A Two-Barrier Compartment Model for Volume Flow across Amphibian Skin

Running title: volume flow across amphibian skin

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ABSTRACT

The amphibian skin has long been used as a model tissue for the study of ion transport and osmotic water movement across tight epithelia. To understand the mechanism of water uptake across amphibian skin, we model the skin as a well-stirred compartment bounded by an apical barrier and a tissue barrier. The compartment represents the lateral intercellular space between cells in the stratum granulosum. The apical barrier represents the stratum corneum, the principal/mitochondria-rich cells and the junctional area between cells. This barrier is hypothesized to have the ability to actively transport solutes through Na\(^+\)-K\(^+\)-ATPase. The actively transported solute flux is assumed to satisfy the Michaelis-Menten relationship. The tissue barrier represents a composite barrier comprising of the stratum spinosum, the stratum germinativum, the basal lamina and the dermis. Our model shows that: 1) the predicted rehydration rates from apical bathing solutions are in good agreement with the experiment results in Hillyard and Larsen [J. Comp. Physiol. 171: 283-292, 2001]; 2) under their experimental conditions, there is a substantial volume flux coupled to the active solute flux and this coupled volume flux is nearly constant when the osmolality of the apical bathing solution is greater than 100 mOsm; 3) the molar ratio of the actively transported solute flux to the coupled water flux is about 1:160, which is the same as that reported in Nielsen [J. Membrane Biol., 159: 61-69, 1997].

Key words: tight epithelium, active solute transport, Michaelis-Menten equation, coupled-water transport
INTRODUCTION

Most amphibian species (toads/frogs) obtain water primarily by osmotic absorption across their skin and are able to absorb Na\(^+\) and Cl\(^-\) across their skin from very dilute solutions [2, 19, 27]. Because of these properties, the amphibian skin has long been used as a model tissue for the study of ion transport and osmotic water movement across epithelia [14].

Amphibian skin is a multi-layered and a very “tight” epithelium ([21], Fig. 1). The outermost layer of the skin is the highly permeable stratum corneum (the cornified cells), followed by the stratum granulosum and the stratum spinosum. The innermost layer is the stratum germinativum that faces the basal lamina. The cells in the stratum granulosum, the stratum spinosum and the stratum germinativum communicate with each other via the gap junctions and are held together by the desmosomes. Tight junctions exist in the stratum granulosum. Amphibian skin is also a heterocellular epithelium. Two types of cells, principal cells (majority) and mitochondria-rich cells (MR cells, minority) are present in the stratum granulosum and stratum spinosum. The MR cells constitute a highly specialized pathway for chloride transport across skin epithelium (epidermis) [21]. They are few in number. In toads, the MR cells only occupy less than two percent of the epithelial cell volume. Their apical membrane area adds up to less than one percent of the total epidermal surface area [21]. Both principal and MR cells have Na\(^+\)-K\(^+\)-ATPase on their basolateral cell membranes and have the ability to actively transport sodium with the presence of ATP [20, 30].

In living toads, the driving force for water reabsorption is believed to be the osmotic gradient across skin. Recently, Sullivan et al. [35] observed that the toad, *Bufo punctatus*, was able to rehydrate more rapidly from a 50 mM NaCl solution than from deionized water despite there being a reduced osmotic gradient for water uptake from the salt solution. A similar
phenomenon was observed by Hillyard and Larsen [13] in experiments using *Bufo marinus*. They dehydrated toads 10-15 percent of their standard weights and allowed them to rehydrate from either deionized water or from 10 to 120 mM NaCl solutions. They found that nearly fully immersed toads could rehydrate from 50 mM NaCl at a rate that is ~1.45 times that from deionized water. In addition, the rehydration rate from 10 mM NaCl bathing solution was comparable to that from 50 mM NaCl bathing solution. However, water uptake from 100 mM sucrose and 50 mM Na gluconate was reduced relative to deionized water by a fraction predicted from the osmotic gradient. To explain the mechanism for enhanced water gain from dilute salt solutions, we postulate that there is water transport coupled with active solute transport (specifically, active Na⁺/Cl⁻ transport) provided that both Na⁺ and Cl⁻ are present in the bathing solutions.

Active solute transport can serve as a driving force for water uptake and is the main driving force in nearly isotonic transport in leaky epithelia such as the proximal tubule epithelium in kidneys [40] and the intestine [22]. This active solute transport also exists in tight epithelia of the frog skin [18, 39, 16, 28]. Nielsen [28] simultaneously measured the short-circuit current (Na⁺ flux) and the transepithelial water flux across isolated frog skin, *Rana esculenta*, bathed with identical Ringer’s on either side. He found a linear correlation between transepithelial Na⁺ transport and the water movement, which corresponded to 160 ± 15 molecules of water following each Na⁺ across the skin.

Figure 2 shows a simplified model for water and solute transport across amphibian skin. Na⁺ transport across the skin occurs through two pathways, transcellular and paracellular. In transcellular transport, Na⁺ first enters the cytoplasm via epithelial sodium channels (ENaCs) on the apical cell membrane, and then Na⁺ in cytoplasm is actively pumped into the intercellular
space by Na\(^+\)-K\(^+\)-ATPase at the basolateral cell membranes of both principal and MR cells [21]. In paracellular transport, sodium/chloride can traverse the tight junction area between cells due to concentration gradients and solvent drag [21]. Water transport is also through two pathways. The transcellular water transport is suggested to occur through water channels at the apical and basolateral cell membranes [36] and the paracellular water transport is through the tight junction area.

