**REVIEW**

**The lateral intercellular space as osmotic coupling compartment in isotonic transport**

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**Abstract**

Solute-coupled water transport and isotonic transport are basic functions of low- and high-resistance epithelia. These functions are studied with the epithelium bathed on the two sides with physiological saline of similar composition. Hence, at transepithelial equilibrium water enters the epithelial cells from both sides, and with the reflection coefficient of tight junction being larger than that of the interspace basement membrane, all of the water leaves the epithelium through the interspace basement membrane. The common design of transporting epithelia leads to the theory that an osmotic coupling of water absorption to ion flow is energized by lateral Na+/K+ pumps. We show that the theory accounts quantitatively for steady- and time dependent states of solute-coupled fluid uptake by toad skin epithelium. Our experimental results exclude definitively three alternative theories of epithelial solute–water coupling: stoichiometric coupling at the molecular level by transport proteins like SGLT1, electro-osmosis and a ‘junctional fluid transfer mechanism’. Convection-diffusion out of the lateral space constitutes the fundamental problem of isotonic transport by making the emerging fluid hypertonic relative to the fluid in the lateral intercellular space. In the Na+ recirculation theory the ‘surplus of solutes’ is returned to the lateral space via the cells energized by the lateral Na+/K+ pumps. We show that this theory accounts quantitatively for isotonic and hypotonic transport at transepithelial osmotic equilibrium as observed in toad skin epithelium in vitro. Our conclusions are further developed for discussing their application to solute–solvent coupling in other vertebrate epithelia such as small intestine, proximal tubule of glomerular kidney and gallbladder. Evidence is discussed that the Na+ recirculation theory is not irreconcilable with the wide range of metabolic cost of Na+ transport observed in fluid-transporting epithelia.

**Keywords** isotonic transport, Na+ recirculation theory, physical-mathematical modelling of epithelial transport, metabolic cost of active sodium transport, solute-coupled fluid transport, toad skin epithelium.

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Solute-coupled water transport is a basic function of transporting epithelia. Associated herewith is the capacity to produce a transportate that is isotonic with the external solutions of identical composition. Transepithelial movement of bulk water against an adverse osmotic gradient (uphill) is another general feature of transporting epithelia. Elucidating the mechanisms has been a major challenge for understanding...
fluid handling by kidney, nutrient absorption by the digestive system, the respiratory defence system of upper airways, formation of cerebrospinal fluid and the composition of secretions by exocrine glands. Before dealing with the question of how the epithelium at transepithelial osmotic equilibrium transports fluid of adjusted tonicity, clarification of the nature of the mechanism that couples transepithelial ion fluxes with the flow of water is required. Four mechanisms have been suggested and are still considered in the literature: (1) osmotic coupling in intraepithelial compartments (Curran 1960, Curran & MacIntosh 1962, Diamond & Bossert 1967); (2) stoichiometric coupling at the molecular level by specific transport proteins as for example the Na\(^{+}\)-glucose transporter, SGLT1 (Loo et al. 1996, Meinhild et al. 1998); (3) electro-osmotic coupling in a channel-forming protein of tight junctions (Fischbarg et al. 2006), and (4) a paracellular junctional fluid transfer mechanism (Hill & Shachar-Hill 2006). The primary aim of the present review is to discuss previously published and new experiments on the coupling mechanism in a tight epithelium. We provide compelling evidence that the lateral intercellular space is the coupling compartment which osmotically forces water through the epithelium energized by lateral Na\(^{+}\)/K\(^{+}\) pumps. Our studies lead to the more general conclusion that the three other coupling mechanisms listed above (2–4) can be excluded definitively for this type of epithelium. Finally, our conclusions are developed for discussing implications for solute-coupled fluid absorption by other vertebrate epithelia.

Method

Recording of water volume flow and ion transport

The epithelium of toad skin (Bufo bufo) was isolated by collagenase according to the method of Willumsen et al. (1992). Transepithelial fluid flow (J\(_{\text{v}}\)), current (I\(_{\text{T}}\), inward current positive) and voltage (V\(_{\text{T}}\), serosal bath grounded) were recorded in preparations exposed to amphibian Ringer’s solution on both sides (mm), 117.4 Na\(^{+}\), 2.0 K\(^{+}\), 1.0 Ca\(^{2+}\), 114 Cl\(^{-}\), 2.4 HCO\(_{3}^{-}\), 5 CH\(_{3}\)COO\(^{-}\), 5 glucose with a nominal osmotic concentration of 252.8 mOsm, and pH = 8.2 when equilibrated with atmospheric air (Nielsen & Larsen 2007). The osmotic concentrations of the bathing fluids were measured before and after each experiment using a VAPRO-5520 vapour pressure osmometer (Wescor, Logan, UT, USA) to check that the osmolarity of the bathing solutions was not changed to any measurable extent during the experiment. The transepithelial conductance (G\(_{\text{T}}\)) was calculated by the response of the clamping current to a ±10 mV voltage pulse (voltage clamp), or by the voltage response to a ±10 μA current pulse (current clamp). The current carried by the active Na\(^{+}\) flux was measured as the short circuit current, I\(_{\text{SC}}\) (V\(_{\text{T}}\) = 0) or expressed as the equivalent short-circuit current defined by:

\[
I_{\text{SC}}^{\text{rev}} = -G_{\text{T}} \times V_{\text{T}}.
\]

where V\(_{\text{T}}\) is the spontaneous transepithelial potential difference (I\(_{\text{T}}\) = 0).

In open circuit mode (I\(_{\text{T}}\) = 0) the active Na\(^{+}\) flux is accompanied by a flux of Cl\(^{-}\) of similar magnitude. With the transepithelial water flow (J\(_{\text{v}}\)) measured simultaneously, the osmotic concentration of the transported fluid could therefore be estimated by:

\[
2 \times \frac{I_{\text{SC}}^{\text{rev}}}{F} = \frac{J_{\text{v}}}{F}
\]

I\(_{\text{SC}}^{\text{rev}}\)/F always tends to overestimate the active Na\(^{+}\) flux at open circuit conditions, and the more so in preparations with a large shunt resistance, i.e. a low Cl\(^{-}\) conductance. Hence, the true osmotic concentration of the transported fluid is less than the estimates given by the method above. At standard physiological conditions the overestimation amounts to 10–12% (Larsen et al. 2007). Our major conclusion is that the epithelium has the capability of producing an isotonic as well as a hypertonic absorbate. Obviously, this conclusion is uninfluenced by the overestimation mentioned.

