How do small hydrophilic nonelectrolytes cross cell membranes? Which pathways are most important for small lipid-insoluble molecules to cross cell membranes? These are questions that have been basic to membrane transport physiology for decades. More importantly, these are questions whose answers have changed significantly within the last 10 years. This review discusses the evidence that pathways other than the lipid bilayer itself exist for the transport across cell membranes of specific small hydrophilic nonelectrolytes. The description begins with briefly analyzing the relevance of well accepted basic mathematical models for transport for understanding the permeability of representative physiologically important molecules across actual cell membranes. Particular emphasis is placed on describing recently discovered proteins that facilitate the transport of some of the smallest physiologically important lipid-insoluble molecules, water, and urea. Evidence also exists for transport proteins that selectively enhance the transmembrane transport of other small lipid-insoluble molecules. Do nonselective pores for small molecules exist in cell membranes?

Key words: aquaporins; urea transporters; nonselective pores; membrane transport

How do small hydrophilic nonelectrolytes cross cell membranes? Which pathways are most important for small lipid-insoluble molecules to cross cell membranes? These are questions that have been basic to membrane transport physiology for decades. More importantly, these questions have changed significantly within the last 10 years. Two of the most important small molecules that need to be able to cross cell membranes are water and urea. Water is the main component of the human body, and urea is one of the main excretory products of the metabolism of nutrients for cellular energy. Formerly, physiologists taught that very small lipid-insoluble molecules crossed cell membranes primarily by slipping between the lipids in the lipid bilayer or by using small, nonselective pores (or holes within the lipid bilayer). Within the last 10 years, highly specific transport proteins have been identified that are vitally important for the physiology of water or urea movement across certain cell membranes.

Water (in the various compartments of an organism) is one of the major variables controlled physiologically by homeostasis. Thus there are sensors, like the osmoreceptors in the brain of mammals, that sense osmotic changes in the extracellular fluid compartment, and effectors, like changes in water fluxes across cell membranes and the production of either concentrated or dilute urine by mammalian kidneys. To accomplish osmotic homeostasis, water must rapidly equilibrate across cell membranes.
Aquatic animals excrete nitrogenous wastes from protein and nucleic acid metabolism as toxic ammonia released into the water. Other terrestrial animals, like insects, reptiles, birds, and some amphibians excrete nitrogenous wastes as uric acid in solid or semisolid form. Mammals excrete urea (~10 times less toxic than ammonia and quite soluble in water), allowing waste products to be excreted in a lower volume of urine than ammonia would require.

Although urea is often thought of primarily as a waste product to be excreted, the conservation of urea in the medullary interstitium of the kidney is essential for the production of hyperosmotic urine. In various segments of the nephron, urea is reabsorbed out of the tubular fluid and back into the interstitium. Urea reabsorption, combined with the countercurrent multiplier and exchange systems in the kidneys, creates an osmotic gradient deep in the medullary interstitium. This NaCl and urea concentration enhances the osmotic reabsorption of water out of the tubule when the tubule epithelia have pathways for water transport.

Basic water reabsorption in the early nephron of mammalian kidneys is constitutive, based on the presence of appropriate water channels, the tightness of the junctions between cells, and the simultaneous reabsorption of nutrient solutes. The water permeability of distal tubule and collecting duct epithelia is regulated by vasopressin (antidiuretic hormone). Urea transporters and water channels are regulated by vasopressin to work together to conserve water in the body as shown (Fig. 1). Thus movements of urea and water molecules across cell membranes are essential for the maintenance of homeostasis in the body fluid compartments.
DIFFUSION ACROSS BIOLOGICAL MEMBRANES

Diffusive fluxes of uncharged molecules across lipid bilayer membranes are usually represented by Fick’s First Law of Diffusion

\[ J = \frac{dn}{dt} = -DA\frac{dC}{dx} \]

where \( D \) = diffusion coefficient for molecule in the barrier (\( \text{cm}^2/\text{sec} \)), \( A \) = surface area of the barrier (\( \text{cm}^2 \)), \( dC/dx \) = change in concentration over distance in the barrier (\( \text{mol} \cdot \text{cm}^{-5} \cdot \text{cm}^{-1} \)), and \( J = \frac{dn}{dt} \) = change in number of moles per unit time (\( \text{mol}/\text{s} \)).