There have been numerous studies on epithelial NaCl and water transport across amphibian epidermis since the revolutionary work by Ussing and his colleagues [37, 38, 18, 39]. These studies are summarized in [17, 16, 15, 20, 21, 32, 24, 29, 33, 34].

The classical two-membrane theory for amphibian skin epithelium or KJU-model proposed by Koefoed-Johnsen and Ussing [18] separated the surface of one epithelial cell into apical and basolateral membranes with Na\(^+\) pumps at the basolateral membrane (Fig. 2). The compartment model for transepithelial transport was first proposed by Curran and MacIntosh [3] to explain isotonic fluid transport across leaky epithelia in the absence of external osmotic gradients. Since then the original compartment model has been refined by many researchers to explain the fluid transport across leaky epithelia such as in the proximal tubule of the kidney, the ileum and the small intestine where the transport is nearly isotonic [4, 5, 40, 34, 22, 23]. However, there is no quantitative compartment model that is applied to the tight epithelial non-isotonic transport like that of the amphibian skin where there is a passive osmotic gradient in addition to solute-coupled water flux.

In this study we propose a two-barrier functional compartment model to examine the volume flux across amphibian skin driven by both the active solute transport and the osmotic gradient. The classical two-membrane model for one epithelial cell has been modified to describe
water and solute transport across amphibian skin consisting of various types of epithelial cells. The amphibian skin is modeled as a well-stirred compartment bounded by an apical barrier and a tissue barrier (Fig. 3). The compartment represents the intercellular spaces between cells in the stratum granulosum. The apical barrier includes both transcellular and paracellular (tight junction) pathways for water and solute transport. The transcellular component of the apical barrier is hypothesized to have the ability to actively transport solutes through Na\(^+\)-K\(^+\)-ATPase. We first postulated that the actively transported solute flux satisfies the Michaelis-Menten equation (Fig. 4), i.e., the flux is nearly saturated when the apical bathing salt solution is \(\sim\)100 mOsm. In addition, we first estimated the permeability properties of the tissue barrier comprising of stratum spinosum, the stratum germinativum, the basal lamina and the dermis, based on the knowledge for the endothelial barrier forming the microvessel wall [1, 7, 8]. Our model predicts a transepithelial volume flux across amphibian skin that is in good agreement with the experimental measurements in Hillyard and Larsen [13]. Further, the volume flux coupled with the active transport is nearly constant when the osmolality of the apical bathing solution is greater than 100 mOsm. The molar ratio between the transported solutes and the coupled water molecules is 1:156, around the value reported in Nielsen [28]. In summary, we proposed a two-barrier compartment model that can quantitatively predict various experimental measurements in Hillyard and Larsen [13] and Nielsen [28] for water and solute transport across tight epithelium of the amphibian skin. This model can also be easily modified to explain transport phenomena in other types of epithelia.

**GLOSSARY**

\(C_i\) solution osmolality. \(i = E\) (transported solution), \(I\) (lateral intercellular space), \(L\) (the apical bathing solution) and \(T\) (solution at the tissue side)
\( \bar{C}_L \) mean salt osmolality across the apical barrier

\( \bar{C}_T \) mean salt osmolality across the tissue barrier

\( D_S \) salt diffusion coefficient in bulk flow

\( J_S \) transepithelial solute flux

\( J_{Si} \) solute flux. \( i = C \) (the cell barrier), \( L \) (the apical barrier), \( T \) (the tissue barrier) and \( TJ \) (the tight junction barrier).

\( J_V \) transepithelial volume flux

\( J_{Vi} \) volume flux. \( i = C \) (the cell barrier), \( L \) (the apical barrier), \( T \) (the tissue barrier) and \( TJ \) (the tight junction barrier).

\( L_{Pi} \) hydraulic conductivity. \( i = C \) (the cell barrier), \( L \) (the apical barrier), \( T \) (the tissue barrier) and \( TJ \) (the tight junction barrier).

\( N \) actively transported solute flux

\( N_{max} \) maximal actively transported solute flux

\( p_i \) hydrostatic pressures, \( i = I \) (lateral intercellular space), \( L \) (the apical side) and \( T \) (the tissue side)

\( P_i \) solute permeability. \( i = C \) (the cell barrier), \( L \) (the apical barrier), \( T \) (the tissue barrier) and \( TJ \) (the tight junction barrier).

\( r \) average radius of the cells in the tissue barrier

\( R \) universal gas constant

\( T \) room temperature in Kelvin

\( W \) average gap width of the lateral intercellular space

\( \delta \) depth of the paracellular route in the tissue barrier

\( \mu \) water viscosity

\( \sigma_i \) reflection coefficient for salt. \( i = C \) (the cell barrier), \( L \) (the apical barrier), \( T \) (the tissue barrier) and \( TJ \) (the tight junction barrier).
MODEL

Model Description

Our functional compartment model for water transport across amphibian skin is depicted in Fig.3. Amphibian skin is modeled as a well-stirred compartment bounded by an apical barrier and a tissue barrier, which are in series and face the bathing side and the tissue side respectively. Physically, the compartment represents the lateral intercellular space under the tight junction (TJ) between cells in the stratum granulosum.

The apical barrier is a heterogeneous barrier comprising of the cell barrier and the TJ barrier. It represents the stratum corneum, the principal/MR cells in the stratum granulosum and the TJ between the cells. The resistance of the stratum corneum is neglected because it is highly permeable to water and solutes as small as \( \text{Na}^+ \) or \( \text{Cl}^- \). The cell barrier represents both the principal and MR cells and the difference between principal and MR cells is neglected. In our idealized model, the cell barrier has the ability to actively transport solutes. The solute flux by active transport is denoted by \( N \), with the unit of nMol s\(^{-1}\) cm\(^{-2}\). The TJ barrier represents the TJ area between the cells in the stratum granulosum. Estimations for sodium and chloride permeability of this barrier are available in Larsen [21]. In our model, the contribution of the TJ or the paracellular pathway to water transport is carefully examined (please see later sections).