Physical–mathematical modelling of epithelial transport

The mathematical description of Larsen et al. (2002, 2006) was introduced in the present study with the following modifications:

(1) In the Na\(^{+}\)/K\(^{+}\) pump flux equations the reversal potential of the pump (E\(_{\text{pump}}^{\text{rev}}\) = −200mV) was included:

\[
J_{\text{Na}}^{\text{pump}} = f_{\text{Na}}^{\text{max}} \left( \frac{C_{\text{Na}}^{\text{cell}}}{C_{\text{Na}}^{\text{cell}} + C_{\text{Na}}^{\text{out}}} \right)^{3} \left( \frac{C_{\text{K}}^{\text{out}}}{C_{\text{K}}^{\text{cell}} + C_{\text{K}}^{\text{out}}} \right)^{2} \times (V_{m} - E_{\text{pump}}^{\text{rev}})
\]

(2) The mitochondria-rich (MR) cells were modelled as a separate pathway between the principal cells conducting Cl\(^{-}\) between the apical solution and the lateral intercellular space with a voltage- and time-dependent electrodiffusive permeability for Cl\(^{-}\).

(3) The set of equations were solved for time-dependent states, which will be discussed elsewhere. Generally, the transport equations for water and solutes are written as:
\[
\frac{dV}{dt} = \sum_j J_V \\
\frac{d(V_{G3})}{dt} = \sum_j J_s
\]

where \( V \) denotes the volume of the cell compartment or the lateral intercellular space, and \( J_V \) and \( J_s \) denote the water and solute fluxes, respectively, through the various membranes, with \( j \) indicating the membrane considered (\( j = 1-5 \)). In the steady case the left-hand side is zero and the solution of the system of equations proceeds as explained in Larsen et al. (2002, 2006). When studying transients, however, the time-dependent behaviour of Eqs. 2 and 3 needs to be simulated. To solve the equations in time we utilize second-order accurate, three-point backward difference schemes (Taylor expansion) as follows:

\[
\frac{1}{\Delta t} \left[ 3V^{(n)} - 4V^{(n-1)} + V^{(n-2)} \right] = \sum_j J_v^{(n)}
\]

\[
\frac{1}{\Delta t} \left[ 3(V_{G3})^{(n)} - 4(V_{G3})^{(n-1)} + (V_{G3})^{(n-2)} \right] = \sum_j J_s^{(n)}
\]

where index \( n \) refers to time \( t^n \), and \( \Delta t \) is the time-step, such that \( t^{n+1} = t^n + \Delta t \). Thus, the equations are solved for all variables with index \( n \) at time \( t = t^n \), leaving the remaining terms as known from the former time step. The equations are solved together with the equations for electro-neutrality and the compliance model. The solution of the total system of equations is carried out using Newton’s method employing the technique described in Larsen et al. (2002).

**Results**

**General remarks**

In this study, our experimental results are discussed with reference to the theory of solute-coupled water transport via osmosis in a lateral intercellular coupling compartment. In some cases its theoretical mechanistic behaviour is understood in non-mathematical intuitive terms. For predicting less obvious quantitative behaviour we employ physical–mathematical modelling programmed for *in silico* experiments.

In vertebrate epithelia the \( \text{Na}^+\text{/K}^+ \) pump is abundantly expressed in plasma membranes lining the lateral intercellular space, which has been demonstrated for frog skin (Mills et al. 1977), small intestine (Stirling 1972), gallbladder (Mills & DiBona 1978), urinary bladder (Mills & Ernst 1975), exocrine glands (Ernst & Mills 1977) and kidney proximal tubule. In the latter mentioned epithelium plasma membranes of basolateral infoldings display \( \text{Na}^+\text{/K}^+ \) pumps, as well (Kashgarian et al. 1985, Pihakaski-Maunsbach et al. 2003). Figure 1a which shows the intraepithelial distribution of \( \text{Na}^+\text{/K}^+ \) pumps in amphibian skin is reproduced from a study employing \([\text{H}]\)-ouabain for localizing the pump sites (Mills et al. 1977). It is noted that \( \text{Na}^+\text{/K}^+ \) pumps are found exclusively in the plasma membranes lining the lateral intercellular space, which is emphasized in Figure 1b showing that the transepithelial active \( \text{Na}^+ \) flux passes the lateral intercellular space (lis). With \( n \) osmolytes the water flux, \( J_V \), across the barriers between the lateral intercellular space and the bathing solutions (tight junction, \( tj \), and interspace basement membrane, \( ibm \)) is given by:

\[
J_V = L_P R T \sum_{j=1}^{n} \sigma_j AC_j - \Delta P
\]

\( L_P \) is the hydraulic conductance of \( tj \) or \( ibm \), \( R \) the universal gas constant, \( T \) the absolute temperature, \( \sigma_j \) the reflection coefficients of \( tj \) or \( ibm \), and \( \Delta P \) and \( AC_j \) the hydrostatic pressure difference and the concentration difference, respectively, across the barrier considered. With identical solutions and similar hydrostatic pressures on the two sides of the epithelium it is immediately recognized that: (1) The direction of water transport depends on the relative magnitude of the reflection coefficients of the membranes delimiting lis; in absorbing epithelia, \( \sigma_j > \sigma_{ibm} \), which is considered here. (2) Water flows into the epithelial cells from both sides while all of the water entering the epithelium exits across the interspace basement membrane \( (\sigma_j > \sigma_{ibm}) \).

**Relationship between \( \text{Na}^+ \) pumping and fluid transport at transepithelial osmotic equilibrium**

According to the theory it is hydrolysis of ATP by the \( \text{Na}^+\text{/K}^+ \) ATPase in the plasma membrane lining the lateral intercellular space (lis) that delivers the energy for driving fluid through the epithelium. The steady-state \( C_{lis}^{in} \) is given by the balance between pump influx across the lateral plasma membrane and leak fluxes across the tight junction and the interspace basement membrane. With constant leak permeabilities, as a first approximation \( C_{lis}^{in} \) is expected to increase linearly with the pump rate, and as \( C_{lis}^{in} \) (together with the concentration of a matching anion) provides the osmotic force for water uptake, we expect, and find (Windhager et al. 1959, Curran 1960, Nielsen 1997, Nielsen & Larsen 2007) a linear relationship between active \( \text{Na}^+ \) flux \( (J_{Na}) \) and volume flow \( (J_V) \). This is shown in Figure 2 with data obtained from 12 different preparations bathed on both sides with Ringer’s solution, i.e. the osmotic
Figure 1 Functional organization of transporting epithelia. (a) Localization of Na\(^+/K^+\) pump sites in ventral pelvic skin of *Rana catesbeiana* by \[^3^H\]ouabain labelling and autoradiography. The distribution of grains supports a model with the living cells of all layers constituting a functional syncytium and the sodium ions being pumped into the lateral intercellular space before they enter the serosal bath. Gr, granular layer; Sp, spiny layers; Ger, germinal layer. From Mills et al. (1977). (b) General model of solute-coupled water transport of a fluid absorbing epithelium exposed on the two sides to physiological saline of identical composition. Water enters the epithelial cell compartment from both sides and leaves the epithelium through ibm. This principle is independent of the mechanisms by which solutes enter the epithelium. To avoid crowding of symbols, Na\(^+\) and Cl\(^-\) enter the epithelium via two different cells and K\(^+\) is omitted. The apical, lateral and serosal plasma membrane are indicated by am, lm and sm respectively. tj, tight junction; ibm, interspace basement membrane; lis, lateral intercellular space.

