

THE EQUATIONS FOR CELLULAR RESTING POTENTIALS
ACCORDING TO THE SURFACE ADSORPTION THEORY,
A COROLLARY OF THE ASSOCIATION-INDUCTION HYPOTHESIS

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• *Failure of previous equations to account for the decline of the cellular resting potentials at low external K^+ concentration has led to a revision of the equation for the cellular resting potential based on the surface-adsorption theory, a corollary of the association-induction hypothesis. This revised equation, which takes into account cooperative interaction among the surface anionic sites adsorbing K^+ or Na^+ , is capable of explaining the entire profile of the resting potential at high as well as low external K^+ concentration (and at high external Na^+ concentrations).*

In 1959 I presented briefly,¹ and later in detail, the surface adsorption theory of cellular resting potential.²⁻⁸ In this theory the resting potential (ψ) is an equilibrium potential; the nature, polarity, and density of fixed ionic sites on the cell surface along with the nature and concentrations of external ions are what determine the polarity and magnitude of the potential. Thus the equation describing the cellular resting potential becomes:

$$\psi = \text{constant}_1 - RTF^{-1} \ln \left\{ \sum_{i=1}^n K_i [p_i]_{\text{ex}} \right\} \quad (1)$$

where K_i is the adsorption constants in $(M)^{-1}$ of the i th ion (bearing opposite electric charge to that of the surface fixed sites) at a concentration $[p_i]_{\text{ex}}$ among a total of n types of ions of the same charge in the external medium.

In normal frog muscle cells the surface sites are anionic (i.e., isolated β - and γ -carboxyl groups) (see ref. 3, p. 278). There is evidence that these isolated sites of carboxyl groups have little or no affinity for

divalent cations but do adsorb monovalent K^+ and Na^+ as well as other monovalent cations.⁵ In that case,

$$\psi = \text{constant}_2 - RTF^{-1} \ln \{ K_K [K^+]_{\text{ex}} + K_{Na} [Na^+]_{\text{ex}} \}. \quad (2)$$

The relations described in Eq. 2 between each of the variables and ψ have been experimentally verified under a variety of conditions.^{3,6-8*} Thus Eq. 2 (as well as the Hodgkin-Katz equation based on the membrane theory¹⁰) are capable of explaining most of the experimental data on the effect of varying external K^+ including: (1) the steady decrease of ψ with increases of $[K^+]_{\text{ex}}$ in the range of $[K^+]_{\text{ex}}$ higher than that found in the cell's natural environment (e.g., 2.5 mM for frog muscle cells);³ (2) the stabilization of ψ toward a constant value as $[K^+]_{\text{ex}}$ decreases to values below that found in the cell's normal environment with either $[Na^+]_{\text{ex}}$ or $([Na^+]_{\text{ex}} + [K^+]_{\text{ex}})$ held constant;³ and

* Edelman, Edelman, and Baldauf in 1974 derived a variant of Eq. 2, in which a term 1 is included in the sum within the bracket?

(3) the continual steady increase of ψ when $[K^+]_{ex}$ is held constant at zero or near zero while $[Na^+]_{ex}$ is steadily lowered.¹¹

However, neither the Hodgkin-Katz equation nor Eq. 2 explains the observation that in the presence of high external Na^+ concentration, ψ may in certain types of cells decrease with lowering of external K^+ concentration beyond that found in the cell's normal environment.¹²⁻¹⁴

Now, Eqs. 1 and 2 were derived on the basis of two assumptions: (1) The percentage of vacant surface anionic sites is small compared to that of the occupied sites; evidence for this assumption based on the magnitude of the electrical potentials is given in the footnote below.** (2) The sites are

** Let us consider that at the cell surface the vacant anionic sites and counterion K^+ form a Helmholtz double layer; the voltage across this double layer is then $V = 4\pi od/\eta$, where d is the distance between the two layers and is about 50 Å or 5×10^{-9} meter. η , assumed to be 1.11×10^{-10} Coulomb/volt-m is equal to that of free space. The question then becomes: what is the density of surface vacant sites to yield a voltage seen in living cells; i.e., 0.1 V? The answer is $\sigma = V\eta/4\pi d = (0.1 \times 1.11 \times 10^{-10})/(4 \times 3.14 \times 5 \times 10^{-9}) = 1.77 \times 10^{-4}$ Coulomb. Since each Faraday is equal to 96,500 Coulomb, $\sigma = (1.77 \times 10^{-4})/(9.65 \times 10^4) = 1.83 \times 10^{-9}$ Faraday. This amount of charge is carried by 1.83×10^{-9} moles of vacant sites per cm^2 of cell surface.

