DIRECT EFFECTS ON THE MEMBRANE POTENTIAL DUE TO "PUMPS" THAT TRANSFER NO NET CHARGE

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ABSTRACT The effects of active ionic transport are included in the derivation of a general expression for the zero current membrane potential. It is demonstrated that an active transport system that transfers no net charge (nonrheogenic) may, nevertheless, directly alter the membrane potential. This effect depends upon the exchange of matter within the membrane between the active and passive diffusion regimes. Furthermore, in the presence of such exchange, the transmembrane active fluxes measured by the usual techniques and the local pumped fluxes are not identical. Several common uses of the term "electrogenic pump" are thus shown to be inconsistent with each other. These inconsistencies persist when the derivation is extended to produce a Goldman equation modified to account for active transport; however, that equation is shown to be limited by less narrow constraints on membrane heterogeneity and internal electric field than those previously required. In particular, it is applicable to idealized mosaic membranes limited by these requirements.

INTRODUCTION

All plasma membranes apparently transport ions actively and ionic pumps are frequently suggested as possible direct contributors to the transmembrane electrical potential difference. The term "direct" here implies that the effect is not achieved indirectly as a result of changes in the bounding concentrations.

Ionic pumping systems have been divided into the categories of electrogenic and nonelectrogenic. This division appears to have arisen in response to two distinct needs. The first was for insight into possible coupling between different active fluxes. The second was to obtain information concerning direct effects on the membrane potential. Several uses of the term "electrogenic pump" have accordingly appeared on the assumption that they are consistent with each other.

Perhaps the most basic use of this term was to refer to active transport systems that transfer net charge at the pump sites (see, for instance, Adrian and Slayman, 1966, p. 1011; Kotyk and Janáček, 1970). Such systems might be more accurately described as "rheogenic," or current generating. Two operational definitions have emerged. One is based on the measurement of transmembrane active fluxes with tracers. This definition assumes that the measured fluxes and those occurring at the pump sites are identical. The other operational definition uses the term "electrogenic" to describe any pumping system that directly affects the membrane potential (see, for instance, Kotyk and Janáček, 1970). This definition assumes that the local active transfer of net electric charge is necessary to produce a direct contribution to the membrane potential.

The actual relationships connecting the fluxes at the pump sites, the externally measureable transmembrane active fluxes, and the zero current membrane potential are derived in this paper.[1] It is demonstrated that these simple assumptions about electrogenic pumps are, in general, not justified. The consequences are examined and the discussion is then extended to determine the effects of active ionic transport on Goldman's (1943) equation for the zero current membrane potential. In addition the constraints usually placed on the applicability of that equation are reexamined and shown to be narrower than required.

THEORY

Direct Pump Effects on the Membrane Potential

I will assume the membrane to be both bounded by parallel planes and homogeneous in the y-z plane (Fig. 1). The diffusion regime is thus unidimensional.[2] If it is also isothermal and isobaric, and active transport occurs, the total flux density of a univalent cation or anion at any point in the membrane can be described by

$$j_k = j_k^m - \omega_k c_k^p \left[RT \frac{\partial}{\partial x} \ln c_k^p + F \frac{\partial \varphi}{\partial x} \right],$$

and (1)

$$j_a = j_a^m - \omega_a c_a^p \left[RT \frac{\partial}{\partial x} \ln c_a^p - F \frac{\partial \varphi}{\partial x} \right]. \tag{1}$$

Here, R, F, and T are, respectively, the gas and Faraday constants and the absolute temperature. The ionic mobilities are denoted by ω_i and the c_i are concentrations. The subscripts a and k denote anions and cations, and φ is the electrical potential. The superscripts m and p denote, respectively, quantities in the active or passive regimes. The local active flux densities j_k^m and j_a^m are introduced kinematically;

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[1] Preliminary reports of this work have appeared (Schwartz, 1969, 1970, 1971).

[2] This constraint is identical to that required by Ussing (1949) in his derivation of the flux ratio equation. It is nevertheless irritating if one wishes to use these relationships with epithelia. It would be more satisfying to examine the properties of a generalized three-dimensional membrane. This work is in process.

