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Passive Sodium Fluxes Across Toad Bladder in the Presence of Simultaneous Transepithelial Gradients of Concentration and Potential

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Summary. Bidirectional sodium fluxes were measured across toad bladder sacs after eliminating active transport with ouabain. Transepithelial potential was clamped to 100 mV or the Nernst potential, ψ_{eq}, at varying sodium concentrations, C_m, in the mucosal medium. Serosal sodium concentration, C_s, was held constant. Equations were derived for permeability, partial ionic conductance, and unidirectional fluxes as functions of C_m and C_s, based in part on the assumption that the ratio, Q, of bulk sodium permeability to tracer sodium permeability is a constant, independent of concentration and potential. The results conformed closely to these equations.

Starting with the theoretical formulations given by Kedem and Essig [5] and Krämer and Meares [6], we have recently derived a set of equations which describe satisfactorily ion fluxes in the passive transport path [2] and sodium fluxes in the active transport path [3] of toad bladder during voltage-clamping. The derivation of these equations was based on the assumption of a constant value for the empirical constant, Q, in the equations for change in flux ratio, f, with change in transepithelial potential, ψ, viz.: $\partial \ln f = Q(zF/RT)\,\partial\psi$. This constant can be shown theoretically to be equal to the ratio of bulk permeability coefficient, P, to tracer permeability coefficient, P^*. Thus Q expresses a membrane property that reflects the interaction between ions of the test species or between the test species and other solutes or reactants.

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We subsequently applied this model to passive sodium fluxes across a sodium concentration gradient in ouabain-poisoned toad bladder clamped to 0 mV [4]. We found that if the serosal sodium concentration is fixed, Q remains constant as mucosal sodium concentration is reduced. Observed unidirectional fluxes corresponded closely to those predicted by these equations.

In the present work, a set of equations is derived on the basis of constant Q for passive transport in the presence of simultaneous gradients of potential and concentration. These equations were found to describe sodium fluxes across ouabain-poisoned toad bladder satisfactorily under these conditions.

Materials and Methods

Toad bladder sacs were prepared by the method previously described [2]. Agar-KCl salt bridges were used. Both sides of the hemibladders were first bathed with amphibian Ringer's solution (in mM: NaCl, 111; $NaHCO_3$, 3; $CaCl_2$, 2.7; $MgCl_2$, 2; KCl, 3.4) for 60–120 min to allow equilibration. Bladders with spontaneous potentials of less than 60 mV were then rejected.

After spontaneous potentials was completely eliminated by ouabain (1.89×10^{-3} M) added to the serosal bath, the bladder sac was emptied, rinsed and filled with the test solution. The same Ringer's solution containing ouabain was used in the serosal bath in all experiments, while [NaCl] in the mucosal medium was varied, resulting in Na concentrations of 114, 40, 12 or 3 mM (attributable to $NaHCO_3$). Transepithelial potential was then clamped to either 100 mV or ψ_{eq}. A steady current was observed after 15–30 min. Bidirectional sodium fluxes were measured by using ^{22}Na and ^{24}Na simultaneously. The methods of measurement of electrical parameters and isotope fluxes of sodium were as previously described [2, 11].

Symbols

C_m	Sodium concentration in the mucosal bathing medium (M)
C_s	Sodium concentration in the serosal bathing medium (M)
$\bar{C}$	Logarithmic mean of the sodium concentrations in two bathing media (M), defined as $(C_m - C_s)/\ln(C_m - C_s)$
β^{ψ}	Serosal-to-mucosal sodium flux (μA/mg) (β^0 = flux at $\psi = 0$)
Φ^{ψ}	Mucosal-to-serosal sodium flux (μA/mg) (Φ^0 = flux at $\psi = 0$)
ψ_{eq}	Potential difference at which $\beta = \Phi(V)$
$zF\overset{\Delta}{J}$	Net sodium flux (μA/mg) defined as $\beta - \Phi$ ($zF\overset{\Delta}{J}^0$ = net flux at $\psi = 0$)
zFJ_{eq}	Unidirectional sodium flux in both directions at ψ_{eq} when $C_m \neq C_s$ (μA/mg)
zFJ^0	Unidirectional sodium flux in both directions at 0mV when $C_m = C_s$ (μA/mg)
D	Ordinary bulk diffusion coefficient for sodium in the transport path
D^*	Tracer diffusion coefficient for sodium in the transport path
P	Bulk sodium permeability coefficient (μA/mg · M)
P^*	Tracer sodium permeability coefficient (μA/mg · M)
Q	Ratio of P/P^* (also D/D^*)
I	Current, μA/mg

