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Contributions of Secondary Active Transport Processes to Membrane Potentials

Lyndsay G.M. Gordon and Anthony D.C. Macknight
Department of Physiology, University of Otago Medical School, Dunedin, New Zealand

Summary. Equations are developed to examine the effects of secondary active transport processes on the steady-state membrane potential of symmetrical cells. It is shown that, with suitable modifications, equations of the type developed by Goldman, Hodgkin and Katz may be derived to accommodate the contributions to the membrane potential of both electroneutral and electrogenic transporters. Where the membrane potential is a function of the dominant medium ions (Na, K, and Cl), other contributions can come only from an electrogenic Na pump and from neutral co- and counter-transporters if, and only if, these involve the dominant ions. Experimental approaches to measure the parameters necessary to solve the equations developed here are discussed.

Key Words membrane potentials · secondary active transport · membrane permeability

Introduction

Equations of the type derived by Hodgkin and Katz (1949) from the array of current-voltage relations of individual species described by Goldman (1943) have provided considerable insights into ion movements across plasma membranes and their relative contributions to the potential difference across the plasma membrane (V). As initially formulated, it is assumed in these equations that ions move by simple passive diffusion through the membrane. In addition, only ions that are not distributed at electrochemcial equilibrium contribute to the membrane potential under steady-state conditions. However, a variety of ions can cross the membrane through coupled pathways (Fig. 1). Where there is direct coupling of metabolic energy to ion movement (e.g., the transport of Na by the (Na-K)-ATPase), this is referred to as primary active transport. Alternatively, where the transport of ions is coupled to fluxes of other ionic species or of neutral solutes, this is referred to as secondary active transport. Such transport can be neutral or electrogenic and can be either cotransport (where the solutes move in the same direction across the membrane) or counter-transport (where the solutes move in opposite directions across the membrane). Equations are therefore needed to show how the various forms of active transport contribute to the membrane potential.

The purpose of this paper is to examine the effects on membrane potential of a number of secondary active transport processes, both neutral and electrogenic, identified in a variety of cells: Na-Cl, K-Cl and Na-K-2Cl (Geck et al., 1980; Geck & Heinz, 1986; O'Grady, Palfrey & Field 1987), Na-glucose (Crane, 1962; Schultz & Curran, 1970), Na-amino acid (Schultz & Curran, 1970) and Na-HCO_3 (Yoshitomi, Burckhardt & Frömter 1985) cotransport, and Cl-HCO_3 (Cabantchik, Knauf & Rothstein, 1978; Boron, 1986; Knauf, 1986), Na-H (Murer, Hopfer & Kinne, 1976; Grinstein & Rothstein, 1986) and Ca-Na (Schatzmann, 1985) counter-transport. Our analysis shows that, even though a transporter may itself be neutral, its contribution to the steady-state distribution of ions between medium and cell can influence V.

Unless stated otherwise, in examining the effects of a variety of processes on V, we have solved equations developed here for the model situation given in Table 1 in which cell chloride is out of electrochemical equilibrium at usual membrane potentials (-45 to -85 mV), and have used for the n, the ratio K : Na on the sodium pump, the value of 2 : 3 (0.6̇). In all equations, R,T, and F are the usual physical quantities.

Membrane Potentials Consequent on Electrodiffusion of Ions

Typically, the contributions of different permeant univalent ions to V have been expressed in terms of their concentrations and the membrane permeabilities, e.g.,

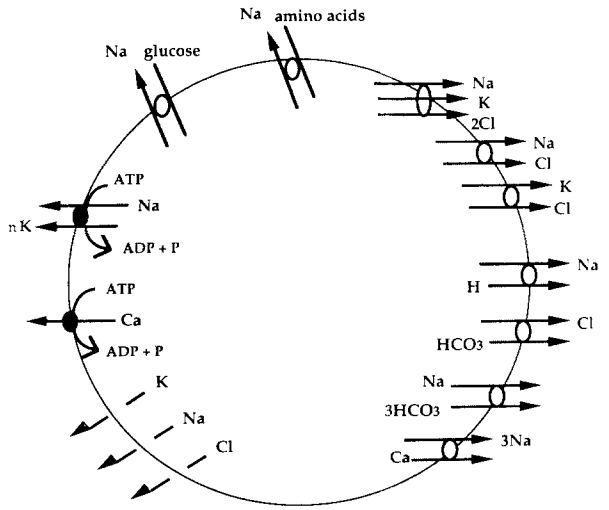

Fig. 1. A diagrammatic representation of the plasma membrane of a cell and the imbedded transport units considered in the text. The arrows show the direction of *positive* currents (always outwards). In this formalism actual individual currents may be negative, (i.e., ions may flow in the opposite direction to that shown by the arrows). Solid ● with solid arrows represent primary active transporters, open ○ with solid arrows represent secondary active transporters, and dotted arrows represent movements through conductance pathways (ion channels)

Table 1. Standard cell and conditions

	Na	K	Cl
Medium ion concentrations (mmol · liter^{-1})	117	3.5	120
Cell ion concentrations (mmol · liter^{-1})	17	140	47
Membrane permeabilities (10^{-7}cm · sec^{-1})	6.4	80	16

$$V_{io} = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o} \quad (1)$$

where the subscripts o and i indicate the solution outside (reference) and inside the cell, respectively. (Having defined the reference solution for Eq. (1), we shall omit subscripts on V in all subsequent equations.) It is common to use ion concentrations in solving these equations, since the more correct parameter, ion activity, is not always readily available, particularly for the intracellular ions. For simplicity, activity coefficients are not included in any of the equations presented here.

In deriving Eq. (1), Hodgkin and Katz (1949) assumed that the concentrations of each ionic species in the membrane surface were in constant proportion with the concentrations of ions in the adjacent surface layer of the aqueous phase and used a flux equation (Goldman, 1943; Hodgkin & Katz, 1949), which has the form

$$I = z^2 PF\phi \frac{c_i \exp\{z\phi\} - c_o}{\exp\{z\phi\} - 1} \quad (2)$$

where z is the ionic charge, $\phi = FV_{io}/RT$ and subscripts i and o designate the fluids bathing the membrane, of which o, as before, is the reference solution to which the current I flows. This is often referred to as the Goldman-Hodgkin-Katz (GHK) flux equation.

Although the original assumption of a constant electric field used in the derivations of Eqs. (1) and (2) is not necessary (Teorell, 1953), it is assumed that ions are free to move independently and, in addition, in Eq. (1), that no processes other than passive ion diffusion contribute directly to the generation of the membrane potential.