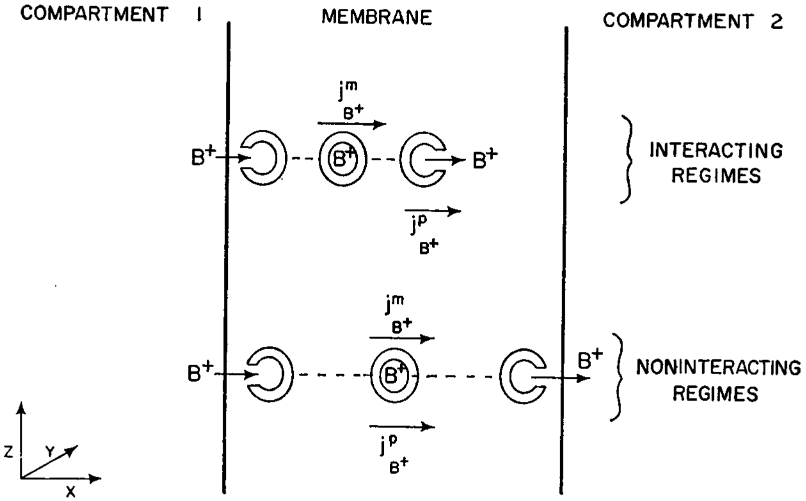

Figure 1 The membrane is bounded by parallel planes and is macroscopically homogeneous in the y-z plane. The y direction is into the plane of the figure. Inhomogeneities may be encountered in the x direction. A possible way in which the active and passive regimes may interact inside the membrane is depicted. A carrier molecule complexes with cation B^+ at the compartment 1-membrane interface. Passive diffusion and active carrier-mediated transport will then occur together in the same region. If the complex dissociates at some internal point, more B^+ then becomes available for passive diffusion. Matter will thus be exchanged within the membrane between the active and passive transport systems. If the complex does not dissociate until it reaches the opposite face of the membrane, the regimes will not interact. Note that both interacting and noninteracting regimes have been depicted in this one figure even though in the text they have been assumed to define two types of membrane.

that is, no attempt to specify force-flux relationships is made. Finally, solvent drag and thermodynamic coupling between flows are assumed negligible, and the solutions are taken to be ideal.[3]

Since

$$\frac{\partial}{\partial x}\left[c_i^p \exp\left(\frac{z_i F\varphi}{RT}\right)\right] = \exp\left(\frac{z_i F\varphi}{RT}\right)\left[\frac{\partial c_i^p}{\partial x} + c_i^p \frac{z_i F}{RT}\frac{\partial \varphi}{\partial x}\right], \qquad (2)$$

rearrangement of equations 1 enables us to write

$$\frac{j_i}{\omega_i}\exp\left(\frac{z_i F\varphi}{RT}\right) = \frac{j_i^m}{\omega_i}\exp\left(\frac{z_i F\varphi}{RT}\right) - RT\frac{\partial}{\partial x}\left[c_i^p \exp\left(\frac{z_i F\varphi}{RT}\right)\right];$$
$$i = k, a; \qquad z_k = 1, \qquad z_a = -1. \qquad (3)$$

When the membrane is in a steady state, intramembranous concentrations and fluxes no longer vary in time, but this can occur in two distinct ways depending on

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[3] Alternatively, these coupling effects can be considered as included in the j_i^m.

the nature of the membrane (Schwartz, 1969, 1970, 1971). If ions can be transferred within the membrane between the active and the passive regimes, the conservation of matter requires that

$$\begin{aligned} \frac{\partial c_i^m}{\partial t} &= -\frac{\partial j_i^m}{\partial x} + \rho_i = 0, \\ \frac{\partial c_i^p}{\partial t} &= -\frac{\partial j_i^p}{\partial x} - \rho_i = 0; \qquad i = a, k; \end{aligned} \tag{4}$$

during a steady state. Local concentrations of a particular species contained in the active and passive systems are denoted by c_i^m and c_i^p. The term ρ_i gives the local transfer of material from the passive to the active regime. This situation corresponds to the case in which a membrane-bound carrier involved in the active system binds or releases the carried species at points within the membrane, both at and away from its boundaries.

Adding equations 4 yields the steady-state relationships

$$\frac{\partial j_i}{\partial x} = \frac{\partial}{\partial x}[j_i^m + j_i^p] = 0; \qquad i = a, k. \tag{5}$$

Thus, in this type of steady state it is only the total flux density of each ion that does not vary across the membrane. The active and passive flux densities, individually, are not constrained in this way. They may be functions of x. This kind of steady state is known to occur in epithelia. The localization of the sites of active transport in such tissues to the plasma membranes necessitates an internal exchange between the active and passive systems. This exchange occurs both within the constituent cells and in the intercellular space. Until more is known about the details of active transport mechanisms, the possible occurrence of such a steady state also within a plasma membrane cannot be ruled out.

If there is no transfer of matter within the membrane between the active and passive systems, a steady state will occur when

$$\frac{\partial c_i^m}{\partial t} = -\frac{\partial j_i^m}{\partial x} = 0,$$

and (6)

$$\frac{\partial c_i^p}{\partial t} = -\frac{\partial j_i^p}{\partial x} = 0; \qquad i = a, k;$$

for each ion in the active and passive systems separately. This corresponds to the case that a membrane-bound carrier in the active regime can combine with or release the carried species only at the membrane-solution boundaries. The active and passive systems may then be viewed as parallel. In this case then, the active and passive flux densities are individually constrained not to vary across the membrane.

