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Application of Membrane Potential Equations to Tight Epithelia

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

Summary. It is shown that equations developed to analyze the contributions of secondary active transport processes to symmetrical cells (Gordon, L.G.M., Macknight, A.D.C., 1991, *J. Membrane Biol.* **120:**139–152) can be used, with minor modifications, to analyze the steady-state membrane potential in epithelia under the unique situation of short circuiting. Only under such conditions is there a single intracellular potential relative to both the mucosal and serosal media. The equations are investigated in relation to a model tight epithelium—the toad urinary bladder. It is shown that the properties of the membrane transport pathways are such that the intracellular potential under short-circuit conditions must be more negative than often reported. Given measurements of membrane potential and of voltage-divider ratio, it is possible to use the equations to estimate the absolute values of the membrane permeabilities and conductances under short-circuit conditions.

Key Words membrane potentials · epithelia · secondary active transport

Introduction

As discussed and analyzed in the preceding paper (Gordon & Macknight, 1991), the contributions of different permeant ionic species to the plasma membrane potential difference (V) can be expressed in terms of ion concentrations and membrane permeabilities, with allowance for effects of primary and secondary active transport pathways, e.g.,

$$V = \frac{RT}{F} \ln \frac{P_K K_o + nP_{Na} Na_o + qP_{Cl} Cl_i}{P_K K_i + nP_{Na} Na_i + qP_{Cl} Cl_o} \qquad (1)$$

where n represents the ratio of K/Na ions transferred in each cycle of the pump and q is a function of n and the cation and anion currents carried by a Cl cotransporter. For example, it was shown that for a cell with a single type of Cl cotransporter $q = (1 + n)/2$ for a Na-K-2Cl cotransporter, $q = 1$ for a K-Cl cotransporter and $q = n$ for a Na-Cl cotransporter.

Application of Eq. (1) to functionally nonpolarized cells can provide considerable insights into the relative contributions of different ions to membrane potentials. In the preceding paper, we used this equation, and modifications thereof, to express the effects on membrane potential of several co- and counter-transporters.

Whereas Eq. (1) and related equations can be applied to functionally nonpolarized cells, they cannot be applied to those epithelia that under physiological conditions generate a transepithelial potential difference. Any such potential difference is distributed unevenly between the two plasma membranes—the apical and basolateral—and thus the two membrane potentials differ. However, as Ussing and Zerahn (1951) demonstrated, it is possible to abolish the transepithelial potential difference by voltage clamping. Under such short-circuited conditions, the potential differences across the two membranes must be of equal magnitude. In this unique situation only, the epithelial cell can be treated exactly as any other nonpolarized cell and the relative contributions of the different ions to the membrane potential can then be assessed using equations of the type developed in Gordon and Macknight (1991). The aim of this paper is to examine aspects of membrane potentials in short-circuited, Na-transporting tight epithelia, using as a model of these tissues the toad urinary bladder about which there is much experimental information (Macknight et al., 1980; Macknight, 1990).

Since with epithelial cells there are two extracellular compartments—mucosal and serosal—which in vivo and under open circuit conditions in vitro have different electrical potentials, it is not possible to use a single extracellular compartment as a reference. A general approach to the study of electrical events in epithelia requires a reference system different from that used for nonpolarized cells. A self-consistent reference system can be developed based on the *direction* of the short-circuit current (I_{sc}) which is one of the characteristic electrical features

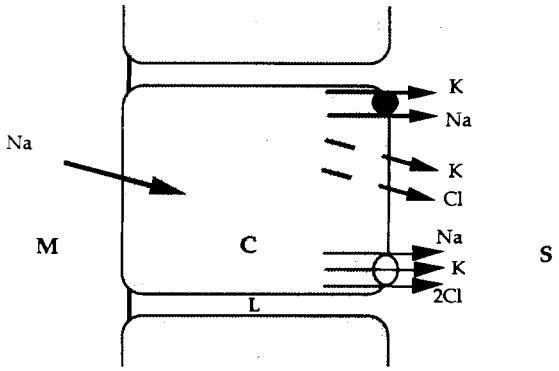

Fig. 1. A model of a tight epithelium. The arrows indicate the positive direction of current through the cell (*C*) and lateral intercellular spaces (*L*) from the mucosa (*M*) to the serosa (*S*). Actual currents may be positive or negative depending on the conditions

of epithelial cells that distinguishes them from nonpolarized cells. The flow of positive ions from the mucosal to the serosal compartment results in the cellular compartment serving as the reference for the mucosal extracellular fluid and the serosal extracellular fluid serving as the reference for the cellular compartment, as illustrated in Fig. 1. In this reference system, positive ions flowing in the opposite direction-from the serosa to the mucosa through the paracellular pathway—or, alternatively, across one of the plasma membranes, produce a negative current, as required for the open-circuit condition where *net* current flow is zero (*see* Fig. 1). [Note that if, instead of this proposed reference system, the cellular compartment were chosen as the reference for both extracellular compartments then, when the transepithelial voltage was changed, there would be a decrease in current across one membrane and an increase in current across the other. This must lead to confusion, although such a reference system more closely resembles that used for functionally nonpolarized cells.]