The diffusion coefficient \( (D) \) is derived from the Stokes-Einstein equation that models the diffusion of spherical particles in a continuous fluid medium and represents the mobility of the solute in the membrane. The diffusion coefficient

\[ D = \frac{kT}{6\pi r \eta} \]

where \( k \) = Boltzmann’s constant, \( T \) = absolute temperature (\( ^\circ \text{K} \)), \( r \) = molecular radius, and \( \eta \) = viscosity of solution.

The partition coefficient (solubility of the solute in the membrane) is the ratio of the concentration of solute in the membrane to the concentration of solute in water. It is difficult to determine the partition coefficient and the diffusion coefficient for a given solute in cell membranes and the thickness of individual living membranes. Thus these three variables are frequently lumped together into the term “permeability,” where \( P = (D_{\text{mem}} \times B_{\text{mem}})/(\text{membrane thickness}) \) such that flux \( J = PA\Delta C \). Thus molecule size and solubility taken together should completely account for the basal permeability (in \( \text{cm/s} \)) of solute molecules across cell membranes.

Basal permeability across lipid bilayer membranes takes advantage of the free van der Waals volume (or holes) in the hydrocarbon interior (25). Van der Waals forces between lipids lead to many different small transient holes of various sizes that continuously form and reform within the lipid bilayer. Elongated molecules have the highest diffusion rates across lipid bilayers due to the greater probability of finding adjacent holes and thus being able to “snake” through the membrane. In many cells, the cell membrane treated as only a lipid bilayer explains the basal permeabilities for small nonelectrolytes. Thus small lipid-insoluble molecules are capable of crossing lipid bilayers by snaking or random walking between the lipids and do not require continuous aqueous channels or pores. This pathway for crossing the lipid bilayer does not obey the Stokes-Einstein equation and thus has been called non-Stokesian (25). For cell membranes, it is known that lipid bilayers are predominantly fluid laterally in the plane of one monolayer of the membrane. However, in the direction in which transmembrane transport occurs, lipid hydrocarbon chains may be partly immobilized by cholesterol and may not flow easily past the diffusing molecules. It has been known since the early 1970s that water moves across mammalian red blood cell membranes much faster under an osmotic gradient than by simple diffusion (25). This observation implies that osmotic flow of water may be laminar through aqueous channels (all water molecules moving in the same direction) and therefore faster than the random walking of individual water molecules diffusing across a lipid bilayer.

Determination of the true basal permeability of small lipid-insoluble molecules across cell membranes required the development of accurate experimental methods of measurement and the recognition of all possible pathways for transport (25). Measurements of the basal permeability of red blood cell membranes to water are made with and without inhibitors of the transport routes other than the lipid bilayer. When appropriate measurements were made, the true basal permeability of mammalian red blood cell membranes to water was \( 1.2 \times 10^{-5} \text{ cm/s} \) (25). In comparison, measurements were made of the true basal permeability of mammalian red blood cell membranes to molecules of small, relatively lipid-insoluble urea. Urea basal permeability measured with inhibitors (phlorizin, organic mercurials, nitrophenols, and urea analogs like thiourea) was \( 7.7 \times 10^{-7} \text{ cm/s} \) (25). The basal permeability of urea molecules through the lipid bilayer alone is considerably lower than the urea permeabilities that have been measured across many cells in the body. For example, urea permeability across human red blood cell membranes is \( 1.2 \times 10^{-5} \text{ cm/s} \) (16).
WATER TRANSPORT

Water molecules are very polar and are thus unlikely to use hydrophobic pathways for crossing membranes. If water molecules do not traverse cell membranes through the lipid bilayer, what other routes are there for the transport of water molecules?

Evidence for Water Channels

In the 1950s, Goldstein and Solomon (5a) demonstrated that the high permeability of mammalian red blood cells to water was strongly inhibited by mercurial sulfhydryl compounds like mercuric chloride (HgCl₂). Subsequently, specific nephron segments in mammalian kidneys were also shown to have high water permeabilities. Water permeability in the collecting duct was low in an unstimulated state and high in the presence of antidiuretic hormone (vasopressin). This led to the "membrane shuttle hypothesis" for vasopressin-regulated water permeability, whereby vasopressin induces insertion of water transporters in apical membranes of collecting duct epithelial cells (25). It was unclear whether these transporting "pores" consisted of protein, lipid, or a combination of protein and lipid components.