Consider the first, mathematically less restrictive of these two steady states. Equations 3 can then be integrated to yield

$$\begin{aligned} j_k \int_1^2 \frac{1}{\omega_k} \exp\left(\frac{F}{RT}\varphi\right) \mathrm{d}x \\ &= \int_1^2 \frac{1}{\omega_k} j_k^m \exp\left(\frac{F}{RT}\varphi\right) \mathrm{d}x - RT\left\{c_k(2)\exp\left[\frac{F}{RT}\varphi(2)\right]\right. \\ &\qquad \left. - c_k(1)\exp\left[\frac{F}{RT}\varphi(1)\right]\right\}, \\ j_a \int_1^2 \frac{1}{\omega_a} \exp\left(-\frac{F}{RT}\varphi\right) \mathrm{d}x \\ &= \int_1^2 \frac{1}{\omega_a} j_a^m \exp\left(-\frac{F}{RT}\varphi\right) \mathrm{d}x - RT\left\{c_a(2)\exp\left[-\frac{F}{RT}\varphi(2)\right]\right. \\ &\qquad \left. - c_a(1)\exp\left[-\frac{F}{RT}\varphi(1)\right]\right\}, \end{aligned} \tag{7}$$

since, at the boundaries

$$c_i^p = c_i.$$

The transmembrane electrical potential

$$\Delta\varphi \equiv \varphi(2) - \varphi(1)$$

is introduced by multiplying the cationic equation by exp $[-(F/RT)\varphi(1)]$ and the anionic equation by exp $[(F/RT)\varphi(2)]$. For convenience we define

$$\begin{aligned} Q_k &\equiv \int_1^2 \frac{1}{\omega_k} \exp\left\{\frac{F}{RT}[\varphi - \varphi(1)]\right\} \mathrm{d}x, \\ Q_a &\equiv \int_1^2 \frac{1}{\omega_a} \exp\left\{\frac{F}{RT}[\varphi(2) - \varphi]\right\} \mathrm{d}x, \\ M_k &\equiv \int_1^2 \frac{1}{\omega_k} j_k^m \exp\left\{\frac{F}{RT}[\varphi - \varphi(1)]\right\} \mathrm{d}x, \end{aligned} \tag{8}$$

and

$$M_a \equiv \int_1^2 \frac{1}{\omega_a} j_a^m \exp\left\{\frac{F}{RT}[\varphi(2) - \varphi]\right\} \mathrm{d}x.$$

Equations 7 then yield

$$j_k = \frac{1}{Q_k}\left\{M_k - RT\left[c_k(2)\exp\left(\frac{F}{RT}\Delta\varphi\right) - c_k(1)\right]\right\}$$

and $$\tag{9}$$

$$j_a = \frac{1}{Q_a}\left\{M_a - RT\left[c_a(2) - c_a(1)\exp\left(\frac{F}{RT}\Delta\varphi\right)\right]\right\}.$$

If free diffusion prevails there will be no electrical current. Thus

$$\sum_k j_k - \sum_a j_a = 0. \tag{10}$$

A summation over the fluxes and a regrouping of terms then gives[4]

$$\Delta\varphi = \frac{RT}{F}\ln\left\{\frac{\sum_k\left(\frac{c_k(1)}{Q_k}\right) + \sum_a\left(\frac{c_a(2)}{Q_a}\right) + \frac{1}{RT}\left[\sum_k\left(\frac{M_k}{Q_k}\right) - \sum_a\left(\frac{M_a}{Q_a}\right)\right]}{\sum_k\left(\frac{c_k(2)}{Q_k}\right) + \sum_a\left(\frac{c_a(1)}{Q_a}\right)}\right\}. \tag{11}$$

The active fluxes will alter the membrane potential directly unless

$$\sum_k \frac{M_k}{Q_k} = \sum_a \frac{M_a}{Q_a}, \tag{12}$$

a condition which will not generally be met. If the pumps transfer no net charge locally within the membrane, they can be considered collectively nonrheogenic and

$$\sum_k j_k^m = \sum_a j_a^m \equiv J_m. \tag{13}$$

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[4] The membrane-solution interface can be treated as a continuum if the ω's are allowed to be continuous functions of x and the entire region is aqueous. Otherwise equation 11 is, strictly, valid only within the membrane phase. In that case the relationship between the concentrations in the bathing media and those just within the boundaries of the membrane must be taken into account. If we assume local equilibrium at the membrane boundaries (Kirkwood, 1954)

$$\mu_i^0(s) + RT\ln c_i(s1) + z_iF\varphi(s1) = \mu_i^0(1) + RT\ln c_i(1) + z_iF\varphi(1)$$

$$\mu_i^0(s) + RT\ln c_i(s2) + z_iF\varphi(s2) = \mu_i^0(2) + RT\ln c_i(2) + z_iF\varphi(2).$$

The (s) denotes a point in the external solution. Then

$$RT\ln\frac{c_i(s2)}{c_i(s1)} + z_iFV = RT\ln\frac{c_i(2)}{c_i(1)} + z_iF(\Delta\varphi),$$

where

$$V \equiv \varphi(s2) - \varphi(s1).$$

Equation 12, however, will not even then be satisfied in general, and the pump fluxes may still alter the membrane potential. This situation changes only if the active and passive transport regimes do not interact, so that equations 6 apply. Since the j_k^m and j_a^m are then no longer functions of x, they may be removed from under the integral signs in equations 8. Equation 12 then simplifies to

$$\sum_k j_k^m = \sum_a j_a^m, \tag{14}$$

which is precisely the condition for no local charge transfer. Therefore, if the membrane is one in which internal ionic transfer between the active and passive regimes