Using the reference system described above, the equations developed in Gordon and Macknight (1991) can be adapted and applied to explore several related questions in a model tight epithelium—the toad urinary bladder—under short-circuit conditions. The only necessary modification is to redefine the positive direction of current for the apical membrane. By definition, positive current flowed out of the nonpolarized cell. For the epithelial cell this remains the direction for current flow across the basolateral membrane but for the apical membrane the positive direction of current is current flow into the cell since, by definition, positive current flows in the direction mucosa to serosa. Thus, although Eq. (10) in Gordon and Macknight (1991) is similar for isolated cells and epithelial cells, I_p^{Na}, which is a component of the total *outward* current across the two membranes, I_T^{Na}, must be written with a negative sign, since it represents an *inward* apical current for epithelial cells. Therefore, for epithelia, Eq. (10) in Gordon and Macknight (1990) becomes

$$I_T^{Na} = -I_p^{Na} + I_c^{Na} + I_a^{Na}. \tag{2}$$

However, the equations in the preceding paper (Gordon & Macknight, 1991) in which V is the subject [e.g., Eqs. (19), (30), (32), (33), (38), (41) and (64)] now represent the voltage V^b across the basolateral membrane of short-circuited epithelial cells and have identical form to those for functionally nonpolarized cells. In contrast, under short-circuit conditions V^a, the voltage across the apical membrane, is of the same magnitude but of opposite sign so that $V^a + V^b = 0$.

Equations additional to those in the preceding paper (Gordon & Macknight, 1991) can also be written. In microelectrode studies, the ratio of the slope resistances or conductances of the apical to basolateral membranes can be obtained from the changes in current (voltage) in response to small imposed voltage (current) pulses. The values of the slope conductances may differ appreciably from the chord conductances which are properly related to the Nernst potentials of the ions (*see* Thompson, 1986). Slope conductances, however, must be used in the equations here since, experimentally, small voltage pulses are used.

It is common practice in microelectrode studies to measure the changes in V^a and V^b in response to current pulses and so define a voltage-divider ratio $\left(\frac{\Delta V^a}{\Delta V^b}\right)$ which is then equated with the ratio of the corresponding membrane slope resistances, i.e.,

$$\frac{\Delta V^a}{\Delta V^b} = \frac{r^a}{r^b} \tag{3}$$

where the superscripts a and b identify the apical and basolateral membranes and r represents the slope resistance of the appropriate membrane. It is more useful here to express these relationships in terms of slope conductances, g. In an epithelium in which the apical membrane conductance is dominated by Na and where the basolateral membrane contains conductances for K and Cl, as in toad urinary bladder (Macknight, DiBona & Leaf, 1980), the conductance ratio is defined as

$$\frac{r^b}{r^a} = \frac{g^a_{Na}}{g^b_K + g^b_{Cl}}. \tag{4}$$

In general (Thompson, 1986), slope conduc-

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

tances for each ionic species (derivatives of the Goldman-Hodgkin-Katz (GHK) flux equation) can be expressed in terms of permeabilities.

$$g = \frac{z^2F^2P}{RT(\exp\{\phi_{12}\} - 1)^2}(C_1\exp\{\phi_{12}\}(\exp\{\phi_{12}\} - 1 - \phi_{12}) + C_2(\phi_{12}\exp\{\phi_{12}\} + 1 - \exp\{\phi_{12}\})) \quad (5)$$

where z is the ionic charge, F is the Faraday, $\phi = zFV/RT$ and subscripts 1 and 2 designate the fluids bathing the membrane, of which 2 is the reference solution.

Since in toad urinary bladder epithelial cells, as well as in cells of other tight epithelia, cell Cl is greater than would be predicted for passive distribution of this ion (Macknight, 1980), we will use Eq. (1), which, through q, provides the opportunity to introduce secondary active transport of Cl into the analyses which follow.

