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Passive Ion Fluxes Across Toad Bladder

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Summary. Toad bladders mounted as sacs, with edge damage eliminated (mean spontaneous potential = 101 mV) were treated with sufficient ouabain (1.89×10^{-3} M) to eliminate active sodium transport. Fluxes of ^{22}Na, ^{36}Cl and $^{35}SO_4$ across the epithelium in both directions were measured at 0 mV and with potential clamped to 100 mV in various media. The results were analyzed by a set of equations, derived from previous work of others. Transport numbers are obtained from unidirectional isotope fluxes measured at 0 mV and 100 mV. Deviation from the Ussing flux ratio equation is expressed in terms of a constant ratio of bulk diffusion coefficient to tracer diffusion coefficient. Tracer flux in either direction at zero potential is the logarithmic mean of bidirectional fluxes at any potential. These equations were tested by comparing fluxes at zero potential predicted from fluxes at 100 mV with observed values, and by comparing transport numbers calculated from average net flux and current. The correspondence was within experimental error. Constant ratio of bulk diffusion coefficient to tracer diffusion coefficient for Na, Cl and SO_4 ions in a given medium is more consistent with the data than constant values for exchange diffusion, independent of potential. Conductance measurements indicate that Na transport is facilitated by (or coupled with) transport of Cl and/or phosphate. Bicarbonate apparently contributes substantially to passive conductance.

In analyzing the fluxes of ions across biological membranes, as measured by tracers, the use of the flux ratio equation [1] was pioneered by Ussing [13]. This equation has proved extremely useful in describing ionic fluxes, but deviations are nevertheless observed in some instances. These deviations have been ascribed to a number of mechanisms, including solvent drag, concentration polarization, interaction between different ionic species, and interaction between tracer and abundant species. Additional mechanisms, admittedly ill-defined, that have been postulated to occur in living membranes include exchange diffusion and single-file pore diffusion.

In previous reports from this laboratory, fluxes of sodium and chloride across toad bladder have been measured under voltage-clamp conditions

in the presence of graded doses of ouabain [16] and in its absence [15]. An attempt [15] to analyze these fluxes in terms of active and passive components, using the techniques described by Shapiro and Candia [12], led to a variety of alternative solutions that could only be expressed as limiting values.

In the present work, fluxes of sodium, chloride and sulfate ions have been measured in toad bladders treated with sufficient ouabain to inhibit active sodium transport completely, at potentials of 0 and 100 mV. The results have been analyzed by a set of equations, derived from previous work of others.

Materials and Methods

Large Dominican toads (*Bufo marinus*) were obtained from National Reagent Company, Bridgeport, Connecticut, and kept in sphagnum moss with access to tap water until use. They were killed by decapitation and pithing. Hemibladders were mounted as sacs on glass cannulas and bathed on both sides with test Ringer's solutions. Both serosal and mucosal baths were vigorously stirred. Initial mucosal bath volume was 20 to 25 ml. Edge damage was avoided by lowering the outer fluid level 1 to 2 mm below the mounting ring [14]; the fluid level was readjusted as necessary throughout the experiment as samples were removed. All experiments were performed at room temperature (25 to 27 °C).

Three different media were used. In all cases, the same medium was used in both serosal and mucosal baths. In Group I, amphibian Ringer's solution (in mM: NaCl, 111; $NaHCO_3$, 3; $CaCl_2$, 2.7; $MgCl_2$, 2; KCl, 3.4) was used (referred to in the tables as HCO_3, Cl). In Group II, sulfate Ringer's solution (in mM: Na_2SO_4, 55; $NaHCO_3$, 3; $CaSO_4$, 1; K_2SO_4, 3; $MgSO_4$, 1) was used (referred to in the tables as HCO_3, SO_4). In Group III, phosphate-buffered sulfate Ringer's solution (in mM: Na_2SO_4, 55; $CaSO_4$, 1; $MgSO_4$, 1; K_2SO_4, 3; Na_2HPO_4, 2.4; NaH_2PO_4, 0.6) was used (referred to in the tables as HPO_4,SO_4). Current-passing and potential-sensing bridges were inserted as previously described [15] but the bridges used varied depending on the test solutions. Agar-NaCl (3 M) bridges were used in the experiments in Group I. In the experiments in Group II and III, agar was dissolved in the medium to be used. The validity of the spontaneous potential was further examined in some experiments in Group I by replacing the agar-NaCl bridges with agar-Ringer's bridges. Differences between potential as measured by these two techniques were negligible in comparison with the applied potential (100 mV).

