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Effect of Transepithelial Concentration Gradients on the Passive Fluxes of Sodium across Toad Bladder

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Summary. Bidirectional sodium fluxes across toad bladder were measured after eliminating active transport with ouabain. Mucosal sodium concentration, C_m, was progressively reduced (from 114 to 3 mM) while serosal sodium remained constant. Potential difference was maintained at zero by current passage. The ratio, Q, of the bulk permeability coefficient for sodium, P, to the tracer sodium permeability coefficient, P^*, was found to remain constant as C_m decreased. Equations were derived on this basis for bidirectional fluxes and for P and P^* as functions of C_m, which corresponded closely to the observed data. The explanation for the observed value of Q and its constancy under these conditions is uncertain.

The use of isotopic tracers to characterize the active and passive transport properties of membranes usually entails the assumption that the permeability coefficient of the membrane for the tracer is the same as the bulk permeability coefficient for the test solute, whether the driving force for bulk movement of solute across the membrane is a chemical or electrical gradient [7]. However, substantial differences between tracer and bulk diffusion coefficients for various solutes have been observed in both artificial [3] and biological [1] membranes. Such differences can be produced by (1) coupling between the flows of tracer and abundant species, (2) unstirred layers, (3) coupling between the flow of the test species and other solutes or the solvent, (4) the presence of fixed or mobile carriers within the membrane, (5) electroosmosis, (6) concentration polarization, or (7) other unknown mechanisms. Often it is not possible to differentiate experimentally between these possibilities.

Nevertheless, quantitative information about membrane properties can be obtained from unidirectional tracer flux measurements provided

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that the ratio, Q, of bulk diffusion coefficient, D, to tracer diffusion coefficient, D^*, remains constant under the conditions of the experiment. Starting with theoretical formulations given by Kedem and Essig [4] and Krämer and Meares [5], we have derived expressions [1, 2] that permit the calculation of partial ionic conductance, transport number, and Q from the measurement of unidirectional flux of an ion at two values of transmembrane potential difference, ψ, provided that (1) conductance is voltage independent and (2) all parallel transport paths share a single value of ψ at which net flux is zero.

In the ouabain-poisoned toad bladder, these equations were shown to be applicable to passive fluxes of Na, Cl, and SO_4 ions between identical media. In other words, Q for each of these three ions was a constant [1], independent of potential, in any given medium. This is not incompatible with two (or more) parallel passive routes of permeation, as noted above. However, Q for both Na and Cl ions changed when the medium composition was altered, suggesting that coupling between flows of different ions was contributing to the observed differences between tracer and bulk solute permeation. Subsequently we demonstrated [2] that sodium fluxes in the active transport pathway of toad bladder (measured as ouabain-inhibitable fluxes) also followed these equations, at least for values of ψ less than E, the value of ψ at which net sodium flux through the active transport path is zero. Thus, if more than one parallel active sodium transport path exists, they share a common value of E.

In the present study, these equations are applied to measured passive fluxes of sodium across toad bladder in the presence of concentration gradients produced by progressively lowering Na concentration in the mucosal medium. Q is again shown to be constant under these conditions, although both tracer permeability and bulk sodium permeability change in a manner consistent with these formulations.

Materials and Methods

The methods for the preparation of toad bladder sacs and measurement of electrical parameters and isotopic fluxes of sodium were as previously described [1, 2]. Hemibladders were first bathed on both sides 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. Bladders with spontaneous potentials less than 60 mV were rejected.

Spontaneous potential and active sodium transport were completely eliminated within 30 min after adding ouabain (1.89×10^{-3} M) to the serosal bath. The mucosal bathing solution was then removed and the bladder sac was rinsed and filled with the test Ringer's solution. The serosal medium remained constant. NaCl in the mucosal medium was varied, resulting

in mucosal Na concentrations of 114, 40, 12 and 3 mM (attributable to $NaHCO_3$). A sodium-free medium was not employed because of the theoretical and experimental difficulties attendant on its use. Agar-KCl (3 M) salt bridges were used. Solvent drag was assumed to be negligible.

Bidirectional sodium fluxes were measured while using each of these media with voltage clamped to 0 mV.

Symbols

The following symbols are modifications of the symbols defined in ref. [1]. The subscript $_i$ is omitted since only sodium ions are considered here.

