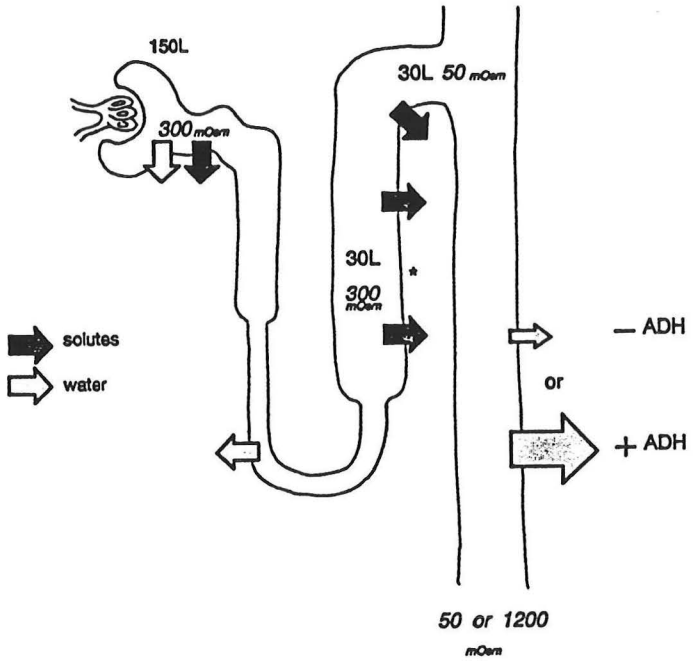
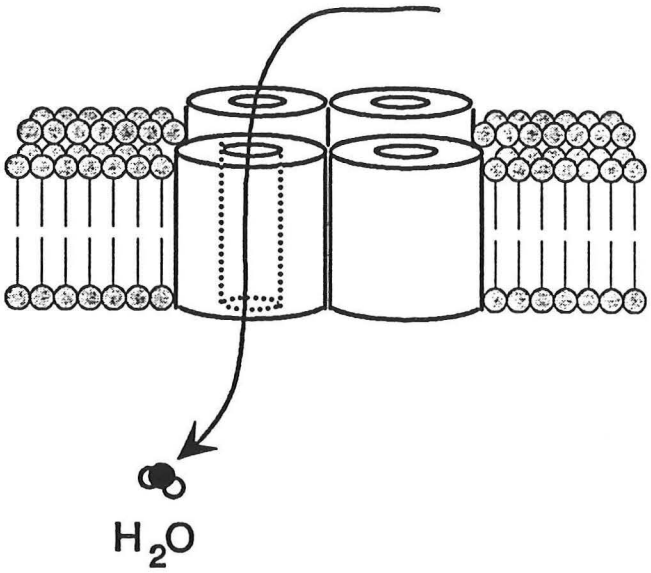
Understanding Diabetes Insipidus: From Water Clearance to Water Channels





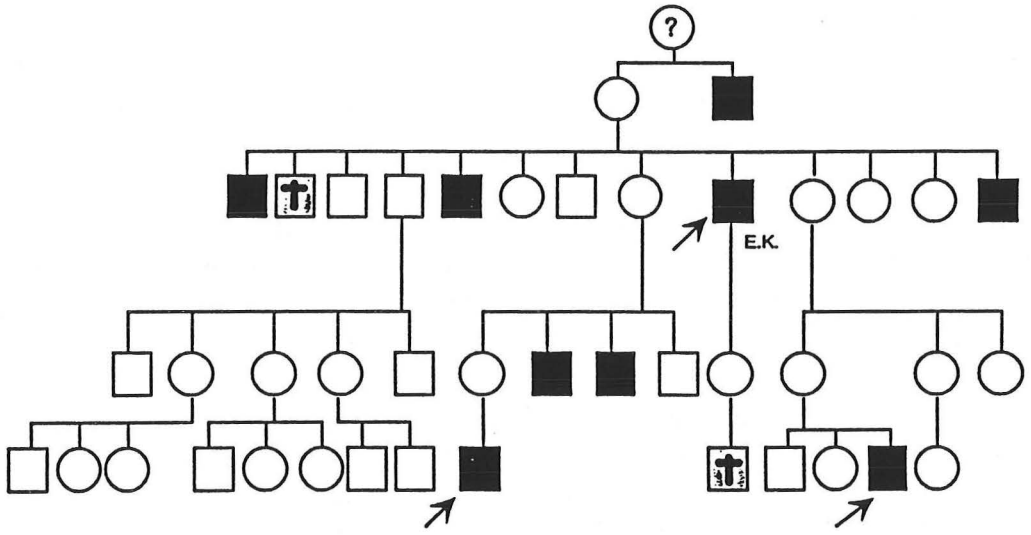
Orson W. Moe, M.D.
August 21, 1997
Medical Grand Rounds
Department of Internal Medicine
University of Texas Southwestern Medical Center

Name:	Orson W. Moe, M.D.
Rank:	Assistant Professor
Division:	Nephrology
Interests:	Renal Physiology, Acid-Base and Electrolyte Metabolism, Epithelial Biology.

HISTORICAL TRIVIA

The first documentation of congenital diabetes insipidus was around the turn of the century based solely on clinical history. A giant German genealogical tree spanning over four generations of patients was compiled by three generations of physicians all within Dr. Weil's family (Weil 1884, Weil 1908, Camerer 1935). These reports stated clearly that congenital diabetes insipidus was inherited as an autosomal dominant trait and such became the axiom in medical textbooks.

The Swedish physician Hans Forssman is believed by many to be the first person to scientifically study the physiology and genetics of congenital diabetes insipidus (Forssman 1942, Forssman 1945). Although he was not quite qualified at the time, Hans Forssman was called upon by the Chief of Medicine during the war to serve as house officer at the University Hospital at Uppsala. One of his first duties was to attend to Mr. E.K, a young man with polyuria. His urine tested positive for sugar and the diagnosis of diabetes mellitus was made. However Forssman noticed that although the diabetes mellitus was of recent onset, the polyuria and polydipsia dated back to childhood. In addition, he noted that despite glycosuria, his urine specific gravity was remarkably low. A revised diagnosis was made of diabetes mellitus superimposed on the background of diabetes insipidus. A careful interview with family members reviewed a classical X-linked recessive inheritance pattern that contradicted the dogma of autosomal dominant inheritance.



Forssman's first kindred ↗ marks the 3 encounters

This encounter prompted a lifelong search and study of this disease. Dr. Forssman tracked down families and study patients using the clinical investigative tools available at the time. He came to the

the time, He came to the conclusion that there are two forms of congenital diabetes insipidus. One with autosomal dominant inheritance and one with X-linked recessive with fundamentally distinct underlying pathophysiology and he termed them central vs. nephrogenic diabetes insipidus. Late in Dr. Forssman career, he had two interesting serendipitous encounters (Forssman 1975). On each of these independent episodes, Dr. Forssman heard from his pediatrician colleague, about a challenging case on the ward of an infant with fever of unknown origin. He recognized that they suffered from congenital diabetes insipidus and was requested to serve as consultant. As he traced the family histories on these new patients, he found out that they were related to his first proband Mr. E.K., the first patient he saw with congenital diabetes insipidus.

Contemporaneously in the United States, similar advances were made most likely independent of Forssman by Waring (Waring 1945), Williams (Williams 1947), and Dancis (Dancis 1948). If one looks beyond the medical literature, Dr. Forssman was actually not the first to described the X-linked recessive pattern of the disease. The existence of "water-drinkers" as they were called, was well documented in Scottish folklore dating back to the 17th century; two hundred years before Gregor Mendel described his garden pea experiments and the basic laws of genetics. A modern day translation of an old passage reads as a perfect description of X-linked recessive inheritance (Rogers 1919).

> *A gypsy woman and her son were travelling the road and became thirsty. Pausing at a well in front of the next house, the gypsy requested water for her son; the house wife refused, whereupon, the gypsy woman cast upon her a curse. Henceforth, the story goes, the woman's sons would be afflicted with a craving for water. The curse would be passed on by her daughters and revisited upon their sons for generations to come.*

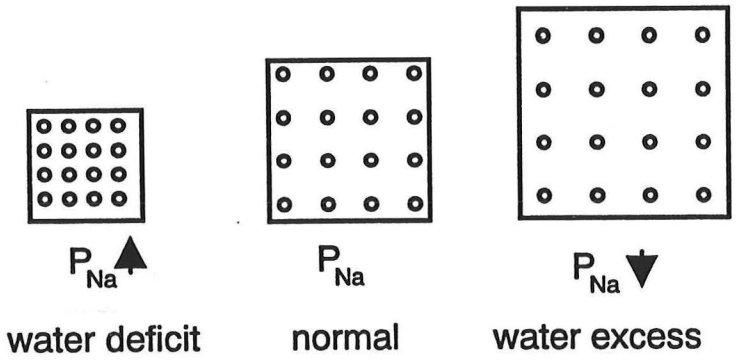
It is fair to ascribe the beginning of scientific study of diabetes insipidus to the early 1940's. In the short span of less than half a century, extraordinary advances were made in the understanding of diabetes insipidus first through well conducted experiments in clinical physiology, and in the last decade through molecular genetics. This manuscript will review the following:

1. The physiology of water homeostasis and the pathophysiology of diabetes insipidus.
2. The genetic lesions of congenital diabetes insipidus treading the "pituitary-collecting duct axis" from the neurosecretory cells that produce vasopressin to its receptor in the collecting duct, and finally the effector of transepithelial water movement in the kidney.

PHYSIOLOGY OF WATER HOMEOSTASIS

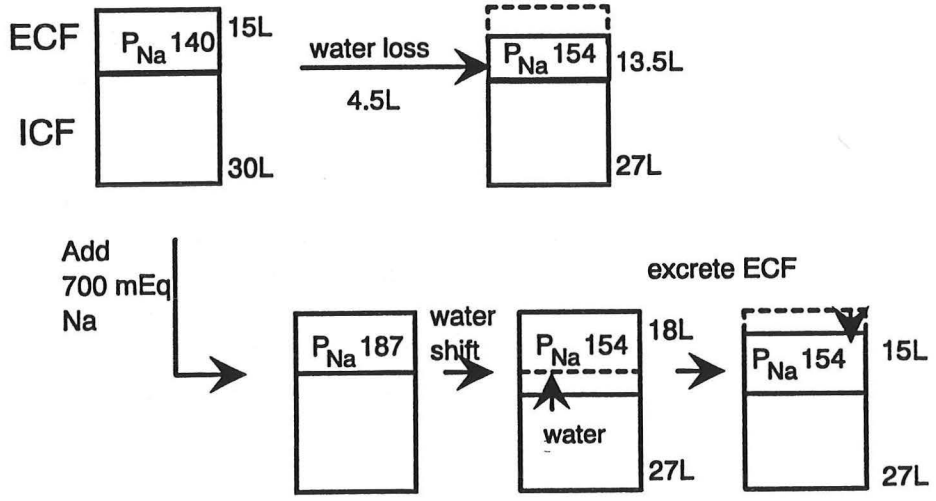
Plasma Na concentration: a marker for total body water

Body fluid osmolarity can be viewed as the ratio of total solutes to total body water. At equilibrium, intracellular fluid osmolarity must equal extracellular fluid osmolarity; which is approximated by plasma Na concentration. Therefore plasma Na concentration is used as a clinical marker for disturbances of total body water.

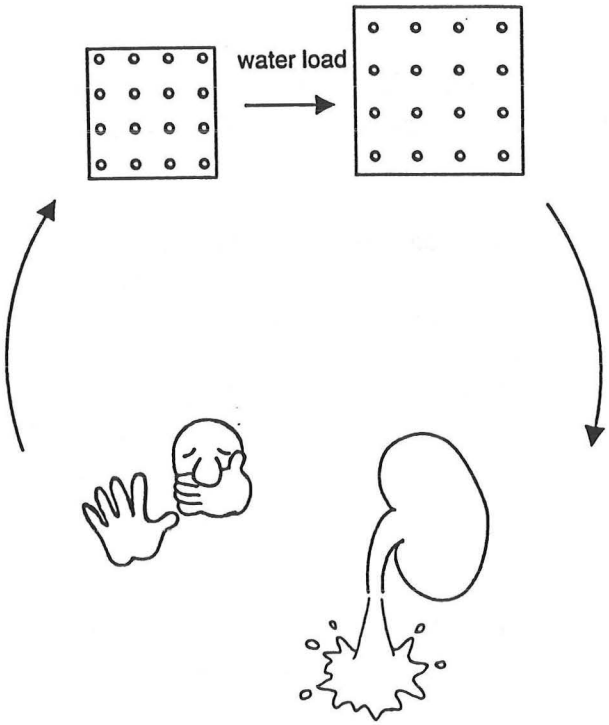


Disturbances in plasma osmolarity or plasma [Na] should be viewed as a "denominator disease". Water excess in general leads to low plasma osmolarity and plasma [Na] and conversely, water deficiency in general leads to high plasma osmolarity and plasma [Na].

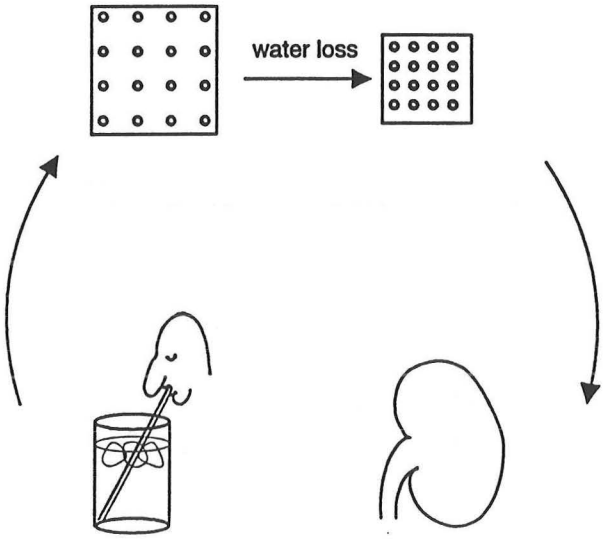
Although the numerator, i.e. Na content in the ECFV can alter plasma [Na]. Changes in plasma [Na] even in that situation still represent a disturbance in total body water. One can consider two examples of hypernatremia from either pure NaCl gain or pure water loss. If one loses 4.5 L of water proportionately from the ICF and ECF, plasma [Na] will be increased from 140 to 154 mEq/L. Now consider the same degree of hypernatremia from pure NaCl gain. An acute load of 700 mEq's of NaCl would raise plasma [Na] to 187 mEq/L transiently followed by rapid movement of water from the ICF to the ECF. This will lead to ECFV expansion from 15 to 18 L's and volume homeostatic mechanisms would return the ECF towards 15 L's. The end result is a plasma [Na] of 154 mEq/L but with a loss of 3L's of water primarily from the ICF.



