Topical Review

Aquaporin water channels in gastrointestinal physiology

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Fluid transport is a major function of the gastrointestinal (GI) tract with more than 9 litres of fluid being absorbed or secreted across epithelia in human salivary gland, stomach, the hepatobiliary tract, pancreas, small intestine and colon. This review evaluates the evidence that aquaporin-type water channels are involved in GI fluid transport. The aquaporins are a family of small (~30 kDa) integral membrane proteins that function as water channels. At least seven aquaporins are expressed in various tissues in the GI tract: AQP1 in intrahepatic cholangiocytes, AQP4 in gastric parietal cells, AQP3 and AQP4 in colonic surface epithelium, AQP5 in salivary gland, AQP7 in small intestine, AQP8 in liver, pancreas and colon, and AQP9 in liver. There are functional data suggesting that some GI cell types expressing aquaporins have high or regulated water permeability; however, there has been no direct evidence for a role of aquaporins in GI physiology. Recently, transgenic mice have been generated with selective deletions of various aquaporins. Preliminary evaluation of GI function suggests a role for AQP1 in dietary fat processing and AQP4 in colonic fluid absorption. Further study of aquaporin function in the GI tract should provide new insights into normal GI physiology and disease mechanisms, and may yield novel therapies to regulate fluid movement in GI diseases.

Large quantities of fluid are transported in the gastrointestinal (GI) tract for secretion of saliva, gastric juice, bile and pancreatic fluid, and for absorption of fluid in the intestine. The magnitude of fluid transport in the GI tract is second only to kidney, where ~180 l of fluid per day are filtered by the glomerulus and processed by various nephron segments. In the human GI tract, the salivary glands produce 1·5 l of fluid per day, the stomach secretes 2·5 l of gastric juice, the liver produces 0·5 l of bile, the pancreas produces 1·5 l of enzyme and bicarbonate-rich fluid, the small intestine absorbs 6·5 l of fluid, and the colon absorbs 1·3 l of fluid against significant osmotic gradients (Fig. 1) (Zhang, 1996). These values represent net fluid transport rates and thus can be substantially lower than unidirectional transport rates. Fluids that are transported across epithelia and endothelia consist of salts (~150 mM) and water (~55 000 mM). Although the transporting proteins involved in salt movement have been studied for decades and now most of the major salt transporters have been cloned, only recently has attention been given to the molecular mechanisms of water transport.

A substantial body of data has been published about the molecular genetics, structure, localization and developmental expression of the mammalian water channels (aquaporins); however, there is relatively little information about their importance in mammalian physiology. The only water channel shown to have a role in human disease is the kidney collecting duct water channel AQP2. Mutations in AQP2 cause hereditary non-X-linked nephrogenic diabetes insipidus (Deen *et al.* 1994), a disease characterized by a urinary concentrating defect in which patients consume large quantities of fluid to prevent dehydration. Recent work with transgenic mice as described below indicates roles for AQP1 and AQP4 in kidney and lung function. This review evaluates current information about water transport and aquaporin expression in GI organs, as well as the limited available evidence for a role of aquaporins in GI function.

Fluid transport in the GI tract

As in other organ systems, the general paradigm in the GI tract is that water movement occurs secondary to osmotic driving forces created by active salt transport and to hydrostatic pressure differences. It is likely that this basic mechanism operates in salivary gland, intrahepatic cholangiocytes and pancreatic acini. Based on a substantial body of evidence in the kidney and other epithelia carrying out active near-isosmolar fluid secretion or absorption, a greater cell membrane water permeability produces greater net fluid movement (Spring, 1998).

In small intestine and colon the situation may be more complicated. Early perfusion studies suggested that proximal segments of small intestine have higher osmotic

permeability than distal segments (Fordtran *et al.* 1965). The duodenum and proximal jejunum have been proposed to be highly water permeable to permit rapid osmotic equilibration of intestinal contents (Hindle & Code, 1962; Soergel *et al.* 1963; Powell & Malawer, 1968). The small intestine contains a highly convolved leaky epithelium with low electrical resistance and low reflection coefficients for small solutes. Rapid water movement across the epithelium in small intestine is generally believed to occur by a paracellular pathway. This concept was supported by the finding of low osmotic permeability of brush border membrane vesicles from small intestine (Worman & Field, 1985; van Heeswijk & van Os, 1986); however, these studies did not consider possible differences in water permeability in cell membranes derived from different segments of the small intestine. Recent data indicate expression of at least two different aquaporins in small intestine epithelia (see Table 1). Wright, Zeuthen and coworkers have proposed the interesting hypothesis that Na^+–glucose and other cotransporters in enterocytes are able to transport water actively – each turnover of the transporter is proposed to carry hundreds of water molecules and thus accomplish isosmolar fluid transport (Zeuthen & Stein, 1994; Loo *et al.* 1996; Meinild *et al.* 1998). Although there is evidence in an oocyte expression system for coupled water transport by the Na^+–glucose transport protein (SGLT1) and other cotransporters, the possibility of water transport by these proteins in small intestine is so far unproven.