Theoretical

As before, we consider a barrier (or barriers) of thickness δ separating two electrolyte solutions containing different concentrations of the test species. We deal only with passive transport during steady-state conditions, in which the epithelium is subjected to both transepithelial concentration gradients for sodium and potential gradients, in the range of potentials in which the electrical conductance is independent of potential. under these conditions, the net flux of passively transported ions in the absence of solvent drag has been described by the integrated Nernst-Planck equation [4, 7]:

$$z F \overset{\Delta}{J} = P \bar{C} \left(\ln \frac{C_s}{C_m} + \frac{z F \psi}{RT} \right), \tag{1}$$

where P is the bulk permeability coefficient defined as $P = z FD/\delta$. We again postulate that this equation is applicable to toad bladder, even though the concentration profile of sodium is almost certainly not linear.

As previously described, if we combine Eq. (1) with the defining equations

$$z F J_{eq} = P^* \bar{C} = (P/Q) \bar{C} \tag{2}$$

$$z F \overset{\Delta}{J} = \beta^{\psi} - \Phi^{\psi} \tag{3}$$

$$\psi_{eq} = -\frac{RT}{z F} \ln (C_s/C_m) \tag{4}$$

and postulate again that

$$z F J_{eq} = \frac{\beta^{\psi} - \Phi^{\psi}}{\ln (\beta^{\psi}/\Phi^{\psi})}. \tag{5}$$

We obtain, by appropriate rearranging, the flux ratio equation,

$$\ln (\beta^{\psi}/\Phi^{\psi}) = Q \left(\ln \frac{C_s}{C_m} + \frac{z F \psi}{RT} \right). \tag{6}$$

Eq. (6) can be rearranged, yielding

$$\Phi^{\psi} = \beta^{\psi} e^{-y} \tag{7}$$

where y is defined as

$$y = Q \left(\ln \frac{C_s}{C_m} + \frac{z F \psi}{RT} \right). \tag{8}$$

Combination of Eqs. (5) and (7) gives the expression for $z FJ_{eq}$ in terms of unidirectional flux and y,

$$z F J_{eq} = \frac{\beta^{\psi} (1 - e^{-y})}{y}. \tag{9}$$

Eq. (9) can be used to evaluate the parameter Q for any pair of values of C_m and C_s if both Φ (or β) at ψ_{eq} and Φ (or β) at any other potential ψ can be measured. Alternatively, If Q remains constant, independent of potential, the unidirectional flux β or Φ can be predicted from knowledge of zFJ_{eq} and Q.

In order to obtain unidirectional flux equations, we use mathematical approaches similar to those previously described [4] and rewrite Eq. (9) as

$$\ln zFJ_{eq} = -\frac{Q}{2}\ln\frac{C_s}{C_m} + \ln\{f(y)\, e^{-QzF\psi/2RT}\} \tag{10}$$

where $f(y)$ is defined as

$$f(y) = \beta^{\psi}(e^{y/2} - e^{-y/2})/y. \tag{11}$$

We have previously shown [4] that zFJ_{eq} can be expressed by the equation

$$\frac{zFJ_{eq}}{zFJ^0} = \left(\frac{C_m}{C_s}\right)^{Q/2}. \tag{12}$$

From Eqs. (10), (11) and (12) there follows, by appropriate rearranging, the serosal-to-mucosal flux equation,

$$\beta^{\psi} = \frac{zFJ^0 y}{(e^{y/2} - e^{-y/2})} \cdot e^{QzF\psi/2RT}. \tag{13}$$

