Topical Review

Analysis of the sodium recirculation theory of solute-coupled water transport in small intestine

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Our previous mathematical model of solute-coupled water transport through the intestinal epithelium is extended for dealing with electrolytes rather than electroneutral solutes. A 3Na⁺–2K⁺ pump in the lateral membranes provides the energy-requiring step for driving transjunctional and translateral flows of water across the epithelium with recirculation of the diffusible ions maintained by a 1Na⁺–1K⁺–2Cl⁻ cotransporter in the plasma membrane facing the serosal compartment. With intracellular non-diffusible anions and compliant plasma membranes, the model describes the dependence on membrane permeabilities and pump constants of fluxes of water and electrolytes, volumes and ion concentrations of cell and lateral intercellular space (lis), and membrane potentials and conductances. Simulating physiological bioelectrical features together with cellular and paracellular fluxes of the sodium ion, computations predict that the concentration differences between lis and bathing solutions are small for all three ions. Nevertheless, the diffusion fluxes of the ions out of lis significantly exceed their mass transports. It is concluded that isotonic transport requires recirculation of all three ions. The computed sodium recirculation flux that is required for isotonic transport corresponds to that estimated in experiments on toad small intestine. This result is shown to be robust and independent of whether the apical entrance mechanism for the sodium ion is a channel, a SGLT1 transporter driving inward uphill water flux, or an electroneutral Na⁺–K⁺–2Cl⁻ cotransporter.

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Based on his studies of water and ion fluxes in rat small intestine Curran (1960) devised the first theory of isotonic transport by a leaky epithelium. He assumed that water absorption is passive but dependent on a solute pump that builds up a hypertonic and hyperbaric intraepithelial compartment. Water flows by osmosis from the mucosal bath into the compartment and is forced by the hydrostatic pressure into the serosal bath through a barrier with near-zero reflection coefficient. Whitlock & Wheeler (1964) refined the theory by suggesting that the coupling compartment is identical to the lateral intercellular space (lis). Since then numerous studies have tested the hypothesis that lis is the coupling compartment (reviewed in Tripathi & Boulpaep, 1989; Reuss, 1991; Whittembury & Reuss, 1992; Weinstein, 1992, 1994; Spring, 1998, 2000; Larsen et al. 2000a). In experiments on toad small intestine it was found that 60–70% of the sodium ions pumped from cells into lis are derived from the serosal bath (Nedergaard et al. 1999). Our theoretical analysis of the physical aspects of solute-coupled water transport (Larsen et al. 2000b) revealed that the fluxes of the driving solute and water emerging from lis may represent a strongly hypertonic transportate, even if the osmotic concentration of lis is only slightly above that of the external bath. That is, isotonicity would be achieved if a fraction of the solute entering the serosal bath from lis were recycled via cells back into lis. Our analysis provided a plausible explanation for the relatively high rates of recirculation in intestine and showed that the theory easily handles a number of other puzzling observations, such as uphill water transport,
anomalous solvent drag, pseudo-solvent drag, and the fact that the cost of transepithelial sodium transport varies from being below to being above the metabolic energy consumed by the Na$^{+}$–K$^{+}$ pump itself. The treatment, however, was incomplete. In particular, it was based on transport of an electroneutral solute. This review examines the idea that energy-dependent recirculation of the charged sodium and chloride ions is a prerequisite for generating a truly isotonic or near-isotonic transportate in toad small intestine. This is done by mathematical analysis of a compartment model of cells and lis with compliant plasma membranes, different apical entrance mechanisms for sodium, rheogenic Na$^{+}$–K$^{+}$ pumps, a serosal cotransporter coupling back fluxes of the three diffusible ions, water channels in all membranes, and passive ion flows obeying classic electrodiffusion and convection–electrodiffusion theory. In addition, the bioelectrical features associated with ion-coupled water transport are analysed.

THE MODEL

Compartments and symbols

The model epithelium is shown schematically in Fig. 1. It contains four well-stirred compartments: mucosal or outer (o), cell (cell), lateral intercellular space (lis) and serosal or inner (i). These are separated by the following plasma membranes: apical (am), lateral (lm) and serosal (sm). The lateral intercellular space is delimited from the two external compartments by the tight junction membrane (tm) and interspace basement membrane (bm), respectively. With these symbols, physical variables are defined according to the following notations (see Fig. 1).

Concentrations of diffusible ions, non-diffusible intracellular anions, non-diffusible extracellular molecules, glucose: $C_{j}^{\text{comp}}$, where $j$ is Na$^+$, K$^+$ or Cl$^-$, $C_{A}^{\text{cell}}$, $C_{ND}^{\text{cell}}$, $C_{Glu}^{\text{comp}}$, respectively. The mean charge of the non-diffusible intracellular anion is denoted $n$, and ‘comp’ is one of the above compartments.

 Fluxes of diffusible ions: $J_{j}^{m}$, $J_{j}^{\text{pump},m}$, $J_{j}^{\text{CO},m}$, and $J_{j}^{\text{KCl},m}$, which for membrane, $m$, indicate the passive flux, the pumped flux, the flux carried by the 1Na$^+–1K^+–2Cl^-$ cotransporter, and the flux carried by the KCl cotransporter, respectively.

 Fluxes of glucose (Glu) and water (W): $J_{\text{Glu}}^{m}$, $J_{W}^{m}$, respectively, where ‘m’ is one of the above membranes.

Permeabilities: the membranes’ ion and water permeabilities follow similar notations: $P_{j}^{m}$ and $P_{W}^{m}$.

Electrical ion conductances: $G_{j}^{m}$ is the electrical conductance of membrane $m$ for ion $j$.

Hydrostatic and osmotic pressures are indicated by $p^{\text{comp}}$ and $\pi^{\text{comp}}$, respectively.

Compliance constants of the three plasma membranes are denoted $\mu^{m}$.

Sign convention: fluxes from left to right (Fig. 1) are positive like the flux from cell to lis.

Mathematical description

Steady state criteria. The primary variables of the model are the ion and glucose concentrations and the hydrostatic pressures of the cell and the lateral intercellular space together with the electrical potentials of the mucosal bath, cell and lateral intercellular space. At steady state these 14 variables fulfil the following requirements.

Mass balance, ions and glucose:

$$\sum J_{j}^{am} = \sum J_{j}^{lm} + \sum J_{j}^{im},$$

$$\sum J_{j}^{bm} = \sum J_{j}^{lm} + \sum J_{j}^{im},$$

$$J_{j}^{\text{Glu}}^{am} = J_{j}^{\text{Glu}}^{lm} + J_{j}^{\text{Glu}}^{im},$$

$$J_{j}^{\text{Glu}}^{bm} = J_{j}^{\text{Glu}}^{lm} + J_{j}^{\text{Glu}}^{im},$$

where the summations are taken over all pathways of ion, $j$, in the membrane specified.
Mass balance, water:
\[ J^{\text{wm}}_W = J^{\text{wm}}_W + J^{\text{sm}}_W, \quad (10) \]
\[ J^{\text{sm}}_W = J^{\text{rm}}_W + J^{\text{ln}}_W. \quad (11) \]

Electroneutrality:
\[ C_A^{\text{cell}} = (C_{\text{Na}}^{\text{cell}} + C_{K}^{\text{cell}} - C_{\text{Cl}}^{\text{cell}})/n_s, \quad (12) \]
\[ C_{\text{Cl}}^{\text{lis}} = C_{\text{Na}}^{\text{lis}} + C_{K}^{\text{lis}}. \quad (13) \]

Compliance model:
\[ p^{\text{cell}} = (\mu^{\text{am}}p^o + \mu^{\text{lm}}p^{\text{lis}} + \mu^{\text{pm}}p)/ (\mu^{\text{sm}} + \mu^{\text{lm}} + \mu^{\text{pm}}). \quad (14) \]

Flux equations. In this section we present the relationships between the primary variables and the fluxes fulfilling the above mentioned steady state criteria. Ion transport through water permeable pores in the tight junction and inter-space basement membrane is given by the convection–electrodiffusion equation for a membrane with homogenous pores of uniform length (see Appendix for its derivation):
\[ J_i = \left( \frac{z_i F V}{R T} P_i + J_W (1 - \sigma) \right) \times \exp(z_i F V/(R T)) \frac{C^{(I)}_i (J_W (1 - \sigma)/P_i) + C^{(II)}_i (J_W (1 - \sigma)/P_i) - 1}{\exp(z_i F V/(R T)) \exp(J_W (1 - \sigma)/P_i) - 1}. \quad (15) \]

The corresponding equation for convection–diffusion of the non-charged glucose reads (the Hertz equation):
\[ J_{\text{Glu}} = J_W (1 - \sigma_{\text{Glu}}) C^{(I)}_{\text{Glu}} \exp(J_W (1 - \sigma_{\text{Glu}})/P_{\text{Glu}}) - C^{(II)}_{\text{Glu}} \exp(J_W (1 - \sigma_{\text{Glu}})/P_{\text{Glu}}) - 1. \quad (16) \]

With the definitions of the compartment model (Fig. 1), for the tight junction membrane: I = 0 while II = lis. For the inter-space basement membrane: I = lis and II = i. For electrodiffusion of ions through plasma membrane channels with no water permeability, eqn (15) takes the form (the Goldman–Hodgkin–Katz equation):
\[ J_i = \left( \frac{z_i F V}{R T} P_i \right) C^{(I)}_i \exp(z_i F V/(R T)) - C^{(II)}_i \exp(z_i F V/(R T)) - \frac{1}{\left( \exp(z_i F V/(R T)) - 1 \right)}. \quad (17) \]

with I = o and II = cell for the apical membrane, I = cell and II = lis for the lateral membrane, and I = cell and II = i for the serosal membrane. This equation is derived with similar assumptions as eqn (15), but as usual \( P_i = \beta D_i/\delta \), where \( \beta \) is the partition coefficient. The pump binds three intracellular Na\(^+\) and two extracellular K\(^+\) ions by first-order reaction kinetics (Lew et al. 1979):
\[ J^{\text{pump},m}_{\text{Na}} = J^{\text{max},m}_{\text{Na}} \left( \frac{C_{\text{Na}}^{\text{cell}}}{K_{\text{pump},m}^{\text{Na}} + C_{\text{Na}}^{\text{cell}}} \right)^3 \left( \frac{C_{K}^{\text{K}}}{K_{K}^{\text{pump},m} + C_{K}^{\text{comp}}} \right)^2, \quad (18a) \]
\[ J^{\text{pump},m}_{K} = -\frac{1}{2} J^{\text{max},m}_{\text{Na}} \left( \frac{C_{\text{Na}}^{\text{cell}}}{K_{\text{pump},m}^{\text{Na}} + C_{\text{Na}}^{\text{cell}}} \right)^3 \left( \frac{C_{K}^{\text{K}}}{K_{K}^{\text{pump},m} + C_{K}^{\text{comp}}} \right)^2. \quad (18b) \]