Other forms of Eq. (1) are often employed. First, rather than using absolute permeabilities, the ratios of permeabilities can be introduced, thereby reducing the number of variables inside the log function from 9 to 8; i.e., if

$$P_{Na} = \alpha P_K \quad (3a)$$

and

$$P_{Cl} = \beta P_K \quad (3b)$$

then

$$V = \frac{RT}{F} \ln \frac{K_o + \alpha Na_o + \beta Cl_i}{K_i + \alpha Na_i + \beta Cl_o}. \quad (4)$$

Secondly, in the steady state in cells where Cl is distributed at electrochemical equilibrium (e.g., skeletal muscle,), the Cl terms can be discarded and

$$V = \frac{RT}{F} \ln \frac{K_o + \alpha Na_o}{K_i + \alpha Na_i}. \quad (5)$$

Since Eq. (1) is based on a number of restrictive assumptions, alternative strategies have been employed. One such approach is to replace the GHK flux equation with an expression which highlights the proportionality between the current, I, and the driving force $(V + E)$

$$I = G(V + E) \quad (6)$$

where the proportionality "constant" is the chord conductance, G, and E is the Nernst potential for the ionic species. By summing all the passive currents

given by Eq. (6), a relationship between V and the concentrations of the ions corresponding to that of Eq. (1) can be obtained.

$$V = \frac{G_K E_K + G_{Na} E_{Na} + G_{Cl} E_{Cl}}{G_K + G_{Na} + G_{Cl}}. \quad (7)$$

Equation (7) would appear to be model free and to have an advantage in this respect over Eq. (1). However, this advantage is only superficial since the evaluation of the chord conductances cannot be made without the use of models and, in general, chord conductances are found to be dependent on the concentrations of the diffusing ions and also on the voltage. Without information concerning the chord conductances, and in particular their relationship to the slope conductances measured in experiments, it is difficult to apply this representation to analyze data obtained experimentally. Therefore, the presentation that follows is restricted to equations involving permeabilities and ion concentrations.

Contribution of Primary Active Transport to V

The equations have been modified to allow for the contribution to the membrane potential of the current flow through the Na-K pump by introducing the coupling ratio n (Moreton, 1969) which represents the ratio of K/Na ions transferred in each cycle of the pump. With the active Na current represented by I_a^{Na}, the total current flow through the pump is given by $[(1 - n)I_a^{Na}]$. By introducing the total pump current into the derivation of the equation for the membrane potential, Moreton (1969) showed that

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i + (1 - n) I_a^{Na} RT/F^2 V}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o + (1 - n) I_a^{Na} RT/F^2 V}. \quad (8)$$

This equation, which is derived later (Eq. 30) under more general conditions in that neutral ion cotransporters are accommodated, requires the introduction of two extra variables (n and I_a^{Na}) but has the advantage over many other forms of the equation in that it holds for both the steady state and the nonsteady state.

In an earlier treatment for the steady state, Mullins and Noda (1963) expressed V more simply with the introduction of only the one extra variable n. More recently, Jacquez and Schultz (1974) followed a similar approach.

$$V = \frac{RT}{F} \ln \frac{P_K K_o + n P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + n P_{Na} Na_i + P_{Cl} Cl_o}. \quad (9)$$

V appears on both sides of Eq. (8) and thus requires iterative procedures for its determination, whereas Eq. (9) shows V explicitly. [Equations (8) and (9) are derived later in this paper for a more general case—Eqs. (31) and (19), respectively.]

The Combined Contribution of Primary and Secondary Active Transport to V

Coupled Cl Fluxes

Cotransporters Involving Cl with Na and/or K

Jacob et al. (1984) developed equations to examine effects of electroneutral transporters on membrane potential and included consideration of a K-Cl cotransporter in their paper. The approach followed in this section differs from theirs and provides more general equations applicable to Na-Cl, and K-Cl cotransporters alone and in combination. Furthermore, the equations developed here are explicit in V.

As represented in Fig. 1, the total currents (I_T) of Na, K and Cl *out of the cell* are, respectively,

$$I_T^{Na} = I_p^{Na} + I_c^{Na} + I_a^{Na} \quad (10)$$

$$I_T^{K} = I_p^{K} + I_c^{K} + I_a^{K} \quad (11)$$

$$I_T^{Cl} = I_p^{Cl} + I_c^{Cl} \quad (12)$$

where I_p represents current flow through passive (p) conductance pathways in the plasma membranes, I_c represents current flows through secondary active coupled (c) pathways, and I_a represents current flow through primary active (a) pathways.

Besides these equations, there are others that relate to the relationships within the transport systems. Using the ratio n of K current to Na current through the Na-K pump,

$$n I_a^{Na} + I_a^{K} = 0. \quad (13)$$

For a neutral co-transporter involving Na, K and Cl ions

$$I_c^{Na} + I_c^{K} + I_c^{Cl} = 0. \quad (14)$$

Given that the only appreciable currents are due to the flows of Na, K and Cl ions, then by Kirchoff's first law

$$I_T^{Na} + I_T^K + I_T^{Cl} = 0. \tag{15}$$

Equations (10)–(15) are all valid for both steady state and nonsteady-state conditions, provided that the capactitive currents can be neglected.

The *steady-state condition* is given by

$$I_T^{Na} = I_T^K = I_T^{Cl} = 0. \tag{16}$$

This condition is more restrictive than Eq. (15). It fixes the relative passive currents of the ions (I_p) and, as follows, establishes a unique intracellular potential for functionally nonpolarized cells.

We now define a variable q, such that

$$q = \frac{-(nI_c^{Na} + I_c^K)}{I_c^{Cl}}. \tag{17}$$

This variable is chosen so that in the steady state we may obtain from Eqs. (10) to (17) a relationship exclusively between the passive currents, i.e.,

$$nI_p^{Na} + I_p^K + qI_p^{Cl} = 0. \tag{18}$$

Substitution of Eq. (2) into Eq. (18) for each ionic species allows the intracellular potential to be expressed explicitly as

$$V = \frac{RT}{F} \ln \frac{P_K K_o + nP_{Na} Na_o + qP_{Cl} Cl_i}{P_K K_i + nP_{Na} Na_i + qP_{Cl} Cl_o} \tag{19}$$

or, using $\alpha = P_{Na}/P_K$ and $\beta = P_{Cl}/P_K$ (from Eqs. 3a and 3b)

$$V = \frac{RT}{F} \ln \frac{K_o + n\alpha Na_o + q\beta Cl_i}{K_i + n\alpha Na_i + q\beta Cl_o}. \tag{20}$$

Note that, since q is defined in terms of current flows through electrically neutral cotransporters (Eq. 17), it is independent of V and Eq. (19) is of the same type as Eq. (9). In this regard our approach differs from that of Jacob et al. (1984) whose equations included a factor g which was a function of V so that their equations were transcendental in V.

Before applying these equations, the driving force for cotransport (i.e., the gradient of free energy for the species transported) must be considered. For electroneutral transporters this gradient is independent of membrane potential and is simply related to the chemical potentials of the substrates. (Note, however, that the *rate* of cotransport, which is determined by activation energy barriers, may be affected by membrane potential.) All cotransport involving Na, K, and Cl ions can be considered to result from a combination of Na-Cl and K-Cl cotransporters, where the two transporters are either free to move independently or are coupled together. For example, an Na-K-2Cl cotransporter could be regarded as a combination of completely coupled 1 : 1 Na-Cl and K-Cl cotransporters, where the coupling is such that the two transporters drive the cations in the same direction across the membrane. In theory, Na-Cl and K-Cl cotransport may be coupled in any combination (e.g., Na and K sites not exclusive for Na or K) and the transport system can be described by xNa-(2-x)K-2Cl, where x refers to the contribution of NaCl cotransport to the total cotransported flux and $0 \leq x \leq 2$. The overall direction of transport is indicated by the net chloride flux through the cotransporter. Using the convention shown in Fig. 1, this flux will be negative (net influx) when $B > 1$ and positive (net efflux) when B < 1 where

$$B = \frac{Na_o^x K_o^{2-x} Cl_o^2}{Na_i^x K_i^{2-x} Cl_i^2}. \tag{21}$$

Thus the standard free energy driving the coupled cotransport system is $RT \ln B$. (Note that Eq. (21) is not applicable for a combination of uncoupled, independent K-Cl and Na-Cl cotransporters.)