In the colon water must move out of the lumen against substantial opposition because of the high effective osmolality of faeces. Several mechanisms have been proposed involving 3-compartment models with standing gradients, as well as a countercurrent concentration mechanism (Edmonds, 1981; Jodal & Lundgren, 1983). However, the anatomic location of the compartments, if they exist, has not been defined, nor is there direct evidence for a countercurrent multiplication mechanism as in renal medulla. The colon is a tight epithelium with substantially higher electrical resistance than the small intestine, and probably a much lower paracellular water permeability. Several studies have suggested that colonic crypts are the major site of both fluid absorption and agonist-stimulated fluid secretion (Welsh *et al.* 1982; Pedley & Naftalin, 1993; Naftalin, 1994; Singh *et al.* 1995). An interesting hypothesis proposed by Naftalin is that solutes are transported actively out of the crypt lumen across a relatively water-impermeable crypt barrier to create a hypertonic interstitial space (Naftalin & Pedley, 1990; Naftalin, 1994). A negative pressure created in the crypt then 'sucks' water out of faeces to produce compact dehydrated faecal matter. Recent studies suggest that the ability of the distal colon to generate a hypertonic absorbate may rely on the barrier properties of the myofibroblast-like peripheral sheath to maintain a hypertonic pericryptal interstitium (Naftalin *et al.* 1999; Naftalin & Pedley, 1999). It is not clear whether surface epithelial cells in the colon play a role in fluid movement.

Hepatic bile formation involves active secretion of primary bile by hepatocytes followed by modification in the bile duct by cholingiocytes. Although large volumes of water are transported across hepatocytes during bile secretion, a recent study indicated that water transport across the hepatocyte plasma membrane was slow and occurred by a non-channel-mediated pathway (Yano *et al.* 1996). This result appears to be inconsistent with the recent molecular identification of two aquaporin water channels whose transcripts are expressed strongly in hepatocytes: AQP8 (Ma *et al.* 1997*b*; Koyama *et al.* 1997; Ishibashi *et al.* 1997) and AQP9 (Ishibashi *et al.* 1998*a*; Tsukaguchi *et al.* 1998). However, the amount of aquaporin protein expression in hepatocytes has not been determined, nor is there information about whether hepatocyte water permeability is regulated. Since AQP9 appears to transport many small

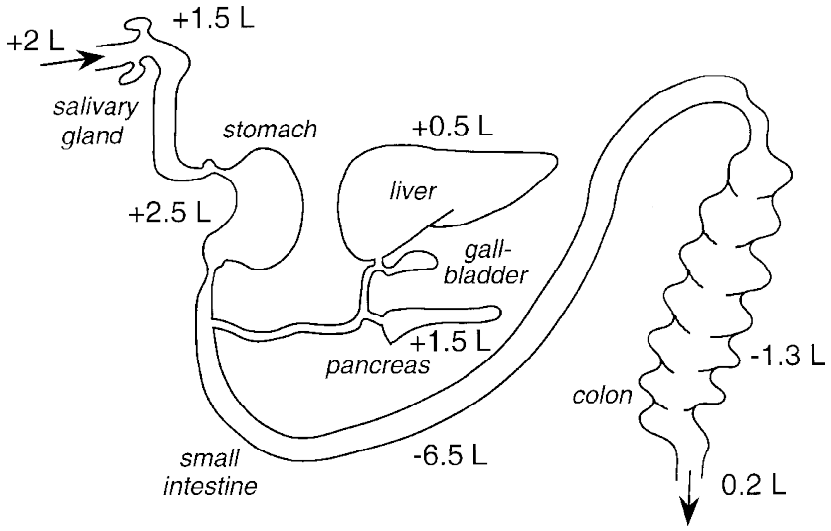

Figure 1
Schematic of the GI tract showing daily fluid secretion and absorption in humans.

solutes as well as water (Tsukaguchi *et al.* 1998), AQP9 may be involved in the transport of metabolites and small solutes produced by hepatocytes.

The saliva secreted by the salivary gland is the first fluid with which ingested food comes into contact. The interstitial to luminal transport of Na^+ and Cl^- across the acinar epithelium is the driving force for osmotic water movement (Nauntofte, 1992). The salivary duct epithelium is believed to be relatively water impermeable, in which the Na^+ and Cl^- are reabsorbed and K^+ and HCO_3^- secreted to produce a hypotonic saliva. The salivary gland can secrete saliva at high rates upon stimulation (up to 50 ml min^{-1} (100 g tissue)$^{-1}$ in humans), which relies on rapid water movement from serosa to mucosa across the capillary endothelium and acinar cells. The possible involvement of aquaporin water channels in this process has been proposed based on the expression of AQP1 in microvascular endothelial cells of salivary gland (Li *et al.* 1994), AQP5 in apical membrane of acinar cells (Nielsen *et al.* 1997), and AQP8 in acinar cells (Koyama *et al.* 1997).

