J Membrane Biol (2010) 234:57–73
DOI 10.1007/s00232-009-9216-y

TOPICAL REVIEWS

Water-Transporting Proteins

Thomas Zeuthen

Received: 21 September 2009 / Accepted: 20 October 2009 / Published online: 30 November 2009

Abstract Transport through lipids and aquaporins is osmotic and entirely driven by the difference in osmotic pressure. Water transport in cotransporters and uniporters is different: Water can be cotransported, energized by coupling to the substrate flux by a mechanism closely associated with protein. In the K^+/Cl^- and the $Na^+/K^+/2Cl^-$ cotransporters, water is entirely cotransported, while water transport in glucose uniporters and Na^+-coupled transporters of nutrients and neurotransmitters takes place by both osmosis and cotransport. The molecular mechanism behind cotransport of water is not clear. It is associated with the substrate movements in aqueous pathways within the protein; a conventional unstirred layer mechanism can be ruled out, due to high rates of diffusion in the cytoplasm. The physiological roles of the various modes of water transport are reviewed in relation to epithelial transport. Epithelial water transport is energized by the movements of ions, but how the coupling takes place is uncertain. All epithelia can transport water uphill against an osmotic gradient, which is hard to explain by simple osmosis. Furthermore, genetic removal of aquaporins has not given support to osmosis as the exclusive mode of transport. Water cotransport can explain the coupling between ion and water transport, a major fraction of transepithelial water transport and uphill water transport. Aquaporins enhance water transport by utilizing osmotic gradients and cause the osmolarity of the transportate to approach isotonicity.

T. Zeuthen (✉)
Nordic Center for Water Imbalance Related Disorders, Institute of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Blegdamsvej 3C, 2200N Copenhagen, Denmark
e-mail: tzeuthen@sund.ku.dk

Keywords Cotransporters · Uniporter · Aquaporin · Water · Epithelia · Absorption · Cellular water homeostasis

Introduction

In humans almost 200 l of water are transported each day across cell layers and epithelia in order to maintain a steady-state level of about 50 l. It is well established that water transport is secondary to ion transport, but how can the transport of a relatively small number of ions or molecules lead to the transport of a large number of water molecules—in mammals, an impressive 175 water molecules per ion or molecule? To set up a general model, it is essential first to have a picture of the molecular mechanism behind water transport across cell membranes; second, these building blocks must be combined into a model of whole cells and organs. In the first part of this review, the water transport properties of aquaporins, cotransporters and uniporters are reviewed and compared. In the second part, general models of epithelial water transport and cellular water homeostasis are set up based upon the properties of these proteins.

Water transport across cell membranes takes place via several routes. Transport via the lipid bilayer and the water channels, the so-called aquaporins, is well established; but there is also general agreement that water is transported by cotransporters and uniporters (Agre et al. 2004; King et al. 2004), proteins usually associated with other functions. What are the transport mechanisms of the various pathways? The transport via lipid and specific water channels is osmotic; water is driven entirely by the difference in water chemical potential and can be hydraulic or diffusive. The transport mechanism in the cotransporters and uniporters is different. The flux of water is coupled to the flux of

substrates by a mechanism closely associated with the protein. The ratio between the various fluxes is a fixed property of the protein, and energy for the water transport can be derived from the transport of the nonaqueous substrates. In other words, water is cotransported and can even be moved uphill against the osmotic gradient. The various modes of water transport are illustrated in Fig. 1.

In terms of whole-body water homeostasis, it is becoming increasingly clear that models based simply upon aquaporins and osmosis are inadequate. Individuals without the major aquaporin AQP1 (Coulton-null phenotypes) suffer little discomfort except being more liable to be dehydrated under extreme conditions (Preston et al. 1994; King and Agre 1996; Agre et al. 2002). The discrepancy between the presence of aquaporins and the ability to maintain water homeostasis is also encountered in simpler organisms. In a study of the nematode worm *Caenorhabditis elegans*, the four existing types of aquaporins were knocked out individually, in combinations or even entirely, without any effect on the phenotype (Huang et al. 2007). Similar puzzles are encountered in a number of mammalian organs and tissues: The reduction in passive permeability achieved by removing the aquaporins is not generally matched by a similar degree of reduction in the rate of transport (Hill et al. 2004; Verkman 2008). It would appear that other water-transporting proteins are involved, and there are several reasons why the contributions from cotransporters and uniporters may be significant. First, the water transport capacity per protein can be considerable. In fact, the protein with the highest osmotic water permeability is not an aquaporin but a urea transporter (Table 1). Second, the number of copies of a given cotransporter or uniporter is dictated by the requirements for the substrate; the water transport capacity that goes along with proteins can be appreciable. Third, water transport in cotransporters and uniporters must be relevant for tissues with no or very few water channels, such as small intestine and gallbladder. Fourth, water can be transported uphill across epithelia against the osmotic gradient, a behavior which is difficult to explain on the basis of passive transport in aquaporins. In this context, cotransporters and uniporters may play an important role since they have the unique property of coupling the water flux to the flux of the ions or substrate by a mechanism closely associated with the protein itself.

Properties of Water-Transporting Proteins

Membrane proteins transport water by two fundamentally different processes, osmosis and cotransport. Some proteins transport by osmosis, others by cotransport, while some use both cotransport and osmosis. In water channels, such as aquaporins, water transport is entirely osmotic, driven by the transmembrane difference in water chemical potential. In some cotransporters, e.g., the NKCC1, KCC and MCT1 (Table 1), water transport proceeds entirely as cotransport, coupled tightly to the transport of the other substrates by a mechanism within the protein. In other cotransporters, e.g., the Na^+-coupled transporters of organic substrates such as glucose, both modes of transport occur: They cotransport water but also have water channel properties. Accordingly, water transport in these proteins is bimodal; osmosis and cotransport of water proceed in parallel. The three types are illustrated in Fig. 1 and the water transport properties are compiled in Table 1.

The first column in Table 1 gives the short name and the second column, the substrates and the stoichiometry, i.e., the number and kind of ions and molecules transported per turnover of the protein. The third column gives the coupling ratio (n), the number of water molecules cotransported per

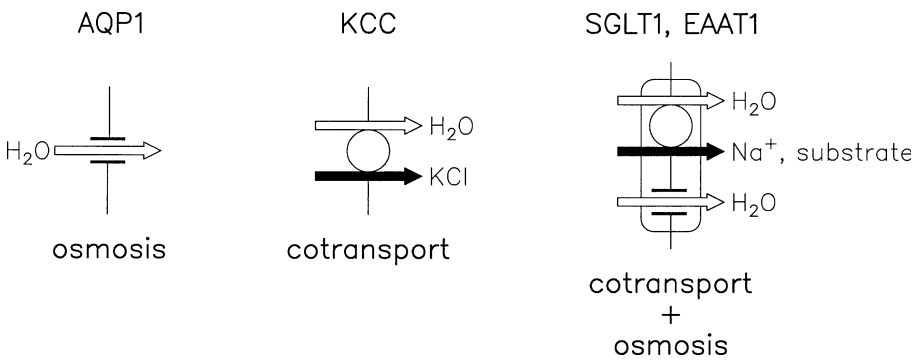

Fig. 1 Molecular mechanism of water transport across cell membranes. Water crosses membranes by diffusion in the lipid bilayer, by osmosis in channels and by cotransport in cotransporters and uniporters. Diffusion and osmosis are driven by the water chemical potential difference. The cotransporters and the uniporters function as molecular water pumps in which free energy contained in the substrate gradient can be transferred to the transport of water; i.e., a downhill transport of substrate can energize an uphill transport of water. Some cotransporters, such as the KCC, employ only cotransport; others, such as the SGLT1 and the EAAT1, employ both cotransport and osmosis

Table 1 Water-transport properties of cotransporters, uniporters and channels

Protein	Substrates	Number of water molecules transported per turnover	Osmotic water permeability per transporter (10^{-14} cm^3 s^{-1})	References
Cotransporters				
KCC4	K^+/Cl^-	500	NA	Zeuthen (1991a, b, 1994)
NKCC1	$Na^+/K^+/2Cl^-$	590[a]	NA	Hamann et al. (2005); see also Appendix
NKCC2	$Na^+/K^+/2Cl^-$	0	NA	Hamann et al. (2005)
MCT1	H^+/lactate	500	NA	Zeuthen et al. (1996), Hamann et al. (2003)
hSGLT1	$2Na^+$/glucose	235	ND	Meinild et al. (1998), Zeuthen et al. (2006)
rSGLT1	$2Na^+$/glucose	380	1.4	Meinild et al. (1998), Loo et al. (1996, 1999), Zeuthen et al. (1997, 2006), Zampighi et al. (1995), Loike et al. (1996)
GAT-1	$2Na^+/Cl^-$/GABA	330	0.7	Loo et al. (1999), MacAulay et al. (2002b)
EAAT1	$2Na^+/H^+/K^+$/glutamate	440	0.2	MacAulay et al. (2001)
NaDC1	$2Na^+$/dicarboxylate	175	1.5	Meinild et al. (2000)
NIS	$2Na^+/I^-$	160	ND	Loo et al. (1996), Zeuthen et al. (2006)
Uniporters and water channels				
GLUT1	Glucose	40	0.2[b]	Fischbarg et al. (1990), Zeuthen, unpublished
GLUT2	Glucose	40–110	0.1	Zeuthen et al. (2007)
UT-B	Urea	ND	7.3	Yang and Verkman (2002)
AQP0	H_2O	NA	0.03	Yang and Verkman (1997)
AQP1	H_2O	NA	4	Zeidel et al. (1992b), Yang and Verkman (1997), Zampighi et al. (1995)

NA not applicable, *ND* not determined

[a] See Appendix

[b] Calculated from the glucose uptake, the glucose permeability, the water permeability and a turnover rate of 151 s^{-1} (Simpson et al. 2007). Measurements were performed in analogy to the study of GLUT2 (Zeuthen et al. 2007)

turnover of the protein. In SGLT1, e.g., two Na^+ molecules and one glucose molecule are accompanied by 235 water molecules. The fourth column gives the passive osmotic water permeability per protein, i.e., the amount of water transported by osmosis per protein per second, given a unit osmotic gradient.

Cotransport of Water

Cotransport of water takes place in close association with the transport of other substrates (Fig. 1b). The properties suggest that the coupling takes place by a mechanism directly associated with the function of the protein: External factors, such as concentration and electrical potentials, only affect the cotransport of water by changing the driving forces of the cotransport process. In summary:

1. Cotransport of water takes place in a strict stoichiometric relationship with the other substrates. The coupling is defined by the coupling ratio (n) and can be described by the Gibbs equation (Meinild et al. 1998; Zeuthen and MacAulay 2002a) (see Appendix).
2. The ratio between the fluxes of water and the nonaqueous substrates is the same irrespective of whether cotransport is driven by a concentration gradient, an electrical gradient or an osmotic gradient (Zeuthen 1982, 1991a, b, 1994; Zeuthen et al. 1996, 2006; Meinild et al. 1998; Zeuthen and MacAulay 2002a).
3. The rate of cotransport of water is altered abruptly by abrupt changes in the driving force of the substrates. Importantly, the coupling ratio (n) is the same before and after the change. These experiments have been performed at high resolution in *Xenopus* oocytes (20 pL, 1 s), (Zeuthen et al. 2006).
4. Cotransport of water can proceed uphill against the osmotic gradient. For example, the KCC can transport water against opposing osmotic gradients of up to 300 mOsm (see Fig. 3) (Zeuthen 1994).
5. The coupling ratio is specific for a given isoform; compare NKCC1 with NKCC2 and rSGLT1 with hSGLT1 in Table 1.
6. For a given transporter, the coupling ratio is smaller for larger substrates. Sugars of different sizes were tested in SGLT1 and anions in NIS (Zeuthen et al. 2006).