Bladders were rejected in which initial open-circuit potential (ψ_{spont}) was less than 60 mV. Fourteen ml of 10^{-2} M ouabain, dissolved in the appropriate solution, was then added to the serosal bath, final ouabain concentration being 1.89×10^{-3} M. Short-circuit current fell to zero within 30 min. The subsequent current-voltage relationship was linear from 0 mV to +150 mV (not shown). Isotopes (^{22}Na, ^{36}Cl or $^{35}SO_4$) were then added to either serosal or mucosal baths. After 20 min or more equilibration, unidirectional fluxes were measured with voltage clamped to 0 mV and 100 mV, or in the reverse sequence. Clamp current at 100 mV remained constant. In some experiments, measurements were made at only one of these potentials. At each potential, 3 to 5 samples were obtained at 10-min intervals. One-milliliter samples for ^{22}Na and ^{36}Cl were counted as previously described [15]; for $^{35}SO_4$, 1-ml samples were counted in PCS (Amersham/Searle Company, Arlington Heights, Illinois). Fluxes were calculated by a computer

program in which the linear regression of cumulative counts crossing the membrane (corrected for diminishing bath volume at each interval) against time was calculated as well as the standard error of the regression slope. Fluxes were expressed as μA/mg wet weight, the latter being obtained after blotting the bladders on filter paper at the conclusion of each experiment.

In four experiments, bidirectional sodium fluxes were measured using ^{24}Na and ^{22}Na, These isotopes were determined as described previously [15].

Symbols

ψ Transepithelial potential difference, serosa minus mucosa

ψ_{spont} Spontaneous potential (V) before ouabain

I Electric current from an external source (μA/mg wet weight), taken as positive when flowing from serosa to mucosa

F Faraday's constant

R Gas constant

T Absolute temperature

g^p Ouabain-insensitive phenomenological conductance, defined as I/ψ_{clamp} (μmho/mg wet weight) after ouabain (g^p is positive because I and ψ are so defined as to have the same sign)

Φ_i^ψ Unidirectional mucosal-to-serosal flux of the i^{th} ion at potential ψ (μA/mg wet weight)

β_i^ψ Unidirectional serosal-to-mucosal flux of the i^{th} ion at potential ψ (μA/mg wet weight)

f_i Flux ratio, Φ_i^ψ/β_i^ψ

z_i Valence of the i^{th} ion

$z_i F\overset{\Delta}{J}_i^\psi$ Net ionic flux in the same direction and units as I at potential ψ, $\beta_i^\psi - \Phi_i^\psi$

$z_i FJ_i^0$ Average of unidirectional fluxes at zero potential, $(\Phi_i^0 + \beta_i^0)/2$ (μA/mg wet weight)

t_+ Transport number of cations defined as $z_i F\overset{\Delta}{J}_i^\psi/I$

t_- Transport number of anions defined as $z_i F\overset{\Delta}{J}_i^\psi/I$

g_+ Passive phenomenological conductance of cations defined as $g^p t_+$ (μmho/mg wet weight)

g_- Passive phenomenological conductance of anions defined as $g^p t_-$ (μmho/mg wet weight).

Theoretical

Kedem and Essig [8] derived an equation which expresses deviations from Ussing's flux ratio equation [13] in terms of coupling in the flow of tracer and abundant species. Their equation differs from that of Ussing in the exponential term by a factor of R^x/R, where R is the phenomenological resistance coefficient, and R^x is the exchange resistance. This equation has been shown to be applicable to a synthetic membrane separating a single-electrolyte solution [4], subjected to voltage-clamping.

More recently, Meares and Sutton [10] and Krämer and Meares [9] have obtained equations for passive flux which are useful for interrelating

conductance and transport number. Their equations have also been shown to be applicable to synthetic membranes. However, they have not been tested in biological systems separating a multi-electrolyte solution.

It is therefore worthwhile to extend the work of Kedem and Essig [8] and Meares and Sutton [10] to derive a modified flux ratio equation which is experimentally more useful and is applicable to the analysis of passive fluxes and flux ratios in a membrane, artificial or biological, separating a multi-electrolyte solution.