For the pump in the serosal membrane, m = sm and \text{comp} = lis. Fluxes carried by 1Na\(^+\)–1K\(^+\)–2Cl\(^-\) cotransporters are calculated as:
\[ J^{\text{CO,sm}}_i = r K^{\text{CO,sm}} \left[ C_{\text{Na}}^{(I)} C_{K}^{(I)} (C_{\text{Cl}}^{(I)})^2 - C_{\text{Na}}^{(II)} C_{K}^{(II)} (C_{\text{Cl}}^{(II)})^2 \right]. \quad (19) \]

Here, \( r = 1 \) for Na\(^+\) and K\(^+\), and \( r = 2 \) for Cl\(^-\). For m = am, I = o and II = cell, and for m = sm, I = cell and II = i. Thus we assume, for simplicity, that the transport system does not saturate, which is a reasonable approximation when the ion concentrations are kept close to their standard values, as they are in the present study. An electroneutral K\(^+\)–Cl\(^-\) cotransporter can be switched on in the lateral and serosal membrane:
\[ J^{\text{KCl,lm}}_i = K^{\text{KCl,lm}} [C_{K}^{(I)} C_{\text{Cl}}^{(I)} - C_{K}^{(II)} C_{\text{Cl}}^{(II)}], \quad (20) \]
where I = cell and II = lis for m = lm, and I = cell and II = i for m = sm. An SGLT1 system can be switched on in the apical membrane which couples uptake of glucose and water to that of Na\(^+\) with the following stoichiometry (Loo et al. 1996):
\[ J^{\text{am},\text{Glu}}_{\text{Na}} = 0.5 J^{\text{am}}_{\text{Na}}, \quad (21a) \]
\[ J^{\text{am}}_{W} = 210 J^{\text{am}}_{\text{Na}}. \quad (21b) \]

For simplicity and without losing anything of importance, the voltage dependence of Na\(^+\) uptake is here given by the constant field equation (eqn (17)), and since we run the model with standard solutions in the external compartments (and cell) only, in the computations presented in this paper we do not incorporate apical saturation kinetics. Furthermore, eqns (21a and b) assume that an outward glucose gradient cannot drive the coupled fluxes outward. This simplification does not introduce problems in the computations presented below since under the prevailing conditions, with the transport system operating far from electrochemical equilibrium (see Fig. 9), the coupled fluxes will always be carried in the inward direction driven by the very large \( m^{\text{am}}_{\text{Na}} \). Exit fluxes of glucose across lateral and serosal membranes are calculated by (e.g. Stein, 1967):
\[ J^{\text{m}}_{\text{Glu}} = \frac{J^{\text{m}\text{max}}_{\text{Glu}} K^{\text{m}}_{\text{Na}} (C_{\text{cell}}^{\text{Glu}} - C_{\text{comp}}^{\text{Glu}})}{(K_{\text{Glu}}^{\text{m}} + C_{\text{cell}}^{\text{Glu}}) (K_{\text{Glu}}^{\text{m}} + C_{\text{comp}}^{\text{Glu}})}. \quad (22) \]

Here \text{comp} = lis for m = lm and \text{comp} = i for m = sm. Water fluxes through the water channels of the three plasma membranes are calculated from:
\[ J^{\text{W},\text{am}}_{\text{W}} = P^{\text{am}}_{\text{W}} V^{\text{W}}_{\text{W}} \left[ (C_{\text{Na}}^{\text{cell}} + C_{\text{K}}^{\text{cell}} + C_{\text{Cl}}^{\text{cell}} + C_{A}^{\text{cell}} + C_{\text{Glu}} - C_{O}^{\text{Na}}) - C_{K}^{\text{cell}} - C_{O}^{\text{Cl}} - C_{O}^{\text{ND}} - C_{O}^{\text{Glu}} + RT (p^o - p^{\text{cell}}) \right], \quad (23a) \]
\[ J^{\text{W},\text{lm}}_{\text{W}} = P^{\text{lm}}_{\text{W}} V^{\text{W}}_{\text{W}} \left[ (C_{\text{Na}}^{\text{lis}} + C_{\text{K}}^{\text{lis}} + C_{\text{Cl}}^{\text{lis}} + C_{\text{Glu}} - C_{\text{Na}}^{\text{cell}}) - C_{K}^{\text{cell}} - C_{A}^{\text{cell}} - C_{\text{Glu}} + RT (p^o - p^{\text{lis}}) \right], \quad (23b) \]
\[ J^{\text{W},\text{am}}_{\text{W}} = P^{\text{am}}_{\text{W}} V^{\text{W}}_{\text{W}} \left[ (C_{\text{Na}}^{\text{cell}} + C_{\text{K}}^{\text{cell}} + C_{\text{Cl}}^{\text{cell}} + C_{\text{ND}} + C_{\text{Glu}} - C_{\text{cell}}^{\text{Na}}) - C_{K}^{\text{cell}} - C_{A}^{\text{cell}} - C_{\text{Glu}} + RT (p^o - p^{\text{cell}}) \right]. \quad (23c) \]
$V_W$ is the molar volume of water, and ND a non-diffusible molecule that can be added to the outer or inner bath for studying water transport in the presence of transepithelial osmotic gradients. With similar notations, water fluxes through the tight junction and the interspace basement membrane are given by:

$$J_{W}^{in} = P_{W}^{in}V_{W}[\sigma_{Na}^{in}(C_{Na}^{in} - C_{Na}^{o}) + \sigma_{K}^{in}(C_{K}^{in} - C_{K}^{o}) + \sigma_{Cl}^{in}(C_{Cl}^{in} - C_{Cl}^{o}) + \sigma_{H_2O}^{in}(C_{H_2O}^{in} - C_{H_2O}^{o}) + RT(p^0 - p^{H_2O})], \quad (23a)$$

$$J_{W}^{out} = P_{W}^{out}V_{W}[\sigma_{Na}^{out}(C_{Na}^{out} - C_{Na}^{o}) + \sigma_{K}^{out}(C_{K}^{out} - C_{K}^{o}) + \sigma_{Cl}^{out}(C_{Cl}^{out} - C_{Cl}^{o}) + \sigma_{H_2O}^{out}(C_{H_2O}^{out} - C_{H_2O}^{o}) + RT(p^{H_2O} - p^0)]. \quad (23b)$$

After the set of equations has been solved we proceed by calculating unidirectional fluxes, intraepithelial volumes and electrical conductances of individual ion pathways.

**Unidirectional fluxes.** Unidirectional paracellular fluxes are calculated following the principles indicated in eqns (21–25) of Larsen et al. (2000b). The expression for the ratio of unidirectional fluxes through a single membrane with homogenous pores, as described above by eqn (15), is:

$$J_{i,m}^{in}/J_{i,m}^{out} = \frac{C_j^{in}}{C_j^{out}}\exp\left(\frac{zF(\psi_{in} - \psi_{out})}{RT}\right)\exp\left(\frac{J_{in}^{in}(1 - \sigma_{i,m}^{in})}{P_j^{in}}\right). \quad (24a)$$

In the model, I = o and II = lis for m = tm, and I = lis and II = i for m = bm. With a similar method, the ratio of unidirectional paracellular fluxes that cross the two serially arranged barriers, tm and bm, is given by:

$$j_{i,par,in}^{in}/j_{i,par,out}^{in} = \frac{C_j^{in}}{C_j^{out}}\exp\left(\frac{zFV_i}{RT}\right)\times \exp\left(\frac{J_{in}^{in}(1 - \sigma_{i,m}^{in})}{P_j^{in}} + \frac{J_{out}^{in}(1 - \sigma_{i,m}^{out})}{P_j^{out}}\right). \quad (24b)$$

With vanishingly small paracellular volume flows, eqn (24b) has as its limit Ussing’s flux-ratio equation for a multi-membrane system. It should be noted, however, that Ussing’s equation was derived with no assumptions regarding variation of membrane variables with distance in the composite membrane, such as ion mobility, electrical potential and ion concentration (Ussing, 1949).

**Volumes of intraepithelial compartments.** The volume of the cell ($Vol_{cell}$) is calculated from:

$$Vol_{cell} = D_{cell}M_{cell}^{m}/C_{cell}^{m}, \quad (25)$$

where $D_{cell}$ is the cell density and $M_{cell}^{m}$ is the amount of non-diffusible anions in the cell. Assuming a finite volume of the lateral intercellular space ($Vol_{lis,ref}$), in the absence of water transport through lis its volume, $Vol_{lis}$, is given by:

$$Vol_{lis} = Vol_{lis,ref}[1 + \mu^{lm}(p_{lis} - p^{cell})]. \quad (26)$$

**Electrical circuit analysis.** With assumptions similar to eqn (17), individual ion conductances ($G_{j,m}^{m}$) are calculated as integral conductances (Sten-Knudsen, 1978). The membrane conductances are then given by, $G_{j,m}^{m} = \Sigma G_{j,m}^{m}$, where $j = Na^+, K^+$ and $Cl^-$ and $m = sm, sm, tm$ or bm. The five membranes of the epithelium constitute a bridge circuit that cannot be analysed by using the rules for series and parallel conductors. The conductance of the epithelium ($G_i$) was found, therefore, by simulating a transepithelial current injection, $\Delta I$, and using Kirchhoff’s rules to the analogue circuit of membrane conductances (see Fig. 2) for calculating the associated $\Delta V_i$. The transepithelial conductance was obtained as: $G_i = \Delta I/\Delta V_i$.

**Computation procedures**

The 14 unknowns are found by solving eqns (1–14), which constitutes a set of non-linear, strongly coupled equations. While a conventional iterative Newton–Raphson method is used, because of the complexity of the associated eqns (15–23), in forming the Jacobian matrix we did not differentiate the equations analytically, but employed a simple difference scheme. The equations were solved to machine accuracy. In this mode of the model the transport constant of the serosal cotransporter, $K_{CO_{2,sm}}$, is an independent variable that enters the input list together with all the other independent variables (Table 1). Thus, the toxicity of the net transportate, $TON$, becomes a...
The derived quantity, which is to be calculated after the solution to eqns (1–14) has been found:

$$TON = \frac{\sum J_{\text{in}}^m + J_{\text{out}}^m + \sum J_{\text{in}}^h + J_{\text{out}}^h}{J_{\text{in}}^w + J_{\text{out}}^w},$$

where $j$ is $Na^+$, $K^+$ and $Cl^-$ and the summations are taken over all pathways.