Terrestrial animals encounter disproportionate gains and losses of solutes and water and are constantly subjected to threats in plasma osmolarity. Maintenance of plasma osmolarity in mammals is achieved within a very narrow range via the partnership of two systems: THIRST and KIDNEY



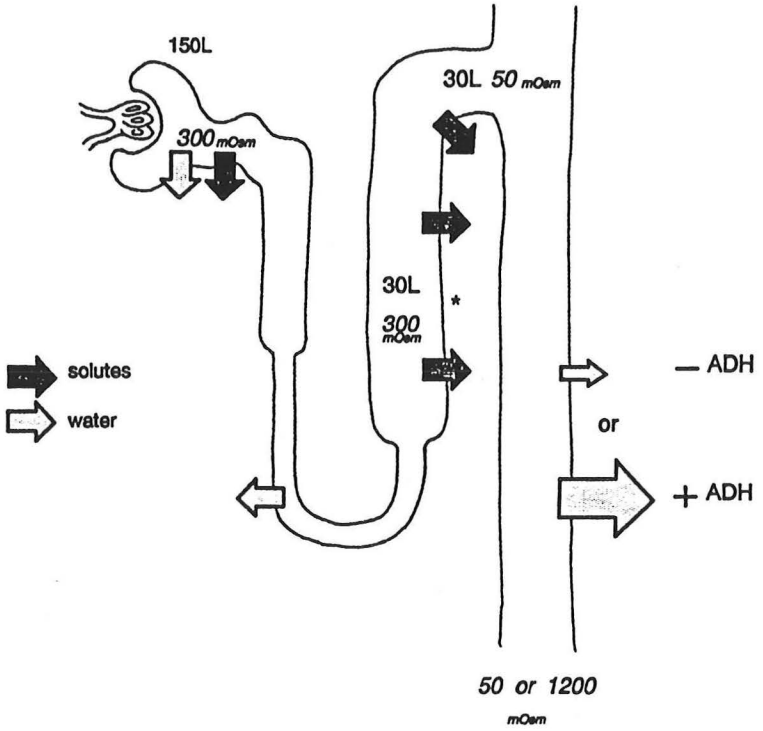
1. Cessation of water intake which prevents further aggravation but does not correct the problem.
2. The major player in this defense is the kidney's ability to excrete dilute urine.



1. The elaboration of a scanty and highly concentrated urine to the point where not only there is water conservation but water is actually added to the organism.
2. By far the strongest defense is water drinking.

Renal water handling

The mammalian kidney can both excrete or generate water as urine is generated from plasma. This figure shows the amount and the osmolarity of urine from the glomerular filtrate to the renal pelvis.

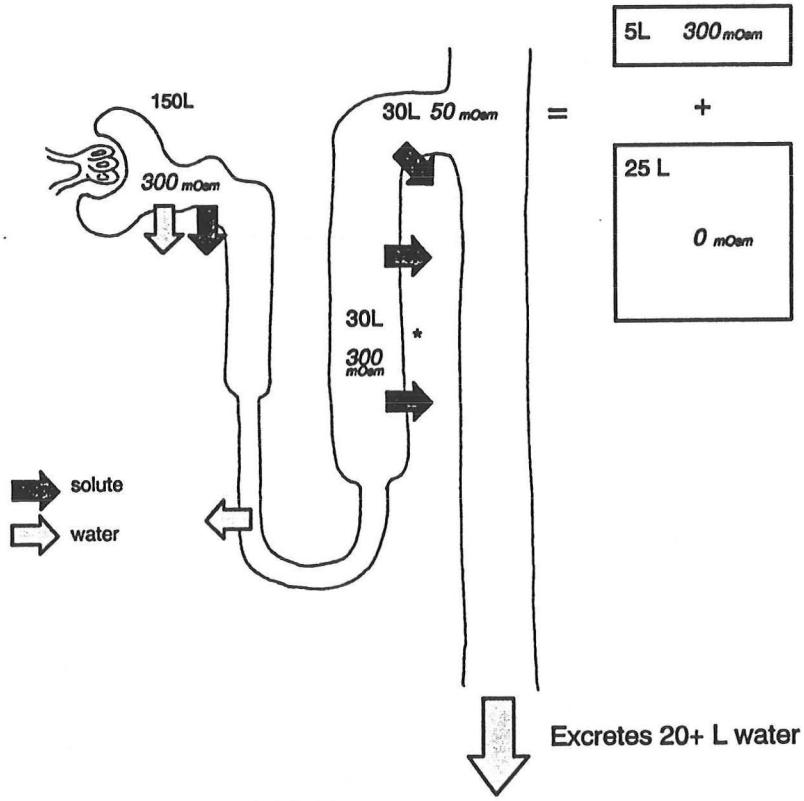


Glomerular filtration and proximal reaborption are both isotonic therefore no significant net solute-free water exchange results. When urine traverses the loop of Henle, it is concentrated. Upon arrival in the ascending limb, because of solute absorption unaccompanied by water movement, the urine becomes progressively more dilute until some point in the thick ascending limb, it reaches isotonicity (*). During euvolemia, of the 150 L's of water filtered at the glomerulus daily, approximately 30 L's reach this point of isotonicity. An excellent discussion of the quantitative estimate of the fluid delivery to the isotonic point by clearance methods can be found in the monograph by Shuster and Seldin (Shuster 1993). Beyond this point, the tubule becomes impermeable to water and as more solutes are reabsorbed, the urine becomes increasingly more dilute reaching its minimal tonicity at the distal nephron. The osmolarity of the 30 L's of urine that came from the isotonic point (*) is now down to about 50 mOsm. The thick ascending limb and distal convoluted tubule that absorb solutes without water is termed the diluting segment. From this point on, the fate of the water in the urine depends on the water permeability of the collecting duct. If one desires to rid the body of water, one would render the collecting very impermeable like a lead pipe. The dilute urine will then leave the kidney and water excretion is effected. Conversely, if one wishes to conserve water, one would make the collecting duct very permeable such that as the urine navigates through the hypertonic medulla, water is abstracted back into the systemic circulation. The singular most important regulator of collecting duct water permeability is antidiurectic hormone or

vasopressin (Grantham 1966, Sands 1987). In addition to increasing water permeability of the collecting duct, vasopressin also enhances the medullary tonicity by stimulating solute transport in the medullary thick ascending limb (Hebert 1987) and cortical collecting duct (Tomita 1985).

Limits of renal water excretion

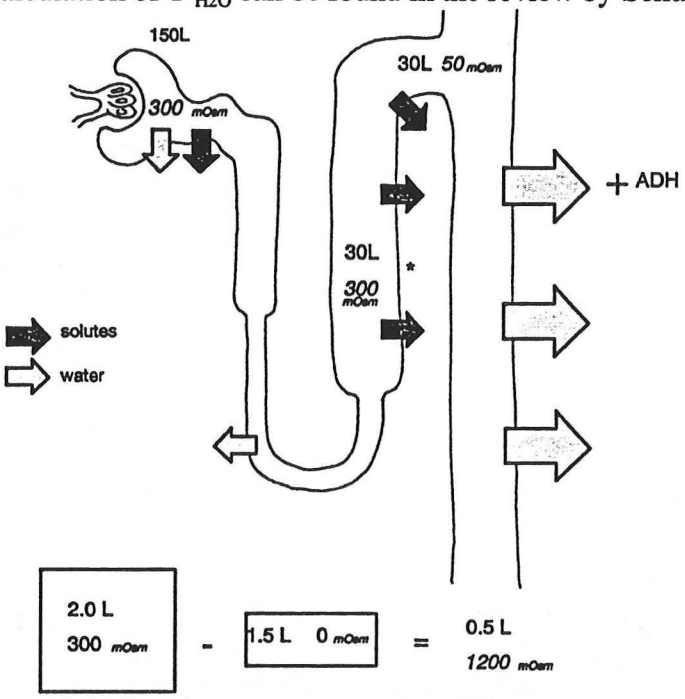
Disturbances in total body water results when water excess or deficit exceeds the homeostatic compensation. To understand disturbances in water one needs to know the limits of this homeostatic system .



Remember that cessation of drinking can only stop further dilution but cannot correct an existing hypotonic state. Defense of water excess therefore depends on water excretion by the kidney. For the sake of this discussion, one can assume that 30 L's of isotonic urine reaches the point of isotonicity in the thick ascending limb. If the diluting segment then renders this urine maximally hypotonic to 50 mOsm, the 30 L's at the end pf the diluting segment in essence can be viewed as being partitioned into 5 L's of isotonic fluid and 25 L's of pure water. Although one cannot turn the collecting duct into a real "lead-pipe", one can indeed render this segment relatively impermeable to water. Even in the absence of vasopressin, some finite water permeability remains (Berliner 1957, Jamieson 1971), particularly at the terminal portion of the inner medullary collecting duct (Sands 1987). Nonetheless, if "near-lead pipe" conditions are achievable, one can theoretically excrete some 20+ L's of pure water every day. This is a high capacity system and this is the reason why it is very difficult to cause hyponatremia by polydipsia alone. Solute-free water excretion is called water clearance or C_{H2O}. This review will not address the mathematical calculation of C_{H2O}. An excellent discussion is found in Schuster and Seldin (Schuster 1993). [See footnote below re: C_{H2O}]

Limits of renal water generation

In the face of water deprivation, antidiuresis is desired. In this instance, the collecting tubule is highly permeable to water. One elaborates a highly concentrated low volume urine of say 0.5 L of 1200 mOsm. In producing this urine, one has taken 2 L's of 300 mOsm plasma, extracted 1.5 L's of electrolyte-free water from it to remain in the plasma, and excreting 0.5 L of urine of 1200 mOsm. The extraction of electrolyte-free water to remain in plasma is termed free water generation or T^c_{H2O}. A discussion of the calculation of T^c_{H2O} can be found in the review by Schuster and Seldin (Schuster 1993).



Generates 1.5 L water

As one can see instantly that the capacity of this system is rather low compared to the 20 L's capacity of water excretion. However, providing the thirst mechanism is intact and water is available, the ability to drink water is virtually limitless so 1.5 L's of free water generation is adequate. The kidney becomes a minor player in the defense against water deficit.

Footnote: Some investigators prefer the use of "effective water clearance" (EWC) rather than free water clearance based on the premise that not all solutes are effective osmoles.

$C_{H2O} = V - C_{osm}$ $\quad C_{osm} = U_{osm} \cdot V / P_{osm}$

$EWC = V - C_{lytes}$ $\quad C_{lytes} = U_{(Na+K)} \cdot V/P_{(Na+K)}$

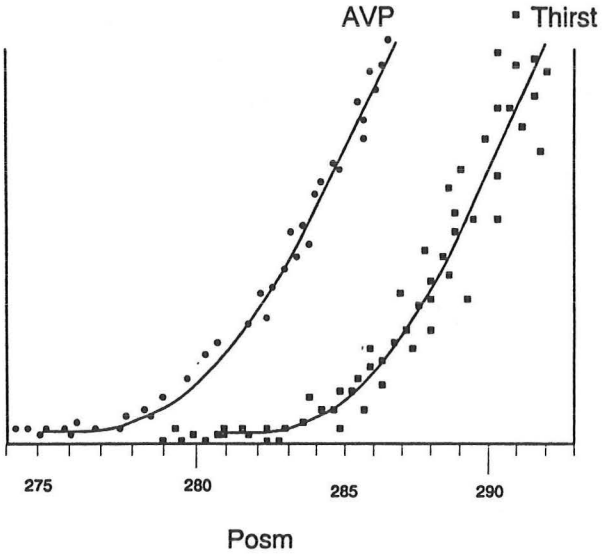
A similar argument can be made for T^c_{H2O} to substitute C_{osm} by C_{lytes}

Bankir L. 1996 Urea and the kidney. In: Brenner and Rector's the kidney Ed BM Brenner 571

Mallie JP, Bichet DG, Halperin ML. 1997. Effective water clearance and tonicity balance: the excretion of water revisited. Clin Invest Med 20:16.

Thirst and renal water handling: Integrated system

In conjunction, these two systems work to control plasma osmolarity within very tight limits. At hyperosmolar states, AVP is stimulated and thirst is turned on. The kidneys generate solute-free water and the organism drinks. When plasma osmolarity falls towards normal but not quite reached normal, the organism ceases drinking. At this point, the water that is already in the GI tract will be absorbed and plasma osmolarity will be rectified. In case when too much water is absorbed such as overdrinking or as a deliberate experimental overcorrection, AVP is suppressed to zero and the powerful renal water excretion mechanism comes into play to bring the plasma osmolarity back up to normal. Note that the inflection point or the threshold of activation of the two curves are about 5-7 mOsm apart. This minimizes overcorrection in either direction.



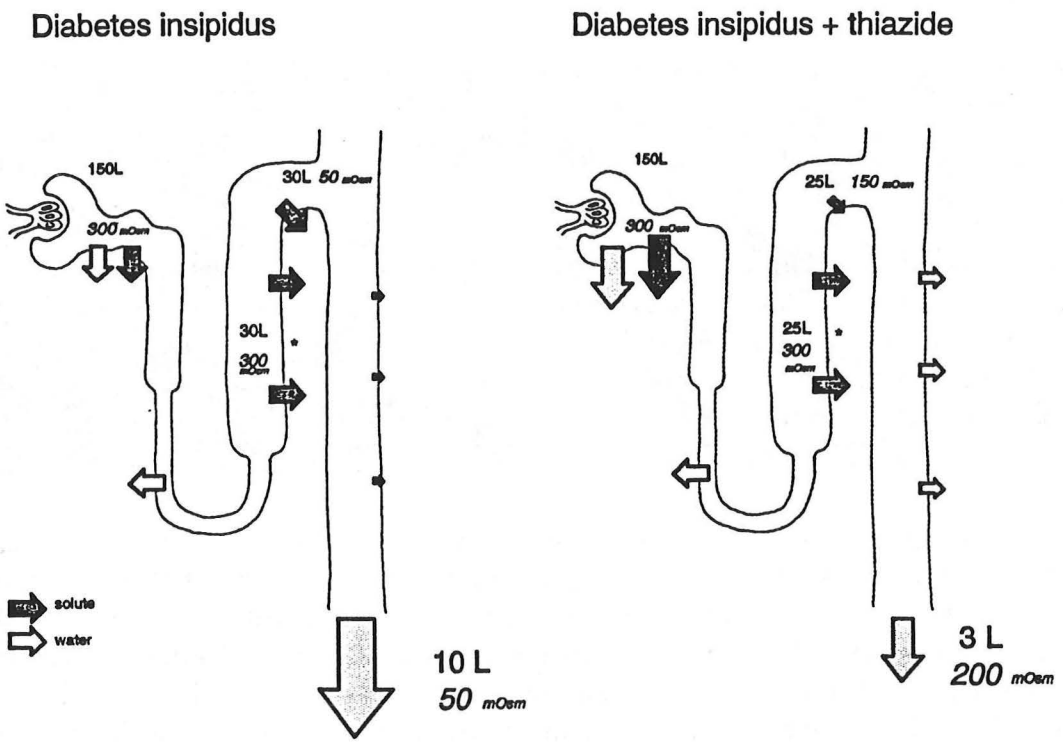
In summary, the kidney is the major player in correcting water excess while thirst is far more important than the kidney in defending against water deficit.