When $x = 0$, 1 or 2, K-Cl, Na-K-2Cl or Na-Cl are transported, respectively. Given that Cl is distributed out of electrochemical equilibrium and the steady-state Cl distribution is governed only by (i) the Cl conductance channel (characterized by P_{Cl}) and (ii) the cotransporter under consideration here, we can find the restrictions on x. If cell Cl is above its equilibrium value, then Cl will leave the cell via the conductance channel and in the steady state there must be an influx of chloride via the transporter, i.e., $B > 1$ and by using Eq. (21)

$$x > 2 \ln \left\{ \frac{Cl_i K_i}{Cl_o K_o} \right\} \Big/ \ln \left\{ \frac{Na_o K_i}{Na_i K_o} \right\}. \tag{22}$$

As shown in Table 1, coupled cation-Cl cotransport requires that $0.99 < x \leq 2$. If cell Cl were less than the equilibrium value, then the inequality (22) would be reversed.

Also, the ratio of cation currents through the cotranporters (coupled or independent) can be represented by y, that is

$$y = \frac{I_c^{Na}}{I_c^K}. \tag{23}$$

Thus, in general, where cell Cl concentration exceeds that required for equilibrium and cell Na is low and K high, and where the cotransporters are not coupled, the value of y will be negative. If it were not for cellular constraints, y could take values from $-\infty$ to $+\infty$, and, since from Eqs. (14), (17) and (23)

$$q = \frac{ny + 1}{y + 1} \tag{24}$$

q could also take values from $-\infty$ to $+\infty$. However, the inequality (Eq. 22) which confines the value of x to a particular range of values, also, by Eq. (24), restricts the ranges of y and q. This restriction is due to the energetics of cellular transport. Obviously, to maintain steady-state conditions, the cotransporters cannot transport against their chemical potentials when uncoupled nor against their summed chemical potentials when coupled.

In addition, it is not possible to have a negative argument in the log function in Eq. (19). Thus q may not take values which produce opposite signs for the numerator and denominator of the argument. Therefore, under our model conditions, q must lie outside the range -1.036 to -5.872 when $n = 2/3$ (the corresponding range of y is given in Table 2). Thus, Table 2 summarizes the allowable cotransport systems, both coupled and uncoupled, for the model cell with $n = 2/3$ and using the permeabilities and ion distributions given in Table 1. Table 2 and Fig. 2 show the importance of the neutral cotransport systems in the establishment of the membrane potential and indicate the 'forbidden' values of y. It is possible that some oscillatory behavior observed in tissues in response to perturbations of transport reflects a shift in the value of y into a 'forbidden' region. This would result in marked instability and force radical changes in cellular parameters before an allowable steady state could be achieved.

We now define values of q for three common cation-coupled Cl cotransporters operating within the permitted constraints. The Na-K-2Cl cotransporter is defined by

$$I_c^{Na} = I_c^{K} = -0.5\, I_c^{Cl} \tag{25}$$

and, on substituting into Eq. (17)

$$q = (1 + n)/2. \tag{26}$$

Similarly, for an independent K-Cl cotransporter

$$q = 1 \tag{27}$$

and, for an independent Na-Cl co-transporter

$$q = n. \tag{28}$$

To illustrate the magnitudes of the effects of possible Cl cotransporters on plasma membrane potentials and also the consequences of a variable stoichiometry of the (Na-K)-ATPase, we have plotted V against n for a Na-Cl and K-Cl cotransporter or mixtures coupled and free thereof (including a Na-K-2Cl) in Fig. 3. It is apparent that at any fixed pump stoichiometry, with $n < 1$, a wide range of V's would be possible depending on the balance between the Na-Cl and K-Cl cotransporters. With the cotransporters coupled so that all ions are moved in the same direction (i.e., into the cells given the driving force used in the example), the effect on V is relatively small (the region between $y = 0.98$ and $y = 2$. However, if the two transporters can move independently of one another through the membrane, much larger effects on membrane potential may occur. Indeed, V can become much more negative than the K equilibrium potential. The reason for this lies in the fact that cell Cl concentration in our model cell is held constant and above its equilibrium distribution. Therefore, there must be a net influx of Cl on the cotransport system at all times to maintain the specified conditions. It must be stressed that potentials more negative than the K equilibrium potential are a consequence of the constraints placed on the model and may not be found in any cell under steady-state conditions simply because cell ion concentrations and permeabilities would change before such values were reached. Nevertheless, the principle remains that under steady-state conditions combinations of electroneutral cotransporters, if coupled together, can depolarize the membrane potential whereas when uncoupled they can result in hyperpolarization which may exceed the K equilibrium potential.

We can also examine the effects on V of particular cell Cl concentrations at different values of P_{Cl}'s (Fig. 4). With any cell Cl concentration, the higher the P_{Cl}, the less negative the cell potential. Indeed, with relatively low P_{Cl} (1×10^{-7} cm · sec^{-1}) there is little effect on V over a wide range of Cl concentrations. In addition, it can be seen that the nature of the cotransporter (Na-Cl or coupled Na-K-2Cl) has only a small effect at any P_{Cl}.

Equations (19) and (20) are only applicable to the steady state. A value of the intracellular potential may also be obtained for the *nonsteady state* but now only as an implicit function. The left-hand side of Eq. (15), which is applicable to the nonsteady state as well as to the steady state, contains within its terms only passive (I_p) and active (I_a) currents, since it is simply the summation of Eqs. (10)–(12) and, with electroneutral cotransporters, the sum of

Table 2.

	V (mV)	I_c^{Na} (μA)	y[1]	x	q	Cotransporter
	−57.1	−1.36	−∞		0.667	Pure NaCl
	−57.5	−1.53	−10		0.629	
	−61.2	−3.00	−2		0.333	
	−66.8	−5.15	−1.5		0.000	
	−78.8	−9.23	−1.3		−0.444	↑
	−146.0	−25.32	−1.2		−1.000	Uncoupled
	−176.0	−31.03	−1.197		−1.025	↓
	−∞		−1.196		−1.036[2]	
Forbidden log						
	+∞		−1.051		−5.872[3]	
	−23.4	+16.25	−1.00..1		−∞	
Forbidden	−23.4	+16.21	−0.99..9		+∞	
on	−51.2	+1.15	−0.5		1.333	
energetics	−53.8	0	0.0	0	1	Pure KCl[4]
	−54.8	−0.43	0.5	0.67	0.888	
	−55.3	−0.64	0.98	0.99[5]	0.835	↑
	−55.4	−0.65	1.0	1	0.833	Coupled
	−55.9	−0.88	2.0	1.33	0.777	↓
	−56.7	−1.23	10	1.82	0.696	
	−57.1	−1.36	∞	2	0.667	Pure NaCl

↓
Cycle to beginning of Table.

[1] $y = \dfrac{I_c^{Na}}{I_c^{K}} = \dfrac{1-q}{q-n}$.

[2] $q = -\dfrac{(P_K K_o + nP_{Na}Na_o)}{P_{Cl}Cl_i}$.

[3] $q = -\dfrac{(P_K K_i + nP_{Na}Na_i)}{P_{Cl}Cl_o}$.

[4] Continued activity of a pure KCl cotransporter is not possible in the steady state within the constraints of our model system because Cl is retained in the cell above its equilibrium distribution whereas a pure KCl cotransporter would produce a net efflux of chloride.