We start from the flux ratio equation derived by Meares and Sutton [10] and rewrite it to give

$$\ln(\Phi_i^{\psi}/\beta_i^{\psi}) = -I t_i \delta / z_i F \bar{C}_i^0 D_i \tag{1}$$

where $\bar{C}_i^0$ is the constant uniform total concentration in the membrane, D_i is the tracer diffusion coefficient of the test species, and δ is the thickness of the membrane.

Our purpose here is to transform Eq. (1) into a more convenient form for use in the analysis of passive fluxes and ratios of tracer fluxes. To this end, we make use of the unidirectional flux equations given by the authors, and rearrange them to give

$$\beta_i^{\psi} = z_i F k \bar{C}_i^0 / [1 - \exp(-k\delta/D_i)] \tag{2}$$

and

$$\Phi_i^{\psi} = -z_i F k \bar{C}_i^0 / [1 - \exp(k\delta/D_i)] \tag{3}$$

where

$$k = I t_i / z_i F \bar{C}_i^0. \tag{4}$$

Note that, since the fluxes Φ and β are defined in units of current, they are negative for anions. Since there is no current flowing when the potential difference is zero, β_i^0 and Φ_i^0 are not defined by Eqs. (2), (3) and (4).

To obtain β_i^{ψ} and Φ_i^{ψ} at $I=0$, we apply L'Hospital's rule by differentiating both numerator and denominator with respect to k, and there follows immediately

$$\beta_i^0 = \Phi_i^0 = z_i F J_i^0 = z_i F D_i \bar{C}_i^0 / \delta. \tag{5}$$

By substituting Eq. (5) into Eq. (1) we obtain

$$\ln(\Phi_i^{\psi}/\beta_i^{\psi}) = -I t_i / z_i F J_i^0. \tag{6}$$

In Eq. (6), however, $I t_i$ can be substituted by $\beta_i^{\psi} - \Phi_i^{\psi}$. Hence, Eq. (6) can be rearranged, yielding

$$\ln(\Phi_i^{\psi}/\beta_i^{\psi}) = (\Phi_i^{\psi} - \beta_i^{\psi})/\Phi_i^0 = (\Phi_i^{\psi} - \beta_i^{\psi})/\beta_i^0. \tag{7}$$

This equation can be stated as follows: the unidirectional flux (of a passively transported ion) at zero potential is the logarithmic mean of the bidirectional fluxes at any other potential. This equation can also be readily obtained from the equations of Kedem and Essig [8].

Although the transport number of the test species for passive flow (t_i) can be calculated from the net flux, Krämer and Meares [9] have shown that t_i can also be calculated from the unidirectional tracer fluxes. However, the method of formulating the equations was not described explicitly, and the application of the equations to biological systems has not been previously attempted.

It is necessary, therefore, to reexamine their equations before we can use them. By rearranging Eq. (6) we immediately have

$$t_i = \frac{z_i F J_i^0}{I} \ln\left(\frac{\beta_i^\psi}{\Phi_i^\psi}\right). \tag{8}$$

By substituting β_i^ψ by $I t_i + \Phi_i^\psi$ and $z_i F J_i^0$ by Φ_i^0 and rearranging the resulting equation, there follows

$$t_i = (\Phi_i^0/I) \ln(1 + I t_i/\Phi_i^\psi). \tag{9}$$

Moreover, if we rewrite Eq. (6) as

$$t_i = -\frac{z_i F J_i^0}{I} \ln(\Phi_i^\psi/\beta_i^\psi) \tag{10}$$

and substitute Φ_i^ψ by $\beta_i^\psi - I t_i$ and $z_i F J_i^0$ by β_i^0, we obtain, by appropriate rearranging,

$$t_i = -(\beta_i^0/I) \ln(1 - I t_i/\beta_i^\psi). \tag{11}$$

For the case of cations for which $\beta_i^\psi > \beta_i^0$ and $\Phi_i^\psi < \Phi_i^0$, Eqs. (9) and (11) become

$$t_+ = (\Phi_+^0/I) \ln(1 + I t_+/\Phi_+^\psi) \tag{12}$$

and

$$t_+ = -(\beta_+^0/I) \ln(1 - I t_+/\beta_+^\psi). \tag{13}$$

For the case of anions for which $|\beta_i^\psi| < |\beta_i^0|$ and $|\Phi_i^\psi| > |\Phi_i^0|$,

$$t_- = -(|\Phi_-^0|/I) \ln(1 - I t_-/|\Phi_-^\psi|) \tag{14}$$

and

$$t_- = (|\beta_-^0|/I) \ln(1 + I t_-/|\beta_-^\psi|). \tag{15}$$

A graphical method of finding t_i from these equations is given by Krämer and Meares [9].

