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# Charge Pulse Studies of Transport Phenomena in Bilayer Membranes

## II. Detailed Theory of Steady-State Behavior and Application to Valinomycin-Mediated Potassium Transport

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*Summary.* The charge-pulse technique is applied to a study of valinomycin-mediated potassium transport across glycerol monooleate (GMO) bilayers. The theory, based on the Läuger-Stark model, is developed for the steady-state domain. The voltage dependences of the surface complexation reactions are also considered. The analysis of the data yields the following values for the rate constants:

$$k'_R = 2.6 \times 10^8 \times \exp\left[0.045 \frac{F}{RT} V\right] \text{cm}^3 \text{ moles}^{-1} \text{sec}^{-1},$$

$$k'_D = 2.6 \times 10^5 \times \exp\left[-0.045 \frac{F}{RT} V\right] \text{sec}^{-1}, \quad k_s = 2.6 \times 10^4 \text{ sec}^{-1} \text{ and } k_{MS}/k_D > 4.$$

With the exception of this last ratio, all the values agree well with previously published data. The implication of the exponential term, 0.045, is that the plane of reaction for the surface complexation actually occurs a small distance within the membrane dielectric. If one presumes that the reaction plane is about half way between the plane of adsorbed complex and the membrane-water interface, one deduces that the complex "feels" only about 80% of the applied voltage across the membrane.

| *Symbol* | *Units* | *Definition* |
|---|---|---|
| $c_M$ | moles/cm$^3$ | Concentration of aqueous permanent ion. |
| $N_s^0$, $N_{MS}^0$ | moles/cm$^2$ | Membrane bound free carrier and carrier complex at equilibrium with aqueous permeant ion concentration, $c_M$. |
| $N'_s$, $N''_s$, $N'_{MS}$, $N''_{MS}$ | moles/cm$^2$ | Membrane bound free carrier and carrier complex on left (′) and right (″) sides of the membrane. |
| $k'_{MS}$, $k''_{MS}$ | sec$^{-1}$ | Voltage dependent translocation rate constants for $N'_{MS}$ and $N''_{MS}$. |
| $k_{MS}$ | sec$^{-1}$ | Voltage independent translocation rate constant – see Eqs. (13), (14). |
| $k_R$ | cm$^3$ mole$^{-1}$ sec$^{-1}$ | Voltage independent "on" rate constant. |

| *Symbol* | *Units* | *Definition* |
|---|---|---|
| $k_D$ | $sec^{-1}$ | Voltage independent "off" rate constant. |
| $k'_R$, $k''_R$ | $cm^3\ mole^{-1}\ sec^{-1}$ | Voltage dependent rate constants for |
| $k'_D$, $k''_D$ | $sec^{-1}$ | left (′) and right (″) side "on-off" processes. |
| $K_h$, $K'_h$, $K''_h$ | $cm^3$/mole | Ratio $k_R/k_D$, $k'_R/k'_D$, $k''_R/k''_D$, respectively. |
| $k_s$ | $sec^{-1}$ | Translocation rate constant for $N'_s$ and $N''_s$. |
| $A_m$ | $cm^2$ | Membrane area. |
| $q$ | coulombs | Charge injected during charge pulse. |
| $q^0$ | coulombs/$cm^2$ | Charge density injected during pulse. |
| $F$ | coulombs/mole | The Faraday. |
| $RT$ | joules/mole | Gas constant-temperature product. |
| $f$ | $volts^{-1}$ | $f=F/RT$ |
| $R_e$ | ohms | External resistance. |
| $V_0$ | volts | $V_0=q^0/C_m$. |
| $V_{t,\infty}$, $V^+_{t,\infty}$ | volts | Steady state and nonsteady-state voltages in high voltage region. |
| $V_{i,\infty}$ | volts | Zero time intercept of high voltage steady-state transient. |
| $\Delta V_{i,\infty}$ | volts | *See* Eq. (7). |
| $V_{t,0}$ | volts | Low-voltage steady-state transient. |
| $V_{i,0}$ | volts | Zero time intercept of low-voltage (steady-state) transient. |
| $\Delta V_{i,0}$ | volt | *See* Eq. (22). |
| $J_{MS}$, $J^*_{MS}$ | moles $cm^{-2}\ sec^{-1}$ | Steady-state and nonsteady-state fluxes. |
| $r$ | $volts^{-1}$ | *See* Eq. (22). |
| $b$ | dimensionless | Location of "on-off" reaction plane: fraction of distance between plane of closest approach of the aqueous permeant ion and the intra BLM residence plane of the ion complex. |
| $u$ | dimensionless | Normalized voltage: $u=fV$. |
| $A$ | dimensionless | *See* Eq. (17). |
| $B$ | dimensionless | *See* Eq. (4). |
| $T$ | dimensionless | *See* Eq. (18). |
| $W$ | dimensionless | *See* Eq. (16). |
| $z$ | dimensionless | *See* Eq. (47). |
| $\beta$ | dimensionless | Fraction of membrane voltage between plane of closest approach of aqueous ions and the intramembrane residence plane of the ion complex. |

Part I of this series (Feldberg & Kissel, 1975) dealt with the application of the charge pulse method to the evaluation of steady-state carrier phenomena in black lipid membranes (BLMs). In this paper we present a more detailed and quantitative approach to the steady-state analysis. The theory is based upon the kinetic analysis of Stark, Ketterer, Benz, and Läuger (1971). For that reason we use their nomenclature. The theory for time-dependent (presteady-state) behavior at high voltages is presented here. The recent work of Benz and Läuger (1976) has demonstrated how a single low-voltage transient may be analyzed to give the kinetic parameters *if* amplitudes and time constants for all relaxations are measurable.

We shall show here how some simple considerations of high-voltage and low-voltage data allow one to deduce the values of the same parameters – even if the time constants of the first and second relaxations are too fast to be analyzed. To avoid confusion it should be pointed out that Benz and Läuger (1976) discuss three relaxations for the carrier model in the context of the charge-pulse method: the first two are the relaxations usually associated with the model, while the third is the time constant of the effective[1] capacitance as it is discharged through the steady-state conduction mechanism.

## Basic Premises

Several simplifying assumptions are made for the Läuger-Stark carrier model (Läuger & Stark, 1970; Stark & Benz, 1971), i.e.:

1. All species are membrane bound.

2. All membrane bound species are located in two planes at each membrane surface. Implicit in the kinetic analysis of Stark *et al.* (1971) is the assumption that these two planes also define the boundaries of the membrane dielectric. In other words, the charged species "feels" the entire potential drop as it moves from one side of the membrane to the other. This is not precisely true and has been considered and demonstrated by a number of workers (Stark & Benz, 1971; Markin, Grigor'ev & Yermishkin, 1971; Hladky, 1972; Andersen & Fuchs, 1975; Hladky, 1975; Eisenman, Krasne & Ciani, 1975; Andersen *et al.*, 1976[2]).