As the quest continued for complete understanding of how water molecules cross cell membranes, analysis emerged of the biophysical characteristics of water transport via various pathways. The Kedem-Katchalsky nonequilibrium thermodynamic equations for coupled water and solute movement model water movement across membranes in response to either osmotic or hydrostatic pressure gradients (27). The osmotic (hydraulic) permeability coefficient ($P_f$) describes water movement as the amount of volume (water) flowing across the membrane in response to driving forces of osmotic and hydrostatic pressures. For cell membranes, $P_f < 0.005 \text{ cm/s}$ when water moves by solubility/diffusion across the lipid bilayer, and $P_f > 0.01 \text{ cm/s}$ when water can also move through continuous aqueous channels (27). On the other hand, the diffusional permeability of water ($P_d$) was modeled by Fick's First Law as the movement of tracer-labeled water molecules across cell membranes in the absence of other driving forces. $P_f$ should equal $P_d$ for a simple lipid bilayer membrane that does not contain water channels or unstirred layers (27). For a narrow channel in which single-file movement of water occurs, $P_f / P_d \sim N$ where $N$ is the number of water molecules in the channel at one time (27). Thus measurement of osmotic vs. diffusional water permeability across cell membranes (with and without osmotic and hydrostatic pressure gradients) predicts whether water channels are present in the cells or not.

The activation energy ($E_a$) for water permeability is also a useful parameter for predicting the presence of water channels in cell membranes (27). $E_a$ is determined by the Arrhenius equation from the temperature dependence of $P_f$. $E_a$ is $>10 \text{ kcal/mol}$ if water is moving by a channel-independent solubility/diffusion pathway and is $<6 \text{ kcal/mol}$ if water is moving through aqueous pores. Higher activation energy for water movement through lipid relates to the formation and breaking of hydrogen bonds between polar water and lipid. Movement of water through aqueous pores is likely to involve fewer bond formation and breaking events between water and surrounding substrate. Thus measurement of the activation energy for water permeability across cell membranes can predict water transport through channels.

Experimental measurements have been developed to distinguish osmotic water permeability from diffusive water permeability (27). Osmotic water permeability can be determined from changes in cell volume in response to osmotic gradients by measurement of light scattering or fluorescence quenching in cells and vesicles. Diffusional water permeability can be determined by the diffusional movement of "labeled" water molecules in the absence of other gradients. Deuterated water has been used as isotopically labeled water molecules to determine isotope differences in fluorophore quantum yields or infrared absorbance. Magnetically labeled water molecules have also been used for analysis by nuclear magnetic resonance.

In the mid-1980s, determination by radiation inactivation of the target size for water transporters (in red blood cells and apical membrane vesicles from kidney proximal tubule cells) indicated a protein of 20–30 kDa (27). In 1988, Agre and coworkers (4a, 18a) isolated and cloned a 28-kDa integral membrane protein from mammalian red blood cells that they called...
CHIP28 for channel-forming integral protein of 28 kDa. Subsequently in 1992, Agre and coworkers (4a, 18a) showed that CHIP28 encoded a water channel. *Xenopus* oocytes do not normally have water channels; however, when the CHIP28 protein was cloned into the oocytes, there was a >10-fold increase in water permeability compared with control oocytes without CHIP28. The increased water permeability was strongly inhibited by HgCl₂. Thus strong evidence for water channels in cell membranes would include one or more of the following characteristics: \( P_f > 0.01 \text{ cm/s} \), \( E_a < 6 \text{ kcal/mol} \), \( P_f / P_d > 3.0 \), and strong inhibition of water permeability by mercurial compounds (27).