Water handling in diabetes insipidus

In diabetes insipidus, there is a breakdown in the homeostatic loop because the ability to generate water is lost. In fact the kidney is constantly operating in the large capacity water excess mode clearing free water regardless of the plasma osmolarity. This perpetually brings the organism to a water deficient state. One must keep in mind that even when one is relentlessly excreting free water, the capacity for this system although high, does have an upper limit. The thirst mechanism on the other hand is infinite. Therefore, correction of water deficit should always be possible if thirst is intact. For this reason, the awake patient who walks into the office with diabetes insipidus usually presents with a normal or only slightly elevated P_{Na} and a Hx of polyuria and polydipsia. The slightly elevated P_{Na} is due to a single (thirst) rather than a dual (thirst and kidney) homeostatic system and that the thirst threshold (Posm) is slightly higher than the AVP threshold. It is only upon withholding water that water deficiency and severe hypernatremia develops.

Physiologic basis for treating diabetes insipidus with thiazides

This review will not be an inclusive account of the treatment of diabetes insipidus. Excellent reviews are available on this topic (Star 1990, Howard 1992, Star 1993). For central diabetes insipidus, hormonal replacement is no doubt the main therapy. For both central and nephrogenic diabetes insipidus, other measures can be taken to minimize the urinary water loss. While it is true that water drinking alone should suffice, it is desirable to lower the obligatory water requirement. One very effective therapy is the use of thiazide diuretics.



Thiazides are aimed at several targets along the cascade of free water clearance. Consider a patient with diabetes insipidus with a normal diluting segment but absence of water permeability in the collecting duct. This patient is excreting 600 mOsm of solutes with a obligatory water volume of about 12 L's. With adequate volume contraction, compensatory increase in proximal absorption will decrease delivery to the diluting segment; hence less filtrate is available for free water formation. The main action of thiazides is impairment of NaCl transport and urinary dilution in the distal convoluted tubule. This will impair urinary dilution without disturbing urinary concentration. Lastly, as we mentioned before, even in the absence of AVP, the collecting tubule is not entirely impermeable to water; particularly the terminal IMCD. If urinary flow can be slowed as in the case with volume contraction, urinary osmolarity can actually rise significantly (Berliner 1957, Jamison 1971, Valtin 1987). In this patient on thiazides, the 600 mosmoles is excreted with 2 rather than 12 L of water. This patient is therefore a lot less prone to dehydration if water access were limited in any way.

AUTOSOMAL DOMINANT CENTRAL DIABETES INSIPIDUS

Clinical and physiologic studies

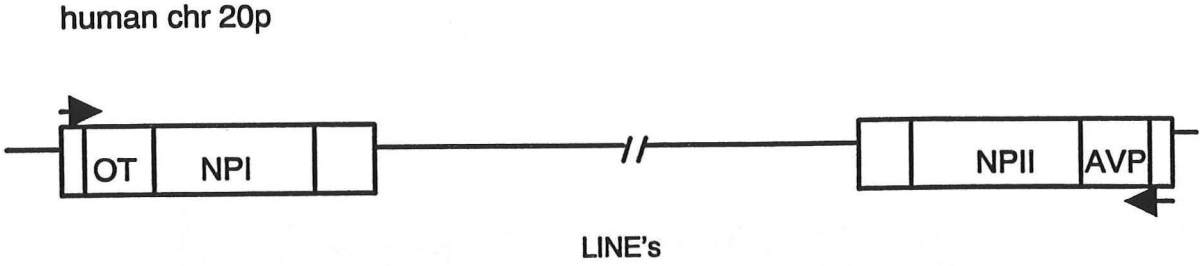
The very first kindred of congenital diabetes insipidus described was of the autosomal dominant variety (Weil 1884, Weil 1908, Camerer 1935). If one were to combine world wide literature to date, this will probably still be the commonest form of congential diabetes insipidus. A vast number of clinical and laboratory observations were made over about three decades. They can be briefly summarized:

1. Autosomal dominant.
2. Slow onset.
3. Milder and incomplete but progressive.
4. Intact renal response to exogenous administered arginine vasopressin (AVP).
5. Cell death and gliosis limited to the magnocellular neurons in the supraoptic and paraventricular nucleii where AVP is synthsized.
6. In patients with established disease, plasma AVP levels are virtually undetectable.
7. Linkage analysis mapped the locus to 20p: very close to AVP gene.

The patients present more often in later childhood or adolescence rather than in infancy (Pender 1953, Levinger 1955, Kaplowitz 1982). An occasional patient will not receive medical attention till his or her twenties. The disease is often incomplete and the patients retain some ability to concentrate their urine. When AVP analogues such as des-amino-8-D-arginine vasopressin (DDAVP) was adminstered, these patients concentrated their urine appropriately (Cobb 1978, , Harris 1989). The kidneys are considered to be completely normal. Post mortem findings show a progressive degeneration of the neurons in the supraoptic and paraventricula nucleii where AVP is produced (Braverman 1965). Now we know this form of the disease is due to mutations in the AVP gene leading to defects in AVP secretion by the posterior pituitary.

AVP genomic organization

The AVP gene encodes the prepro form of the enzyme which includes a 19 amino a cids leader signal sequence, the 9 amino acid AVP sequence itself followed by a 95 aminoa acids carrier protein called neurophysin II (NPII). C-terminal to NPII is a 39 aminoa acid glycoprotein of yet undefined function. In close proximity on chromosome 20 separated by about 10,000 base pairs from the AVP gene is a sister gene oxytocin (OT) (Schmale 1983, Land 1982, Land 1983, Schmitz 1991). Intercalated between OT and AVP is a region of repetitive DNA sequences called Linear Interspersed repetitive Nucleiic acid Elements. or LINE's. The function of these sequences are unclear but they appear to hybridize to a poly-A- fraction of cellular RNA that is localized in the nucleus and contains multiple potential reading frames (Schmitz 1991).



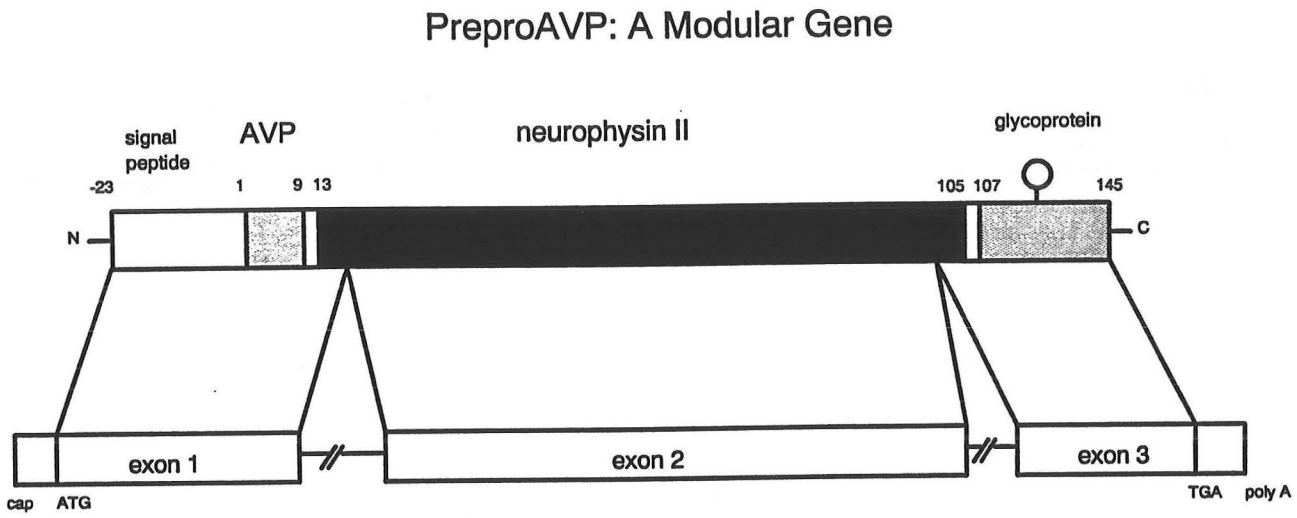
The AVP and OT genes are similar in 3 aspects.

1. OT and AVP peptides are identical to each other in seven out of nine amino acids.
2. In its prepro form, the OT gene has a similar genomic organization as AVP. The carrier protein for OT is neurophysin I (NPI). .
3. In addition to the homology between OT and AVP, there are long regions (197 bps to be exact) of NPI and NPII that are identical not only in amino acids but in nucleic acid sequence in several species.

It has been suggested that these genes are the result of recent gene conversion (Rupert 1984, Baltimore 1981). Although a lot of duplicated genes are on the same strand of the DNA (Miller 1983), OT and AVP are actually read off complementary DNA strands (Schmitz 1991). OT and AVP are both synthesized and secreted by the magnocellular neurons of the paraventricular and supraoptic nucleii which are neurosecretory cells in the hypothalamus. It turns out that a given magnocellular neuron secrete either AVP or OT but not both (Mohr 1988, Mohr 1990). Expression of OT is maximally suppressed in AVP-secreting neurons and vice versa. Theses two peptide hormones serve very different purposes and are regulated by different stimuli (Richter 1988, Mohr 1990). It makes intuitive sense that OT and AVP are transcribed off opposite strands so they can have a 5'-flanking promoter region away from each other and away from the LINE's to secure cell-specific expression and regulation.

The processing of AVP-NPII

The AVP-NPII gene is a modular gene. The structure of the polyprotein preprohormone is shown here.



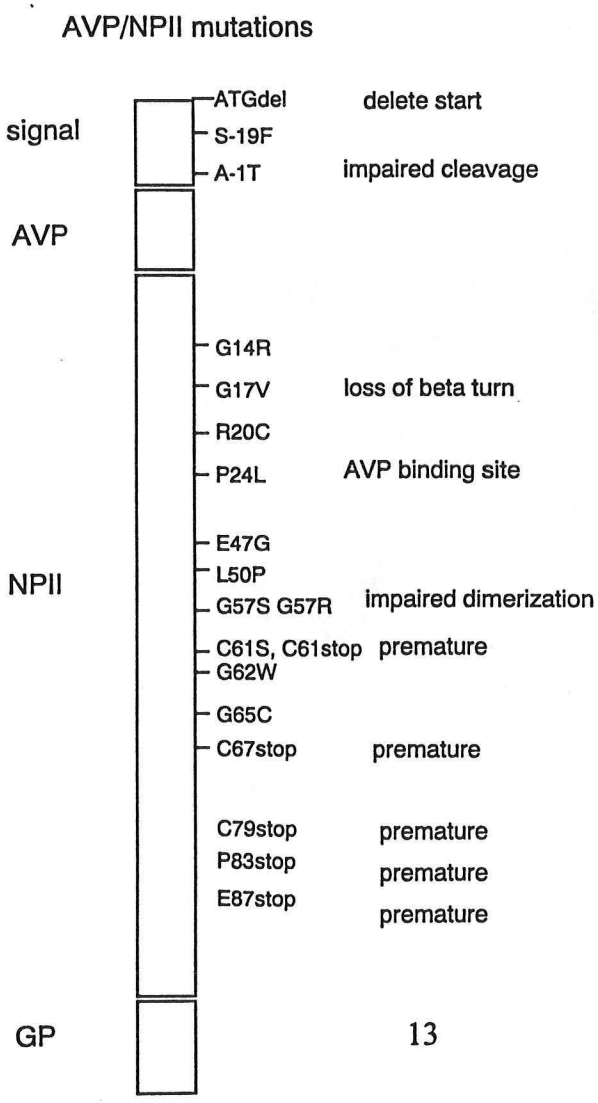
The mature transcript harbors four polypeptides in a single reading frame. These are the signal peptide, AVP itself, the carrier protein NPII, and a glycoprotein of unknown function (Schmale 1983). The three parts of the preprohormone is represented by 3 exons each dedicated to approximately one of the proteins. AVP is synthesized in the magnocellular neurons in the paraventricular and supraoptic nucleii under the control of plasma tonicity (Richter 1988). These neuroendocrine cells send their axons down to the posterior pituitary where AVP is released into the circulation.

The processing of AVP/NPII has been studied in detailed (Gainer 1985, Cohen 1987, Richter 1988) After synthesis as a pre-pro-hormone, AVP/NPII is translocated to the ER, targeted to the Golgi where the signal peptide is cleaved off. Within the Golgi, the prohormone is cleaved into AVP and NPII. Both proteins undergoes extensive disulphide bridge formation and secondary folding. From the Golgi, secretory vesicles are packaged and transported down the axon to the posterior pituitary. Inside these vesicles, AVP and NPII is cleaved and are reassembled into a hormone:carrier complex. The crystal structure of these complexes revealed that NPII forms dimers. Dimer-dimer contacts then allow formation of NPII tetramers. The biochemical studies suggest that each NPII tetramer is associated with 4 AVP molecules however crystallographic studies suggest 5 AVP analogues are bound to each NPII tetramer (Chen 1991). Four of each are at the major hormone binding site while one straddles one of the monomer-monomer interfaces. NPII oligomerization allosterically increases the binding affinity of AVP to NPII. In turn, AVP binding to NPII also allosterically stabilized oligomerization. The secretory vesicles are transported down the axon to the posterior pituitary, stored in neurosecretory granules. Changes in ambient tonicity as small as 1% causes changes in cell volume of the neurosecretory neuron and activates mechanosensitive cation channels which transduce the mechanical signal to an electrical one (Oliet 1993, Bourque 1994).