3. The membrane capacitance is constant over the voltage range of interest. Recent work of Requena, Haydon and Hladky (1975), Benz, Frohlich, Läuger and Montal (1975), and Bamberg and Benz (1976), indicates that voltage-induced capacitance changes of GMO/*n*-decane and GMO/*n*-hexadecane BLMs have a time constant of $\sim 3 \times 10^{-2}$ sec and that at times shorter than $10^{-3}$ sec there is virtually no change in capacitance. Furthermore, the amplitude of these changes is considerably less (approximately one-third) if the BLM is formed with *n*-hexadecane rather than with *n*-decane (Bamberg *et al.*, 1976).

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1 The effective capacitance may be larger than the bare membrane capacitance because the distribution of complex within the BLM is a function of voltage. This point will be clarified in subsequent discussion and in the Appendix.

2 O.S. Andersen, S. Feldberg, H. Nakadomari, H. Levy, S. McLaughlin (1976). A new type of electrostatic boundary potential in lipid bilayers. (*Presented at:* 20th Annual Meeting of the Biophysical Society.)

## Theory

### *Behavior in the High-Voltage Limit*

When the voltage is sufficient to establish the following conditions:

$$\frac{k'_{MS}-k''_{MS}}{k'_{MS}+k''_{MS}} \cong 1 \tag{1}$$

$$\frac{k'_{MS}+k''_{MS}}{k_D} \gg 1. \tag{2}$$

If we assume that the complex feels all the voltage across the membrane, it is straightforward to derive the following time dependent equation:

$$V^{\pm}_{t,\infty} = \frac{q^0}{C_m} - \frac{F}{C_m} \cdot N_s^0 \left\{ \left( \frac{B}{1+B} \right)^2 \left( 2k_s \frac{(1+B)\,t}{B} - (e^{-(2k_s + k_R c_M)t} - 1) \right) + K_h c_M \right\}, \tag{3}$$

where

$$B = \frac{k_R c_M}{2k_s}. \tag{4}$$

The simplifying assumption in obtaining this equation is that at high voltage all of the charged complex ($N^0_{MS} = K_h c_M N_s^0$) moves across the membrane instantaneously. At steady state (i.e., when the exponential term in Eq. (3) goes to zero)

$$V_{t,\infty} = \frac{q^0}{C_m} - \frac{F}{C_m} N_s^0 \left( \left( \frac{B}{1+B} \right)^2 \left( 2k_s \frac{(1+B)\,t}{B} + 1 \right) + K_h c_M \right). \tag{5}$$

Eq. (5) predicts a linear voltage-time decay for a system at high voltage and at steady state. Extrapolation of the linear decay curve back to zero time gives the intercept voltage

$$V_{i,\infty} = \frac{q^0}{C_m} - \frac{FN_s^0}{C_m} \left( \left( \frac{B}{1+B} \right)^2 + K_h c_M \right). \tag{6}$$

The intercept discrepancy is defined

$$\Delta V_{i,\infty} = \frac{q^0}{C_m} - V_{i,\infty} = V_0 - V_{i,\infty} = \frac{FN_s^0}{C_m} \left( \left( \frac{B}{1+B} \right)^2 + K_h c_M \right). \tag{7}$$

It is clear that $\Delta V_{i,\infty}$ is the sum of two relaxation amplitudes

$$\Delta V_{i,\infty} = \Delta V_{1,\infty} + \Delta V_{2,\infty} \tag{8}$$

where

$$\Delta V_{1,\infty} = \frac{F}{C_m} K_h c_M N_s^0 \tag{9}$$

and

$$\Delta V_{2,\infty} = \frac{F}{C_m} N_s^0 \left(\frac{B}{1+B}\right)^2. \tag{10}$$

Eq. (9) derives directly from Eq. (3) with the condition $t=0$. Another useful equation is the time derivative of Eq. (5)

$$-\frac{dV_{t,\infty}}{dt} = \frac{F}{C_m} \frac{2k_s B N_s^0}{(1+B)} = \frac{F}{C_m} \frac{k_R c_M N_s^0}{\left(1+\frac{k_R c_M}{2k_s}\right)}. \tag{11}$$

Since

$$J_{MS} = -\frac{C_m}{F} \frac{dV_{t,\infty}}{dt} = \frac{k_R c_M N_s^0}{1+\frac{k_R c_M}{2k_s}}, \tag{12}$$

Eq. (11) is simply a manifestation of the well-known steady-state expression for the Läuger-Stark Model in the high-voltage limit.

### *Behavior at Low Voltages*

The following approximations are valid at low voltages:

$$k'_{MS} + k''_{MS} = 2k_{MS}; \tag{13}$$

$$k'_{MS} - k''_{MS} = k_{MS} u. \tag{14}$$

Unlike the high-voltage situation where the charge distribution on the membrane surfaces does not change once the steady-state condition is achieved, there is a constant readjustment of those concentrations as the voltage changes. Thus, as the voltage decays, one can envision two fluxes: (1) the flux due to the voltage driving ions from the aqueous phase on the left of the membrane to the aqueous phase on the right, and (2) a counter flux due to the adsorbed ions piled up on the right surface of the membrane drifting back to the left surface as the voltage decays to zero.

The steady-state expression for this low-voltage behavior is easily (albeit, tediously) derived from the kinetic equations of Stark *et al.* (1971) (*see* Appendix for details). One obtains:

$$\frac{d \ln V_{t,0}}{dt} = -\frac{\dfrac{F^2}{RTC_m} \cdot \dfrac{k_R c_M N_s^0 W}{1+A}}{1+T} \tag{15}$$

where

$$W = \frac{k_{MS}}{k_D}, \tag{16}$$

$$A = 2W(1+B) = \frac{2k_{MS}}{k_D}\left(1 + \frac{k_R c_M}{2k_s}\right), \tag{17}$$

and

$$T = \frac{1}{2}\frac{F^2 N_s^0}{RTC_m}\left(\frac{A}{1+A}\right)^2\left[\left(\frac{B}{1+B}\right)^2 + K_h c_M\right]. \tag{18}$$

Thus, a plot of $\ln V_{t,0}$ *vs.* time will be linear. Only in the limit when $T \to 0$, does

$$\frac{d \ln V_{t,0}}{dt}_{T \to 0} = -\frac{F^2}{RTC_m}\frac{k_R c_M N_s^0 W}{1+A}. \tag{19}$$

Then, since

$$G = \frac{F^2}{RT}\frac{k_R c_M N_s^0 W}{1+A}, \tag{20}$$

one can write

$$\frac{d \ln V_{t,0}}{dt}_{T \to 0} = -\frac{G}{C_m}. \tag{21}$$

Eq. (15) is valid even when the transient began in the high-voltage region, and some useful relationships may be derived relating high and low voltage data from the same transient.

$$r = \frac{\dfrac{d \ln V_{t,0}}{dt}}{\dfrac{dV_{t,\infty}}{dt}} = \frac{\dfrac{1}{2}\dfrac{F}{RT}A}{(1+A)(1+T)}. \tag{22}$$

Combining with Eqs. (7) and (18) gives

$$r = \frac{\dfrac{1}{2}\dfrac{F}{RT}A}{1+A+\dfrac{1}{2}\dfrac{F}{RT}\dfrac{A^2}{(1+A)}\Delta V_{i,\infty}}. \tag{23}$$