The large quantity of gastric fluid produced by the mammalian stomach is thought to be secreted mainly by fundic glands in the mucosa of the stomach body. These glands contain mucus cells, chief cells and parietal cells that secrete mucus, pepsinogen and hydrochloric acid, respectively. It was reported that during agonist-stimulated acid secretion, gastric juice is transported from the mucosal interstitium into the human gastric lumen at a rate of ~0·7 ml min^{-1} (100 g tissue)$^{-1}$ (Granger *et al.* 1983). There is little information about the relative contributions of different cell types involved in gastric fluid secretion. So far, AQP4 is the only aquaporin identified in the stomach. Rat AQP4 was immunolocalized to the basolateral membrane of parietal cells (Frigeri *et al.* 1995*a*). Subsequently, a human AQP4 homologue was cloned from stomach and immunolocalized to both parietal cells and chief cells (Misaka *et al.* 1996). It has been postulated that AQP4 is involved in gastric acid and pepsinogen secretion and/or cell volume regulation.

The exocrine pancreas secretes a large volume of digestive juice into the duodenum for the digestion of proteins, carbohydrates, fat and nucleic acids. The basal rate of pancreatic secretion in humans is ~0·25 ml min^{-1}, which can increase to 4·5 ml min^{-1} when maximally stimulated (for review, see Argent & Case, 1994). Pancreatic secretions contain contributions from acinar and ductal epithelial cells. The acinar cells secrete a plasma-like fluid containing digestive enzymes. Fluid secretion by acinar cells can be stimulated by cholinergic and other agonists, but quantitatively much larger volumes of fluid are secreted by the ductal cells. The ductal cells secrete a bicarbonate-rich fluid at rates up to 4 ml min^{-1} when stimulated by secretin. The high bicarbonate content of pancreatic secretions appears to require a chloride–bicarbonate exchanger and a CFTR chloride channel at the ductal cell luminal membrane. The water permeability characteristics of acinar and ductal cells have not been studied, but it is likely that the ductal epithelium is highly water permeable. Water channels AQP1 and AQP3 are expressed in human pancreas, and AQP8 in rat and mouse pancreas (see Table 1). The cellular localization of these water channel proteins and their role in pancreatic fluid transport remain uncharacterized.

The aquaporin family of molecular water channels

Nine aquaporin-type water channels have been identified in mammalian tissues (AQP1–AQP9) and many more in plants and lower organisms. Each of the proteins is small (~30 kDa) and related by homology to MIP, the major intrinsic protein of lens fibre (Reizer *et al.* 1993). The aquaporins contain six membrane-spanning domains with cytoplasmically oriented N- and C-termini. Ultrastructural analysis of AQP1 indicates that individual monomers associate as tetramers in membranes (Verbavatz *et al.* 1993) in which each monomer probably contains a pore-like pathway for water (Shi *et al.* 1994). Recent crystallographic studies indicate that AQP1 monomers contain six tilted helices that appear to surround a central opening that might constitute the water pathway (Cheng *et al.* 1997; Li *et al.* 1997; Walz *et al.* 1997). Many of the aquaporins appear to transport water selectively and exclude even small solutes. Reconstitution of AQP1 in proteoliposomes increases water permeability 100-fold without transporting protons, urea or small polar solutes (van Hoek & Verkman, 1992; Zeidel *et al.* 1992). No ion currents have been detected in *Xenopus* oocytes expressing any of the aquaporins. It is thought that AQPs 1, 2 and 4–6 are water selective, whereas AQPs 3 and 7–9 also transport certain small polar solutes to some extent including glycerol and urea. Recently, it has been suggested that AQP1 is able to transport small amounts of CO_2 when expressed in *Xenopus* oocytes (Nakhoul *et al.* 1998); however, the physiological relevance of this observation has not been established. In terms of regulation, the collecting duct water channel AQP2 is subject to phosphorylation-dependent vesicle trafficking which is responsible for increased kidney collecting duct water permeability and the formation of a concentrated urine (Nielsen *et al.* 1995; Knepper & Inoue, 1997). The other aquaporins, with the possible exception of AQP1 in cholangiocytes (see below), appear to function as constitutively active water channels expressed at cell plasma membranes.