[5] The lowest value of x allowed in coupled cotransport is governed by the inequality (22).

the I_c components is zero. Thus, in the nonsteady state, Eq. (15) can be expanded to

$$I_p^{Na} + I_p^{K} + I_p^{Cl} + I_a^{Na} + I_a^{K} = 0 \tag{29}$$

which, of course, is also applicable to the steady state. After substituting for I_p's from Eq. (2) and on rearranging

$$V = \frac{\dfrac{RT}{F}\ln P_K K_o + P_{Na}Na_o + P_{Cl}Cl_i + (I_a^{Na} + I_a^{K})RT/F^2V}{P_K K_i + P_{Na}Na_i + P_{Cl}Cl_o + (I_a^{Na} + I_a^{K})RT/F^2V} \tag{30}$$

which by use of Eq. (13) yields Eq. (8) first derived by Moreton (1969) but without consideration of the possibility of secondary active transport. As q does not appear in Eq. (30), this formulation can be considered to be independent of the type of neutral cotransport present, and, as it is also applicable to both the steady state and nonsteady-state conditions, it is, in theory, relatively general.

Alternatively, Eq. (30) can be rearranged to fit the format of the GHK equations for current.

$$I_a^{Na} = F\phi\frac{C\text{-}D\exp\{\phi\}}{(\exp\{\phi\} - 1)(1\text{-}n)} \tag{31}$$

where $C = P_K K_o + P_{Na}Na_o + P_{Cl}Cl_i$ and $D = P_K K_i + P_{Na}Na_i + P_{Cl}Cl_o$.

We can now use Eq. (30) to derive different expressions for the *steady-state* potential difference. The following formulation of V (for the steady state) can be obtained from Eqs. (19), (30) and (13).

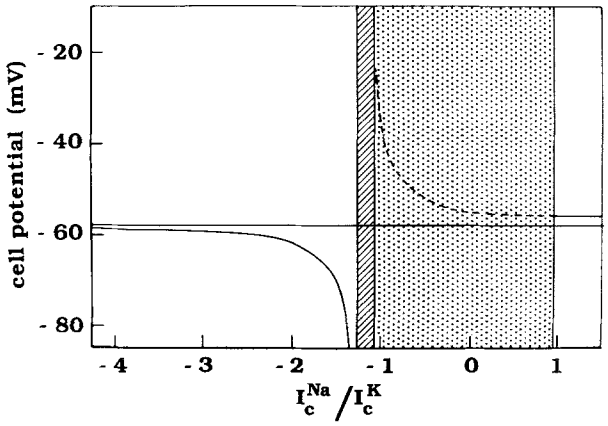

Fig. 2. The relationship between the ratio of the cotransported cation currents I_c^{Na}/I_c^K and the membrane potential (V) for Cl cotransporters. Using the membrane permeabilities P_K, P_{Na}, and P_{Cl} and the corresponding intra- and extracellular ion activities as given, the values of I_c^{Na}/I_c^K are found to be restricted to two ranges. For the standard cell, these ranges are $\infty < I_c^{Na}/I_c^K < -1.2$ for independent Na-Cl and K-Cl cotransporters, and $0.98 < I_c^{Na}/I_c^K < \infty$ for coupled Na-Cl and K-Cl cotransporters. The forbidden range of I_c^{Na}/I_c^K (....) on energetic grounds is dependent on the value of n (*see* Table 2 and Fig. 3). The hatched area shows the forbidden range consequent upon a negative argument in the log function

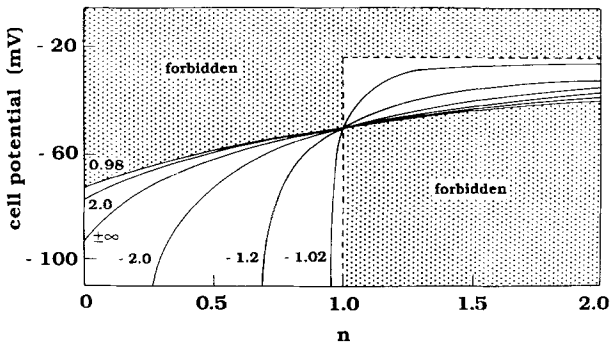

Fig. 3. The relationship between the membrane potential (V) and n (the K to Na pump ratio) for Cl cotransporters at fixed values of $I_c^{Na}/I_c^N = y$ which are given beside the appropriate lines. For $n = 2/3$, Table 2 and Fig. 2 show V as a function of y. At $n = 1$, the value of V is independent of y and invariant at -50.13 mV. As n increases, all lines approach the Nernst potential for Cl. Allowable values for y lie only within the range encompassed by unshaded areas on the Figure

$$V = \frac{RT}{F} \ln \frac{P_{Na}Na_o + \dfrac{yP_{Cl}Cl_i}{(1+y)} + I_a^{Na}RT/F^2V}{P_{Na}Na_i + \dfrac{yP_{Cl}Cl_o}{(1+y)} + I_a^{Na}RT/F^2V}. \quad (32)$$

Curiously, Eq. (32), which concerns only the steady state, does not involve either I_a^K or n explicitly or, indeed, any other direct reference to the K system, except through the parameter y. The reason for this is that both I_a^K and n are fixed through their relationships to the other terms in the equation. A similar curiosity is the nonappearance of any reference throughout this discussion to the absolute magnitude of cotransport. Instead, the number of cotransporter sites and the mobility of ions through the cotransporter are reflected here in the magnitudes of the concentrations of the ions. It should be noted that the currents through the pump and through the cotransporters are not related specifically in the equation to their driving forces, nor is there any reference to their mechanisms except through their association with the GHK requirements. However, $y/(1 + y)$ the coefficient of the terms $P_{Cl}Cl$ in Eq. (32) has a range of values (that can be calculated from Table 2) as the integrated cotransport system in the membrane changes in type from K-Cl to Na-Cl. Here cotransport is in association with Cl, but, indeed, the equation is more general and is independent of the presence of other netural cotransporters, as shown below.

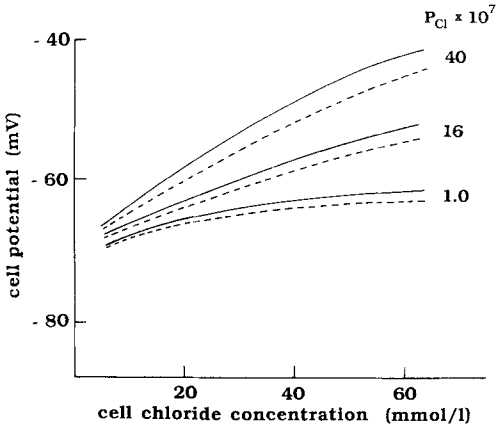

Fig. 4. The relationship between the membrane potential (V) and the intracellular Cl concentration at three different P_{Cl}'s. For each P_{Cl}, the solid line shows V for a Na-Cl cotransporter and the dotted line shows V for a Na-K-2Cl cotransporter. Other parameters relate to the standard cell

If the only important pathways for Na through the membrane were a simple conductance and the Na pump, then $q = 1$ and since from Eq. (24) $y = (1 - q)/(q - n)$, Eq. (32) would become independent of both the K and Cl pathways. i.e.,

$$V = \frac{RT}{F} \ln \frac{P_{Na}Na_o + I_a^{Na}RT/F^2V}{P_{Na}Na_i + I_a^{Na}RT/F^2V}. \quad (33)$$

Here, the active pump current of Na is equiva-

lent to the current through the conductance pathway and, as such, Eq. (33) can be rewritten in the simple format of Eq. (2). As discussed in the following paper (Gordon & Macknight, 1989), this may be the situation in some 'tight' epithelia under certain experimental conditions.