The tissue barrier is in series with the apical barrier. It represents the stratum spinosum, the stratum germinativum, the basal lamina and the dermis, i.e. the layers after the stratum granulosum. We first assume that the dermis is much more permeable to water and solute than other layers and its resistance to water and solute transport is neglected. The permeability of the tissue barrier to water and solutes are estimated later in parameter section.
In this model we hypothesize that the main resistance to water and solute transport comes from the apical barrier. The outflow from the tissue barrier mixes together at the exit to the tissue space. The osmolality of the exit flow can be different from that in the tissue.

Mathematical Formulation

Denote the hydrostatic pressure and the osmolality in the lateral intercellular space $p_l$ and $C_l$, respectively. The volume flux across the cell barrier on unit surface area, $J_{VC}$, from the apical side to intercellular space, is

$$J_{VC} = L_{PC} (p_L - p_i + \sigma_C RT (C_i - C_L)).$$  \hspace{1cm} (1)

Here $L_{PC}$ is the hydraulic conductivity of the cell barrier and $\sigma_C$ its reflection coefficient for salt solutes. $C_L$ is the osmolality of the apical bathing solution and $p_L$, the hydrostatic pressure in the apical side. $R$ is universal gas constant and $T$ is temperature. Similarly, the volume flux across the TJ barrier on unit surface area, $J_{VTJ}$, from the apical bathing solution to intercellular space, is

$$J_{VTJ} = L_{PTJ} (p_L - p_i + \sigma_{TJ} RT (C_i - C_L)).$$  \hspace{1cm} (2)

$L_{PTJ}$ is the hydraulic conductivity of the TJ barrier and $\sigma_{TJ}$ its reflection coefficient for salt solutes.

Denote

$$L_{PL} = L_{PC} + L_{PTJ},$$

$$\sigma_L = \frac{L_{PC} \sigma_C + L_{PTJ} \sigma_{TJ}}{L_{PC} + L_{PTJ}}$$

The total volume flux across the apical barrier is

$$J_{VL} = J_{VC} + J_{VTJ} = L_{PL} (p_L - p_i + RT \sigma_L (C_i - C_L)).$$  \hspace{1cm} (3)

The volume flux across the tissue barrier on unit surface area, $J_{VT}$, is
\( J_{VT} = L_{PT}(p_T - p_L + \sigma_T RT(C_T - C_I)). \) (4)

\( L_{PT} \) is the hydraulic conductivity of the tissue barrier and \( \sigma_T \) its reflection coefficient to salt solutes. \( C_T \) is the osmolality of the well-stirred tissue compartment. \( p_T \) is the hydrostatic pressure in the tissue side, which is assumed to be the same as that in the apical side \( p_L \). Both \( p_L \) and \( p_T \) are set to be zero in our model.

Under steady state the flux into and out of the intercellular space is equal.

\[ J_V = J_{VL} = J_{VT}. \] (5)

Equation 5 can be rewritten to provide a constraint between \( p_L \) and \( C_I \).

\[ p_L = \frac{L_{pl} \sigma_T RT(C_I - C_L) - L_{PT} \sigma_T RT(C_T - C_I)}{L_{pl} + L_{PT}}. \] (6)

Applying Eq. 6, the total volume flux, \( J_V \), can be expressed only in terms of \( C_I \).

\[ J_V = \frac{L_{pl} L_{PT}}{L_{pl} + L_{PT}} RT \left( \sigma_T (C_T - C_I) + \sigma_L (C_I - C_L) \right). \] (7)

\( J_{VC} \) and \( J_{VT} \) can be also expressed only in term of \( C_I \).

The solute flux across the cell barrier on unit surface area, \( J_{SC} \), is

\[ J_{SC} = J_{VC} (1 - \sigma_C) \bar{C}_I + P_C (C_L - C_I) + N. \] (8)

Here \( P_C \) is the solute permeability of the cell barrier. \( \bar{C}_I \) is defined as

\[ \bar{C}_I = \frac{(C_L - C_I)}{\ln \left( \frac{C_L}{C_I} \right)}. \] (9)

\( N \) is the actively transported solute flux. We assume that \( N \) satisfies Michaelis-Menten equation [6],

\[ N = N_{max} \frac{C_L}{C_L + K}. \] (10)
$N_{\text{max}}$ is the maximal flux; $K$ is the Michaelis-Menten constant, which depends on the concentrations of salt solutions and the function of Na$^+$-K$^+$-ATPase.

The solute flux across the TJ barrier on unit surface area, $J_{STJ}$, is

$$J_{STJ} = J_{VTJ} (1 - \sigma_C) \overline{C}_L + P_{TJ} (C_L - C_I). \quad (11)$$

$P_{TJ}$ is the solute permeability of the TJ barrier.

The solute flux across the tissue barrier on unit surface area is

$$J_{ST} = J_{VT} (1 - \sigma_T) \overline{C}_T + P_I (C_I - C_T). \quad (12)$$

$P_T$ is the solute permeability of the tissue barrier. $\overline{C}_T$ is defined as

$$\overline{C}_T = \frac{(C_T - C_I)}{\ln \frac{C_T}{C_I}}. \quad (13)$$

Under steady state the solute flux into and out of the lateral intercellular space is equal.

$$J_S = J_{SC} + J_{STJ} = J_{ST}. \quad (14)$$

Equation 14 provides another constraint for $p_I$ and $C_I$.

