

Multiple Membrane Systems as Biological Models Current-Voltage Behavior

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Summary. The current-voltage equations for double, triple, and quadruple membrane systems are derived in closed form from the flow equations of irreversible thermodynamics. Numerical examples show that the behavior of these systems is very similar to that of nerve and muscle membranes. Multiple membrane systems exhibit resting potentials which do not have a characteristic Nernst concentration dependence; nonpermeant ions play a significant role in this nonlogarithmic behavior. Furthermore, multiple membrane systems have rectification properties similar to those of biological membranes. The direction of rectification is determined by the polarity of the membrane systems, not by the ionic concentrations in the bathing solutions.

The non-Nernst concentration dependence of resting potentials and the rectification properties of nerve and muscle membranes apparently cannot be analyzed by a straightforward application of the integrated form of the phenomenological equations of irreversible thermodynamics, in which the flows are given as linear functions of the differences of the forces across the membrane. However, this limitation lies not within the thermodynamic equations themselves, but with an assumption implicit in their application: that the membrane is homogeneous. There is no doubt that biological membranes are not homogeneous. The mathematical difficulties inherent in a calculation of the current-voltage behavior of an inhomogeneous membrane appear to preclude simple analytical solutions. Nevertheless, inhomogeneous membranes can be approximated by complex structures built up of discrete homogeneous components. In such a model the linear flow equations can be applied to each component, and the overall behavior of the system found algebraically. In this paper we shall consider inhomogeneous membranes which are systems of components arranged in series array: i.e., the membrane is a sandwich of several different permselective components.

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The flows of nonelectrolytes and water through bimembrane systems have been analyzed by Patlak, Goldstein, and Hoffman (1963) and more recently by Mikulecky in the Appendix to Sha'afi, Rich, Mikulecky, and Solomon (1970). Tasaki and Takenada (1964) proposed that their measurements of resting potentials, which did not vary logarithmically with concentration, might be explained by potentials across unstirred layers next to the membrane. In effect, they proposed a triple membrane system with the stagnant layers as two components. In a later article Singer and Tasaki (1968) proposed a composite membrane with an external layer containing a high density of negative fixed-charge sites and an internal layer with relatively few fixed anionic sites. In a fundamental paper on the permeability of composite membranes, Kedem and Katchalsky (1963) derived the overall *practical* parameters for membranes in series array. With these parameters it is possible to calculate the voltage behavior of a composite membrane. However, this behavior is not immediately apparent from the parameters themselves. An explicit current-voltage function given in terms of quantities measured in the external bathing solutions is not presented; the calculations required are not straightforward. In later discussions of rectification in biological membranes, Katchalsky (1967, 1968) presented the current-voltage function for a bimembrane, given as Eqs. (11) and (13) in the following section. This solution, as it stands, does not indicate that the permeabilities ω^{α} and ω^{β} appearing in Eq. (13) are functions of C_s^* , the concentration between the two components making up the bimembrane, and are therefore current dependent. Here, in an extension of this basic work, I shall derive the current-voltage equation in a more explicit form and present numerical evaluations of several multiple membrane systems bearing a strong resemblance in their behavior to nerve and muscle membranes. Equations for double, triple, and quadruple membrane arrays are derived.¹ The analysis is based upon the assumption that the membrane potential is determined by the distribution of a mono-monovalent salt; bi-ionic potentials are not considered. This is not to imply that biological membrane potentials may not arise from the interdiffusion of two salts, but is to simplify the system in order to demonstrate unequivocally that membrane structure itself may give rise to unusual and unexpected potentials. It will also be shown that the presence of nonpermeant ions may have a strong effect upon membrane potentials. In the numerical examples sodium represents nonpermeant cations. Water flow through the membrane is assumed to be negligible.

¹ In these derivations it is assumed that the reader is familiar with the Kedem-Katchalsky notation.

The picture of the biological membrane as it is drawn by the accretion of data from researches in many diverse fields is one of constantly increasing complexity. The analysis offered here takes no account of the dynamic structural changes and chemical processes occurring in biological membranes and is thus limited in scope. Nevertheless, it should be of some value to see that seemingly complex behavior may arise in membranes composed of simple passive components.

Rectification in Salt Systems

In multiple membrane systems, rectification occurs because of differences in the transport properties of the components making up a series system. It is a fundamental property of the membrane, not of the bathing solutions, in that the membrane alone determines the direction of rectification. To show clearly that rectification *per se* in these systems is not a property of the ionic concentrations in the bathing solutions, we shall initially consider salt systems rather than ionic (colloidal) systems, where nonpermeant ions are present in the bathing solutions. Therefore, to start we shall consider the rectification properties of a multiple membrane system separating two solutions of a neutral mono-monovalent salt, such as KCl. A general current-voltage equation for ionic systems will be derived in the following section.