7. The activation energy (E_a) of water cotransport is high, around 25 kcal mol^{-1} (Loo et al. 1996, 1999; Meinild et al. 1998; Hamann et al. 2005).
8. Small hydrophilic molecules such as urea are cotransported in the SGLT1 (Leung et al. 2000).

The tight coupling between water and substrate in cotransporters allows these proteins to function as molecular water pumps; i.e., the free energy contained in the substrate gradient can be transferred to the water fluxes. In this way, a downhill flux of substrate can be used for driving an uphill flux of water and vice versa: Water is cotransported by secondary active transport. The independence of the coupling ratio on external factors points toward a mechanism closely associated with the cotransporters itself and excludes the influence of external factors such as concentrations and conventional unstirred layers effects (see below). The findings are independent of methods applied and of the biological expression system used. The investigations have been carried out by a variety of methods ranging from fluorescence, ion-selective microelectrodes, and two-electrode voltage-clamp combined with high-resolution volume measurements for proteins in their native tissue, tissue cultures as well as proteins expressed heterologously in *Xenopus laevis* oocytes (for references see Table 1; some reviews are Zeuthen 1996; Zeuthen and MacAulay 2002a, b; MacAulay et al. 2004).

Passive Water Permeability in Cotransporters and Uniporters

In addition to the cotransport of water, several Na^+-coupled cotransporters and uniporters support osmotic water transport (Fig. 1c, Table 1). The properties of this pathway are compatible to those of aquaporins:

1. The passive water permeability is relatively high compared to that of AQP1.
2. The Arrhenius activation energy (E_a) is in most cases similar to that of aquaporins. E_a values for UT-B and SGLT1 are in the range 2–5 kcal mol^{-1} (Yang and Verkman 1998, 2002; Loo et al. 1996, 1999; Meinild et al. 1998). In contrast, E_a for GLUT1 is relatively high, between 10 and 13 kcal mol^{-1} (Zeidel et al. 1992a).
3. The passive water permeability can be inhibited by specific inhibitors (Loo et al. 1999; MacAulay et al. 2002a; Fischbarg et al. 1990; Zeuthen et al. 2007; Yang and Verkman 1998).
4. The presence of the substrate may increase the passive water permeability (MacAulay et al. 2002a).
5. Some cotransporters and uniporters support passive transport of small hydrophilic molecules (MacAulay et al. 2002a; Leung et al. 2000; Yang and Verkman 1998).
6. The passive water permeability measured by smaller osmolytes can be larger than that measured by larger osmolytes (MacAulay et al. 2002a).

These data are good evidence that a continuous aqueous pathway exists through some of the Na^+-coupled cotransporters and uniporters: The activation energy of the pathway is low, it can be blocked and it allows the passage of small hydrophilic molecules. The more complicated properties can be understood against a background of conformational changes of the protein. The function of these proteins depends upon a cycle of conformational states, and the overall passive water permeability of the protein can be seen as the time average of the permeability of each of these states. The presence of a substrate (glutamate in case of EAAT1) may change the occupancy of the various states and thereby the overall permeability. Inhibitors can have several effects. They may lock the cotransporters in a water-impermeable conformation or in a water-permeable conformation, or they may block, fully or partially, the aqueous pathway. It should be noted that the measurement of passive water permeability is not affected measurably by changes in the cotransport component of water transport. First, the osmotic gradients used are small, about 20 mOsm. Second, it can be ascertained from the Gibbs equation (Appendix) that osmotic gradients of around 20 mOsm do not alter the cotransport component of water transport measurably. In other words, the osmotic and the cotransport pathways function relatively independently.

It is possible to assign values of passive water permeability to the NKCC1, the KCC and the MCT1, defined as the ratio between water flux and the osmotic gradient. It must be emphasized, however, that the water transports induced are not osmotic but coupled directly to the conformational changes associated with the cotransport process (see above). The operational water permeability that can be assigned to NKCC1, KCC and MCT1 differs from that of osmotic or channel-mediated transport in three respects: The osmotically induced water flux is coupled to ion movements, has high activation energy and saturates at osmotic gradients of around 100–200 mOsm (Hamann et al. 2005). The water fluxes induced by KCl, e.g., were half-saturated at KCl concentrations of 25 mM, close to the K_m determined for KCC transporters (Mercado et al. 2000).

What Are the Molecular Mechanisms of Water Transport?

Water transport in aquaporins is osmotic, driven by a hydrostatic gradient within the pore (Mauro 1957). Aquaporins are small, membrane-spanning, pore-forming molecules equipped with two filters that prevent permeation of ions. The central NPA filter is found in almost all

aquaporins. It is made up of the residues of asparagine, proline and alanine and has been shown experimentally to prevent passage of ions such as Na^+, K^+ and, to some extent, H^+. The second filter, the so-called aromatic argine filter (ar/R), faces the outward solution and is important in the complete prevention of H^+ permeation (Beitz et al. 2006; Wu et al. 2009). The amino acid composition of the ar/R filter is highly variable among various types of aquaporins and determines whether an aquaporin is permeable to small hydrophilic molecules such as urea and glycerol. In the present context, only water-specific aquaporins will be considered, in particular AQP1.

The molecular mechanism of water cotransport is entirely different from that of osmosis and is probably related to the way cotransporters function as membrane-bound enzymes. Aqueous as well as membrane-bound enzymes have structures and exhibit conformational changes that are relevant for water transport. It is well established that enzymes have large aqueous cavities or vestibules that connect the binding sites with the external solution (Parsegian 2002; Rand 2002). Aqueous cavities with linear dimensions of up to 50 Å are indeed present in cotransporters as revealed from high-resolution structures (Hirai et al. 2002; Abramson et al. 2003; Huang et al. 2003; Yernool et al. 2004; Yamashita et al. 2005; Faham et al. 2008). A similar motif is found in ATPases (Olesen et al. 2007). During enzymatic activity, the cavity may close in order to avoid hydrolysis and open again in order to release the product. This leads to changes in the size of the water-filled cavities and to changes in the amount of loosely bound surface water (Parsegian 2002; Rand 2002). The total amount of water shifted between different conformational states ranges from 10 in the cytocrome *c* oxidase (Kornblatt 1998; Kornblatt and Kornblatt 2002) to 1,320 in a voltage-gated anion channel (Zimmerberg and Parsegian 1986). The number of cotransported water molecules per turnover, 40–590, is clearly within this range (Table 1). Compared to the size of cotransporters, these numbers of water molecules are not excessive. The volume occupied by 500 water molecules is about 15,000 A^3. Given a molecular weight of the KCC of about 125 kDa (Russell 2000), this water would constitute <10% of the protein volume. A similar calculation applies for the SGLT1 (Loo et al. 2002).

Three different models have been suggested for cotransport of water (Fig. 2). The hyperosmolar-cavity model and the alternating-access model build upon the enzymatic properties outlined above (Zeuthen and Stein 1994; Zeuthen 1994; Naftalin 2008). The third model is based upon unstirred layer effects, i.e., retarded diffusion of the substrates in the external bathing solutions (Duquette et al. 2001; Gagnon et al. 2004). In the hyperosmolar-cavity model the osmolarity increases in the cavity as the substrate leaves its binding site and becomes thermodynamically free; this hyperosmolarity persists as long as the substrate remains inside the cavity (Fig. 2b). If the separation between the extracellular solution and the cavity functions as a semipermeable membrane, water will enter the cavity from the outside solution by osmosis and be driven farther into the inside solution by the hydrostatic pressure in the cavity. Given reasonable parameter values, the model describes the coupling of water and substrates in GLUT2 and SGLT1 (Naftalin 2008). The model is analogous to the three-compartment model developed for cells (Curran and Macintosh 1962). The alternating-access model relates to the way the substrate gains access to a binding site in combination with occlusion of substrate and water (Fig. 2a). When the substrate is presented at the outside of the transporter, it binds inside an aqueous cavity. This is followed by conformational changes in which the substrate and a number of water molecules are occluded. Finally, the cavity opens to the inside and the substrate

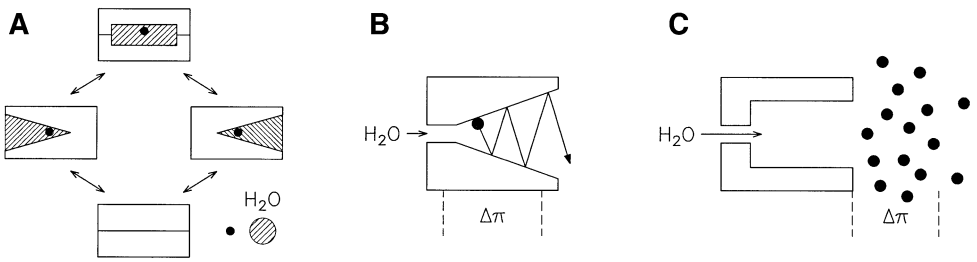

Fig. 2 Molecular models of water cotransport. **a** The alternating-access model adapted for cotransport of both nonaqueous substrates (*black spheres*) and water (*hatched*). Hydration of the access cavity allows the substrate to enter from the outside and to bind in the cavity (*left*). Subsequently, water and substrate are occluded (*top*) and conformational changes shift the opening of the cavity toward the inside (*right*). Substrate and water enter the inside solution and the protein attains a closed conformation (*bottom*). **b** In the hyperosmolar-cavity model, the substrate generates an intramolecular osmotic driving force ($\Delta\pi$) while occupying the vestibule open to the inside. Water is transported by osmosis via an aqueous pathway open in this conformation. As discussed in the text, the models (A and B) can be combined. **c** In the conventional unstirred layer model, the substrate generates a hyperosmolar zone in the cytoplasm adjacent to the membrane. The resulting osmotic difference ($\Delta\pi$) drives water through an aqueous channel in the protein. The model requires unphysiologically low diffusion coefficients in the cytoplasm in order to work (see text)

exits. In the absence of the substrate molecule, the cavity closes and, as a consequence, a number of water molecules follow the substrate. The osmotic problems associated with the exit of a substrate described for the hyperosmolar-cavity model are also encountered in the alternating-access model. Thus, the two models could be combined. Both models explain why larger substrates lead to less water being cotransported per turnover. A large substrate would be less osmotically active and would take up more space in an aqueous cavity (Zeuthen et al. 2006).