For the limiting condition $c_M \to 0$: $B \to 0$, $A \to 2W$ and $\Delta V_{i,\infty} \to 0$. Thus, from Eq. (23)

$$r \xrightarrow[c_M \to 0]{} \frac{\frac{F}{RT} W}{1+2W}. \tag{24}$$

In the other limit, when $A \gg 1$, which can occur as a result of large values of $W$ and/or $B$, one obtains

$$r \xrightarrow[A \gg 1]{} \frac{\frac{1}{2}\frac{F}{RT}}{1+\frac{1}{2}\frac{F}{RT}\Delta V_{i,\infty}}. \tag{25}$$

If the low-voltage condition obtains from the beginning of the transient once can determine the low-voltage intercept

$$V_{i,0} = \frac{V_0}{1+T} \tag{26}$$

and the intercept discrepancy

$$\Delta V_{i,0} = V_0 - V_{i,0} = \frac{TV_0}{1+T}. \tag{27}$$

Here, $V_{i,0}$ is the zero-time intercept of the linear $\ln V_{t,0}$ *vs.* $t$ plot. Eq. (15) may be rewritten substituting from Eq. (7)

$$\frac{d \ln V_{t,0}}{dt} = -\frac{\frac{F^2}{RTC_m} k_R c_M N_s^0 W}{1+A+\frac{1}{2}\frac{F}{RT}\frac{A^2}{(1+A)}\Delta V_{i,\infty}}, \tag{28}$$

or substituting from Eqs. (20) and (26)

$$\frac{d \ln V_{t,0}}{dt} = -\frac{V_{i,0}}{V_0}\frac{F^2}{RTC_m}\frac{k_R c_M N_s W}{(1+A)} = -\frac{V_{i,0}}{V_0}\frac{G}{C_m}. \tag{29}$$

Thus, one has a direct, testable correlation between the low-voltage decay and the low-voltage conductance, $G$.

### *Effect of Voltage Dependence of $k_R$ and $k_D$*

The theory thus far has dealt only with an ideal and unrealistic limitation, i.e., assumption 2, that the carrier complexes "feel" the entire voltage

across the BLM. If we presume that $N_s$ and $N_{MS}$ lie in a plane within the membrane it is clear that one necessarily introduces a voltage dependence of the surface rate processes since the reactions

$$c_M + N'_s \underset{k'_D}{\overset{k'_R}{\rightleftharpoons}} N'_{MS} \tag{30}$$

$$c_M + N''_s \underset{k''_D}{\overset{k''_R}{\rightleftharpoons}} N''_{MS} \tag{31}$$

move charge through a dielectric. As Hladky (1974) has shown, the equations for all rate constants except $k_s$ are redefined:

$$k'_R = k_R\, e^{b\beta fV}, \tag{32}$$

$$k'_D = k_D\, e^{(b-1)\beta fV}, \tag{33}$$

$$k'_{MS} = k_{MS}\, e^{(\frac{1}{2}-\beta)fV}, \tag{34}$$

$$k''_R = k_R\, e^{-b\beta fV}, \tag{35}$$

$$k''_D = k_D\, e^{(1-b)\beta fV}, \quad \text{and} \tag{36}$$

$$k''_{MS} = k_{MS}\, e^{(\beta-\frac{1}{2})fV}. \tag{37}$$

Implicit in these equations is the presumption that the charge absorbed within the membrane will not significantly modify the electric field. The term $\beta$ is the fraction of the membrane voltage $V$ between the plane of closest approach of aqueous ions $P_1$, and the intramembrane residence plane, $P_2$, of the ion complex. The term $b$ is the fraction of the voltage drop ($0 \leqq b \leqq 1$) between planes $P_1$ and $P_2$ felt by the reaction plane. In the limiting case where $c_M \to 0$, Hladky (1974) has shown that the steady-state flux equation becomes

$$J_{MS} = c_M N_s \frac{\dfrac{k'_R k'_{MS}}{k'_D} - \dfrac{k''_R k''_{MS}}{k''_D}}{1 + \dfrac{k'_{MS}}{k'_D} + \dfrac{k''_{MS}}{k''_D}}. \tag{38}$$

Substituting from Eqs. (32)–(37) gives

$$J_{MS} = \frac{K_h c_M N_s k_{MS}(e^{\frac{1}{2}fV} - e^{-\frac{1}{2}fV})}{1 + \dfrac{k_{MS}}{k_D}(e^{(\frac{1}{2}-b\beta)fV} + e^{(-\frac{1}{2}+b\beta)fV})}. \tag{39}$$

At low voltage this reduces to

$$J_{MS}=\frac{K_h c_M N_s k_{MS} f V_{t,0}}{1+2\dfrac{k_{MS}}{k_D}}=\frac{k_R c_M N_s W f V_{t,0}}{1+2W}, \tag{40}$$

which is independent of the location parameters, $b$ and $\beta$. At high voltage Eq. (39) simplifies to become

$$J_{MS}=k_R c_M N_s^0 e^{b\beta f V_{t,\infty}} \tag{41}$$

$$\frac{dV_{t,\infty}}{dt}=-\frac{F J_{MS}}{C_m}=-\frac{F}{C_m} k_R c_M N_s^0 e^{f b \beta V_{t,\infty}}. \tag{42}$$

The parameter $b\beta$ is the fraction of the membrane voltage modifying the rate constant, $k_R$. Rearranging and integrating gives

$$e^{-f b \beta V_{t,\infty}}=\frac{F f b \beta}{C_m} k_R c_M N_s^0 t+e^{-f b \beta V_{i,\infty}}. \tag{43}$$

By guessing an appropriate value for the parameter $b\beta$ and plotting the LHS of Eq. (43) *vs.* $t$, a straight line is obtained. One can then estimate $k_R c_M N_s^0$ and deduce the corresponding $\dfrac{dV_{t,\infty}}{dt}$ that would be observed if $k_R$ were truly voltage independent. Combining with the low-voltage value for $\dfrac{d \ln V_{t,0}}{dt}$, one can estimate $r$ and therefore $W$ since for these conditions [see Eq. (24)]

$$W=\frac{r}{\left(\dfrac{F}{RT}-2r\right)}. \tag{44}$$

At high concentrations the equations become considerably more complex. Hladky (1972) has presented a very useful generalized form of the steady-state equation. Substituting into his Eq. (87) the definitions for $k'_R$, $k''_R$, $k'_D$, and $k''_D$, assuming $k_s=k'_s=k''_s$ gives for *low voltages*

$$J_{MS}=\frac{k_R c_M N_s^0 f V_{t,0}}{1+A}. \tag{45}$$

Thus, the expression for the steady-state low-voltage flux, even at high concentrations, is independent of the location parameters, $b$ and $\beta$, and is identical (as it should be) to the expression derived by Läuger and Stark (1970). Unfortunately, the expression for the steady-state high-voltage flux does not simplify. Nevertheless, even without a detailed analysis we

find that a plot of the same form as that used for the low concentration limit [based on Eq. (43)] will also give a linear plot albeit with different values for the exponential parameter. Thus we have an empirical method for establishing the value of $dV_{t,\infty}/dt$ corresponding to a voltage independent $k_R$ and $k_D$. We cannot ascribe a physical interpretation to the value of the exponential parameter ($b\beta$), but we can demonstrate that the theoretical values of $r$ [Eqs. (22) and (23)] are consistent with this technique. For large values of $c_M$ and low carrier concentrations, one estimates that $r$ will limit

$$r \xrightarrow[\substack{N_S^0 \to 0 \\ A \to \infty}]{} \frac{1}{2}\frac{F}{RT}. \tag{46}$$

Such an equation is easily tested.