The model can also be run in another mode in which $TON$ enters the input list of independent variables. In this mode, during the iterations the transport constant of the serosal cotransporter of eqn (19), $K^{CO,sm}$, is adjusted to obtain:

$$TON(J_{\text{in}}^w + J_{\text{out}}^h) - (\sum J_{\text{in}}^m + J_{\text{out}}^m + \sum J_{\text{in}}^h + J_{\text{out}}^h) = 0. \quad (27)$$

Thus, by solving eqns (1–14 and 27), we find the value of $K^{CO,sm}$, which provides a tonicity of the net transportate equal to $TON$. In the computations of the present article eqn (27) was included, and unless otherwise indicated (Fig. 6) $TON$ was equal to the osmolarity of the bathing solutions; that is, truly isotonic transport was demanded.

We use the phrase ‘sodium recirculation’ to indicate the fraction of sodium ions pumped across the lateral membrane that is derived from the serosal bath and that has been transported into the cell via the cotransporter in the serosal membrane:

$$\text{Na}^+ \text{ recirculation} = \frac{J_{\text{Na}}^{CO,sm}}{J_{\text{Na}}^{\text{pump},lm}} \quad (28)$$

With this definition the $Na^+$ recirculation is a dimensionless quantity between zero and one.

**Independent variables of the model**

The ‘reference state of the model’ refers to a preparation bathed with Ringer solution on either side with transport activity corresponding to that of toad small intestine (Table 2; Fig. 3). Its independent variables are listed in Table 1. They were found by five independent or weakly coupled steps.

I. Ion permeabilities and maximum pump rates were chosen to obtain the following four general features:

1. Intracellular concentrations and serosa membrane potential in agreement with values for cells of vertebrate intestinal mucosa (Friszell et al. 1973; Gunter-Smith et al. 1982; Hudson & Schultz, 1984; White et al. 1984; White & Ellingsen, 1989; Sullivan & Field, 1991). In the literature mentioned these numbers vary somewhat between preparations, and they may also vary with season (White, 1977). Generally, however, they obey the rule of a relatively low $Na^+$ concentration and a $Cl^-$ concentration that is above its electrochemical equilibrium value.

2. The number of pumps is relatively large in lateral membrane (Sterling, 1972).

3. With a conductive uptake of $Na^+$ across am, we assume asymmetric distribution of cation permeabilities with small $P_A^{in}$ and $P_A^{out}$ being relatively large. In the computations with an electroneutral cotransporter in the apical membrane $P_A^{in}$ is relatively large whereas $P_A^{out}$ is small (Halm et al. 1985a).

4. $Na^+$ fluxes resemble those of toad small intestine taken from the experimental study of Nedergaard et al. (1999). While it is quite easy to find independent variables that satisfy a selected set of cellular concentrations and membrane potentials, it is clear that other sets could also have been selected for. Within reasonable limits, however, their precise values do not influence our conclusions (e.g. whether we decide that $C_{Na}^{cell}$ is about 5 or 30 mm is of no principal significance).

II. The osmotic water permeability of the apical membrane ($P_A^{w}$) was set to a value comparable with that of the brush border membrane of rat small intestine (Worman & Field, 1985). The number arrived at in their study, $1.2 \times 10^{-3}$ cm s$^{-1}$, was obtained by stopped-flow nephelometry (light scattering) of vesicles formed by isolated brush border membranes and can be assumed to be little contaminated by unstirred layer effects. Next we assumed $P_A^{in} = P_A^{w}$ and $P_A^{out} = 4P_A^{w}$, corresponding to cube-formed cells with four sides facing lis and one side facing each of the external compartments. These assumptions are further discussed in the Results section.

III. With a ratio $P_A^{in}/P_A^{out} = 100$ (Welling & Grantham, 1972), water permeability, $Na^+$ reflection coefficient and $Na^+$ permeability of tm and bm were chosen to obtain paracellular $Na^+$ fluxes, including their ratio, similar to those of toad small intestine (Nedergaard et al. 1999). During the search for appropriate values of $\sigma_{Na}$ and $P_{Na}$ we assumed $\sigma_K = \sigma_{Cl} = \sigma_{Na}$ for both membranes and cation permeability ratio of the two membranes corresponding to the ratio of their diffusion coefficients in water (see also paragraph IV). The chosen values of reflection coefficients, $\sigma^{in} = 0.7$ and $\sigma^{out} = 10^{-5}$, fulfill the requirement of fluid absorption at transepithelial equilibrium conditions.

IV. Considering that the serosal cotransporter maintains $C_{Cl}^{cell}$ above equilibrium and the model in its reference state has only anion channels in the apical membrane, the net inward flux of $Cl^-$ would have to pass between cells ($P_{Cl}^{in} \gg P_{Cl}^{out}$). $P_{Cl}^{in}$ was chosen to provide the fairly small transepithelial potential difference of toad intestine of about $-4$ mV (Nedergaard et al. 1999). Taken together with the assumptions given in paragraph III above, tm becomes anion selective: $P_{Na}^{in}: P_{K}^{in}: P_{Cl}^{in} = 1: 1.47: 28.9$. For a bm with small reflection coefficient (large pores), the selectivity would probably be identical to the ratios of the diffusion coefficients in water. Therefore, $P_{Na}^{in}: P_{K}^{in}: P_{Cl}^{in} = 1: 1.47: 1.52$ (Robinson & Stokes, 1970). See the Results section for further discussion.
The two other compliance factors are given by mechanisms for sodium entrance in the apical membrane:

We will take advantage of this by incorporating three alternative

RESULTS

We will investigate general principles for solute-coupled water flow across leaky epithelia together with the conditions for isotonic transport in toad small intestine. The computing strategy has been designed, therefore, to provide the model with much flexibility regarding choice of transport mechanisms in the membranes. We will take advantage of this by incorporating three alternative mechanisms for sodium entrance in the apical membrane: a channel, a rheogenic, water-transporting SGLT1 system, and an electroneutral cotransporter. The results are discussed as they are presented. In the Discussion section questions of a more general nature are considered.

Sodium channels in apical membrane

Table 1 lists the independent variables and Table 2 contains a selected number of variables derived from the associated mathematical solution to eqns (1–14 and 27). For comparison, the experimental values are also given to illustrate how well the model simulates the small intestine. Further details of the model epithelium are given in Fig. 3. At this stage the only comment is that all pertinent experimental fluxes are reproduced sufficiently well with a net transportate that is in osmotic equilibrium with the external solutions (truly isotonic transport). The predicted sodium recirculation flux is remarkably similar to that obtained in experiments on isolated toad small intestine. In the following we will analyse these results in more detail to see whether they are compatible with a transporting leaky epithelium. Most important is the predicted requirement of ion recirculation for isotonic transport. We will therefore analyse how robust this particular result is, and discuss its significance from physical principles of solute-coupled water transport.

Fluxes and their driving forces

Sodium, potassium and chloride. The flux entering the epithelium via the apical membrane is \( J_{Na}^{in} = 801 \text{ pmol s}^{-1} \text{ cm}^{-2} \), while the flux entering via tight junctions is \( J_{Na}^{in} = 282 \text{ pmol s}^{-1} \text{ cm}^{-2} \) (Fig. 3). Thus, the net flux of sodium of 1083 pmol s \(^{-1} \) cm \(^{-2} \) contains a major cellular component, like toad small intestine (Table 2). In rabbit ileum about two-thirds of the \(^{22}\text{Na}^{+} \) influx was confined to

### Table 1. Values of independent variables of reference state with a Na\(^+\) channel in apical membrane. For all membranes both hydraulic conductance \( (L_w, \text{ first row}) \) and osmotic water permeability \( (P_w, \text{ second row}) \) are indicated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>am</th>
<th>sm</th>
<th>lm</th>
<th>tm</th>
<th>bm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( L_w )</td>
<td>cm (^3) N (^{-1}) s (^{-1})</td>
<td>( 1.0 \times 10^{-7} )</td>
<td>( 1.0 \times 10^{-7} )</td>
<td>( 4.0 \times 10^{-7} )</td>
<td>( 8.0 \times 10^{-6} )</td>
<td>( 8.0 \times 10^{-4} )</td>
</tr>
<tr>
<td>( P_w )</td>
<td>cm s (^{-1})</td>
<td>( 1.35 \times 10^{-3} )</td>
<td>( 1.35 \times 10^{-3} )</td>
<td>( 5.41 \times 10^{-3} )</td>
<td>( 0.108 )</td>
<td>( 10.8 )</td>
</tr>
<tr>
<td>( P_{Na} )</td>
<td>cm s (^{-1})</td>
<td>( 2.5 \times 10^{-6} )</td>
<td>(&lt; 10^{-10})</td>
<td>(&lt; 10^{-10})</td>
<td>( 1.9 \times 10^{-6} )</td>
<td>( 3.5 \times 10^{-4} )</td>
</tr>
<tr>
<td>( P_x )</td>
<td>cm s (^{-1})</td>
<td>(&lt; 10^{-10})</td>
<td>( 5.0 \times 10^{-5} )</td>
<td>( 6.0 \times 10^{-5} )</td>
<td>( 2.8 \times 10^{-6} )</td>
<td>( 5.1 \times 10^{-4} )</td>
</tr>
<tr>
<td>( P_{K^{O}} )</td>
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<td>( 1.0 \times 10^{-7} )</td>
<td>( 5.0 \times 10^{-5} )</td>
<td>( 5.5 \times 10^{-5} )</td>
<td>( 5.3 \times 10^{-4} )</td>
</tr>
<tr>
<td>( K^{Cl} )</td>
<td>cm (^3) mol (^{-1}) s (^{-1})</td>
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<td>( 0 )</td>
<td>( — )</td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
<td>( F_{K^{Cl}} )</td>
<td>mol s (^{-1}) cm (^{-2})</td>
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<td>( 4.5 \times 10^{-9} )</td>
<td>( — )</td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
<td>( K_{Na}^{max} )</td>
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<td>( 3.4 )</td>
<td>( — )</td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
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<td>( 0.75 )</td>
<td>( — )</td>
<td>( — )</td>
<td>( — )</td>
</tr>
<tr>
<td>( \sigma_{Na} )</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.7</td>
<td>( 10^{-5} )</td>
<td>—</td>
</tr>
<tr>
<td>( \sigma_{K} )</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.7</td>
<td>( 10^{-5} )</td>
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<tr>
<td>( \sigma_{Cl} )</td>
<td>—</td>
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<td>0.7</td>
<td>( 10^{-5} )</td>
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</tr>
<tr>
<td>( \mu )</td>
<td>1/Pa</td>
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<td>( 4.15 \times 10^{-4} )</td>
<td>( 2.5 \times 10^{-4} )</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\( p^o = p^i = 1 \text{ atm}; TON = 240 \text{ mosmol l}^{-1}. \)

\( D_{\text{eff}} = 1.6 \times 10^5 \text{ cm}^2; A^o = 1.5 \times 10^{-12} \text{ mol cell}^{-1} \), \( n = -1.6; \text{Vol}_{\text{lis,ref}} = 300 \text{ nl cm}^{-2}. \)

\( C_{Na} = 118 \text{ mm}; C_{K} = C_{Cl} = 2 \text{ mm}; C_{Na}^{out} = C_{Cl}^{out} = 120 \text{ mm.} \)

\( F = 96485 \text{ C mol}^{-1}; R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}; T = 293 \text{ K.} \)
The cellular pathway (Frizzell & Schultz, 1972). Using an optical video-imaging method to measure cell volume during reversible abolition of transport, Spring & Hope (1979) calculated for *Necturus* gallbladder that the transepithelial Na$^+$ absorption occurred mainly via cells. This indicates that, with respect to the relative significance of Na$^+$ pathways, the model reproduces a general feature of low resistance fluid-transporting epithelia.