If the apical bathing solution osmolality $C_L$ and the tissue osmolality $C_T$ are given and the parameters for both the apical barrier and tissue barrier, $L_{PC}$, $P_C$, $\sigma_C$, $L_{PTJ}$, $P_{TJ}$, $\sigma_{TJ}$, $L_{PT}$, $P_T$ and $\sigma_T$, are all known, there are only two unknowns left, $C_I$ and $p_I$. There are also two constraints, Eqs. 5 and 14. To solve this problem, we first substitute the hydrostatic pressure $p_I$ in terms of $C_I$, using Eq.6, in Eqs. 1, 2 and 4. In this way, the volume fluxes, $J_{VC}$, $J_{VTJ}$ and $J_{VT}$, are expressed only in term of $C_I$, as in Eq. 7. We then put the revised forms for $J_V$’s in Eqs. 8, 11 and 12 to express all $J_S$’s only in terms of $C_I$. The conservation condition for solute flux, Eq.14, provides an implicit constraint on $C_I$. The meaningful solution for $C_I$ is found by using a commercial software MathCAD. After $C_I$ is determined, the hydrostatic pressure $p_I$ can be determined using Eq. 6.
The volume fluxes across the cell barrier, the TJ barrier and the tissue barrier can be determined using Eqs. 1, 2, and 4. Finally, the solute fluxes, $J_{SC}$, $J_{STJ}$ and $J_{ST}$, can be determined by using Eqs. 8, 11 and 12.

One important variable of interest is the osmolality of the transported solution. In very leaky epithelia such as kidney proximal tubule and intestine, transepithelial transport is nearly isotonic and the osmolality of the transported solution is close to that for serum in the interstitial tissue space [40, 22, 23]. For water transport across amphibian skin (tight epithelium), the osmolality of the transported solution is likely to be different from the serum osmolality provided that toads can rehydrate from deionized water where there is no reabsorptive solute flux [13]. The osmolality of the transported solution is defined as

$$C_E = \frac{J_S}{J_V}. \quad (15)$$

If $C_E$ is different from $C_T$, it may induce a perturbation on $C_T$. However, the volume flux across amphibian skin, in our case, is small, compared to total blood/lymph flux to skin. This perturbation is negligible and the assumption, that $C_T$ is constant, is valid.

**Parameters**

The parameter values used in our model are listed in Table 1. Room temperature (25°C) is used in all calculations. $C_T$ is the tissue osmolality and its value is set to be 250 mOsm. This value is a typical one for the blood/lymph osmolality of toads based on the experimental measurements [13]. $C_L$ is the osmolality of the apical bathing solution. Based on the concentrations of NaCl bathing solutions (0-120 mM) used in Hillyard and Larsen [13], we choose $C_L$ from 0 to 250 mOsm.
$N_{\text{max}}$ is the maximum of the actively transported solute flux used in Michaelis-Menten equation. In order to fit the experimental results in Hillyard and Larsen [13], $N_{\text{max}} = 4.0 \text{ nMol s}^{-1} \text{ cm}^{-2}$ (see later section in RESULTS). $K$, the Michaelis-Menten constant, is estimated by fitting the experimental results in Hillyard and Larsen [13]. Figure 4 shows the dependence of $N/N_{\text{max}}$ on $K$. $K$ of 20 mOsm is used in our calculation.

The resistance of each barrier in the compartment model to the transepithelial water transport is not completely known. We hypothesize that the apical barrier offers most of the resistance to water transport. In this way, the hydraulic conductivity of the apical barrier ($L_{\text{PL}}$) should be close to the transepithelial hydraulic conductivity ($L_P$, see Eq.16). The transepithelial hydraulic conductivity, $L_P = 54.2 \times 10^{-7} \text{ cm/s/Atm} \ (7.1 \times 10^{-9} \text{ cm/s/mmHg})$, for isolated frog skin was measured in Nielsen [28], in the presence of antidiuretic hormone (AVT), a hormone which can increase the transepithelial hydraulic conductivity. This measured value is adopted for $L_{\text{PC}}$, the hydraulic conductivity of the cell barrier for the toad skin during rehydration. There is no measured value for the hydraulic conductivity of the TJ barrier, $L_{\text{TJ}}$. Because amphibian skin is a tight epithelium, we assume that $L_{\text{TJ}}$ is much smaller than $L_{\text{PC}}$. In fact, in our calculation $L_{\text{TJ}}$ is set to be one tenth of $L_{\text{PC}}$. This ratio will be further examined later in the DISCUSSION.

The salt permeability of the cell barrier, $P_C$, is set to be $2.4 \times 10^{-8} \text{ cm/s}$, an averaged value for measured inner and outer membrane permeability of amphibian principal cells [21]. The TJ solute permeability, $P_{\text{TJ}}$, has a value of $5 \times 10^{-8} \text{ cm/s}$, a reported value for sodium permeability of the junctional membrane in Larsen [21].

The hydraulic conductivity and solute permeability of the tissue barrier have never been reported. We thus construct a simple model here to provide an estimate for them. We hypothesize that water and solute transfer across the tissue barrier via the intercellular space of
epithelia. However, the detailed dimensions of the intercellular space for toad skin are not available. The best-known ultrastructure of the intercellular space is that between endothelial cells forming capillary walls. Adamson and Michel [1] showed that the interendothelial cleft appears to be a small slit around endothelial cells. The gap width of the cleft is 20 nm and the depth is 0.5 µm (thickness of the wall) in frog mesenteric capillary. This value has been used in a series of research papers [7, 8, 9, 10, 11] to successfully predict water and solute transport across capillary walls. In this study we shall use a slit geometry to provide an estimate for the hydraulic conductivity and salt permeability of the tissue barrier.