Combination of Eqs. (13) and (7) yields the mucosal-to-serosal flux equation,

$$\Phi^{\psi} = \frac{zFJ^0 y\, e^{-y}}{e^{y/2} - e^{-y/2}} \cdot e^{QzF\psi/2RT}. \tag{14}$$

Eqs. (13) and (14) are useful in analyzing and predicting the unidirectional fluxes observed at any clamping potential ψ in the presence of concentration gradients provided that Q is constant and that zFJ^0 can be accurately determined. At $\psi = 0$, Eqs. (13) and (14) reduce to the same equations as described previously [4],

$$\beta^0 = \frac{zFJ^0 n}{e^{n/2} - e^{-n/2}} \tag{15}$$

and

$$\Phi^0 = \frac{zFJ^0 n\, e^n}{e^{n/2} - e^{-n/2}}, \tag{16}$$

where $n = Q\ln(C_m/C_s)$.

In practice, it may be more convenient to express the unidirectional flux at any potential in terms of the unidirectional flux at 0 mV. From

Eq. (5)

$$zFJ_{eq} = \frac{\beta^{\psi} - \Phi^{\psi}}{\ln(\beta^{\psi}/\Phi^{\psi})} = \frac{\beta^{0} - \Phi^{0}}{\ln(\beta^{0}/\Phi^{0})}. \tag{17}$$

From ref. [4],

$$\ln \Phi^{0}/\beta^{0} = Q \ln(C_m/C_s) = n \tag{18}$$

from which

$$\Phi^{0} = \beta^{0} e^{n}. \tag{19}$$

Introducing Eqs. (18) and (19) into Eq. (17), there follows, by appropriate rearranging,

$$\Phi^{\psi} = \Phi^{0} \{(1 - e^{-n})\, y/(e^{-y} - 1)\, n\} \tag{20}$$

and

$$\beta^{\psi} = \beta^{0} e^{Qz F\psi/RT} \{(1 - e^{-n})\, y/(e^{-y} - 1)\, n\}. \tag{21}$$

Eqs. (20) and (21) can be used to evaluate the ratio Q from unidirectional fluxes, β^{ψ} and β^{0} (or Φ^{ψ} and Φ^{0}). If Q is known, β^{ψ} or Φ^{ψ} at any potential can be predicted from β^{0} or Φ^{0}. Note that Eqs. (20) and (21) are applicable for any values of C_s and C_m; that is, it is not necessary to fix C_s as described earlier [4].

As in the case of passive ion transport in the absence of transepithelial potential difference [4], the bulk permeability coefficient P can also be expressed in terms of zFJ^{0} and y. If we combine Eqs. (1), (3), (13) and (14) there follows, after appropriate rearrangement,

$$P = \frac{zFJ^{0} Q (C_m/C_s)^{Q/2} \ln(C_s/C_m)}{C_s - C_m}. \tag{22}$$

Eq. (22) is identical to the permeability equation we derived previously in the absence of a transepithelial potential difference [4]. Thus, we have theoretically predicted that the bulk permeability coefficient, P, and also the tracer permeability coefficient, P^*, are independent of clamp potential, if our assumptions are valid.

The partial conductance of a passively transported ion in the presence of a concentration gradient can be obtained as follows: if we introduce $\bar{C} = (C_s - C_m)/\ln(C_s/C_m)$ into Eq. (1) we obtain

$$zF\overset{\Delta}{J} = P\Delta C + (P\bar{C}\, zF/RT)\, \psi. \tag{23}$$

Since, as Eq. (22) shows, P is independent of potential, it follows from Eq. (23) that

$$zF\overset{\Delta}{J}^{0} = P\Delta C. \tag{24}$$

From Eqs. (23) and (24),

$$(zF\overset{\Delta}{J} - zF\overset{\Delta}{J}^{0})/\psi = P\bar{C}\, zF/RT. \tag{25}$$