Aquaporin expression in the GI tract

In many cases aquaporins are expressed in tissues where high water permeability is thought to be important such as in epithelia and endothelia; however, there are many exceptions. In kidney, an organ where large amounts of water continuously move across tubules, AQP1 is expressed in epithelial cell plasma membranes of the proximal tubule and the thin descending limb of Henle, as well as in microvascular endothelia (reviewed in Yamamoto & Sasaki, 1998; Verkman, 1998). AQP3 and AQP4 are coexpressed at the basolateral membrane of principal cells in collecting duct (Frigeri *et al.* 1995*b*). In extrarenal tissues, various aquaporins are expressed in fluid-transporting tissues (choroid plexus where cerebrospinal fluid is made, ciliary

Table 1. Expression of mammalian aquaporins in gastrointestinal tissues

AQP	Species	Tissue	Cell type	Method	Reference
AQP1	Human	Liver	Epithelium of intrahepatic bile duct	IH	Nielsen *et al.* 1993
			Endothelium of peribiliary capillaries	IH	Nielsen *et al.* 1993
		Gallbladder	Neck epithelium	IH	Nielsen *et al.* 1993
		Pancreas	Exocrine acinar cells	IF	Hasegawa *et al.* 1994
		Colon	Crypt epithelium	IF	Hasegawa *et al.* 1994
	Rat	Salivary gland	Capillary endothelium	IH,IG	Li *et al.* 1994
		Liver	Cholingiocytes	IH	Roberts *et al.* 1994
		Pancreas	Capillary endothelium	IH	Nielsen *et al.* 1993
		Oesophagus	Lymphatic endothelium	IH	Koyama *et al.* 1999
		Small intestine	Endothelium of central lacteal	IH	Nielsen *et al.* 1993
		Colon	Lymphatic endothelium	IH	Nielsen *et al.* 1993
AQP3	Human	Colon, liver, pancreas and small intestine	?	NB	Ishibashi *et al.* 1995
	Rat	Colon	Villus epithelium	IH	Frigeri *et al.* 1995*b*
		Oesophagus	Squamous epithelium	IH	Koyama *et al.* 1999
AQP4	Human	Stomach	Parietal cells and chief cells	IH	Misaka *et al.* 1996
	Rat	Salivary gland	Epithelium of excretory duct	IH	Frigeri *et al.* 1995*a*
		Stomach	Parietal cells	IH	Frigeri *et al.* 1995*a*
		Ileum	Crypt epithelium	IH,IS	Koyama *et al.* 1999
		Colon	Villus epithelium	IH	Frigeri *et al.* 1995*b*
AQP5	Rat	Salivary gland	Secretory epithelium	IH	Nielsen *et al.* 1997
			Secretory lobule	IS	Raina *et al.* 1995
AQP7	Human	Small intestine	?	NB	Kuriyama *et al.* 1997
	Rat	Small intestine	?	NB	Ishibashi *et al.* 1998*b*
AQP8	Rat	Salivary gland	Acinar cells	IS	Koyama *et al.* 1997
		Liver	Hepatocytes	IS	Koyama *et al.* 1997
		Pancreas	Acinar cells	IS	Koyama *et al.* 1997
		Jejunum	Villus epithelium	IS	Koyama *et al.* 1999
		Colon	Villus epithelium	IS	Koyama *et al.* 1997
	Mouse	Liver, pancreas and colon	?	NB	Ma *et al.* 1997*b*
		Salivary gland	?	RT-PCR	Ma *et al.* 1997*b*
AQP9	Human	Liver	?	NB	Ishibashi *et al.* 1998*a*
	Rat	Liver	Hepatocytes	IS	Tsukaguchi *et al.* 1998

Abbbreviations: IH, immunohistochemistry; IF, immunofluorescence; IG, immunogold; IS, *in situ* hybridization; NB, Northern blot; RT-PCR, reverse transcription-polymerase chain reaction.

body where aqueous fluid is made, airways, exocrine glands, etc.). In addition, aquaporins are expressed in many cell types that are not obviously involved in rapid fluid movement (AQP4 in brain astrocytes, AQP3 in urinary bladder and skin, AQP7 in adipocytes, and AQP9 in leukocytes). The reader is referred to several papers and recent reviews for further information about aquaporin expression in non-GI organs (Nielsen *et al.* 1993, 1997; Hasegawa *et al.* 1994; Brown *et al.* 1995; Frigeri *et al.* 1995*a*,*b*; Verkman *et al.* 1996).

The expression of specific aquaporins in the GI tract provides clues to possible functional roles. Table 1 provides a comprehensive review of published information about aquaporin expression in GI organs. Information is provided about cellular localization, the type of evidence (e.g. immunohistochemistry, immunofluorescence, *in situ* hybridization) and the species. In many cases there appears to be conflicting evidence from different groups that will need to be resolved by immunocytochemistry. Several well-documented expression patterns suggest a role of aquaporins in these tissues, including: AQP1 in intrahepatic bile duct epithelium (bile formation), intestinal lacteals (fat absorption), and salivary gland microvascular endothelium (saliva secretion); AQP4 in the basolateral membrane of parietal cells in the stomach (acid/fluid secretion); AQP3 and AQP4 in the basolateral membrane of colon surface epithelium (faecal dehydration); AQP5 in the apical membrane of acinar cells in salivary glands (saliva secretion); and AQP8 in jejunal villi. AQP8 and AQP9 transcripts were localized to hepatocytes by *in situ* hybridization, suggesting