## Experimental

### *Materials and Methods*

The concept of the charge-pulse technique has been discussed in Part I. A Chronetics Pulse Generator Model P32A produces a voltage pulse of adjustable height ($\leqq 25$ volts) and width (as small as $2\times10^{-7}$ sec). An adjustable resistance ($0$–$5\times10^4$ ohms) in series controls the current and a 2N4416 FET transistor with the drain and source shorted prevents the voltage across the membrane from discharging back into the pulser. A precision capacitor ($C_p = 2.000\times10^{-8}$ F) in series with the cell allows accurate measurement of the charge injected in each transient.[3] The voltage follower employs a 46J Analog Devices FET operational amplifier. Data is acquired with a Nicolet Model 1090 digital scope with 12-bit resolution and a maximum acquisition rate of one point per 0.5μ sec. The total system response is such that only data at times longer than 2μ sec are acceptable. The charge-pulse technique can be much faster as has been recently demonstrated by Benz and Läuger (1976) who were able to produce acceptable data after 0.2μ sec. The Chronetics Pulse Generator and the Nicolet were triggered by single or multiple pulses from a Wavetec Pulse Generator, Model 116. Trigger levels were adjusted so that the Nicolet was triggered first.

Membrane capacitance in the absence of ionic transport (bare membrane, or membrane with carrier but no permeant ion) is easily measured by shorting the electrodes with a 5000 ohm precision resistor, $R_e$. Since the capacitance is about $5\times10^{-9}$ F (area $\cong 0.01$ cm$^2$) the time constant for the decay will be about $2.5\times10^{-5}$ sec. Thus the transient is complete before voltage dependent capacitance changes are effected. Furthermore, because the membrane is exposed to a given voltage for a very short time, a larger charge pulse (and therefore higher initial voltage) may be used, thus giving capacitance information over a

3 By triggering the digital scope with a separate pulse generator (Wavetec Pulse Generator, Model 116) before the charge pulse a pre-baseline is obtained. The transient will decay to a post-baseline. The voltage difference between these two baselines is proportional to the charge injected and

$$q = \Delta V_b / C_p.$$

The capacitor must be momentarily shorted prior to each experiment.

greater voltage range. The membrane capacitance may be evaluated from the slope of the $\ln V$ *vs.* $t$ plot and/or from the zero-time intercept of that plot ($V_i$).

$$A_m C_m = \frac{1}{R_e}\left[\frac{d \ln V}{dt}\right]^{-1} \quad \text{or} \quad A_m C_m = q/V_i, \quad \text{where } q \text{ is the charge injected.}$$

Classical steady-state data were obtained using a 9 V battery in series with an appropriately large precision resistor connected to two silver-silver-chloride electrodes. The current is "turned on" simply by dipping the electrodes into the cell solutions (negative electrode on the ground side of the membrane), thus effecting a four electrode measuring system. The change in voltage ($\sim 10$ mV) is observed with the Nicolet digital scope in a slow sweep mode. The exact voltage of the battery is also measured using the scope.

For certain experiments a series of pulses were injected. By inserting a small capacitor in series with the cell (and precision capacitor) the charge injected during each succeeding pulse decreases.

The cell and membrane formation technique are similar to that described in Part I. The membrane forming material was 1.8% by weight glycerol monooleate (Matheson, Coleman, and Bell) in *n*-hexadecane (Eastman). Valinomycin, obtained from Calbiochem, was dissolved in the lipid phase. The KCl, CsCl and LiCl are reagent grade. All chemicals were used without further purification. Triply distilled water was used for all preparations.

The membrane forming material was pre-equilibrated with the aqueous phase by prolonged (24 hr) vigorous agitation of 0.2 ml of the membrane forming material with 250 ml of LiCl ($3.0 \times 10^{-3}$ moles/cm$^3$) or 25 ml KCl ($3.0 \times 10^{-3}$ moles/cm$^3$). Solutions were filtered prior to use to remove undissolved organics. The hydrocarbon-aqueous partition coefficient of valinomycin is about $2 \times 10^4$. Assuming a formation constant of $10^3$ for the aqueous valinomycin-potassium complex, we estimate that the concentration of valinomycin in the hydrocarbon phase is diminished by about $2\frac{1}{2}$% with KCl and by about 6% with LiCl. The virtues of pre-equilibration have been discussed in detail by Hladky (1973) and by Benz, Stark, Janko and Läuger (1973). Except where noted, experiments were carried out with the aqueous ionic strength maintained at $3.0 \times 10^{-3}$ moles/cm$^3$. In spite of changes in the activity coefficients as KCl is substituted for LiCl, we presume that the aqueous concentrations of the carrier and carrier-complex adequately approximate the concentration that would result from pre-equilibrating that KCl-LiCl mixture.

All experiments were carried out at $25 \pm 0.5$ °C.

## *Results and Conclusions*

The validity of the interpretation of charge pulse data will depend in part on the voltage independence of the membrane capacitance. The decay of charge through an external resistance should be a classical $RC$ decay. This is demonstrated by the data in Fig. 1. Both the slope and the zero-time intercept correspond to a capacitance of $6.3 \times 10^{-7}$ F/cm$^2$. Note that the time constant was kept less than $10^{-3}$ sec to avoid time-dependent changes as described by Requena *et al.* (1975), Benz *et al.* (1975), or Bamberg and Benz (1976). The capacitance of the BLM with no valinomycin is the same within experimental error.

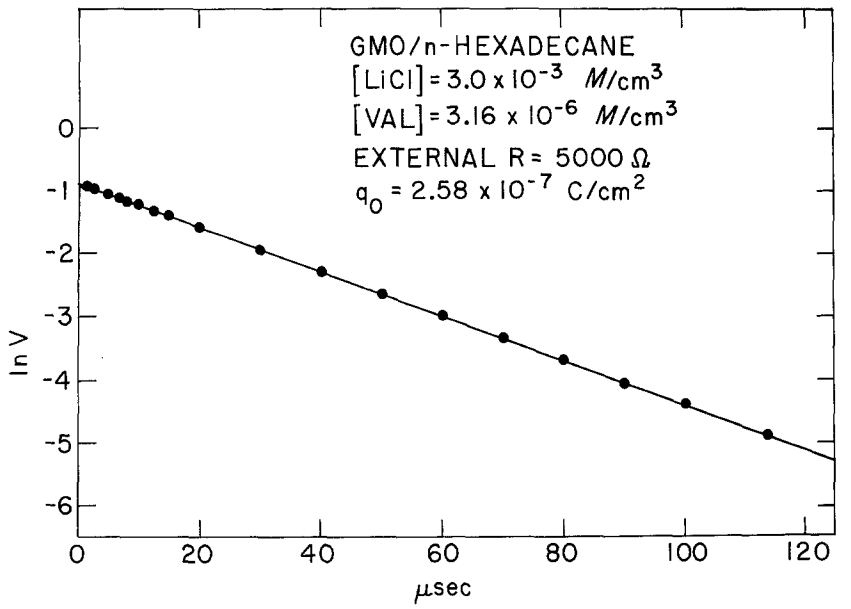


Fig. 1. Ln $V$ *vs.* $t$ plot for membrane charge decaying through an external resistance. Intercept and slope each correspond to $C_m = 6.3 \times 10^{-7}$ F/cm$^2$