Both the cellular and the paracellular net inward flux of Na$^+$ depend on the activity of the Na$^+-$K$^+$ pump, but in different ways. The cellular flux is supposed to be coupled to ATP hydrolysis. With the cation concentrations and membrane potential indicated in Fig. 3, and a stoichiometry of 3Na$^+$ : 2K$^+$, at steady state this mechanism performs thermodynamic work of 46 kJ per pump cycle. This is fully compatible with a free energy of hydrolysis of ATP of $\Delta G_{\text{ATP}}^{\text{cell}} \approx -60$ kJ mol$^{-1}$. The paracellular flux is driven by the transepithelial potential difference ($V_t = -3.69$ mV, Table 2) and by solvent drag with the sum of the two forces being $RT \log_e (J_{\text{Na,par,in}}/J_{\text{Na,par,out}}) = 3.13$ kJ mol$^{-1}$ (Table 2). Here, the electrical driving force is $FV_t = -0.36$ kJ mol$^{-1}$, showing that solvent drag overcomes the electrical force by a significant margin, whereby the net flux of Na$^+$ between cells becomes inward rather than outward. The transepithelial fluxes of K$^+$ and Cl$^-$ contain vanishingly small cellular components (not shown, but compare input permeabilities of Table 1). These small cellular back fluxes are somewhat arbitrary and will not be considered further here, but will be discussed in the section below where Na$^+$ enters the cell via a cotransporter rather than a channel. The computed paracellular fluxes, as given in the following, are associated with the forces:

$$RT \log_e (J_{\text{Na,par,in}}/J_{\text{Na,par,out}}) = RT \log_e (8.17/3.56) = 2.02 \text{ kJ mol}^{-1},$$

and

$$RT \log_e (J_{\text{Cl,par,in}}/J_{\text{Cl,par,out}}) = RT \log_e (6629/5361) = 0.517 \text{ kJ mol}^{-1},$$

respectively. Thus, for all three ions solvent drag is of significance. The large paracellular unidirectional Cl$^-$ fluxes are associated with a net inward flux of this ion through tm of $J_{\text{Cl,tm}} = 1088$ pmol s$^{-1}$ cm$^{-2}$ (Fig. 3). With a driving force smaller than those of the cations, the flux of Cl$^-$ reflects the relatively high Cl$^-$ permeability of tm. Thus

![Figure 3. Reference state of the model furnished with apical Na$^+$ channels](image-url)

All fluxes indicated are net fluxes. Solute fluxes are given in units of pmol s$^{-1}$ cm$^{-2}$, and water flows in nl s$^{-1}$ cm$^{-2}$. See Tables 2 and 3 for further information and the text for detailed discussion.

<table>
<thead>
<tr>
<th>Independent variables are listed in Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_t$ (mV)</td>
</tr>
<tr>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Experiment *</td>
</tr>
<tr>
<td>Model</td>
</tr>
</tbody>
</table>

See Fig. 3 for further details. Computed intracellular volumes are as follows: $V_{\text{cell}} = 2513$ nl cm$^{-2}$ ($\sim 22000$ $\mu$m$^3$ cell$^{-1}$), and $V_{\text{lis}} = 329$ nl cm$^{-2}$, respectively. *From Nedergaard et al. (1999). **Na$^+$ recirculation indicates the fraction of sodium ions pumped into the lateral space that is derived from the serosal bath (eqn (27)), i.e. 60–70% of the sodium ions pumped into lis comes from the serosal side of the epithelium. ***Assuming Cs$^+$ fluxes are confined to the paracellular pathway with permeability given by $P_{\text{Cs}}/P_K = 1.05$, for both tm and bm.
our model reproduces paracellular solvent drag. This may not be a general feature of water-transporting epithelia (Kovbasnjuk et al. 1998; Spring. 2000). However, in experiments on small intestine evidence has been obtained that nutrients like glucose and amino acids are absorbed not only by cellular mechanisms, but also by convection through tight junctions (Pappenheimer, 1993). The transepithelial net fluxes of the three ions are \( J_{\text{am}}^{\text{net}} + J_{\text{lm}}^{\text{net}} \) of Fig. 3), \( J_{\text{am}}^{\text{net}} = 1083, J_{\text{lm}}^{\text{net}} = 4 \) and \( J_{\text{lm}}^{\text{net}} = 1087 \) pmol s \(^{-1} \) cm \(^{-2} \), respectively. As required, under open circuit the transepithelial ion movement is electroneutral. The net transport of \(^{134}\text{Cs}^+\) is also inward with a predicted flux ratio similar to that measured in experiments on toad small intestine (Table 2). In the model this result is obtained by assuming passage of \(^{134}\text{Cs}^+\) through the paracellular route, with the ratio of permeabilities of the alkali metal ions to both membranes being identical to the ratio of their mobilities in water.

**Water.** With a small apical membrane water permeability, as indicated in the experimental study of Worman & Field (1985), and a water permeability of \( \text{tm} \) resulting in measured paracellular convection–electrodiffusion fluxes of cations \( (P_{\text{w}}^m/P_{\text{w}}^m = 1.25 \times 10^{-2}, \text{Table 1}) \), it is computed that just a small fraction of the transepithelial water influx is translateral \( J_{\text{w}}^{\text{am}}/J_{\text{w}}^{\text{am}} = 0.107/8.954 \) (Fig. 3). The flux of 107 n l \(^{-1} \) cm \(^{-2} \) s \(^{-1} \) is driven into the cell by an osmotic pressure difference of \( \Delta \pi_{\text{am}} = 4.4 \) mosmol l \(^{-1} \) (Fig. 3). With \( P_{\text{w}}^m = P_{\text{w}}^m = 0.25P_{\text{w}}^m \) (Table 1) and water fluxes of similar magnitude entering the cell from both sides (Fig. 3), the force driving water from the cell into lis amounts to \( \Delta \pi_{\text{lm}} = 2.2 \) mosmol l \(^{-1} \). Evidently, with a translateral water flux via channels the cell must be hyperosmotic at steady state. With a similar bath composition on the two sides of the epithelium \( (\Delta \pi_{\text{am}} = \Delta \pi_{\text{lm}}) \), the ratio \( \Delta \pi_{\text{am}}/\Delta \pi_{\text{lm}} \) will decrease with increasing \( (P_{\text{w}}^m + P_{\text{w}}^m)/P_{\text{w}}^m \), while the sum is given by \( \Delta \pi_{\text{am}} + \Delta \pi_{\text{lm}} = \Delta \pi_{\text{lm}} \). The osmotic pressure of the cell, therefore depends also on tight junction water permeability, and, as will be discussed below, on the active \( \text{Na}^+ \) flux. A small osmotic force of 6.6 mosmol l \(^{-1} \) drives the flux of water into lis via \( \text{tm} \). This hyperosmolarity of lis is less than 3% of the osmolarity of the baths. In the reference state of the model the water permeability of the \( \text{sm} \) membrane is set to a value similar to that of the apical membrane. There is no justification for this. If \( P_{\text{w}}^m \) is increased to a much larger value, arbitrarily by a factor of 1000, the osmotic pressure of the cell will be reduced to a value very close to that of the bathing solutions \( (\Delta \pi_{\text{am}} = \Delta \pi_{\text{lm}} = 0.05 \) mosmol l \(^{-1} \) \) with literally no effect on the osmotic pressure of lis \( (\Delta \pi_{\text{lm}} = 6.6 \) mosmol l \(^{-1} \) \). It follows that the recirculation is unaffected by the water permeability of the \( \text{sm} \) (provided the mucosal and serosal solutions are in osmotic equilibrium with each other). These computed results are also intuitively expected from the considerations above, and it follows that the assumption of a low osmotic water permeability of the serosal membrane is not critical for the conclusions arrived at in the present paper.

With the ratio of \( P_{\text{w}}^\text{bm}/P_{\text{w}}^\text{tm} = 100 \) (Welling & Grantham, 1972), the hydrostatic pressure head driving water from lis into the serosal bath is small as well: \( \Delta p = 1.15 \) cmH \(_2\)O (Fig. 3).