Rectification in a Double Membrane

The first multiple membrane system to be analyzed is the double membrane, or bimembrane, as depicted in Fig. 1*a*. The two components of the composite membrane are labeled α and β . They are each homogeneous and have a uniform distribution of fixed-charge sites. The physical properties determining the flows through each component are specified by the *practical* parameters, ω , κ , and τ_1 . Since the analysis will be limited to those biological membranes for which the assumption of negligible water flow is reasonable, the steady-state equations for the salt flow and the current through either component of the bimembrane are

$$J_s^i = \omega^i \Delta \pi_s^i + \frac{\tau_1^i}{F} I^i \quad (1)$$

and

$$I^i = \frac{\kappa^i \tau_1^i}{F} \Delta \mu_s^i + \kappa^i E^i, \quad i = \alpha, \beta. \quad (2)$$

The superscripts refer to the particular membrane component under consideration. By convention the differences $\Delta \mu_s^i$, $\Delta \pi_s^i$, and E^i are taken from

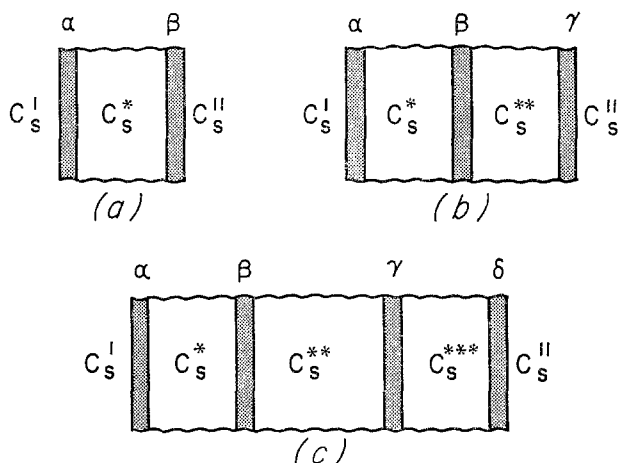


Fig. 1. (a) Double membrane or bimembrane. (b) Triple membrane. (c) Quadruple membrane

left to right: e.g.,

$$\Delta \pi_s^\alpha = 2RT(C_s^I - C_s^*) \quad (3)$$

and

$$\Delta \mu_s^\beta = 2RT \ln \frac{C_s^*}{C_s^{II}}. \quad (4)$$

The superscript I refers to region I on the left of membrane α , and the superscript * refers to the space between the two components α and β . This space is merely a mathematical device which allows us to express all forces as differences of potentials evaluated in aqueous solution. It has no physical significance. The electrochemical potentials in the intermembrane aqueous region are set equal to the actual values in the membrane phase at the interface between the two components. This use of an equivalent system is analogous to the method of images in electrostatics. There the image charge does not actually exist, and likewise the intermembrane aqueous region does not exist.

The two flow equations depend upon the salt concentration and the voltage in the region * between the two components, and these are unknown quantities. The only data available for determining the overall flows through the bimembrane system are the concentrations C_s^I and C_s^{II} and the total potential drop E between regions I and II. Nevertheless, the intermembrane concentration can be calculated using the practical equations for each separate component and then making use of the continuity of the flows. By *continuity* we mean that at all points of the system a flow has the same magnitude and direction; this is a consequence of being in steady state in a

one-dimensional system. In other words, as time increases there can be no accumulation of salt or charge between components α and β ; therefore, the flow into the intermembrane region through membrane component α must equal the flow out through component β . We have

$$J_s^\alpha = J_s^\beta = J_s \quad (5)$$

and

$$I^\alpha = I^\beta = I. \quad (6)$$

The analysis depends upon one additional fact: the forces across the complete membrane system are equal to the sum of the forces across its components: e.g.,

$$\Delta \pi_s = \Delta \pi_s^\alpha + \Delta \pi_s^\beta. \quad (7)$$

This follows from Eq. (3). Similarly, the emf E and the difference in the chemical potential $\Delta \mu_s$ are found by summing across the membrane components.

The current equation (2) is in terms of an emf determined by electrodes reversible to the anion. Electrophysiological data is often presented in terms of $\Delta \psi$, the emf determined by calomel electrodes; for this data we write Eq. (2) as

$$I \frac{1}{\kappa^\alpha} = \frac{1}{F} (\tau_1^\alpha \Delta \mu_1^\alpha - \tau_2^\alpha \Delta \mu_2^\alpha) + \Delta \psi^\alpha \quad (8)$$

where $\Delta \mu^\alpha$ is the concentration-dependent part of the electrochemical potential. This follows from the fact that $E^\alpha = -\Delta \tilde{\mu}_2^\alpha / F$.

There is a similar current equation for the β component. Summing these two equations gives the important result

$$\Delta \psi = I \rho - \frac{1}{F} (\tau_1^\alpha \Delta \mu_1^\alpha - \tau_2^\alpha \Delta \mu_2^\alpha + \tau_1^\beta \Delta \mu_1^\beta - \tau_2^\beta \Delta \mu_2^\beta) \quad (9)$$

where we have introduced the notation

$$\rho = \frac{1}{\kappa^\alpha} + \frac{1}{\kappa^\beta}. \quad (10)$$

Since here there are no charged species in the bathing solutions aside from the mobile salt ions, electro-neutrality dictates that $\Delta \mu_1^\alpha = \Delta \mu_2^\alpha$; Eq. (9) can be simplified to

$$\Delta \psi = I \rho - \frac{RT}{F} \left[(2\tau_1^\alpha - 1) \ln \frac{C_s^I}{C_s^*} + (2\tau_1^\beta - 1) \ln \frac{C_s^*}{C_s^{II}} \right] \quad (11)$$

where C_s^* is as yet undetermined. In the following paragraphs we shall see that C_s^* is a function of the current, and thus by Eq. (11), $\Delta \psi$ is a nonlinear function of I .