Eq. (43) is directly tested by carrying out the charge pulse experiment at as low a concentration of KCl as possible while keeping the decay time constant less than a msec. Some typical data are shown in Fig. 2. The optimum value of the parameter $z$ where

$$z = b\beta \underset{c_M \to 0}{=} 0.045 \tag{47}$$

was computed using a least squares fit of the data for $V_{t,\infty} > 0.2$ V. The presumption that $b \approx \frac{1}{2}$ (implying that the reaction plane for the complexation is approximately half way between the $P_1$ and $P_2$ planes) implies that $\beta \approx 0.09$. This suggests that the adsorbed valinomycin-potassium complex "feels" about 80% of the applied voltage – a number that agrees well with Andersen's recent experiments with pro-valinomycin (O. Andersen, 1976, *private communication; see also* Ting-Beall *et al.*, 1974). From the slope of this plot we obtain an estimate of the "limiting" value of $dV/dt$ that would exist at high voltage if the complexation rate were not voltage assisted. Similar plots are obtained for higher concentrations of KCl (*see* Fig. 3, for example) and even though the theoretical basis is not well founded, the limiting values of $dV/dt$ are deduced in a similar manner. Over a wide range of valinomycin concentrations the values of $z$ appear to depend only upon the concentration of KCl. With a constant valinomycin concentration in the membrane-forming material (and with the

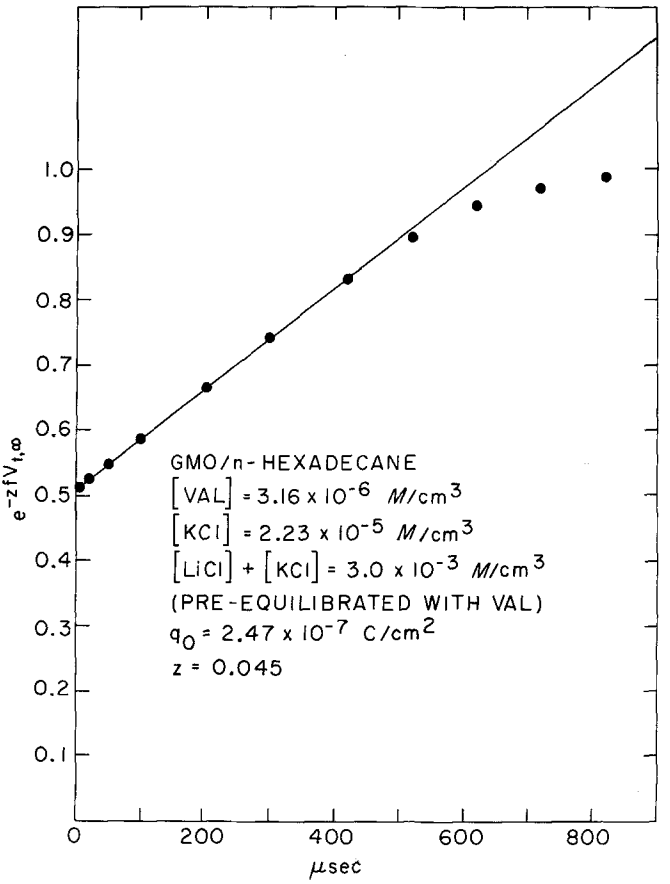


Fig. 2. Example of linearization of high voltage, low concentration data as predicted by Eq. (43). Limiting $dV/dt = 4.37 \times 10^2$ V sec$^{-1}$

aqueous phases pre-equilibrated) Eq. (12) predicts that a plot of $(dV_{t,\infty}/dt)^{-1}_{\text{lim}}$ *vs.* $(c_M)^{-1}$ (Fig. 4) should give a linear plot with

$$\text{slope} = \frac{C_m}{F k_R N_s^0} \text{ sec cm}^3 \text{ volt}^{-1} \text{ mole}^{-1} \tag{48}$$

and

$$\text{intercept} = \frac{C_m}{2F k_s N_s^0} \text{ sec volt}^{-1}. \tag{49}$$

On the basis of this plot we deduce $\frac{k_R}{2k_s} = 5.3 \times 10^3 \frac{\text{cm}^3}{\text{mole}}$, $k_R N_s^0 = 1.45 \times 10^{-4}$ cm sec$^{-1}$ and $k_s N_s^0 = 1.36 \times 10^{-8}$ moles sec$^{-1}$ cm$^{-2}$. Another variable that is easily measured is $\Delta V_{i,\infty}$ – the difference between the "expected" and observed zero-time voltage intercept [*see* Eq. (7)]. The plot of $\Delta V_{i,\infty}$ *vs.* $c_M$ (Fig. 5) can be fit by a theoretical curve based on Eq. (7). In order to optimize the fit, we use the previously determined

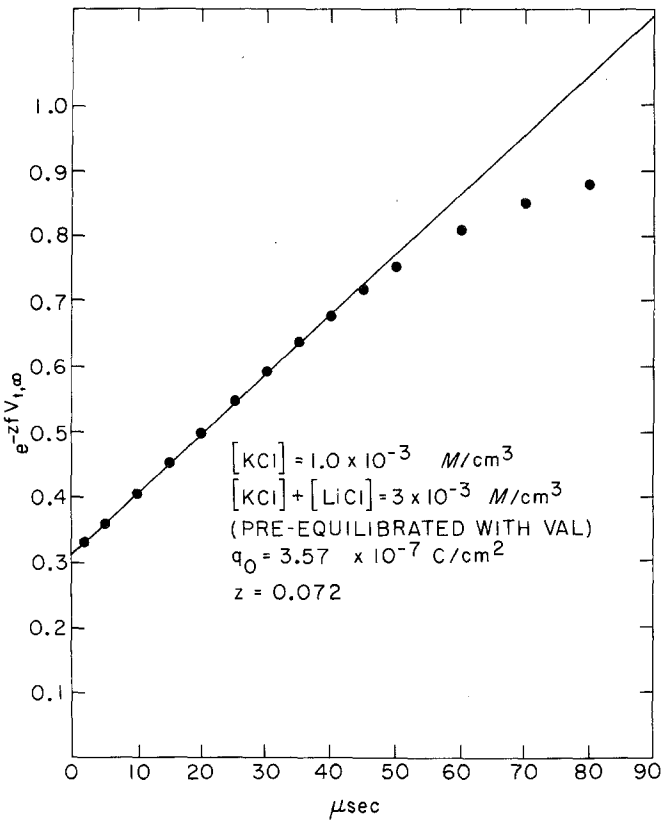


Fig. 3. Example of linearization of high voltage, high concentration data. Limiting $dV/dt = 3.28 \times 10^3$ V sec$^{-1}$