Unlike the situation in a range of leaky epithelia, a Na-glucose cotransporter is not a prominent pathway in tight absorptive epithelia and its effects, therefore, will not be considered in this paper. Also, although it is likely that the regulation of pH in the Na-transporting cells involves dual Na-H and Cl-HCO_3 transporters, since their contributions can be effectively incorporated within a Na-Cl cotransporter (Gordon & Macknight, 1991), this possibility is included implicitly within the analyses which follow.

We will examine six questions concerning the properties of a short-circuited epithelium and since $V^b = -V^a$, $V\,(= V^b)$ will be used to represent the cell potential under these conditions.

1. Given the available estimates of cellular ion concentrations, what relative ion conductances or permeabilities will be associated with what range of V?
2. What effects would different Cl cotransporters have on the steady-state V?
3. How would a variable pump stoichiometry affect the transport properties of the epithelium?
4. As the rate of transepithelial Na transport is altered, how may V change?
5. How do changes in P_{Cl} and P_K affect I_{sc} and V?
6. What are the absolute values of the passive permeabilities of the membrane for Na, K and Cl?

Table 1 gives the standard values of the parameters used to solve the equations in the analysis of the behavior of a model tight epithelium. Note that, as illustrated in Fig. 2, the calculations are relatively insensitive to the individual cell ion concentrations used. Even large changes in cell ion concentrations could have relatively little effect on calculated V. Thus changes in ion concentrations associated with changes in the conditions of incubation or even substantial errors in the estimates of the concentrations, will not materially affect the conclusions drawn.

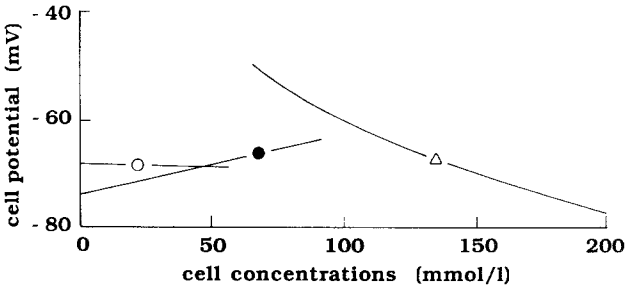

Fig. 2. The cell potential (V) of the standard cell (Table 1) as a function of the intracellular concentrations changed for each ion progressively [K_i(△), Na_i(○), Cl_i(●)] with the ratios of the ion permeabilities and the concentrations of the other ions held constant. This figure illustrates the relative insensitivity of the calculations to possible errors in the magnitudes of the cell ion concentrations

The reason for this apparent theoretical indifference of V to cellular ionic concentrations is due to the manner in which the ions contribute to V. These contributions are through the terms P_KK_i, $P_{Na}Na_i$ and $P_{Cl}Cl_i$ and of these P_KK_i is the major term. For this reason even large changes in Na_i have little effect, percentage-wise, on the denominator of Eq. (1) and hence on V. The contribution of the K component is increased by the fact that, in our chosen conditions with the pump coupling ratio at 2K : 3Na and a Na-K-2Cl cotransporter, the values for both n and q are less than unity. Thus changes in Na_i and Cl_i have insignificant effects on V. Also, since V is a logarithmic function of K_i, changes in K_i need to be very large to modify V significantly.

Membrane Potentials Associated with a Range of Relative Ion Permeabilities

First (Fig. 3), we examine relationships between the conductance ratios and cell potential over a range of values of the Na : K passive permeability ratio (dotted lines) at several different Cl : K passive permeability ratios (solid lines). Here we include the Na-K-2Cl cotransporter, so that $(1 + n)/2$ (Gordon & Macknight, 1991), is substituted for q in Eq. (1).