To determine the value of the intermembrane concentration C_s^* , we use the continuity of salt flow, Eq. (5). Recalling that $\Delta \pi_s = \Delta \pi_s^\alpha + \Delta \pi_s^\beta$, we sum the flow equations (1) for salt flow through components α and β to obtain

$$J_s = \frac{1}{\frac{1}{\omega^\alpha} + \frac{1}{\omega^\beta}} \left[\Delta \pi_s + \frac{I}{F} \left(\frac{\tau_1^\alpha}{\omega^\alpha} + \frac{\tau_1^\beta}{\omega^\beta} \right) \right]. \quad (12)$$

We set this expression for J_s equal to J_s^α as given by Eq. (1) and solve this equation for $\Delta \pi_s^\alpha$. Finally, we can determine C_s^* because $\Delta \pi_s^\alpha = 2RT(C_s^I - C_s^*)$. Hence,

$$C_s^* = \frac{\omega^\alpha C_s^I + \omega^\beta C_s^{II}}{\omega^\alpha + \omega^\beta} - \frac{I}{2RTF} \frac{\tau_1^\beta - \tau_1^\alpha}{\omega^\alpha + \omega^\beta}. \quad (13)$$

From the natural condition $C_s^* \geq 0$, we see that I must be bounded. It is demonstrated that the bimembrane system rectifies and that the direction of rectification depends upon the signs of the fixed charges in the components since the magnitudes of τ_1^α and τ_1^β depend primarily upon fixed-charge polarity. Eq. (13) can be used in Eq. (11) to obtain the current-voltage equation for a bimembrane. It must be stressed that Eq. (13), as it stands, is not an explicit solution for C_s^* ; the *practical* parameters, as shown in Kedem and Katchalsky (1963) and Richardson (1971), are functions of the salt concentrations of the external solutions. For example, ω^α and τ_1^α are functions of C_s^I and C_s^* . To obtain the quantitative results presented in the discussion on rectification in biological systems, Eq. (13) was solved numerically.

Rectification in a Triple Membrane

The triple membrane system has two intermembrane concentrations C_s^* and C_s^{**} , which must be determined in order to evaluate the emf $\Delta \psi$ across the complete system. As shown in Fig. 1*b*, the component membranes are labeled α , β , and γ . The same calculation scheme as used in the preceding section will be used here; the calculations here will be abbreviated. The salt flow through each component is given by Eq. (1). By summing the salt flow equations for all three components, we find

$$J_s \Sigma \frac{1}{\omega^i} = \Sigma \Delta \pi_s^i + \frac{I}{F} \Sigma \frac{\tau_1^i}{\omega^i} \quad (14)$$

where the summation over the index i is a sum over components α , β , and γ . Equating J_s as given by Eq. (14) to J_s^α as given by Eq. (1) gives an expression

for $\Delta \pi_s^\alpha$. Since $\Delta \pi_s^\alpha = 2RT(C_s^I - C_s^*)$, we can solve this expression for

$$C_s^* = \frac{1}{\omega^\alpha \Sigma \frac{1}{\omega^i}} \left[\left(\omega^\alpha \Sigma \frac{1}{\omega^i} - 1 \right) C_s^I + C_s^{II} - \frac{I}{2RTF} \left(\frac{\tau_1^\beta - \tau_1^\alpha}{\omega^\beta} + \frac{\tau_1^\gamma - \tau_1^\alpha}{\omega^\gamma} \right) \right]. \quad (15)$$

By considering J_s^γ we can find the analogous equation for C_s^{**} . From the natural conditions $C_s^* \geq 0$ and $C_s^{**} \geq 0$, we see that it is possible for the triple membrane to be a double rectifier; that is, it is possible for the current to be bounded from above and below. Similar to Eq. (11), the current-voltage equation is

$$I\rho = \frac{1}{F} \Sigma (2\tau_1^i - 1) \Delta \mu_1^i + \Delta \psi \quad (16)$$

where $\rho = \Sigma \frac{1}{\kappa^i}$ and the summations run over all three components.

Rectification in a Quadruple Membrane

Although this system is complex, its properties are interesting, and it is perhaps no more complicated than the elementary structure of an axon membrane for which it might serve as a model. A diagram of the quadruple membrane is presented in Fig. 1c. The equations derived in the section on triple membranes are actually general equations which can be extended to systems comprised of any number of components. Here the summations will now run over α , β , γ , and δ . Since they have the exact form of the preceding equations, the expressions for C_s^* , C_s^{**} , and C_s^{***} will not be presented.

Like the triple membrane it is possible for the quadruple membrane system to be a double rectifier. The current-voltage equation is Eq. (16) with the summation going over all four components. It must be stressed again that these solutions are not reductions to closed form; the *practical* parameters ω and τ_1 are functions of the intermembrane concentrations. Solutions are achieved numerically.