a possible role of these aquaporins in hepatocyte volume regulation and bile secretion. It is noted that by Northern blot analysis, strong expression of the AQP3 transcript was found in human small intestine and the AQP7 transcript in rat small intestine. The possible involvement of these aquaporins in fluid transport across small intestine requires investigation. Figure 2 shows examples of aquaporin protein localization in the GI tract, with AQP1 in central lacteals of small intestine (panel *A*), AQP4 at the basolateral membrane of surface epithelial cells in the colon (panel *B*), and AQP4 at the basolateral membrane of gastric parietal cells (panels *C* and *D*). Together, the cellular expression pattern of aquaporins in the GI tract supports a role of aquaporins in GI fluid transport, although expression evidence alone does not prove functional significance.

AQP1 and cholangiocyte water permeability

Several lines of evidence have suggested that AQP1 may play a role in secretin-regulated fluid transport in intrahepatic bile duct epithelial cells (cholangiocytes). Immunocytochemistry showed AQP1 expression on the apical and basolateral membrane domains of cholangiocytes (Nielsen *et al.* 1993). Semi-quantitative water permeability measurements in isolated cholangiocytes and intrahepatic bile ducts suggested aquaporin-mediated water transport based on weakly temperature-dependent water transport and inhibition by $HgCl_2$ (Roberts *et al.* 1994). Membrane fractionation studies suggested that secretin caused a redistribution of AQP1 from intracellular vesicles to the cell plasma membrane, and that the redistribution was blocked by colchicine and low temperature (Marinelli *et al.* 1997, 1999). If correct, these

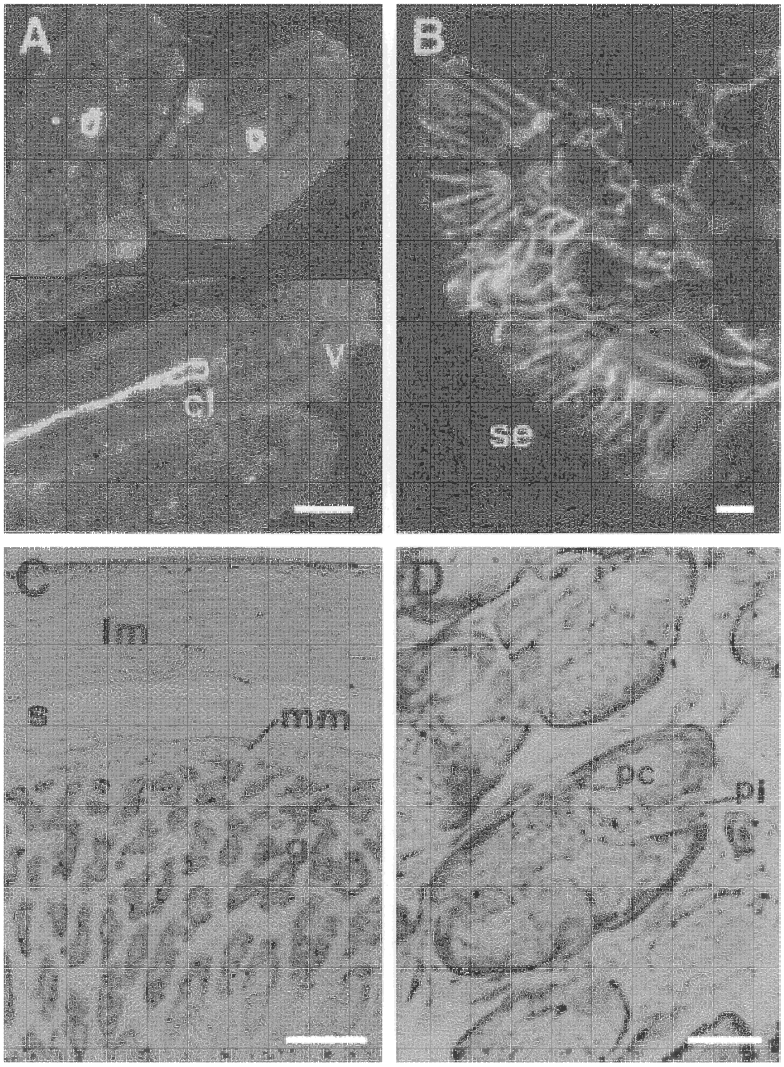

Figure 2. Immunocytochemistry of aquaporin expression in the GI tract

A, immunofluorescence of mouse small intestinal villi showing AQP1 protein in central lacteals. *B*, immunofluorescence of mouse colon with AQP4 antibody showing basolateral membrane labelling of surface epithelium. *C* and *D*, immunoperoxidase at low (*C*) and high (*D*) magnification showing basolateral membrane labelling of parietal cells in the rat stomach. Abbreviations: pc, parietal cell; pi, peptic cells; s, submucosa; lm, longitudinal muscle layer; mm, muscularis mucosa; v, villus; cl, central lacteal; se, surface epithelial cells. Scale bars: *A*, 50 μm; *B*, 50 μm; *C*, 70 μm; *D*, 7 μm.