From Fig. 1, we can estimate that on average, the cells are cylindrical with radii r from 5 to 10 µm; and the depth δ of the paracellular pathway varies from 50 to 100 µm. Further, we assume that the gap width W of the paracellular pathway in the tissue barrier is around 50-100 nm, larger than that for interendothelial space in frog mesenteric capillary walls. Using a slit model for the paracellular pathway [26], the hydraulic conductivity and the solute permeability of the tissue barrier, $L_{PT}$ and $P_T$, are calculated as,

$$L_{PT} = \frac{W}{r} \frac{W^2}{12 \mu \delta}$$

$$P_T = \frac{W}{r} \frac{D_s}{\delta}$$

Here $D_s$ is the salt (Na⁺ or Cl⁻) diffusion coefficient in bulk flow at 25°C, $D_s = 1.4 \times 10^{-5}$ cm²/s [8]. $\mu$ is solution viscosity at 25°C, $\mu = 1.01$ cp [11]. The calculated $L_{PT}$ varies from 1.4 to $45 \times 10^{-7}$ cm/s/mmHg and the $P_T$ varies from 0.7 to $5.6 \times 10^{-5}$ cm/s, which are of the same magnitudes of those for frog mesenteric capillary wall, respectively. In this study, we choose $L_{PT} = 4 \times 10^{-7}$ cm/s/mmHg and $P_T = 1.1 \times 10^{-5}$ cm/s. Compared to permeability of the apical barrier, the tissue barrier provides much less resistance to water and solute transport.
The tissue barrier is in series with the apical barrier. The total resistance of amphibian skin (inverse of the transepithelial hydraulic conductivity) can be approximated by the sum of the resistance from the apical barrier and from the tissue barrier.

\[
\frac{1}{L_p} = \frac{1}{L_{PL}} + \frac{1}{L_{PT}}. \tag{16}
\]

Using \(L_{PL} = L_{PC} + L_{PTJ} = 7.8 \times 10^{-9} \text{ cm/s/mmHg}\) (Table 1) for hydraulic conductivity across the apical barrier, and \(L_{PT} = 4.0 \times 10^{-7} \text{ cm/s/mmHg}\) for that across the tissue barrier, the hydraulic conductivity across the skin \(L_p = 7.65 \times 10^{-9} \text{ cm/s/mmHg}\). This value is almost the same as that reported in Nielsen [28].

Water and salt traverse the apical cell membrane in the stratum granulosum via specific channels. Sodium enters into the cytoplasm via the epithelial sodium channels (ENaCs) and is pumped out into the intercellular space by \(\text{Na}^+\)-\(\text{K}^+\)-ATPase, while water transfers via exclusive water channels (aquaporins) [36]. In this study we assume ENaCs are nearly impermeable to water and water channels are impermeable to sodium ion. The reflection coefficient for solute of the cell barrier \(\sigma_C\) is set to be 0.95. This value has been reported for KCl in Larsen [21] for both MR cells and principal cells. We also assume a typical TJ reflection coefficient \(\sigma_{TJ}\) of 0.80. The high values chosen for \(\sigma_C\) and \(\sigma_{TJ}\) allow the apical barrier tight enough to build the osmotic gradient for water transport. In the calculation, we try to vary \(\sigma_C\) from 0.95 to 0.99 and vary \(\sigma_{TJ}\) from 0.40 to 0.95. It is found that the transepithelial volume flux is not sensitive to these values. The reflection coefficient \(\sigma_T\) for the tissue barrier is set to be 0.01, the measured value for frog mesenteric capillaries [26] because the tissue barrier is as permeable as the capillary wall to solutes as small as \(\text{Na}^+\) or \(\text{Cl}^-\).
RESULTS

Before we present the model predictions, we first discuss two characteristic values for volume flux across amphibian skin. The first is the measured volume flux in Hillyard and Larsen [13]. They reported that nearly immersed toads, *Bufo marinus*, could increase their body weight by 10-12 percent in 120 minutes through rehydration from 50 mM NaCl. Assuming that the standard weight of a toad is ~200 g and the estimated reabsorption area is ~185 cm$^2$ by a cylinder model for the toad body, the volume flow across toad skin can be estimated as $\sim 1.7 \times 10^{-5}$ cm/s.

The second characteristic value is the measured transepithelial volume flux across isolated frog skin (*Rana esculenta*) in Nielsen [28], $2.2 \times 10^{-6}$ cm/s, at the presence of AVT. Under the same condition, the actively transported solute flux was measured as 0.765 nMol s$^{-1}$ cm$^{-2}$. Another characteristic value reported by Nielsen [28] is that there is a linear correlation between actively transported solute flux and the volume flux, which corresponds to that $160 \pm 15$ molecules of water follow each Na$^+$ across the skin.