Rectification in Ionic Systems

The *practical* parameters ω and τ_1 are concentration dependent, and this greatly complicates any analysis of multiple membrane systems using the *practical* equation for salt flow because the intermembrane concentrations depend upon the current. Fig. 3 shows that the variation of ω is significant and cannot be ignored; but τ_1 is very nearly either unity or zero, depending upon the sign of the fixed charges. Therefore, we can safely

put $\tau_1 = 1$ or $\tau_1 = 0$ in our calculations. And under this condition we can solve the current-voltage equation in closed form for a system of any number of components.

The Current-Voltage Equation for a Double Membrane

The equation for the cation flow through a single homogeneous membrane separating two ionic solutions has been derived in an earlier paper (Richardson, 1971). The cation flow through the α component is given as

$$J_1^\alpha = \lambda^\alpha (C_1^I C_2^I - C_s^{*2}) + \tau_1^\alpha \frac{I}{F} \quad (17)$$

where

$$\lambda^\alpha = \frac{\phi_w^2 RT}{(f_{im} + f_{iw} + X \xi_{12}) X \Delta x} \quad (18)$$

and $f_{iw} = f_{2w}$ if the α component is negatively charged or $f_{iw} = f_{1w}$ if it is positively charged. The same convention holds for f_{im} . Also, τ_1^α takes the values 1 or 0, respectively. There is a similar equation for the β component. From the steady state condition $J_1^\alpha = J_1^\beta$ we obtain

$$C_s^{*2} = \frac{\tau_1^\alpha - \tau_1^\beta}{(\lambda^\alpha + \lambda^\beta) F} (I - I_0) \quad (19)$$

where the limiting current I_0 is

$$\begin{aligned} I_0 &= I(C_s^{*2} = 0) \\ &= \frac{\lambda^\alpha C_1^I C_2^I + \lambda^\beta C_1^{II} C_2^{II}}{(\tau_1^\beta - \tau_1^\alpha)} F. \end{aligned} \quad (20)$$

The condition $C_s^{*2} \geq 0$ implies that I is bounded, and the sign of the difference $(\tau_1^\alpha - \tau_1^\beta)$ determines whether I is bounded from above or below. The limiting current I_0 is this bound; the bimembrane is a rectifier. We see that the direction of rectification does not depend upon the values of the ionic concentrations in the bathing solutions but upon the signs of the fixed-charge groups in the two components. It is possible for a bimembrane to rectify in the direction of decreasing salt concentration; thus the bimembrane may exhibit anomalous rectification.

By Eq. (9) we finally obtain the current-voltage equation for a bimembrane:

$$\Delta\psi = \Delta\psi_0 + I\rho + \frac{RT}{F} (\tau_1^\alpha - \tau_1^\beta) \ln \left(1 - \frac{I}{I_0} \right) \quad (21)$$

where the resting potential is

$$\Delta\psi_0 = \frac{RT}{F} \left[\ln \frac{C_2^I}{C_2^{II}} + \tau_1^\alpha \ln \frac{\lambda^\alpha C_1^I C_2^I + \lambda^\beta C_1^{II} C_2^{II}}{(\lambda^\alpha + \lambda^\beta) C_1^I C_2^I} - \tau_1^\beta \ln \frac{\lambda^\alpha C_1^I C_2^I + \lambda^\beta C_1^{II} C_2^{II}}{(\lambda^\alpha + \lambda^\beta) C_1^{II} C_2^{II}} \right]. \quad (22)$$

The potential $\Delta\psi$ across the bimembrane is made up of three contributions: (1) the resting potential $\Delta\psi_0$, (2) a linear portion $I\rho$, and (3) a nonlinear portion which becomes infinite as I approaches the limiting value I_0 .

Eq. (21) gives an adequate representation of the current-voltage behavior of multiple membrane systems made up of ion-exchanger membranes (Y. Katz, *unpublished results*). Using the Nernst-Planck equation for ion flow, Spiegler (1971) has recently investigated the limiting currents which occur in electro dialysis stacks. He considered a system consisting of an ion-exchanger membrane and its two boundary layers and arrived at a current-voltage equation similar to Eq. (21), with a linear resistive term and two logarithmic terms. From the viewpoint of the present analysis the two log terms occur because his membrane system is composed of three components, having two interfaces separating components of differing transference numbers. Spiegler points out that a complete description of limiting currents in electro dialysis stacks must take into account side effects such as water-splitting.

The Current-Voltage Equation for Triple and Quadruple Membranes

In a triple membrane system there are three cation flow equations [see Eq. (17)]; the steady-state condition is $J_1^\alpha = J_1^\beta = J_1^\gamma = J_1$. We combine these equations to obtain

$$J_1 = \bar{\lambda} (C_1^I C_2^I - C_1^{II} C_2^{II}) + \bar{\tau}_1 \frac{I}{F} \quad (23)$$

where

$$\bar{\lambda} = \left(\sum \frac{1}{\lambda^i} \right)^{-1} \quad \text{and} \quad \bar{\tau}_1 = \left(\sum \frac{1}{\lambda^i} \right)^{-1} \sum \frac{\tau_1^i}{\lambda^i}, \quad i = \alpha, \beta, \gamma. \quad (24)$$

