12)$$

and

$$M^{K}_{out} = \frac{v_{do} \cdot v_{ai}}{v_{do} + v_{di}}. \qquad (13)$$

From equations 12, 13, and 5—8, the flux ratio becomes

$$\frac{M^{K}_{in}}{M^{K}_{out}} = \left(\frac{v_{ao}}{v_{do}}\right) \cdot \left(\frac{v_{di}}{v_{ai}}\right) = \frac{[K^+]^{n}_{oj}}{[K^+]^{n}_{ij}} \cdot \frac{[C^z]_{om}}{[C^z]_{im}} \cdot \frac{[K_n C^{z+n}]_{im}}{[K_n C^{z+n}]_{om}}. \qquad (14)$$

Finally, substituting equations 1–4 into equation 14, one has

$$\frac{M_{\text{in}}^{\text{K}}}{M_{\text{out}}^{\text{K}}} = \exp\left[-(V_i - V_K)nF/RT\right]. \tag{15}$$

The interest and importance of the flux ratio equation 15 stems from the fact that all but one of the adjustable parameters of the model cancel out; only the order of the reaction, n, for K^+ remains. For the experiments reported on in this paper, n is clearly 2. It is also of some interest that equation 15 is of the same mathematical form as that resulting from the assumptions of a single-file mechanism (Hodgkin and Keynes, 1955).

From the assumptions outlined above, the individual fluxes can be expressed in terms of the parameters of the model, V_i, $[K^+]_o$, and $[K^+]_i$. They are given by

$$M_{\text{out}}^{\text{K}} = A \cdot \frac{([K^+]_i^n \exp\left[-\eta_i nF/RT\right]) \cdot \exp\left[-(V_i + \eta_i - \eta_o)zF/RT\right]}{([K^+]_o^n \exp\left[-\eta_o nF/RT\right] + K_a) + B} \tag{16}$$

$$M_{\text{in}}^{\text{K}} = A \cdot \frac{([K^+]_o^n \exp\left[-\eta_o nF/RT\right]) \cdot \exp\left[-(V_i + \eta_i - \eta_o)(z+n)F/RT\right]}{([K^+]_o^n \exp\left[-\eta_o nF/RT\right] + K_a) + B} \tag{17}$$

$$M_{\text{net}}^{\text{K}} = M_{\text{out}}^{\text{K}} - M_{\text{in}}^{\text{K}} \tag{18}$$

where $A = \dfrac{K_a \cdot Q \cdot a}{(1 + \exp\left[-(V_i + \eta_i - \eta_o)(z+n)F/RT\right])}$

and $B = ([K^+]_i^n \exp\left[-\eta_i nF/RT\right] + K_a) \exp\left[-(V_i + \eta_i - \eta_o)zF/RT\right]$.

A reasonable fit of the data given above can be obtained with $n = 2$, $z = -3$, $\eta_o = 0$, $\eta_i = 54$ mv, and with the association constant, K_a, taken as negligible compared with $[K^+]_{ij}^2$ and $[K^+]_{oj}^2$ (see Fig. 5). The resting potassium efflux estimated at 100 pmoles/cm^{-2} sec^{-1} in 100 mM K^+, 217 mM Cl^-, and $V_i = -18$ mv suggests that the value of the product $(K_a \cdot Q \cdot a)$ is 650 pmoles cm^{-2} sec^{-1}. A function which reasonably fits the activation of the carrier system is $Q = 1.6[K^+]_o/(60 \text{ mM} + [K^+]_o)$ (see Fig. 1). Such an equation can be developed from a model having a nonmobile control site which, when it associates with K, liberates a carrier molecule. The carrier is assumed to be inactivated when the control site is not associated with potassium. At present, however, it merely represents a convenient formula for summarizing the effects of $[K^+]_o$ on K^+ efflux for the range of values studied.

A few comments may be made about the values for the adjustable parameters used to fit the data. The parameter n can only be 2. If one allows z to range over the integers, either -3 or $+1$ will fit the data. On physicochemical grounds, one might expect that $z = -3$ is the more reasonable possibility. The value of η_i is to a certain extent arbitrary and depends on the values chosen for η_o, K_a, and z.

Finally, on the basis of these data, the calculated slope conductance for potassium at $V_i = -18$ mv has a value of 770 μmho/cm^2. This value is of the same order of magnitude found by previous experimenters (Adrian and Freygang, 1962 *b*; Hodgkin and Horowicz, 1959).

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