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It follows that

$$\frac{c_k(s2)}{c_k(s1)}\frac{c_k(1)}{c_k(2)} = \exp\left(\frac{F}{RT}[\Delta\varphi - V]\right)$$

and

$$\frac{c_a(s2)}{c_a(s1)}\frac{c_a(1)}{c_a(2)} = \exp\left(-\frac{F}{RT}[\Delta\varphi - V]\right).$$

Substituting into equation 11 for $c_k(2)$ and $c_a(1)$ yields

$$\exp\left(\frac{FV}{RT}\right) = \frac{\sum_k\left(\frac{\beta_k c_k(s1)}{Q_k}\right) + \sum_a\left(\frac{\beta_a c_a(s2)}{Q_a}\right) + \frac{1}{RT}\left[\sum_k\left(\frac{M_k}{Q_k}\right) - \sum_a\left(\frac{M_a}{Q_a}\right)\right]}{\sum_k\left(\frac{\beta_k c_k(s2)}{Q_k}\right) + \sum_a\left(\frac{\beta_a c_a(s1)}{Q_a}\right)},$$

where

$$\beta_k \equiv \frac{c_k(1)}{c_k(s1)}, \qquad \beta_a \equiv \frac{c_a(2)}{c_a(s2)}.$$

This expression is analogous to equation 11. If the membrane-solution equilibrium can be described as a partition, the β's are the partition coefficients. They can be absorbed into the Q's without altering the form of equation 11 (Hodgkin and Katz, 1949). If the membrane contains fixed charges and the interface must be described by a Donnan equilibrium (Teorell, 1953), the β's will describe the equilibrium. Indeed

$$\beta_k = \frac{1}{r(1)}$$

and

$$\beta_a = r(2),$$

where the r's denote the Donnan ratios at the boundaries. If these ratios are maintained constant in the course of experiment they can again be absorbed into the Q's, leaving the form of equation 11 unchanged. Problems may develop if the Donnan ratios are not maintined constant. The dependence of membrane permeability both on internal mobility and on membrane-solution selectivity is here revealed.

can occur, even a locally noncharge-transferring pumping system can directly alter the membrane potential. This is the result both of the displacement of the internal concentration profiles from their passive configurations, and of the pattern of active and passive intramembranous fluxes required by the steady-state constraint (equation 5).

The oft made assumption that only net charge–transferring pumping systems can exert a direct influence on the potential is thus incorrect. In general, a locally nonrheogenic pump will fail to affect the membrane potential directly only if the active and passive regimes do not interact and are thus, in effect, parallel. This should not be surprising. The model first suggested by Koefoed-Johnsen and Ussing (1958) to explain the frog skin potential included a pumping system that transferred no net charge. I have already noted that, in a membrane of that kind, the active and passive regimes cannot be regarded as separate. At the very least, matter will exchange between them inside the epithelial cells; but, as was also noted, the possible occurrence of such interacting regimes within plasma membranes in general and neural membranes in particular cannot at this point be dismissed. Thus a nonrheogenic pump may also directly alter the membrane potential in these membranes.

The Physical Significance of the Quantities M_i/Q_i : Two Different Kinds of Active Flux Densities

The local j_k^m and j_a^m are the actual pumped flux densities. They are therefore the fluxes most relevant to any discussion of events, such as coupled ionic pumping, thought to occur at the pump sites, but they cannot, in general, be measured by existing techniques.

The quantities M_i/Q_i, which determine whether there is a direct effect on the membrane potential (equations 11 and 12), contain these fluxes in an integrated form. They define a weighted average set of different active flux densities, $\hat{j}_i^m$, such that[5]

$$\hat{j}_i^m = \frac{M_i}{Q_i} = \frac{\int_1^2 \frac{1}{\omega_i} j_i^m \exp\left(\frac{z_i F\varphi}{RT}\right) \mathrm{d}x}{\int_1^2 \frac{1}{\omega_i} \exp\left(\frac{z_i F\varphi}{RT}\right) \mathrm{d}x}; \qquad i = a, k; \; z_k = 1, z_a = -1. \quad (15)$$

It is precisely these $\hat{j}_i^m$ that are measured with tracers. This is evident from equations 9, and can be most clearly seen in the following two cases. The passive terms in those equations vanish when the membrane is short-circuited (Ussing and Zerahn,

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[5] The weighting factor is proportional to $[(1/\omega_i) \exp(z_i F\varphi/RT)]$. The physical significance of this term is evident from equation 3. The quantity $[RT\omega_i \exp(-z_i F\varphi/RT)]$ can be seen to serve as an equivalent diffusion coefficient for charged species when the diffusion force is written in terms of the gradient of $[c_i \exp(z_i F\varphi/RT)]$. This equivalent diffusion coefficient assumes the form of the usual diffusion coefficient when uncharged species are involved.