The third model is based upon conventional unstirred layer effects in which osmotic driving forces are generated in the bulk solutions outside the transport protein (Fig. 2c). After transport through the protein, the substrate diffuses into the cytoplasm. If this diffusion is sufficiently slow, significant substrate concentrations/osmolarities will arise at the inside of the membrane and water will be dragged by osmosis through the protein. At present, however, there is general agreement that diffusion coefficients in cytoplasm are relatively high (Zeuthen et al. 2002, 2006; Charron et al. 2006; Zeuthen and Zeuthen 2007; Lapointe 2007; Naftalin 2008). Consequently, substrate concentrations at the inside of the membrane can be calculated to be too low to induce significant passive osmotic water transport. The question has been investigated in detail for the Na^+-coupled glucose transporter (SGLT1) and the glucose uniporter (GLUT2) expressed in *Xenopus* oocytes. The diffusion coefficient for Na^+ and glucose in the cytoplasm is one-half to one-fifth of the free solution diffusion coefficient, in agreement with what is found in other cell types (Zeuthen et al. 2002, 2006, 2007; Charron et al. 2006; Zifarelli and Pusch 2009). This is much too high to support significant unstirred layer effects, which would require the diffusion coefficients to be two to three orders of magnitude lower than in free solutions. This limit can be ascertained by correlating water and Na^+ fluxes at a high resolution, 20 pL and 1 s (Zeuthen et al. 2006). Independent arguments for no unstirred layer effects have been forwarded in studies of the KCC (Zeuthen 1994; Zeuthen and Stein 1994). To explain water transport by unstirred layer effects, the required concentrations of K^+ and Cl^- in the unstirred layer would have to be so large as to abolish or even reverse the driving force for K^+/Cl^- transport (see Fig. 3b). Similar arguments apply for the MCT1 (Zeuthen et al. 1996) and the NKCC1 (Hamann et al. 2005) (Fig. 4). In conclusion, cotransport of water is best explained by a mechanism directly associated with the properties of the transport protein.

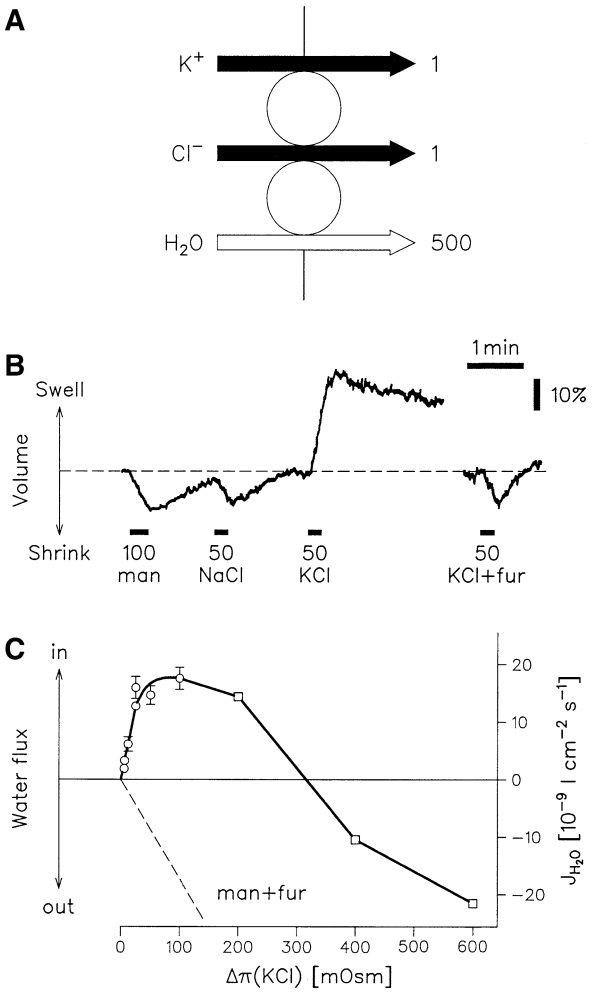

Fig. 3 Water transport by the KCC. **a** The KCC couples the fluxes of K^+, Cl^- and H_2O in a ratio of 1:1:500. **b** Uphill transport of water by the KCC. Addition of 100 mM of mannitol or 50 mM of NaCl to the bathing solution caused immediate cell shrinkage. In contrast, addition of 50 mM of KCl caused cell swelling, despite the external osmolarity being 100 mM higher than the intracellular osmolarity. The intracellular concentrations of K^+ and Cl^- only changed a few millimoles during the exposure to KCl (not shown). When the KCC was blocked by furosemide (fur), the cell shrank in response to the addition of 50 mM of KCl. **c** Uphill influx of water as a function of the osmotic gradient implemented by KCl (experiments as in **b**). The uphill influx increased for KCl gradients of up to 50 mOsm (equivalent to 25 mM of KCl) and saturated at higher values. Around 300 mOsm the inward cotransport of water and the osmotic efflux induced by the KCl balanced each other, and the net flux was zero. *Dashed line* (man + fur) indicates normal osmometric behavior as found with mannitol or when the KCC is blocked by furosemide. Measurements by ion-selective microelectrodes in choroid plexus epithelium of *Necturus maculosus* (Zeuthen, 1991a, 1991b, 1994)

Physiological Roles of Water-Transporting Proteins

The properties of water-transporting proteins are studied most conveniently in epithelia such as small intestine and kidney proximal tubule. In spite of variations in anatomy, location and environments, all epithelia share two important properties that any general model will have to explain. First, it has long been known that water can be transported uphill, against the direction of the transepithelial osmotic gradient (Ludwig 1861; Reid 1892). The situation in the small intestine after a meal is illustrative. Due to enzymatic

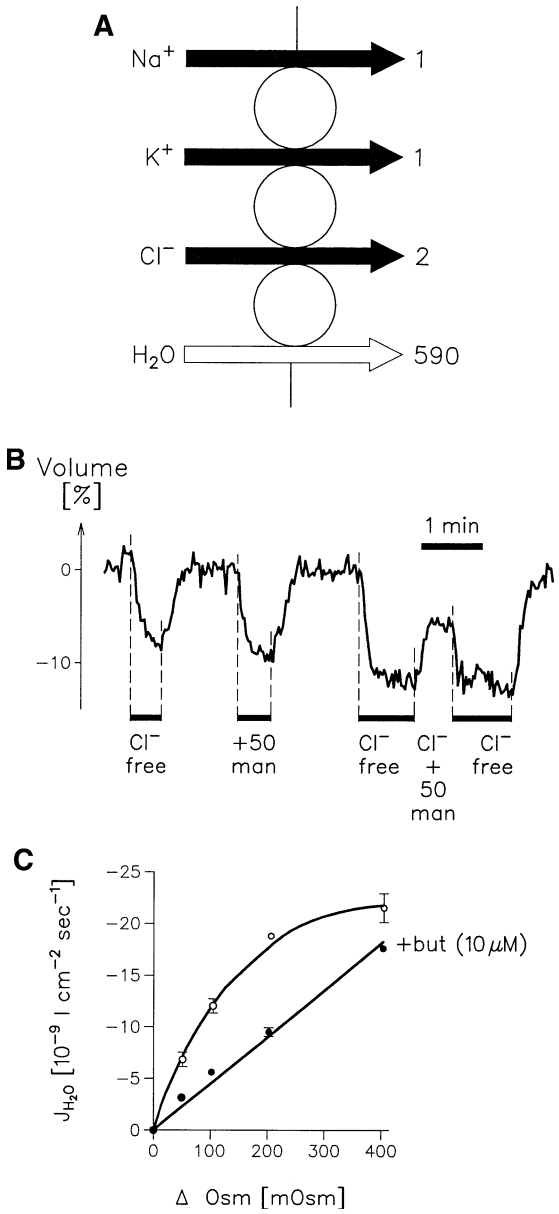

Fig. 4 Water transport by the NKCC1. **a** NKCC1 couples the fluxes of Na^+, K^+, Cl^- and H_2O in a ratio of 1:1:2:590. **b** Uphill water transport by the NKCC1. Cells were exposed to abrupt changes in bathing solution composition and the resulting volume changes were recorded. In the first experiment, isosmotic substitution of Cl^- by the inert anion (Cl^--free) caused rapid cell shrinkage, which is efflux of water; when Cl^- was returned, there was a rapid influx of water that caused the cell to return to its normal volume. In the second experiment, addition of 50 mOsm of mannitol (+50 man) produced rapid cell shrinkage. In the last experiment, cells were first shrunk by removal of Cl^-. Then, Cl^- was returned, simultaneously with the addition of 50 mOsm of mannitol (Cl^- + 50 man). This combined experiment tests whether the Cl^--dependent influx of water can overcome the osmotic efflux generated by the mannitol. The experiment demonstrates that the Cl^--dependent influx can proceed inward against the osmotic gradient of 50 mOsm imposed by the mannitol. **c** Water transport across a cell membrane is increased in the presence of NKCC1. The permeability is derived from the cell shrinkage (J_{H2O}) induced by addition of mannitol; it saturates at increasing osmotic gradients and is abolished in the presence of bumetanide (+but). The water permeability has high activation energy and is closely associated with ion movements. Accordingly, the water transport is not osmotic but derives from cotransport of water (see text). Data from the apical membranes of pigmented ciliary epithelial cells, Hamann et al. (2005) and Hamann et al. unpublished

cleavage of the longer-chained sugars and proteins, the osmolarity in the lumen may reach values some 250 mOsm higher than that of the blood plasma (Pappenheimer 1998); yet water is being moved from the lumen into the blood against this high osmotic gradient (Fig. 5). A similar yet less extreme situation is encountered in so-called isotonic transport, where epithelia transport water apparently without any external osmotic gradient. Second, it is well established that water transport derives its energy from the transport of ions, but how the coupling takes place is not understood at all (House 1974). How can the properties of the water-transporting proteins contribute to explain these two facts? It should be emphasized that the specific problems associated with the vasopressin-induced expression of AQP2 in the collecting duct of the kidney (Kwon et al. 2009) are outside the scope of this review. Water transport in this epithelium is driven by large transepithelial osmotic gradients.

Uphill Water Transport in Epithelia

All water-transporting epithelia investigated so far have the ability of uphill transport. This applies irrespective of whether the transepithelial water permeability is high or low or whether the epithelium is absorptive or secretory. If the osmolarity of the luminal solution is increased moderately, there will be a reduction in the rate of water absorption, but the increases have to be quite high to stop transport completely. In mammals, the maximal adverse gradient for the small intestine is 150–250 mOsm (Pappenheimer and Reis 1987; Hakim et al. 1963; Parsons and Wingate 1961; Pappenheimer 1998); gallbladder, 80 mOsm (Diamond 1964); choroid plexus, 150 mOsm (Heisey et al. 1962); kidney proximal convoluted tubulus, 30–60 mOsm (Green et al. 1991; Bomsztyk and Wright 1986; Frömter et al. 1973; Alpern et al. 1985). In *Necturus maculosus* gallbladder the maximal gradient is 30 mOsm (Persson and Spring 1982) and in fish gallbladder, 40 mOsm (Diamond 1962; for a review, see Zeuthen 2002). Clearly, the epithelia with the lowest passive water permeabilities are able to transport against the largest adverse osmotic differences, but even the very water-permeable mammalian kidney proximal tubule is able to counter gradients as large as 60 mOsm. There is less experimental material for secretory epithelia. It has long been known, however, that glandular secretion could proceed against significant hydrostatic pressures—in the cat salivary gland, against 300 cm H_2O (Ludwig 1861). Similar