**Structure of Water Channels**

Since the initial discovery that a protein acts as a water channel in mammalian red blood cell membranes, additional water channels have been discovered in other tissues, with currently 10 (or 11) known mammalian water channels. CHIP28 [also known as aquaporin-1 (AQP1)] is now identified as a representative of this new class of transport proteins known as aquaporins. AQP1 has 269 amino acids forming two tandem repeats of three membrane-spanning \( \alpha \)-helices plus two short helical loops (B and E loops) within the lipid bilayer (31). Figure 2 shows the “hourglass” model of AQP1. The carboxy and amino termini are both cytoplasmic. The B loop connects helices 2 and 3 and the E loop connects helices 5 and 6. The connecting loops each contain an Asn-Pro-Ala (NPA) motif that appears to be the site of the channel for water. Sui et al. (26) conducted definitive analysis of AQP1 by X-ray crystallography down to a resolution of 2.2 Å and in so doing clarified the selectivity of the pore region for water molecules. The short helical B

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**FIG. 2.**
The hourglass model of a monomer of AQP1, which includes the 6 transmembrane-spanning \( \alpha \)-helices and the short helical B and E loops with their Asn-Pro-Ala (NPA) motifs within the membrane. The hydropathy plot in the membrane is shown on top, and on the bottom is the 3-dimensional plot that shows the juxtaposed NPA motifs forming a single aqueous pore [from Jung et al. (9a)].
and E loops are two membrane-inserted non-membrane-spanning helices capped by Asn residues. The centrally located channel adjacent to these loops has a constriction of ~2.8 Å (water molecules have a radius of 2.8 Å). Thus the pore itself consists of an extracellular and a cytoplasmic vestibule connected by an extended narrow pore with a long hydrophobic core and a minimal number of solute-binding sites. Residues in the region of the constriction (particularly histidine-182, which is conserved in the aquaporin family) are critical for the selectivity of the channel for water molecules. The hydrophilic face of the pore provides the chemical groups for displacing waters of hydration to establish a pathway for coordinating the transport of water molecules. It appears that the formation of hydrogen bonds between water molecules and the pore residues causes the specificity of the channel for water. Water molecules permeate the channel single file and break hydrogen bonds with each other to form one and then the other hydrogen bonds with residues of the B and E loops within the channel.

Mammalian aquaporins have been broken down into two groups (9): the CHIP group, simply known as aquaporins, and the GLP group, known as aquaglyceroporins (GLP). Primary structures of both groups are similar in size (250–290 amino acids). For both groups, the NH₂ terminus is variable in length, there are six putative transmembrane α-helices, the COOH terminus is variable in length and hydrophilic, both termini are found in the cytoplasm, and there are two highly conserved NPA boxes. The CHIP group proteins (aquaporins) have generalized water channel activity, whereas the GLP group proteins are functionally specialized for the transport of glycerol across cell membranes. The groups are divided on the basis of the length of the extracellular sequences between the NPA boxes with the GLP group having 13–21 additional amino acids between helices 3 and 4 and 11–13 additional amino acids between helices 5 and 6. Aquaglyceroporins are less selective than aquaporins and may carry glycerol, water, and/or urea. Glycerol is used as an organic osmolyte as well as an energy shuttle hypothesis for facilitating the production of concentrated or dilute urine. AQP3 is found in basolateral membranes of epithelia in multiple organs. AQP4 (previously known as mercurial-insensitive water channel, or MIWC) is also found in basolateral membranes of epithelia in multiple organs and is constitutively active in red blood cells. AQP2 is found in apical membranes and intracellular vesicles of collecting duct and principal cells of the kidney and participates in the vasopressin-regulated membrane shuttle hypothesis for facilitating the production of concentrated or dilute urine. AQP3 is found in apical membranes of epithelia in multiple organs and is unique for its insensitivity to inhibition by mercurials. AQP5 is found in apical membranes of epithelia in salivary glands, lungs, and eyes. AQP6 is found in intracellular vesicles in intercalated cells of the collecting ducts of the kidneys and may be involved in acid-base balance. AQP7 is found in apical membranes of testes and kidney proximal tubules and in adipocytes. AQP8 is found in intracellular vesicles in multiple organs. AQP9 is found in apical membranes in multiple organs. Recently, preliminary data have identified a new protein of the GLP group, called AQP10, which is abundantly expressed in human duodenum and jejunum and may function as an absorptive pathway for water (but not glycerol or urea) (7). Studies to correlate tissue-specific expression of aquaporins with their functions have shown that expression does not necessarily imply major physiological importance. Thus aquaporin function needs to be evaluated on a physiologic channel. The mammalian family of aquaporins includes representatives of both the CHIP and the GLP groups, with numbers chosen primarily by chronology of discovery.