The left-hand side of Eq. (25) is clearly equal to the partial ionic conductance, g_i, in the presence of a concentration gradient. Hence, g_i can be expressed in terms of bulk permeability coefficient, P, and average bath concentration, $\bar{C}$, for any pair of values of C_m and C_s, as

$$g_i = P\bar{C}\, z\, F/RT. \tag{26}$$

Alternatively, g_i can be calculated from Q and $z\,FJ_{eq}$ obtained at ψ_{eq} by the equations described previously [4],

$$g_i = (Q\, z\, F/RT)\, z\, FJ_{eq}. \tag{27}$$

Substituting Eq. (12) into Eq. (27), we obtain g_i as a function of the concentration of the test species in the bathing media,

$$g_i = (Q\, z\, F/RT)\, z\, FJ^0 (C_m/C_s)^{Q/2}. \tag{28}$$

The transport number, t, of passively transported ions in the presence of a concentration gradient is given by

$$t = z\,\overset{\Delta}{FJ}/I = g_i/g, \tag{29}$$

where g is the total electrical conductance in the passive transport path.

Results

Bladders Clamped to 100 mV

The results are shown in Table 1. Both unidirectional fluxes increase with increasing mucosal sodium concentration. This countertransport effect is similar to that observed in the absence of a transepithelial potential gradient (4), but more pronounced. The unidirectional fluxes, $z\,FJ_{eq}$, calculated by Eq. (5) from bidirectional fluxes at 100 mV are not significantly different from those previously calculated from bidirectional fluxes at $\psi = 0$ [4].

Both the net sodium fluxes, $z\,\overset{\Delta}{FJ}^{100}$, and the electric current, I^{100}, also increased with increasing mucosal sodium concentration. The transport number of sodium ions, t, estimated by Eq. (29) is reduced by increasing mucosal sodium concentrations because the rate of increase of electrical current with respect to the change in mucosal sodium concentration is greater than that of net sodium flux. Thus sodium conductance, g_i, rises. Reuss and Finn [10] have reported that the shunt conductance

Table 1. Bidirectional sodium fluxes in ouabain-poisoned toad bladders clamped to 100 mV at varying mucosal sodium concentrations[a]

C_m	Observed data			Derived data						
	β^{100}	Φ^{100}	I^{100}	$zF\overset{\Delta}{J}^{100}$	zFJ_{eq}[b]	t	g_i $(=PzF\bar{C}/RT)$	P	P^*	P/P^*
mM	μA/mg	μA/mg	μA/mg	μA/mg	μA/mg	*ratio*	μmho/mg	μA/mg · M	μA/mg · M	*ratio*
3	0.241 ±0.062 (6)	0.005 ±0.001 (6)	0.355 ±0.039 (6)	0.236 ±0.061 (6)	0.060 ±0.013 (6)	0.64 ±0.11 (6)	1.22 ±0.32 (6)	1.026 ±0.266 (6)	1.977 ±0.419 (6)	0.50 ±0.03 (6)
12	0.294 ±0.042 (8)	0.012 ±0.002 (8)	0.576 ±0.060 (8)	0.282 ±0.042 (8)	0.087 ±0.012 (8)	0.51 ±0.08 (8)	1.79 ±0.26 (8)	1.014 ±0.267 (8)	1.933 ±0.267 (8)	0.52 ±0.03 (8)
40	0.336 ±0.048 (7)	0.026 ±0.004 (7)	0.658 ±0.096 (7)	0.310 ±0.047 (7)	0.119 ±0.015 (7)	0.49 ±0.02 (7)	2.44 ±0.37 (7)	0.887 ±0.135 (7)	1.690 ±0.208 (7)	0.52 ±0.04 (7)
114	0.354 ±0.026 (11)	0.048 ±0.012 (11)	0.914 ±0.065 (11)	0.317 ±0.023 (11)	0.152 ±0.017 (11)	0.353 ±0.024 (11)	3.17 ±0.23 (11)	0.713 ±0.053 (11)	1.332 ±0.148 (11)	0.57 ±0.04 (11)

[a] Serosal sodium concentration fixed at 114 mM.
[b] Estimated from β^{100} and Φ^{100} using Eq. (17).