Discharge of vesicles is triggered by cell depolarization (Bourque 1994). AVP is released into the circulation dissociated from NPII and the glycoprotein. Although secreted, no specific effects of NPII or the glycoprotein in the periphery has been described.

Mutations of the AVP gene: more aptly called a disease of neurophysin II

This is a catalogue of the mutations as of a recent search (Ito 1991, Pan 1992, van den Ouweland 1992, Bahnsen 1992, Lolaite 1992, Ito 1993, Yuasa 1993, McLeod 1993, Krishnamani 1993, Repaske 1994, Nagasaki 1995). The sequelae of some but not all of these mutations have been worked out. A mutation abolishing the removal of the signal peptide prevents conversion from prepro- to prohormone and trapping of the preprohormone in the ER. *Note that not a single mutation has been described in the AVP sequence itself.* The majority of the mutations reside in the carrier protein NPII. Congenital central diabetes insipidus is really a genetic disease of NPII and not AVP. These mutation perturb NPII structure/function by either gross deletions as premature stop condons, by disruption of the secondary folding, or by impairment of AVP binding.

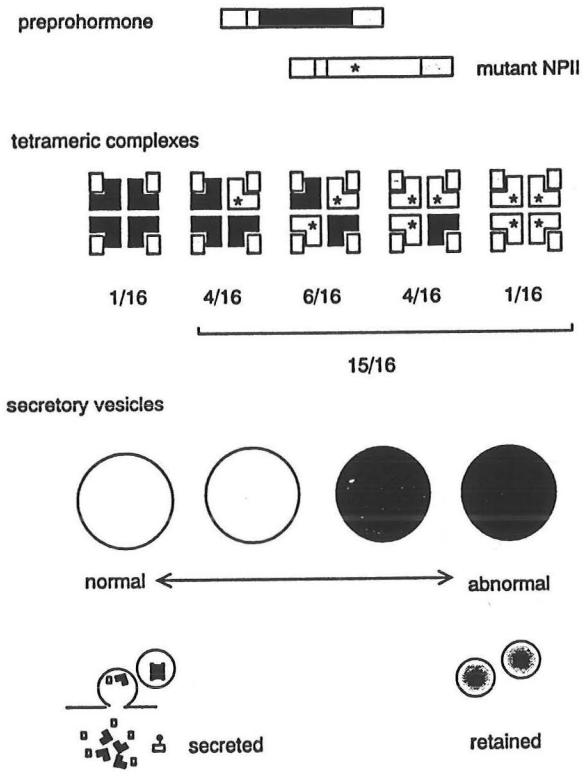


Genetic basis for the clinical manifestations

A heterozygous mutant alleles of the NPII sequence actually explains most of the clinical findings. Recall the cardinal clinical features of the disease are:

1. Autosomal dominance
2. Late onset of disease
3. Incomplete diabetes insipidus but progressive to complete more severe disease.

In general, there are two reasons why an inherited trait is autosomal dominant. One is bi-allelic dependence. The second is gain-of-function or dominant negative function of the mutant protein. For the AVP/NPII gene, one can envision the following mechanisms at play. First is that since the hormone:carrier complex exist as tetramers, one mutant NPII subunit may ruin the whole complex and impair processing down the axon. A dominant negative effect. However, since there is sill one normal allele, there is a $(1/2)^4$= 1/16 chance of assembling a completely normal tetramer. In addition, tetramers with only one abnormal NPII may function close enough to normal that it is actually secreted. This explains why early in the disease, the phenotype is that of partial central diabetes insipidus. However, since 15/16 complexes are abnormal, the chronically retained complexes may be toxic to the magnocellular neurons. So a few trapped complexes accumulated over time is sufficient to lead to neuronal degeneration. This explains the progressive nature of the disease and the autosomal dominance pattern.



X-LINKED RECESSIVE NEPHROGENIC DIABETES INSIPIDUS

Clinical and physiologic findings

The second commonest form is the X-linked variety. Recall that in certain communities in Eastern Canada, this is not considered a rare disease (Bode 1969). There is a large body of data on the clinical features and pathophysiology of this disease (reviewed in Reeves 1989, Howard 1992, Bichet 1994). A brief summary is presented.

1. Onset in infancy.
2. Resistance to AVP.
3. Locus mapped to Xq28

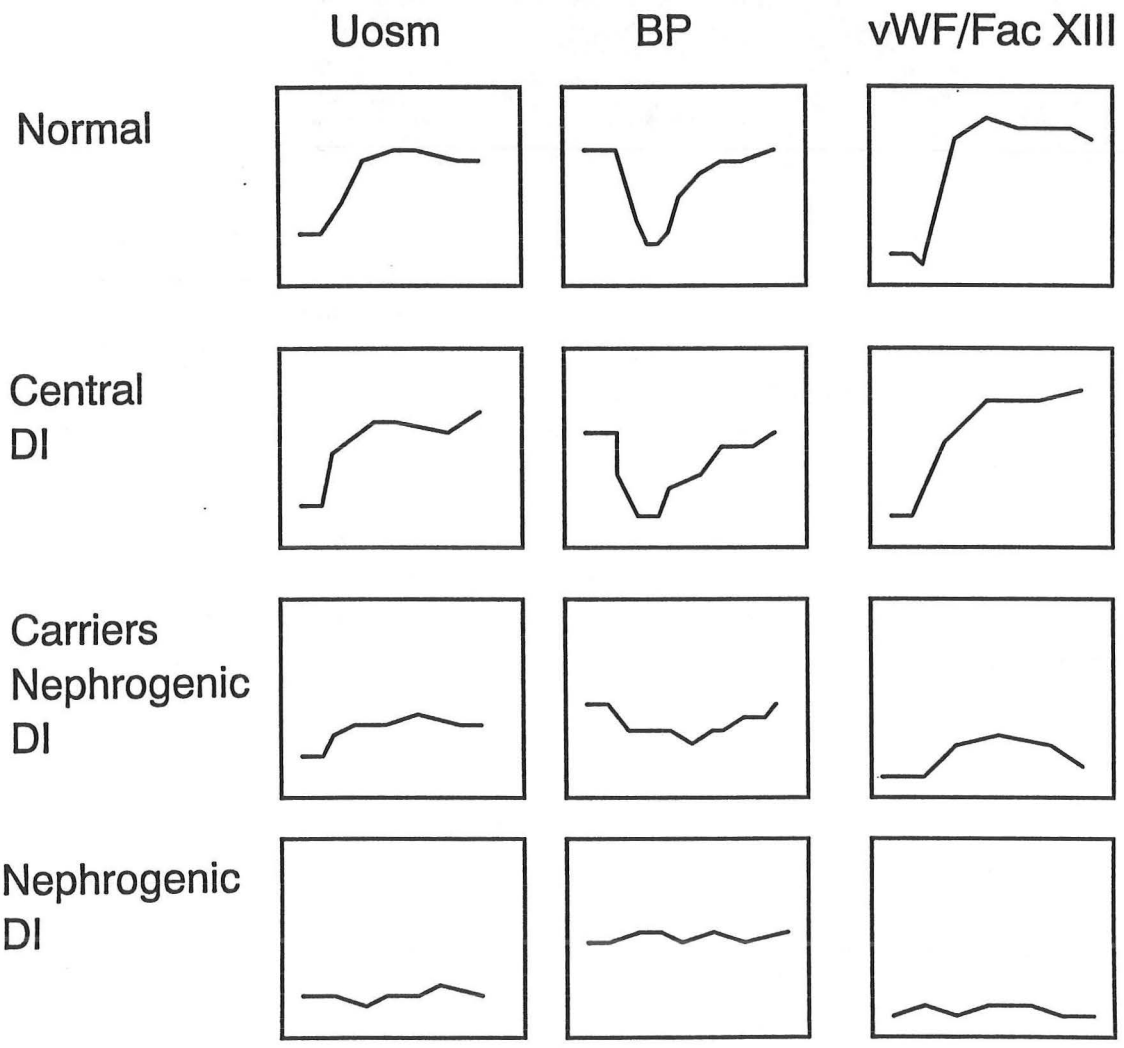
Unlike the autosomal dominant variety, this is truely a neonatal disease. The infants are irritable, eager to feed but will vomit milk unless adequate water is given. Constipation and fever of unknown origin is common. Neonatal mortality is extremely high unless ample water is given. Failure to thrive is common. Because of frequent repeated bouts of hypertonicity causing cerebral dehydration, mental retardation is extremely common if the child survives infancy (Reeves 1989). At one point in the 1950's, one out of ten males in insitutions for the mentally retarded in Nova Scotia were believed to be suffering from this disease.

Before the vasopressin receptors were even cloned, the implication of the V2R as a candidate gene for this disease was extremely strong based entirely on clincal physiology (Bichet 1988, Bichet 1989, Bichet 1991, Bichet 1991). AVP receptors were classified according to their pharmacologic characteristics before the cDNA's were obtained (Jard 1988).

	Signalling coupling:	Predominant tissue:	Functional role:
V1a	PLC/Ca^{2+}	Vascular	Vasoconstriction
V1b	PLC/Ca^{2+}	Pituitary	Regulation of ACTH release
V2	Aden Cyclase	Renal	Regulation of water permeability of collecting duct
		Extrarenal	Vasorelaxation
			Release of endothelial procoagulants

One seminal paper by Bichet and coworkers in Montreal illustrates the power of clinical physiology (Bichet 1988). They examined the renal and extrarenal effects of V2R agonism in 4 groups of patients: Normal controls, patients with central diabetes insipidus, obligatory carriers, i.e. mothers of affect males, and patients with X-linked nephrogenic diabetes insipidus. The AVP analogue DDAVP which has a 4000-fold higher affinity for the V2R compared to V1R was used.

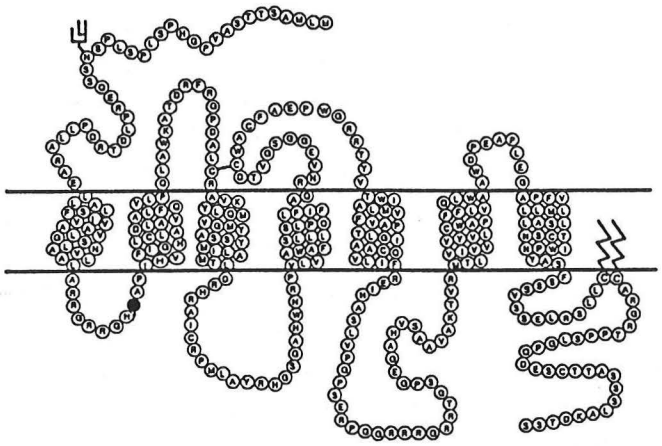
The renal effect of increasing urinary and antidiuresis (urinary concentration) was normal in controls and patients with central diabetes insipidus, completely absent in nephrogenic diabetes insipidus. It is interesting that an intermediate response was noted in obligatory carriers; an effect compatible with X chromosome inactivation. V2R stimulation is known to cause slight hypotension and is also a potent stimulator for Factor and VIII and vWF release via V2R in the endothelium. Both of these extra-renal effects showed absence of response in affected males and an intermediate response in the carriers, an identical pattern to the renal effects of V2R agonism. These results are graphically depicted below.



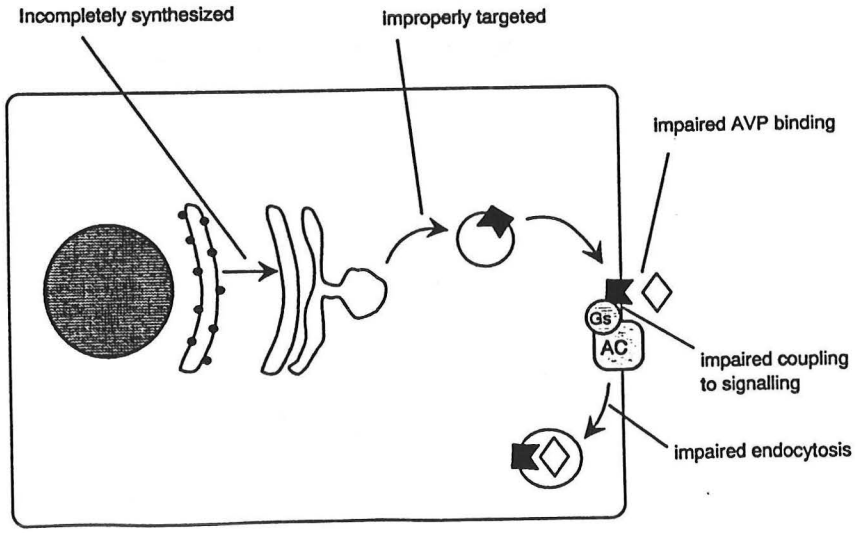
The clinical evidence was overwhelmingly strong in supporting the V2R as the candidate gene for X-linked nephrogenic diabets insipidus. When the V2R gene was cloned and mapped to Xq28 (Birnmaumer 1992, Siebold 1992, van Den Ouweland 1992), it was not surprising that V2R mutations was uniformly found in all patients tested and cosegregated with the nephrogenic diabetes insipidus phenotype (Bichet 1996).

V2R structure and mutations

The V2R is a typical G-protein coupled receptor of 371 amino acids with 7 transmembrane domains. To date, 87 mutations has been described in 116 families (partially reviewed in Bichet 1996). These mutations involve missense, nonsense, frameshift, small (<20 bps),and large deletions.



These families were gathered from extremely diverse ethnic groups originated from Northern European, Mediterranean, Middle Eastern, South Pacific, Asian, and African ancestry. This unequivocally disproved the Hopewell hypothesis (Bode 1969) of a single founder bringing this disease to North American on board the ship Hopewell. The famous Hopewell mutation is in amino acid 71 where a tryptophan is mutated to a stop codon resulting in a truncation of the protein. W71X is still one of the more common mutation in North America. The study of the function of these mutant receptors turned out to be extremely educational in understanding the biology of V2R itself. Multiple functional defects of the V2R has been described:



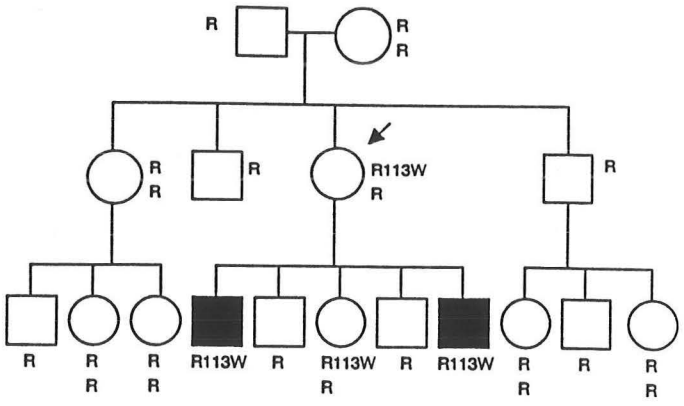
V2R mutations appear to be in genetic equilibrium

In selected communities in Nova Scotia, the incidence of the disease can be as high as 24 in 1,000 males. Most of the patients with the original Hopewell mutation resided within 2 villages in Colchester county in Nova Scotia. It is difficult to asses the stability of the allele frequency with any accuracy in such small communities since minor migration changes will have significant impact on the results. However, in the province of Quebec with a much larger population and relatively slow migration patterns, the prevalence of the disease has been very constant at 4 in 1,000,000 males for decades (Bichet 1992).