The cation flow through the composite membrane is thus given as a function of measurable quantities (the current and the ionic concentrations in the bathing solutions) and has the same form as the flow equation for a single membrane. This expression for J_1 is equated to that for J_1^* and C_s^* determined as a function of the measurable quantities. We obtain

$$C_s^{*2} = \frac{\tau_1^\alpha - \bar{\tau}_1}{\lambda^\alpha F} (I - I_0^*) \quad (25)$$

where the limiting current for the intermembrane region is

$$I_0^* = \frac{\lambda^\alpha C_1^I C_2^I - \bar{\lambda}(C_1^I C_2^I - C_1^{II} C_2^{II})}{\bar{\tau}_1 - \tau_1^\alpha} F. \quad (26)$$

In the same manner we find

$$C_s^{**2} = \frac{\tau_1^\beta - \bar{\tau}_1}{\lambda^\beta F} (I - I_0^{**}) \quad (27)$$

where

$$I_0^{**} = \frac{\lambda^\beta C_s^{*2} - \bar{\lambda}(C_1^I C_2^I - C_1^{II} C_2^{II})}{\bar{\tau}_1 - \tau_1^\beta} F. \quad (28)$$

These intermembrane concentrations are then put into Eq. (9) to give a current-voltage equation in closed form similar to Eq. (21).

Eqs. (27) and (28) are recursion formulas which can be used to find the intermembrane concentrations for a multiple membrane system of any number of components. The current-voltage equation will have the form of Eq. (21) with a $\ln(1 - I/I_0)$ term for each intermembrane region. As the algebra is straightforward, the quadruple membrane equations will not be presented.

Rectification in Biological Systems

Rectification is a well-established phenomenon in muscle and nerve membranes; the membrane exhibits nonlinear and asymmetric behavior in that the resistance of the membrane depends upon the direction and the magnitude of the current (Cole & Baker, 1941). As a possible explanation for their rectification curves in frog muscle, Adrian and Freygang (1962) proposed a multiple barrier, one component of which exhibited anomalous rectification. In experiments with muscle bathed in artificial seawater which had all sodium replaced by potassium, Gilbert and Ehrenstein (1966) found that the current was limited in both the positive and negative direction: i.e., the muscle was a double rectifier.

In our analysis we have already demonstrated that bimembranes rectify in one direction and that triple and quadruple membranes can be double rectifiers. To illustrate more concretely the current-voltage behavior of multiple membrane systems, several numerical examples are presented. The system parameters chosen for these examples are of the order of magnitude of those found in biological membranes; however, no attempt is made to fit biological data exactly by a variation of the rather large number of parameters specifying the system.

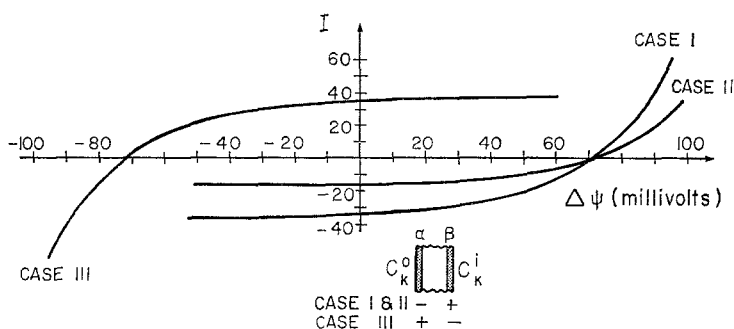


Fig. 2. Rectification in bimembranes. All cases: $C_K^0 = C_{Cl}^0 = 10^{-5} \text{ M/cm}^3$; $C_K^i = C_{Cl}^i = 3.5 \times 10^{-4} \text{ M/cm}^3$. Case II: Fixed charge density is twice that of Case I. Case III: Structural polarity is reverse that of Case I. The current is given as amps per cm^2 of aqueous channel

By presenting several multiple membrane systems with reasonable, but rather arbitrarily chosen, physical properties, we shall demonstrate that these models offer a possible explanation for experimental observations. In this manner it will be possible to determine in what ways a biological membrane resembles a single, double, triple, or quadruple membrane system. We shall assume that the membrane components are permeable solely to potassium and chloride ions. The single homogeneous membrane can be immediately discarded since it neither fits the concentration-dependent behavior of the resting potential nor predicts rectification. In our formulation the Nernst equation represents the steady-state potential of a single very highly charged negative membrane; it is just Eq. (8) with $\tau_2 = 0$.

There are several reasons to suspect that biological membranes are highly charged, but a detailed discussion of this particular subject is outside the scope of this paper. The *practical* parameters and the ionic flow equations derived in Kedem and Katchalsky (1963) and Richardson (1971) are restricted to highly charged membranes. We shall make the assumption of high charge density in the membrane components and then see in what way these membrane systems resemble biological membranes.