1951) while identical solutions bathe both of its faces. They also vanish in the equation for a single ion when the membrane is held at the Nernst potential for that ion. A determination of the net transmembrane flux j_i will yield $\hat{j}_i^m$ under either of these conditions.

These $\hat{j}_i^m$ are, in general, not equivalent to the actual pumped flux densities j_i^m. Only if the active and passive transport regimes do not interact are $\hat{j}_i^m$ and j_i^m equal. The measurable $\hat{j}_i^m$ may therefore be used for the modeling of events at the pump sites only with caution.

In a heterogeneous membrane within which the active and passive regimes do interact, each $\hat{j}_i^m$ will be a function of the structures of j_i^m, ω_i, and φ. This dependence on ω_i suggests a possible tool for determining whether such interaction indeed exists. A pharmacological agent is required that changes only the x dependence of ω_i, but by more than a constant factor. Interaction within the membrane should be suspected if the application of this agent alters $\hat{j}_i^m$ when it is measured under short circuit conditions. A short circuit is desirable so that complicating effects arising from any attendant transmembrane potential changes may be eliminated.

The Goldman Equation

If the reciprocals of the Q's are regarded as permeabilities and the active transport terms are discarded, equation 11 formally resembles the expression

$$\Delta\varphi = \frac{RT}{F} \ln \left\{ \frac{\sum_k \omega_k c_k(1) + \sum_a \omega_a c_a(2)}{\sum_k \omega_k c_k(2) + \sum_a \omega_a c_a(1)} \right\} \qquad (16)$$

derived by Goldman (1943) and rederived by Hodgkin and Katz (1949).[6] This simple relationship lends itself well to studies of membrane permeability. It has therefore been used extensively; but in its derivation Goldman, as well as Hodgkin and Katz, ignored fluxes resulting from active transport. In addition the electrical field in the membrane had to be constant, and permeable membrane regions were assumed to be entirely homogeneous so that the mobilities would be constant in space. These constraints were not required to derive equation 11; however, equations 11 and 16 share the following requirements: the membrane is in a steady state for each permeant ion; there is no electrical current; all permeant ions are univalent and each is permitted to diffuse in response only to the gradient of its own electrochemical potential as in a dilute free solution.

Several of the passive constraints on the Goldman equation have been reexamined. It can be shown that the requirement of univalence is too rigid; it is sufficient for all the permeant ions to have the same absolute valence. Sjodin (1961) has dis-

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[6] Hodgkin and Katz accounted for the partition of each solute between the solution and membrane phases by introducing modified mobilities as permeability coefficients. These P's appear in place of the ω's in their version of this equation (also, see footnote 4).

cussed some problems related to the assumption of free uncoupled diffusion. Barr (1965) demonstrated that in a homogeneous membrane the constant field is a sufficient but not a necessary requirement. Sandblom and Eisenman (1967) showed a constant field to be unnecessary for a membrane homogeneous in the y-z plane only (Fig. 1), provided the total ionic concentrations at the two membrane faces are equal. Mullins and Noda (1963) showed that if ions of only one sign are permeant, and the mobilities are constant throughout the membrane, the structure of the electric field does not have to be specified at all. Sandblom and Eisenman (1967) have demonstrated the validity of this conclusion under very general conditions. They have also extensively discussed the limitations imposed by the requirement of constant ratios of ionic permebabilities.

The effects of active fluxes, however, have barely been considered. Both Barr (1965) and Geduldig (1968) confined themselves to pumps that are either collectively nonrheogenic, or almost so. Moreton (1969) inserted a term into the Goldman equation to account for a charge-transferring (rheogenic) pump, but he assumed a constant field in the membrane and made no distinction between the actual local pumped fluxes and the integrated transmembrane active fluxes. In so doing he confined his discussion entirely to a membrane within which no exchange between the active and passive transport systems can occur.

A more thorough investigation of the effects of active fluxes on the Goldman equation is thus in order. This can be achieved readily by examining the relationship between equations 11 and 16. The Q's in equation 11 are dependent on the structure of the electric field in the membrane (equations 8). Their reciprocals, therefore, are not membrane parameters to be determined by perturbing the bathing media as are the ω's of equation 16.[7] Further constraints must be imposed to arrive at the simpler form of the Goldman equation. These constraints will be shown to involve the electric field and the mobility. I will demonstrate that these constraints may be made less narrow than was previously supposed. In addition, it will become clear that their imposition does not basically alter the conclusions drawn from equation 11 about pump effects on the membrane potential.

The following two conditions must be met for the passive terms in equation 11 to take on the form of those in equation 16.

(*a*) For any pair of cations, k and j, it is necessary that

$$\frac{Q_k}{Q_j} = A_{kj}, \tag{17}$$

and for any pair of anions a and b, it is necessary that

$$\frac{Q_a}{Q_b} = A_{ab}, \tag{18}$$

where the A_{nm} are constant in x.