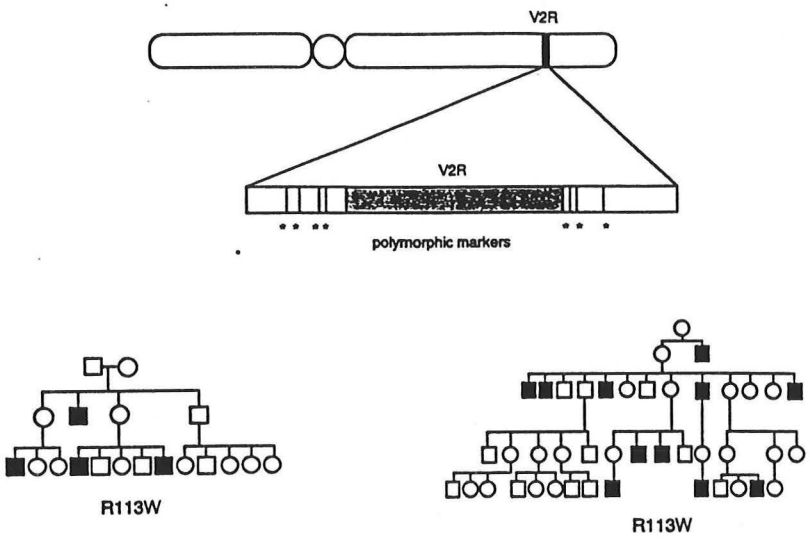
Up until recently, this disease was often lethal for affected males. Even males who survived into reproductive age had a very high incidence of mental retardation (Bode 1969). A great majority of these individuals were confined to institutions and were thus deprived of opportunities to bear offsprings. For these mutations to evade gradual unavoidable attrition, one of two things have to occur. First is a remarkably strong survival and/or reproductive advantage for the female heterozygous carrier. There is no reason to believe that to be true. In fact, the heterozygous females exhibit variable degrees of impaired urinary concentration due to skewed X chromosome inactivation. An alternative explanation appears more likely. The loss of mutant alleles are constantly balanced by a high spontaneous mutation rate thereby maintaining genetic equilibrium. In fact, if one assumes no males reproduce and mutation rates are the same in males and females, one-third of new cases should be due to new mutations. If this true, the recent advances in prenatal and perinatal diagnoses along with aggressive water replacement may unmask the high mutation rate and one should witness an escalating prevalence of X-linked nephrogenic diabetes insipidus. This data is not currently available.

De novo mutations have been shown in two ways. First way is to actually document within a given kindred that the mutation was absent in the grandmother's but present in the mother's somatic cells implying a germ line mutation has occurred in the grandmother. This type of documentation is not commonly available.

De novo mutation in V2R within a pedigree



A more indirect approach is often used. It is not uncommon to encounter the exact identical mutation in seemingly unrelated families. These likely represent independent de novo mutations in "hot spots" if the families are indeed unrelated. To rule out the possibility of distant unrecognized relations, closely linked highly polymorphic markers flanking the V2R gene were examined. If the common mutation in the families were genuinely identical by descent from a common ancestor, then they most likely will share identical alleles in the flanking polymorphic markers.



Using this method, Bichet and coworkers have demonstrated a number of de novo mutations in 18 families to date (Bichet 1994, Bichet 1996). What is the reason for the high susceptibility to spontaneous mutations?

Molecular basis for high mutation rate in V2R

The predisposing factors lie within the nucleiic acid sequence of the V2R gene. There were a total of 9 mutations described at CpG dinucleotides in the V2R gene. Of these, 7 have been documented to be de novo mutations (Bichet 1994, Bichet 1996). These 7 point mutations are shown below.

Amino acid change	Nucleotide change	
V88M	CGTG ⇨	CATG
R113W	CGG ⇨	TGG
R137H	CGC ⇨	CAC
S167L	TCG ⇨	TTG
R181C	CGT ⇨	TGT
R202C	CGT ⇨	TGT

Note that all of these point mutations are either G-to-A or C-to-T changes in the context of CpG dinucleotides. The V2R gene contains 58 CpG's (~5% of dinucleotides) compared to a predicted number of 12-14 CpG's in 1113 bp's of coding sequence. CpG's have long been recognized to be mutational hotspots since an excessive frequnecy of C-to-T mutations were

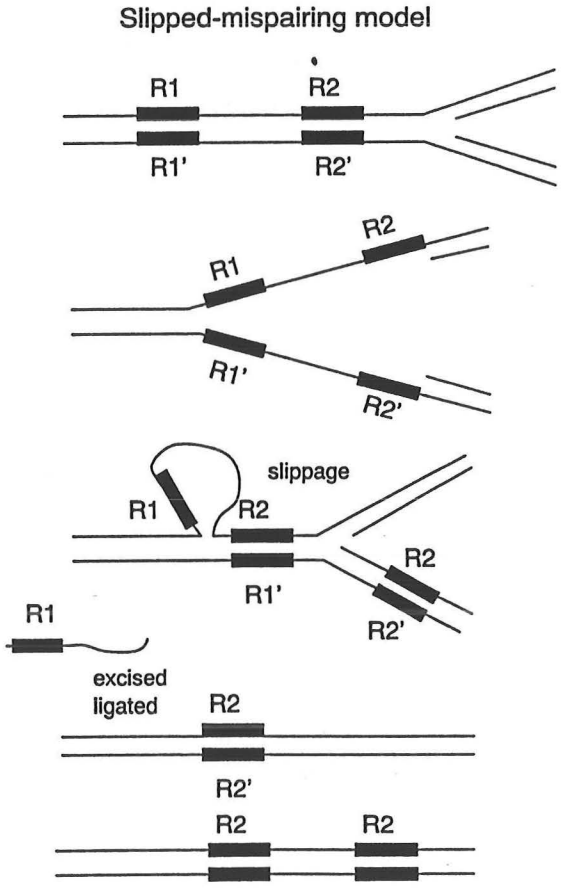
first reported in hemoglobin variants in humans (Vogel 1965, Vogel 1977). The cytosine in CpG in the vertebrate genome often exist as 5-methylcytosine(5mC) which can be deaminated to a thymidine; hence the C-to-T transition. The G-to-A transition occcurs from a 5mC-to-T transition on the antisense strand followed by a miscorrection of G to A on the sense strand. As pointed out by Cooper and Krawczak (Cooper 1990), CG to TG or CA subsitutions are 18 times more frequent than any other mutations at CpG dinucleotides.

Another group of mutations of the V2R that are known hot spots are the small deletions. Of the 18 small deletions, 13 involved direct or complementary repeats (2-9 bps) or strings of 4-6 nucleotides that are either direct repeats or palindromic repeats (Bichet 1994). These sequences carry their own inherent mechanism for mutability (Krawczak 1991, Sinden 1992). Of the 13 small deletions based on this mechanism, 2 are shown here to illustrate the mutation mechanism. First one involves 2 direct repeats.

5'....GCCTT**CCTGGG**GCTGGT**CCTGGG**AGCCA...
---

12 bp's deleted
including one repeat

Two replication-based models can be used to explain these deletions (Cooper 1990, Krawczak 1991). First is the *slipped mispairing* model. During replication of a DNA strand containing R1 and R2 direct repeats, the duplex becomes single-stranded at the replication fork. If R2 "slips" and pairs with R1' instead of R2', a single stranded loop containing R1 is transiently created. The error can be corrected if R2 dissociate with R1' and pairs with R2'. However, the loop may exist long enough for DNA repair enzymes to excise and ligate the loop. If this comes to pass, one replicated daughter strand will be missing

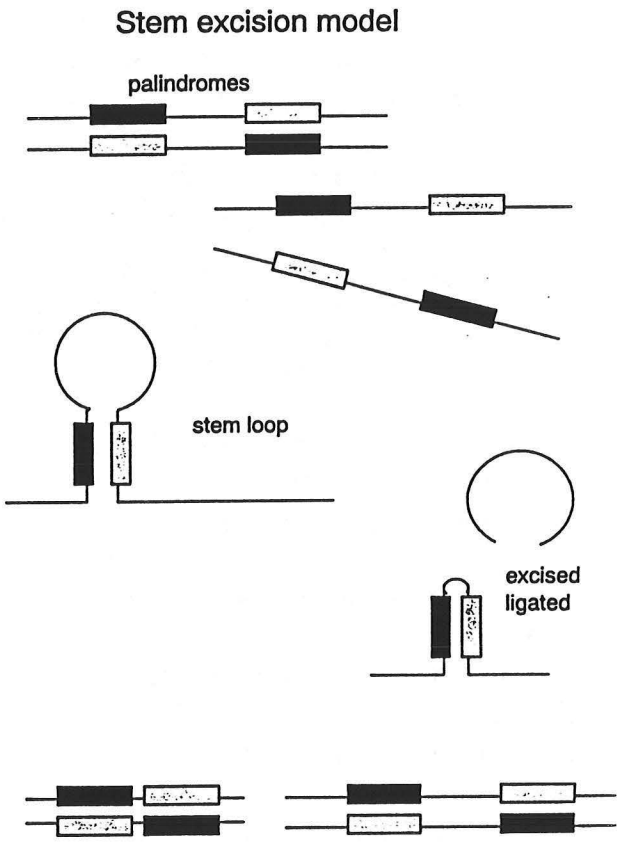


The second model explains palindromic repeats such as this sequence.

5'....GC**GGCCC**TAGCTCGGCG**GGGCC**GGCGGGG...

10 bp's deleted
between two repeats

In this second model, when DNA strands separate during replication. One strand pairs with itself on account of the inverted repeats or palindromes and create a stem loop structure. The single straned loop is then excised and ligated. When the stem releases itself to resume DNA replication, one daughter strand will have a deletion of the sequence between the two inverted repeats.



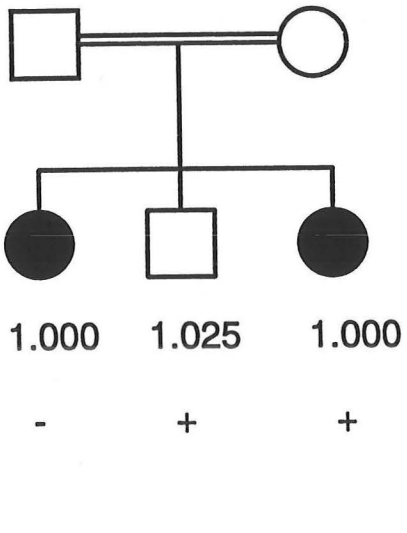
AUTOSOMAL RECESSIVE NEPHROGENIC DIABETES INSIPIDUS

Identification of another form of nephrogenic diabetes insipidus

The third genetic form of the disease, which is the rarest form documented to date, is the autosomal recessive variety. In certain families suffering from nephrogenic D.I. Two discrepancies were repeatedly observed. First, some families did not exhibit classical X-linked inheritance. Second, some patients have near normal or completely normal urinary cAMP and/or plasma vWF in resposne to exogenous AVP (Zimmerman 1975, Monn 1976, Ohzeki 1984, Brenner 1988, Moses 1988, Knoers 1991). This suggested that the V2R is normal in these patients yet, AVP failed to ellicit antidiuresis in any of these patients. Lacking a pathophysiologic explanation, these patients were labeled to have "type II" nephrogenic diabetes insipidus.

1. Autosomal recessive
2. Normal extrarenal response to exogenous AVP
3. Renal response to AVP:
Urinary cAMP- normal
Urinary concentration- none

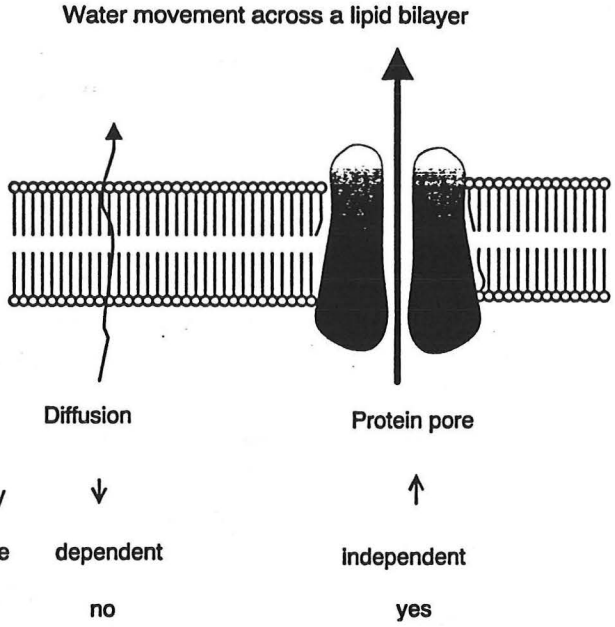
The first definitive genetic evidence of a disease distinct from the V2R mutations was a case that came from Sick Children's Hospital in Toronto (Langley 1991). This report actually predated the identification of the V2R genes as the cause for X-linked nephrogenic diabetes insipidus. Two girls born of a cosanguinous marriage had clinical diabetes insipidus but not their brother. Simple genotyping using a marker (DXS52) that was known to be tightly linked to the Xq28 nephrogenic diabetes insipidus locus provided definitive proof. If these sisters had the X-linked form of disease, then they should both inherit the same DXS52 allele since DXS52 cosegregates very tightly with the disease. This was not true. The sisters did not share a particular RFLP fragment of DXS52. The boy who has the same marker allele as one of the affected sisters did not inherit the diease. This dissociation between phenotype and genotype unequivocally proved that the disease locus was not on Xq28. In conjunction with the physiologtic data, particularly the normal urinary cAMP response to DDAVP, a lesion downstream from the V2R seemed inevitable. We now know that this is an autosomal recessive genetic disease of one of the water channels or the aquaporins (van Lieburg 1994, Deen 1994, Oksche 1996).