Concentrations on the two sides of the epithelium are identical. The two fluxes decrease with similar time courses after the addition of ouabain to the serosal bath confirming the dependence of \(j_V\) on the activity of the Na\(^+/K^+\) pump (see Fig. 3). Very likely, the slow time course of inhibition reflects that ouabain has to diffuse against an intraepithelial flow of fluid before binding to the pumps in the plasma membranes lining the labyrinth of intercellular spaces (cf. Fig. 1a).

It is easy to understand that as water is at thermodynamic equilibrium across the epithelium increasing the hydraulic conductance of the plasma membranes or the interspace barriers does not affect the rate of volume transport provided the Na\(^+\) pump flux and Na\(^+\) leak fluxes remain constant. Importantly, such an increase results in a smaller steady-state \(C_{NaCl}^{lis}\) which is of significant physiological importance as it reduces the diffusion flux of Na\(^+\) across ibm and thereby the hypertonicity of the fluid emerging from lis relative to the tonicity of lis (Larsen et al. 2002). Similarly, it is intuitively comprehended that with constant pump rate and hydraulic conductances the ratio of \(j_V/j_{Na}\), which is a measure of the efficiency of coupling, is affected by the leak permeabilities for Na\(^+\). For example, \(j_V/j_{Na}\) decreases with increasing Na\(^+\) permeability of tj and ibm because this manoeuvre leads to a smaller \(C_{NaCl}^{lis}\), that is the osmotic driving force of the water flux from the outside solution into lis is being reduced.
The membrane-impermeable non-electrolyte sucrose was used for reducing the water activity of the outer bath (corneal side) of the epithelium (Nielsen & Larsen 2007). Figure 4a shows that while $J_{Na}$ stayed near its control value prior to the osmotic challenge, the simultaneously recorded $J_V$ was reduced stepwise in response to a stepwise increase in the external sucrose concentration. In this experiment the water flow reversed at a transepithelial osmotic concentration difference of −34 mOsm (Fig. 4b). From the slope of the linear relationship and a molar water volume of $\sim$18 cm$^3$ mol$^{-1}$ H$_2$O (20 °C) we calculate an apparent water permeability of, $P_t = 8.6 \times 10^{-3}$ cm$^3$ s$^{-1}$. Obviously, this is not the true water permeability of the epithelium as the osmotic driving force for water flow into the epithelium is given by the difference in osmotic concentration between liss and the outside solution (cf. Eqn 6 above). This is an unknown quantity which has never been estimated precisely in experiments with transporting epithelia, but it is supposed much smaller than the transepithelial osmotic gradient (Weinstein & Stephenson 1981, Larsen et al. 2000).

The concentration of the transported fluid

Figures 3 and 6a show that stimulation of $J_{Na}$ by the $\beta$-adrenergic agonist isoprotenerol results in a parallel stimulation of $J_V$. Likewise, inhibition of $J_{Na}$ with amiloride is associated with inhibition of $J_V$ (Fig. 6a) confirming that $J_V$ is depending on and coupled to $J_{Na}$. At open circuit conditions ($I_T = 0$), the active flux of Na$^+$ generates a transepithelial electrical potential...
Figure 4 Uphill water transport by toad skin epithelium. (a) Effect on active Na⁺ flux and fluid transport of adding increasing concentrations of sucrose (numbers on the graph in mM) to the solution bathing the outside of the epithelium at open-circuit conditions. (b) Variation of steady-state rate of fluid transport with the difference in osmotic concentration between outside and inside solution. \( \Delta C = C_{\text{inside}} - C_{\text{outside}} \) with excess osmotic concentration of the outside bath obtained by adding sucrose to the Ringer’s solution. The direction of fluid flow was estimated to reverse at \( \Delta C_{\text{rev}} = -34 \) mOsm.

Figure 5 Model computations simulating the experiment shown in Figure 4a,b. (a) A non-permeant electroneutral solute was added to the external ‘Ringer’s solution’ in steps of 5 mM with \( F_I \) clamped at 0 (open-circuit conditions). At each step, the concentration was increased exponentially with a time constant of 5 s. Shown are the time courses of the active Na⁺ flux and the rate of fluid transport given by the model. (b) Computed steady-state dependence of rate of fluid transport on transepithelial osmotic concentration difference. \( \Delta C = C_{\text{inside}} - C_{\text{outside}} \) with excess osmotic concentration of the outside compartment obtained by adding non-permeant electroneutral molecules on top of the ‘Ringer’s solution’ of the outside compartment [see (a)]. The direction of fluid flow reverses at \( \Delta C_{\text{rev}} = -31 \) mOsm.

difference that drives Cl⁻ inwardly at a rate similar to that of Na⁺. As no other solutes pass the epithelium, the osmotic concentration of the transported fluid can be estimated by \( 2J_{\text{Na}}/J_{V} \). The values thus obtained overestimate the true osmotic concentration of the transported fluid by 10–12% prior to and following isoproterenol stimulation (see Method section). The osmotic concentrations calculated by the above method are indicated in Figures 3 and 6a with a summary given in Figure 6b. On average, prior to hormone stimulation the tonicity of the transported fluid tends to be hypertonic, whereas the fluid becomes near-isotonic following isoproterenol stimulation. With amiloride in the outside bath the transepithelial active Na⁺ flux decreased relatively more than the flux of water resulting in a new steady state with a highly significant hypotonic transportate at the transepithelial osmotic equilibrium (Fig. 6a,b and comments to Table 1 below). In other words, the transported fluid is significantly diluted (< 62 ± 12 mOsm, mean ± SEM, \( n = 7, P < 10^{-3} \)) with respect to the bathing solutions of Ringer’s strength (253 mOsm). As discussed in Nielsen & Larsen (2007) and below, this indicates that a fraction of the sodium ions pumped into the lateral space is derived from the serosal bath, which therefore does not contribute to the transepithelial net uptake of Na⁺ and electrical charge flux.