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[7] The permeabilities defined by Patlak (1960) appear also to suffer from this difficulty.

(*b*) For any cation k and anion a, it is necessary that

$$\frac{Q_k}{Q_a} = A_{ka}. \tag{19}$$

The ratio of any two Q's would then be constant in x.

The first condition is satisfied if the mobilities are of the form

$$\begin{aligned} \omega_k &= \Omega_k f(x) \\ \text{and} \qquad & \\ \omega_a &= \Omega_a g(x), \end{aligned} \tag{20}$$

where the Ω's are constants, but f and g may be functions of x. A single f must serve for all cations, and a single g for all anions. All the cations would then have one spatial variation and all the anions another. A degree of membrane inhomogeneity is thus possible. The significance of this freedom when treating biological membranes should depend on the membrane type. In an epithelium the permeabilities to ions of the same sign seem to change in respect to each other as the membrane is crossed. This could be in part because of mobility variations. Equations 20 will not allow for this, but in a plasma membrane not enough is known to make a judgement. In any case, the mobilities are not required to be constants in the x direction in order to satisfy the above conditions.

The ensuing derivation is simplified by using the average of the potentials at the membrane boundaries as a reference level. A potential Ψ is therefore defined such that

$$\Psi \equiv \varphi - \left[\frac{\varphi(1) + \varphi(2)}{2}\right]. \tag{21}$$

Equations 8 then become

$$\begin{aligned} Q_k &= \frac{1}{\Omega_k} \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right) N_+, & N_+ &\equiv \int_1^2 \frac{1}{f} \exp\left(\frac{F}{RT}\Psi\right) \mathrm{d}x; \\ Q_a &= \frac{1}{\Omega_a} \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right) N_-, & N_- &\equiv \int_1^2 \frac{1}{g} \exp\left(-\frac{F}{RT}\Psi\right) \mathrm{d}x; \\ M_k &= \frac{1}{\Omega_a} \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right) L_k, & L_k &\equiv \int_1^2 \frac{1}{f} j_k^m \exp\left(\frac{F}{RT}\Psi\right) \mathrm{d}x; \end{aligned} \tag{22}$$

and

$$M_a = \frac{1}{\Omega_a} \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right) L_a, \qquad L_a \equiv \int_1^2 \frac{1}{g} j_a^m \exp\left(-\frac{F}{RT}\Psi\right) \mathrm{d}x.$$

Substitution into equation 11 yields

$$\Delta\varphi = \frac{RT}{F}\ln\left\{\frac{\frac{1}{N_+}\sum_k \Omega_k c_k(1) + \frac{1}{N_-}\sum_a \Omega_a c_a(2) + \frac{1}{RT}\exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right)\left[\frac{1}{N_+}\sum_k L_k - \frac{1}{N_-}\sum_a L_a\right]}{\frac{1}{N_+}\sum_k \Omega_k c_k(2) + \frac{1}{N_-}\sum_a \Omega_a c_a(1)}\right\} \tag{23}$$

We need require only that

$$N_+ = N_- \tag{24}$$

for the second condition (equation 19) to be satisfied.[8] Except for the active terms, equation 23 will then take on the form of equation 16. This means that

$$\int_1^2 \left[\frac{1}{f}\exp\left(\frac{F}{RT}\Psi\right) - \frac{1}{g}\exp\left(-\frac{F}{RT}\Psi\right)\right] dx = 0 \tag{25}$$

must relate the membrane structure to the internal potential. This appears to be the minimal required constraint on the electrical potential. Rewriting equation 23 for this kind of field yields

$$\Delta\varphi = \frac{RT}{F}\ln\left\{\frac{\sum_k \Omega_k c_k(1) + \sum_a \Omega_a c_a(2) + \frac{1}{RT}\exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right)\left[\sum_k L_k - \sum_a L_a\right]}{\sum_k \Omega_k c_k(2) + \sum_a \Omega_a c_a(1)}\right\}, \tag{26}$$

an expression formally identical to the Goldman equation when no terms are

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[8] If ions of only one sign are permeant, and, for any reason, the contribution of the active terms vanishes, an equation of the form

$$\Delta\varphi = \frac{RT}{F}\ln\frac{\sum_i \Omega_i c_i(\alpha)}{\sum_i \Omega_i c_i(\beta)};$$

$$i = k, \quad \alpha = 1, \quad \beta = 2;$$

$$i = a, \quad \alpha = 2, \quad \beta = 1;$$

appears. The Goldman equation is then valid without any assumption regarding the nature of the electric field, as Mullins and Noda (1963) demonstrated. The second condition (equations 19 and 24) is then not pertinent.

included for the active transport fluxes. Equation 25 implies the existence of a large group of functions $\Psi(x)$ which will yield the Goldman equation.[9]