Counter-Transporters Involving Cl

Examples of counter-transport can be found in the expulsion of the products of metabolism, H^+ and HCO_3^-, from cells. In some cells, these transport systems involve a 1 : 1 HCO_3-Cl exchanger and a 1 : 1 Na-H exchanger (e.g., some epithelia—some epithelia—Boron, 1986). Jacob et al. (1984) considered a system in which Na-H, HCO_3-Cl and K-Cl transporters were available and resulted effectively in Na-K exchange. Though their original analysis of Na-H exchange was in error (Scriven & Mundel, 1985), they subsequently modified their approach and analyzed the effects of this exchanger on V in model systems where an organic anion generated with H ions during metabolism left the cell by a diffusive pathway and where there was a significant passive permeability of the plasma membrane to H ions (Jacob et al., 1985). Our approach here differs from theirs in that we assume that CO_2 which is produced in cells from the oxidation of metabolites diffuses out of the cells as a neutral species or is ejected in ionic form as HCO_3^- and H^+ through the exchangers and that, compared to the fluxes of Na, K, and Cl, there is little flux of either H^+ or HCO_3^- through conductance pathways in the membrane. Thus the steady-state currents out of the cell are given, as before [from Eqs. (10)–(12) and (16)].

By definition, in the steady state, the effluxes of H^+ and HCO_3^- must be equal.

$$I_c^{\mathrm{H}} + I_c^{\mathrm{HCO_3}} = 0 \tag{34}$$

where the subscript c here indicates counter-transport.

The conditions imposed by 1 : 1 transporters imply that

$$I_c^{\mathrm{Cl}} + I_c^{\mathrm{HCO_3}} = 0 \tag{35}$$

and

$$I_c^{\mathrm{H}} + I_c^{\mathrm{Na}} = 0. \tag{36}$$

Since $(nI_a^{\mathrm{Na}} + I_a^{\mathrm{K}})$ is zero (Eq. 13), it is simple to show using Eqs. (10–(12) (16), and (34)–(36) that

$$nI_p^{\mathrm{Na}} + I_p^{\mathrm{K}} + nI_p^{\mathrm{Cl}} = 0 \tag{37}$$

and by substituting the appropriate GHK flux equations we obtain the following equation:

$$V = \frac{RT}{F} \ln \frac{P_{\mathrm{K}}\mathrm{K}_o + n(P_{\mathrm{Na}}\mathrm{Na}_o + P_{\mathrm{Cl}}\mathrm{Cl}_i)}{P_{\mathrm{K}}\mathrm{K}_i + n(P_{\mathrm{Na}}\mathrm{Na}_i + P_{\mathrm{Cl}}\mathrm{Cl}_o)} \tag{38}$$

which is identical to the case of the NaCl cotransporter [combining Eqs. (19) and (28)].

Indeed, an important conclusion arises from this analysis. Where the membrane potential is a function of the dominant medium ions, Na, K, and Cl, other contributions can come only from the Na pump (if $n \neq 1$; Fig. 3) and from neutral co- and counter-transporters if, and only if, these involve the dominant ions. In the steady state, minor ions must exist in conjugate pairs since no one type of ion can accumulate *ad infinitum*. In the example above, the conjugate pair is H^+ and HCO_3^-. They influence the distribution of Na and Cl ions across the membrane, and thus their effect on membrane potential may be examined simply in terms of a Na-Cl cotransporter. In general, neutral transporters may be reduced simply to NaCl and KCl cotransporters and Eqs. (19) and (30) remain applicable. In contrast, current-carrying co- and counter-transporters may affect the membrane potential directly [e.g., Eq. (42)] and, although Eqs. (19) and (30) remain true, the permeability terms then require extension [Eq. 43)].

Electrogenic Cotransporters

A description of electrogenic cotransport at the microscopic level has been given by Läuger and Jauch (1986). Their treatment is based on Transition Rate Theory, and, although it is limited to a system in which the binding site on the transport protein may switch from inward-facing to outward-facing only when empty or full (i.e., containing both neutral molecule and ion), it provides a more flexible model for an electrogenic cotransport system than the GHK approach presented here. However, their approach does not address the question we are concerned with here of how the transporter affects cell potential but rather the converse question of how the cell potential affects the cotransport. Other models have been postulated by Kaunitz and Wright (1984) and Restrepo and Kimmich (1985).

As an example of the GHK approach to electrogenic cotransport, we will first examine a 1 : 1 Na^+-glucose cotransporter. We will assume, for simplicity, that the Na-glucose cotransporter can be represented by a transporter in which one Na ion and one glucose molecule are transported only as a pair, and that the partition coefficient for this Na-glucose pair is independent of concentrations and given by

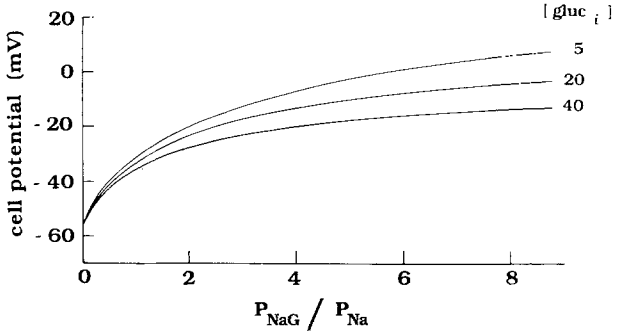

Fig. 5. The effects on membrane potential (V) of increasing P_{NaG} relative to a constant P_{Na} at different cell glucose concentrations

$$\frac{[Na\ gluc]}{[\mathrm{Na}]\,[\mathrm{gluc}]} \tag{39}$$

where [*Na gluc*] indicates the membrane concentration of the solute. (Note that we assume (i) [*Na*] = [*gluc*] in the membrane but [Na] ≠ [gluc] in the bulk solution, i.e., the activity of the substrate pair [*Na gluc*] in the membrane surface is proportional to the product of the activities in the bulk aqueous phase and (ii) that the fluxes are independent; conditions used by Hodgkin and Katz (1949) for simple ion transport.)

In the GHK formalism, the cotransport current is given by

$$I_{NaG} = P_{NaG} F\phi \frac{\mathrm{Na}_o \mathrm{gluc}_o \exp\{\phi\} - \mathrm{Na}_i \mathrm{gluc}_i}{\exp\{\phi\} - 1} \tag{40}$$

where P_{NaG} represents the permeability of the membrane to the cotransported product (Na-glucose), i.e., the flux through the membrane per unit driving force. [The glucose influx would be given by $J_{NaG} = I_{NaG}/F$. Since the permeance of Na moving through the cotransporter is proportional to the glucose concentration in the solution, P_{NaG} has the units $cm^4 \cdot s^{-1} \cdot mol^{-1}$. P_{NaG} can be considered to be invariant only when this cotransport system is far from saturation. Otherwise P_{NaG} no longer increases linearly with the product of the concentrations of Na and glucose. In that situation a more complex treatment is required which will not be developed here. Thus P_{NaG} as a constant should be regarded as a first approximation only.