### Table 3. Bioelectrical properties of the model epithelium with Na\(^+\) channels in apical membrane

<table>
<thead>
<tr>
<th>( V_i ) (mV)</th>
<th>( G_i ) (mS cm(^{-2}))</th>
<th>( I_{sc} ) (( \mu A ) cm(^{-2}))</th>
<th>( V_{\text{cell}} ) (mV)</th>
<th>( V_{\text{lis}} ) (mV)</th>
<th>( J_{\text{am}}^{\text{net}} ) (pmol s(^{-1}) cm(^{-2}))</th>
<th>( R_{\text{am}} ) (k( \Omega ) cm(^{-2}))</th>
<th>( R_{\text{lm}} ) (k( \Omega ) cm(^{-2}))</th>
<th>( R_{\text{sm}} ) (k( \Omega ) cm(^{-2}))</th>
<th>( R_{\text{tm}} ) (k( \Omega ) cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-3.69)</td>
<td>25.4</td>
<td>93.7</td>
<td>(-67.0)</td>
<td>(-0.13)</td>
<td>1.658</td>
<td>0.380</td>
<td>0.108</td>
<td>0.038</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*This is the instantaneous short-circuit current calculated as, \( G_i (-V_i) \). The steady state short-circuit current is \( 92.9 \) \( \mu A \) cm\(^{-2}\) (computations not shown) and it contains a significant shunt component (13%) driven by paracellular solvent drag as discussed in the text.*
of Frizzell & Schultz (1972), who arrived at the conclusion that the shunt of rabbit ileum exhibits a ratio of $P_{Na} : P_{Cl} = 0.55$. In their experiments, however, glucose was not included in the mucosal bath. Pappenheimer et al. (1994) provided evidence that glucose-stimulated fluid absorption in rat small intestine is associated with a significant increase in paracellular permeability and solvent drag of solutes. They found that even octapeptides (synthesized from D-amino acids) are absorbed by paracellular solvent drag in the stimulated state of the intestine. With this information, it may not be unlikely that paracellular Cl$^-$ permeability is increased also during stimulated fluid absorption, but direct experimental evidence is lacking. An alternative way of obtaining the small transepithelial potential difference would be to assume a Na$^+$-coupled secondary active uptake of Cl$^-$ across the apical membrane. However, this will not affect the general conclusion of this paper, i.e. that the fluid emerging from lis is predicted to be strongly hypertonic, thus requiring recirculation if isotonic transport is demanded (not shown, but see section III below).

It was discussed above that the ion fluxes between cells are driven by $V_i$, as well as by water flow. As an interesting consequence, in the short-circuited epithelium the shunt current is not zero, as is usually assumed in experimental studies. The short-circuited epithelium generates paracellular flux ratios of Na$^+$, K$^+$ and Cl$^-$, which at steady state are equal to 3.40, 2.29 and 1.06, respectively (not shown). Thus, the associated convection forces are 31.1, 21.0, and 1.5 mV, respectively. Computations further show that these forces drive an inward shunt current of 12.3 $\mu A$ cm$^{-2}$ ($V_i = 0$ mV). This current is contained in $I_{sc}$ and with $V_i$ clamped at 0 mV it amounts to about 13% of the steady state $I_{sc}$.

**Ion recirculation**

**Isotonic transport.** The measured paracellular fluxes of Na$^+$ with a ratio of ~3.66 are well reproduced by the model

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**Figure 4. Computations showing effects of varying the osmotic water permeability of tight junction membrane while keeping $P_{W,tm}/P_{W,bm}$ constant**

The dashed vertical line indicates the reference value ($P_{W,tm}$ of Table 1). Isotonic transport with all other input variables as indicated in Table 1. A, the driving force for water uptake from the mucosal solution into lis increases significantly with decreased $P_{W,tm}$. B, this increases the diffusion fluxes across bm as indicated by the significantly 'up-regulated' Na$^+$ recirculation for maintaining isotonic transport, that is, $J_{Na,sm}/J_{Na,pump,lm}$ increases with decreasing $P_{W,tm}$. C, with the increased return of Na$^+$ into the cells via the serosal cotransporter, the net flux of Na$^+$ decreases, which in turn causes the isotonic fluid uptake to decrease. It is because of the decreased paracellular water flow that the flux ratio of Na$^+$ decreases with $P_{W,tm}$, cf. eqns (24a, b). D, the most dramatic effect of decreasing the water permeability of the barrier separating the coupling compartment from the mucosal solution is a very significant decrease in the efficiency of transport. In the computations shown the ratio of the net flux of Na$^+$ and the pumped flux of Na$^+$ spans a range from 0.23 to 1.12, corresponding to a transport of 4.1 and 20 mol Na$^+$, respectively, per mole of oxygen consumed by the Na$^+$–K$^+$ pumps. Thus, the thermodynamic effectiveness of isotonic transport increases quite dramatically with increasing water permeability, while the water flux changes relatively little.
(Table 2). According to these computations the convection–electrodiffusion fluxes out of the lateral space are \( J_{Na}^{bm} = 1974 \), \( J_{K}^{bm} = 118 \) and \( J_{Cl}^{bm} = 3200 \text{ mol s}^{-1} \text{cm}^{-2} \) (Fig. 3). With \( J_{w}^{bm} = 9.168 \text{ nl s}^{-1} \text{cm}^{-2} \) the virtual osmotic concentration of the fluid leaving isl is strongly hypertonic, i.e. 577 mosmol l\(^{-1}\). This indicates that with the rates of transport characterizing small intestine, the mass transfer of ions out of the lateral space is small compared with their diffusion fluxes. In the computations shown, the activity of the cotransporter was adjusted to provide a toxicity of absorbed fluid of 240 mosmol l\(^{-1}\), that is, truly isotonic transport was demanded. It can be seen (Table 2) that the Na\(^+\) recirculation necessary for obtaining this limiting condition is literally identical to the experimental value for toad small intestine. Necessarily, all three ions must be recirculated and the cotransporter of the serosal membrane is well designed for the purpose.

The significance of the \( P_{K}^{lm}/P_{K}^{sm} \) and the \( P_{Cl}^{lm}/P_{Cl}^{sm} \) ratio. The K\(^+\) recirculation flux depends on the ratio of K\(^+\) permeabilities of lateral and serosal membrane, \( P_{K}^{lm}/P_{K}^{sm} \). Increasing this ratio (keeping the sum constant) results in a larger isl [K\(^+\)] and, therefore, in a larger convection–electrodiffusion flux through the interspace basement membrane (not shown). But as this has little effect on the other features of the model, there is no need to consider this parameter choice further. In the reference state of the model lm constitutes the major exit pathway for passive flow of Cl\(^-\) out of the cell. This means that the major component of the Cl\(^-\) flux taken up by the serosal cotransporter is returned to isl.

Nor does the ratio \( P_{Cl}^{lm}/P_{Cl}^{sm} \) significantly affect the demand of ion recirculation. As an example, if \( P_{Cl}^{lm}/P_{Cl}^{sm} \) is inverted with respect to its reference value (Table 1), the cellular concentrations of Na\(^+\) and Cl\(^-\) increase to 13.5 and 70.6 mm, respectively, while the cell depolarizes to \(-50.1\text{ mV}\). The significantly larger C\(^{lis}\) results in increased pump fluxes across basolateral membranes. As the transepithelial water uptake decreases (explained below), the Na\(^+\) recirculation increases from 0.63 (Table 3) to 0.81 (the return flux of this ion, \( J_{Na}^{CO,sm} \), increases from \(-1060\) (Table 3) to \(-1874 \text{ pmol s}^{-1} \text{cm}^{-2}\)). Another effect is the (expected) decrease of C\(^{lis}\) which is now 122.7 mm. This new value of C\(^{lis}\) together with C\(^{Na}\) = 120.1 mm and C\(^{K}\) = 2.6 mm, result in a decreased \( \pi^{isl} \). Thus, the water uptake from the mucosal bath into isl is being reduced too, and so is the ratio of paracellular unidirectional Na\(^+\) fluxes, which decreases from 3.65 to 2.91 (cf. eqn (24)). The smaller C\(^{lis}\) also results in a reduction of Cl\(^-\) flux out of isl from 3200 (Fig. 3) to 890 pmol s\(^{-1}\) cm\(^{-2}\), which is now much closer to the Cl\(^-\) flux via tm: \( J_{Cl}^{tm} = 878 \text{ pmol s}^{-1} \text{cm}^{-2}\). Thus, the relative magnitude of return pathways for Cl\(^-\) has virtually no effect on how the isotonic absorbate is generated. This conclusion still holds if the tight junction Cl\(^-\) permeability is reduced to give a ratio of \( P_{Cl}^{lm}/P_{Cl}^{sm} \) of 1.52 (cf. above). In this case the much larger transepithelial electrical driving force (now \( V_{i} = -25 \text{ mV}\)) maintains the Cl\(^-\) flux through isl (not shown).

The sodium recirculation associated with a small osmotic driving force is a robust result. Water absorption by our model of intestinal mucosa follows the theory of Curran (1960). With input variables chosen to simulate experimentally estimated cellular and paracellular Na\(^+\) fluxes, the forces for fluid transport are predicted to be so small that they may escape measurement. With TON of eqn (27) included among the independent variables, the Na\(^+\) recirculation flux becomes a computed quantity (see Figure 5). Effect of exit permeabilities of isl on Na\(^+\) recirculation and paracellular Na\(^+\) flux ratio

The ratio of exit permeabilities was kept constant and equal to the ratio given by the reference state (Table 1, \( P_{Na}^{lm} : P_{Na}^{sm} : P_{Cl}^{lm} = 1 : 1.47 : 1.52\)), but their absolute values were reduced in steps down to 5 % of the respective standard values. A, the concentration of the three diffusible ions in isl increases as their exit permeabilities are reduced. Shown here is the resulting increase of their sum, which is the osmotic pressure of isl (\( \pi^{isl} \)). Given the large reduction of the permeabilities, one might have expected that \( \pi^{isl} \) would have increased much more. As explained in the text (and below), since the recirculation flux is also decreased, the flux of Na\(^+\) pumped into isl is reduced accordingly. The overall effect is, therefore, a fairly shallow dependence of \( \pi^{isl} \) on the \( P_{Na}^{lm} \) values. B, the major effect of reducing \( P_{Cl}^{lm} \) is smaller diffusion flux out of isl, which reduces the requirement for recirculation to maintain osmotic equilibrium between the net transportate and the bathing solutions. The increase of the paracellular Na\(^+\) flux ratio with decreasing \( P_{Na}^{lm} \) follows from eqns (24a, b).
‘Computation procedures’). Thus, it is noteworthy also that the computed and experimental recirculation fluxes are virtually identical (Table 2). The robustness of these results will now be examined.

The significance of \( P^w \) for energetic effectiveness of transport and rate of fluid absorption. The osmotic water permeability of tight junctions is of major significance for the ratio of paracellular Na\(^+\) fluxes. Since this permeability is a parameter for which there are no direct experimental estimates, it is important to consider how our choice affects the general results. In the computations shown in Fig. 4 the osmotic water permeability of tight junction spans a fairly large range about its standard value of \(-0.11 \text{ cm s}^{-1}\). As above, the computations were performed with truly isotonic transport (eqn (27)). With decreasing water permeability the force driving water into the coupling compartment increases (Fig. 4A). As discussed above, this is reflected also in the osmotic pressure of the cell. The osmotic concentrations are monotonic functions of the water permeability and they attain values consistent with a living epithelium. Within the large range of water permeabilities investigated the paracellular Na\(^+\) flux ratio varies from 2.1 to 5.5 (Fig. 4B), while the unidirectional paracellular Na\(^+\) fluxes attain values (pmol cm\(^{-2}\) s\(^{-1}\)) from 314 to 460 (influx), and from 150 to 84 (outflux), respectively (not shown). All these numbers span ranges that are somewhat larger that those of the respective experimental data. Nevertheless, as can be seen from Fig. 4B, all mathematical solutions contain significant recirculation fluxes, ranging from 0.90 to 0.20.