observations would indicate that unlike other cell types where AQP1 is expressed constitutively at the plasma membrane, the cholangiocyte possesses a unique regulatory mechanism for AQP1. The cell biology of regulated AQP1 trafficking will require elucidation, since AQP1 (unlike AQP2; Fushimi *et al.* 1997) does not contain a consensus phosphorylation site at its C-terminus; in fact, a chimeric AQP1 with its C-terminus replaced by that of AQP2 undergoes regulated trafficking in transfected renal epithelial cells, whereas a chimeric AQP2 with its C-terminus replaced by that of AQP1 does not (Toriano *et al.* 1998). As has been done for AQP2 in kidney, proof for regulated AQP1 trafficking will require immunogold localization of AQP1 in control and stimulated cholangiocytes, and direct evidence that cholangiocyte water permeability is increased by secretin.

Aquaporins and salivary gland function

As reported in Table 1, AQP1 protein is expressed in the microvascular endothelium of salivary gland, AQP5 in the apical membrane of the secretory acinar cells, and AQP8 transcript in acinar cells. Recently the Baum group studied the relationship between adenovirus-mediated aquaporin expression and salivary gland fluid secretion (Delporte *et al.* 1997). Administration of an AQP1 adenovirus to irradiated rat submandibular glands by retrograde ductal instillation produced significant AQP1 expression in acinar and ductal cells and a 2- to 3-fold increase in saliva secretion. *In vitro* studies using rat and human salivary epithelial cells and other polarized cell lines indicated that net transcellular fluid movement in response to an osmotic gradient was increased after adenovirus and adenoassociated virus-induced expression of AQP1 or AQP5 (Delporte *et al.* 1996, 1998; Braddon *et al.* 1998). These studies suggested that aquaporin expression can increase fluid movement across epithelial cells. It was proposed that aquaporin gene transfer may be a potential approach for the treatment of post-radiation salivary gland hypofunction.

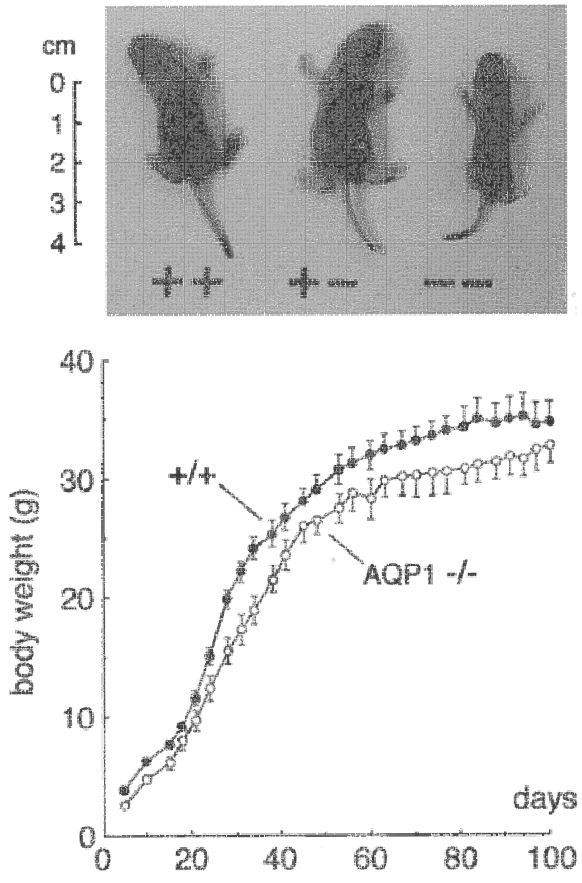

Figure 3. Growth of AQP1 knockout mice

Top, photograph of litter-matched mice of indicated genotype. Bottom, growth curves of wild-type *vs.* AQP1 knockout mice.

Transgenic aquaporin knockout mice

The functional data and aquaporin expression patterns in the GI tract provide indirect evidence for a role of aquaporins in GI physiology. Direct evidence requires phenotype information on humans or other mammals in which selected aquaporins have been functionally deleted by inhibitors, natural mutations or genetic manipulation. As mentioned above, naturally occurring mutations of the kidney-specific water channel AQP2 produce the non-X-linked form of hereditary nephrogenic diabetes insipidus. Rare humans with AQP1 mutations have been identified with reportedly normal phenotype (Preston *et al.* 1994), but these individuals have not been subject to any clinical studies. Natural mutations of the other aquaporins have not been identified in man or other mammals, nor do suitable non-toxic inhibitors exist for any of the aquaporins. Our approach has been to generate transgenic knockout mice lacking specific aquaporins by targeted gene disruption, and to compare the phenotype of litter-matched wildtype, heterozygous and homozygous knockout mice.