Based on the aforementioned experimental results, we can determine $K$ and $N_{\text{max}}$ in the Michaelis-Menten equation that is assumed for the active solute transport across the apical barrier. Figure 4 shows the relation between dimensionless $N/N_{\text{max}}$ and $K$. We choose $K = 20$ mOsm and plot in Fig. 5 the volume flux across the skin $J_V$ as a function of $N$ at various apical bathing solution concentration $C_L$. These lines are nearly parallel to each other with the slope $2.8-3.0$ nL/nMol. $J_V$ shown here is the combined result from the active solute transport and the osmotic gradient across the skin except at $C_L = 250$ mOsm when the osmotic gradient disappears. The larger the $N$, the higher the $J_V$. The slope of the line for $C_L = 250$ mOsm, 2.8 nL/nMol, corresponds to that 156 molecules of water follow each solute across the skin. This ratio is in the range of $160 \pm 15$ reported in Nielsen [28]. Therefore, by properly choosing $K$, we can fit the
experimental data. To satisfy the observed $J_V$ of $\sim 1.7 \times 10^{-5}\text{cm/s}$ in Hillyard and Larsen [13] at $C_L = 100\text{mOsm}$ (50 mM NaCl solution), from Fig. 5, $N = 3.5, N_{\text{max}}$ should be $\sim 4\text{nMol}_2\text{cm}^2$. This value is also comparable to that measured by Larsen [21]. When $N = 0.765\text{nMol/cm}^2$, corresponding $J_V$ in Fig. 5 is about $2.2 \times 10^{-6}\text{cm/s}$ under isotonic condition at $C_L = 250\text{mOsm}$. It is the measured value across the frog skin in Nielsen [28].

Figure 6 shows the model predictions and the experimental results for the volume flux $J_V$ as a function of osmolality of the apical bathing solution $C_L$. The results are expressed as the ratio to the volume flux when $C_L = 0$ (bathing solution is deionized water). Lines are model predictions and symbols are experimental data. Upper curve shows the result when $N$ satisfies Michaelis-Menten equation with $K = 20\text{mOsm}$ and $N_{\text{max}} = 4\text{nMol}_2\text{cm}^2$. Lower one is when $N = 0$, representing that the driving force is osmotic gradient only. We can see that under both cases the model predictions are in good agreement with the experimental data.

The volume flux in the presence of the active solute transport (upper curve) is always larger than that in the absence of the active solute transport (lower curve). The difference between these two curves, namely, the volume flux coupled with the active solute transport, is of the same order of the magnitude as the volume flux from the deionized water due to the osmotic gradient.

When $C_L$ is greater than 100 mOsm, the coupled volume flux is nearly constant because the two curves in Fig. 6 are nearly parallel to each other. This indicates the saturation of active solute transport when $C_L \geq 100\text{mOsm}$ (See Fig. 4 at $K = 20\text{mOsm}$). When $C_L < 100\text{mOsm}$, this model predicts a nearly constant transepithelial water flux from both active and passive transport. This is in agreement with the experimental measurements in Hillyard and Larsen [13] because
they suggested that the hydration rate across the toad skin is nearly constant when the apical salt solution was dilute (< 100 mOsm).

In Fig. 7A, we plot the intercellular osmolality $C_I$ as a function of the apical bathing solution osmolality $C_L$ in the presence and the absence of active solute flux $N$. When $N = 0$, $C_I$ is always less than tissue osmolality $C_T$ (250 mOsm) but greater than $C_L$ at each $C_L$. This induces an osmotic gradient driving water from the apical side to the tissue side. However, when $N$ is not zero but satisfies the Michaelis-Menten equation with $K = 20$ mOsm and $N_{\text{max}} = 4$ nMol s$^{-1}$cm$^{-2}$, $C_I$ can be either hypotonic or hypertonic relative to $C_T$ depending on $C_L$. When $C_L$ is less than 150 mOsm, $C_I$ is hypotonic to $C_T$. When $C_L$ is more than 150 mOsm, $C_I$ is hypertonic to $C_T$.

We plot in Fig. 7B the hydrostatic pressure $p_I$ in the intercellular space as a function of the apical bathing solution osmolality $C_L$ in the presence and the absence of active solute flux $N$. When $N = 0$, $p_I$ is negative, but very close to both pressures in the apical and tissue sides that are set to zero. This pressure difference will draw water into the intercellular space from both sides. However, compared to osmotic gradient that drives water from the intercellular space to the tissue side (~1500 mmHg at $C_L = 20$ mOsm), the contribution to $J_V$ from the negative hydrostatic pressure difference can be neglected. When $N \neq 0$, $p_I$ ranges from 22 to 41 mmHg when $C_L$ ranges from 20 to 250 mOsm. Again, the volume flux contributed from the hydrostatic pressure difference is negligible compared to the contribution from the osmotic gradient, which is ~1500 mmHg at $C_L = 250$ mOsm.

In Fig. 7C, we plot the transepithelial solute flux $J_S$ as a function of $C_L$ in the presence and the absence of active solute flux $N$. We notice that $J_S$ is almost completely contributed by $N$. When $N = 0$, $J_S$ is close to zero at each $C_L$. 
Figure 7D shows the osmolality of the transported solution $C_E = J_S/J_V$ as a function of the apical bathing solution osmolality $C_L$ in the presence and the absence of active solute flux $N$. When $N = 0$, $C_E$ is almost zero due to negligible $J_S$ (Fig. 7C). When $N \neq 0$, $C_E$ increases with increasing $C_L$. $C_E$ is greater than $C_T = 250$ mOsm when $C_L > 150$ mOsm. Higher $C_E$ would induce a disturbance in $C_T$ at the exit of the tissue barrier. However, compared to the much greater amount of water and solutes in the tissue region, this disturbance can be neglected.

**DISCUSSION**

**Effects of Uncertainty in Parameters**

The uncertainty that resides in the estimated parameters would induce deviations in the model predictions. Therefore, we first discuss the sensitivity of the model to chosen parameters.

The hydraulic conductivity of the apical barrier $L_{PL}$ is the summation of the transcellular permeability $L_{PC}$ and the paracellular permeability $L_{PTJ}$. In our model, we use the measured value by Nielsen [28] for $L_{PC}$, $7.1 \times 10^{-9}$ cm/s/mmHg, which was the measurement in the presence of a hormone AVT. In the absence of AVT, it was measured as $9.3 \times 10^{-10}$ cm/s/mmHg, which is roughly one tenth of that in the presence of AVT.