Within this fairly large range, where \( P^w \) is increased by a factor of forty from its lowest to its highest value, the water absorption is stimulated only by a factor of about two, from 5.7 to 11.6 nl cm\(^{-2}\) s\(^{-1}\) (see Fig. 4C). The explanation for this is as follows. Since we are studying a system that couples passive water transport to active solute pumping, the flux of water across the intestinal mucosa is governed by the active net uptake of Na\(^+\). As the osmotic concentrations of lis decreases with increasing \( P^w \), the diffusion fluxes across the interspace basement membrane are diminished quite significantly (not shown) whereby the recirculation fluxes are reduced correspondingly (Fig. 4B). Thus, with increasing \( P^w \) the net flux of Na\(^+\) contains an increasingly larger fraction of the pumped Na\(^+\) flux. The overall result is that the net flux of Na\(^+\) increases somewhat and so does the rate of water absorption (see Fig. 4C). Thus, the thermodynamic effectiveness of isotonic transport must increase quite significantly with increasing \( P^w \). That this is so can be seen from the graph depicted in Fig. 4D showing how the ratio of the net flux of Na\(^+\) and the pumped flux, i.e. \( J_{\text{Na}}^{\text{net}}/(J_{\text{Na}}^{\text{pump,lm}} + J_{\text{Na}}^{\text{pump,sm}}) \), varies with \( P^w \). The lowest value of \( J_{\text{Na}}^{\text{net}}/(J_{\text{Na}}^{\text{pump,lm}} + J_{\text{Na}}^{\text{pump,sm}}) \), 0.23, corresponds to no more than 0.69 mol of Na\(^+\) transported per mole of ATP hydrolysed by the Na\(^+\)–K\(^+\)-ATPase, while

\[
\text{the highest value of this ratio, 1.12, corresponds to 3.36 mol of Na}^+ \text{ transported across the epithelium per mole of ATP hydrolysed. The latter mentioned high effectiveness, which is greater than that of the pump itself, is obtained because a relatively large paracellular uptake of Na}^+, J_{\text{Na}}^{\text{pump,lm}} = 375 \text{ pmol cm}^{-2} \text{ s}^{-1}, \text{ exceeds the Na}^+ \text{ flux recirculated via the serosal cotransporter, } J_{\text{Na}}^{\text{recirc}} = -222 \text{ pmol cm}^{-2} \text{ s}^{-1}. \text{ Thus, still with these computations as an example, which are not shown in the figures, as } J_{\text{Na}}^{\text{pump,lm}} + J_{\text{Na}}^{\text{pump,sm}} = 1228 \text{ is the resulting net uptake of Na}^+, J_{\text{Na}}^{\text{net}} = 1381 \text{ pmol cm}^{-2} \text{ s}^{-1}. \text{ Effectivities of sodium transport that exceed that of the Na}^+–K^+ \text{ pump have been reported for kidney (Lassen & Thaysen, 1961; Lassen et al. 1961) and gallbladder under physiological conditions (Martin & Diamond, 1966; Frederiksen & Leyssac, 1968). Furthermore, effectiveness lower that that of the pump have been reported also, e.g. in gallbladder exposed to diluted external solutions (Frederiksen & Leyssac, 1968). Thus, upon dilution of the external bath the junctional uptake of Na}^+ \text{ must decrease relatively more than the concomitantly reduced recirculation flux. Regarding this peculiar response there is agreement between experimental and computed results (Larsen et al. 2000b).}

The significance of \( P^w \) for energetic effectiveness of transport and rate of fluid absorption. The above computations show that within a large range of osmotic water permeabilities of tight junctions, the small ion-concentration differences between lis and serosal bath generate ion fluxes out of lis so large that recirculation is required for isotonic transport. The large ion fluxes out of lis find their physical explanation in the large diffusion permeabilities of the interspace basement membrane. Since we have no direct experimental information on these permeabilities, we will

![Figure 6](image)

The tonicity of the net transportate was varied from being 60 % hyposmotic to 200 % hyperosmotic with respect to the bathing solutions of 240 mosmol l\(^{-1}\) (TON in Table 1, and eqn (27)). The graph shows that within this large range of tonicities of the absorbate the associated Na\(^+\) recirculation fluxes are quite significant. This is another way of demonstrating that the diffusion fluxes out of lis dominate the fluxes carried by bulk transport. Furthermore, it can be concluded that ion recirculation is to be expected also for the case of ‘near-isotonic’ transport.
analyse this point in more detail. The computed results shown in Fig. 5 were obtained by reducing in large steps the three diffusion permeabilities of bm in such a way that their ratios were kept constant and equal to those of the reference state. As discussed in Larsen et al. (2000b), the lateral intercellular-space concentration increases slightly as the exit permeability is reduced. This is summarized in Fig. 5A, which shows the resulting shallow dependence of the osmotic pressure differences between bath and the two epithelial compartments on $P_J^{bm}$. The water flux into lis by osmosis increases, but not by much (not shown). Therefore, it is the fairly large decrease of $P_j^{bm}$ that will govern the change of the paracellular flux ratio. As a result, from eqns (24a, b) it follows that $J_{Na}^{bath}/J_{Na}^{lis}$ must increase significantly as the exit permeability is being reduced (see Fig. 5B). Another effect of reducing $P_j^{bm}$ is, of course, that the convection–diffusion fluxes out of lis are decreased. This readily explains the parallel reduction of the flux of Na+ that is being recirculated for maintaining an isotonic transportate ($J_{Na}^{para,in}/J_{Na}^{para,out}$ in Fig. 5B). Also in these computations, all other computed parameters (i.e. intracellular concentrations, membrane potentials, volumes, transepithelial Na+ fluxes, etc.) are within ranges compatible with physiological steady states of the living tissue (not shown).

We conclude that the small osmotic concentration difference between lis and bath, as well as the associated demand for ion recirculation given by the model, are robust results. This important point has been investigated further by varying several other membrane parameters about their standard values. The results are so similar to those obtained with the ‘electroneutral’ model (Larsen et al. 2000b) that there is no reason to present them in the figures.

Near-isotonic transport. The demand for ion recirculation was investigated above for the limiting case of truly isotonic transport. Here we will deal briefly with the more general case of absorption of fluid that is not in osmotic equilibrium with the bathing solutions. Keeping to the model’s reference state with the tonicity of the baths at 240 mosmol l$^{-1}$, the net transportate was varied from hypotonic to hypertonic for computing the resulting recirculation fluxes (eqn (27)). All mathematical solutions are characterized by cell parameters and fluxes fully compatible with physiological values (not shown). As can be seen from Fig. 6, not only the 60 %-diluted but also the 200 %-concentrated transportate depend on significant ion recirculation: 0.87 and 0.19, respectively. This result emphasizes the general conclusion of our analysis above: that is, the diffusion flux from the lateral space into the serosal bath is relatively large compared with the mass flow of ions.

Relationship between active sodium flux and associated isotonic water absorption

The rate of transepithelial water uptake by leaky epithelia increases with the transepithelial active Na+ flux. Curran (1960) observed this in his studies on small intestine, thus confirming the results of previous studies on kidney proximal tubule (Windhager et al. 1958). Subsequently, Diamond (1962a, b; 1964a, b) generalized the observation to vertebrate gallbladder. In the computations shown in Fig. 7, the transepithelial active Na+ flux was varied by varying the Na+ permeability of the apical membrane. It can be seen that the model reproduces the above general feature of leaky epithelia. The physical mechanism is as

![Figure 7](Image)

Figure 7

The recirculation model reproduces a most pertinent feature of leaky epithelia, that is, that water absorption increases with active net uptake of Na+. The active absorption of Na+ was varied by varying $P_j^{apm}$. The proportionality between the active Na+ flux and rate of water uptake follows simply from the demand of isotonic transport (eqn (27)). See text for further discussion.

![Figure 8](Image)

Figure 8

With Na+ passing the cells via a translateral route and with water uptake coupled to the active flux of Na+ via lis, it follows that the volume of cells as well as of the lateral intercellular space is dependent on the rate of fluid absorption. This is illustrated in the computations shown here in which fluid uptake was abolished by replacing 117 mmol of luminal NaCl with 234 mmol of a non-diffusible electroneutral solute (‘sucrose’). By this manoeuvre the cell volume decreased from its standard value of 3513 to 3154 nl cm$^{-2}$ ($\Delta Vol^{apm} = -10\%$), while the volume of lis decreased from 329 to 48 nl cm$^{-2}$ ($\Delta Vol^{lis} = -85\%$). The compliance constants of the plasma membranes of the model were taken from an experimental study of gallbladder (Spring & Hope, 1978). The computed $\Delta Vol^{lis}$ is similar to their measured value (Spring & Hope, 1979).
follows: The sodium concentration in the cell is reduced with the influx of this ion across the apical membrane. In turn, the rate at which Na$^+$ is pumped into lis decreases, whereby its osmotic pressure is being reduced. With reduced driving force across the tight junction the water uptake is reduced too.

**Volume response to abolition of fluid absorption**

Replacement of luminal NaCl with an equiosmotic concentration of a non-permeant solute decreases cell and interspace volumes to 89.8 and 14.5 %, respectively, of their control volumes (Fig. 8). Similar reductions of volumes were obtained in studies on gallbladder exposed to sucrose on the mucosal side (Spring & Hope, 1979). The loss of lis volume is caused by the reduced pump flux into the interspace, which leads to a reduced hydrostatic pressure of lis.

With compliant membranes, volume is lost according to eqn (26). The loss of cell volume of the model is caused by a reduction of the intracellular pools of diffusible ions counteracted, however, by the increased coupled inflow of these ions across the serosal membrane pari passu with reduced $C_{\text{Na}}^\text{cell}$. Therefore, the cell volume loss depends on the leak permeabilities and on how much $C_{\text{Cl}}^\text{cell}$ is above its equilibrium value prior to the ion replacements on the mucosal side.

**Water entering epithelial cells via an SGLT1 system**

It has been suggested that water uptake in intestinal mucosa is tightly linked to cotransport with sodium and glucose via the apical SGLT1 transporter (Wright & Loo, 2000; Zeuthen, 2000). This has been tested in experiments on heterologous expressed SGLT1 in *Xenopus* oocytes, showing that a phlorizin-inhibitable water uptake occurs when the transporter is activated by glucose. The stoichiometry of the three substrates was fixed and independent of the work done (Loo *et al*. 1996; Meinild *et al*. 1998).