Additional useful expressions for unidirectional fluxes in terms of ψ and g_i can be obtained by combining the definitions

$$t_i = z_i F \overset{\Delta}{J}_i^{\psi} / I = g_i / g_p \tag{16}$$

and

$$z_i F \overset{\Delta}{J}_i^{\psi} = \psi g_i \tag{17}$$

with Eqs. (12)–(15). For cations,

$$\Phi_i^{\psi} = \psi g_i / [\exp(\psi g_i / \Phi_i^0) - 1] \tag{18}$$

$$\beta_i^{\psi} = \psi g_i / [1 - \exp(-\psi g_i / \beta_i^0)] \tag{19}$$

and for anions

$$|\Phi_i^{\psi}| = \psi g_i / [1 - \exp(-\psi g_i / |\Phi_i^0|)] \tag{20}$$

$$|\beta_i^{\psi}| = \psi g_i / [\exp(\psi g_i / |\beta_i^0|) - 1]. \tag{21}$$

Furthermore, combining Eqs. (18) and (19) or Eqs. (20) and (21) we immediately obtain, by appropriate rearranging,

$$\ln(\Phi_i^{\psi} / \beta_i^{\psi}) = -Q_i z_i F \psi / RT \tag{22}$$

where Q_i is defined, for generality, as

$$Q_i = \frac{RT g_i}{z_i^2 F^2 J_i^0} \tag{23}$$

which is positive since $z_i^2 F^2 J_i^0$ is always positive for all i. Clearly, Eq. (22) differs from Ussing's flux ratio equation by a factor of Q_i.

Q_i can also be expressed in terms of a constant ratio of bulk diffusion coefficient to tracer diffusion coefficient. If we compare Eq. (22) with Eq. (1), there follows immediately

$$Q_i = \frac{I t_i \delta RT}{z_i^2 F^2 \psi \bar{C}_i^0 D_i} = \frac{1}{D_i} \cdot \frac{I t_i \delta RT}{\bar{C}_i^0 z_i^2 F^2 \psi}. \tag{24}$$

Our next task is to relate the parameter $(I t_i \delta RT / \bar{C}_i^2 z_i^0 F^2 \psi)$ to the bulk diffusion coefficient for the test species D. D is related to the net flux of the test species $\overset{\Delta}{J}_i^{\psi}$ by the Nernst-Planck equation, for systems with gradients

of concentration and electrical potential, in the absence of electroosmotic solvent drag,

$$\overset{\Delta}{J}_i^{\psi} = D\left(\frac{dC_i}{dx} + \frac{\bar{C}_i^0 z_i F}{RT}\frac{d\psi}{dx}\right) \tag{25}$$

from which

$$z_i F \overset{\Delta}{J}_i^{\psi} = D z_i F\left(\frac{dC_i}{dx} + \frac{\bar{C}_i^0 z_i F}{RT}\frac{d\psi}{dx}\right). \tag{26}$$

In Eq. (26), D is assumed to be a constant, independent of x. However, D could also be a function of x, in which case D must be included in the integration of Eq. (26). Since at steady state $z_i F\overset{\Delta}{J}_i^{\psi}$ is constant, we integrate Eq. (26) over the thickness of the membrane from $x=0$ to $x=\delta$. There follows

$$z_i F \overset{\Delta}{J}_i^{\psi} = D z_i F\left(\frac{\Delta C_i}{\delta} + \frac{\bar{C}_i^0 z_i F\psi}{RT\delta}\right). \tag{27}$$

In the absence of a concentration gradient, $\Delta C_i = 0$ and hence Eq. (27) becomes

$$z_i F \overset{\Delta}{J}_i^{\psi} = \frac{D\bar{C}_i^0 z_i^2 F^2 \psi}{RT\delta} = g_i \psi. \tag{28}$$

In Eq. (28), however, $z_i F\overset{\Delta}{J}_i^{\psi} = I t_i$ and hence we obtain, by rearranging,

$$D = \frac{I t_i \delta RT}{\bar{C}_i^0 z_i^2 F^2 \psi}. \tag{29}$$

Substitution of Eq. (29) into Eq. (24) finally yields

$$Q_i = D/D_i. \tag{30}$$

Eq. (30) indicates that Q_i can be alternatively expressed as a function of the two parameters, the bulk diffusion coefficient D and the tracer diffusion coefficient D_i.