To date AQP1 and AQP4 knockout mice have been generated and analysed most extensively in terms of renal and lung phenotype. Initial evaluation of the transgenic mice included determination of genotype distribution from mating of heterozygous mice, and general observations of survival, appearance and growth. For AQP1, the number of knockout mice when genotyped at 5 days after birth was significantly below the predicted Mendelian 1 : 2 : 1 ratio of wildtype : heterozygous : knockout mice (distribution to date 257 : 511 : 202). This finding indicates impaired survival of AQP1 knockout mice *in utero* and/or in the early neonatal period. The AQP1 knockout mice were 10–15% smaller by total body weight than litter-matched heterozygous or wildtype mice (Fig. 3), but had normal postnatal survival and no other obvious differences in appearance. For AQP4, the genotype distribution conformed to the predicted Mendelian ratios, and the AQP4 knockout mice were grossly indistinguishable from wildtype mice.

Several interesting phenotype observations in kidney and lung will be briefly mentioned. In kidney, AQP1 is expressed in epithelia of proximal tubule and thin descending limb of Henle, and in microvascular endothelia

of descending vasa recta. The AQP1 knockout mice are polyuric and polydipsic, and upon water deprivation they become severely dehydrated because of a severe defect in urinary concentrating ability (Ma *et al.* 1998). Mechanistic studies indicated low transepithelial water permeability and impaired near-isosmolar fluid reabsorption in the proximal tubule (Schnermann *et al.* 1998), and very low water permeability in the thin descending limb of Henle (Chou *et al.* 1999). The major effect of AQP1 deletion in kidney is disruption of the countercurrent multiplication system that is responsible for generating a hypertonic medullary interstitium and a concentrated urine. AQP4 is expressed at the basolateral membrane of the medullary kidney collecting duct. In contrast to results for AQP1 knockout mice, the AQP4 knockout mice manifest only a mild defect in maximal urinary concentrating ability (Ma *et al.* 1997*a*) despite a 4-fold reduction in transepithelial water permeability in the inner medullary collecting duct (Chou *et al.* 1998). These data have provided new insights into renal physiology (reviewed in Verkman, 1999). In lung, AQP1 is expressed in alveolar microvascular endothelia and AQP4 in airway epithelia. Osmotically driven water permeability between the airspace and capillaries was ~10-fold reduced by AQP1 deletion but decreased little by AQP4 deletion (Bai *et al.* 1999). AQP1 deletion slowed fluid accumulation in a model of hydrostatic interstitial oedema, but did not affect active isosmolar reabsorption of alveolar fluid. The AQP1 and AQP4 knockout mice have also been useful in ultrastructure studies to prove that AQP4 is the orthogonal array protein (Verbavatz *et al.* 1997) and that AQP1 is responsible for the high density of intramembrane particles in the thin descending limb of Henle (Chou *et al.* 1999).

We have begun to investigate the role of aquaporins in GI physiology using the knockout mice. Dietary fat misprocessing was recently observed in AQP1 knockout mice (Ma *et al.* 1999). Due to the expression of AQP1 in several GI organs involved in fat processing (cholangiocytes, gallbladder epithelium, pancreas, intestinal lacteals), the ability of mice to tolerate a high fat diet was studied. Matched wildtype AQP1 knockout mice were placed on a diet containing 50% animal fat. Whereas wildtype mice gained 36 ± 5% (S.E.M., $n = 45$) body weight in 8 days, the knockout mice lost 2 ± 1% body weight and developed steatorrhea (Fig. 4*A*). The weights became similar 6 days after the return to a 6% fat diet. Despite a 50% decrease in *ad libitum* food intake in the knockout mice, averaged serum triglyceride concentrations were 137 mg dl^{-1} in wildtype and 66 mg dl^{-1} in knockout mice on the high fat diet. Semi-quantitative analysis of stool fat content by a lipid extraction method showed elevated stool fat in the knockout mice on the high fat diet. Addition of porcine pancreatic enzymes (lipase, amylase and protease) to the 50% fat diet partially corrected the weight loss and the increased stool fat content in the AQP1 knockout mice. These results provide evidence for a role of an aquaporin in