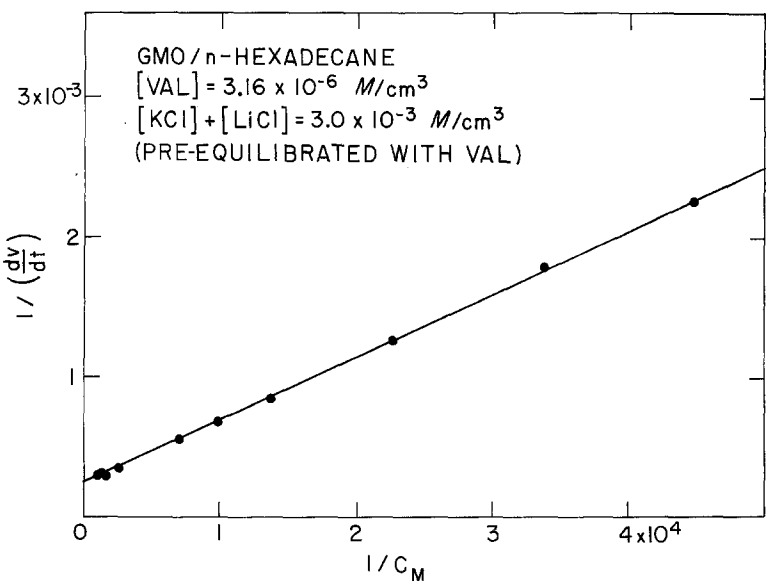


Fig. 4. Plot of limiting $(dV/dt)^{-1}$ *vs.* $c_M^{-1}$

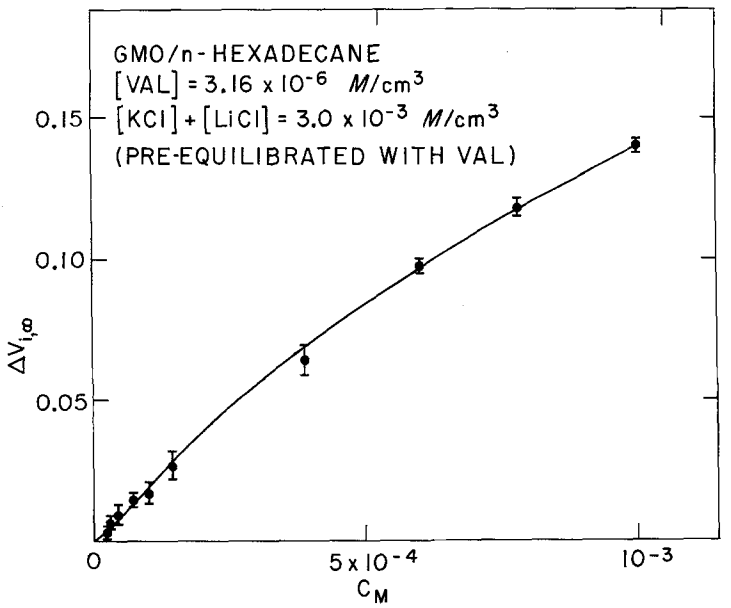


Fig. 5. Plot of $\Delta V_{i,\infty}$ *vs.* $c_M$. Solid line calculated according to Eq. (7) for $C_m = 6.4 \times 10^{-7}$ F/cm$^2$, $K_h = 10^3$ cm$^3$ moles$^{-1}$, $k_R/k_s = 1.08 \times 10^4$ cm$^3$ moles$^{-1}$, $N_s^0 = 5.5 \times 10^{-13}$ moles/cm$^2$

values $C_m = 6.4 \times 10^{-7}$ F cm$^{-2}$, $k_R/(2k_s) = 5.3 \times 10^3$ cm$^3$ mole$^{-1}$, and assume $K_h = 1 \times 10^3$ cm$^3$ mole$^{-1}$ and $N_s^0 = 5.5 \times 10^{-13}$ moles cm$^{-2}$. From these parameters, it is possible to estimate the values of all the rate constants except $k_{MS}$. These are given in Table 1 along with values recently obtained by Benz and Läuger (1976) and Laprade, Ciani, Eisenman, and Szabo (1974) for valinomycin-mediated potassium transport across GMO/*n*-decane bilayers.

An estimate of $k_{MS}$ requires a determination of $W$. We attempted this by evaluating the parameter $r$ [Eq. (22)] as a function of concentration of KCl (Fig. 6). Using the rate constants in Table 1, it is possible to evaluate the concentration dependence of $r$ from Eq. (22) for various values of $W$. These theoretical curves are also shown in Fig. 6. It would seem that

Table 1. Rate parameters for the valinomycin-mediated potassium transport

| | Units | This work | Benz & Läuger | Laprade *et al.* |
|---|---|---|---|---|
| $k_R$ | cm$^3$ moles$^{-1}$ sec$^{-1}$ | $2.6 \times 10^8$ | $2.9 \times 10^8$ | $7.4 \times 10^7$ |
| $k_D$ | sec$^{-1}$ | $2.6 \times 10^5$ | $2.7 \times 10^5$ | $7.4 \times 10^4$ |
| $k_s$ | sec$^{-1}$ | $2.6 \times 10^4$ | $3.8 \times 10^4$ | $9.0 \times 10^4$ |

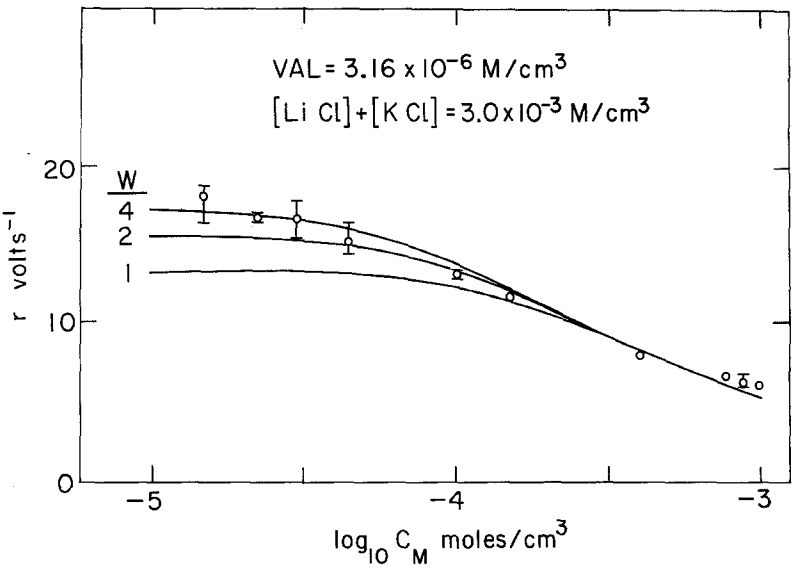


Fig. 6. Plot of $r$ *vs.* $c_M$. Error bars indicate range of 3–5 measurements, and point indicates average. Absence of error bar indicates a range smaller than the "point". Solid lines are computed from Eq. (22) using our values of constants given in Table 1 and indicated values of $W$