In this discussion of rectification, we consider bathing solutions of potassium chloride with no nonpermeant ions present. The friction between water and the mobile ions is the only one considered. The current is given as amperes per square centimeter of aqueous channel in the membrane. Using the following expression for the conductance,

$$\kappa = \frac{F^2 X}{f_{iw}^0 \Delta x} \quad (29)$$

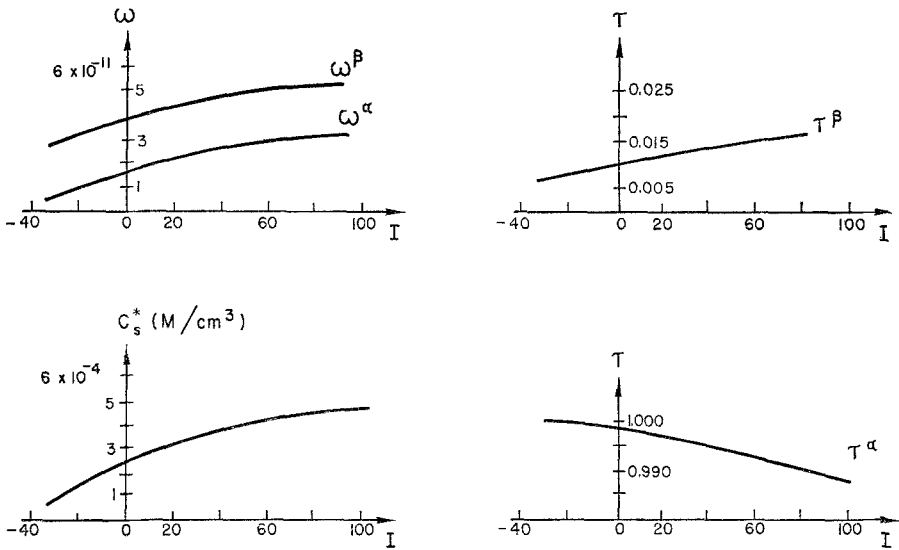


Fig. 3. Intermembrane concentration C_s^* , permeabilities ω^α and ω^β , and transference numbers τ_1^α and τ_1^β as a function of the current for the bimembrane given in Case I, Fig. 2. The current is given as amps per cm^2 of aqueous channel

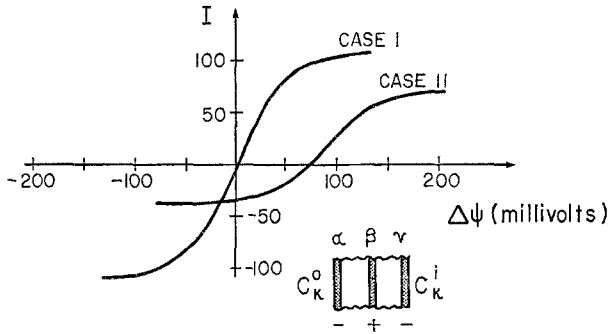


Fig. 4. Rectification in a triple membrane. *Case I*: $C_K^0 = C_{C1}^0 = 3.5 \times 10^{-4} \text{ M/cm}^3$; $C_K^i = C_{C1}^i = 3.5 \times 10^{-4} \text{ M/cm}^3$. *Case II*: $C_K^0 = C_{C1}^0 = 10^{-5} \text{ M/cm}^3$; $C_K^i = C_{C1}^i = 3.5 \times 10^{-4} \text{ M/cm}^3$. The current is given as amps per cm^2 of aqueous channel

$i=1$ or 2 depending upon whether the component is negative or positive, the curves in Figs. 2, 3, and 4 were calculated using a numerical computer program. The magnitude of the fixed-charge concentration was set at $X=3 \times 10^{-3} \text{ M/cm}^3$ in the calculations. This unusually large value is Passow's (1969) "effective" concentration for erythrocyte membranes. The thickness of each component was set at $\Delta x=2 \times 10^{-6} \text{ cm}$.

Two *practical* parameters, the permeability and the transference number, are functions of external concentrations. In a multiple membrane system this means that they depend upon the values of the intermembrane concentrations, which in turn are functions of the current through the system. Fig. 3 shows the dependence of these parameters and of the intermembrane concentration upon the current. It also shows that the assumption that τ_1 is nearly unity for a highly charged negative membrane is valid. Therefore, the numerical calculation shows that we can safely use the closed-form I vs. $\Delta\psi$ [Eq. (21)], with τ_1 assuming values of 1 or 0.

Resting Potentials in Ionic Systems

The resting potential for any multiple membrane system is given by the appropriate current-voltage equation from the penultimate section under the condition that $I=0$. For clarity and simplicity in the numerical examples, λ will, in general, assume the same value for all membrane components in a system. This would be the case for the permeation of KCl through water-swollen components of equal thickness and with equal fixed-charge densities (with differing signs, however). The membrane-solute friction would be negligible; and $f_{s,w}$ is the same for K and Cl ions in free solution.

The resting potential for a double membrane is given explicitly by Eq. (22). For a triple membrane with $\lambda^\alpha = \lambda^\beta = \lambda^\gamma$ we can use Eq. (25) to obtain

$$C_s^{*2} = \frac{2C_1^I C_2^I + C_1^{II} C_2^{II}}{3}, \quad (30)$$

and similarly we have

$$C_s^{**2} = \frac{C_1^I C_2^I + 2C_1^{II} C_2^{II}}{3}. \quad (31)$$

These values of the intermembrane concentrations are then used in Eq. (9) to obtain $\Delta\psi$ for $I=0$. In the quadruple membrane we have