**ENaC inhibition by amiloride**

The experiments shown above make it evident that the volume flow is coupled to the transepithelial active flux of Na⁺ (Figs 2, 3 and 6a). It turned out that these variables could be dissociated temporarily following fast block of the apical ENaC by amiloride, which was associated with slow decrease in \( J_{V} \) with a half time of ~8 min (Fig. 7a). The rate at which \( J_{\text{Na}} \) decays depends...
on how fast amiloride blocks ENaC. Essentially, this is determined by the unstirred layer at the apical plasma membrane. According to the theory, the time course of $J_V$ inhibition depends on the rate at which the intracellular Na$^+$ pool is emptied by the lateral Na$^+/K^+$ pumps. The model computations shown in Figure 7b confirm that with a physiological value of the cellular Na$^+$ pool, the half-time of the pool would be relatively slow, $T_{50} = 5.8$ min. In the model, the Na$^+$ pool of the syncytium of principal cells prior to 'amiloride' treatment was 76 nmol cm$^{-2}$, corresponding to a total cell water volume of 7.2 $\mu$L cm$^{-2}$ with $C_{Na^+}^{cell} = 10.6$ mm.

The reduced activity of the lateral Na$^+/K^+$ pumps results in depletion of the NaCl pool of lis associated with a decrease in the volume of lis from 516 to 353 nL cm$^{-2}$ and a parallel decrease in its excess hydrostatic pressure from 0.863 to 0.220 cmH$_2$O. These numbers cannot be verified experimentally. They are mentioned here to show that with a hydraulic conductance of ibm that is 1–2 orders of magnitudes larger than that of tj, and with the experimentally estimated compliance factor of epithelial plasma membranes (Spring & Hope 1979) the hydrostatic pressure forcing fluid out of lis is expected to be small.

**Water flow associated with a passive Cl$^-$ flux through mitochondria-rich cells energized by transepithelial voltage and current clamps**

The apical plasma membrane of principal cells is impermeable to Cl$^-$ even in preparations with maximally stimulated transepithelial Cl$^-$ conductance (Willumsen & Larsen 1986, Willumsen et al. 1992). The major physiological flux of Cl$^-$ goes through the MR cells. These bottle-shaped cells are located in the

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**Figure 6** (a) Time course of $F_{sc}^{EVP} / F$ (an estimate of $J_{Na^+}$) and rate of fluid transport in response to adding a $\beta$-adrenergic agonist to the serosal bath and – subsequently – amiloride to the outside bath. The upper graph shows the time course of $2 F_{sc}^{EVP} / (F \times J_V)$ as an estimate of the osmotic concentration of the transported fluid. Data from (Nielsen & Larsen 2007). As mentioned in the text $2 F_{sc}^{EVP} / (F \times J_V)$ represents a 10–12% overestimation of the true osmotic concentration prior to amiloride treatment (Larsen et al. 2007). See also Table 1. (b) Summary of results obtained by the experimental protocol indicated in (a) (mean ± SEM, $n = 7$).

**Figure 7** Transient dissociation of the volume flow from the active Na$^+$ flux. (a) Time course of active Na$^+$ flux and rate of fluid absorption in short-circuited toad skin epithelium following addition of 100 $\mu$m amiloride to the external solution. (b) Results given by the model with a protocol similar to that of the experiment shown in (a). The apical Na$^+$ permeability was reduced exponentially with a time constant of 10 s to 1% of its control value prior to ‘amiloride’. The much slower decrease in $J_V$ proceeds with a half time of ~6 min.
outer region of the epithelium with the neck between the outermost principal cells so that the apical plasma membrane faces the subcorneal space like the ENaC expressing apical plasma membrane of principal cells (see Fig. 8a). The apical plasma membrane of the most abundant type of MR cells, the γ-type MR cell (Larsen 1991), displays a population of Cl− selective channels that is controlled by the apical membrane potential. The Cl− channels are slowly activated by membrane depolarization brought about by transepithelial hyperpolarization, which results in a strongly rectified steady-state transepithelial Cl− current (Fig. 8b). The definitive proof that this is a specific function of this minority cell type was obtained by voltage clamp experiments with isolated MR cells (Fig. 8c,d), which generated currents accounting quantitatively for the macroscopic voltage-activated currents with respect to their magnitude and time course (Larsen et al. 2001) confirming earlier studies relating macroscopic Cl− fluxes to the density of MR cells (Vouite & Meier 1978, Willumsen & Larsen 1986, Devuyst et al. 1990). The apical Cl− channels exhibit a large single channel conductance of 150–300 pS identified by noise analysis of voltage-activated Cl− currents in whole-cell patch-clamp studies (Larsen & Harvey 1994) and by single-channel studies on cell attached and inside-out patches (Sorensen & Larsen 1996). In the open state the channel is noisy with several substrates (Fig. 8e). The channel has been observed to open and close in a stepwise fashion. It has been difficult to study and is not characterized at the molecular level.

In the present context the above macroscopic features of the MR cells are useful for an independent way of testing the theory of the lateral space as an osmotic coupling compartment. From Figure 8a it can be concluded that a current flowing through the MR cells passes lis before entering the serosal bath. Therefore, following amiloride block of the transepithelial active Na+ flux we should be able to generate a graded flow of fluid in the inward direction by activating the apical Cl− channels of MR cells by transepithelial voltage or current clamping. The results shown in Figure 9a,b confirm this notion; both during activation in voltage clamp mode and at steady state during current clamps a direct dependence of Jv on JCl is observed. To a fair extent these experimental observations are reproduced by in silico experiments applying similar protocols (Fig. 9c,d). The other important outcome of these computations is that the Cl− concentration of the lateral space is predicted to be so close to that of the bathing solutions that it would be impossible to measure the difference with available methods.

**Discussion**

Historically, epithelial fluid transport in the absence of external driving forces for water was discovered more than 100 years ago by Edward Warmouth Reid in his...
**Figure 9** Cl$^-$ flux-generated transepithelial volume flows. (a) Variation of $J_V$ with $J_{Cl}$ during development of the depolarization activated Cl$^-$ conductance of the apical membrane of mitochondria-rich (MR) cells of a toad skin epithelium by a transepithelial voltage clamp from 0 to $-50$ mV (grounded serosal bath). The protocol was repeated five times as indicated by different colours. (b) Steady-state relationship between inward transepithelial Cl$^-$ flux through MR cells and transepithelial volume flow (data from one preparation, mean $\pm$ SEM, $n = 8$–11 data points). The straight line is the regression line according to the equation, $J_V = (1.98 \pm 0.15) \times 10^{-3} \times J_{Cl} + 0.68 \pm 0.22, R^2 = 0.993$. (c) Relationship between volume flow and chloride flux given by the model produced by shifting $V_T$ from 0 to $-50$ mV, thus simulating the experiments of (a). (d) Computed steady-state variation of $J_V$ with the Cl$^-$ flux through MR cells in transepithelial current clamp mode at a constant chloride permeability of the MR cell pathway. The steady-state Cl$^-$ concentration of lis is given for each clamping current.