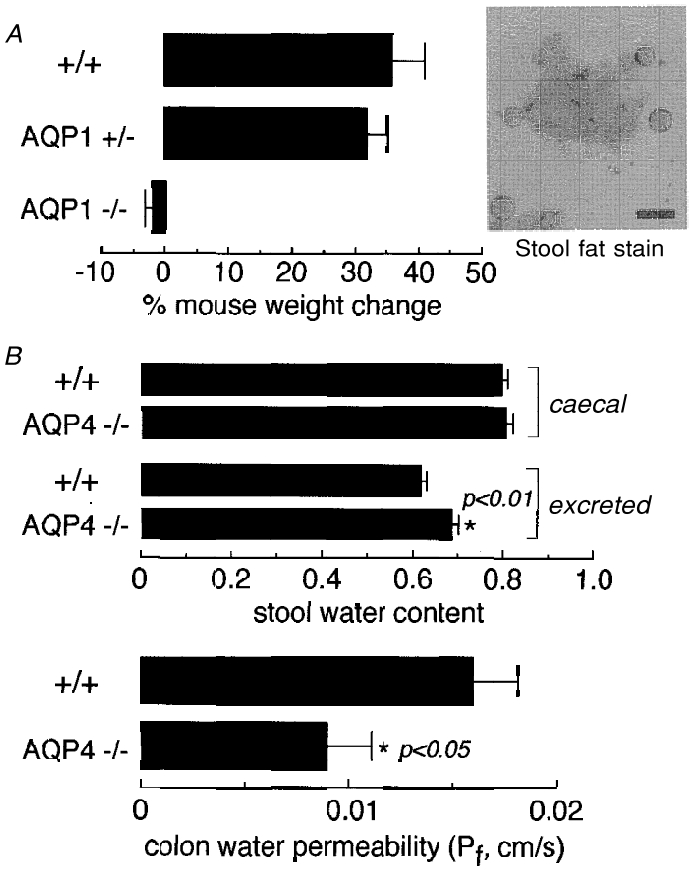

Figure 4. GI phenotype of AQP1 and AQP4 knockout mice

A, dietary fat misprocessing in AQP1 knockout mice. Body weight change in +/+, +/− and −/− mice on day 8 after a 50% animal fat diet. Inset, Sudan IV staining of faecal fat from a −/− mouse on the high fat diet (from Ma *et al.* 1999). Scale bar, 80 μm. *B*, top, defective faecal dehydration in AQP4 knockout mice. Data (means ± S.E.M.) obtained by measurement of wet-to-dry weights in caecal and defecated stool samples. Bottom, transepithelial osmotic water permeability in the *in situ* perfused colon (from Wang *et al.* 1999).

GI function. The fat malabsorption and steatorrhea in AQP1 null mice may involve defective intrahepatic bile production, pancreatic secretion and lipid uptake in the small intestine. Studies of bile and pancreatic fluid composition and flow may define the mechanism(s) by which AQP1 deletion produces defective dietery fat processing.

The AQP4 knockout mice were recently used to test the hypothesis that AQP4 is involved in colon fluid transport and faecal dehydration (Wang *et al.* 1999). AQP4 was localized to the basolateral membrane of surface colonic epithelial cells with strongest expression in the proximal colon. Analysis of stool water content indicated no differences in caecal stool from wildtype *vs.* AQP4 knockout mice, but significantly greater water content in defecated stool from the knockout mice (Fig. 4*B*). The transepithelial osmotic water permeability coefficient (P_f) of the *in vivo* perfused colon was measured using [^{14}C]-polyethylene glycol as a volume marker. The measured P_f of wildtype mice was $0{\cdot}016 \pm 0{\cdot}002$ cm s^{-1}, and was independent of osmotic gradient magnitude and direction as well as the solute used to induce osmosis. P_f was significantly lower in the AQP4 knockout mice (Fig. 4*B*). The absorptive response to intravenous peptide YY was significantly reduced in the AQP4 knockout mice compared with wildtype mice, but the colonic fluid secretory response to intraluminal aminophyllin was not different. These results suggest that AQP4 is involved in colonic fluid absorption and thus implicates a role of the colonic surface epithelium in this process. Further studies with knockout mice lacking the other colonic water transporters, AQP3 and AQP8, should be helpful in understanding the role of water channels in faecal dehydration.

Perspective and directions

There remain many basic questions to be addressed about water-transporting mechanisms in GI physiology. The role of aquaporins in salivary gland, biliary and pancreatic secretions will need to be addressed by phenotype analysis of single and multiple knockout mice deficient in the various GI aquaporins. The role of Na^+-coupled solute transporters in fluid absorption in the small intestine needs to be resolved, as does the possibility of regulated AQP1 trafficking in cholangiocytes. The expression of additional as yet unidentified aquaporins in the small intestine warrants investigation. A major function of the GI tract, the dehydration of faeces by the colon despite large opposing osmotic gradients, remains incompletely understood. One can also think about the roles of aquaporins in diseases of the GI tract and the development of novel therapies. For example, is aquaporin expression altered in biliary and pancreatic disease, and in various diarrhoeas? Can modulation of aquaporin function by novel pharmacological agents or gene delivery alter the course of diarrhoeas, inflammatory bowel disease, hepatic fibrosis, Sjorgren's syndrome, and other GI disorders? From the considerations discussed in this review, we believe that the investigation of molecular water-transporting mechanisms in the GI tract has been an understudied area with considerable potential for significant advances.

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