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	Serosal-to-mucosal sodium flux (μA/mg) (β^0 = flux at $\psi = 0$)
Φ	Mucosal-to-serosal sodium flux (μA/mg) (Φ^0 = flux at $\psi = 0$)
ψ_{eq}	Potential difference at which $\beta = \Phi$ (V)
zFJ	Net sodium flux (μA/mg) defined as $\beta - \Phi$
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 0 mV, 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 to P^* (also $= D/D^*$)

Theoretical

Net flux and permeability are related as follows:

$$P\Delta C = \beta^0 - \Phi^0 = zFJ \tag{1}$$

where

$$\Delta C = C_s - C_m. \tag{2}$$

Eq. (1) can be modified by introducing the parameter of zFJ_{eq}, yielding

$$(\beta^0 - \Phi^0)/zFJ_{eq} = P\Delta C/zFJ_{eq}. \tag{3}$$

As described previously [1], the unidirectional tracer flux in both directions at equilibrium, zFJ_{eq}, is defined by

$$zFJ_{eq} = P^* \bar{C}. \tag{4}$$

Note that this equation was derived without the restriction of equal concentrations in the bathing media.

The average concentration $\bar{C}$ can be obtained by combining the Nernst-Planck equation [1] under the condition of zero net flux

$$zF\overset{\Delta}{J}=P\left(\Delta C+\frac{zF\bar{C}\psi}{RT}\right)=0 \tag{5}$$

and the Nernst equation,

$$\psi_{eq}=-\frac{RT}{zF}\ln\left(C_s/C_m\right) \tag{6}$$

to give

$$\bar{C}=\Delta C/\ln\left(C_s/C_m\right). \tag{7}$$

In the following, we assume that this expression for $\bar{C}$ is also applicable when steady-state net flux exists. From Eqs. (3), (4) and (7), we obtain

$$(\Phi^0-\beta^0)/zFJ_{eq}=Q\ln\left(C_m/C_s\right). \tag{8}$$

By analogy from previous studies [1, 2], we postulate that the tracer flux zFJ_{eq} at ψ_{eq} is the logarithmic mean of bidirectional fluxes Φ and β at any value of ψ when C_m and C_s are constant and unequal, i.e.

$$zFJ_{eq}=(\Phi-\beta)/\ln\left(\Phi/\beta\right). \tag{9}$$

The limiting flux at ψ_{eq} is defined as zFJ_{eq} and is clearly equal in both directions. I.e.,

$$\beta^{\psi=\psi_{eq}}=\Phi^{\psi=\psi_{eq}}=zFJ_{eq}. \tag{10}$$

In the present derivation we employ only the hypothetical limiting flux zFJ_{eq}; further work to establish the validity of Eq. (9) at values of $\psi\neq 0$ remains to be carried out.

From Eqs. (8) and (9), the flux ratio in the presence of a concentration gradient, but in the absence of an electrical gradient, can then be expressed as

$$\ln\left(\Phi^0/\beta^0\right)=(P/P^*)\ln\left(C_m/C_s\right)=Q\ln\left(C_m/C_s\right). \tag{11}$$

When $C_m=C_s$ and $\psi\neq 0$, the flux ratio is given by the equation previously derived [1]

$$\ln\left(\Phi/\beta\right)=-Q(zF\psi/RT)=(\Phi-\beta)/zFJ^0. \tag{12}$$

zFJ_{eq}, the unidirectional flux in both directions at ψ_{eq}, can also be expressed in terms of C_m, C_s, and Q. If we combine Eq. (8) with Eq. (11) and rearrange the resulting equation, there follows

$$zFJ_{eq}/\beta^0=(e^n-1)/n \tag{13}$$

where n is defined as

$$n = Q \ln (C_m/C_s). \tag{14}$$

Eq. (13) provides an expression for estimating Q by measuring unidirectional flux at $\psi = 0 (\beta^0)$ and $\psi = \psi_{eq} (zFJ_{eq})$, for any given pair of concentrations, C_m and C_s. A simpler expression that permits calculation of Q from bidirectional fluxes at $\psi = 0$, when one of the two bath concentrations (C_m, for example) is varied and the other is held constant, can be proposed as follows. Expanding Eq. (13),

$$zFJ_{eq}/\beta^0 = e^{n/2}(e^{n/2} - e^{-n/2})/n, \tag{15}$$

from which

$$\ln zFJ_{eq} = n/2 + \ln [\beta^0 (e^{n/2} - e^{-n/2})/n]. \tag{16}$$

We now postulate that the second term on the right-hand side of Eq. (16) is a constant, independent of C_m, when C_s is fixed. Since, according to L'Hospital's rule,

$$\lim_{C_m \to C_s} [(e^{n/2} - e^{-n/2})/n] = 1 \tag{17}$$

and since β^0 becomes zFJ^0 (by definition) when $C_m = C_s$, then Eq. (16) becomes