**in vitro** studies on rabbit small intestine and frog skin. Reid concluded: ‘It would appear [...] that it is possible [...] to gain distinct, positive, evidence of vital absorptive action in the intestine’ (Reid 1892a, his accentuations) and, ‘there is present in the living skin of the frog a vital absorptive force dependent upon protoplasmic activity [...] and by virtue of this vital action the skin is actually able to cause a stream of fluid to pass from its outer to its inner surface’ (Reid 1892b). With his method and interpretations Reid was far ahead of his time, and more than half a century passed before similar **in vitro** studies were performed on other transporting epithelia that confirmed and generalized Reid’s findings, e.g. toad skin (Kalman & Ussing 1955), rat small intestine (Curran & Solomon 1957, Parsons & Wingate 1958, Curran 1960), proximal tubule of the glomerular nephron (Windhager et al. 1959), gallbladder (Diamond 1962, 1964), exocrine glands (Thayse & Thorn 1954) and upper airways (Grubb et al. 1997).

The coupling mechanism

A wide range of experimental observations provide strong evidence that the lateral space serves as the site of solute–water coupling in the amphibian skin epithelium.

(1) The dependence of water flow on active Na$^+$ transport indicates that Reid’s *vital absorptive action* is generated by Na$^+$,K$^+$-ATPase. This enzyme is localized in the plasma membranes lining the lateral intercellular space (Mills et al. 1977) (see Fig. 1a).

(2) The flow of water decreases following application of the Na$^+$,K$^+$ pump inhibitor ouabain (Fig. 3).

(3) The linear relationship between the active flux of Na$^+$ and the rate of transepithelial water transport follows logically when it is appreciated that lis is a pump-leak system. With constant leak permeabilities, $C_{Na}$ would increase linearly with the pump rate, whereby $J_V$ must also increase linearly with the pump rate (Fig. 2).

(4) Stimulation of $J_{Na}$ via intracellular cAMP-dependent protein kinase (PKA) signalling is paralleled by an increase in $J_V$ (Figs. 3 and 6a,b).

(5) It was predicted that the coupling of the transepithelial Na$^+$ flux and $J_V$ becomes temporarily dissociated by amiloride application because the time course of $J_{Na}$ inhibition is governed by the rate at which amiloride binds to the population of apical ENaC while the time course of $J_V$ inhibition is governed by the turnover of the intracellular Na$^+$ pool. The experimental results confirmed this notion (Fig. 7).

(6) A further test of the theory came from the experiments with voltage activation of the Cl$^-$ flux via...
MR cells. We reasoned that if the lateral space constitutes an osmotic coupling compartment we should be able to generate an inward flow of water by forcing Cl\textsuperscript{−} into the space by an external electromotive force. Our experiments made it plain that this reasoning was correct (Fig. 9).

(7) With no further assumptions uphill water transport follows logically from the theory of a lateral intercellular coupling compartment (Figs. 4 and 5).

(8) Our experimental results exclude the other common theories on the mechanism of solute-solvent coupling in epithelia. The flux of Na\textsuperscript{+} into the cellular compartment of amphibian skin depends on apical sodium channels with no contributions from Na\textsuperscript{+}-dependent co-transport mechanisms (Fuchs et al. 1977, Van Driessche & Lindemann 1979). This rules out the idea of Na\textsuperscript{+} gradient-driven molecular water pumps as the mechanism of epithelial fluid absorption (Zeuthen et al. 2001). The demonstration of water flow in the short-circuited epithelium with current flowing entirely through the cellular compartment (Fig. 2) and the dissociation of \( f_{\text{V}} \) from the ion flux during non-steady state (Fig. 7) rule out the theory of paracellular electro-osmosis developed by Fischbarg et al. (2006). The localization of Na\textsuperscript{+}K\textsuperscript{+} pumps in lateral plasma membranes (Fig. 1a), the demonstration of a Cl\textsuperscript{−} flux-generated water flow (Fig. 9) and lack of evidence for an ATP-driven ‘junctional fluid transfer mechanism’ in any known epithelium make it obvious that the theory of Hill & Shachar-Hill (2006) would have to be abandoned as well.

**Tonicity of transported fluid**

It is often believed that the physiological solution to isotonic transport is the development of a hydraulic conductance of the epithelium that is relatively large compared to the active flux of Na\textsuperscript{+}. Our experimental results and theoretical analyses indicate that it is not quite so simple.

The efficiency with which the steady-state ion flux drives water through the epithelium varies with the ion species (Figs 2 and 9b) and the physiological state of the preparation (Fig. 6b). For example, the slopes of 7.7 \( \times \) \( 10^{-3} \) nL H\textsubscript{2}O pmol\textsuperscript{−1} Na\textsuperscript{+} (Fig. 2) and 2.0 \( \times \) \( 10^{-3} \) nL H\textsubscript{2}O pmol\textsuperscript{−1} Cl\textsuperscript{−} (Fig. 9b) correspond to coupling ratios of 428 H\textsubscript{2}O/Na\textsuperscript{+} and 110 H\textsubscript{2}O/Cl\textsuperscript{−} respectively. A simple interpretation of the pump-leak model of the lateral space would suggest that the leak permeabilities of the barriers delimiting lis are different for the two ions. As the unidirectional Cl\textsuperscript{−} fluxes via the voltage-activated MR cells obey the flux-ratio equation (Larsen 1991), the activation of the Cl\textsuperscript{−} flux into the lateral space inevitably leads to a simultaneous activation of the leak permeability for Cl\textsuperscript{−}. A similar type of interpretation would be quite insufficient for explaining the different experimental results of Figure 6b with mean ratios of 304 (control), 487 (isoproterenol) and 1787 H\textsubscript{2}O/Na\textsuperscript{+} (isoproterenol + amiloride) respectively. The clue comes from the observation that the epithelium has the capacity to generate an isotonic as well as a hypotonic absorbate.