In the computations presented below the apical SGLT1 system was activated with 5 mM glucose in the two external compartments. With $P_{\text{am}}^{\text{sm}}$ kept equal to its experimentally estimated value (Table 1) water exchange across the apical plasma membrane is now controlled both by channels and by coupling to the downhill Na$^+$ entrance. Paracellular transport of glucose is allowed for by assuming a reflection coefficient for glucose in bm identical with that of the ions, and $\sigma^{\text{bm}}_{\text{Glu}} = 0.8$, $P_{\text{Glu}}^{\text{bm}} = 0.5P_{\text{Na}}^{\text{bm}}$ and $P_{\text{Na}}^{\text{bm}} = 0.5P_{\text{Na}}^{\text{am}}$. Thus, a minimum of changes of the input list have been imposed with the new independent variables listed in Table 4.

**Table 4. Independent variables with a SLGT1 system in apical membrane were taken from Table 1 except for the variables indicated here**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>am</th>
<th>sm</th>
<th>lm</th>
<th>tm</th>
<th>bm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{\text{am}}^{\text{Na}}/J_{\text{am}}^{\text{Glu}}$</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$J_{\text{W}}^{\text{am}}/J_{\text{am}}^{\text{Na}}$</td>
<td>—</td>
<td>210</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$P_{\text{Glu}}^{\text{am}}$</td>
<td>cm s$^{-1}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>$9.5 \times 10^{-7}$</td>
<td>$1.75 \times 10^{-4}$</td>
</tr>
<tr>
<td>$J_{\text{Glu}}^{\text{max,am}}$</td>
<td>mol s$^{-1}$ cm$^{-2}$</td>
<td>300</td>
<td>3000</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$\sigma_{\text{Glu}}^{\text{am}} = 0.8; \sigma_{\text{Glu}}^{\text{bm}} = 10^{-5}; C_{\text{Glu}}^{\text{am}} = 5 \text{ mM}; \text{TON} = 245 \text{ mosmol L}^{-1}$.

**Figure 9. Transport features of the model with an apical SGLT1 system in the apical membrane according to Loo *et al*. (1996)**

![Figure 9](image_url)

All fluxes indicated are net fluxes. Solute fluxes are given in units of pmol s$^{-1}$ cm$^{-2}$, and water flows in units of nl s$^{-1}$ cm$^{-2}$. With (secondary) active uptake of water across the apical membrane, cell water is above equilibrium and water is leaking out through the water channels of the apical and serosal membranes. These fluxes are of similar magnitude ($P_{\text{Glu}}^{\text{am}} = P_{\text{Na}}^{\text{am}}$, Table 1). Thus, with the active uptake of water equal to 3.061 nl s$^{-1}$ cm$^{-2}$ and $-J_{\text{W}}^{\text{am}} = J_{\text{W}}^{\text{bm}} = 0.421$ nl s$^{-1}$ cm$^{-2}$, $J_{\text{W}}^{\text{sm}} = 2.219$ nl s$^{-1}$ cm$^{-2}$ ($J_{\text{W}}^{\text{sm}}$ is not indicated in the figures). See Table 4 for further information and the text for detailed discussion.
Pertinent results are given in Fig. 9. The Na⁺ fluxes generated by the model are still within the range of experimental values. The steady state concentration of glucose in the cell of $9.7 \text{m}\text{M}$ is maintained above equilibrium by the electrochemical gradient for Na⁺ across the apical membrane. With the osmotic pressure of the two external solutions at 245 mosmol l⁻¹, this gradient also drives water uphill across the apical membrane so that the cell water becomes above equilibrium with $p_{\text{cell}} = 228 \text{mosmol l}^{-1}$ associated with a 10% volume expansion from $22.0 \times 10^3$ to $24.1 \times 10^3 \text{m}^3 \text{cell}^{-1}$ and dilution of intracellular [K⁺]

Since the external solutions have identical compositions, water now recycles across the outer border of the epithelium ($0.421 \text{nl s}^{-1} \text{cm}^{-2}$, Fig. 9). A water flux of similar magnitude exits the cell across the serosal plasma membrane. There are no surprises here.

The significantly smaller osmotic pressure of the cell is reflected also in a smaller osmotic pressure of lis ($\Delta p_{\text{tm}} = 5.5 \text{mosmol l}^{-1}$ in Fig. 9 versus $\Delta p_{\text{tm}} = 6.6 \text{mosmol l}^{-1}$ in Fig. 3). This results in a reduced influx of water across tm and, therefore, also in a smaller ratio of paracellular Na⁺ fluxes: $J_{\text{Na}}^{\text{para,in}}/J_{\text{Na}}^{\text{para,out}} = 2.98$ (Fig. 9). But as this flux ratio is not incompatible with the experimental flux ratio of $3.66 \pm 0.34$ (Table 2) there is no need to readjust tight junction variables. It is noteworthy that a significant ion recirculation, 0.56, is still required for obtaining a truly isotonic net transportate, which is 245 mosmol l⁻¹ with glucose in the external baths. The transepithelial water flux of $10.257 \text{nl s}^{-1} \text{cm}^{-2}$ ($J_{W_{\text{sm}}} + J_{W_{\text{bm}}}$, Fig. 9) is about the same as with apical Na⁺ channels.

In Fig. 9 the component of the active uptake of water that proceeds to the serosal bath via the serosal plasma membrane ($J_{W_{\text{sm}}} = 0.421 \text{nl s}^{-1} \text{cm}^{-2}$) amounts to 4% of the net water flux. The relatively very small value is given by the small osmotic water permeability of the serosal membrane. By letting a larger fraction of the water flux be transcellular rather than translateral, recirculation will decrease. However, as the SGLT1 system generates a primary transportate with a virtual Na⁺ concentration of 265 mm, recirculation will always be required if isotonic transport is demanded. For example, if $P_{W_{\text{sm}}}$ is increased by a factor of 1000 from its standard value (not shown), as expected the osmotic pressure of the cell now approaches that of the baths ($\Delta p_{\text{cell}} = 244.9 \text{mosmol l}^{-1}$) with just small changes in the net sodium uptake ($J_{\text{Na}}^{\text{net}} = 1115 \text{pmol s}^{-1} \text{cm}^{-2}$) and transepithelial water absorption ($10.065 \text{nl s}^{-1} \text{cm}^{-2}$). A larger fraction of the transepithelial water flow is now transcellular: $J_{W_{\text{sm}}} = 2.780 \text{nl s}^{-1} \text{cm}^{-2}$ (~28%). Also in this case the paracellular water flow generates large paracellular convection fluxes of Na⁺ with a ratio of 2.95, and with a Na⁺ recirculation flux of 0.47. Thus, these numbers (which are not shown in the figures) would be compatible, as well, with the experimental data for small intestine, including the recirculation flux of Na⁺. The general conclusion is that with an apical SGLT1 system ion recirculation is necessary if the net transportate is to be in osmotic equilibrium with the bathing solutions, as observed in the first studies of Curran (1960).

**Sodium entering epithelial cells via an electroneutral cotransporter**

The small intestine of the marine teleost plays a significant role in whole body osmoregulation. The small intestine of the winter flounder, *Pseudopleuronectes americanus*, expresses a Na⁺–K⁺–2Cl⁻ cotransporter in the apical membrane, and via this system epithelial salt uptake takes

---

**Table 5. Independent variables with a 1Na⁺–1K⁺–2Cl⁻ transport system in apical membrane were taken from Table 1 except for the values indicated below**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>am</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{Na}$</td>
<td>cm s⁻¹</td>
<td>$&lt; 10^{-10}$</td>
</tr>
<tr>
<td>$P_{K}$</td>
<td>cm s⁻¹</td>
<td>$2.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>$K^{\text{CO}}$</td>
<td>cm¹⁰ mol⁻³ s⁻¹</td>
<td>$2.4 \times 10^{8}$</td>
</tr>
</tbody>
</table>

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**Figure 10. Transport features of the model with an apical 1Na⁺–1K⁺–2Cl⁻ cotransport system in the apical membrane according to Halm et al. (1985a)**

All fluxes indicated are net fluxes. Solute fluxes are given in units of pmol s⁻¹ cm⁻², and water flows in units of nl s⁻¹ cm⁻². See Table 5 for further details and the text for discussion.
place in the absence of glucose in the luminal solution (Halm et al. 1985a, b; O’Grady et al. 1986). In this section, as above with the SGLT1 system, with a minimum of changes to the reference state we will study the effects of an apical cotransporter on bioelectrical features, paracellular convection and ion recirculation.

The results shown in Fig. 10 were obtained with the new input variables indicated in Table 5. The activity of the cotransporter was adjusted to provide transepithelial Na+ fluxes close to those of the experimental values of the toad. Furthermore, we have been guided by the study in Frizzell’s laboratory of the winter flounder, which reported a significant K+ conductance in the apical membrane. They concluded that the potassium ions taken up by the cotransporter are recycled back into the luminal bath (Halm et al. 1985a). Since both Cl– and Na+ are transported across the epithelium, and because the apical uptake of Cl– is larger than that of Na+, with little apical recycling (Fig. 10), the short-circuit current is now outward, −21 μA cm−2, with a reversed transepithelial potential difference of 0.82 mV. While these values are incompatible with experimental data for toad intestine, they are in better agreement with those of flounder intestinal mucosa: 

\[
\begin{align*}
I_{sc} &= -66 \pm 8 \mu A \text{cm}^{-2} \\
V_{cell} &= 2.5 \pm 0.2 \text{mV}
\end{align*}
\]

respectively (Halm et al. 1985b). Given the few adjustments of input variables simulating the experimental values of toad intestinal mucosa (Table 5), the other bioelectrical parameters are also fairly well reproduced by our model, e.g. \(G_i = 26.5 \text{mS cm}^{-2}\) (flounder: 28.0 ± 1.6), and \(V_{cell} = -63 \text{mV}\) (flounder: −56 ± 2), with both \(C_{K_r}^{\text{cell}}\) and \(C_{Cl}^{\text{cell}}\) being above their respective electrochemical equilibrium concentrations in the model, as in the flounder. The fractional resistance of the apical membrane of flounder intestine is no more than 0.24 ± 0.04, however, which is significantly smaller than that of the model with \(f_R = 0.88\). This is partly due to a somewhat smaller apical membrane conductance of the model (2.7 versus 5.2 ± 1.2 mS cm−2 in flounder). But it also has to do with the experimental finding that significant components of the exit fluxes of K+ and Cl– seem to take place via an electroneutral K+–Cl− transport system in the basolateral membrane (Halm et al. 1985a). In the model these ion fluxes are channel mediated and thus conductive. If we switch on the 1 : 1 K+–Cl− cotransporter in serosal and lateral membranes (see eqn (20)) and direct large components of the exit fluxes of K+ and Cl– through these systems, the fractional resistance drops to significantly lower values, while the ion concentration of lis increases slightly. This has insignificant effects on other computed quantities. Since it is outside the scope of our study to investigate the physiological significance of such a system, we will just note that the major conclusions hold if a basolateral 1 : 1 K+–Cl− cotransporter is in operation. The paracellular Na+ fluxes, and their ratio, are well reproduced by the model (see Fig. 10). The computations further show that with the apical uptake fluxes of Na+ and Cl– being tightly coupled, truly isotonic transport is associated with fairly large recirculation of all ions. Thus, all pertinent features regarding solute-coupled water transport are retained after replacing the apical Na+ channel with an electroneutral cotransport mechanism.