**Physiology and Pathophysiology of Water Channels**

Aquaporins have been identified in cells of the eyes and blood-brain barrier, salivary glands, lungs, heart, spleen, pancreas, colon, and red blood cells and various parts of the nephron. Functional roles of aquaporins include facilitating water reabsorption by the kidneys, assisting in fluid balance in various systems via rapid transepithelial transport, and minimizing the osmotic gradient and volume changes between cells and interstitium in the kidney medulla (9, 18). AQP0 appears to be a gap junction protein limited to lens fiber cell membranes of the eye. AQP1 is widely distributed in endothelia and in apical and basolateral membranes of epithelia in multiple organs and is constitutively active in red blood cells. AQP2 is found in apical membranes and intracellular vesicles of collecting duct and principal cells of the kidney and participates in the vasopressin-regulated membrane shuttle hypothesis for facilitating the production of concentrated or dilute urine. AQP3 is found in basolateral membranes of epithelia in multiple organs. AQP4 (previously known as mercurial-insensitive water channel, or MIWC) is also found in basolateral membranes of epithelia in multiple organs and is unique for its insensitivity to inhibition by mercurials. AQP5 is found in apical membranes of epithelia in salivary glands, lungs, and eyes. AQP6 is found in intracellular vesicles in intercalated cells of the collecting ducts of the kidneys and may be involved in acid-base balance. AQP7 is found in apical membranes of testes and kidney proximal tubules and in adipocytes. AQP8 is found in intracellular vesicles in multiple organs. AQP9 is found in apical membranes in multiple organs. Recently, preliminary data have identified a new protein of the GLP group, called AQP10, which is abundantly expressed in human duodenum and jejunum and may function as an absorptive pathway for water (but not glycerol or urea) (7). Studies to correlate tissue-specific expression of aquaporins with their functions have shown that expression does not necessarily imply major physiological importance. Thus aquaporin function needs to be evaluated on a...
tissue-by-tissue basis (28). The CHIP group of aquaporins includes the generalized water channels AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8. The GLP group of aquaglyceroporins contains the less selective channels that transport glycerol, water, and/or urea, AQP3, AQP7, AQP9, and AQP10.

Elucidation of the physiological and pathophysiological significance of the various aquaporins in mammals has been enhanced by numerous studies in knockout mice and by studies in humans with known mutations. In some cases, differences in the severity of the pathophysiological defect have been found between aquaporin mutations or in the absence of aquaporins in humans and in mice. Different aquaporins are present in apical and basolateral membranes of epithelia in various segments of the kidneys. Water permeability across individual nephron segments is highly correlated with expression of aquaporins (13). Thus specific aquaporin blockers are likely to become useful as novel diuretic agents (28). Diuresis resulting from a major defect in urinary concentrating ability (primarily due to reduced fluid absorption in the collecting ducts) has been found in AQP1 knockout mice (28). Discovery of the Colton blood group antigen on AQP1 has allowed identification of humans who are rare Colton-null individuals and lack AQP1. Overtly, these individuals have no obvious clinical phenotype. However, recent studies of AQP1-null humans show that, although they do not have polyuria similar to the AQP1 knockout mice, they do have impaired ability to concentrate urine maximally when deprived of water (11). In addition, these individuals have evidence of decreased pulmonary vascular permeability in response to fluid challenge (12). AQP2 deficiency produces nephrogenic diabetes insipidus with renal resistance to vasopressin and the excretion of large volumes of dilute urine. Four different aquaporins have been identified in the respiratory tract and are involved in appropriate handling of water in the vascular, interstitial, and airspace compartments of the lungs. AQP1 is found mostly in pulmonary microvascular endothelia, AQP4 in airway epithelia, and AQP5 in apical membranes of type 1 alveolar epithelial cells (15). In AQP1 and AQP4 knockout mice, AQP1 was shown to facilitate hydrostatically driven lung edema but not be required for active absorption of alveolar fluid (1). In AQP5 knockout mice, alveolar fluid clearance was unimpaired, suggesting that, although AQP5 is important for osmotically driven water movement out of alveolar spaces, it does not facilitate hydrostatically driven lung edema or active alveolar fluid absorption (15). AQP1 is colocalized in the choroid plexus with Na<sup>+</sup>-K<sup>+</sup>-ATPases and is likely involved in cerebrospinal fluid production. Mice lacking AQP4 have reduced brain swelling after acute hyponatremia and ischemic stroke (28). Mutations in AQP0 (which functions as a low-capacity water channel in lens fiber cells of the eye) have been implicated in congenital cataracts. AQP1 and AQP5 are likely involved in reducing the water content of corneal and lens epithelia of the eye, as has already been shown for the cornea in knockout mice (13). AQP5 is abundant in secretory cells of salivary and lacrimal glands and thus may be involved in Sjogren’s disease, which presents with immunologically dry eyes and mouth and desiccation of tracheobronchial secretions (13). Thus aquaporins have been shown to be extremely important for maintaining water balance in many tissues and appear to be the primary pathways for the movement of water molecules across cell membranes. If the ubiquitous and polar water molecules need protein-mediated transport to cross cell membranes, it is likely that urea molecules also have proteins that mediate their transport across the cell membranes of the compartments in which they are found.