The following points emerge from consideration

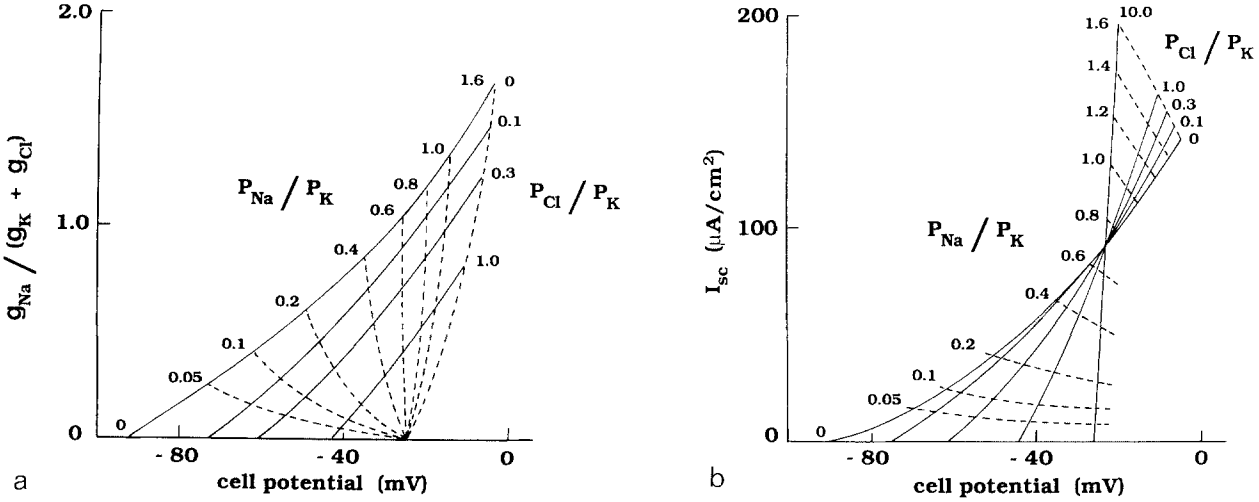

Fig. 3. (*a*) The relationship between the ratio of the apical to basolateral conductance and cell potential (V) with variations in P_{Na}/P_K (dashed lines) and P_{Cl}/P_K (solid lines). (*b*) The relationship between I_{sc} and cell potential (V) with variations in P_{Na}/P_K (dashed lines) and P_{Cl}/P_K (solid lines)

of Fig. 3. First, as is well known, membrane potentials more negative than -80 mV require very low relative Na and Cl permeabilities, i.e., where V is close to the K equilibrium potential (E_K). As all data points in Fig. 3 represent steady-state values, a low Cl passive permeability demands that the activity of the Cl transporter also be low. This combination of a negligible Cl passive permeability and inactivity of a Na-K-2Cl cotransporter, with a greater cell Cl concentration than predicted for passive distribution of the ion, seems to be the situation in the frog skin, a tight epithelium where the basolateral membrane potential approximates E_K (Ussing, 1986).

Second, with the pump stoichiometry of 2K : 3Na used in generating the data in Fig. 3, membrane potentials under short-circuit conditions can only be less negative than -20 to -30 mV, as reported for toad urinary bladder in several microelectrode studies (e.g., Frazier, 1962; Civan & Frazier, 1968; Reuss & Finn, 1974), when $P_{Na} > 0.6 P_K$. Such a high ratio would result in an $I_{sc} \sim 80\ \mu A \cdot cm^{-2}$ or more (Fig. 3*b*). Such high currents are never observed experimentally in this tissue, an I_{sc} of 10 to 20 $\mu A \cdot cm^{-2}$ being a representative value for the toad bladder. This indicates that P_{Na}/P_K ratios of 0.2 or less are typical for this tissue. Thus it appears that in most reported microelectrode studies of toad bladder epithelial cells, membrane potential has been appreciably underestimated. Note also that changes in Cl permeability relative to K permeability, with P_{Na}/P_K constant and below 0.6, are without appreciable effect on I_{sc} (Fig. 3*b*).

Third, in contrast to earlier studies of toad bladder, membrane potentials greater than -50 mV, as estimated from the distribution of isotopically labeled lipophilic cations (Leader & Macknight, 1982) and recorded in recent microelectrode studies (Donaldson, Leader & Macknight, 1987; Nagel & van Driessche, 1989), require tht P_{Cl}/P_K does not exceed 0.3 (Fig. 3*b*). Given a voltage-divider ratio of 5 (equivalent to a conductance ratio of 0.2) and a membrane potential of -55 mV (Donaldson et al., 1987), it can be seen (Fig. 3*a*) that P_{Na}/P_K approximates 0.08 and P_{Cl}/P_K about 0.2. Such estimates are consistent with the known properties of the two membranes in this epithelium. They would be associated with an I_{sc} of $\sim 15\ \mu A \cdot cm^{-2}$, a realistic value for this parameter.