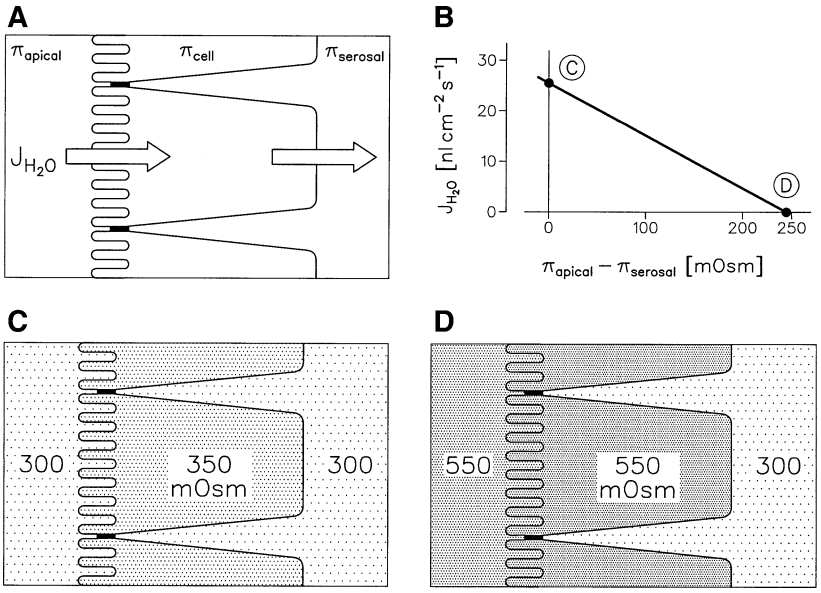

Fig. 5 Water transport across epithelia. **a** If water crosses epithelia by the cellular route, it has to cross the apical and the serosal membranes. **b** In water-transporting epithelia the rate of transport (J_{H2O}) is a linear function of the osmotic gradient across the epithelium $\pi_{apical} - \pi_{serosal}$. *Solid line* summarizes the data from rat small intestine (Parsons and Wingate, 1961); in case of no gradient, the transport is maximal ($\pi_{apical} - \pi_{serosal} = 0$, point C), when the opposing gradient is 250 mOsm ($\pi_{apical} - \pi_{serosal} = 250$), net transport stops. **c** Osmotic profile across the epithelium in case of no external osmotic gradient (point C in **b**). **d** Osmotic profile when the osmolarity of the apical solution is increased from 300 to 550 mOsm, e.g., in the lumen of the small intestine after a meal (point D in **b**)

effects have been demonstrated in dog and rat salivary gland, which could transport against 60 mOsm or more (Imai et al. 1973; Nakahari et al. 1997; Murakami et al. 2006).

Isotonic transport can be considered a simplified case of uphill transport. In isotonic transport, water is transported without any transepithelial osmotic gradient and the transported solution has an osmolarity equal to the bathing solutions (Fig. 5c). However, if water crosses the epithelium by the cellular route, the transport has to proceed uphill at least part of the way. If water enters the cell by osmosis, it is required that the cell be hyperosmolar relative to the apical solution (Fig. 5c); this profile has in fact been confirmed experimentally in gallbladder epithelium (Zeuthen 1982, 1983). However, if the water is to leave the cell by osmosis into the serosal bath, the osmolarity of this bath must be larger than that of the cell and, therefore, even larger than that of the apical compartment ($\pi_{apical} < \pi_{cell} < \pi_{serosal}$). Such a stepwise osmotic profile is incompatible with isotonic transport, i.e., the osmolarity of the serosal solution being equal to that of the apical bath ($\pi_{apical} = \pi_{serosal}$). The stepwise profile is also in conflict with experimental findings which confirm that the serosal solution and the solution in the lateral intercellular spaces are indeed equal to that of the apical bath (Zeuthen 1983; Ikonomov et al. 1985). In the simple osmotic model, the serosal solution will always be hyperosmolar relative to the apical bath. Similar problems have led to models where water is assumed to cross epithelia by a paracellular pathway (for reviews, see Reuss 2006; Fischbarg et al. 2006; Hill 2008).

Models that view transepithelial water transport as a result of high passive water permeability and small transepithelial osmotic gradients are inadequate. The models ignore the fact that epithelia are able to transport water uphill against gradients of several hundred milliosmoles. In a simple osmotic model, such adverse gradients would not only abolish water transport but also cause massive backfluxes of water. The osmotic model is usually presented in a version in which water transport is driven by small intraepithelial osmotic differences, e.g., the lateral intercellular spaces being hyperosmolar relative to the cell. This in combination with high passive water permeabilities should drive water transport. Neither of these two assumptions has been supported by experiments. Water permeabilities of epithelial cell membranes are generally not very high (Zeuthen 1996; Hill 2008), and despite significant experimental efforts, no intraepithelial hyperosmolar compartments have been demonstrated. On the contrary, detailed studies with ion-selective microelectrodes in intact lateral intercellular spaces have shown that the concentration of the solution in the lateral intercellular spaces is isosmotic with the bathing solutions during isotonic transport (Zeuthen 1983; Ikonomov et al. 1985). This agrees with the fact that lateral intercellular spaces are generally too short and too wide to restrict solute diffusion

(Hill 1975) and that subepithelial structures are not tight enough to significantly impede the movement of salt away from the epithelium (Zeuthen 2002). In fact, any significant role of ultrastructural aspects such as lateral intercellular spaces and microvilli is doubtful. One problem is that none of these anatomical features correlates with the direction of water transport. In the absorptive epithelia of the small intestine and proximal convoluted tubule, water enters across the microvillous apical membrane and leaves the cell across the basolateral membrane, which defines the lateral intercellular spaces. In contrast, in the choroid plexus epithelium, water proceeds in the opposite direction, from the lateral spaces and leaving the cells across the microvillous apical membrane. In secretory systems, such as gland acini and the airway epithelia, water leaves the cell across the microvillous apical membrane, while in the retinal pigment epithelia water enters across this membrane (Zeuthen 1996).

The Role of Aquaporins in Epithelial Transport

The fact that epithelia can transport water uphill against the osmotic gradient rules out osmosis as the only driving force and aquaporins as the only pathway. Osmosis, by its very nature, transports water in the direction of the osmotic gradient; in a simple osmotic model, therefore, water would be transported in the wrong direction when faced by adverse osmotic gradients. A high water permeability resulting from a high density of aquaporins would only accentuate the problem. The role of aquaporins is also unclear in relation to whole-body water homeostasis: Individuals without the major water-transporting aquaporin AQP1 suffer no visible discomfort except under extreme conditions such as water deprivation (for references, see "Introduction"). What, then, are aquaporins for (Hill et al. 2004)?

Epithelia that operate in an environment of well-controlled osmolarity are usually equipped with aquaporins at both the apical and serosal membranes in order to utilize small or relatively constant transepithelial osmotic gradients. Examples are the corneal endothelium and the kidney proximal tubule (Borgnia et al. 1999; Verkman and Mitra 2000; Kwon et al. 2009). In contrast, epithelia that have to cope with highly variable external hyperosmolarities have no or only few aquaporins as a high water permeability could result in large, futile back-fluxes. Examples are the small intestine and the gallbladder (Nielsen et al. 1993; Ma and Verkman 1999; Ramírez-Lorca et al. 1999), as well as the retinal pigment epithelium (Hamann et al. 1998). The latter transports salt and water away from the subretinal space, which becomes hyperosmolar during high rates of retinal lactate production.

The role of aquaporins can be analyzed quantitatively by comparing the kidney proximal tubule and the small intestine, two epithelia that have similar functions but different distributions of aquaporins. The epithelia reabsorb NaCl and water at roughly similar rates: In rat, the small intestine transports water at rates between 25 and 70 nl cm^{-2} s^{-1} (Pappenheimer and Reis 1987; Pappenheimer 1998, 2001), in proximal tubule values between 10 and 65 nl cm^{-2} s^{-1} are reported (Green and Giebisch 1984, 1989; Bomsztyk and Wright 1986; Weinstein and Windhager 1985). In both tissues, the absorption of Na^+ is around 4×10^{-9} mol cm^{-2} s^{-1} (data reviewed in Zeuthen 1996). In terms of water permeability, however, the tissues are different. The kidney proximal tubule has a high transepithelial water permeability (0.2 cm s^{-1}), which allows small luminal hyposmolarities to drive a significant osmotic component of absorption that may account for around 30% of the uptake of water from the filtrate in vivo (Green and Giebisch 1984, 1989; Green et al. 1991). In contrast, the small intestine has relatively low passive water permeability (0.02 cm s^{-1}) (Pappenheimer and Reis 1987). The comparison hints at why the presence of aquaporins in the kidney proximal tubule is important but not vital. The aquaporins will increase transepithelial transport in the proximal tubule by using external osmotic gradients, but otherwise the epithelium transports in a manner of a small intestine, i.e., relying on the innate ability of the cells to transport water. This can be tested quantitatively in mice in which the principal water channel AQP1 is deleted by genetic engineering. The passive water permeability of the tubule wall in $AQP1^{-/-}$ phenotypes is reduced by 80% compared to wild-type animals. The reabsorption of water, however, is reduced by only 50% (Ma et al. 1998; Schnermann et al. 1998; Vallon et al. 2000), and a large fraction of this can be explained simply by the removal of the transepithelial transport driven by external osmotic gradients. In knockout animals there is a concomitant reduction in salt reabsorption of about 40% compared to wild-type animals. If this is taken into account as well, the reabsorption rate ought to have been reduced by around 90%, if aquaporins were entirely responsible for transport. It follows that the epithelium of the proximal tubule has an innate capacity for water transport that is independent of the presence of aquaporins and sufficient to sustain life. The results also show that there is no simple relation between the ability to transport water and the passive aquaporin-dependent water permeability. A similar lack of correlation is found for most other water-transporting epithelia (Hill et al. 2004). The specific problems encountered in the choroid plexus epithelium are discussed in MacAulay and Zeuthen (2009).

Although the presence of AQP1 is not vital in the kidney proximal tubule, the aquaporins do increase the absorption rate in vivo and cause the absorbed solution to have an osmolarity close to that of plasma; if the AQP1s are

removed, the luminal solution becomes markedly hypertonic (Vallon et al. 2000; Verkman 2009). In other words, the coupling between salt and water transport becomes more effective in the presence of aquaporins. If the entry step of absorption is partly osmotic, it will be energized by an elevated intracellular osmolarity (Fig. 5c). In the absence of aquaporins, the water permeability is low and the entry of water can only be achieved by increased values of intracellular hyperosmolarity, which is costly due to the increased leaks of ions involved (Zeuthen et al. 2001).

Secretory systems typically have an asymmetrical distribution of aquaporins: The aquaporins associated with water transport are usually found in the membrane across which water leaves the cells. Salivary glands express AQP5 at their apical membrane, across which water is secreted, and AQP1 and AQP4 at their serosal side (Raina et al. 1995). However, whereas transgenic mice lacking AQP5 showed reduced capacity for fluid and hypertonic secretion, AQP1 and AQP4 knockout mice showed no defects (Ma et al. 1999). It would appear that the apical localization of AQP5 and the serosal NKCC1 complies with the model outlined in Fig. 5b: Water enters the cell by cotransport and leaves the cell by osmosis. A similar situation exists in the epithelium of the ciliary body of the eye, where water leaves the epithelium via AQP1 and AQP4 (Hamann 2002; Zhang et al. 2002; Verkman 2009).

The Role of the KCC in Absorptive Epithelia

In a molecular model of absorption, the KCC has three functions: it explains the coupling between ion and water fluxes, it is responsible for a large fraction of the transepithelial water flux and it gives an explanation of how the water flux can proceed uphill against transepithelial osmotic gradients. In most absorptive epithelia, the KCC is located in the serosal (or exit) membrane together with the Na^+/K^+-ATPase (Fig. 6a). This applies to gallbladder, kidney proximal tubule, small intestine and choroid plexus epithelium (for reviews, see Zeuthen 1996; Adragna et al. 2004). In epithelial cells K^+ and Cl^- ions are accumulated above equilibrium, the K^+ ions being supplied directly by the Na^+/K^+-ATPase. Accordingly, there is a major driving force for cotransport of K^+, Cl^- and water by the KCC out of the cell in the same direction as that of the water transport of the whole epithelium (Zeuthen 1996).