We test the influence of $L_{PC}$ on the volume flux $J_V$, which is shown in Fig. 8. The 10 times higher $L_{PC}$ would increase $J_V$ by up to ~3.5 times of that for the lower $L_{PC}$ in both cases of $N = 0$ and $N \neq 0$. We choose the higher $L_{PC}$ to predict the experimental results in Hillyard and Larsen [13] because the toads in the experiments were under dehydration conditions and there should be some hormonal effect [12, 25].
In Fig. 9, we test the influence of hydraulic conductivity of the TJ barrier $L_{PTJ}$ on $J_V$. Three $L_{PTJ}$ values, 0.02$L_{PC}$, 0.1$L_{PC}$, and 0.5$L_{PC}$, are chosen for the comparison. $L_{PC} = 7.1 \times 10^{-9}$ cm/s/mmHg. The 25 times change in $L_{PTJ}$, from 0.02$L_{PC}$ to 0.5$L_{PC}$, would only induce up to 18% increase in $J_V$. The difference in lines of $L_{PTJ} = 0.02L_{PC}$ and $L_{PTJ} = 0.1L_{PC}$ is negligible.

The influence of other estimated parameters, $L_{PT}$, $P_T$, $\sigma_C$, $\sigma_{TJ}$, and $\sigma_T$, has been discussed correspondingly in the parameter section. Although there is a lack of experimental sources for these parameters, the estimates used in our model are shown to be reasonable.

**Compartment models**

Compartment models are widely used to model “leaky” epithelia for the coupled water transport, such as in the proximal tubule of the kidney, in the ileum and in the small intestine, where transport is nearly isotonic [3, 4, 5, 34, 22, 23, 40]. Transport across amphibian skin, the “tight” epithelia, is far from isotonic as shown in Hillyard and Larsen [13]. The concentration of the apical bathing solution can be as low as zero (deionized water) while the lymph osmolality under subcutaneous skin varies from 220 mOsm to 260 mOsm [13]. Therefore, unlike the isotonic transport in leaky epithelia, both the osmotic gradient and the active solute transport can act as driving forces for transepithelial water uptake.

We tried to adopt the four compartments-five membranes model by Larsen et al. [22] for the coupled water transport in toad intestine to model the volume flow across the amphibian skin. We failed due to the lack of permeability data for each membrane and being unable to simplify a series of non-linear equations because there is no isotonic condition in our case.

In the current study, we are able to functionally simplify the skin into three compartments-two barriers structure with the active solute transport mechanism at the apical
barrier. All the needed permeability parameters are either from measured data or from simple model estimations. We first proposed to use Michaelis-Menten equation for the active solute transport by Na⁺-K⁺-ATPase, which is a commonly used relationship in this type of molecular event [6]. We show that this assumed relationship for the active solute transport of epithelia in amphibian skin can lead us to a good prediction for a variety of experimental observations.

There is an observation in Hillyard and Larsen’s experiment [13] showing that the total solute content after rehydration increased by ~8% compared to that in the hydrated and dehydrated states, when the apical NaCl solution was 120 mM. There was only slight increase (~2 %) when the apical solutions were 10 mM and 50 mM, no increase when it was deionized water. From Figs. 6, and 7C, we notice that when the apical solution \( C_L \) increases from 100 mOsm to 250 mOsm, the solute flux \( J_S \) increases but the volume flux \( J_V \) decreases. In this way, there would be a net increase in salt content when \( C_L = 240 \) mOsm (or 120 mM NaCl) compared to that when \( C_L = 100 \) mOsm (or 50 mM NaCl) if the toad uptakes same amount of the volume.

Another observation in Hillyard and Larsen [13] was that adding amiloride (a pyrazine diuretic) to block the epithelial sodium channels (ENaC, Fig. 2) had no effect on the rehydration degree when \( C_L = 20 \) mOsm and 100 mOsm. But the addition of amiloride significantly reduced the degree of rehydration relative to the same toads when \( C_L = 240 \) mOsm or 120 mM NaCl. Where is the source for the salt that is pumped into the intercellular space to induce an osmotic gradient for water uptake, if the apical sodium channel is blocked by amiloride? The sodium recirculation theory proposed in Larsen et al. [22, 23] may be employed in the current model to elucidate the amiloride effect in the future.

So far, it is still under debate that if the transepithelial water flow coupled to ion transport is driven by local osmosis, or electro-osmosis, or molecular water pumping under nonosmotic
conditions [34]. A recent study by Sanchez et al. [31] showed the role of electro-osmosis in fluid transport across corneal endothelium (a leaky epithelium). Nielsen [28] in his study showed that the coupled water flow to Na\(^+\) transport across frog skin epithelium was due to local osmosis. For this reason, we neglect the contribution of the transepithelial potential difference to the transepithelial water flux (electro-osmosis). However, our model can be easily modified to include the electro-osmosis contribution for other types of epithelia where electro-osmosis is important.

In summary, we have developed a new two-barrier compartment model with the active solute transport mechanism for the water flux across amphibian skin (tight epithelia). This model can successfully explain the experimental results in Hillyard and Larsen [13] and Nielsen [28]. This model may also be applied in other cases for water transport across tight epithelia, such as bladder and colon epithelia.
ACKNOWLEDGEMENT

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REFERENCES


Table 1 Parameters and their values used in the model

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<td>$T$ (°K)</td>
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<td>$C_T$ (mOsm)</td>
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<td>$N_{max}$ (nMol/s/cm²)</td>
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<tr>
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<td>0.01</td>
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FIGURE CAPTIONS

**Fig. 1** Sketch of amphibian skin epithelium. 1, stratum corneum; 2, stratum granulosum and stratum spinosum; 3, stratum germinativum; 4, basal lamina; 5, mitochondria-rich cell; j.m., the tight junction; o.m., outward facing membrane; i.m., the membrane lining lateral intercellular spaces and tissue-facing membrane of germinativum cells. (From [21], with permission of EH Larsen).