$$\ln zFJ_{eq} = \frac{Q}{2} \ln C_m/C_s + \ln zFJ^0, \tag{18}$$

which can also be expressed as

$$\frac{zFJ_{eq}}{zFJ^0} = \left(\frac{C_m}{C_s}\right)^{Q/2}. \tag{19}$$

Elimination of zFJ_{eq} between Eqs. (16) and (18) gives the expression for serosal-to-mucosal unidirectional flux, β^0, in terms of zFJ^0 and n,

$$\beta^\circ = zFJ^0 [n/(e^{n/2} - e^{-n/2})]. \tag{20}$$

Thus, if C_s is held constant and if the ratio Q of P to P^* is independent of C_m, then zFJ_{eq} can be expressed by Eq. (19), and serosal-to-mucosal sodium flux, β^0, is a function of C_m.

Similar expressions for the mucosal-to-serosal flux, Φ, in the presence of a concentration gradient at 0 mV can also be obtained by combining Eqs. (20) and (11), yielding

$$\Phi^0 = zFJ^0 [n e^n/(e^{n/2} - e^{-n/2})]. \tag{21}$$

According to Eqs. (20) and (21), if zFJ^0 and Q can be accurately determined from experiments in which $C_m = C_s$, then Φ and β can be predicted at any other value of C_m with transmembrane potential difference clamped to zero.

In addition, the bulk permeability coefficient P and the tracer permeability coefficient P^* can also be expressed in terms of C_m, C_s, Q, and the unidirectional flux zFJ^0 measured when $C_m = C_s$. If we combine Eqs. (1), (14), (20), and (21) there follows

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

The expression for P^* as a function of C_m and C_s can be simply derived by substituting $P^* = P/Q$ into the equation.

From a single measurement of β^0 (when $C_m \neq C_s$), P can be calculated from the following expression derived by combining Eqs. (1) and (11)

$$P = \frac{\beta^0}{C_s - C_m}[1 - (C_m/C_s)^Q]. \tag{23}$$

Obviously, use of this equation requires knowledge of Q. We have used Eq. (22) instead of Eq. (23) in order to test the validity of our formulation, because it permits prediction of P at varying values of $C_m (< C_s)$ from measurement of Q and zFJ^0 when $C_m = C_s$, providing that Q remains constant.

Results

Observed Unidirectional Sodium Fluxes in Ouabain-poisoned Toad Bladder Clamped to 0 mV *at Varying Mucosal Sodium Concentrations; Serosal Sodium Concentration Fixed at* 114 mM

The results are shown in Table 1. Since ouabain does not alter passive permeability of the membrane [2], sodium fluxes measured after ouabain in this study represent the components of sodium fluxes flowing through the passive path. As shown in Table 1, when the same medium (amphibian Ringer's) was used in the serosal bath, mucosal-to-serosal sodium flux at 0 mV (Φ) in this study was increased, to a greater extent, by increasing mucosal sodium concentration, while serosal-to-mucosal sodium flux at 0 mV (β) was increased only slightly. Both the bulk permeability coefficient (P) and the tracer permeability coefficient (P^*) of sodium ions were also modified by varying mucosal sodium concentrations. However,

Table 1. Bidirectional sodium fluxes in ouabain-poisoned toad bladders clamped to 0 mV at varying mucosal sodium concentrations. Serosal sodium concentration fixed at 114 mM

		Observed data			Derived data			
C_m (mM)	No. of Expts.	β^0 (μA/mg)	Φ^0 (μA/mg)	zFJ^0 (μA/mg)	zFJ_{eq} (μA/mg)	P	P^* (μA/mg−M)	P/P^* ratio
3	15	0.131 ±0.015	0.018 ±0.004	0.112 ±0.017	0.053 ±0.005	1.01 ±0.15	1.75 ±0.15	0.58 ±0.07
12	6	0.147 ±0.025	0.037 ±0.003	0.110 ±0.022	0.079 ±0.010	1.07 ±0.22	1.73 ±0.23	0.59 ±0.05
40	15	0.151 ±0.012	0.088 ±0.009	0.063 ±0.009	0.116 ±0.010	0.85 ±0.12	1.64 ±0.14	0.56 ±0.09
114	17	0.154 ±0.013	0.154 ±0.013	0	0.154 ±0.013	0.71 ±0.05	1.35 ±0.11	0.57 ±0.04

the ratio, Q, of P to P^* appears, within experimental error, to be constant. It should be noted that P was calculated from Eq. (1), Q from Eq. (11), and P^* from P/Q. The observed data for $P/P^*(Q)$, as calculated from Eq. (11) at varying mucosal sodium concentrations, were very close to 0.57, the value of Q observed when $C_m = C_s$. Constant Q may be interpreted as the result of constant g_i/zFJ_{eq} ratio, where g_i is the partial sodium conductance in the passive pathway at any given mucosal sodium concentration, since as previously described [1], the ratio Q can also be expressed in terms of $RTg_i/z^2F^2J_{eq}$.