The rate of water cotransport by the KCC is compatible to the total water transport rate of the epithelium. From transport parameters determined for the KCC situated in the choroid plexus of the salamander it can be estimated that the KCC could account for a water efflux of 3.5–18 nl cm^{-2} s^{-1}, given the intra- and extracellular concentrations of K^+ and Cl^- (see Appendix). This would explain the range of water fluxes measured in amphibian epithelia, 0.6–4 nl cm^{-2} s^{-1} (Windhager et al. 1959; Diamond 1962; Grandchamp and Boulpaep 1974; Sackin and Boulpeap 1981; Whittembury and Hill 1982; Zeuthen 1982; Persson and Spring 1982; Reuss 1985). Another estimate based upon mass balance suggests that the KCC could account for two-thirds of isotonic transepithelial transport: The efflux of K^+ by the KCC is close to that of the K^+ influx of the Na^+/K^+-ATPase (Fig. 6a); the channel-mediated K^+ fluxes can be disregarded in this context as they have been shown to be comparatively small (Zeuthen 1994). The KCC transports 500 water molecules per 1 K^+ and 1 Cl^-, so the KCC produces a solution of a tonicity of 110 mM, close to isotonicity (data from amphibians). If the overall rate of isotonic transport of the epithelium is defined by the rate of the Na^+ transport of the Na^+/K^+-ATPase, the combined effect of the Na^+/K^+-ATPase and the KCC is an extrusion of Na^+ but only two-thirds of the water required for isotonic transport defined by the Na^+ flux.

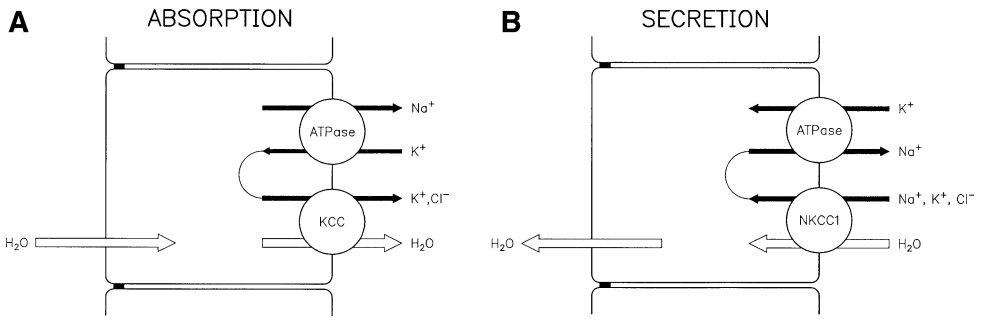

Fig. 6 Molecular models of absorption and secretion. **a** In the model of absorption, the coupling between salt and water transport takes place in the KCC located in the serosal membrane. The secondary active transport of K^+, Cl^- and H_2O in the KCC is energized by the K^+ gradient built up by the Na^+/K^+-ATPase. The intracellular Cl^- concentration is also above equilibrium due to the combined action of secondary active Na^+- and Cl^--dependent acid/base transporters (not shown). **b** In the model of secretion, the coupling between salt and water transport takes place in the NKCC1 located in the serosal membrane. The secondary active transport of Na^+, K^+, Cl^- and H_2O is energized by the Na^+ gradient built up by the Na^+/K^+-ATPase. The two models show only the key proteins responsible for the coupling between salt and water. Clearly, additional transporters are required to describe specific cells

The presence of the KCC in the exit membrane suggests how water can be transported uphill across absorptive epithelia. The problem was investigated in gallbladder and choroid plexus epithelium (Zeuthen 1982, 1991a, b, 1994) where it was demonstrated experimentally that the flow of 1 K^+ and 1 Cl^- was strictly coupled to the flow of 500 water molecules in the KCC and that a downhill flux of K^+ and Cl^- could drive an uphill flux of water. An example is shown in Fig. 3b, where a KCl-driven water flux proceeds into the cell despite the extracellular osmolarity being about 100 mOsm higher than that of the intracellular solution. In fact, osmotic gradients of 300 mOsm could be matched by KCl-induced influxes of water (Fig. 3c). Under physiological conditions, there is sufficient energy contained in the outwardly directed gradients of K^+ and Cl^- to mediate an efflux of water against osmotic gradients of up to 300 mOsm (for calculations, see Appendix). Such values are required when epithelia transport against externally applied osmotic differences, e.g., in the small intestine after a meal (see Figs. 5d, 7). In case of isotonic transport (Fig. 5c), the KCC has to energize the exit of water from the cell against osmotic gradients of around 20 mOsm, clearly within the working range of the KCC.

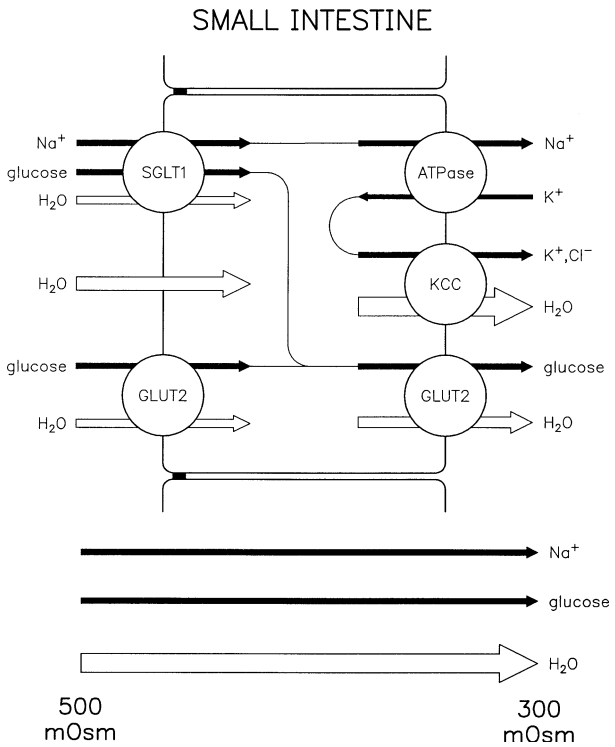

Fig. 7 Cotransport of water in the small intestine just after a meal. After a meal, the osmolarity of the luminal solution increases markedly due to the presence of glucose and other nutrients. Water absorption can proceed even if the osmolarity is increased by about 200 mOsm above that of plasma, i.e., when the total luminal osmolarity is about 500 mOsm (see Fig. 5). Water is cotransported together with Na^+ and glucose in the luminal SGLT1 and GLUT2. In the serosal membrane water is cotransported with the ions in KCC and with glucose in GLUT2. These components of water transport can be considered as secondarily active; the energy is ultimately derived from the Na^+/K^+-ATPase. GLUT2 is present in both the apical and serosal membranes during a meal (Kellett, 2001)

In summary, the anatomical location and direction of transport of ions and water in the KCC suggest a pivotal role of this protein in transepithelial water transport. In a simple molecular model, the properties of the KCC explain a large fraction of epithelial water transport and how water can be transported uphill. This complies with the fact that furosemide inhibits water absorption from small intestine (MacKenzie et al. 1975; Humphreys 1976). Clearly, the presence and interaction of other transporters are required to explain the final composition of the transportate and other specific features, such as isotonic transport. In analogy to other cells, the transport properties of the various transporters are expected to be orchestrated be a system of sensors and signaling pathways (Zeuthen 1996; Shachar-Hill and Hill 2002; MacAulay et al. 2009).

The Role of the NKKC1 in Secretory Epithelia

In many respects the NKCC1 fills the same role as a coupling device between ion and water transport in secretory cells as the KCC had in the absorptive cells. Unfortunately, there is not the same amount of experimental data available for the NKCC1 as for the KCC, so the molecular model for secretion (Fig. 6b) must be seen as a working hypothesis. In most secretory epithelia, NKCC1 is localized unilaterally at the basal membrane together with the Na^+/K^+-ATPase, i.e., the membrane across which water enters the cell (Fig. 6b). The low intracellular Na^+ maintained by the Na^+/K^+-ATPase energizes the inwardly directed electroneutral cotransport of $Na^+/K^+/2Cl^-$. Thus, the cotransport of ions takes place in the same direction as the transepithelial secretion of salt and water (Silva et al. 1977). Examples are acini of the salivary glands and airway epithelia (for reviews, see Boucher 1999; Russell 2000; Haas and Forbush III 2000; Matthews 2002).

The coupling ratio between ions and water in the NKCC1 can be estimated from the ability of osmotic gradients implemented by NaCl to pull water through the protein (Hamann et al. 2005). When the *inward* driving force for the NKCC1 is increased by adding NaCl to the outside bath, this could be balanced by the *outward* driving force that arises from the simultaneous increase in osmolarity. For the NKCC1 expressed in the ciliary epithelia from the mammalian eye, the two effects could be made to match each other precisely. By means of the Gibbs equation, this enables an estimate of the coupling ratio (n) of around 590 water molecules per 1 Na^+, 1 K^+ and 2 Cl^- (see Appendix).

The cotransport of water by the NKCC1 is compatible with a significant fraction of the overall water transport of secretory cells. In secretory models, the flux of the 2 Cl^- ions carried by the NKCC1 proceeds through the cell and defines the rate of ion secretion by the cell layer. The Na^+ ions are assumed to be transported paracellularly by the transepithelial electrical potential at the same rate as the paracellular transport of Cl^- (Silva et al. 1977). By cotransport of water, 295 water molecules are transported for each Cl^-. It follows that the cotransported component of water in the NKCC1 could supply about 80% of the water found in the final secretion, where there are 370 water molecules per Na^+ and Cl^-.

Water transport by the NKCC1 may also explain the ability of secretory cells to transport water uphill. The ability of the NKCC1 for uphill transport is illustrated in Fig. 4c, where the application of Cl^- ions to a cell deprived of Cl^- energizes the uphill transport of water against osmotic gradients of 50 mOsm. Given the coupling ratio of 590 water molecules per 1 Na^+, 1 K^+ and 2 Cl^- and typical values for intracellular and extracellular ion activities, it can be calculated that the NKCC1 under physiological conditions could transport against an osmotic gradient of 100 mOsm equivalent to a hydrostatic pressure of 22 m of H_2O (see Appendix). This value is compatible to those demonstrated in salivary gland (Ludwig 1861; Imai et al. 1973; Nakahari et al. 1997; Murakami et al. 2006).

SGLT1 and GLUT2 in the Small Intestine

While the KCC is relevant for the exit of water and ions from absorptive cells, the importance of the sugar transporters SGLT1 and GLUT2 lies in their role in uptake, particularly across the brush border in the small intestine and the kidney proximal tubule. In the small intestine SGLT1 is constitutively present in the brush border and GLUT2 in the basolateral membranes. Interestingly, just after a meal, i.e., high luminal glucose concentrations, GLUT2 is expressed in the apical membrane as well (Kellett 2001; Kellett and Helliwell 2000; Helliwell and Kellett 2002) (Fig. 7). It has become clear that the transport capacity of SGLT1 is insufficient to explain glucose uptake under conditions of high luminal glucose conditions (Madara and Pappenheimer 1987; Pappenheimer 1998). The transport capacity supplied by the apical GLUT2, however, suffices to explain the majority of the glucose uptake under these conditions (Kellett et al. 2008).