**DISCUSSION**

As the first step in a theoretical analysis of sodium-coupled water transport in a multi-membrane system, one would have to consider the Smoluchowski–Hertz equation integrated through a water and ion permeable pore (eqn (15)), together with its associated equations for unidirectional paracellular fluxes (eqns (24a, b)). We see, with these equations, that the concentration of the transported fluid is governed by the ratio of bulk flow and the permeability coefficient of the solute (see also the discussion in Larsen et al. (2000b)). Considering transport of Na+ from lis to serosa with \(C_{Na}^{lis}/C_{Na}^{serosa} > 1\) and \(V^{bm} \approx 0\), for \(J^{bm} \rightarrow 0\), \(J^{lis-serosa}/J^{serosa-lis} \rightarrow J^{lis}/C_{serosa}\). At the limit, the Na+ concentration of the transportate is \(J_{Na}^{bm}/J_{w}^{bm} \rightarrow +\infty\). In contrast, for \(J_{w}^{bm} \rightarrow +\infty\) the mass flow of ions becomes dominating so that the virtual concentration of the fluid emerging from lis is \(J_{Na}^{bm}/J_{w}^{bm} \rightarrow C_{Na}^{lis} (1 - \sigma^{bm})\). Since \(C_{Na}^{lis}\) is not much above \(C_{Na}^{serosa}\), in this other limit with \(\sigma^{bm} \approx 0\), the concentration of the transportate is so close to that of the bath that ‘near isotonic transport’ is achieved with no requirement for recirculation. The above conclusions are valid also when two membranes in series, i.e. tm and bm, are being considered where \(C_{Na}^{lis} > C_{Na}^{lumen} = C_{serosa}^{serosa}\) (eqn (24b)). The general and useful conclusion is that the transepithelial paracellular flux ratio is a measure of the relative significance of diffusion and mass transport in determining the concentration of the fluid emerging from the lateral space. Thus, if the paracellular flux ratio under equilibrium conditions is close to unity, then the ‘primary transportate’ is strongly hypertonic, that is, recirculation is required for generating an isotonic net transportate. These considerations emphasize the importance of an experimental study of paracellular Na+ fluxes, and the significance of their ratio for estimating those physical variables of the paracellular pathway that govern ion-coupled water transport, which are difficult – if not impossible – to estimate by other methods.

The significance of this important principle governing ion-coupled water transport in leaky epithelia has been illustrated by the above example with small intestine. The most important result of our treatment is the prediction that, generally, ion recirculation is necessary for the formation of a transportate in osmotic equilibrium with the external bath. The significance of diffusion across the interface between lis and the serosal bath for generation of a hypertonc fluid emerging from lis has already been
emphasized in the theoretical study of Diamond & Bossert (1967). In our study, the large virtual concentration of fluid emerging from lis is derived from experimental results as it follows, simply, from the measured unidirectional paracellular Na+ fluxes and the water flux that is given by the experimentally measured active net flux of Na+. Thus, with the transport rates prevailing in toad intestinal mucosa, the diffusion fluxes out of lis overrule the ion fluxes carried by bulk transport. This readily explains the fairly large recirculation fluxes of Na+ estimated in the experiments on toad small intestine (Nedergaard et al. 1999). We can conclude that our treatment of paracellular fluxes based on convection–electrodiffusion theory removes the contradiction between a near-isotonic lateral intercellular-space fluid and large recirculation fluxes associated with isotonic or near-isotonic transport.

This implies that the osmolarity of the net transportate is tightly regulated and indicates that the epithelial cells are ‘osmotic sensors’. Since water channels in the serosal membrane serve rapid exchange of water between the cell and serosal bath, changes of cell volume may signal whether the rate of recirculation is properly adjusted to provide an isotonic net transportate. Our theory predicts that cell shrinkage caused by the formation of a hypertonic transportate would result in the stimulation of the cotransporter in the serosal membrane. In this connection it is interesting to note that in Ehrlich ascites tumour cells (Hoffmann et al. 1983) and in epithelial cells of the shark rectal gland (Greger et al. 1999) the loss of cell volume is the signal for activation of a cotransporter.

With similar active sodium fluxes the volume flows were fairly identical and independent of the entrance membrane (Figs 3, 9 and 10). This conforms to the principle governing solute-coupled water transport that the rate of water uptake is primarily dependent on the active flux of Na+. The same principle also explains the proportionality between active transepithelial Na+ transport and fluid absorption (Fig. 7), which is characteristic of isotonic transport in leaky epithelia.

Our analysis has shown that there is no contradiction between transcellular water uptake via the apical SGLT1 transporter and ion recirculation (Fig. 9). In a straightforward way our model accommodates this mechanism, but our analysis showed that the problem of isotonic transport is not being solved by this manoeuvre. There is still requirement for ion recirculation unless additional as yet unidentified mechanisms, alternative to water channels, are postulated for bringing water out of the cell, notably in such a way that the transepithelial absorbate is tightly regulated about its osmotic equilibrium value.

At equilibrium the energetic cost of transepithelial water transport depends on the water permeability of the pathway(s) separating the mucosal bath from lis (Fig. 4D). A similar rule was deduced from our ‘electroneutral’ model (Larsen et al. 2000b), and it is logical for a recirculation model coupling water transport to an actively transported solute via a hyperosmotic subcompartment. Our present computations with the ion model show that with high water permeability of the barrier separating the mucosal bath from the coupling compartment the recirculation flux associated with isotonic transport attained a small value, −0.2 (Fig. 4B), with an osmotic pressure of lis that was < 1 % above that of the bathing solutions (Fig. 4A). Therefore, our treatment covers the case of fluid transport in the absence of measurable osmotic pressure differences.

Although model predictions satisfy a large number of observations it is important to emphasize that a major assumption of the model has not yet been tested experimentally. While the recirculation flux of Na+ was estimated in experiments on toad small intestine (Nedergaard et al. 1999), we have not obtained experimental evidence that it is mediated by a 1Na+–1K+–2Cl− cotransporter, as postulated in the present theoretical treatment. It should be mentioned also that our model implies that the ‘basolateral membrane’ is divided into two functional membrane domains, i.e. the membrane lining the lateral intercellular space (lm) and the membrane facing the serosal space (sm). If our theory is correct we predict that, for example, inhibition of the putative cotransporter in the serosal membrane would result in the formation of a hypertonic transportate. Such a testing would be a powerful way of distinguishing between the Na+ recirculation theory and other theories of isotonic transport.

**APPENDIX**

The differential equation for electrodiffusion with superimposed convection can be written (Smoluchowski, 1915; Hertz, 1922):

\[
J_j = -D_j \frac{dC_j}{dx} - \frac{z_j F}{RT} \frac{d\psi}{dx} + J_W C_j. \tag{A1}
\]

Here \(J_j\) and \(J_W\) are the flux of the ion, \(j\), and water, respectively, \(D_j\) is the diffusion coefficient of \(j\) in water, \(C_j\) is the concentration of \(j\), \(\psi\) is the electrical potential, while \(z_j\), \(F\), \(R\) and \(T\) have their usual meanings. Following Staverman (1952) by introducing the reflection coefficient, \(\sigma\), the product \(J_W(1 - \sigma)\) represents the convection velocity of the ion, which replaces \(J_W\) of eqn (A1). With the assumption of constant electric field in the pore of length \(\delta\) and conventional sign convention,
i.e. \( \frac{dy}{dx} = -V/\delta \), and with stationary transmembrane fluxes, eqn (A1) takes the form:

\[
dC_j/dx + aC_j = b,
\]

where the constants \( a \) and \( b \) are:

\[
a = \left( \frac{J_w(c_1 - \sigma)}{D_p} + \frac{z_F V \delta}{RT} \right),
\]

\[
b = -J/D_p,
\]

and the general solution is:

\[
C_i = \exp(-ax) \left( \frac{b}{a} \exp(ax) + K \right).
\]

With boundary conditions, \( C_i = C_i(0) \) for \( x = 0 \) and \( C_j = C_j(\delta) \) for \( x = \delta \), the particular solution is given by:

\[
K = C_j(0) - (b/a),
\]

which leads to:

\[
J_i = \left( \frac{z_F V D_p}{RT} + J_w(1 - \sigma) \right) \left( \frac{C_j(\delta) - C_i(0) \exp(z_F V/(RT) + J_w(1 - \sigma)\delta/D_p)}{1 - \exp(z_F V/(RT) + J_w(1 - \sigma)\delta/D_p)} \right).
\]

We assume partition coefficients of unity at the interfaces between pore water and the well-stirred external fluid compartments, (I) and (II), so that \( C(0) = C^{(i)} \), \( C(\delta) = C^{(ii)} \), and:

\[
J_i = \left( \frac{z_F V}{RT} + J_w(1 - \sigma) \right) \left( \frac{C^{(i)} \exp(z_F V/(RT)) \exp(J_w(1 - \sigma)/P)}{\exp(z_F V/(RT)) \exp(J_w(1 - \sigma)/P) - 1} \right).
\]

Fluxes directed from (I) to (II) are positive, \( V = \psi^{(i)} - \psi^{(ii)} \) and \( P = D/P_\delta \). Equation (A5) is the convection–electrodifussion equation for a membrane with homogenous pores of uniform length with the Goldman–Hodgkin–Katz equation for electrodiffusion (Hodgkin & Katz, 1949) and the Hertz equation for convection–diffusion (Hertz, 1922), respectively, as limiting cases.

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