**UREA TRANSPORT**

Urea is found in the blood, tubular fluid, and interstitium of mammalian kidneys as well as in the liver (where ureagenesis occurs) and in various tissues that synthesize polyamines in physiological or pathophysiological conditions like the heart muscle during cardiac hypertrophy, the testis during spermatogenesis, and the brain. Thus numerous cells in an organism must be able to clear toxic urea.

Filtered urea moves out of the proximal tubule of the kidneys and into the interstitium; however, much of the rest of the tubule is relatively impermeable to urea. Thus urea is increasingly concentrated in the tubular fluid as water leaves in the loop of Henle and distal tubule. In the inner medullary portion of the collecting duct (IMCD), urea moves into the interstitium, adding to the hyperosmolality there. The movement of urea out of the IMCD is regulated by vasopressin. Conversely, when dilute tubular fluid (no
vasopressin) reaches the IMCD, urea moves from the interstitium into the tubular lumen, and the osmotic gradient in the interstitium is reduced. Urea movement in and out of the tubule leads to a mechanism that allows the formation of either concentrated or dilute urine. Via the countercurrent multiplier and exchange systems in the kidneys, an osmotic gradient develops deep into the medullary tissue that is composed of NaCl (due to a variety of transport systems in the tubular segments) and urea in the interstitium. The amount of urea in the urine and in the medullary interstitium of mammalian kidneys varies with the amount of urea filtered at the glomerulus, which in turn varies with the dietary intake of protein. A low-protein diet prevents an individual from producing concentrated urine.

Evidence for Urea Transporters

Numerous students have conducted experiments on red blood hemolysis in various osmotic solutions to investigate the principles of diffusion and osmosis. Red blood cells lyse very rapidly (too fast to measure the time) when placed in an isosmotic solution of urea (300 mosM). Red blood cells lyse measurably less rapidly when placed in an isosmotic solution (300 mosM) of the urea analog thiourea. The explanation for the lysis that was commonly taught was that urea and thiourea were able to permeate the red blood cell membranes through nonspecific pores to cause osmotically driven swelling and rupture of cells. However, identification of transport proteins for select small molecules has shown that red blood cells have numerous aquaporins (AQP1) that facilitate rapid water movement under osmotic gradients and numerous urea transporters (UT-B) that facilitate rapid urea movement by diffusion under concentration gradients. Thiourea molecules utilize the same urea transporters to cross red blood cell membranes as urea but permeate more slowly.

In the early 1970s, evidence was first obtained that urea transport in human red blood cells saturates and can be inhibited (by phloretin) without significantly altering simultaneous water transport (29). Subsequently, using a fast flow system, Mayrand and Levitt (17) confirmed that urea transport across human red blood cell membranes was a saturable facilitated diffusion process that was competitively inhibited by a large number of urea analogs. Early studies found species differences between human and chicken red blood cells in the kinds of facilitated diffusion transporters likely available for the movement of urea and water molecules (3). However, the mechanisms for urea permeability across red blood cell membranes were not further investigated at that time. Even though urea is a highly polar molecule that should have low permeability across lipid bilayers, renal physiologists and most textbooks continued until the late 1990s to describe urea transport as primarily simple diffusion through the lipid bilayer.