The water-transport properties of the proteins have been studied by heterologous expression in *Xenopus laevis* oocytes. For the human SGLT1, the transport of two Na^+ and one glucose is coupled to the cotransport of 240 water molecules, while for the rabbit isoform 380 water molecules are cotransported (for references, see Table 1). Glucose transport by GLUT2 in the inward direction is accompanied by 30–40 water molecules (Zeuthen et al. 2007). SGLT1 transports a hypertonic solution; there are 175 water molecules per ion or molecule in mammalian plasma, but the cotransporter only transports between 70 and 120 water molecules per ion or molecule. Given these differences, it can be estimated that in the small intestine under resting conditions (no GLUT2 in the apical membrane) about one-third of the influx of water takes place by cotransport by the SGLT1, one-third by osmosis via the SGLT1 and one-third by osmosis across the lipid bilayer (Zeuthen et al. 2001). Just after a meal, however, the situation is different due to the activation of luminal GLUT2. Under this condition of high luminal glucose it can be estimated that the water transported by SGLT1 and GLUT2 in unison could account for most of the water transport across the brush border (Zeuthen et al. 2007; Kellett et al. 2008). The glucose that accumulates in the cell leaves to the serosal side via the GLUT2 located in the exit membrane. Glucose transport by the GLUT2 in this direction (inside to outside) may cotransport as much as 110 water molecules per glucose molecule (Zeuthen et al. 2007). In addition to the component of water transported by the KCC, this could account for a majority of the exit of water from the cell (Fig. 7). In the above estimate only glucose transport is considered. The brush border, however, contains Na^+- and H^+-coupled cotransporters of amino acids, which have been shown also to cotransport water (Loo et al. 1996; MacAulay et al. 2001). In a more physiological setting this extra component of water cotransport will have to be included.

The strict coupling between Na^+, glucose and water in the SGLT is described by a Gibbs equation, which relates the transmembrane differences in concentrations of Na^+, glucose and the osmotic gradient with that of the electrical potential (Appendix, Eq. 8). Under physiological conditions, it can be estimated that the inwardly directed cotransport of Na^+, glucose and water can proceed despite adverse osmotic gradients of up to 1,700 mOsm (Meinild et al. 1998). It follows that the rate of Na^+, glucose and water cotransport is relatively insensitive to luminal hyperosmolarities of the order of 100–200 mOsm encountered during a meal. The cotransport model for the transport of water glucose and salt across the small intestine gives a straightforward explanation for the mechanism of oral rehydration therapy (Loo et al. 2002; Zeuthen 2008) (Fig. 7). With high Na^+ and glucose concentrations in the lumen, the influx of these substrates will be optimized. The transport of each of these substrates will be coupled directly to water transport in membrane proteins, SGLT1 and GLUT2 at the apical membrane and KCC and GLUT2 at the serosal membrane. This will result in an obligatory

influx of water which compensates for the secretory efflux induced in, e.g., cholera infections.

Conclusions

Cotransporters of the symport type function as molecular water pumps. Energy is transferred between substrate fluxes and the water fluxes by a mechanism inside the protein; water can even be transported uphill against the direction of the osmotic gradient. In contrast, water transport in aquaporins and the lipid bilayer is passive and always downhill. In epithelia, the two kinds of transport can be combined to explain a major fraction of transepithelial water transport and how this flux can proceed uphill. Obviously, transport by ion channels and the paracellular pathway must be taken into account in more refined epithelial models.

The presence of aquaporins, such as AQP1, increases the rate of transepithelial transport, causes the ratio of salt to water transport to approach that of plasma and may reduce the metabolic cost of transport. Yet, the presence of the aquaporin is not vital. Apparently, the capacity for water transport by other proteins is sufficient to sustain life. Some epithelia have few, if any, water-transporting aquaporins. This applies to the small intestine, which has low water permeability to enable uphill water transport. It is noteworthy that the small intestine and the proximal tubule epithelium transport water at similar rates, despite the respective absence or presence of aquaporins.

The properties of the water-transporting proteins can be combined into a pump-leak model for cellular water homeostasis. In this model, the cell may not be precisely isosmolar relative to the surroundings in a steady state; water that enters by osmosis or diffusion can be expelled by the cotransporters. Accordingly, there is no need for the cell to become hyposmotic in order to lose water. Steady-state hyperosmolarities have been demonstrated by ion-selective microelectrodes in living cells (Zeuthen 1981, 1982, 1983). Most cells contain both the NKCC1 and KCC, and the activities of the transporters are regulated by phosphorylation in response to external stimuli (Adragna et al. 2004; Gamba 2005). In the epithelial model the NKCC1 and the KCC have key roles for coupling between ion and water transport; in a nonpolarized cell coupling between salt and water transport in the NKCC1 and the KCC leads to cellular water homeostasis. It appears that cellular volume regulation and transepithelial water transport are closely related and that regulation of these transporters may turn out to be an important aspect of epithelial transport (Zeuthen 1996; Shachar-Hill and Hill 2002; MacAulay et al. 2009).

Acknowledgements The support of the Nordic Center of Excellence in Water Imbalance-Related Disorders, The Danish Medical Research Council, The Lundbeck Foundation, The Novo Nordic Foundation and Merck-Sharpe-Dome is gratefully acknowledged. Svend Cristoffersen is thanked for the artwork, and Dr. Nanna MacAulay for critical reading.

Appendix

Thermodynamics of Water Cotransport

The rate of water cotransport across a membrane can be described by

$$J_{H_2O} = B(\mu_i - \mu_o) \quad (1)$$

For the KCC, μ equals R·T· (ln [K] + ln [Cl] + n · ln [C_w]) taken on the inside (i) and the outside (o) of the membrane; [K], [Cl] and [C_w] are the concentrations of K^+, Cl^-. The number of water molecules coupled to the transport of one K^+ and one Cl^- ion (n) equals 500 for the KCC; in Table 1 this parameter is called "CR." R is the gas constant, T the absolute temperature and B a constant that can be determined experimentally. At equilibrium, the rate of water cotransport is zero and the Gibbs criterion for equilibrium of water, $\mu_i = \mu_o$, can be written

$$[K_i][Cl_i]\left[C_{w,i}\right]^n = [K_o][Cl_o]\left[C_{w,o}\right]^n \quad (2)$$

Since [C_w] is proportional to exp ($-osm_i/n_w$), where osm is the osmolarity and n_w is the molarity of water, 55 M, Eq. 2 can be written as

$$[K_i][Cl_i]\exp(-n\, osm_i/n_w) = [K_o][Cl_o]\exp(-n\ osm_o/n_w) \quad (3)$$

This equation can be used to predict how high adverse osmotic gradients are required to stop transport under physiological conditions. For amphibians, [K_i] and [Cl_i] are typically 100 and 40 mM and [K_o] and [Cl_o] are 2 and 120. For n = 500, an osmotic difference, $osm_i - osm_o$, of 310 mOsm is required. For mammals, where [K_o] and [Cl_o] are 5 and 120 mM, an osmotic difference of 200 mOsm would be required to stop transport. These values are in good agreement with experiments in epithelia (see text).

The water flux mediated by the KCC $\left(J_{H_2O}^{KCC}\right)$ given in Eq. 1 can be written as

$$J_{H_2O}^{KCC} = B \cdot R \cdot T \cdot [\ln(K_i/K_o) + \ln(Cl_i/Cl_o) + n/n_w(osm_i - osm_o)] \quad (4)$$

The validity of this equation is supported by data from the choroid plexus epithelium from *Necturus maculosus*. Here, $J_{H_2O}^{KCC}$ was a linear function of the external osmolarity (osm_o) when this was increased from its normal value of

200 mOsm to values up to 400 mOsm by addition of mannitol (Zeuthen 1994); the proportionality factor $B \cdot R \cdot T$ was 6×10^{-6} cm s^{-1} in this range. Given this value, it can be estimated that the KCC in the exit membrane could be responsible for a major component of the transepithelial water transport. In amphibians under steady-state conditions, K^+ and Cl^- concentrations are typically 80 and 40 mM intracellular and 2 and 110 mM extracellular, with intra- and extracellular osmolarities around 200 mOsm. With these values $J_{H_2O}^{KCC}$ calculates as 18 nl cm^{-2} s^{-1}. $J_{H_2O}^{KCC}$ has previously been estimated by a less exact approach to 3.5 nl cm^{-2} s^{-1} (Zeuthen 1995); this is smaller than the estimate obtained from Eq. 1 but still a good approximation to the measured values (see text).

For the NKCC1, μ in Eq. 1 equals $R \cdot T \cdot (\ln[Na] + \ln[K] + 2 \cdot \ln[Cl] + n \cdot \ln[C_w])$. At equilibrium, the Gibbs equation becomes

$$[Na_i][K_i][Cl_i]^2[C_{w,i}]^n = [Na_o][K_o][Cl_o]^2[C_{w,o}]^n \quad (5)$$

With C_w proportional to $\exp(-n \cdot osm/n_w)$:

$$[Na_i][K_i][Cl_i]^2\exp(-n\ osm_i/n_w) = [Na_o][K_o][Cl_o]^2\exp(-n\ osm_o/n_w) \quad (6)$$

The coupling ratio (n) for the NKCC1 can be determined by comparing two different situations which give the same rate of water transport. In the pigmented epithelium of the ciliary body of the eye, hyperosmolar addition of NaCl to the outside solution did not alter the transport rate for water by the NKCC1 (Hamann et al. 2005); when 37.5 mM of NaCl was added to the outside solution, the osmotic effect of the extra NaCl, i.e., 75 mOsm, was exactly matched by the increased chemical driving force originating from the extra 37.5 mM of Na^+ and 37.5 mM of Cl^-. If the rates of water transport in the NKCC1 are the same in the two situations, the chemical potential given by the outside solution μ_o must also be the same. If these are called o,1 and o,2, it follows from Eq. 1 that $\mu_{o,1} = \mu_{o,2}$, or

$$[Na_{o,1}][K_{o,1}][Cl_{o,1}]^2\exp(-n\ osm_{o,1}/n_w) = [Na_{o,2}][K_{o,2}][Cl_{o,2}]^2\exp(-n\ osm_{o,2}/n_w)] \quad (7)$$

Given values of $[Na_{o,1}]$, $[K_{o,1}]$, $[Cl_{o,1}]$, $osm_{o,1}$ of 120, 2, 120 mM and 290 mOsm and values of $[Na_{o,2}]$, $[K_{o,2}]$, $[Cl_{o,2}]$, $osm_{o,2}$ of 157.5, 2, 157.5 mM and 365 mOsm, n calculates as 590. Eq. 7 can now be used to predict how high adverse osmotic gradients are required to stop transport under physiological conditions. For mammals $[Na_i]$, $[K_i]$ and $[Cl_i]$ are typically 10, 100 and 60 mM and $[Na_o]$, $[K_o]$ and $[Cl_o]$ are 150, 5 and 120. For $n = 590$, an osmotic difference, $osm_i - osm_o$, of 100 mOsm is required.