The direct contribution of the active fluxes to the membrane potential must still be taken into account. This contribution now depends on the term

$$\sum_k L_k - \sum_a L_a = \int_1^2 \left[\frac{1}{f}\exp\left(\frac{F}{RT}\Psi\right)\sum_k j_k^m - \frac{1}{g}\exp\left(-\frac{F}{RT}\Psi\right)\sum_a j_a^m\right] dx. \quad (27)$$

Consider the nonrheogenic case. Equation 13 describes this condition. Equation 27 then yields

$$\sum_k L_k - \sum_a L_a = \int_1^2 J_m \left[\frac{1}{f}\exp\left(\frac{F}{RT}\Psi\right) - \frac{1}{g}\exp\left(-\frac{F}{RT}\Psi\right)\right] dx. \quad (28)$$

In this form equation 28 describes a membrane with interacting active and passive regimes. It will not, in general, be equal to zero, but if the active and passive regions are separate, J_m is not a function of x (equations 6 and 13). Equation 28 then becomes

$$\sum_k L_k - \sum_a L_a = J_m \int_1^2 \left[\frac{1}{f}\exp\left(\frac{F}{RT}\Psi\right) - \frac{1}{g}\exp\left(-\frac{F}{RT}\Psi\right)\right] dx = 0 \quad (29)$$

with the aid of the constraint on the potential specified in equation 25. In general the active contribution vanishes only under these conditions.[10] The validity of this

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[9] An equation of the Goldman form also results in the special steady-state case that f and g are equal and the total ionic concentrations at both membrane faces are the same (Sandblom and Eisenman, 1967). Although Sandblom and Eisenman did not indicate any constraint on the structure of the internal potential, it can be shown that it will automatically satisfy equation 25 under these conditions (Schwartz, unpublished notes).

In the case that the membrane is entirely homogeneous

$$f(x) = g(x) = 1.$$

Equation 25 then requires that

$$\int_1^2 \sinh\left(\frac{F}{RT}\Psi\right) dx = 0$$

as Barr (1965) showed for that special case. (This equation differs from Barr's equation 14, which contains an error.) The functions $\Psi(x)$ specified by this equation include a set having odd symmetry about a midplane through the membrane. The particular Ψ which yields a constant field is only one such odd function, but the complete group is not confined to odd functions.

[10] If only ions of one sign are actively transported, the nonrheogenic condition becomes

$$J_m = 0.$$

Thus, in a membrane governed by equations 20, direct active effects on the membrane potential will also vanish in this special case.

conclusion, which was drawn earlier in the discussion, has thus not been altered by the subsequent assumptions about Ψ and ω.

It is unfortunately impossible to say too much more about the active contribution to the potential in either type of membrane regime without detailed knowledge of Ψ, f, and g. This is evident from equations 27, 28, and 29. The problem is that equation 25 specifies only that $\int_1^2 (1/f) \exp [(F/RT)\Psi]dx$ and $\int_1^2 (1/g) \exp [-(F/RT)\Psi]dx$ be equal. While this is sufficient to eliminate functions of Ψ from the passive terms in equation 23, it is insufficient to do so for the active terms. Permeabilities calculated from the Goldman equation must therefore contain errors unless the active terms vanish; however, if the influence of the active terms is small, the errors should also be small.

The extent of this influence may be examined as follows. Equation 26 is of the form

$$\Delta\varphi = \frac{RT}{F} \ln \frac{a + b + m \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right)}{c + d}. \tag{30}$$

The meaning assigned to each symbol is obvious. It follows that

$$\exp\left(\frac{F}{RT}\Delta\varphi\right) = \frac{a + b + m \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right)}{c + d}, \tag{31}$$

and

$$\exp\left(\frac{F}{RT}\Delta\varphi\right) - \frac{m}{c + d}\exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right) - \frac{a + b}{c + d} = 0. \tag{32}$$

If

$$\xi \equiv \exp\left(\frac{F}{RT}\frac{\Delta\varphi}{2}\right)$$

$$\xi^2 - \frac{m}{c + d}\xi - \frac{a + b}{c + d} = 0. \tag{33}$$

Thus

$$\xi = \frac{m}{2(c + d)} + \left[\left(\frac{m}{2(c + d)}\right)^2 + \left(\frac{a + b}{c + d}\right)\right]^{1/2}, \tag{34}$$

since the second root is physically meaningless. This yields

$$\exp\left(\frac{F}{RT}\Delta\varphi\right) = \left\{\frac{m}{2(c + d)} + \left[\left(\frac{m}{2(c + d)}\right)^2 + \left(\frac{a + b}{c + d}\right)\right]^{1/2}\right\}^2. \tag{35}$$

The active terms will have little effect if

$$m^2 \ll 4(a + b)(c + d). \tag{36}$$

Then

$$\exp\left(\frac{F}{RT}\Delta\varphi\right) \simeq \frac{a+b}{c+d}, \qquad (37)$$

as required by equation 16.