Results

Observed Undirectional Fluxes after Ouabain

Table 1 summarizes the results. Replacement of chloride by sulfate ions did not change open-circuit potential, but replacement of bicarbonate by phosphate ions (in sulfate Ringer's) was associated with a significant fall (from 101 mV to 86 mV). Fluxes of sodium ions at zero potential after ouabain treatment were the same in both directions in all three media, as were fluxes of chloride in normal Ringer's and fluxes of sulfate in the chloride-

Table 1. Sodium, chloride and sulfate fluxes at 0 mV and 100 mV after ouabain. Observed data

Medium	Electrical parameters		Sodium fluxes (μA/mg)				Anion fluxes [c] (μA/mg)			
	ψ_{spont} [a] (mV)	I [b] (μA/mg)	Φ^0	β^0	Φ^{100}	β^{100}	Φ^0	β^0	Φ^{100}	β^{100}
HCO_3,Cl	101 ±3 (27)	0.905 ±0.053 (27)	0.15 ±0.01 (7)	0.14 ±0.01 (7)	0.05 ±0.01 (5)	0.37 ±0.02 (6)	0.19 ±0.03 (5)	0.18 ±0.03 (12)	0.39 ±0.02 (4)	0.08 ±0.03 (12)
HCO_3,SO_4	101 ±4 (16)	0.51 ±0.02 (16)	0.13 ±0.01 (6)	0.13 ±0.02 (5)	0.05 ±0.01 (5)	0.24 ±0.03 (5)	0.061 ±0.014 (3)	0.055 ±0.013 (3)	0.166 ±0.055 (3)	0.019 ±0.006 (3)
HPO_4,SO_4	86 ±4 (15)	0.61 ±0.04 (15)	0.14 ±0.02 (8)	0.13 ±0.02 (8)	0.04 ±0.01 (4)	0.36 ±0.04 (5)	0.071 ±0.020 (3)	0.077 ±0.008 (3)	0.203 ±0.030 (3)	0.014 ±0.005 (3)

Values given are means ± SEM. Number of observations in parentheses.

[a] Before ouabain.

[b] I = current required to clamp potential to 100 mV after ouabain.

[c] Cl or SO_4.

Table 2. Sodium, chloride and sulfate conductances and transport numbers

Medium	Conductances (μmho/mg)			Transport numbers [a]			
	g^p	g_+	g_-	t_+		t_-	
				(1)	(2)	(1)	(2)
HCO_3, Cl	9.05 ± 0.53 (27)	3.12 ± 0.22 (11)	3.14 ± 0.34 (15)	0.35 ± 0.02 (11)	0.35	0.36 ± 0.03 (16)	0.35
HCO_3, SO_4	5.12 ± 0.02 (16)	1.91 ± 0.24 (10)	1.59 ± 0.35 (6)	0.37 ± 0.04 (10)	0.37	0.28 ± 0.04 (6)	0.29
HPO_4, SO_4	6.11 ± 0.40 (15)	3.28 ± 0.34 (9)	2.15 ± 0.20 (6)	0.50 ± 0.03 (9)	0.52	0.35 ± 0.04 (6)	0.31

[a] Calculated from (1) unidirectional fluxes and current, and (2) mean net flux and mean current.

free media. Thus none of these ions was subject to active transport under these conditions.

Derived data from these experiments are summarized in Table 2. Ouabain-insensitive conductance was reduced 43% ($p < 0.01$) on replacing chloride by sulfate but was scarcely affected on replacing bicarbonate by

Table 3. Sodium and chloride transport numbers calculated from serosal-to-mucosal fluxes (t^{β}) compared to those from mucosal-to-serosal fluxes (t^{Φ}) in normal bicarbonate Ringer's

	t^{β}_{Na}	t^{Φ}_{Na}	t^{β}_{Cl}	t^{Φ}_{Cl}
Mean	0.35	0.36	0.34	0.36
SEM	±0.01	±0.03	±0.02	±0.03
n	(7)	(6)	(6)	(6)

phosphate. Ionic conductance not accounted for by the measured ions is considered below.