As the glucose component is neutral, the effect of the cotransporter (Na^+-glucose) on the steady-state membrane potential given above by Eq. (19) is

$$V = \frac{RT}{F} \ln \frac{P_K K_o + n(P_{Na} + P_{NaG}\mathrm{gluc}_o)\mathrm{Na}_o + qP_{Cl}\mathrm{Cl}_i}{P_K K_i + n(P_{Na} + P_{NaG}\mathrm{gluc}_i)\mathrm{Na}_i + qP_{Cl}\mathrm{Cl}_o}. \tag{41}$$

Figure 5 illustrates the effects on V of increasing P_{NaG} relative to P_{Na} at different cell glucose concentrations. The greater the Na movement through the cotransporter, the greater the membrane depolarization even though the increased Na influx must be compensated for by increased Na efflux through the electrogenic Na pump.

Equation (41) can be extended to a more general case to include all secondary active transport in which neutral molecules are co- or counter-transported with Na. Here, we define P^o_{Na} and P^i_{Na} which express the overall permeabilities for Na from the outside to the inside, and the inside to the outside of the cell, respectively.

$$P^o_{Na} = P_{Na} + P'_{Na}\mathrm{gluc}_o + P''_{Na}aa_o + \ldots P'''_{Na}z_i \tag{42}$$

and

$$P^i_{Na} = P_{Na} + P'_{Na}\mathrm{gluc}_i + P''_{Na}aa_i + \ldots P'''_{Na}z_o \tag{43}$$

where *aa* represents the concentrations of neutral amino acids cotransported with Na and *z* represents neutral molecules counter-transported with Na. Note that these 'permeability' terms are no longer symmetrical since they contain within them concentration terms which may be different for cell and extracellular fluids. Introducing these terms into Eq. (19) we obtain

$$V = \frac{RT}{F} \ln \frac{P_K K_o + nP^o_{Na}\mathrm{Na}_o + qP_{Cl}\mathrm{Cl}_i}{P_K K_i + nP^i_{Na}\mathrm{Na}_i + qP_{Cl}\mathrm{Cl}_o}. \tag{44}$$

Combinations of Electrogenic and Electroneutral Transporters

A more complex situation arises when we consider combinations of electrogenic and electroneutral co- and counter-transporters. As an example, consider the possible combination of an electrogenic Na-HCO_3 cotransporter and electroneutral Na-K-Cl cotransporter and Cl-HCO_3 counter-transporter. Here, the steady-state currents for K are given by

$$I^K_p + I^K_c + I^K_a = 0 \tag{45}$$

but the Na currents are now

$$I^{Na}_p + I^{Na}_c + I^{Na}_a + I^{Na}_{sb} + I^{Na}_{sh} = 0 \tag{46}$$

where I_{sb} indicates current carried on the Na-HCO_3 exchanger and I_{sh} indicates the current carried on the Na-H exchanger. The Cl currents become

$$I^{Cl}_p + I^{Cl}_c + I^{Cl}_{bc} = 0 \tag{47}$$

where I_{bc} indicates current carried on the HCO_3-Cl exchanger. In addition, the currents involved in CO_2 efflux as HCO_3 and H ions are represented by

$$I_{sb}^{HCO_3} + I_{sh}^{H} + I_{bc}^{HCO_3} = 0. \tag{48}$$

The currents associated with the various membrane elements are

$$\text{Na-K pump: } nI_a^{Na} + I_a^{K} = 0 \tag{13}$$
$$\text{Na-}r\text{HCO}_3\text{: } rI_{sb}^{Na} + I_{sb}^{HCO_3} = 0 \tag{49}$$

(where r is the ratio of HCO_3 to Na transported by the exchanger in each cycle).

$$\text{Na-H: } I_{sh}^{Na} + I_{sh}^{H} = 0 \tag{50}$$
$$\text{HCO}_3\text{-Cl: } I_{bc}^{HCO_3} + I_{bc}^{Cl} = 0 \tag{51}$$
$$\text{Na-K-Cl: } I_c^{Na} + I_c^{K} + I_c^{Cl} = 0 \tag{14}$$

and

$$I_c^{Na} - y I_c^{K} = 0. \tag{23}$$

Under these conditions, V is given by Eq. (19), but now

$$q = \frac{I_{sh}^{H} n\left(\frac{1}{r} - 1\right) + I_{bc}^{HCO_3}\left(\frac{n}{r}\right) - I_c^{Cl}\left(\frac{ny+1}{y+1}\right)}{I_{bc}^{HCO_3} - I_c^{Cl}}. \tag{52}$$

If $r = 1$, then

$$q = \frac{nI_{bc}^{Cl} + I_c^{Cl}\left(\frac{ny+1}{y+1}\right)}{I_{bc}^{Cl} + I_c^{Cl}}. \tag{53}$$

In the absence of a Na-K-Cl cotransport system, I_c^{Cl} in the above equations is zero and

$$q = \frac{n}{r}\left\{1 - (1-r)\frac{I_{sh}^{H}}{I_{bc}^{HCO_3}}\right\}. \tag{54}$$

Electrogenic Counter-Transporters

Sjodin (1983) has developed equations to describe Na^+-Ca^{2+} counter-transport. To his analysis we shall add the treatment of neutral chloride cotransporters. The procedure can be summarized as follows. In the steady state the passive Ca current, I_p^{Ca}, and the counter-transported Ca current, I_{cs}^{Ca}, will have the same magnitude but different signs. The ratio of currents, Na to Ca, through the Na-Ca cotransporter is assumed to be constant to that a number, f, can be defined by

$$f = \frac{-nI_{cs}^{Na}}{I_{cs}^{Ca}}. \tag{55}$$

The following four equations represent the steady state for all values of n, q and f.

$$n(I_p^{Na} + I_c^{Na} + I_a^{Na} + I_{cs}^{Na}) = 0 \tag{56}$$
$$I_p^{K} + I_c^{K} + I_a^{K} = 0 \tag{57}$$
$$q(I_p^{Cl} + I_c^{Cl}) = 0 \tag{58}$$
$$f(I_p^{Ca} + I_{cs}^{Ca}) = 0. \tag{59}$$

However, n, q and f are defined according to Eqs. (13), (17) and (55), and the summation of Eqs. (56)–(59) leads to

$$nI_p^{Na} + I_p^{k} + qI_p^{Cl} + fI_p^{Ca} = 0. \tag{60}$$

Since Ca is divalent, the GHK flux Eq. (2) has to be reformatted before the Ca term can be combined with those for the univalent ions. Following Sjodin (1983), this operation can be done by simultaneously converting the divalent cation to an apparent univalent cation and P_{Ca} into P'_{Ca} and P''_{Ca} (apparent permeabilities for influx and efflux, respectively), where

$$P'_{Ca} = \frac{2P_{Ca}\exp\{\phi\}}{\exp\{\phi\}+1} = \frac{2P_{Ca}}{\exp\{-\phi\}+1}$$

and

$$P''_{Ca} = \frac{2P_{Ca}}{\exp\{\phi\}+1}. \tag{61}$$

From this analysis, we see that presenting a divalent ion flux in an expression for univalent positive ions requires the apparent permeabilities for forward and reverse currents to be both voltage dependent and different.

Alternatively, we may retain the true value of P_{Ca} and encompass the voltage dependence within the "concentration terms" so that

$$Ca'_i = \frac{2\,Ca_i}{\exp\phi + 1}$$

and

$$Ca'_o = \frac{2\,Ca_o}{\exp\{-\phi\}+1}. \tag{62}$$

Equation (19) then becomes

$$V = \frac{RT}{F}\ln\frac{P_K K_o + nP_{Na}Na_o + qP_{Cl}Cl_i + fP_{Ca}Ca'_o}{P_K K_i + nP_{Na}Na_i + qP_{Cl}Cl_o + fP_{Ca}Ca'_i} \tag{63}$$

where $f = \frac{ns}{2}$ [Eq. (55)] and s is the ratio of Na/Ca ions transferred by the Na-Ca counter-transporter. Only at $V = 0$, do $Ca'_o = Ca_o$ and $Ca'_i = Ca_i$.