The average number of fixed anionic sites per cm^2 to a depth of 50 Å in frog muscle cells can be roughly estimated as $1.5 \times 10^{-1} \times 5 \times 10^{-7} = 7.5 \times 10^{-8}$ moles, assuming that this site density is the same in the bulk phase as in the cell surface. The number of vacant sites is thus only $(1.83 \times 10^{-9})/(7.5 \times 10^{-8})$ or 2.5% of the total surface-fixed anionic sites. Even if the vacant sites are entirely confined to a "monomolecular layer" of surface anionic sites, the percentage of vacant sites will be increased only by a factor of 2 since the average charge-to-charge and hence layer-to-layer distance is about 20 Å (see ref. 3, p. 48). All these calculations were based on a permittivity η of the vacuum. In truth, the system is aqueous, containing fixed ionic sites and counter ions. Thus the value of η (dielectric constant) must be considerably higher. Consequently, the percentage of vacant sites must be still lower than the values estimated.

independent and show no cooperative interaction.

I shall demonstrate that by retaining the first assumption but relaxing the second, and by introducing the concept of cooperative interaction among the surface protein anionic sites—a concept that has been most useful in explaining bulk phase K^+ and Na^+ accumulation in living cells—the decrease of ψ with decreasing $[K^+]_{ex}$ at high $[Na^+]_{ex}$ can also be explained.

In 1965 I showed that the adsorption of K^+ and Na^+ in the bulk phase cytoplasm of whole cells with nearest-neighbor interaction energy equal to $-\gamma/2$ can be described by the following isotherm derived on the basis of the one-dimensional Ising model:¹⁵⁻¹⁸

$$[K^+]_{ad} = [f]/2 \left\{ 1 + \left(\frac{\xi - 1}{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)} \right)^{1/2} \right\}, \quad (3)$$

where $[K^+]_{ad}$ is the concentration of adsorbed K^+ , and $[f]$ is the concentration of adsorption sites for the i th (and j th) species. ξ is defined as follows:

$$\xi = ([K^+]_{ex}/[Na^+]_{ex}) \cdot K_{Na \rightarrow K}^{oo}, \quad (4)$$

where $[K^+]_{ex}$ and $[Na^+]_{ex}$ are the concentration of free Na^+ and K^+ in the external solution and $K_{Na \rightarrow K}^{oo}$ is the intrinsic equilibrium constant for the $Na \rightarrow K$ solute exchange. $K_{Na \rightarrow K}^{oo}$ is related to the intrinsic free energy of exchange $\Delta F_{Na \rightarrow K}^{oo}$ by the relation:

$$-\Delta F_{Na \rightarrow K}^{oo} = RTF^{-1} \ln K_{Na \rightarrow K}^{oo}. \quad (5)$$

It is to be noted that $\Delta F_{Na \rightarrow K}^{oo}$ refers to the free energy change in $Na^+ \rightarrow K^+$ exchange of adsorption, which involves no change in the total number of $Na-K$ pairs of nearest neighbors within the system. This is the case when the exchange on the middle site occurs in a triad of sites:

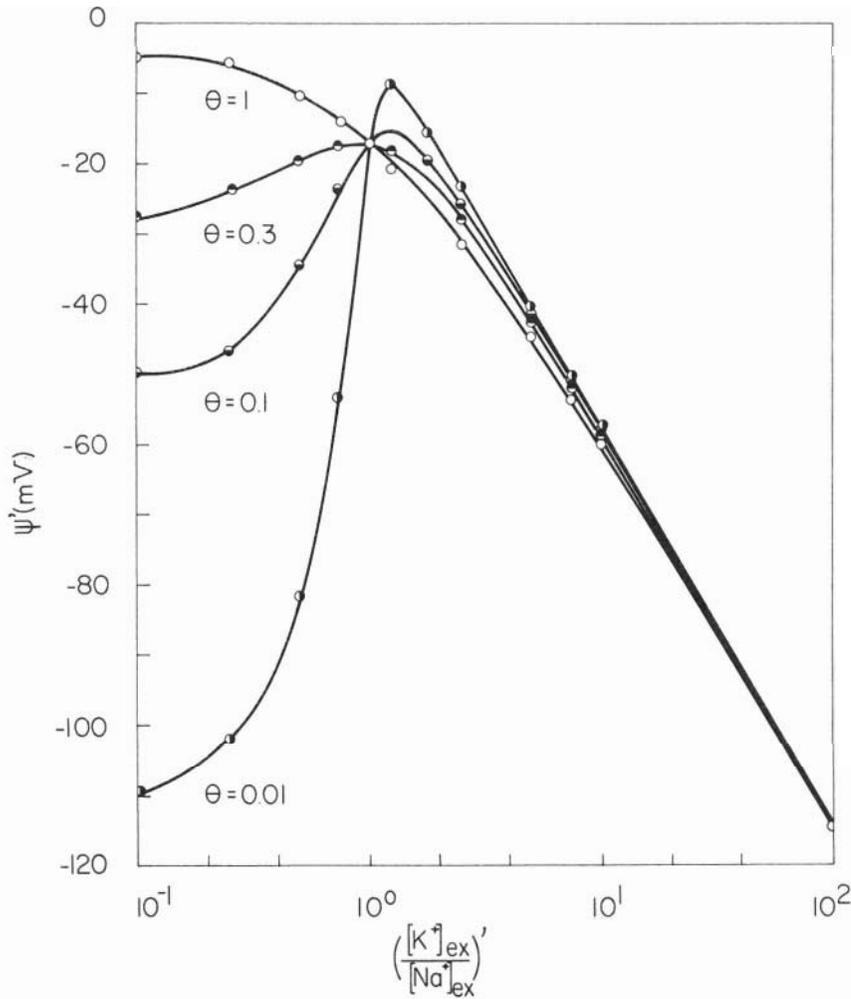


FIGURE 1. A plot of the resting potential against external K^+ and Na^+ concentration ratio according to Eq. 13. Ordinate represents ψ' which is equal to $\psi - \text{constant}$; abscissa represents $\{[K^+]_{ex}/[Na^+]_{ex}\}'$ which is $([K^+]_{ex}/[Na^+]_{ex} \cdot K_{Na \rightarrow K}^{oo})$. For experiments carried out in the presence of a constant concentration of Na^+ (e.g., 100 mM, the abscissa is then $[K^+]_{ex} \cdot (K_{Na \rightarrow K}^{oo}/0.1)$).

$KNaNa \rightarrow KKNa$. A total of one KNa neighboring pair exists before the exchange and afterward.

On the other hand, in an exchange of $NaNaNa \rightarrow NaKNa$, two $Na-K$ neighboring pairs are created. The creation of each additional mole of new $Na-K$ entails another energy term equal to $-(\gamma/2)$. Thus, in this case, the total free energy change is not merely $\Delta F_{Na \rightarrow K}^{oo}$ but

$$\Delta F_{Na \rightarrow K}^{oo} + 2[-(\gamma/2)] = \Delta F_{Na \rightarrow K}^{oo} - \gamma. \quad (6)$$

Consider the equilibrium distribution of K^+ on the cell surface (only). That surface contains a microscopically thin layer of fixed anionic sites which adsorbs K^+ (and Na^+). The electrochemical potential of K^+ in the adsorption phase (Phase I), μ_K^I , is then

$$\mu_K^I = \mu_K^{0(I)} + F\psi_I + RT \ln f_K x_K^I, \quad (7)$$

where $\mu_K^{0(I)}$ is the standard chemical potential of K^+ in Phase I, ψ_I is the electrical potential in Phase I and x_K^I is the mole fraction

of the adsorbed \mathbf{K}^+ in Phase I. $f_{\mathbf{K}^+}^{\text{I}}$ is the activity coefficient of the adsorbed \mathbf{K}^+ . In the contiguous external solution phase (Phase II),

$$\mu_{\mathbf{K}^+}^{\text{II}} = \mu_{\mathbf{K}^+}^{\text{o(II)}} + F\psi_{\text{II}} + RT \ln f_{\mathbf{K}^+}^{\text{II}} [\mathbf{K}^+]_{\text{II}}, \quad (8)$$