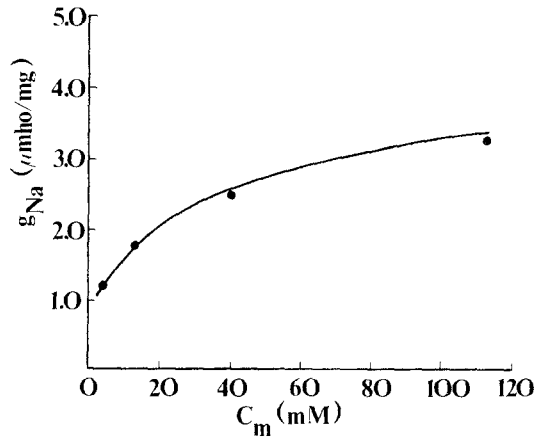

Fig. 1. Observed and predicted sodium conductance as a function of mucosal sodium concentration in toad bladder. The points are from Table 1. The curve is Eq. (28) with $zFJ^0 = 0.154$ μA/mg and $Q = 0.57$ [4]

of toad bladder for sodium was increased by increasing mucosal sodium concentration. Fig. 1 shows a comparison between the observed sodium conductance and that predicted by Eq. (28) at varying mucosal sodium concentrations. A close agreement was seen. The total electrical conductance after ouabain (g), as estimated by $\Delta I/\Delta\psi$, was found to be 2.19 ± 0.29 (6), 3.50 ± 0.31 (8), 5.66 ± 0.77 (7) and 9.14 ± 0.10 (11) μA/mg at $C_m = 3$, 12, 40 and 114 mM, respectively. Hence, g is also shown to be increased with increasing mucosal sodium concentration.

The observed values of bulk permeability coefficient, P, as estimated by Eq. (1) and the tracer permeability coefficient, P^*, as estimated by Eq. (2) at varying mucosal sodium concentrations do not differ significantly from those estimated in the absence of a potential gradient [4]. The estimated values of Q do not change significantly with C_m.

Bladders Clamped to the Nernst Potential

The results are shown in Table 2. At the two mucosal sodium concentrations employed, unidirectional sodium fluxes measured with potential clamped to ψ_{eq} are nearly equal. The values so obtained at these two concentrations are not significantly different from the values of zFJ_{eq} in Table 1, estimated using Eq. (5) from Φ^{100} and β^{100}.

Table 2. Bidirectional sodium fluxes in ouabain-poisoned bladders clamped to the Nernst potential ψ_{eq} at two mucosal sodium concentrations[a]

C_m mM	ψ_{eq} mV	I μA/mg	β μA/mg	Φ μA/mg	zFJ μA/mg
3	−94	0.287 ±0.110 (7)	0.051 ±0.009 (7)	0.055 ±0.007 (7)	−0.004 ±0.018 (7)
12	−58	0.330 ±0.032 (10)	0.079 ±0.014 (10)	0.081 ±0.007 (10)	−0.002 ±0.017 (10)

[a] Serosal sodium concentration fixed at 114 mM.