Though this approach allows us to account for the contribution of a Na-Ca counter-transporter within the confines of the GHK formalism, this modification to the equation is not always required. Although important for the maintenance of cellular ionized Ca^{2+} activity, in many cells the Na-Ca exchanger produces negligible fluxes across the membrane since P_{Ca} is exceedingly small in comparison with the other permeabilities.

Finally, we may develop an equation which defines the steady-state membrane potential when we have a range of co- and counter-transported solutes. This is achieved by replacing P_{Na} in Eq. (63) with P^o_{Na} and P^i_{Na} as defined in Eqs. (42) and (43).

$$V = \frac{RT}{F}\ln\frac{P_K K_o + nP^o_{Na}Na_o + qP_{Cl}Cl_i + fP_{Ca}Ca'_o}{P_K K_i + nP^i_{Na}Na_i + qP_{Cl}Cl_o + fP_{Ca}Ca'_i}. \tag{64}$$

Note that Eq. (64) remains valid when q is given by Eqs. (52) to (54), and is thus the most general form of the modified GHK equations developed in this paper.

Experimental Assessment of the Determinants of Resting Membrane Potentials

Major problems arise when attempts are made to obtain experimental data from living cells under steady-state conditions to examine the equations developed in this paper. Though there remain difficulties in determining cell ion activities, the use of double-barrel, ion-sensitive microelectrodes, and the recent developments of fluorescent dyes sensitive to a range of ions, make this task much easier than it has been. Of much greater concern is the measurement of the membrane permeabilities.

Membrane Permeabilities

Most of the equations require one to determine the permeabilities of the conductance pathways through which the ions move by simple electrodiffusion. The fact that the plasma membranes of many cells contain a variety of pathways through which coupled movements occur means that a common method used in the past to determine membrane ion permeability—measurement of unidirectional ion flux using isotopes—is invalid for the purpose of solving most of the equations developed here. This intuitively obvious fact can be illustrated by considering the situation where Cl permeability is measured in a cell possessing neutral KCl and NaCl cotransporters, as well as an electrodiffusive pathway. Here, net Cl currents through the Cl conductance are given by

$$I^{Cl}_p = P_{Cl}F\phi\frac{Cl_i\exp\{-\phi\} - Cl_o}{\exp\{-\phi\} - 1} \tag{65}$$

through the KCl cotransporter by

$$I^{Cl}_{cK} = -k_K F(K_i Cl_i - K_o Cl_o) \tag{66}$$

through the NaCl co-transporter by

$$I^{Cl}_{cNa} = -k_{Na}F(Na_i Cl_i - Na_o Cl_o) \tag{67}$$

where k_K and k_{Na} are the associated rate constants for membrane transit of the ions.

The magnitude of the influx of Cl is, therefore, given by

$$J^i_{Cl} = \left(\frac{P_{Cl}\phi}{1 - \exp\{-\phi\}} + k_K K_o + k_{Na}Na_o\right) Cl_o \tag{68}$$

or

$$J^i_{Cl} = \frac{P^i_{Cl}\phi}{1 - \exp\{-\phi\}} Cl_o \tag{69}$$

where

$$P^i_{Cl} = P_{Cl} + (k_K K_o + k_{Na}Na_o)\frac{1 - \exp\{-\phi\}}{\phi}. \tag{70}$$

Similarly, from the magnitude of the Cl efflux we obtain,

$$P^o_{Cl} = P_{Cl} + (k_K K_i + k_{Na}Na_i)\frac{\exp\{\phi\} - 1}{\phi} \tag{71}$$

i.e., $P^o_{Cl} \neq P^i_{Cl}$ and both P^o_{Cl} and P^i_{Cl} are functions of concentrations and membrane voltages and therefore only pseudo-permeability constants.

The number of terms in the expressions for P^o_{Cl} and P^i_{Cl} depends on the number of pathways for

Cl, both neutral and electrogenic, through the plasma membrane. Thus the permeability for Cl through the conductance pathway, P_{Cl}, can be found only after the additional fluxes have been determined separately. This poses considerable methodological problems, particularly as the use of specific blockers to inhibit pathways is likely to result in difficult steady-state concentrations of the intracellular ions and modifications to the permeabilities of the remaining pathways.

Similar arguments can be developed for the measurements of membrane K and Na permeabilities and for these the fluxes through the Na-K pump provide an additional pathway to be corrected for.

Changes in Membrane Potential.

In a variety of experimental situations, alterations in medium composition will affect the resting membrane potential. Although many of the equations developed here apply only to the steady state, the following approaches may enable one to use the equations to predict the instantaneous change in membrane potential following a step change in either the external concentrations of Na or K, the pump rate, or P_{Na}, P_K or P_{Cl}, *provided that* the only parameters altered are the membrane potential and the parameter changed by the experimenter.

For example, Eq. (8) may be rearranged so that

$$P_K K_o + P_{Na} Na_o = h \tag{72}$$

where h contains the remaining parameters. Since h is a function of V, step changes in Na_o or K_o will immediately affect V. However, by Eq. (72), decreasing Na_o and increasing K_o (or vice-versa) at constant Cl_o would reduce the changes in h compared to those which would follow step changes in concentration for only one of the cations. By trial and error, concentrations of Na_o and K_o could be chosen so that a simultaneous step-wise change in both cation concentrations would give no change in voltage. Thereby h would remain constant. This condition is given by

$$P_K \Delta K_o + P_{Na} \Delta Na_o = 0 \tag{73}$$

or

$$\frac{P_K}{P_{Na}} = -\frac{\Delta Na_o}{\Delta K_o}.$$

This approach thus provides a way of obtaining the ratio of the cation permeabilities. It has a number of disadvantages, however. It cannot be applied to determine anion:cation permeability ratios, and in the presence of electrogenic cotransporters P^o_{Na} [Eq. (42)] would need to be substituted in Eqs. (72) and (73). Also, experiments would need to be performed in the domain where all the values of K_o used would saturate the Na pump so that I_a^{Na} (of which h is a function) remains constant, i.e., changes in K_o could affect I_a^{Na} and invalidate Eq. (73). Since the condition is that V must remain constant during step changes in Na_o and K_o, the problem of P_{Na} or P_K changing with V does not arise using this approach.

Blocking the Na-K pump rapidly changes the membrane potential given by Eq. (30)

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o} \tag{1}$$

the equation with which we began! Note that as ions redistribute, there will be continuing change in the potential which will, however, still be given by this equation.

Instantaneous inhibition of neutral co- or counter-transporters will have no immediate effect on membrane potential since their characteristics are not included, as such, in the nonsteady-state formation [Eq. (30)]. Instead, their contributions are reflected only in the concentration terms which will not change *instantaneously* following inhibition of the transporter.

However, any step changes in membrane permeabilities (as may occur with electrogenic transporters or simple conductances) will lead to immediate changes in membrane potential. Complete inhibition of the Na conductance reduces the potential from that given by Eq. (30) to

$$V = \frac{RT}{F} \ln \frac{P_K K_o + P_{Cl} Cl_i + ((1-n) I_a^{Na}) RT/F^2 V}{P_K K_i + P_{Cl} Cl_o + ((1-n) I_a^{Na}) RT/F^2 V} \tag{74}$$

where I_a^{Na}, as well as the other parameters, apart from V, remain constant.