$W \sim 4$. The data are most sensitive at low $c_M$ where we see $r = 17 \pm 1$ while a value of $W = 0.7$ as determined by Benz and Läuger (1976) and Laprade *et al.* (1974) predicts that $r \approx 11\ V^{-1}$. We have done the analogous experiment with GMO/*n*-decane bilayers; there, too, we obtain high values of $r$, so the difference is not caused by the solvent. We are concerned that our estimate of $W$ could be off considerably if our analysis of $V_{i,\infty}$ were only slightly incorrect. An experiment carried out using high KCl concentration ($= 3 \times 10^{-3}$ moles $cm^{-3}$) while varying the valinomycin concentration fulfilled the prerequisite condition for Eq. (25). The values of $z$ were consistently between 0.07 and 0.08 and a plot of $1/r$ *vs.* $\Delta V_{i,\infty}$ (Fig. 7) gives an experimental intercept $\cong 0.05\ V^{-1}$ and a slope of 0.75. Agreement with theory seems acceptable. The theoretically predicted intercept is $2RT/F$ or 0.051 V and the expected slope is clearly 1.0. Both high and low voltage fluxes exhibit an anomalous increase of about a factor of two as the valinomycin is increased in concentration from $1.1 \times 10^{-7}$ moles/$cm^3$ to $1.74 \times 10^{-6}$ moles/$cm^3$ in GMO/*n*-hexadecane. We have no explanation for this.

The relationship expressed in Eq. (29) allows one to correlate low-voltage charge pulse data and low-voltage clamp data. Values of low

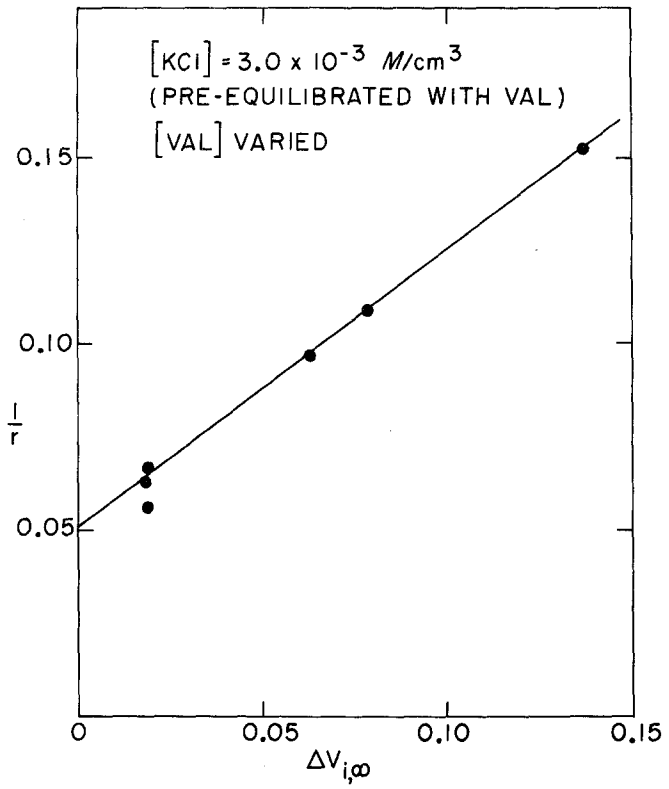


Fig. 7. Plot of $1/r$ *vs.* $\Delta V_{i,\infty}$ (*see* Eq. (25))

voltage conductance $G_0$ measured using both techniques are presented in Table 2.

The basic presumption in the derivation of the equations and consequently in the interpretation of the data is that the rate processes are effectively at steady-state within the accessible time domain ($t > 2\,\mu$sec). From the argument of the exponential of Eq. (3) and from the values of the rate constants (Table 2) we can estimate the time constant for relaxation[4] as a function of concentration. The amplitude of the relaxation may also be estimated. The "instantaneous" $dV/dt$ (after the first relaxation – *see* footnote) may be related to the steady-state value [*see* Eq. (3)]

$$\frac{dV^{+}_{t,\infty/dt}}{dV_{t,\infty/dt}} = 1 + B = 1 + \frac{k_R c_M}{2k_s}$$

None of the high voltage transients exhibits a relaxation having anywhere near the predicted time dependence. In an experiment with $1.43 \times 10^{-4}$ moles/cm$^3$ KCl and $3.16 \times 10^{-6}$ moles/cm$^3$ valinomycin in GMO/*n*-

4 This is really the second relaxation. The first, which is simply the movement of the membrane bound valinomycin-potassium complex, is virtually infinitely fast at high voltage.

Table 2. Correlation of low-voltage charge-pulse and steady-state measurement

| $c_M$ (moles cm$^{-3}$) | $q^0$ (coul cm$^{-2}$) | $V_{i,0}$ (volts) | $d \ln V/dt$ (sec$^{-1}$) | $G$ (mho cm$^{-2}$) | steady-state $G$ (mho cm$^{-2}$) |
|---|---|---|---|---|---|
| $1.43 \times 10^{-4}$ | $1.85 \times 10^{-8}$ | 0.0191 | $2.40 \times 10^{4}$ | $2.32 \times 10^{-2}$ | $2.21 \times 10^{-2}$ |
| $3.91 \times 10^{-4}$ | $2.06 \times 10^{-8}$ | 0.0140 | $2.58 \times 10^{4}$ | $3.80 \times 10^{-2}$ | $3.66 \times 10^{-2}$ |
| $7.78 \times 10^{-4}$ | $3.49 \times 10^{-8}$ | 0.0162 | $2.42 \times 10^{4}$ | $5.21 \times 10^{-2}$ | $4.64 \times 10^{-2}$ |
| $1.00 \times 10^{-3}$ | $3.08 \times 10^{-8}$ | 0.0137 | $2.09 \times 10^{4}$ | $4.69 \times 10^{-2}$ | $4.35 \times 10^{-2}$ |

$[KCl] + [LiCl] = 3.00 \times 10^{-3}$ moles cm$^{-3}$.
Valinomycin $= 3.07 \times 10^{-6}$ moles cm$^{-3}$ in GMO/*n*-hexadecane (no pre-equilibration).

hexadecane repetitive multiple charges were injected – each charge being somewhat smaller than the preceding one and spaced by a time somewhat longer than the time required for a transient to decay to baseline. If there is a relaxation at the beginning of each transient, it should be easily observed. According to Eq. (3) and the data in Table 2, the time constant for the rate processes to reach steady-state is about $10^{-5}$ sec and the ratio of the "instantaneous" $dV/dt$ to the steady state $dV/dt$ is about 1.7. Thus, the relaxation ought to be clearly observed – it is not. Several factors may contribute to this: the effective value of $k_R$ will be increased at higher voltages, although probably no more than a factor of 2 at 0.4 V or about a factor of 1.4 at 0.2 V. If the correct value of $k_s$ were a factor of two larger, this would have the double effect of decreasing both the time constant and the amplitude of the relaxation making it more difficult to measure with our instrumentation.