$$C_s^{*2} = \frac{3C_1^I C_2^I + C_1^{II} C_2^{II}}{4} \quad (32)$$

$$C_s^{***2} = \frac{C_1^I C_2^I + 3C_1^{II} C_2^{II}}{4} \quad (33)$$

and

$$C_s^{***2} = \frac{C_s^{*2} + C_s^{***2}}{2}. \quad (34)$$

The Resting Potential in Biological Systems

Early measurements of biological potentials, determined as injury potentials or by the insertion of micro-electrodes, were found to be in reasonable agreement with values predicted by the Nernst equation if it were assumed that the resting potential was determined by the distribution of the potassium ions and that the membrane was impermeable to sodium ions. In a test of this theory, Hodgkin and Keynes (1955) varied the external potassium concentration and discovered that the resting potential of an intact squid axon did not follow the Nernst equation but fell off rapidly below an external potassium concentration of 50 mM. With the advent of perfusion techniques, it became possible to control the internal environment. For example, in the experiments of Baker, Hodgkin, and Shaw (1962) the external potassium concentration was held fixed while the internal potassium concentration was varied by replacing isotonic KCl with isotonic NaCl. Replotting their data on semi-log coordinates shows clearly that the resting potential does not follow the Nernst equation. A dramatic departure from the Nernst equation was offered by Tasaki and Takenada (1964), who measured both the resting potential and the peak of the action potential under several very interesting combinations of salt concentrations.

Figs. 5 and 6 show the calculated resting potentials for two multiple membrane systems where the internal potassium concentration is varied by replacing isotonic KCl with isotonic NaCl. If sodium is not absolutely non-

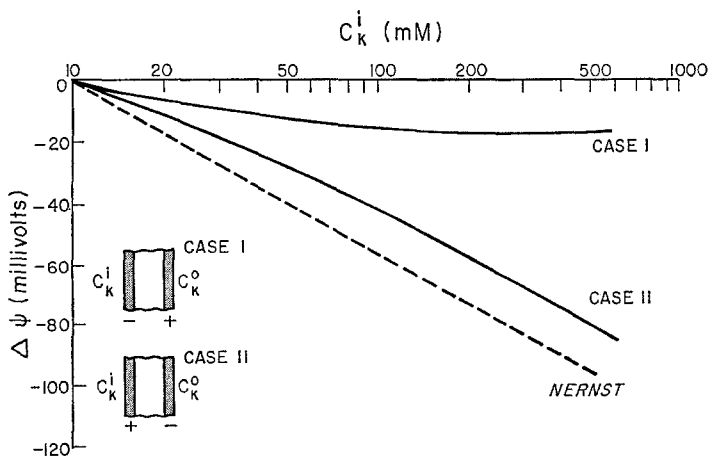


Fig. 5. Resting potential for a bimembrane. Potassium concentration varied by replacing isotonic KCl with isotonic NaCl. Sodium assumed to be nonpermeant. Both cases: $C_K^o = 10^{-5} \text{ M/cm}^3$; $C_{Cl}^o = 6.33 \times 10^{-4} \text{ M/cm}^3$; $C_{Cl}^i = 6.33 \times 10^{-4} \text{ M/cm}^3$

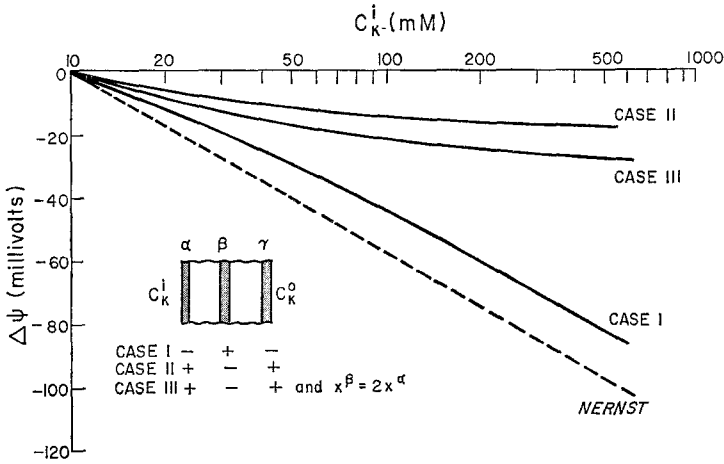


Fig. 6. Resting potential for a triple membrane. Potassium concentration varied by replacing isotonic KCl with isotonic NaCl. Sodium assumed to be nonpermeant. All cases: $C_K^0 = 10^{-5} \text{ M/cm}^3$; $C_{Cl}^0 = 6.33 \times 10^{-4} \text{ M/cm}^3$; $C_{Cl}^i = 6.33 \times 10^{-4} \text{ M/cm}^3$