Water channels in mammalian cells

Cells are composed primarily of water and are bathed in water. Movement of water in and out of cells is fundamental to all cell function. The search for water channel dates back to the early 1950's (Paganelli 1957, Sidel 1957, Solomon 1983, Solomon 1989) when pure biophysical approaches were used. These studies and the models proposed were incredibly accurate even by today's standards in predicting the existence of water channels. However, the actual biological water channel molecules underlying these descriptions remained elusive for decades. Since the purification and cloning of the first water channel, the understanding of water movement through biologic membranes have taken an exponential expansion.

Since the 1950's, biologist have recognized that water can move through lipid bilayers in one of two ways.



Water molecules can indeed enter into the lipid bilayer although the partition coefficient is about 10^{-6}. Given the molar concentration of water being >50 M in the physiologic aqueous phase, there is a small but finite amount of water in the micromolar range within the lipid membrane. This small amount of water is adequate for diffusion across the bilayer. The permeability based on this mode of water transport (Pd) is small in magnitude. This permeability is highly temperature dependent with a relatively high Arrhenius activation energy (>10 kcal/mol). As the lipid fluidity decreases with temperature, water movement becomes progressively more restricted. Third, it is independent of covalent pharmacologic modifications as no proteins are involved.

A second mode of transport as predicted by decades of biophysical measurement is through a protein pore. The notion of a water pore and the supportive experimental data of aqueous pores in RBC membranes emerged mostly from the seminal biophysical studies from Solomon and coworkers (Paganelli 1957, Sidel 1957). This water movement is strongly driven by osmotic

gradients and the permeability (Pf) is several times or an order of magnituide larger than that of diffusional water movement (Pd). In fact, the flux of water is so fast that net fluid absorption appeared completely isotonic such as in the RBC and the proximal tubule. In addition, these putative pores are minimally affected by sub-physiologic temperatures with relatively low Arrhenius activation energies (<5 kcal/mol). They are in general sensitive to inhibition by mercurial sufhydryl compounds. Therefore for decades, epithelial biologists would measure the Pf/Pd ratio in a membrane, the Arrhenius acitvation energy for water movement, and sensitivity to mercurial derivatives and be equipped to concluded whether there is an aqeous pore in a membrane.

The kidney is an ideal organ for studying water transport as all aspects of water transport are present.

Water permeability	*Segment*
Exceedingly high	Proximal tubule and the thin descending limb
Extremely low	Apical membrane of the thick ascending limb
Hormone-dependent	Collecting duct, water permeability can switch from diffusional to osmotic.

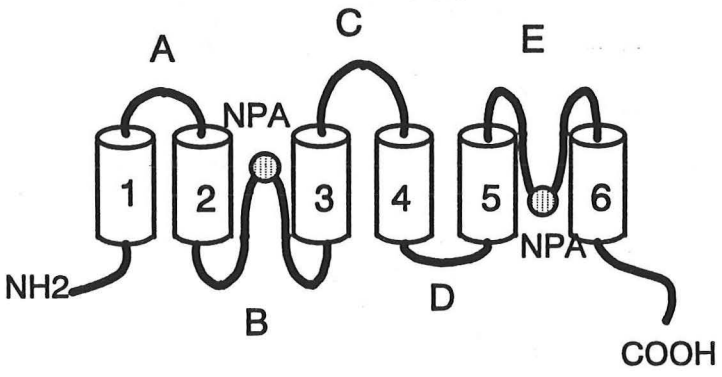
Cloning of the first water channel... by happenstance

By the late 1980's, there was little doubt that water channels exist despite an elusive unfruitful search for three decades. Several other membrane transporters such as the band-3 Cl/HCO3 exchanger (Solomon 1983), the urea transporter (Macey 1984), the facilitative glucose carrier GLUT-1 (Fishbarg 1989, 1990), the vacuolar H pump (Harvey 1991), and the cystic fibrosis transmembrane reglator CFTR (Hasegawa 1992) were all touted as contenders but were all eventually proven to be incorrect. *Xenopus* oocyte expression cloning (Zhang 1990, Tsai 1991) and direct purification out of vasopressin-treated toad bladder epithelium (Harris 1992) failed to yield the desired cDNA. Out of the blue came a classic series of paper by Agre and coworkers who serendipitously encountered the ubiquitous water channel during a search for something completely different (Agre 1987, Saboori 1988, Denker 1988, Smith 1991, Preston 1991). A concise summary of this quest is presented. While purifying the Rhesus (Rh) 32 kD protein from human red cells, they repeatedly encountered what they thought was a 28 kD proteolytic product of the 32 kD Rh polypeptide. Isolation was hampered by a stubborn insolubility of this protein in Triton X-100 suggestive of cytoskeletal association and a peculiar lack of affinity for Coomassie blue staining. Despite these set-backs, their curiosity and motivation soon intensified mainly because this protein has about 150,000 copies per RBC comprising 2.4% of its membrane protein mass. The purification was greatly facilitated by its abundance and a very unusual solubility of this protein in sarcosyl that permitted a one step purification of the protein which can then be easily identified with silver staining rather than Coomassie blue. Cloning using conventional λ-phage expression library screening was unsuccessful as the antisera did not recognize the phage-expressed epitopes. Finally with amino acid microsequence-derived degenerate oligonucleotides, they obtained cDNA's with PCR; from which they use non-degenerate oligo's to screen and clone the full length cDNA. The current nomemclature for this family of proteins is aquaporin (AQP).

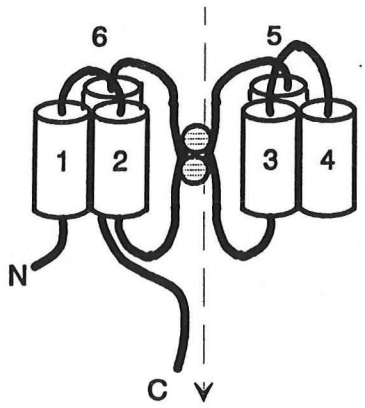
Structure of aquaporins

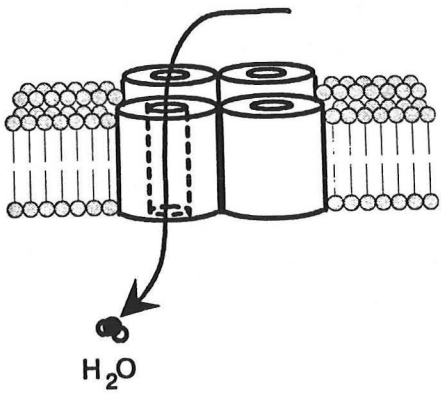
Hydrophobicity analysis predicts six transmembrane segments seperated by five connecting loops. This topology of all AQP's and all known plant and bacterial homologues are very similar (Agre 1993, Chrispeels 1994, Agre 1995). The amino acids are 20-40% identical when the N-termini are aligned. The structure of AQP's have been intensively investigated. A few topologic landmarks will be highlighted here. Extensive reviews are available (Agre 1993, van Os 1994, King 1995, Nielsen 1995, Verkman 1995, Agre 1995, Agre 1996)

- An internal homology between the N-and C-terminal halves of the protein.
- Glycosylation sites are present in one of the extracellular loops.
- The putative mercurial inhibition site (Cys189) in loop E.
- The Colton blood group antigen in loop A.
- A highly conserved Asn-Pro-Ala motif in both loops B and E.



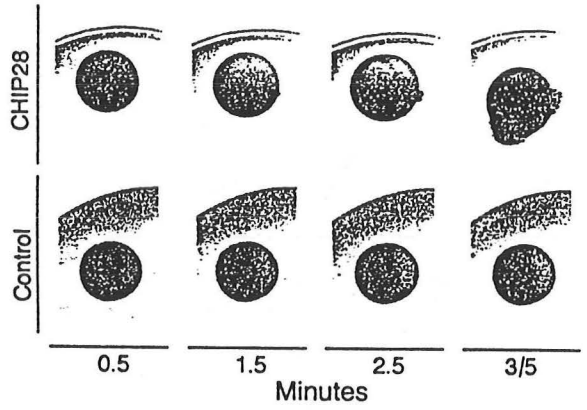
The current consensus appears to be an hourglass model for the protein where loops B and E are imbedded in the membrane from opposite ends of the protein in close proximity creating a critical narrowing through the lipid membrane (Jung 1994). This model places the mercurial covalent site at the critical entry point of the pore. It has also been shown that 4 monomers assemble as homotetramers which results in a characteristic uniform set of intramembrane particles. It appears that each monomeric subunit carries its own functional water channel quite independently of the others.

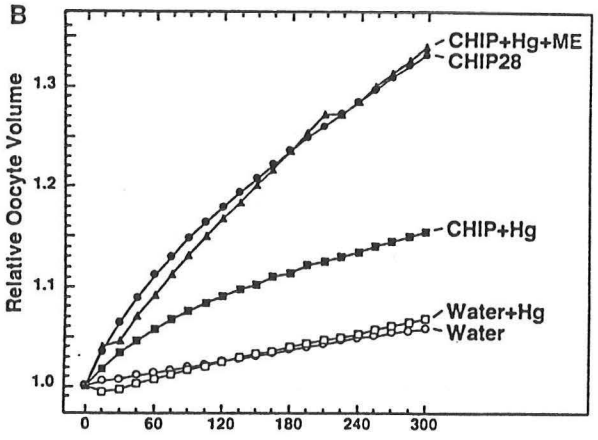




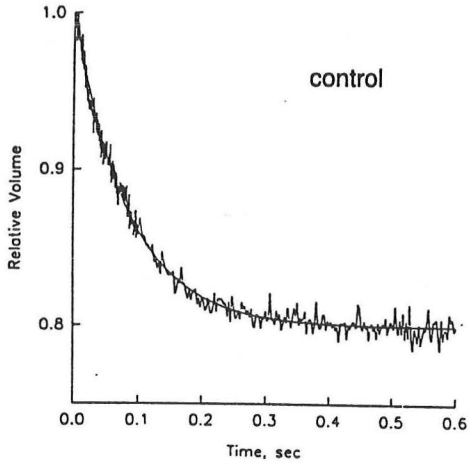
Function of AQP-1 as a water channel

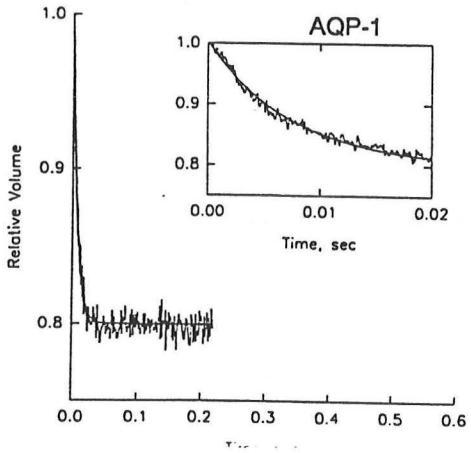
AQP as a water channel was shown in two systems. Expression in Xenopus oocytes (Preston 1992) and in reconstituted proteoliposomes (Zeidel 1992). In both of these systems, AQP expression dramatically increased water permeability.





❑ Oocytes expressing AQP1 swell until they burst when challenged with a hypotonic stimulus.





❑ Proteoliposomes containing AQP1 shrinks a lot faster when presented with a hypertonic stress.

In addition, when osmotic permeabilities (Pf), Arrhenius activation energies (Ea), and sensitivity to mercurial compounds were examined, they were comparable to native highly water permeable membranes. These studies prove that AQP1 is indeed the water channel that has eluded identification for 3 decades.

Gene family of AQP

Since the cloning of the first cDNA, additional cDNA's have now been cloned. A summary of the isoforms to date is tabulated.

Isoform	*Synonyms*	*Main distribution*	*Features*
AQP0	MIP26	Lens fibre cells	Lower water permeability Murine mutation-congenital cataract
AQP1	CHIP28 DER2	Ubiquitous Erythrocyte **Prox tubule** **Thin des limb** **Apical and basolateral** **Vasa recta endothelium**	Constitutively active high permeability Epitope for Colton Ag Human mutation- subclinical (?)
AQP2	WCH-CD AQP-CD WCH2	**Collecting duct** **apical membrane** **subapical vesicles** **principal cells**	AVP-regulated water transport Human mutation- nephrogenic D.I.
AQP3	GLIP BLIP	**Collecting duct** **lateral memb** **principal cells**	Constitutive basolateral exit step No mutations identified
AQP4	MIWC	Eppendymal cells Paraventricular Supraoptic nucleii **Collecting duct** **basolateral memb** **principal cells**	Spinal fluid resorption Osmoreception Constitutive basolateral exit step No mutations identified
AQP5		Salivary & lacrimal glands Cornea Lung	Tears, saliva, sputum

A tremendous amount of data exist for all of these isoforms (reviewed va Os 1994, Agre 1995, Agre 1996). Only a few salient features will be highlighted here.

❑ The current accepted nomenclature enumerates the AQP's according to the order of their identification. AQP0 is named as such because it was recognized only in retrospect to be a water channel when it was cloned. The synonyms represent a haphazard nomenclature that is typical in the early stages of uncovering a gene family. Their use should be discouraged.

❑ AQP0 is major protein that controls fluid balance in the lens. A murine mutation causes congenital cataracts (Muggleton-Harris 1987, Shiels 1993). No human mutations are identified but human AQP0 may play a role in the pathogenesis of senile cataracts in humans.

❑ AQP1 is quite ubiquitous. All the high flux isotonic water movement in the proximal nephron is mediated by AQP1 on both the apical and basolateral membranes (Agre 1995, Agre 1996). Because of its ubiquitous distribution and its abundance in erythrocytes and proximal tubule, one would expect its deletion to be lethal. Worldwide blood group referencing has led to the identification of 5 kindreds whose red cells do not express the Colton antigen (Preston 1994). Analysis of 3 of these individual showed complete or near-complete absence of antigenic and functional AQP1 from homozygous mutations of AQP1. Quite surprisingly, the patient not have any hematologic or renal problems (Preston 1994, Mathai 1995). These five kindreds were found after searching tens of millions of donors and recipients so they are indeed very rare. One possible explanation may be that AQP1 mutations are indeed lethal and these kindreds represent a few rare instances where extraordinary compensatory redundancy exist within the system.