**Fig. 2** A simplified model for amphibian skin. Amiloride-sensitive epithelial sodium channels (ENaCs) on the apical cell membrane allow for sodium transport from the apical bathing solution to cell interior. Na⁺-K⁺-ATPase (P) on the basolateral cell membrane actively pumps sodium into the intercellular space. Chloride transport is not shown. Water transport through exclusive H₂O channels at the cell membranes. Sodium and water can also transport through tight junction (TJ) at the apical barrier. Cₐ, apical bathing solution osmolality; Cₛ, serum osmolality at tissue side; Cᵢ, intercellular space osmolality; pᵢ, hydrodynamic pressures in the intercellular space; Jᵥ, transepithelial water flux; Jₛ, transepithelial solute flux; TJ, the tight junction barrier. (Revised from [21]).

**Fig. 3** The functional compartment model for the amphibian skin. It is modeled as a compartment bounded by an apical barrier and a tissue barrier. The apical barrier comprises of a cell barrier and a tight junction (TJ) barrier in parallel. The cell barrier has the ability to actively transport solute. Lᵢ, water permeability of barriers; Pᵢ, solute permeability of barriers; σᵢ, solute reflection coefficient of barriers; i = C (cell barrier), TJ (tight junction barrier), L (apical barrier)
and T (tissue barrier). N, actively transported solute flux. Jvi, water flux across barriers; JSi, solute flux across barriers. i = C (cell barrier), TJ (tight junction barrier), and T (tissue barrier).

**Fig. 4** The ratio of N to $N_{\text{max}}$ as a function of apical bathing solution osmolality $C_L$ at different $K$. $K = 0, 10, 20$ and $50$ mOsm. Actively transported solute flux $N$ satisfies the Michaelis-Menten equation $N = N_{\text{max}} \times C_L/(C_L+K)$. We choose $K = 20$ mOsm in the current study to satisfy the experimental observations in Hillyard and Larsen [13] and Nielsen [28].

**Fig. 5** The transepithelial water flux $J_V$ as a function of $N$, the actively transported solute flux at different $C_L$. The slopes of the lines are the molar ratio of transported water molecules and salt solutes. To satisfy the experimental observations in Hillyard and Larsen [13], we find $N_{\text{max}} = 4$ nMol s$^{-1}$ cm$^{-2}$ in our model.

**Fig. 6** The ratio of the transepithelial volume flux $J_V$ from salt solutions to that from deionized water (DI) as a function of the apical bathing solution osmolality $C_L$ at the tissue side. When $C_L$ is more than 100 mOsm, these two curves are nearly parallel and the volume flux coupled to the active solute flux (the difference between these two curves) is nearly constant despite the variation in the apical bathing solution osmolality. Symbols are experimental data from Hillyard and Larsen [13]. ■ hydration rates from NaCl bathing solution; △ hydration rates from 50 mM Na Gluconate; ▼ hydration rates from 100 mM Sucrose.
**Fig. 7(A)** The osmolality of the lateral intercellular space $C_I$ as a function of the apical bathing solution osmolality $C_L$ at the tissue side. Solid line is the result for no active solute transport and dashed line for active solute transport satisfying Michaelis-Menten equation.

**Fig. 7(B)** The hydrostatic pressure $p_I$ in the lateral intercellular space as a function of the apical bathing solution osmolality $C_L$ at the tissue side. Solid line is the result for no active solute transport and dashed line for active solute transport satisfying Michaelis-Menten equation.

**Fig. 7(C)** The transepithelial solute flux $J_S$ as a function of the apical bathing solution osmolality $C_L$ at the tissue side. Solid line is the result for no active solute transport and dashed line for active solute transport satisfying Michaelis-Menten equation.

**Fig. 7(D)** The osmolality of the transported solution $C_E$ as a function of the apical bathing solution osmolality $C_L$ at the tissue side. Solid line is the result for no active solute transport and dashed line for active solute transport satisfying Michaelis-Menten equation.

**Fig. 8** The transepithelial water flux $J_V$ as a function of $C_L$ for two measured cell water permeability in Nielsen [28], $L_{PC} = 7.1 \times 10^{-9}$ cm/s/mmHg and $L_{PC} = 9.3 \times 10^{-10}$ cm/s/mmHg under conditions of no active ($N = 0$) and active solute transport.

**Fig. 9** The transepithelial water flux $J_V$ as a function of $C_L$ under condition of active solute transport for three TJ water permeability, $L_{PTJ} = 0.02$, 0.1 and 0.5$L_{PC}$ where $L_{PC} = 7.1 \times 10^{-9}$ cm/s/mmHg.
Fig. 1
Fig. 2
Apical side $C_L$

Cell barrier $L_{PC}, P_C, \sigma_C$

$J_{STJ} \quad J_{VTJ} \quad J_{VC} \quad J_{SC}$

Tissue barrier $L_{PTJ}, P_{TJ}, \sigma_{TJ}$

Lateral intercellular space $C_i, p_i$

$J_{ST} \quad J_{VT}$

Tissue side $C_T$

Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7(A)
Fig. 7(B)
Fig. 7(C)
Fig. 7(D)
Fig. 8
Fig. 9