Transport numbers were calculated in two ways (*see* Table 2): (*1*) from unidirectional fluxes at two potentials, in individual experiments, by Eqs. (12)–(15), and (*2*) from the mean net flux at 100 mV, and mean current. Transport numbers were essentially the same whether calculated by the first or second method, in six comparisons (Table 2 and Table 3).

t_{Na} and t_{Cl} were both 0.35 to 0.36 in normal bicarbonate Ringer's, and g_{Na} and g_{Cl} (calculated in individual experiments as $t_{\pm} g^{p}$) both averaged 3.1 μmho/mg. Replacement of chloride by sulfate reduced g_{Na} significantly ($p<0.01$) but did not alter t_{Na}. However, replacement of bicarbonate by phosphate (in sulfate Ringer's) increased both t_{Na} and g_{Na} significantly. Sulfate conductance was less than chloride conductance and was unaffected on replacement of bicarbonate by phosphate.

Unaccounted-for conductance, calculated as $g_p - g_{Na} - g_{Cl}$ or $g_p - g_{Na} - g_{SO_4}$, was greatest in normal Ringer's solution (2.79 ± 0.67 μmho/mg), was insignificantly reduced by replacing chloride with sulfate (to 1.62 ± 0.42 μmho/mg) but was significantly ($p<0.05$) reduced (to 0.68 ± 0.56 μmho/mg), and made insignificantly different from zero, on replacing bicarbonate by phosphate.

These effects can be rationalized as follows: after ouabain, sodium transport may be facilitated by (or coupled with) the transport of chloride and/or phosphate ions. Passive bicarbonate transport probably accounts for the unidentified conductance, indicating a relatively high permeability for this ion.

Tests of the Flux Ratio Equation

Eq. (7) indicates that the unidirectional flux (in either direction) at zero potential should be the logarithmic mean of the two fluxes at 100 mV. In Fig. 1 is shown a comparison of the mean of the two mean unidirectional fluxes at zero potential with the value predicted from the mean fluxes at 100 mV. The correspondence is within experimental error.

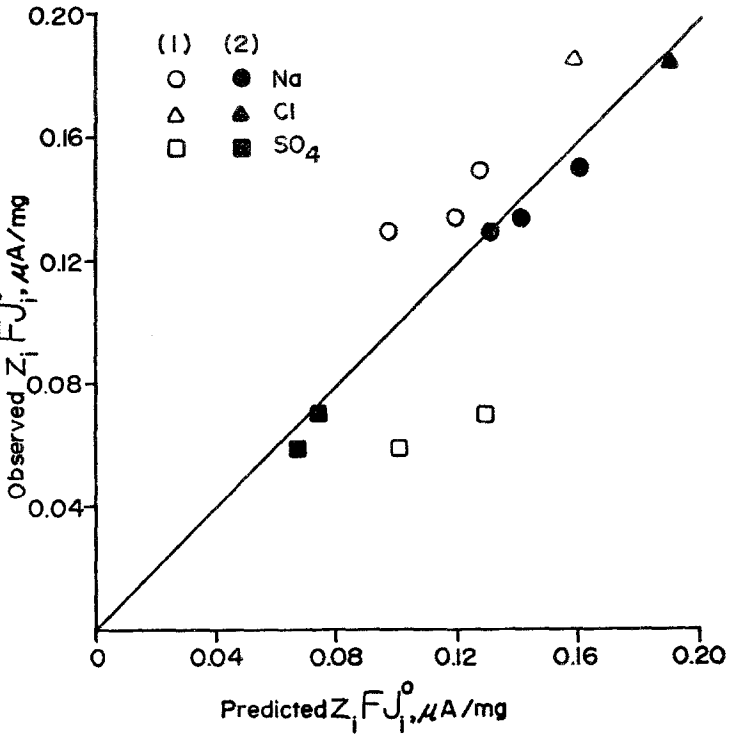

Fig. 1. Mean of bidirectional fluxes at zero potential ($z_i FJ_i^0$) observed (on vertical axis) and predicted (on horizontal axis). Solid symbols indicate values predicted from Eq. (7), representing constant ratio of bulk diffusion coefficient to tracer diffusion coefficient. Open symbols indicate values predicted from Eqs. (34) and (35), representing constant exchange diffusion. The former are consistent with the data but the latter are not