Mosaic Membranes

The evidence in favor of heterogeneous mosaic-type excitable membranes has become compelling (see, for instance, Nakamura et al., 1965; Grundfest, 1966; Hille, 1968, 1970; Narahashi and Haas, 1968; Ruiz-Manresa et al., 1970). An ideal mosaic membrane can be viewed as a parallel array of membrane patches. Each patch may be either impermeable to any ion or permeable to one ion only. All patches of one kind are assumed to be homogeneous in the *y-z* plane. An electrical current will then exist in each permeable patch because of the single ionic flux within it. The zero local current density condition (equation 10) will thus be violated within the individual patches, but total ionic fluxes can be calculated from the flux densities by taking into account the areas available to both the active and passive transport of each ion. Equation 10 may then be rewritten as a constraint on these fluxes. It will be satisfied over the entire membrane area in the absence of external currents. An expression having the same form as equation 11 can then be derived for a patch membrane. The procedure is analogous to that described in equations 1–11. Our earlier conclusions concerning active transport effects on the membrane potential thus also apply to this idealized mosaic membrane. An equation formally similar to equation 26 can also be derived for this membrane. The Goldman equation taken with its constraints is thus applicable to mosaic membranes.

The Goldman Equation and Epithelia

The required constraints on the ω's (equations 20) seem to invalidate equation 26 for use with epithelia; but an epithelium is often modeled as a single internal compartment contained between two parallel homogeneous "plasma membranes." This model is obviously an approximation. If the active terms are ignored, the resulting Goldman equation may be applicable to each of the plasma membranes taken separately. It has sometimes been used in this manner (see, for instance, Koefoed-Johnsen and Ussing, 1958; Lindley and Hoshiko, 1964). The validity of this usage depends on the applicability of the model, but it does not result in an equation of the Goldman form when taken over the whole membrane.

SUMMARY AND CONCLUSIONS

(*a*) Both rheogenic and nonrheogenic pumping systems can, in general, directly alter the membrane potential. A nonrheogenic pump can do this if the active and passive transport systems interchange matter within the membrane. This would occur if the active system utilized a carrier able to transport only partway across the membrane. One cannot be certain whether such systems actually occur within the plasma membrane until more detailed information about active transport mechanisms becomes available; but, when evaluating data, the possibility of a nonrheogenic pump directly altering the membrane potential must be considered. Conversely, one should not reason from the lack of a direct effect on the membrane potential that the pumps are necessarily locally electroneutral. Such reasoning is valid only in the case in which no interchange of matter between the active and passive systems occurs within the membrane. This would occur if the active system utilized a carrier constrained to pick up or release the transported species at the membrane boundaries only. In that case a nonrheogenic pump cannot have a direct effect on the membrane potential.

(*b*) Direct pump effects on the membrane potential are demonstrated to depend on a set of quantities M_i/Q_i that correspond to weighted average active flux densities. It is these average flux densities that are measured in tracer experiments. The vanishing of their sum implies the lack of any direct effect on the membrane potential, but these average measurable active flux densities do not, in general, correspond to the actual pumped fluxes. It is thus incorrect to conclude that if their sum vanishes, the pumps are necessarily locally nonrheogenic. It is also generally incorrect to use these measured fluxes for the purpose of modeling pump site activity. These difficulties vanish when there is no interchange of matter between the active and passive systems within the membrane. In that case the average transmembrane active flux densities and the pump flux densities are identical.

(*c*) A type of experiment is suggested whereby it might be possible to determine if interchange between the active and passive transport systems actually exists within a heterogeneous membrane. This experimental test is based on the fact that the measurable average active flux densities are dependent on the ω_i in the presence of interchange.

(*d*) I suggest that, to avoid confusion, the term rheogenic (current-creating) be used to designate local charge-transferring pumps. A direct effect on the membrane potential and its correlated sum over the measureable M_i/Q_i can then be unambiguously characterized as electrogenic in agreement with present popular operational usage. This terminology is used in this paper.

(*e*) All of the above effects are shown to appear in the Goldman equation when active transport is taken into account. Membrane permeabilities calculated from the unmodified equation when active transport is present may therefore be in error. The error depends on the relative magnitude of a designated term.

(*f*) The constraints actually required to obtain the Goldman equation are shown not to be as narrow as was previously thought. Two minimal conditions must be met. First, all the cationic and all the anionic mobilities must be respectively covariant as the membrane is crossed. Second, a designated function, containing both the internal electrical potential and the expressions describing the spatial dependence of the mobilities, must vanish when integrated across the membrane. Membrane homogeneity, as required by Barr (1965) is unnecessary. Constant total concentration does not have to be maintained across the membrane, nor do the cationic and anionic mobilities have to be mutually covariant as required by Sandblom and Eisenman (1967). Indeed, these can be demonstrated to be special cases of the more general conditions specified in this paper.

(*g*) It is indicated that both the modified Goldman equation in which active transport terms are included, and several of the more general expressions derived in this paper, are applicable to an idealized mosaic membrane.

(*h*) Caution is required in applying the Goldman equation to epithelia.

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