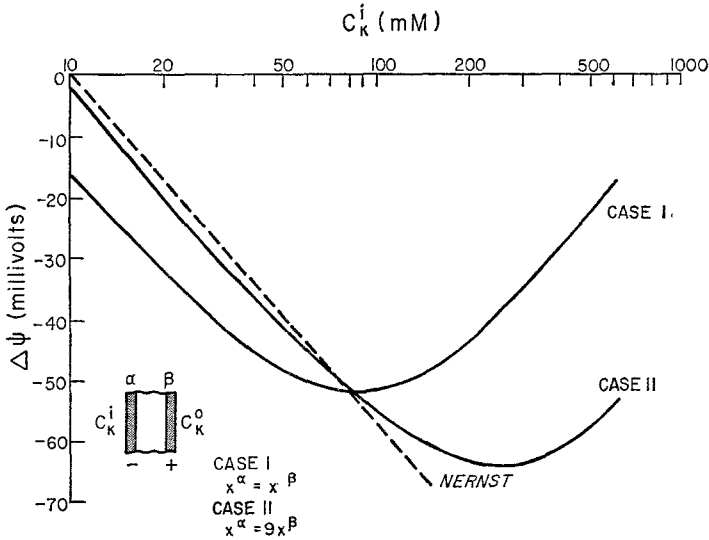


Fig. 7. Resting potential for a bimembrane. Ratio of the Na/K concentrations held fixed at 1/10. Sodium assumed to be nonpermeant. Both cases: $C_K^0 = 10^{-5} \text{ M/cm}^3$; $C_{Cl}^0 = 6.33 \times 10^{-4} \text{ M/cm}^3$; $C_{Cl}^i = 1.1 C_K^i$

permeant, caution should be taken in comparing this particular model calculation with physiological data since here as C_K^i increases from 10 mM, C_{Na}^i decreases from 623 mM to zero.

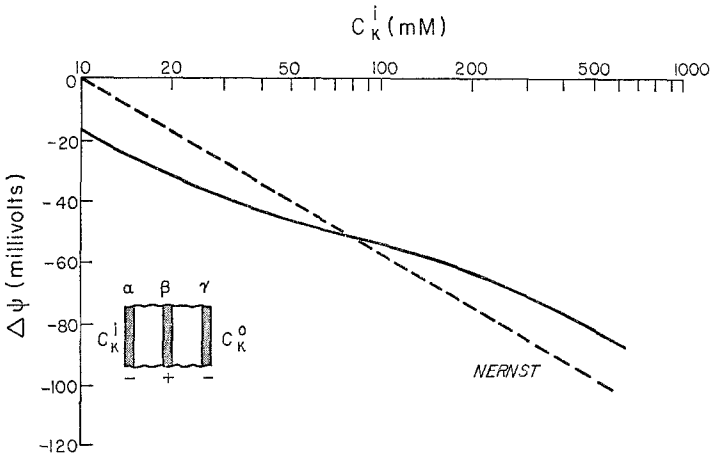


Fig. 8. Resting potential for a triple membrane. Ratio of the Na/K concentrations held fixed at 1/10. Sodium assumed to be nonpermeant. $C_K^o = 10^{-5}$ M/cm³; $C_{Cl}^o = 6.33 \times 10^{-4}$ M/cm³; $C_{Cl}^i = 1.1 C_K^i$

In their measurements of the resting potential, Tasaki and Takenada (1964) kept the relative concentrations of sodium and potassium constant: $C_{Na}^i = 1/10 C_K^i$. Consequently, if sodium is slightly permeant, its relative contribution to the cationic flow will not vary greatly with changes in C_K^i . The outer solution was sea water. For the calculation we set $C_K^o = 10$ mM and $C_{Cl}^o = 633$ mM. Fig. 7 shows the resting potential calculated for a bimembrane. Fig. 8 shows the resting potential for a triple membrane. The resting potential for a quadruple membrane is very similar to that of the triple membrane and therefore not presented.

Symmetry and Polarity in Multiple Membrane Systems

Hodgkin (1964) mentions that if the inner and outer solutions in a perfused axon are interchanged, the sign of the resting potential reverses but the magnitude does not change significantly. This is a highly unnatural state for the axon, and perhaps this phenomenon should not be considered as a definite criterion for judging models for biological membranes. Nevertheless, it is worth noting. If, for example, in the bimembrane system given as Case II, Fig. 5, the inner and outer solutions at the point where $C_K^i = 500$ mM are interchanged, then the resting potential $\Delta\psi_0$ goes from -82 mV to $+17$ mV. It must be concluded that the bimembrane does not fulfill the symmetry requirement. A triple membrane with components α and γ identical has a geometric symmetry which assures a reversal of the sign of the potential

and no change in its magnitude upon an interchange of solutions. This is also true of a quadruple membrane with components α and δ identical and components β and γ identical.

The couplet of transference numbers for cations, $(\tau_1^{\alpha}, \tau_1^{\beta})$, indicates the structural polarity of a bimembrane. The state of polarity of Case I in Fig. 2 is thus (1, 0), with a corresponding resting potential of $\Delta\psi_0 = -72$ mV. If by some means the sign of the fixed charges of the two membrane components were interchanged, causing the state of polarity to become (0, 1), then the resting potential would jump to $\Delta\psi_0 = +72$ mV. Thus, in a system where KCl is the only permeant salt and where $C_K = C_{Cl}$ in the bathing solutions (a salt system), a reversal of polarity reverses the sign of the resting potential. Fig. 5 shows that in systems where nonpermeant ions are in the bathing solutions (ionic systems), a reversal of polarity does not necessarily reverse the resting potential. At $C_K^i = 500$ mM, a reversal of polarity causes the resting potential to jump from $\Delta\psi_0 = -82$ mV to $\Delta\psi_0 = -17$ mV. In general, the effects of polarity reversals must be found by an examination of Eqs. (20), (21), and (22).

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