On the basis of evidence from mammalian red blood cells, specific facilitated urea transport was first proposed in 1987 for the terminal IMCD of mammalian kidneys (22). Measured urea permeability in the absence of vasopressin was 85 times greater than predicted for simple lipid phase diffusion or for paracellular transport. Subsequent experiments verified that urea transport in the perfused terminal IMCD showed saturation kinetics (4), supporting the hypothesis of a protein-mediated transport process.

These phenomenological studies set the stage for the subsequent molecular biological investigations searching for urea transporters. Expression cloning in Xenopus oocytes of the cDNA clone of a renal urea transporter from rabbit inner medulla was first reported in 1993 (30). The oocytes expressed functional transporters that facilitated the transport of urea. Urea transport activity of Xenopus oocytes injected with mRNA from human or rat kidney papilla or rabbit reticulocytes was enhanced about threefold compared with water-injected controls (6). This expressed urea transport could be completely inhibited by phloretin or p-chloromercuribenzenesulfonate (pCMBS).

Structure of Urea Transporters

Structural analysis of urea transporters is incomplete. Some of the urea transporters are predicted to be ~43-45 kDa, whereas one is predicted to be ~95 kDa (21). Figure 3 shows a current model of urea transporter UT-A1 found in apical membranes of the IMCD in rat kidneys. All isoforms contain several sequences of amino acids that are known to encode for sites for N-glycosylation or N-myristoylation or for protein kinases, suggesting that the proteins are
highly regulated. The family of urea transporters appears to be remarkably well conserved among various species and various isoforms. Proposed models for the structure of urea transporters predict that the number of transmembrane segments ranges between 8 and 16, with intracellular carboxy termini, an extracellular N-glycosylation site located in a relatively hydrophilic segment in the middle of the protein, and several internal tandem repeats (24).

**Physiology and Pathophysiology of Urea Transporters**

Early studies of urea transport in human red blood cell membranes showed saturation, competition, and asymmetry, suggesting that a transport protein was likely to be involved (17). Because urea transport could be inhibited by phloretin and pCMBS (known inhibitors of facilitated diffusion transport), urea transporters in red blood cells were likely facilitated diffusion transporters. The turnover number for molecular transport via urea transporters was estimated to be from 0.3 to $1.0 \times 10^{-5}$ molecules/s, suggesting that facilitated urea transporters are likely to act as channels instead of carriers (14).

The major functional advantage for having facilitated diffusion transporters for urea in red blood cells is known as the “Macey hypothesis.” In modeling the passage of red blood cells through the human renal medulla, which may have up to 0.6 M urea, urea transporters likely play two critical roles (16). In passing along the vasa recta, red blood cells shrink by...
osmosis as they approach the hyperosmotic regions of the kidney and then swell as they return toward the cortex. In red blood cells with inhibited urea transport (lipid bilayer only), the volume changes due to osmosis are large and may actually cause cell lysis. In red blood cells with functional urea transporters, urea (and water) enters cells as they approach the hyperosmotic regions and urea (and water) leaves cells as they return toward the cortex. Thus fast urea equilibration across red blood cell membranes stabilizes cell volume as cells pass through the renal medulla. The second role is that the rapid urea efflux from red blood cells as they exit the medulla prevents washing out of the medullary interstitial gradient of urea by keeping the cells from being able to carry urea molecules away from the medulla.

In humans, the red blood cell urea transporter (UT-B) is also the Kidd blood group antigen. Humans lacking the Kidd blood group antigen are unable to concentrate urine above 800 mosmol/kgH2O (21). Thus it is likely that facilitated urea transport in red blood cells is necessary to preserve the efficiency of countercurrent exchange in the kidney. UT-B urea transporters are likely also located in the descending vasa recta, where they would be involved in the rapid recycling of urea in and out of the medullary tissue.