Effects of Different Cl Cotransporters on the Steady-State V

The effects of the type of Cl transport process on V is shown in Table 2 for ranges of g_{Cl}/g_K and g_{Na}/g_K. In constructing this table, it was assumed that the cotransporter was either a Na-K-2Cl, or a Na-Cl transporter working alone and that the cell ion concentrations remain unchanged. Note that pure K-Cl cotransport is not allowed in this model of the toad bladder (*see* Table 1, Gordon & Macknight, 1991). Given these assumptions, the nature of the Cl cotransporter has virtually no effect on the membrane potential under short-circuit conditions. However, if the Na-Cl and K-Cl transporters were *independent of each other* and $-2 < I_c^{Na}/I_c^K < -1.2$, then these cotransport systems would play an important role in determining V (Gordon & Macknight, 1991). For

Table 2. Effects of Na-K-2Cl and Na-Cl cotransporters on V

	g_{Na}/g_K	V	
		Na-K-2Cl	Na-Cl
$g_{Cl}/g_K = 0.1$	2	−0.3	−0.6
	1.5	−11	−11
	1	−28	−28
	0.5	−54	−55
	0.05	−82	−84
	0	−92	−92
$g_{Cl}/g_K = 1.5$	2	−11	−9
	1.5	−18	−17
	1	−26	−26
	0.5	−35	−37
	0.05	−45	−48
	0	−92	−92

example, under short-circuit conditions it is impossible to have a *steady-state* V more negative than E_K with coupled Cl cotransporters but, as shown in the preceding paper (Gordon & Macknight, 1991), such steady-state membrane potentials can be achieved in theory with a combination of uncoupled K-Cl and Na-Cl cotransporters provided that the contribution of outward K-Cl transport is only a little smaller than that of inwardly directed Na-Cl cotransport.

Were Cl distributed at electrochemical equilibrium rather than being subject to cotransport, then, with a cellular Cl concentration of 47 mM, the steady-state basolateral membrane potential would be −24.2 mV and independent of membrane Cl conductance. From Fig. 3*a*, this would require P_{Na}/P_K to approximate 0.6 and would result in a short-circuit current of ~ 80 $\mu A \cdot cm^{-2}$ (Fig. 3*b*), a value rarely, if ever, recorded.

Effects of Variable Pump Stoichiometry

The majority of workers favor the view that pump stoichiometry in epithelial cells is the same as reported for other cell types (2K : 3Na, Post & Jolly, 1957) and remains constant whatever the rate of transepithelial Na transport. However, there have been suggestions, based largely on results from studies of isotope exchanges, that the pump stoichiometry may vary rather than remaining fixed (e.g., Robinson & Macknight, 1976; Cox, 1988). The effects of different Na-K pump stoichiometries on the electrical properties of a short-circulated epithelial cell are shown in Fig. 4. Clearly, if the stoichiometry of the pump remains constant, an increase in transepithelial transport (shown in Fig. 4*b* as I_{sc}) can only occur if P_{Na}/P_K increases progressively. This must be associated with an increase in the conductance ratio (Fig. 4*a*) and an appreciable depolarization of the cell.

However, if the pump ratio were variable rather than constant, it would be possible to alter I_{sc} without necessarily changing P_{Na}/P_K. With any given combination of cell ion concentrations and permeability ratio, P_{Cl}/P_K, increasing the ratio above 2 : 3 (0.6) would depolarize V and, with P_{Na}/P_K constant, this depolarization would be associated with a decrease in the conductance ratio (Fig. 4*c*). Membrane depolarization would decrease I_{sc} (Fig. 4*b*) by reducing the driving force for Na entry to the cell across the apical membrane. In contrast, a decrease in the pump ratio would hyperpolarize the membrane and increase transepithelial Na transport.

Effects of Changes in the Rate of Transepithelial Sodium Transport on Membrane Potential

An important determinant of the rate of transepithelial Na transport by tight epithelia is the Na conductance of the apical plasma membrane (Macknight et al., 1980). Were this the only pathway which altered when transepithelial transport was changed, then, with a K : Na pump ratio of 2 : 3 and P_{Cl}/P_K of 0.3, V would vary as shown in Fig. 3*b*. For example, to obtain an increase in I_{sc} in toad bladder of 250% after vasopressin (say from 10 to 25 $\mu A \cdot cm^{-2}$—a common observation), would require P_{Na}/P_K to increase from ~0.05 to ~0.15 with V changing from −54 to −48 mV. This is a relatively small change in V for such a large increase in transport, and it is not surprising that many studies of tight epithelia reveal a relative constancy of membrane potential with fluctuations in Na transport. Also, though data is sparse, there do not appear to be appreciable changes in cellular ion concentrations with large variations in the rate of transepithelial Na transport. For example, Na, K and Cl concentrations in toad urinary bladder epithelial cells do not change detectably between open- and short-circuited conditions (Robinson & Macknight, 1976) and are not markedly affected after maximal stimulation of Na transport by vasopressin (Macknight, Leaf + Civan, 1971). These observations are consistent with there being relatively little change in the basolateral membrane potential following such perturbations.