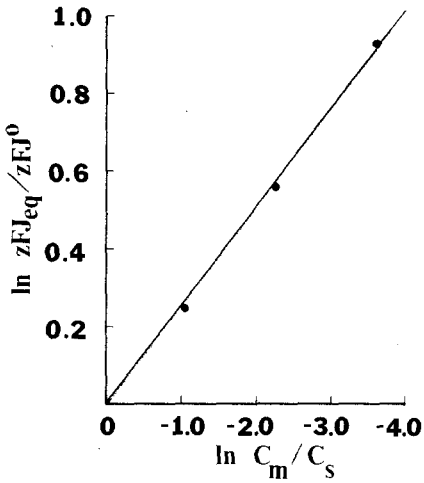

Fig. 2. The ratio of unidirectional fluxes at the Nernst potential (zFJ_{eq}) in the presence of a concentration gradient to unidirectional fluxes observed when $C_m = C_s$ (zFJ^0), plotted against the ratio C_m/C_s. Proportionality is observed, as predicted by Eq. (12). The ratio of the two quantities yields $Q/2$, and is equal to 0.25 ± 0.006 (SEM)

Test of the Unidirectional Flux Equation

In Fig. 2 is shown a log-log plot of the observed ratio zFJ_{eq}/zFJ^0 *vs.* the ratio of C_m/C_s. Q, estimated from the slope of the line ($=Q/2$) is 0.50 ± 0.01 (SEM). Thus, the ratio Q appears to be a constant, independent of ψ, and independent of mucosal sodium concentration.

In order to test the validity of Eqs. (13) and (14), a comparison between the observed unidirectional sodium fluxes at 100 mV plotted against the

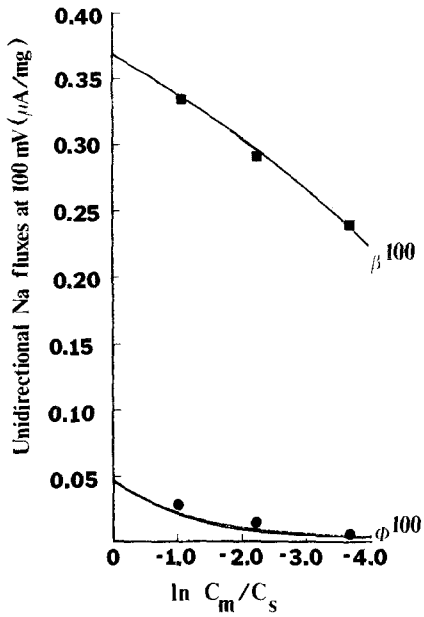

Fig. 3. Observed and predicted unidirectional sodium fluxes at 100 mV, as a function of the ratio C_m/C_s. The points are from Table 1. The curves are Eqs. (13) and (14) with $zFJ^0 = 0.154$ μA/mg $Q = 0.57$ [4]

logarithm of the ratio of C_m to C_s and those predicted by these equations using $zFJ^0 = 0.154$ μA/mg and $Q = 0.57$ [4] is shown in Fig. 3. Close agreement between them is seen. The second term on the right-hand side of Eq. (10) was calculated for $C_m = 3$, 12, and 40 mM using the data shown in Table 1 to be -1.863, -1.842, and -1.841, respectively. Hence, the second term on the right-hand side of Eq. (10) is apparently a constant, independent of mucosal sodium concentration and clamp potential.

Discussion

The mathemetical model of passive ion transport employed in this work appears to describe adequately both net and tracer sodium fluxes under the influence of simultaneous gradients of concentration and potential. The model is based on a number of simplifying assumptions, in particular on the assumption that the ratio, Q, of bulk sodium permeability to isotopic sodium permeability is independent of potential and concentration. The correspondence between the equations so derived and the

observations strongly suggests that this assumption is correct. The other assumptions made in deriving these equations are approximations whose validities have not been so critically tested.

In one sense, these results are not surprising, since we have previously reported that electrical [2] or chemical [4] gradients, when imposed separately, fail to alter Q, according to these equations. However, the general relationships which evolve from these assumptions are surprisingly powerful. Thus, Eq. (22), which describes permeability as a function of ion concentrations in the two bathing media, and was derived previously for concentration gradients without electrical gradient [4], is now seen to be applicable whether an electrical gradient is present or not. Eq. (28), which describes ion conductances as a function of external ion concentrations, also appears to be quite general.