Having discussed some of the experimental difficulties in obtaining membrane permeabilities, we here outline a possible experimental approach. The following parameters must be determined: V, the intra- and extra-cellular concentrations of ions (or, better, the activities), and three independent flux measurements (J^i_{Na} (or J^o_{Na}), J^{iK} (or J^o_K), J^i_{Cl} (or J^o_{Cl})). The measurements must be made when the cell is in a steady state so that the influxes and effluxes of each of the ions can be equated.

As an example, suppose that the cell contains Na,K and Cl conductance pathways, both NaCl and

KCl cotransporters, an Na-K pump, that the fluxes through these pathways are given by equations similar respectively to Eqs. (65) to (67), and that the Na flux through the pump is given by

$$J_a^{Na} = k_p Na_i. \tag{75}$$

Though this equation may seem too simple to provide a representation of the Na flux through the pump, it may be made as complex as required by making k_p a function of the variables of the system. As the experiments are performed under steady-state conditions, k_p will be constant.

For simplicity in formulating the equations, we shall use the following abbreviations:

$$\phi^* = \frac{\phi}{1 - \exp\{-\phi\}} \tag{76}$$

$$\phi' = \frac{\phi}{\exp\{\phi\} - 1}. \tag{77}$$

Effluxes and influxes are represented by the superscripts o and i, respectively.

$$J_{Na}^o = (P_{Na}\phi^* + k_{Na}Cl_i + k_p)Na_i \tag{78}$$

$$= (P_{Na}\phi' + k_{Na}Cl_o)Na_o = J_{Na}^i \tag{79}$$

$$J_K^o = (P_K\phi^* + k_K Cl_i)K_i \tag{80}$$

$$= \left(P_K\phi' + k_K Cl_o + nk_p \frac{Na_i}{K_o}\right)K_o = J_K^i \tag{81}$$

$$J_{Cl}^o = (P_{Cl}\phi' + k_K K_i + k_{Na}Na_i)Cl_i \tag{82}$$

$$= (P_{Cl}\phi^* + k_K K_o + k_{Na}Na_o)Cl_o = J_{Cl}^i. \tag{83}$$

In these six independent linear equations, there are six unknowns P_{Na}, P_K, P_{Cl}, k_{Na}, k_K and k_p. A computer spread-sheet is helpful for diagnostic procedures when solving these equations. If in the analysis either k_{Na} or k_K is found to be negative, this may simply indicate that the NaCl and KCl cotransporters are coupled, perhaps as an Na-K-2Cl cotransporter.

Having developed these equations and illustrated some of their applications and implications for symmetrical cells, we apply them in the following paper to consider membrane potentials in epithelia, illustrating our approach by considering, in particular, a typical simple 'tight' epithelium.

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References

Boron, W.F. 1986. Intracellular pH regulation. *In:* Physiology of Membrane Disorders. (2nd ed.) T.E. Andreoli et al., editors. pp. 423–435. Plenum Medical Book, New York

Cabantchik, Z.I., Knauf, P.A., Rothstein, A. 1978. The anion transport system of the red blood cell: The role of the membrane protein evaluated by the use of 'probes'. *Biochim. Biophys. Acta* **515:**239–302

Crane, R.K. 1962. Hypothesis for mechanism of intestinal active transport of sugars. *Fed. Proc.* **21:**891–895

Geck, P., Heinz, E. 1986. The Na-K-2Cl cotransport system. *J. Membrane Biol.* **91:**97–105

Geck, P., Pietrzyk, C., Burckhardt, B.-C., Peiffer, B., Heinz, E. 1980. Electrically silent cotransport of Na^+, K^+, and Cl^- in Ehrlich cells. *Biochim. Biohys. Acta* **600:**432–447

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27:**37–60

Gordon, L.G.M., Macknight, A.D.C. 1991. Application of membrane potential equations to tight epithelia. *J. Membrane Biol.* **120:**155–163

Grinstein, S., Rothstein, A. 1986. Mechanisms of regulation of the Na^+/H^+ exchanger. *J. Membrane Biol.* **90:**1–12

Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108:**37–77

Jacob, R., Piwnica-Worms, D., Horres, C.R., Lieberman, M. 1984. Theoretical effects of transmembrane electroneutral exchange on membrane potential. *J. Gen. Physiol.* **83:**47–56

Jacob, R., Piwnica-Worms, D., Horres, C.R., Lieberman, M. 1985. Electroneutral Na-H exchange may depolarize the resting membrane potential. *J. Gen. Physiol.* **85:**891–895

Jacquez, J.A., Schultz, S.G. 1974. A general relation between membrane potentials, ion activities, and pump fluxes for symmetric cells in a steady state. *Math. Biosci.* **20:**19–25

Kaunitz, J.D., Wright, E.M. 1984. Kinetics of sodium D-glucose cotransport in bovine intestinal brush border vesicles. *J. Membrane Biol.* **79:**41–51

Knauf, P.A. 1986. Anion transport in erythrocytes. *In:* Physiology of Membrane Disorders. (2nd ed.) T.E. Andreoli et al., editors. pp. 191–220. Plenum Medical Book, New York

Läuger, P., Jauch, P. 1986. Microscopic description of voltage effects on ion-driven cotransport systems. *J. Membrane Biol.* **91:**275–284

Moreton, R.B. 1969. An investigation of electrogenic sodium pump in snail neurones using the constant field theory. *J. Exp. Biol.* **51:**181–201

Mullins, L.J., Noda, K. 1963. The influence of sodium-free solutions on the membrane potential of frog sartorius muscle. *J. Gen. Physiol.* **47:**117–132

Murer, H., Hopfer, U., Kinne, R. 1976. Sodium/proton antiport in brush border membrane vesicles isolated from the rat small intestine and kidney. *Biochem. J.* **154:**597–604

O'Grady, S.M., Palfrey, H.C., Field, M. 1987. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. *Am. J. Physiol.* **253:**C177–C192

Restrepo, D., Kimmich, G.A. 1985. Kinetic analysis of the mechanism of intestinal Na^+-dependent sugar transport. *Am. J. Physiol.* **248:**C498–C509

Schatzmann, H.J. 1985. Calcium extrusion across the plasma membrane by the calcium pump and the Ca^{2+}-Na^+ exchange system. *In:* Marme, Calcium and Cell Physiology. pp. 18–52. Springer-Verlag, Berlin

Schultz, S.G., Curran, P.F. 1970. Coupled transport of sodium and inorganic solutes. *Physiol. Rev.* **50:**637–718

Scriven, D.R.L., Mundel, T. 1985. Electroneutral Na-H exchange does not depolarize the resting membrane potential. *J. Gen. Physiol.* **85:**933–935

Sjodin, R.A. 1983. Contributions of Na/Ca transport to the resting membrane potential. *J. Gen. Physiol.* **76:**99–108

Teorell, T. 1953. Transport processes and electrical phenomena in ionic membranes. *Prog. Biophys. Chem.* **3:**305–369

Yoshitomi, K., Burckhardt, B.C., Frömter, E. 1985. Rheogenic sodium-bicarbonate cotransport in the peritubular cell membrane of rat renal proximal tubule. *Pfluegers Arch.* **405:**360–366

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