Diffusion-convection of solutes at the exit of lis creates a fundamental problem of isotonic transport, which is conveniently analysed by considering Hertz’s diffusion-convection equation (Larsen et al. 2000):

\[
\frac{f_{\text{V}}^{\text{bn}}}{f_{\text{V}}^{\text{bm}}} = \left(1 - \sigma_{\text{S}}^{\text{bn}}\right) \frac{C_{\text{s}}^{\text{lis}} - C_{\text{s}}^{\text{serosa}} \exp[-f_{\text{V}}^{\text{bm}}(1 - \sigma_{\text{S}}^{\text{bn}})/P_{\text{S}}^{\text{bm}}]}{1 - \exp[-f_{\text{V}}^{\text{bm}}(1 - \sigma_{\text{S}}^{\text{bn}})/P_{\text{S}}^{\text{bn}}]}
\]

where \( f_{\text{V}}^{\text{bn}} \) and \( f_{\text{V}}^{\text{bm}} \) are the solute and volume flux across the boundary, \( C_{\text{s}}^{\text{lis}} \) and \( C_{\text{s}}^{\text{serosa}} \) are the solute concentrations on the two sides of the boundary, while \( \sigma_{\text{S}}^{\text{bn}} \) and \( \sigma_{\text{S}}^{\text{bm}} \) are the reflection coefficient and solute permeability respectively. As a numerical example we will consider the rat proximal tubule with \( P_{\text{S}}^{\text{bn}} \approx 6.5 \text{ nL cm}^{-2} \text{s}^{-1} \) and \( R_{\text{b}}^{\text{bn}} \approx 0.6 \text{ cm}^{-2} \text{ cm}^{-2} \) corresponding to \( P_{\text{S}}^{\text{bm}} \approx 1.6 \times 10^{-3} \text{ cm}^{-1} \text{s}^{-1} \) (Frömter 1979, Weinstein 2008). With \( \sigma_{\text{S}}^{\text{bn}} = 10^{-3}, C_{\text{s}}^{\text{serosa}} = 300 \text{ mm} \) and \( C_{\text{s}}^{\text{lis}} = 301.5 \text{ mm}, \) Eqn 7 predicts a concentration of the fluid emerging from lis, \( f_{\text{S}}^{\text{bn}}/f_{\text{V}}^{\text{bm}} = 337.6 \text{ mm} \), which is hypertonic relative to lis and the serosal bath by \( \sim 12.5\% \) and should be compared to the hardly detectable difference in solute concentrations on the two sides of the boundary. In other words, a large \( P_{\text{S}}^{\text{bn}} \) relatively to \( f_{\text{V}}^{\text{V}} \) results in a diffusion flux that is comparable to the convection flux within an order of magnitude, implying that the solute concentration of the appearing fluid is larger than that of lis. With \( x = 0 \) at the tight junction and \( L \) being the length of the lateral space Diamond & Bossert (1967) assumed as boundary condition in their standing-gradient theory that, \( \partial C_{\text{s}}/\partial x \bigg|_{x=L} = 0 \) (see Fig. 10a). When fulfilled the diffusion flux at the boundary is zero and the emerging fluid truly isotonic.

In the Na\textsuperscript{+}-recirculation theory (Nedergraad et al. 1999) it is realized that the diffusion flux cannot be zero and, therefore, the solute concentration of the fluid emerging from lis tends to be hypertonic. The above boundary condition is here replaced by the assumption that isotonic transport is obtained by a simultaneous solute flux from the serosal bath via the epithelial cells into lis (Fig. 10b). Accordingly, the Na\textsuperscript{+}K\textsuperscript{+} pumps in the lateral plasma membrane play a dual role in fluid transport: (1) they make lis hypertonic and hyperbaric, and (2) they pump into lis the sodium ions entering the cells from the serosal bath thus ‘bringing back the diffusion component to the coupling compartment’. 

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In order for this mechanism to be robust over a wide range of physiological conditions we have to assume that the back flux of Na\(^+\) (the recirculation flux) is regulated for achieving the demanded osmotic concentration of the absorbate. Based upon the observation that \(J_V\) decreased in response to serosal bumetanide application (Nielsen & Larsen 2007) we hypothesized that the recirculation pathway of the serosal membrane is the NKCC transporter, which was discovered in studies on the regulation of volume and intracellular Cl\(^-\) concentration of frog skin principal cells (Dörge et al. 1983, 1989, Ussing 1985).

Our detailed experimental analyses of ion concentrations, membrane potentials and membrane conductances have provided sufficient data for modelling with reasonable confidence the amphibian skin epithelium in the resting state and during isoproterenol stimulation and amiloride treatment (see above and Larsen et al. 2007). Here we will focus on the most interesting part of the experimental results, which were obtained with amiloride resulting in a hypotonic absorbate (Fig. 6a,b). The computed results shown in Table 1 are compared with observed physiological variables obtained with double-barrelled microelectrodes in isolated voltage-clamped epithelia mounted in a mini-Ussing chamber on a microscope table (Larsen et al. 1992, Willumsen et al. 1992) and a determination of the Na\(^+\) transport pool with the isotope tracer technique (Nielsen 1982). Our computations confirm that a recirculation flux estimated from the intracellular Cl\(^-\) concentration together with experimentally obtained serosal plasma membrane potential and Cl\(^-\) conductance would generate a transepithelial fluid absorption of a magnitude and tonicity comparable to the measured observables.

**Applicability to other epithelia**

**The coupling mechanism.** All experimental evidence discussed in this paper shows that fluid absorption in the absence of transepithelial driving forces for water is brought about by osmotic coupling in the lateral intercellular compartment by a mechanism essentially conceived by Peter Curran in his studies on mammalian small intestine (Curran 1960). The similar distribution of the Na\(^+\)/K\(^+\) pumps in all studied transporting epithelia of vertebrates (Stirling 1972, Mills & Ernst 1975, Ernst & Mills 1977, Mills et al. 1977, Mills & DiBona 1978,