❑ As we will elaborate further, AQP2 is the one responsible for regulated water transport in the collecting duct (Agre 1996, Nielsen 1996) and AQP2 mutations are responsible for autosomal recessive diabetes insipidus.

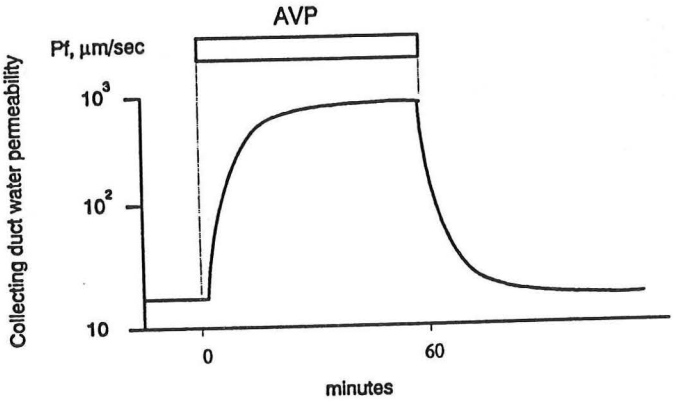
❑ AQP3 is present mostly in the lateral membranes of the collecting duct (OMCDi & outer portion IMCD) that provides the constitutive exit step for water during urinary concentration. It is sensitive to mercurials even though the conserved cysteine is missing. It appears to also transport glycerol and urea to some extent (Echevarria 1994, Ishibashi 1994, Ma 1994). AQP3 may not be entirely constitutive as its expression can be induced by long term administration of AVP (Terris 1996).

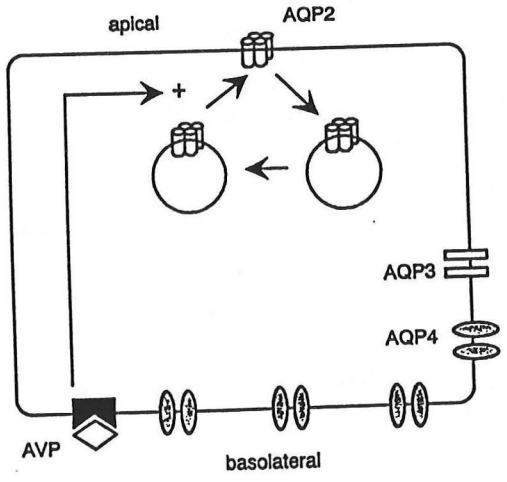
❑ AQP4 is only isoform thus far this is insensitive to mercurials. Its mercurial insensitivity is an enigma because introduction of cys189 does not confer mercurial sensitivity. In the kidney, it is diffusely present in the basolateral membrane throughout the collecting duct and likely mediates the constitutive basolateral exit step in urinary concentration. AQP4 is also abundant in the brain. It has been localized to the magnocellular neurons in the supraoptic and paraventricular nuclei and likely represents the channel that allows magnocellular neurons to response to changes in ambient tonicity (Bourque 1994, Oliet 1993, Jung 1994).

❑ AQP5 is the most recent isoform cloned. The "ubiquitous" AQP1 is absent in most glandular tissues suggesting other water channels are probably functional. AQP5 is mostly epithelial in distribution and likely mediates sweat, tears and lung water homeostasis.

Acute regulation of AQP2 by AVP

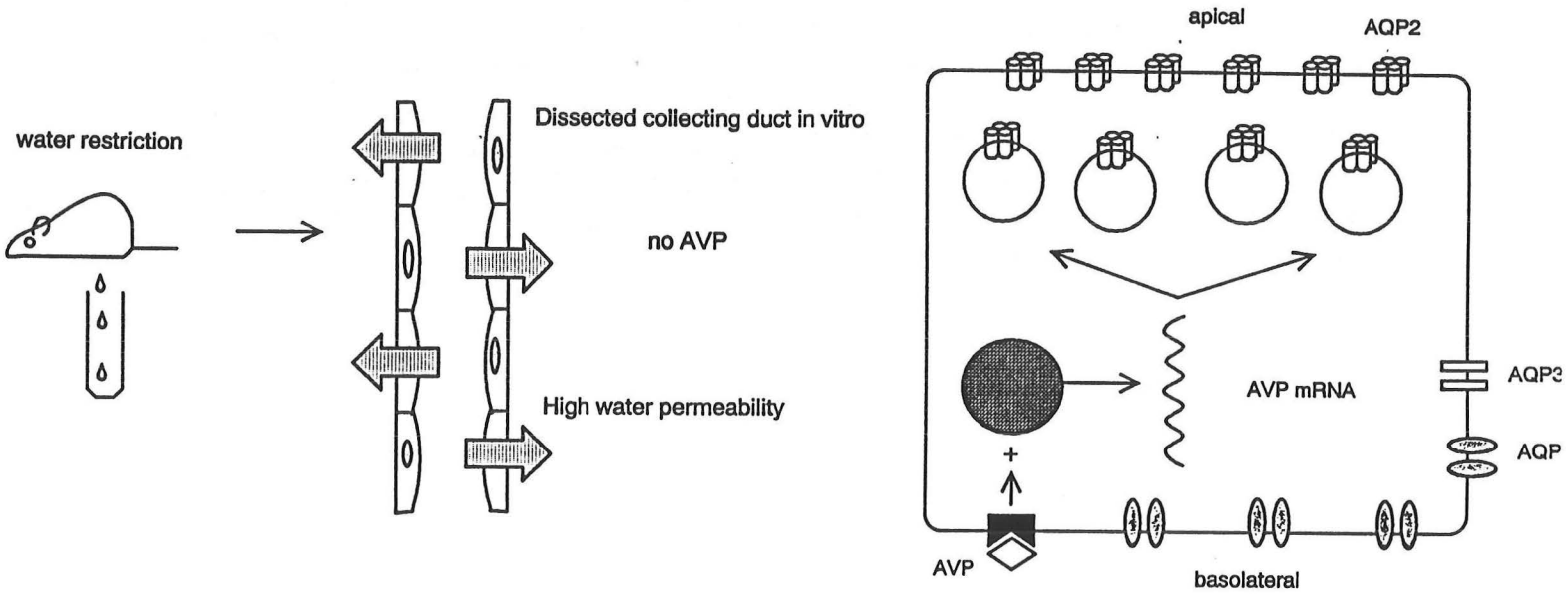
Acute regulation refers changes that occurs in a few minutes. Water permeability of the collecting duct increases within 40 seconds, with a maximal response of an 8-fold increase in about 40 mins (Flamion 1990, Wall 1992). Upon removal of AVP, the response is fully reversible in about the same time frame. These transepithelial permeability changes are due almost entirely to changes in apical membrane and not the basolateral membrane (Flamion 1990). The shuttle hypothesis that were proposed in the early 80's (Wade 1981, Brown 1983) are now confirmed by specific reagents. Subapical vesicle containing AQP-2 are rapidly fused to the surface increasing the number of water channel in the apical membrane (Nielsen 1993, Sabolic 1995, Nielsen 1995). The mechanism responsible for the insertion is currently unknown. Acute phosphorylation by protein kinase A appears to be necessary for increasing AQP2-mediated water permeability (Kuwahara 1995). It is presently not known whether phosphorylation by protein kinase A induces insertion, modulates gating of existing channels, or both. Vesicle-associated membrane protein (VAMP's) or synaptobrevins are believed to determine the specificity of vesicular traffick, docking, and fusion to membrane (Nielsen 1995, Jo 1995).





Chronic regulation of AQP2

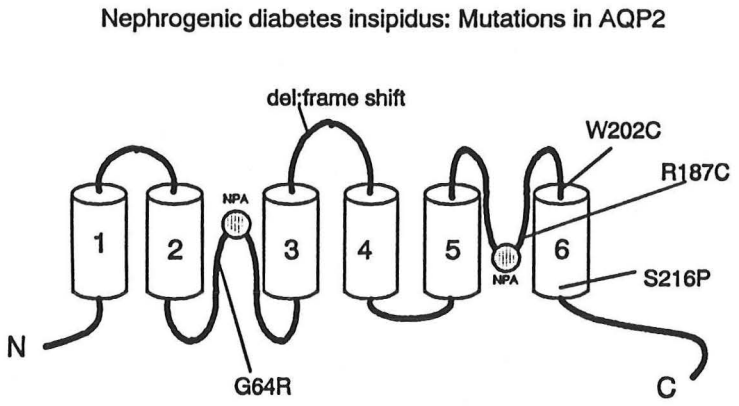
Another mode of regulation takes place over days or at least 24 hrs. It is well known that renal collecting ducts taken from thirsted animals persist to have high water permeability *in vitro* long after the removal of AQP2 (Langford 1991, Knepper 1993, Han 1994). This is due an adaptation in vivo in response to sustained high levels of AVP leading to increase abundance of intramembrane cluster (Wade 1994), increased AQP2 mRNA and protein abundance on the apical membrane and the whole cell (Nielsen 1993, Hayashi 1994, Terris 1996).



In central diabetes insipidus, the absence of AVP is not the only reason why the kidney cannot concentrate urine. When AVP levels are chronically low such as in the Brattle boro rat with congenital diabetes insipidus, AQP2 virtually disappears from the kidney (Digiovani 1994). When the animal is then repleted with exogenous AVP, it takes about one to two days before adequate AQP2 levels are established. The clinical observation is that in patients with established central diabetes frequently do not respond to DDAVP immediately or only have partial responses. Reasons such as medullary "wash-out" have frequently been proposed due to the loss of solutes in the renal medulla after prolonged water diuresis (Valtin 1966, Fanton 1987) and prolonged treatment with vasopressin re-establishes medullary tonicity and antidiuresis (Harrington 1965, Harrington 1968). Suppressed AVP also prevents interstitital urea accumulation in the inner medulla via AVP-sensitive urea transport at the terminal medullary collecting duct (Morgan 1968, Knepper 1987, Knepper 1990). A more attractive or at least a supplementary explanation for the appearently lack of response to AVP after chronincally low AVP from either central diabetes insipidus or water loading is a normal physiologic delay in AVP-induced AQP2 expression.

Autosomal recessive nephrogenic diabetes insipidus from mutations in AQP-2

A number of inactivating mutations have been identified in patients who are either homozygous or compound heterozygotes.



With the exception of the frameshift deletion, all these mutations are situated around or at the region All these mutant proteins are predicted to be either improperly processed, non-functional, or both. The mechamistic details are just being uncovered. Bichet et al have identified defective trafficking as one mechanism (Bichet, personal communication).

Urinary AQP-2 levels

Since apical membrane proteins from renal epithelial cells are detectable in normal urine, a sensitive radioimmunoassay can actually measure AQP2 levels in human urine. Kanno and coworkers studied urinary AQP2 in normals, subjects with central and nephrogenic diabetes insipidus (Kanno 1995). All were given a single dose of DDAVP. Urinary AQP2 levels rose in the first two groups but the profile was completely flat in the patients with nephrogenic diabetes insipidus. This clinical test is currently not widely available it is highly promising as an investigative tool for further human studies of water metabolism.

ACQUIRED NEPHROGENIC DIABETES INSIPIDUS

Much more common than congenital diabetes insipidus is the acquired form. That applies to both central and nephrogenic. Acquired diabetes insipidus with a hypotonic urine (polyuria with paucity of solutes in the urine) can be broadly classified into two pathophysiologic categories:

1. Loss of medullary hypertonicity
2. Lack of permeability of the collecting duct (central vs. nephrogenic)

Since the discovery of aquaporins has enhanced our knowledge of the pathophysiology of nephrogenic diabetes insipidus, the role of AQP2 in three common clinical forms of nephrogenic diabetes insipidus will be described.

Lithium-induced nephrogenic diabetes insipidus

One common acquired form of nephrogenic diabetes insipidus is the Li-induced type which has been described up to 50% of patients on lithium although not all of them have overt clinical presentations (Boton 1987, Walker 1993). The urinary concentration defect is resistant to AVP and likely multifactorial in nature including impairment of cAMP generation (Christensen 1985, Christensen 1988, Sugawara 1988, Goldberg 1988) and enhanced prostaglandin E2 effects (Sugawara 1988, Yamaki 1991). Recently, AQP2 has been shown in an animal model to be an effector in this disorder (Marples 1995). Chronic Li therapy led to a urinary concentration defect, polyuria and polydipsia that was paralleled by a dramatic reduction of collecting duct AQP2 levels. The recovery of AQP2 is slow and incomplete within after one week of cessation of Li therapy. During chronic Li therapy, a 7 days sustained pharmacologic infusion of AVP which normally induces AQP2 protein to supraphysiologic levels only partially restored the AQP2 levels and partially reversed the concentration defect while the animals were on Li. This study suggests that Li has a dramatic effect on AQP2 expression that is more than pure antagonism of tonic levels of AVP.

Nephrogenic diabetes insipidus from K depletion

K depletion has long been known to induce a urinary concentration defect. Like lithium, the urinary concentration defect is multifactorial including loss of medullary solutes (Mannitus 1960), deranged papillary blood flow (Whinery 1979), and stimulation of prostaglandin synthesis (Ferris 1978) although species variations do exist (Berl 1980). In a rat model of K deficiency (Marples 1996), AQP2 levels were significantly suppressed after 2 weeks of a low K diet. In the presence of chronically suppressed AQP2 levels, the kidney failed to mount a urinary concentrating response respond to acute water restriction. Only upon K repletion and restoration of AQP2 levels could normal urinary concentration be operative.

Post-obstructive nephrogenic diabetes insipidus

A diuretic phase after relief of urinary obstruction is a common clinical problem characterized by inability to concentrate urine (Wilson 1951, Wilson 1975). All segments of the nephron appear to be involved in the diuretic phase; proximal tubule, loop of Henle, and the collecting duct (Jaenike 1972, Yarger 1972, McDougal 1972, Wilson 1975, Sonnenberg 1976, Buerkert 1977, Buerkert 1978, Hanley 1982). The role of AQP2 in this disorder was recently shown in a rat model of transient bilateral ureteral ligation (Frøkiær 1996). One day after obstruction was induced, AQP2 levels fell to <30% of control. Upon relief of this obstruction, AQP2 levels remained extremely low for 48 hours. The diuresis in the first 24 hrs was due to solute diuresis with a significantly elevated Cosm. However, 48 hours post obstruction, the diuresis is predominantly a water diuresis; most likely attributable to the low AQP2 levels. Even after 7 days of relief of the obstruction, AQP2 levels still have not returned to baseline. Although there is no significant diuresis at this point, rats with a history of obstruction 7 days ago still cannot respond to an 18 hour water deprivation with antidiuresis likely due to the low AQP2 levels.