Test of Unidirectional Flux Equations in Ouabain-poisoned Bladder Clamped to 0 mV *at Varying Mucosal Sodium Concentrations*

In order to test the validity of Eq. (19), we plot $\ln(zFJ_{eq}/zFJ^0)$ versus $\ln(C_m/C_s)$ (Fig. 1). As predicted by the equation, a straight line is observed. Q, calculated from the slope of the line ($=Q/2$) was found to be 0.58, which is close to the value of 0.57 obtained when $C_m = C_s$. The results also support the postulate (*see above*) that the second term on the right of Eq. (16) is a constant when C_s is fixed and C_m varies: thus the values of this term at $C_m = 3, 12, 40$ and 114 mM are -1.851, -1.845, -1.845, and -1.871, respectively.

A comparison of both unidirectional sodium fluxes with fluxes predicted by Eqs. (20) and (21) using $zFJ^0 = 0.154$ μA/mg and $Q = 0.57$ is shown in Fig. 2. A satisfactory agreement is seen.

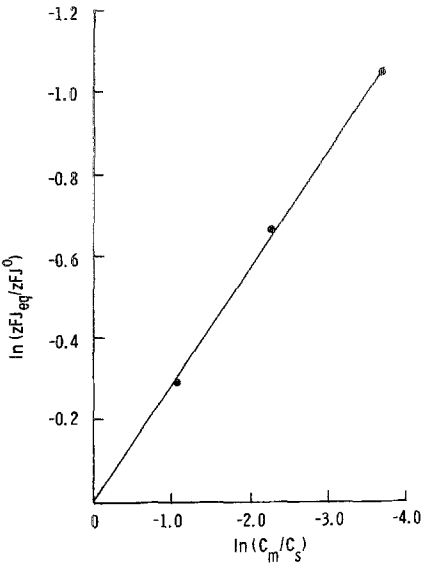

Fig. 1. A log-log plot of the observed ratios zFJ_{eq}/zFJ^0 (from Table 1) to the ratio C_m/C_s. As Eq. (19) predicts, proportionality is observed. The slope of the line, 0.29, is an estimate of $Q/2$

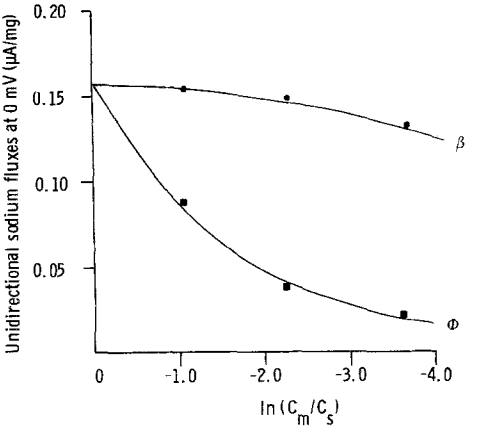

Fig. 2. Observed unidirectional sodium fluxes (Φ and β), shown as solid circles, correspond closely to predictions of Eq. (20) and (21) (smooth curves), using $zFJ^0 = 0.154$ μA/mg and $Q = 0.57$

To test Eq. (22), we predict P at varying mucosal sodium concentrations using $zFJ^0 = 0.154$ and $Q = 0.57$ (values obtained when $C_m = C_s$) and compare the results to observed values of P from Table 1. A satisfactory agreement is seen (Fig. 3).