In actuality, any increase in P_{Na} under short-circuit conditions will tend to increase cell Na concentration and depolarize the cell, thus decreasing the driving force for Na entry. But the increased cell Na concentration will in turn stimulate the Na pump thus tending to increase cell K concentration. Since the cell K concentration remains relatively constant, P_K must increase under these conditions and this,

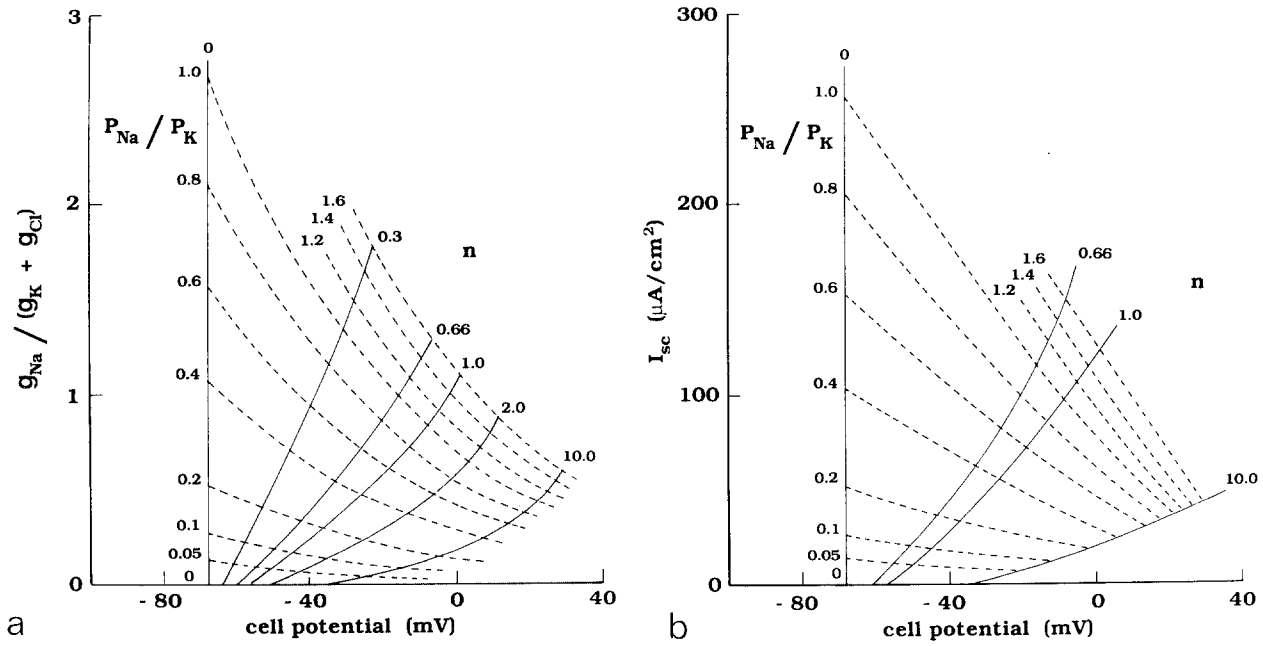

Fig. 4. The relationship between the ratio of the apical to basolateral conductance and cell potential (V) with variations in P_{Na}/P_K (dashed lines) and the Na pump ratio, n (solid lines). (P_{Cl}/P_K = 0.3). (*b*) The relationship between I_{sc} and cell potential (V) with variations in P_{Na}/P_K (dashed lines) and the Na pump ratio, n (solid lines). (P_{Cl}/P_K = 0.3)

together with the stimulation of the electrogenic Na pump, would negate much of the initial fall in V with increased P_{Na} and thus maintain the driving force for Na entry despite the effect of the increased P_{Na} on V. Overall, the values of V, and the cell Na and K concentrations could remain relatively constant but with both P_{Na} and P_K having increased significantly. It is possible that the stimulation of transepithelial Na transport by a hyposmotic serosal medium (Bentley, 1964; Bentley et al., 1973; Finn & Reuss, 1975; Gordon, 1988; Lipton, 1972) and the inhibition of such transport by a hyperosmotic serosal medium (Bentley et al., 1973; Finn & Reuss, 1975; Gordon, 1988) are contributed to by changes in basolateral membrane K conductance.