Time-dependent low-voltage transients (resulting from small charge pulses) are of the expected magnitude and clearly observed. Our analyses (Table 2) only consider the steady-state region, the criterion for this being a linear ln $V$ *vs.* $t$ plot. A detailed analysis of the low-voltage transient has been carried out by Benz and Läuger (1976).

We conclude that the Läuger-Stark model effectively characterizes valinomycin-mediated potassium transport across GMO/*n*-hexadecane bilayers. The single modification would be the introduction of a voltage dependence for the heterogeneous rate constants $k_R$ and $k_D$. High-voltage behavior is consistent with the following formulation:

$$k'_R = k_R e^{0.045 f V_{t,\infty}}$$

$$k''_R = k_R e^{-0.045 f V_{t,\infty}}$$

$$k'_D = k_D e^{-0.045 f V_{t,\infty}}$$

$$k''_D = k_D e^{0.045 f V_{t,\infty}}.$$

The value of 0.045 is also consistent with Hladky's (1975) estimate of $\approx 0.05$ for trinactin-mediated potassium or ammonium ion transport, and with Eisenman, Krasne and Ciani (1975) who find $\approx 0.05$ for actin type carriers and 0.04 for valinomycin-type carriers.

There appear to be a few deviations from the simple model: an anomalously high increase in both high- and low-voltage conductance with increasing valinomycin concentration ($1.1 \times 10^{-7} - 1.7 \times 10^{-8}$ moles $cm^{-3}$) at constant KCl concentration ($3.0 \times 10^{-3}$ moles $cm^{-3}$); high-voltage time dependent transients with less than the expected magnitude; and an anomalously high value of $k_{MS}/k_D$ (a value of approximately 4 as compared to 0.7 as determined by Benz *et al.* (1976) and Laprade *et al.* (1974). These last two discrepancies have in common that they could both be manifestations of an anomalously low high-voltage flux. We emphasize, however, that although a value of $W \sim 4$ might seem to be a spectacular disagreement with the accepted value of $\sim 0.7$, the measured value of $r$ (from which $W$ is deduced) is less than a factor of two too large. Considering that our evaluation of $W$ is based on high-voltage data while the literature values have been determined using low-voltage relaxation techniques, the agreement is surprisingly good.

Comparison of our present results with those obtained in Part I (Feldberg & Kissel, 1975) will show some discrepancies. The earlier work was carried out using GMO/*n*-decane BLMs (which our recent work indicates is not a critical difference) and a somewhat lower valinomycin concentration. The earlier value of $W = 0.8$ (the reported value must be divided by 2 in order to be compared to the $W$ in the present paper) seems in better agreement with the accepted value. The earlier analysis of the high-voltage data did not consider a possible voltage dependency of $k_R$ and $k_D$ or the effect of charge redistribution during low-voltage decay (somewhat reduced because of the low valinomycin concentration). Both these omissions would tend to lower the value of $r$ and thus of $W$. The ratios $k_R/k_D$ and $k_R/k_s$ are virtually the same as that obtained in the present work, although the individual rates $k_R$, $k_D$, and $k_s$ were somewhat higher. This is due to a more precise evaluation of $N_s^0$ in the present work.

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## Appendix

The time-dependent equations of Stark *et al.* (1971) lead directly to a general expression [Eqs. (15), (29)–(32)] describing low-voltage steady-

state decay and intercepts. During a low-voltage decay one must consider not only the steady-state flux, but the contribution to the apparent flux by the redistribution of charged species. The following derivation presumes that $k_R$, $k_D$, and $k_S$ are voltage independent.

Thus

$$-\frac{dV}{dt}=\frac{F}{C_m}\left(J_{MS}+\frac{dN''_{MS}}{dt}\right)=\frac{F}{C_m}\left(J_{MS}+\frac{dV}{dt}\cdot\frac{dN''_{MS}}{dV}\right). \tag{A.1}$$

For a given constant voltage applied to the membrane the charge required to reach a new steady-state is

$$\int_0^{\alpha} J^{\pm}_{MS}\,dt=J_{MS}\int_0^{\infty}1+\alpha_1\,e^{-t/\tau_1}+\alpha_2\,e^{-t/\tau_2}\,dt. \tag{A.2}$$

The portion of this charge movement due to redistribution of $N_{MS}$ is

$$\Delta N''_{MS}=J_{MS}\int_0^{\infty}\alpha_1\,e^{-t/\tau_1}+\alpha_2\,e^{-t/\tau_2}\,dt=J_{MS}[\alpha_1\,\tau_1+\alpha_2\,\tau_2]. \tag{A.3}$$

Since

$$\Delta N''_{MS}=N''_{MS}-N^0_{MS}$$

$$\frac{dN''_{MS}}{dV}=\frac{d}{dV}(J_{MS}[\alpha_1\,\tau_1+\alpha_2\,\tau_2]). \tag{A.4}$$

Rewriting (A.1) gives

$$-\frac{du}{dt}=\frac{\dfrac{F^2}{RTC_m}J_{MS}}{1+\dfrac{F^2}{RTC_m}\dfrac{d}{du}(J_{MS}[\alpha_1\,\tau_1+\alpha_2\,\tau_2])}. \tag{A.5}$$

On the basis of the equations of Stark *et al.* (1971) one obtains

$$\alpha_1\,\tau_1+\alpha_2\,\tau_2=\frac{\dfrac{(k'_{MS}+k''_{MS})}{k_D}\{B^2+K_h\,c_M(1+B)^2\}}{k_R\,c_M\left\{\dfrac{(k'_{MS}+k''_{MS})}{k_D}(1+B)+1\right\}}. \tag{A.6}$$

Since

$$k'_{MS}+k''_{MS}=2k_{MS} \tag{A.7}$$

and

$$A=2W(1+B) \tag{A.8}$$

it is clear that (A.6) is voltage independent and

$$\alpha_1\,\tau_1+\alpha_2\,\tau_2=\frac{A^2\left\{\dfrac{B^2}{(1+B)^2}+K_h\,c_M\right\}}{2Wk_R\,c_M(A+1)}. \tag{A.9}$$

Since

$$J_{MS}=\frac{k_R\, c_M\, N_S^0\, \dfrac{(k'_{MS}-k''_{MS})}{k_D}}{1+\dfrac{k'_{MS}+k''_{MS}}{k_D}(1+B)} \tag{A.10}$$

and

$$k'_{MS}-k''_{MS}=k_{MS}\, u, \tag{A.11}$$

$$\frac{dJ_{MS}}{du}=\frac{k_R\, c_M\, N_s^0\, W}{1+A} \tag{A.12}$$

$\therefore$ Eq. (A.5) becomes

$$-\frac{d\ln u}{dt}=\frac{\dfrac{F^2}{RTC_m}\,\dfrac{k_R\, c_M\, N_s^0\, W}{(1+A)}}{1+\dfrac{F^2}{2RTC_m}\,\dfrac{N_s^0\, A^2}{(1+A)^2}\left\{\dfrac{B^2}{(1+B)^2}+K_h\, c_M\right\}}. \tag{A.13}$$

This equation leads directly to Eq. (15).

We thank Drs. Roland Benz and Peter Läuger for making data available to us prior to publication (Benz & Läuger, 1976).

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