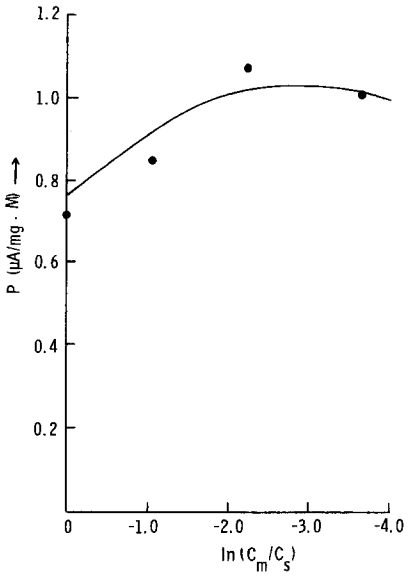

Fig. 3. Observed bulk permeability coefficients for sodium (●) compared to Eq. (22) (smooth curve), using $zFJ^0 = 0.154$ μA/mg and $Q = 0.57$

Discussion

In this initial study of ion fluxes across toad bladder in the presence of concentration gradients we have examined theoretically and experimentally a restricted set of conditions, namely the effect of reduction in the sodium concentration in the mucosal medium while the serosal sodium concentration is held constant. Since Q expresses a variety of coupling phenomena (*see above*), it would not be surprising to find that Q changed as C_m was reduced. Lacking a theoretical explanation of Q we would have been unable to derive theoretical expressions under these conditions.

However, as the results show, Q remains constant when NaCl is progressively removed from the mucosal medium. The significance of this observation is uncertain, and hence it is impossible to predict under what conditions the equations derived may be applicable. For example, reduction in serosal sodium concentration at constant mucosal sodium concentration has not been examined experimentally because the bladder exhibits a progressive rise in conductance under these conditions. Reduction of both serosal and mucosal concentrations to an equal extent remains to be examined.

Furthermore, we have not studied reduction of mucosal sodium to extremely low values because of the technical problems of maintaining a constant and known C_m under these conditions.

Despite these limitations, the results are of interest in showing close correspondence between theory and observation. The equations are simple to use, and give reasonably accurate predictions of both unidirectional fluxes and membrane permeability as mucosal sodium is progressively reduced from 114 to 3 mM.

Eq. (22) is of particular interest in showing that in this system, bulk permeability to sodium rises as C_m is progressively reduced, reaching a maximum at $C_m/C_s \simeq 0.1$ and then falling slowly. This does not imply that sodium conductance also rises as C_m is reduced; on the contrary, conductance should fall in proportion to $(C_m/C_s)^{Q/2}$, in accordance with equation (19), because the conductance of an ion is proportional to zFJ_{eq}, as shown in previous reports [1, 2]. As shown in Table 1, zFJ_{eq} falls as C_m is decreased. Thus the decrease in sodium conductance with C_m attributable to the shunt pathway found by Reuss and Finn [7] is compatible with this formulation. It should also be reemphasized that heterogeneous parallel passive pathways are not inconsistent with a constant value of Q, since the potential at which bidirectional fluxes are equal in all such passive paths is the same (in the absence of solvent drag).

If Q is assigned a value of unity, a very different type of relationship results. Here no increase in permeability occurs, but instead permeability declines progressively, as C_m/C_s is reduced, falling by half at C_m/C_s of approximately 0.01. Whether such behavior can be demonstrated experimentally remains to be seen, but clearly it would have important biological implications.

It should be emphasized that Eq. (22) expresses the relationship between net flux and concentration gradient, and has nothing to do with the presence or absence of isotopes. Thus the coupling coefficient Q expresses a membrane property which, though apparently requiring the use of isotopes to assess, is of major importance in determining the bulk flow of solute across the membrane as the concentration on one side is reduced. Although it is often assumed that tracer flux must accurately reflect transport of the carrier species when the carrier concentration is near zero on the "trans" side of a membrane, it appears from the present work that even a low "trans" carrier concentration may result in substantial differences between tracer and carrier permeability.

The results shown in this study, and in our previous study of passive sodium fluxes with equal sodium concentrations and electrical gradients [1], clearly indicate that electrochemical gradients attributable to differences in chemical concentration exert effects distinct from electrochemical gradients attributable to potential difference. The explanation of this

apparently anomalous finding must lie in different profiles of electrochemical activity within the membrane brought about by the two types of gradients.

Li, DeSousa, and Essig [6] suggest that R and R^x (similar to $1/P$ and $1/P^*$) are unknown functions of the bath concentrations. They suggest measuring R and R^x in the absence of a gradient and then applying these values to observations made in the presence of gradients such that the mean concentration is unaltered. They employed the arithmetic mean rather than the logarithmic mean, but have presented results from flux measurements in an ion exchange membrane to support this proposal. They found close correspondence between observed and predicted flux ratios.

The deviation from unity in the ratio R^x/R ($=P/P^*$) in the experiments of these authors [3, 6] is clearly caused by interactions between the solute particles of a single species ("isotope interaction"), while the deviation seen in toad bladder may not be attributable to this phenomenon because it varies with the nature of the anion accompanying sodium [1]. An adequate explanation for this interesting property of biological membranes is as yet lacking.

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