For the SGLT1 the Gibbs equation takes the following forms (Meinild et al. 1998):

$$[Na_i]^2[G_i][C_{w,i}]^n\exp[2F\Psi_i/RT] = [Na_o]^2[G_o][C_{w,o}]^n\exp[2F\Psi_o/RT] \quad (8)$$

$$RT\ln[Na_o/Na_i]^2 + RT\ln[G_o/G_i] + RT\ln[C_{w,o}/C_{w,i}]^n = 2F[\Psi_i - \Psi_o] \quad (9)$$

Na is the Na^+ concentration, G the glucose concentration and Ψ the electrical potential (other symbols as above). In a physiological situation, where the Na^+ concentration is 10 times higher on the outside than on the inside, the glucose concentrations the same, and the membrane potential is –50 m*V*; it can be calculated that the inward flux of water would proceed in face of an adverse osmotic gradient of up to about 1,700 mOsm.

At constant Na^+ and glucose concentrations, the relation between changes in external water concentration, proportional to $\exp(-osm/n_w)$, and the reversal potential $(\Psi_i - \Psi_o)$ becomes

$$\Delta(\Psi_i - \Psi_o) = -n/n_w\ RT/2F\ \Delta osm_o \quad (10)$$

This relation has been used for a determination of the coupling ratio (n) for the rabbit SGLT1 (Zeuthen and MacAulay 2002a). Here, a shift in the outside osmolarity of 100 mOsm resulted in a shift in the reversal potential of about 5 m*V*, equivalent to an n of 247. This compares well with estimates of n obtained under non-steady-state conditions (Table 1).

References

Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, Iwata S (2003) Structure and mechanism of the lactose permease of *Escheria coli*. Science 301:610–615

Adragna NC, Di Fulvio M, Lauf PK (2004) Regulation of K-Cl cotransport: from function to genes. J Membr Biol 201:109–137

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S (2002) Aquaporin water channels—from atomic structure to clinical medicine. J Physiol 542.1:3–16

Agre P, Nielsen S, Ottersen OP (2004) Towards a molecular understanding of water homeostasis in the brain. Neuroscience 12:849–850

Alpern RJ, Howlin KJ, Preisig PA (1985) Active and passive components of chloride transport in the rat proximal convoluted tubule. J Clin Invest 76:1360–1366

Beitz E, Wu B, Holm LM, Schultz JE, Zeuthen T (2006) Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci USA 103:269–274

Bomsztyk K, Wright FS (1986) Dependence of ion fluxes on fluid transport by rat proximal tubule. Am J Physiol 250:F680–F689

Borgnia M, Nielsen S, Engel A, Agre P (1999) Cellular and molecular biology of the aquaporin water channels. Annu Rev Biochem 68:425–458

Boucher RC (1999) Molecular insights into the physiology of the "thin film" of airway surface liquid. J Physiol 516:631–638

Charron FM, Blanchard MG, Lapointe J-Y (2006) Intracellular hypertonicity is responcible for water flux associated with Na^+/glucose cotransport. Biophys J 90:3546–3554

Curran PF, Macintosh JR (1962) A model system for biological water transport. Nature 193:47–348

Diamond JM (1962) The mechanism of solute transport by the gallbladder. J Physiol 161:474–502

Diamond JM (1964) Transport of salt and water in rabbit and guinea pig gall bladder. J Gen Physiol 48:1–14

Duquette P-P, Bisonnette P, Lapointe J-Y (2001) Local osmotic gradients drive the water flux associated with Na^+/glucose cotransport. Proc Natl Acad Sci USA 98:3796–3801

Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+/sugar symport. Science 321:810–814

Fischbarg J, Kuang K, Vera JC, Arant S, Silverstein SC, Loike J, Rosen OM (1990) Glucose transporters serve as water channels. Proc Natl Acad Sci USA 87:3244–3247

Fischbarg J, Diecke FPJ, Iserovich P, Rubashkin A (2006) The role of tight junctions in paracellular fluid transportacross corneal endothelium. Electro-osmosis as a driving force. J Membr Biol 210:117–130

Frömter E, Rumrich G, Ullrich KJ (1973) Phenomenologic description of Na^+, Cl^- and HCO_3^- absorption from proximal tubules of the rat kidney. Pfluegers Arch 343:189–220

Gagnon MP, Bissonnette P, Deslandes LM, Wallendorff B, Lapointe JY (2004) Glucose accumulation can account for the initial water flux triggered by Na^+/glucose cotransport. Biophys J 86:125–133

Gamba G (2005) Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. Physiol Rev 85:423–493

Grandchamp A, Boulpaep EL (1974) Pressure control of sodium reabsorption and intercellular backflux across proximal kidney tubule. J Clin Invest 54:69–82

Green R, Giebisch G (1984) Luminal hypotonicity: a driving force for fluid absorption from the proximal tubule. Am J Physiol 246:F167–F174

Green R, Giebisch G (1989) Osmotic forces driving water reabsorption in the proximal tubule of the rat kidney. Am J Physiol 257:F669–F675

Green R, Giebisch G, Unwin R, Weinstein AM (1991) Coupled water transport by rat proximal tubule. Am J Physiol 261:F1046–F1054

Haas M, Forbush III B (2000) The Na-K-Cl cotransporter of secretory epithelia. Annu Rev Physiol 62:515–534

Hakim A, Lester RG, Lifson N (1963) Absorption by an in vitro preparation of dog intestinal mucosa. J Appl Physiol 18:409–413

Hamann S (2002) Molecular mechanisms of water transport in the eye. Int Rev Cytol 215:395–431

Hamann S, Zeuthen T, La Cour M, Nagelhus EA, Ottersen OP, Agre P, Nielsen S (1998) Aquaporins in complex tissues: distribution of aquaporins 1–5 in human and rat eye. Am J Physiol 274:C1332–C1345

Hamann S, Kiilgaard JF, La Cour M, Prause JU, Zeuthen T (2003) Cotransport of H^+, lactate and H_2O in porcine retinal pigment epithelial cells. Exp Eye Res 76:1–12

Hamann S, Herrera-Perez JJ, Bundgaard M, varez-Leefmans FJ, Zeuthen T (2005) Water permeability of Na^+-K^+-$2Cl^-$ cotransporters in mammalian epithelial cells. J Physiol 568:123–135

Heisey SR, Held D, Pappenheimer JR (1962) Bulk flow and diffusion in the cerebrospinal fluid system of the goat. Am J Physiol 203:775–781

Helliwell PA, Kellett GL (2002) The active and passive components of glucose absorption in rat jejunum under low and high perfusion stress. J Physiol 544:579–589

Hill AE (1975) Solute-solvent coupling in epithelia: a critical examination of the standing-gradient osmotic flow theory. Proc R Soc Lond B 190:99–114

Hill AE (2008) Fluid transport: a guide for the perplexed. J Membr Biol 223:1–11

Hill AE, Shachar-Hill B, Shachar-Hill Y (2004) What are aquaporins for? J Membr Biol 197:1–32

Hirai T, Heymann JAW, Shi D, Sarker R, Maloney PC, Subramaniam S (2002) Three-dimensional structure of a bacterial oxalate transporter. Nat Struct Biol 9:597–600

House CR (1974) Water transport in cells and tissues. Edward Arnold, London

Huang Y, Lemieux MJ, Song J, Auer M, Wang D-N (2003) Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. Science 301:616–620

Huang CG, Lamitima T, Agre P, Strange K (2007) Functional analysis of the aquaporin gene family in *Caenorhabditis elegans*. Am J Physiol 292:C1867–C1873

Humphreys MH (1976) Inhibition of NaCl absorption from perfused rat ileum by furosemide. Am J Physiol 230:1517–1523

Ikonomov O, Simon MX, Frömter E (1985) Electrophysiological studies on lateral intercellular spaces of *Necturus* gallbladder epithelium. Pfluegers Arch 403:301–307

Imai Y, Nishikawa H, Yoshizaki K, Watari H (1973) Evidence for the osmotic flow across dog submaxillary gland epithelia as a cause of salivary secretion. Jap J Physiol 23:635–644

Kellett GL (2001) The facilitated component of intestinal glucose absorbtion. J Physiol 531:585–595

Kellett GL, Helliwell PA (2000) The passive component of intestinal glucose absorption is mediated by the glucose-inducedrecruitment of GLUT2 to the brush-border membrane. Biochem J 350:155–162

Kellett GL, Brot-Laroche E, Mace OJ, Leturque A (2008) Sugar absorbtion in the intestine: the role of GLUT2. Annu Rev Nutr 28:8.1–8.20

King LS, Agre P (1996) Pathophysiology of the aquaporin water channel. Annu Rev Physiol 58:619–648

King LS, Kozono D, Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5:687–698

Kornblatt JA (1998) The water channel of cytochrome *c* oxidase: inferences from inhibitor studies. Biophys J 75:3127–3134

Kornblatt JA, Kornblatt MJ (2002) Water as it applies to the function of enzymes. Int Rev Cytol 215:49–73

Kwon TH, Nielsen J, Moller HB, Fenton RA, Nielsen S, Frokiaer J (2009) Aquaporins in the kidney. Handb Exp Pharmacol 190:95–132

Lapointe JY (2007) Response to Zeuthen and Zeuthen's comment to the editor: enough local hypertonicity is enough. Biophys J 93:1417–1419

Leung DW, Loo DF, Hirayama BA, Zeuthen T, Wright EM (2000) Urea transport by cotransporters. J Physiol 528:251–257

Loike J, Hickman S, Kuang K, Xu M, Cao L, Vera JC, Silverstein SC, Fischbarg J (1996) Sodium-glucose cotransporters display sodium- and phlorizin-dependent water permeability. Am J Physiol 271:C1774–C1779

Loo DDF, Zeuthen T, Chandy G, Wright EM (1996) Cotransport of water by the Na^+/glucose cotransporter. Proc Natl Acad Sci USA 93:13367–13370

Loo DF, Hirayama BA, Meinild A-K, Chandy G, Zeuthen T, Wright E (1999) Passive water and ion transport by cotransporters. J Physiol 518.1:195–202

Loo DDF, Wright EM, Zeuthen T (2002) Water pumps. J Physiol 542:53–60

Ludwig C (1861) Lehrbuch der Physiologie des Menschen, 2nd edn. C.F. Wintersche Verlagshandlung, Leipzig

Ma T, Verkman AS (1999) Aquaporin water channels in gastrointestinal physiology. J Physiol 517:317–326

Ma TH, Yang BX, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS (1998) Severely impaired urinary concentration ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 273:4296–4299

Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS (1999) Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 274:20071–20074

MacAulay N, Zeuthen T (2009) Water transport between CNS compartments: contributions of aquaporins and cotransporters. J Neurosci (in press)

MacAulay N, Gether U, Klærke DA, Zeuthen T (2001) Water transport by the Na^+-coupled glutamate cotransporter. J Physiol 530:367–378

MacAulay N, Gether U, Klaerke DA, Zeuthen T (2002a) Passive water and urea permeability of a human Na^+-glutamate cotransporter expressed in *Xenopus* oocytes. J Physiol 530:367–378

MacAulay N, Zeuthen T, Gether U (2002b) Conformational basis for the Li^+-induced leak current in the rat gamma-aminobutyric acid (GABA) transporter-1. J Physiol 544:447–458

MacAulay N, Hamann S, Zeuthen T (2004) Water transport in the brain: role of cotransporters. Neurosci 129:1031–1044

MacAulay N, Hamann S, Zeuthen T (2009) Chloride transporters as water pumps. In: Alvarez-Leefmans FJ, Delpire E (eds) Physiology and pathology of chloride transporters and channels in the nervous system: from molecules to diseases. Elsevier, New York