Urea is important for the conservation of body water due to its vital role as a major component of the medullary osmotic gradient that allows production of concentrated urine. To establish the osmotic gradient, large quantities of urea must be delivered to the deepest portions of the inner medullary interstitium (20). Facilitated urea transporters have also been identified by competition and inhibition studies in mammalian terminal IMCD (20). Several urea transporters have been cloned from various kidney tissues, some of which are facilitated urea transporters regulated by vasopressin and some of which are not vasopressin regulated. Functional studies indicate that phloretin-inhibitable facilitated urea transport is present in both apical and basolateral membranes of the rat terminal IMCD, with apical transporters likely being the vasopressin-stimulated, rate-limiting transporters.

Increasing osmolality (by adding NaCl or mannitol) has been shown to increase facilitated urea permeability in perfused rat terminal IMCDs in a manner that is independent of vasopressin (23). Angiotensin II increases vasopressin-stimulated facilitated urea transport in rat terminal IMCDs and thus may play a role in urine concentration by enhancing the change in urea permeability in response to vasopressin (10). Conditions in which the ability to concentrate urine is impaired lead to increased basal urea permeability out of the IMCD and increased abundance of urea transporter proteins (UT-A1) in the cell membranes of the IMCD, indicating that regulated urea transport is used to bring the urine concentration mechanism back to normal (20). The increased number of urea transporters in the IMCD may help with rapid restoration of the medullary urea concentration when urea or protein becomes present.

Recently, three sodium-dependent secondary active transporters for urea have also been found in various segments of the IMCD (20). One is a vasopressin-independent apical sodium-urea cotransporter used for urea reabsorption found in the initial IMCD in rats fed a low-protein diet (8%). This cotransporter is encoded by a longer mRNA than that of the facilitated diffusion urea transporters. The other two are both vasopressin-regulated sodium-urea transporters. One is a basolateral sodium-urea countertransporter involved in urea reabsorption found in the initial IMCD in furosemide-treated rats. The other is an apical sodium-urea countertransporter found in the deep terminal IMCD of rats. These special urea transporters are induced to secrete urea into the urine during conditions associated with reduced ability to concentrate urine. Integrative animal studies with polyclonal antibodies to urea transporters have shown that vasopressin regulation apparently occurs by phosphorylation of urea transport proteins (UT-A1) in rat IMCD.

Expression of urea transporters in renal tissue and red blood cells can be explained physiologically as facilitation of the production of concentrated urine in an organism. Urea transporters are also expressed in rat liver, brain, seminiferous tubules, and heart (J. M. Sands, personal communication). It is likely that the liver urea transporters are present because liver tissue is the primary site for ureagenesis. Urea is produced either from arginine in the urea cycle (liver) or as a
byproduct of ornithine synthesis from arginine via arginase in the polyamine synthesis pathway (other nonrenal tissues). This polyamine pathway is also present in the heart. Polyamine production is known to increase in conditions associated with cardiac hypertrophy. Ornithine decarboxylase inhibitors protect the heart from becoming hypertrophic. Duchesne et al. (5) found that urea transporter proteins are upregulated in the hearts of uremic or hypertensive rats and in dilated cardiomyopathy in humans. This upregulation may be important for facilitating urea exit from the cells in pathological conditions in which urea production is increased. Recently, Ranade and colleagues (19) showed that single nucleotide polymorphisms in human kidney urea transporter UT-A2 appear to be associated with variation in blood pressure in men, but, for as yet unknown reasons, not women.

CONCLUSION

Although physiological studies in the 1970s and 1980s confirmed that water channels are present in human red blood cell membranes, molecular biological studies in the early 1990s with expression cloning in Xenopus oocytes were necessary to identify the proteins involved in water transport. Likewise, convincing evidence for urea transport proteins was made possible by molecular biological techniques in the early 1990s. It appears that many other small, hydrophilic molecules of physiological importance also have selective transport pathways facilitated by specific proteins. Thus the concept of small, nonselective pores in cell membranes has decreased in significance for a complete understanding of membrane transport. With the growing understanding of the consequences of absence or mutations in specific transport proteins, the future will likely lead to specific targeting of particular channels with new drugs to correct general or local disturbances in water homeostasis such as renal, liver, or heart failure, glaucoma, acute hydrocephalus, or Sjogren’s syndrome.

REFERENCES