In a sense, these derivations could have been made without using the parameter Q, since they presumably apply where Q is unity. However, Q is not unity in toad bladder for any path yet examined [2–4], and therefore a derivation which included the assumption that $Q=1$ would not yield relationships which correspond to experimental data obtained from study of this tissue.

According to Eqs. (22) and (28), permeability and conductance can be estimated from knowledge of Q, C_m and C_s. If correct, these relationships point to a role of this parameter in determining bulk ion movements, even though it is basically an isotopic measurement. This must mean that unidirectional flux measurements, which can be made only with isotopes, nevertheless reveal a membrane property which is important in determining bulk ion movement when concentration gradients are present. Intuitively, it is tempting to conclude that unidirectional flux approximates bulk flux as "trans" concentration approaches zero, and that, therefore, isotope fluxes give an indication of the bulk flux to be expected under such conditions, even when "trans" concentration is not zero. However, this is obviously an oversimplification.

The general unidirectional flux Eqs. (20) and (21), were not specifically tested in this work because fluxes were not measured at both 0 and 100 mV in individual experiments. Nevertheless, it is possible to test these equations by comparing mean fluxes at 100 mV in the present work with mean fluxes at 0 mV found in the previous study [4], using the same mucosal sodium concentrations. The similarity of the mean values for Q, zFJ_{eq} and P in the two sets of experiments has already been noted. When the mean fluxes at 0 mV from the previous study [4] are used in Eqs. (20) and (21) to predict fluxes at 100 mV for each mucosal sodium concentra-

tion (3, 12, and 40 mM), all six values are within 10% of the mean fluxes found in the present work. This is to be expected, since these equations are logical extensions of the more limited unidirectional flux equations already tested, Eqs. (15) and (16).

Previous observations in frog bladder [8], frog skin [9] and toad bladder [1, 10] suggest that shunt conductance for sodium is reduced as mucosal sodium concentration is reduced. Furthermore, Mandel and Curran [9] have reported that ion permeability of frog skin is virtually unaffected by hyperpolarizing potentials, although depolarizing potentials increased permeability. Thus our findings are qualitatively in accord with previous work. Apparently no quantitative models of the dependence of these properties on ion concentration have previously been tested in epithelia.

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References

1. Bindslev, N., Tormey, J. M., Pietras, R. J., Wright, E. A. 1974. Electrically and osmotically induced changes in permeability and structure of toad urinary bladder. *Biochim. Biophys. Acta* **332**:286
2. Chen, J. S., Walser, M. 1974. Passive ion fluxes across toad bladder. *J. Membrane Biol.* **18**:365
3. Chen, J. S., Walser, M. 1975. Sodium fluxes through the active transport pathway in toad bladder. *J. Membrane Biol.* **21**:87
4. Chen, J. S., Walser, M. 1976. Effect of transepithelial concentration gradients on the passive fluxes of sodium across toad bladder. *J. Membrane Biol.* **27**:381
5. Kedem, O., Essig, A. 1965. Isotope flows and flux ratios in biological membranes. *J. Gen. Physiol.* **48**:1047
6. Krämer, H., Meares, P. 1969. Correlation of electrical and permeability properties of ion-selective membranes. *Biophys. J.* **9**:1006
7. Kotyk, A., Janacek, K. 1970. Cell Membrane Transport. p. 50. Plenum Press, New York
8. Lindemann, B., Grebhart, U. 1973. Delayed change of Na-permeability in response to steps of (Na) at the outer surface of frog skin and frog bladder. *In:* Transport Mechanisms in Epithelia. H. H. Ussing and N. A. Thorn, editors. p. 115. Munksgaard, Copenhagen
9. Mandel, L. J., Curran, P. F. 1972. Response of the frog skin to steady-state voltage clamping. I. The shunt pathway. *J. Gen. Physiol.* **59**:503
10. Reuss, L., Finn, A. L. 1975. Effects of changes in the composition of the mucosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Membrane Biol.* **20**:191
11. Walser, M. 1972. Components of sodium and chloride flux across toad bladder. *Biophys. J.* **12**:351