**Table 1** Experimental and computed biophysical parameters of the amphibian skin epithelium treated with 5 \(\mu\)M isoproterenol on the serosal side and 100 \(\mu\)M amiloride on the outside. The model is that of Figure 7b, but here computed for open circuit conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_T) (mV)</td>
<td>-1.5 (\pm) 0.7*</td>
<td>1.5 (\pm) 0.2*</td>
<td>1.5 (\pm) 0.8*</td>
<td>1.6 (\pm) 0.1</td>
<td>145 (\pm) 8*</td>
<td>51 (\pm) 3*</td>
<td>-99 (\pm) 3*</td>
<td>0.50 (\pm) 0.15*</td>
<td>62 (\pm) 12*</td>
</tr>
<tr>
<td>(G_T) (mS cm(^{-2}))</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>140</td>
<td>53.2</td>
<td>-96.5</td>
<td>0.43</td>
<td>69*</td>
</tr>
<tr>
<td>(I_{rec}) ((\mu)A cm(^{-2}))</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>140</td>
<td>53.2</td>
<td>-96.5</td>
<td>0.43</td>
<td>69*</td>
</tr>
<tr>
<td>(C_{cell Na}) (mM)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>(C_{cell K}) (mM)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>(C_{cell Cl}) (mM)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>(J_V) (nL cm(^{-2}) s(^{-1}))</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>(2.4) (\mu) (mOsm)</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
<td>69*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sum_{m}) (mOsm)</td>
<td>24*</td>
</tr>
</tbody>
</table>

*a Nielsen & Larsen (2007).
*b Nielsen (1982).
*c Larsen et al. (1992).
*d Willumsen et al. (1992).
*e The true osmotic concentration of the computed absorbate (24 mOsm) is significantly smaller than that calculated from the equivalent short circuit current (69 mOsm). The difference reflects the fact mentioned in the Method section that the active Na\(^+\) flux calculated from the equivalent short-circuit current is an overestimation of the active Na\(^+\) flux at open circuit conditions.
Kashgarian et al. 1985, Pihakaski-Maunsbak 2003) suggests that they share the same basic coupling mechanism. The abundant expression of Na+/K+ pumps in projections from the basal plasma membrane in the proximal tubule of the kidney (Maunsbach & Boulpaep 1991) suggests that this extracellular labyrinth constitutes a parallel coupling compartment. Unlike lis with highly conductive tight junctions (Frømter 1979) the basal infoldings are closed to the luminal solution indicating that a relatively large flow of water takes a transcellular route via aquaporins (Nielsen et al. 2002).

Nevertheless, another coupling mechanism has been suggested (Zeuthen et al. 2001). Zeuthen and co-workers assume that the steady-state isotonic water transport across the brush border of the small intestine and kidney proximal tubule is divided into two components of which one is generated by a stoichiometric coupling to Na⁺ and glucose fluxes via SGLT1, and the other is due to osmosis via a water channel in the SGLT1 protein of the small intestine or the apical AQP1 water channel of the proximal tubule. Common to these two models is the assumption that the epithelial cell becomes hyperosmotic because of the stoichiometry of 264 H₂O : 2 Na⁺ : 1 glucose of bSGLT1. It is true that with a volume of \( \sim 18 \text{ cm}^3 \text{ mol}^{-1} \text{ H}_2\text{O} \) and the above stoichiometry the coupled fluxes of the three substrates represent a hyperosmotic transportate (631 mOsm). It is incorrect, however, to assume that at steady state the cell water is hyperosmotic. On the contrary, the active transport of water into the epithelial cell results in a steady state where water is above thermodynamic equilibrium, i.e. the cell water becomes hyposmotic relative to the bathing solutions. This implies that if a water channel is displayed in the brush border membrane together with the above SGLT1 mechanism water recirculates across the apical plasma membrane. Hence the overall water uptake from the luminal solution becomes smaller than the supposed Na⁺ gradient-driven water flux through SGLT1, which is in contrast to Zeuthen and co-workers’ assumption (discussed with quantitative details in Larsen et al. 2002).

It has been further argued that an osmotic mechanism has to be abandoned or is questionable because attempts to detect a difference in osmotic concentration between lis and the bathing solutions have failed (Zeuthen 1983, Ikonomov et al. 1985). This argument is significantly weakened by our computations showing that with a relatively large ratio of apical water permeability and active Na⁺ flux the excess steady-state lateral intercellular ion concentrations would be too small to be detected with available methods. Our experiments and computations with Cl⁻ as the driving ion lead to the same conclusion. In connection with this, it is recalled that at a given rate of active Na⁺ absorption, a large water permeability of the barriers between lis and the luminal solution inevitably results in small steady-state osmotic concentration of lis. For example, in the highly water-permeable proximal tubule of the rat kidney the excess osmotic concentration was estimated to be less than 1 mOsm (Larsen et al. 2006). As this conclusion is independent of the apical mechanism of Na⁺ absorption, it applies generally, including all reabsorbing segments of the proximal tubule in vertebrate glomerular kidneys.

We conclude that all experimental evidence points to solute–water coupling by osmosis in an intracellular coupling compartment energized by lateral Na⁺/K⁺-pumps.

The tonicity of the absorbate. Hence the puzzles posed by isotonic transport should be attacked by realizing that the fluid leaving the intraepithelial osmotic coupling compartment always tends to be hypertonic relative to the fluid in the coupling compartment and to the bathing solutions of identical composition and, as discussed above, this would be compensated for by regulated Na⁺ recirculation. The separation of cellular and paracellular unidirectional Na⁺ fluxes by the pre-steady-state flux-ratio method (Sten-Knudsen & Ussing 1981) allowed Nedergaard et al. (1999) to estimate the Na⁺ recirculation flux in the small intestine. In this epithelium with low, if any, expression of aquaporins in the brush border membrane the experimental results indicated that about 65% of the Na⁺ pumped into the lateral space is derived from the serosal bath. Subsequent modelling of the small intestine predicted that this relatively water-tight epithelium would build up an osmotic concentration in the lateral space of 6–7 mOsm above that of the bathing solutions, and that the resulting fairly large diffusion component of the Na⁺ flux emerging from the lateral space would have to be compensated for by a recirculation flux of 63% if isotonic transport is demanded (Larsen et al. 2002). With active transport of water across the apical plasma membrane via SGLT1 assuming a stoichiometry of 210 H₂O : 2 Na⁺ : 1 glucose (Loo et al. 1996), the necessary recirculation for isotonic transport decreased just by a small number, from 63% to 56% (Larsen et al. 2002). At the other end of the scale, by in silico experiments it was estimated that truly isotonic transport in the highly water-permeable rat proximal tubule demands less than 5% Na⁺ recirculation for ‘correcting’ an otherwise ~3% hypertonic absorbate (Larsen et al. 2006).

Initial segments of the mammalian proximal tubule pose an interesting problem because reabsorption of Na⁺ is here accompanied by HCO₃⁻ (Weinstein 2008). If further experimental studies indicate that the necessary fine adjustment of the tonicity of the tubular reabsorbate rely on ion recirculation, different molecular mechanisms would have to serve this function in
different segments of the proximal tubule depending on whether Cl− or HCO₃⁻ constitutes the dominating anion accompanying Na⁺.