MacKenzie JF, Cochran KM, Russell RI (1975) The effect of frusemide on water and electrolyte absorption from the human jejunum. Clin Sci Mol Med 49:519–521

Madara JL, Pappenheimer JR (1987) Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. J Membr Biol 100:149–164

Matthews JB (2002) Molecular regulation of Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) and epithelial chloride secretion. World J Surg 26:826–830

Mauro A (1957) Nature of solvent transfer in osmosis. Science 126:252–253

Meinild A-K, Klaerke DA, Loo DDF, Wright EM, Zeuthen T (1998) The human Na^+/glucose cotransporter is a molecular water pump. J Physiol 508:15–21

Meinild A-K, Loo DFF, Pajor A, Zeuthen T, Wright EM (2000) Water transport by the renal Na^+/dicarboxylate cotransporter. Am J Physiol 278:F777–F783

Mercado A, Song L, Vázquez N, Mount DB, Gamba G (2000) Functional comparison of the K^+-Cl^- cotransporters KCC1 and KCC4. J Biol Chem 275:30326–30334

Murakami M, Murdiastuti K, Hosoi K, Hill AE (2006) AQP and the control of fluid transport in a salivary gland. J Membr Biol 210:91–103

Naftalin RJ (2008) Osmotic water transport with glucose in GLUT2 and SGLT. Biophys J 94:3912–3923

Nakahari T, Steward MC, Yoshida H, Imai Y (1997) Osmotic flow transients during acetylcholine stimulation in the perfused rat submandibular gland. Exp Physiol 82:55–70

Nielsen S, Smith BL, Christensen EI, Agre P (1993) Distriburion of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 90:7275–7279

Olesen C, Picard M, Winther A-ML, Gyrup C, Morth JP, Oxvig C, Møller JV, Nissen P (2007) The structural basis of calcium transport by the calcium pump. Nature 450:1036–1039

Pappenheimer JR (1998) Scaling of dimensions of small intestines in non-ruminant eutherian mammals and its significance for absorptive mechanisms. Comp Biochem Physiol 121:45–58

Pappenheimer JR (2001) Intestinal absorption of hexoses and amino acids: from apical cytosol to villus capillaries. J Membr Biol 184:233–239

Pappenheimer JR, Reis KZ (1987) Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. J Membr Biol 100:123–136

Parsegian VA (2002) Protein–water interactions. Int Rev Cytol 215:1–31

Parsons DS, Wingate DL (1961) The effect of osmotic gradients on fluid transfer across rat intestine in vitro. Biochim Biophys Acta 46:170–183

Persson B-E, Spring KR (1982) Gallbladder epithelial cell hydraulic water permeability and volume regulation. J Gen Physiol 79:481–505

Preston GM, Smith BL, Zeidel ML, Moulds JJ, Agre P (1994) Mutations in aquaporin-1 in phenotypically normal humans without functional CHIP water channels. Science 265:1585–1587

Raina S, Preston GM, Guggino WB, Agre P (1995) Molecular cloning and characterization of an aquaporin cDNA from sallivary lacrimal, and respiratory tissues. J Biol Chem 270:1908–1912

Ramírez-Lorca R, Vizuete ML, Venero JL, Revuelta M, Cano J, Ilundáin AA, Echevarría M (1999) Localization of aquaporin-3 mRNA and protein along the gastrointestinal tract of Wistar rats. Pflug Arch 438:94–100

Rand RP (2002) The lipid–water interface: revelations by osmotic stress. Int Rev Cytol 215:33–48

Reid EW (1892) Report on experiments upon "absorbtion without osmosis". BMJ 1:323–326

Reuss L (1985) Changes in cell volume measured with an electrophysiologic technique. Proc Natl Acad Sci USA 82:6014–6018

Reuss L (2006) Mechanisms of water transport across cell membranes and epithelia. In: Alpern RJ, Hebert SC (eds) The kidney, physiology and pathophysiology. Elsevier, Amsterdam

Russell JM (2000) Sodium-potassium-chloride cotransport. Physiol Rev 80:211–276

Sackin H, Boulpeap EL (1981) Isolated perfused salamander proximal tubule: methods, electrophysiology, and transport. Am J Physiol 241:F39–F52

Schnermann J, Chou C-L, Ma T, Traynor T, Knepper MA, Verkman AS (1998) Defective proximal tubular fluid reabsorbtion in transgenic aquaporin-1 null mice. Proc Natl Acad Sci USA 95:9660–9664

Shachar-Hill B, Hill AE (2002) Paracellular fluid transport by epithelia. Int Rev Cytol 215:319–350

Silva P, Stoff J, Field M, Fine L, Forrest JN, Epstein FH (1977) Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. Am J Physiol 233:F298–F306

Simpson IA, Carruthers A, Vannucci SJ (2007) Supply and demand in cerebral energy metabolism: the role of nutrient transporters. J Cereb Blood Flow Metab 27:1766–1791

Vallon V, Verkman AS, Schnermann J (2000) Luminal hypotonicity in proximal tubules of aquaporin-1-knockout mice. Am J Physiol 278:F1030–F1033

Verkman AS (2008) Mammalian aquaporins: diverse physiological roles and potential clinical significance. Expert Rev Mol Med 10:e13

Verkman AS (2009) Knock-out models reveal new aquaporin functions. Handb Exp Pharmacol 190:359–381

Verkman AS, Mitra AK (2000) Structure and function of aquaporin water channels. Am J Physiol 278:F13–F28

Weinstein AM, Windhager EE (1985) Sodium transport along the proximal tubule. In: Seldin DW, Giebisch G (eds) The kidney: physiology and pathophysiology. Sinauer Associates, Sunderland, MA, pp 1033–1062

Whittembury G, Hill BS (1982) Fluid reabsorption by *Necturus* proximal tubule perfused with solutions of normal and reduced osmolarity. Proc R Soc 215:411–431

Windhager EE, Whittembury G, Oken DE, Schatzmann HJ, Solomon AK (1959) Single proximal tubules of the *Necturus* kidney. III. Dependence of H_2O movement on NaCl concentration. Am J Physiol 197:313–318

Wu B, Steinbronn C, Alsterfjord M, Zeuthen T, Beitz E (2009) Concerted action of two cation filters in the aquaporin water channel. EMBO J 28:2188–2194

Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005) Crystal structure of a bacterial homologue of Na^+/Cl^- -dependent neurotransmitter transporters. Nature 437:215–223

Yang B, Verkman AS (1997) Water and glycerol permeabilities of aquaporin 1–5 and MIP determined quantitatively by expression of epitope-tagged constructs in *Xenopus* oocytes. J Biol Chem 272:14146–16140

Yang B, Verkman AS (1998) Urea transporter UT3 functions as an efficient water channel. J Biol Chem 273:9369–9372

Yang B, Verkman AS (2002) Analysis of double knockout mice lacking aquaporin-1 and urea transporter UT-B. J Biol Biochem 277:36782–36786

Yernool D, Boudker O, Jin Y, Gouaux E (2004) Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. Nature 431:811–818

Zampighi GA, Kreman M, Boorer KJ, Loo DDF, Bezanilla F, Chandy G, Hall JE, Wright EM (1995) A method for determining the unitary functional capacity of cloned channels and transporters expressed in *Xenopus laevis* oocytes. J Membr Biol 148:65–78

Zeidel ML, Albalak A, Grossman E, Carruthers A (1992a) Role of glucose carrier in human erythrocyte water permeability. Biochemistry 31:589–596

Zeidel ML, Ambudkar SV, Smith BL, Agre P (1992b) Reconstitution of functional water channels in liposomes containing purified red cell CHIP28 protein. Biochemistry 31:7436–7440

Zeuthen T (1981) Isotonic transport and intracellular osmolarity in the *Necturus* gallbladder epithelium. In: Ussing HH, Bindslev N, Lassen NA, Sten-Knudsen O (eds) Water transport across epithelia. Munksgaard, Copenhagen, pp 313–331

Zeuthen T (1982) Relations between intracellular ion activities and extracellular osmolarity in necturus gallbladder epithelium. J Membr Biol 66:109–121

Zeuthen T (1983) Ion activities in the lateral intercellular spaces of gallbladder epithelium transporting at low external osmolarities. J Membr Biol 76:113–122

Zeuthen T (1991a) Water permeability of ventricular cell membrane in choroid plexus epithelium from *Necturus maculosus*. J Physiol 444:133–151

Zeuthen T (1991b) Secondary active transport of water across ventricular cell membrane of choroid plexus epithelium of *Necturus maculosus*. J Physiol 444:153–173

Zeuthen T (1994) Cotransport of K^+, Cl^- and H_2O by membrane proteins from choroid plexus epithelium of *Necturus maculosus*. J Physiol 478:203–219

Zeuthen T (1995) Molecular mechanisms for passive and active transport of water. Int Rev Cytol 160:99–161

Zeuthen T (1996) Molecular mechanisms of water transport. Springer, Berlin

Zeuthen T (2002) General models for water transport across leaky epithelia. Int Rev Cytol 215:285–317

Zeuthen T (2008) Molecular water pumps—or how water can move uphill across epithelia. Physiol News 68:3–5

Zeuthen T, MacAulay N (2002a) Cotransporters as molecular water pumps. Int Rev Cytol 215:259–284

Zeuthen T, MacAulay N (2002b) Passive water transport in biological pores. Int Rev Cytol 215:203–230

Zeuthen T, Stein WD (1994) Co-transport of salt and water in membrane proteins: Membrane proteins as osmotic engines. J Membr Biol 137:179–195

Zeuthen T, Zeuthen E (2007) The mechanism of water transport in Na^+-coupled glucose transporters expressed in *Xenopus* oocytes. Biophys J 93:1413–1416

Zeuthen T, Hamann S, La Cour M (1996) Cotransport of H^+, lactate and H_2O by membrane proteins in retinal pigment epithelium of bullfrog. J Physiol 497:3–17

Zeuthen T, Meinild A-K, Klaerke DA, Loo DDF, Wright EM, Belhage B, Litman T (1997) Water transport by the Na^+/glucose cotransporter under isotonic conditions. Biol Cell 89:307–312

Zeuthen T, Meinild A-K, Loo DDF, Wright EM, Klaerke DA (2001) Isotonic transport by the Na^+-glucose cotransporter SGLT1. J Physiol 531:631–644

Zeuthen T, Zeuthen E, Klaerke DA (2002) Mobility of ions, sugar, and water in the cytoplasm of *Xenopus* oocytes expressing Na^+-coupled sugar transporters (SGLT1). J Physiol 542:71–87

Zeuthen T, Belhage B, Zeuthen E (2006) Water transport by Na^+-coupled cotransporters of glucose (SGLT1) and of iodide (NIS). The dependence of substratesize studied at high resolution. J Physiol 570:485–499

Zeuthen T, Zeuthen E, MacAulay N (2007) Water transport by GLUT2 expressed in *Xenopus laevis* oocytes. J Physiol 579:345–361

Zhang D, Vetrivel L, Verkman AS (2002) Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. J Gen Physiol 119:561–569

Zifarelli G, Pusch M (2009) Conversion of the $2Cl^-/1H^+$ antiporter ClC-5 in a NO_3^-/H^+ antiporter by a single point mutation. EMBO J 28:175–182

Zimmerberg J, Parsegian VA (1986) Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel. Nature 323:36