A comparison was also made with the flux at zero potential predicted from a model in which two parallel pathways exist, one in which the Ussing flux ratio equation is obeyed and one in which bidirectional fluxes are equal and constant (i.e., independent of potential). This is one model for exchange diffusion [2, 3, 12]. Exchange diffusion fluxes, $z_i FJ_e$, can be estimated from Φ_i^ψ and β_i^ψ. If $\Phi_i^{\psi U}$ and $\beta_i^{\psi U}$ are the unidirectional fluxes obeying the Ussing equation, then

$$\Phi_i^\psi = \Phi_i^{\psi U} + z_i FJ_e \tag{31}$$

$$\beta_i^\psi = \beta_i^{\psi U} + z_i FJ_e \tag{32}$$

with

$$\Phi_i^{\psi U} = \beta_i^{\psi U} \exp(-z_i F\psi/RT). \tag{33}$$

Making use of Eq. (33) we solve Eqs. (31) and (32) for $z_i FJ_e$ and obtain thereby

$$z_i FJ_e = \frac{\Phi_i^\psi - \beta_i^\psi \exp(-z_i F\psi/RT)}{1-\exp(-z_i F\psi/RT)}. \tag{34}$$

Table 4. Bidirectional sodium fluxes in ouabain-poisoned toad bladders at various clamping potentials and at zero potential[a]

Exp. no.	Clamping potential, ψ (mV)	Φ^0 (μA/mg)	β^0 (μA/mg)	Φ^ψ (μA/mg)	β^ψ (μA/mg)	$z_iFJ_i^0$ obs. (μA/mg)	calc. (μA/mg)
1	100	0.07	0.08	0.01	0.27	0.075	0.079
2	50	0.09	0.08	0.03	0.16	0.085	0.078
3	50	0.17	0.10	0.04	0.22	0.135	0.105
4	35	0.19	0.12	0.09	0.24	0.155	0.153

[a] Average of bidirectional fluxes at zero potential, $(\Phi^0+\beta^0)/2$, shown in the column labeled "obs.", is nearly equal to the logarithmic mean of bidirectional fluxes at the clamping potential, $(\Phi^\psi-\beta^\psi)/(\ln \Phi^\psi/\beta^\psi)$, shown in the column labeled "calc.".

Table 5. Ratio Q_i of bulk diffusion coefficients to tracer diffusion coefficients for Na, Cl and SO_4 ions obtained from $Q_i=RTg_i/z_i^2F^2J_i^0$

Medium	Ion	Q_i
HCO_3,Cl	Cl	0.44 ± 0.04 (12)
HCO_3,Cl	Na	0.55 ± 0.04 (10)
HCO_3,SO_4	Na	0.40 ± 0.03 (11)
HPO_4,SO_4	Na	0.76 ± 0.08 (11)
HPO_4,SO_4	SO_4	0.43 ± 0.08 (6)
HCO_3,SO_4	SO_4	0.40 ± 0.10 (7)

Eq. (34) can be used to estimate $z_iFJ_i^0$ from the equation

$$z_iFJ_i^0=g_iRT/z_iF+z_iFJ_e \tag{35}$$

where the parameter, g_iRT/z_iF equals the Ussing unidirectional flux at zero potential.

To illustrate, we calculated z_iFJ_e at 100 mV from Eq. (34) and g_iRT/z_iF and $z_1FJ_i^0$ from Eq. (35) for sodium and chloride ions in HCO_3,Cl medium at 25 °C. For sodium ions, $z_iFJ_e=0.043$ μA/mg; $g_iRT/z_iF=0.80$ μA/mg; and $z_iFJ_i^0=0.123$ μA/mg. For chloride ions, $z_iFJ_e=-0.074$ μA/mg; $g_iRT/z_iF=-0.081$ μA/mg; and $z_1FJ_i^0=-0.155$ μA/mg. Absolute values for $z_iFJ_i^0$ predicted from these equations differ considerably from measured values, as shown in Fig. 1. Thus, constant exchange diffusion is less consistent with the data than constant ratio of bulk diffusion coefficient to tracer diffusion coefficient.

Four experiments in which bidirectional sodium fluxes were measured, using ^{22}Na and ^{24}Na are shown in Table 4. The last two columns show the close correspondence between observed and predicted $z_i F J_i^0$.

From Eqs. (23) and (30) the ratio of D/D_i can be expressed as $Q_i = RTg_i/z_i^2 F^2 J_i^0$. As shown in Table 5, this ratio is less than unity for all three ions, to varying degrees. Q_{Na} is reduced by replacement of chloride by sulfate but is increased when phosphate replaces bicarbonate. These changes may be attributable to coupling between the flux of sodium and of other ions. Q_{SO_4} is the same in bicarbonate-buffered and phosphate-buffered media.