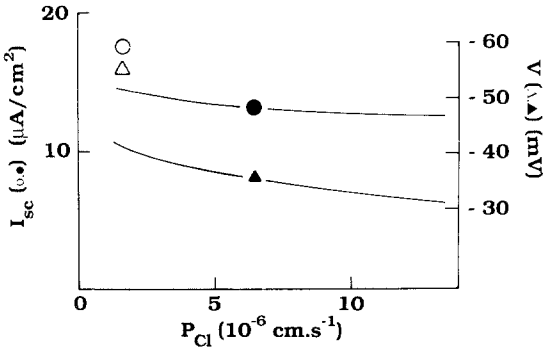

Fig. 5. I_{sc}(○) and V(△) under initial conditions (standard cell, Table 1) and after changing to hypo-osmotic conditions (40% dilution of serosal medium)—I_{sc}(●) and V(▲). The final values reached by I_{sc} and V are dependent on the final value of P_{Cl} which may also change with osmolality

Effects of Changes in P_{Cl} and P_K on I_{sc} and Membrane Potential

The influence of P_{Cl} on the rate of transepithelial Na transport and V can also be assessed. Ussing (1982, 1985) has argued that the partial restoration of volume in frog skin epithelial cells following exposure to a hyposmotic serosal medium is associated with an activation of basolateral membrane Cl channels. If only medium Na and Cl concentrations have been altered and the initial response to a decrease in medium osmolality by, for example, 40%, is a decrease in cell ion concentrations by this percentage as water enters the cells, then the effects of P_{Cl} on I_{sc} and on membrane potential in the new steady state can be determined (Fig. 5). While an increase in P_{Cl} may well contribute to secondary volume regulation in this tissue, it cannot explain the stimulation of I_{sc} under these conditions which is a characteristic of the response of tight epithelia to even a small decrease in serosal medium osmolality (e.g., Gordon, 1988).

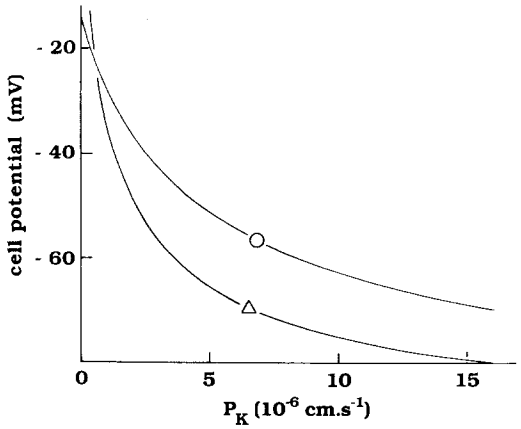

Fig. 6. The relationship between V and P_K at two values of P_{Cl}. [(△)P_{Cl} = 10^{-7} cm · sec^{-1}; (○)P_{Cl} = 16 × 10^{-7} cm · sec^{-1}]

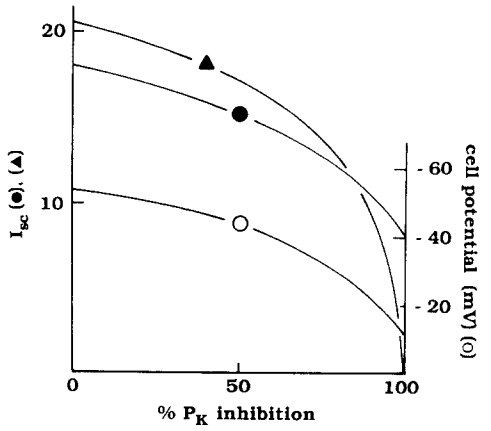

Fig. 8. The variations of I_{sc}(●) and V(○) with progressive inhibition of P_K from the standard conditions (Table 1). Also shown is the effect on I_{sc}(▲) when P_{Cl} is zero

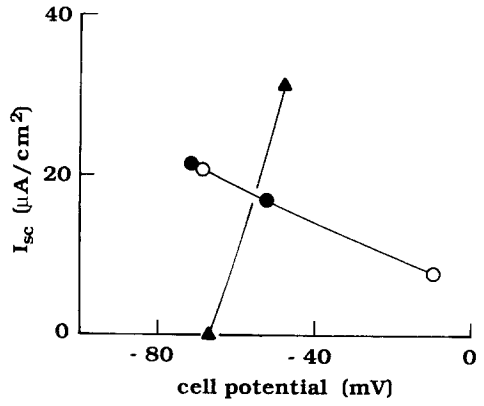

Fig. 7. The inter-relationship between I_{sc} and V as P_{Na}(▲), P_K(○) and P_{Cl}(●) vary from the condition of the standard cell (Table 1) shown at the intersection of the lines

Whereas stimulation of transepithelial Na transport in a tight epithelium may result in comparatively little membrane depolarization (e.g., Nagel & van Driessche, 1989), complete blockage of Na entry from the mucosal medium by amiloride does hyperpolarize the basolateral membrane as predicted in Fig. 3 (e.g., Nagel, 1980; Nagel & van Driessche, 1989). In principle, if the primary effect of reducing P_{Na} with amiloride is established before any secondary alterations occur in cell ion concentrations or in the P_{Cl}/P_K ratio, then the most negative value of V reached at I_{sc} = 0 provides an estimate of P_{Cl}/P_K (Fig. 3*a* and *b*).