where $f_{\mathbf{K}^+}^{\text{II}}$ is the activity coefficient and $[\mathbf{K}^+]_{\text{II}}$ the concentration of \mathbf{K}^+ ion in the external solution and ψ_{II} is the electrical potential in Phase II. At equilibrium, the electrochemical potentials of the two phases are equal: the electric potential difference between these phases (i.e., the resting potential ψ) is then

$$\psi = \psi_{\text{I}} - \psi_{\text{II}} = \left(\frac{\mu_{\mathbf{K}^+}^{\text{o(I)}} - \mu_{\mathbf{K}^+}^{\text{o(II)}}}{F} \right) + \left(RTF^{-1} \ln \left\{ \frac{(f_{\mathbf{K}^+}^{\text{I}} x_{\mathbf{K}^+}^{\text{I}})}{(f_{\mathbf{K}^+}^{\text{II}} [\mathbf{K}^+]_{\text{II}})} \right\} \right). \quad (9)$$

The mole fraction of the adsorbed \mathbf{K}^+ in Phase I is

$$x_{\mathbf{K}^+}^{\text{I}} = [\mathbf{K}^+]_{\text{ad}} / [f^-], \quad (10)$$

where $[f^-]$ is the concentration of fixed surface anionic sites.

From Eqs. 3 and 10, we have

$$x_{\mathbf{K}^+}^{\text{I}} = \frac{1}{2} \left\{ 1 + \left(\frac{\xi - 1}{(\xi - 1)^2 + 4 \xi \exp(\gamma/RT)} \right)^{1/2} \right\} \quad (11)$$

Substituting Eq. 11 into Eq. 9, we obtain

$$\psi = \mu_{\mathbf{K}^+}^{\text{o(I)}} - \mu_{\mathbf{K}^+}^{\text{o(II)}} + RTF^{-1} \ln(f_{\mathbf{K}^+}^{\text{I}} / 2f_{\mathbf{K}^+}^{\text{II}}) + RTF^{-1} \ln(1/[K^+]_{\text{ex}}) \left\{ 1 + \left(\frac{\xi - 1}{(\xi - 1)^2 + 4 \xi \exp(\gamma/RT)} \right)^{1/2} \right\} \quad (12)$$

or

$$\psi = \text{constant} + RTF^{-1} \ln(1/[K^+]_{\text{ex}}) \left\{ 1 + \left(\frac{\xi - 1}{(\xi - 1)^2 + 4 \xi \exp(\gamma/RT)} \right)^{1/2} \right\}, \quad (13)$$

where ξ is that described by Eq. 4. For

consistency with symbols earlier published, the following relation is also given here:

$$\delta = \exp(\gamma/RT). \quad (14)$$

Figure 1 is a set of theoretical plots of ψ' ($= \psi - \text{constant}_3$) against $\{[\mathbf{K}^+]_{\text{ex}} / [\mathbf{Na}^+]_{\text{ex}}\}' = ([\mathbf{K}^+]_{\text{ex}} / [\mathbf{Na}^+]_{\text{ex}}) \cdot K_{\text{Na}}^{\text{o}} \rightarrow \kappa$ according to Eq. 13 with different values for the nearest-neighbor interaction energy: $-\gamma/2 = 0$ kCal/mole ($\delta = 1$); $-\gamma/2 = 0.356$ kCal/mole ($\delta = 0.3$); $-\gamma/2 = 0.682$ kCal/mole ($\delta = 0.1$); and $-\gamma/2 = 1.363$ kCal/mole ($\delta = 0.01$).

It is clear that an increase in the nearest-neighbor interaction energy $-\gamma/2$, tends to bring about a decrease of ψ' as $[\mathbf{K}^+]_{\text{II}}$ decreases beyond the concentration found in the cell's normal environment while $[\mathbf{Na}^+]_{\text{ex}}$ is maintained at more or less constant and relatively high values as experimentally observed.¹²⁻¹⁴ This decrease of ψ' follows from the autocoperative shift from \mathbf{K}^+ adsorption to Na^+ adsorption on the cell surface" anionic sites, a phenomenon of fundamental importance in generation of the action potential according to the association-induction hypothesis (see ref. 3, chapter 10).

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