Discussion

The most important aspect of this work is the evidence pertaining to the validation of a simple theoretical model for tracer fluxes across a biological membrane, derived from previous work [4, 8–10]. The results for three ions, studied in different media, are very close to the predictions of this model. In previous studies of biological membranes, negative deviations (i.e., $Q<1$ or $|\delta \ln f/\delta \psi| < |z_i F/RT|$) from the Ussing flux ratio equation have been interpreted as indicative of exchange diffusion, often with the implication of a mobile carrier. Since such transport cannot carry current, it is generally assumed [2, 3, 12] to be independent of potential. But as Eq. (22) shows, constant diffusive flux of this type is not consistent with constant Q_i. The present data, for example, cannot be described by two parallel pathways, one in which $|\ln f_i| = |z_i \psi F/RT|$ and the other in which bidirectional fluxes are equal and independent of potential. As DeSousa *et al.* [4] have pointed out, what appears to be carrier-mediated exchange could be coupling between tracer and nontracer flows in a fixed-charge membrane.

Positive deviations ($Q_i > 1$) from the Ussing equation have also been observed [6], and have been attributed to single-file pore diffusion. While this may be the correct mechanistic interpretation, it is interesting to note that the equation used to describe such effects, $\ln f = n(C_1 C_2)\, \psi F/RT$, has the same form as Eq. (22), with $Q = n(C_1 C_2)$.

Eq. (7) can be used to obtain the third flux if any two of the three fluxes, Φ_i^ψ, β_i^ψ, and $z_i F J_i^0$ ($= \Phi_i^0 = \beta_i^0$) are known. Ionic conductance g_i is then readily obtained as $g_i = (\beta_i^\psi - \Phi_i^\psi)/\psi$, as well as the coefficient Q_i. It is then possible to predict bidirectional fluxes at any other potential within the range of validity of the equations. In general, this should include the range in which g_i is independent of ψ.

Table 6. Comparison of reported fluxes[a] of passively transported ions with predictions of Eq. (7), and estimates of Q_i

Tissue	Ion	$\lvert\psi\rvert$ (mV)	Φ_i^ψ	β_i^ψ	$z_iFJ_i^0$ obs.[b]	$z_iFJ_i^0$ pred.	Q_i	Ref.
Toad bladder	Cl	75	0.24	0.12	0.18	0.17	0.24	[15]
Frog stomach	Na	60	0.26	1.49	0.73	0.70	0.73	[7]
Frog stomach	K	60	0.20	0.67	0.31	0.39	0.67	[7]
Frog large intestine	Cl	45	2.17	1.17	1.42	1.62	0.66	[2]
Rabbit ileum	Cl	25	4.2	10.5	7.15	6.88	0.94	[11]

[a] Units of fluxes vary among these reports.
[b] *See* Table 4 for explanation of "obs." and "pred.".

Some comparisons of published data with predictions of this model are shown in Table 6. Reasonably close correspondence is seen. It is interesting to note that negative deviation ($D_i > D$) is apparently present in most of these studies, to varying extents. Further analysis of these systems would be required to ascertain whether constant exchange diffusion fits the data less well or better than constant Q_i.

Extension of equations of this general form to actively transported ions has been considered theoretically by Essig [5]. Adequate data are not yet available to permit a test of their applicability under these circumstances. Although previous data are available on sodium fluxes at varying potentials in the toad bladder preparation used here [15, 16], calculation of fluxes in the active pathway by subtraction between these two sets of experiments is hazardous. Among other difficulties, ouabain may have altered passive permeability. Quantitatively, it is evident that Φ_{Na} consists almost entirely of ouabain-inhibitable flux, while β_{Na} is chiefly ouabain-insensitive, at both 0 and 100 mV. Further analysis of the characteristics of the active transport path is under investigation.

It also remains to be determined whether these equations can be applied to biological membranes in the presence of ionic concentration gradients. Despite the thermodynamic equivalence of electrochemical gradients induced by potential difference or concentration difference, it is possible that perturbations of the membrane properties might occur, or the deviation from the Ussing flux ratio equation could change.

These observations also shed some light on ion transport properties of the toad bladder. The relative insensitivity of Φ_{Cl}, β_{Cl} and β_{Na} to applied potential in previous work [15, 16] is now seen to be the result of the low ratio of bulk diffusion coefficient to tracer diffusion coefficient for Na and Cl.

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