As can be seen in Fig. 6, decreasing P_K at constant P_{Na} and P_{Cl}, leads to a depolarization of the cell and this has the effect of reducing I_{sc} (Fig. 7). Increasing P_{Cl} has the same effect (Fig. 7). Barium is a blocker of K channels in a variety of tissues (Lewis, Hanrahan & van Driessche, 1984) and has been used to reduce P_K in toad bladder (Lewis et al., 1985). The I_{sc} is shown in Fig. 8 as a function of P_K to illustrate the effects of an instantaneous reduction in the K permeability with Ba^{2+} in the serosal bathing fluid. Note that the rate of change of current with inhibition becomes greater as the inhibition increases, particularly over the range 95–100% inhibition when P_{Cl} is relatively low. The fractional fall in current with 100% inhibition depends on the value of P_{Cl}. The reason for this is that P_{Cl} is intimately associated with the rate of cotransport which, in turn, affects the transport of Na and K as well as Cl. From this model, complete inhibition of the passive K current cannot result invariably in a reduction of current to 1/3 of the previous value as in the model of Nielsen (1982), and thus the pump stoichiometry cannot be determined from such experiments alone.

Absolute Magnitudes of the Membrane Permeabilities

So far we have presented data in terms of permeability ratios. However, in a tight short-circuited Na-transporting epithelium we have a unique opportunity to characterize the absolute permeabilities given the assumption that $P^a_{Na} \gg P^b_{Na}$ and the following experimental data: I_{sc}, cell ion concentrations, V, the voltage divider ratio, n and q. Since I_{sc} is simply the passive Na current across the apical membrane, we can obtain the permeability, P^a_{Na} from a rearrangement of the GHK flux equation written in the form

$$P^a_{Na} = \frac{I_{sc}(\exp\{\phi^a\} - 1)}{F\phi^a(c_1\exp\{\phi^a\} - c_2)} \qquad (6)$$

where $\phi^a = FV^a/RT = -\phi^b$ and subscripts 1 and 2 designate the mucosal and cell fluids, respectively.

Given intracellular concentrations of these ions, unique values of the permeability ratios P^a_{Na}/P^b_K and P^b_{Cl}/P^b_K exist for any combination of V and conductance ratio (Fig. 3). If we know V and the voltage-divider ratio, we can obtain P^a_{Na} from Eq. (6) and P^a_{Na}/P^b_K from Fig. 3*a*, and thus evaluate P^b_K. Then P^b_{Cl} can be determined from P^b_{Cl}/P^b_K using Fig. 3*a*. We can also derive the absolute values of the membrane conductances from the values for the membrane permeabilities using Eq. (5).

As an example, a bladder with a I_{sc} of 13 $\mu A \cdot cm^{-2}$, a V of -55 mV and a voltage divider ratio of 0.17 must, from Fig. 3, have $P^a_{Na}/P^b_K = 0.7$ and $P^b_{Cl}/P^b_K = 0.25$. Thus the absolute permeabilities ($cm \cdot sec^{-1}$) will be $P^a_{Na} = 4.7 \times 10^{-7}$, $P^b_K = 6.8 \times 10^{-6}$, and $P^b_{Cl} = 1.7 \times 10^{-6}$, and the conductances under short-circuit conditions ($S\ cm^{-2}$) will be $g^a_{Na} = 1.8 \times 10^{-4}$, $g^b_K = 7.5 \times 10^{-4}$, and $g^b_{Cl} = 3.9 \times 10^{-4}$.

In conclusion, we stress that this paper concerns short-circuited tight epithelia in their steady states and that a variety of challenges to the system are analyzed with only one parameter at a time changing. No doubt other modifications to transport parameters are set in train by the original stimulus. Our purpose here is simply to explore some of the determinants of the steady-state potential under short-circuit conditions to provide an indication of some of the consequences for, and limitations on, the system. Open-circuit epithelia are not amenable to this analysis since there is no unique uniform membrane potential, and the paracellular pathway contributes additional unknown parameters which can be ignored when analyzing the short-circuited tissue.

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