The overall conclusion is that the theory of osmotic coupling of water and Na⁺ fluxes in intracellular lateral spaces accounts for the water transport as well as for other essential experimental observations in the small intestine (Larsen et al. 2002) and kidney proximal tubule (Larsen et al. 2006). The demand on Na⁺ recirculation for isotonic transport follows logically from the physics of convection-diffusion of Na⁺ out of the lateral space, which tends to make the Na⁺ concentration of the emerging fluid larger than that of the coupling compartment. Further to this, the theory considering Na⁺ gradient-driven apical water uptake by a SGLT1 protein fails to predict the essential isotonic transport in these two epithelia.

**Metabolic cost of solute-coupled water transport**

At first sight, one might object to the idea of Na⁺ recirculation by pointing out that the energetic efficiency would be too low for serving important energy-consuming body functions. Actually, the theory seems to contradict the observation that in fluid-transporting epithelia the ratio of Na⁺ transport and associated O₂ consumption may be larger than that expected for the Na⁺/K⁺ pump itself, which is ~18 Na⁺/O₂ (this ratio is calculated from a pump stoichiometry of 3 mol Na⁺/mol ATP and a mitochondrial ATP/O₂ ratio of ~6). Table 2 presents a large number of such data. It is seen that efficiencies both above and below the generic ratio have been obtained. Any theory of isotonic transport would have to deal with these numbers in a logical and coherent way.

The statistical means in the two classical studies of amphibian skin preparations are almost identical to the ratio given above (Table 2). It is striking though that both studies report a significant variation of this quantity spanning from 12 to 40 Na⁺/O₂ (Zerahn 1958) and from ~6 to ~35 Na⁺/O₂ (Leaf & Renshaw 1957). This fairly large – and admittedly surprising – range of metabolic cost of active Na⁺ transport was confirmed by two more recent studies on high resistance epithelia (Vieira et al. 1972, Nellans & Finn 1974). In frog skin made ‘leaky’ by exposing the outside of the epithelium to a high osmotic concentration, Ussing (1966) measured a ratio of 12–14.4 Na⁺/O₂. Studies on the mammalian kidney electrolyte and energy metabolism invariably reported values significantly above 18 Na⁺/O₂ (Deetjen & Kramer 1961, Lassen & Thaysen 1961, Lassen et al. 1961, Thurau 1961, Torelli et al. 1966). Two studies on rabbit gallbladder found similar high efficiencies of Na⁺ transport, 24 Na⁺/O₂ (Martin & Diamond 1966) and 30 Na⁺/O₂ (Frederiksen & Leyssac 1969), while dilution of the external baths resulted in a drop of this ratio in the gallbladder to ~13 Na⁺/O₂ (Frederiksen & Leyssac 1969).

While recirculation of Na⁺ would account for the lower-range values, a number of experimentally verified mechanisms by which sodium ions bypass the Na⁺/K⁺ pump account for ratios above 18 Na⁺/O₂. These are: (1) Na⁺ uptake via the paracellular route by convection (solvent drag) in tight junction of the proximal tubule (Frömter et al. 1973, see Larsen et al. 2006 for a quantitative treatment), (2) cellular Na⁺ uptake via the basolateral Na⁺/[HCO₃]⁻ cotransporter of the upper segments of the kidney proximal tubule (Boron & Boulaap 1983, Alpern & Chambers 1987).

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation</th>
<th>mol Na⁺/mol O₂</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. temporaria</td>
<td>Skin in vitro</td>
<td>17.8</td>
<td>12–40</td>
<td>Zerahn (1958)</td>
</tr>
<tr>
<td>R. esculenta</td>
<td>Skin in vitro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. bufo</td>
<td>Skin in vitro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. temporaria</td>
<td>‘Leaky’ skin in vitro</td>
<td>18.2 ± 2.3</td>
<td>5.8–35.4</td>
<td>Leaf &amp; Renshaw (1957)</td>
</tr>
<tr>
<td>B. bufo</td>
<td>‘Leaky’ skin in vitro</td>
<td>No mean</td>
<td>12–14.4</td>
<td>Using (1966)</td>
</tr>
<tr>
<td>R. pipiens</td>
<td>Skin in vitro</td>
<td></td>
<td>7.1–30.9</td>
<td>Vieira et al. (1972)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Kidney slices</td>
<td>25</td>
<td>~21–35</td>
<td>Lassen &amp; Thaysen (1961)</td>
</tr>
<tr>
<td>Dog</td>
<td>Kidney in vivo</td>
<td>~30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Kidney in vivo</td>
<td>28</td>
<td></td>
<td>Deejen &amp; Kramer (1961)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Kidney in vivo</td>
<td>30</td>
<td></td>
<td>Torelli et al. (1966)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Gallbladder in vitro</td>
<td>25</td>
<td></td>
<td>Martin &amp; Diamond (1966)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Gallbladder in vitro</td>
<td>32</td>
<td></td>
<td>Frederiksen &amp; Leyssac (1969)</td>
</tr>
</tbody>
</table>

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Maunsbach et al. 2000), and as the in vivo studies were carried out on whole kidneys, (3) paracellular electro-diffusion in thick ascending limb of Henle’s loop. The point here is that the apical NKCC2 co-transporter in series with the basolateral Na+/K+ pump in the thick ascending limb of loop of Henle imply a ratio of 36 Na+/O2 if the paracellular shunt current is carried exclusively by Na+ (Gregor 1985). About 25% of the primary urine is reabsorbed in this segment; hence the high efficiency of this segment affects measurably the overall energy expenditure of the kidney. With the demand of a very small recirculation of Na+ in the proximal tubule together with the above mechanisms in the kidney the overall energetic efficiency of Na+ reabsorption exceeds significantly 18 Na+/O2 (Larsen et al. 2006). Likewise, model simulation of gallbladder with a ratio of 25 Na+/O2 when exposed to 300 mOsm Ringer’s solution predicted a decrease to 12 Na+/O2 when the external baths were diluted to 50 mOsm. This was caused by a large decrease in the paracellular convection flux relative to the decrease in the recirculation flux required for isotonic transport (Larsen et al. 2000).

In conclusion, the Na+ recirculation theory with the lateral intercellular space as the coupling compartment of solute-coupled water absorption in a logical way handles metabolic costs of active Na+ transport below that of the Na+/K+ pump. Metabolic costs exceeding that of 18 Na+/O2 of the Na+/K+ pump as observed in both high- and low-resistance epithelia follow directly from the observation that a varying fraction of the Na+,K+-ATPase-driven transepithelial Na+ uptake bypasses the Na+/K+ pump.

Conflict of interest

There are no conflicts of interest for our work.

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