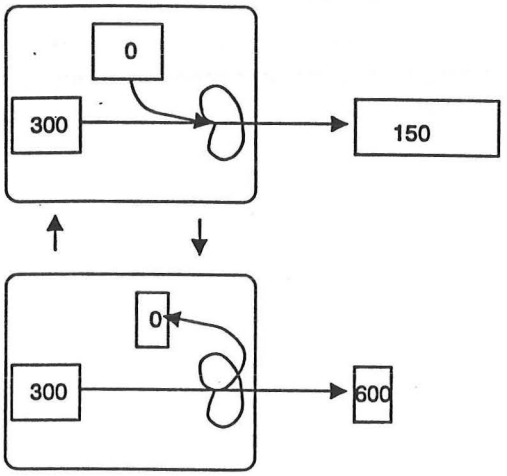
SUMMARY

1. Plasma [Na] is a clinical marker for total body water.

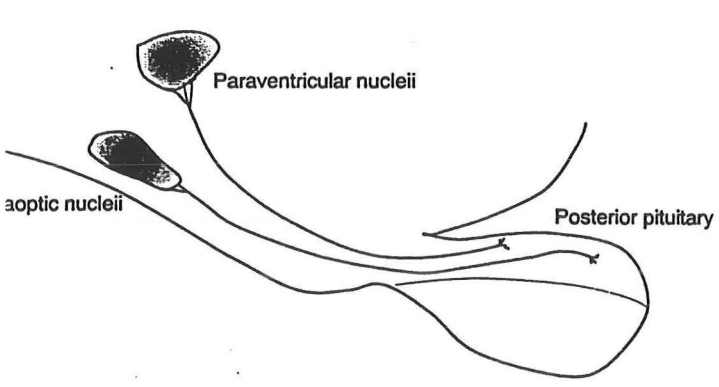
2. Regulation of total body water is achieve by a dual system of thirst and renal water excretion or generation.

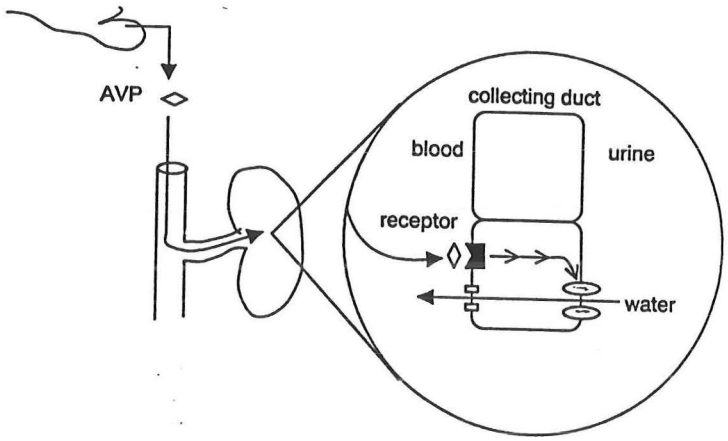
	Major defense
Water excess	Kidney
Water deficit	Thirst

3. Kidney can add or remove water from plasma.



4. Posterior pituitary-collecting duct axis: .Determinant of the fate of water in the kidney.





Congential diabetes insipidus

Mutations in the carrier protein of AVP:	autosomal dominant central
Mutations in the V2 receptor:	X-linked nephrogenic
Mutations in the AQP2 water channel:	autosomal recessive

LITERATURE CITED

Agre P, Saboori AM, Asimos A, Smith BL. 1987. Purification and partial chracterization of the Mr 30,000 integral membrane protein associated with the eruthrocyte Rh(D) antigen. J Biol Chem 262:17497.

Agre P, Preston GM, Smith B, Jung JS, Raina S, Moon C, Guggino W, Nielsen S.. 1993. Aquaporin CHIP: the archetypal molecular water channel. Am J Physiol 265:F463.

Agre P, Brown D, Nielsen S. 1995. Aquaporin water channels: unanswered questions and unresolved controversies. Curr Opinion Cell Bio 7:472.

Bahnsen U, Oosting P, Swaab DF, Nahke P, Richter D, Schmale H. 1992. A missense mutation in the vasopressin-neurophysin gene cosegregates with human autosomal dominant neurohypophyseal diabets insipidus. EMBO J 11:19.

Baltimore D. 1981. Gene conversion: some implications for immunoglobulin genes. Cell 24:592.

Berl T, Aisenbury GA, Linnas SL. 1980. Renal concentrating defect in the hypokalemic rat is prostaglandin independent. Am J Physiol 238:F37.

Berliner RW, Davidson DG. 1957. Production of hypertonic urine in the absence of pituitary antidiuretic hormone. J Clin Invest 36:1416.

Bichet DG, Razi M, Lonergan M, Arthus M-F, Papunka V, Kortas C, Barjon J-N. 1988. Hemodynamic and coagulation reponses to 1-desamino[8-D-arginine] vasopressin in patients with congenital nephorgenic diabetes insipidus. N Engl J Med 318:881.

Bichet DG, Razi M, Arthus M-F. Lonergan M, Tittley P, Smiley RK, Hirsch D. 1989. Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus. Kidney Int 36:859.

Bichet DG, Arthus M-F, Lonergan M. 1991. Platelet vasopressin receptors in patients with congenital nephrogenic diabetes insipidus. Kidney Int 39:693.

Bichet DG, Arthus M-F, Lonergan M. 1991. Is testing with dDAVP useful in detecting carriers of the nephrogenic diabetes insipidus gene. Nephron 58:372.

Bichet DG, Hendy GN, Lonergan M, Arthus M-F, Ligier S, Pausova Z, Kluge R, Zingg H, Saenger P, Oppenhimer E, Hirsh DJ, Gilgenkrantz S, Salles J-P, Pberle I, Mandel J-L, Gregory MC, Fujiwara TM, Morgan K, Scriver CR. 1992. X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. Am J Hum Genet 51:1089.

Bichet DG. 1994. Nephrogenic diabetes insipidus. Sem Nephrology 14:349.

Birnbaumer M, Siebold A, Gilbert S, Ishido M, Barberis C, Antaramian A, Brabet P, Rosenthal W. 1992. Molecular cloning of the receptor for human antidiuretic hormone. Nature 357:333.

Bode HH, Crawford JD. 1969. Nephorgenic diabetes insipidus in North America. The Hopewell hypothesis. N Engl J Med 280:7540.

Boton R, Gaviria M, Batlle DC. 1987. Prevalence, pathogenesis, and treatment of renal dysfunction associated with chronic lithium therapy. Am J Kidney Dis 10:329.

Braverman LE, Mancini JP, McGlodrick DM. 1965. Hereditary diabetes insipidus: A case report with autopsy findings. Ann Int med 63:503.

Brown D, Orci L. 1983. Vasopressin stimulates formation of coated pits in rat kidney collecting ducts. Nature 302:253.

Buerkert J, Head M, Klahr. 1977. Effects of acute bilateral ureteral obstruction on deep nephron and termial collecting duct function n the young rat. J Clin Invest 59:1055.

Buerkert J, Martin D, Head M, Prasad J, Klahr S. 1978. Deep nephron function after release of acute unilateral ureteral obstruction in the young rat. J Clin Invest 62:1228.

Camerer JW. 1935. Eine Ergänzung des Weilschen Diabetes insipidus-Stammbaumes. Arch Rassenbiol. 28:382.

Chen L, Rose JP, Breslow E, Yang D, Chang W-R, Furey Jr WF, Sax M, Wang B-C. 1991. Crystal structure of a bovine neurophysin II dipeptide complex at 2.8Å determined fro, single-wavelength anomalous scattering signal of an incorporated iodine atom. Proc Natl Acad Sci 88:4240.

Chrispeels MJ, Agre P. 1994. Aquaporins: water channel proteins of plant and animal cells. TIBS 19:411.

Christensen S, Kusano E, Yusufi AN, Murayama N, Dousa TP. 1985. Pathogenesis of nephrogenic diabetes insipidus due to Li in rats. J Clin Invest 75:869.

Christensen S. 1988. Vasopressin and renal concentrating ability. In Lithium therapy monographs. Edited by FN Johnson. S Karger Basel. 20.

Cobb WE, Spare S, Reichlin S. 1978. Neurogenic diabetes insipidus: management with DDAVP. Ann Int Med 88:183.

Cohen P. 1987. Proteolytic events in the post-translational processing of polypeptide hormone precursors. Biochime 69:87.

Dancis J, Birmingham JR, Lesli SH. 1948. Congenital diabetes insipidus resistant to treatment with pitressin. Am J Dis Child 75:316.

Deen PMT, Verdijk MAJ, Knoers NVAM, Wieriga B, Monnens LAH, van Os CH, van Oost BA. 1994. Requirement of human renal water channel AQP2 for vasopressin depenedent concnetration of urine. Science 264, 92.

Deen PM, Croes H, van Aubel RAMH, Ginsel LA, va Os CH. 1995. Water channel encoded by mutant aquaporin-2 genes in nephrogenic diabetes insipidus are impaired in their cellular routing. J Clin Invest 95:2291.

Denker BM, Smith BL, Kuhajda FP, Agre P. 1988. Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. J Biol Chem263:15634.

Digiovani SR, Nielsen S, Christensen EI, Knepper MA. 1994. Regulation of collecting duct water channel expresison by vasopressin in the Brattleboro rat. Proc Natl Acad Sci 91:8984.

Echevarria M, Windhager EE, Tate SS, Frindt G. 1994. Cloning and expression of AQP3, a water channel from the medullary collecting duct of rat kidney. Proc Natl Acad Sci 91:10997.

Fanton JW Golden JG, David TD. 1987. Renal medullary solute depletion resulting from psychogenic polydipsia in a rhesus monkey. J Am Vet Med Assoc 191:1011.

Ferris T. 1978. Prostaglandins, potassium and Bartter's syndrome. J Lab Clin med 92:663.

Fishbarg K, Kuang K, Hirsh J, Lecuona S, Rogozinski L, Silverstein SC, Loike J. 1989 Evidence that the glucose transporter serves as a water channel in J774 macrophages Proc Natl Acad Sci 86:8397.

Fishbarg K, Kuang K, Hirsh J, Lecuona S.Vera JC, Arant SM, Silverstein SC, Loike J, Rosen OM. 1990. Glucose transporters serve as water channels. Proc Natl Acad Sci 87:3244.

Flamion B, Spring KR. 1990. Water permeability of apical and basolateral cell membranes of rat inner collecting duct. Am J Physiol 259:F986.

Forssman H. 1942. Om ärftlighetsgången vid diabetes insipidus (On the mode of hereditary diabetes in sipidus) Nord Med 16:3211.

Forssman H. 1945. On diabetes insipidus with special regard to a sex-linked form. Acta Med Scand Suppl: 159.

Forssman H. 1975. Modern medical history: The recognition of diabetes insipidus. Acta Med Scand 197: 1.

Frøklær J, Marples D, Knepper MA, Nielsen S. 1996. Bilateral ureteral obstruction downregulates expression of vasopressin-sensitive AQP2 water channel in rat kidney. Am J Physiol 270:F657.

Gainer H, Russel JT, Peng Loh Y. 1985. The enzymology and intracellular organization of peptide precursor processing: the secretory vesicle hypothesis. Neuroendocrinol 40:171.

Goldberg H, Clayman P, Skorecki K. 1988. Mechanism of Li inhibition of vasopressin-sensitive adenylate cyclase in cultured renal epithelial cells. Am J Physiol 255:F995.

Grantham JJ, Burg MB. 1966. Effect of vasopressin and cAMP on permeability of isolated collecting tubules. Am J Physiol 211:255.

Han JS, Maeda Y, Ecelbarger C, Knepper MA. 1994. Vasopressin-independent regulation of collecting duct water permeability. Am J Physiol 266:F139.

Hanley MF, Davidson K. Isolated nephron segment from rabbit models of obstructive nephropathy. J Clin Invest 69:165.

Harrington AH, Valtin H. 1965. Vasopressin effect om urinary concentration in rats with hereditary hypothalamic diabetes insipidus. Proc Soc Exp Biol Med 118:448.

Harrington AH Valtin H. 1968. Impaired urinary concnetration after vasopressin and its gradual correction in hypothalamic diabetes insioidus. J Clin Invest 47:502.

Harris AS. 1989. Clinical experience with desmopressin: efficacy and safety in central diabetes insipidus. J Pediatr 114:711.

Harris Jr. HW, Hosselet C, Guay-Woodford L, Zeidel ML. 1992. Purification and partial chracterization of candidate antidiuretic hormone water channel proteins of Mr 55,000 and 53,000 from toad urinary bladder. J Biol Chem 267:22115.

Harvey B, Lacoste I, Ehrenfeld J. 1991. J Gen Physiol 97:749.

Hasegawa H, Skach W, Basker O, Calayag MC, Lingappa V, Verkman AS. 1992. A multifunctional aqueous channel formed by CFTR. Science 258:1477.

Hayashi M, Sasaki S, Tsuganezawa H, Monkawa T, Kitajima W, Konishi K, Fushimi K, Marumo F, Saruta T. 1994. Expression and distribution of aquaporin of collecting duct are regulated by vasopressin V2 receptor in rat kidney. J Clin Invest 94:1778.

Hebert SC, Reeves WB, Moloney DA, Andreoli TE. 1987. The medullary thick ascending limb: function and modulation of the single-effect multiplier. Kidney int 31:580.

Howard RL, Bichet DG, Schrier RW. 1992. Hypernatremia and polyuric states. In The Kidney:Physiology and Pathophysiology. Edited by DW Seldin & G Giebisch. Raven Press Ltd. New York. 1753.

Ishibashi K, Sasaki S, Fushimi K, Uchisa S, Kuwahara M, Marumo F. 1994. Moelcular cloning and expression of a member of the aquaporin family with permeability to glycerol and urea in addition to water expressed at the basolateral membrane of kidney collecting duct cells. Proc Natl Acad Sci 91:6269.

Ito M, Mori Y, Oiso Y, Saito H. 1991. Single base substitution in the coding region for neurophysin II associated with central diabetes insipidus. J Clin Invest 87:725.

Ito M, Oiso Y, Murase T, Kondo K, Saito H, Chinzei T, Racchi M, Lively MO. 1993. Possible involvement of inefficient cleavage of preprovasopressin by signal peptidase as a cause for familial central diabetes insipidus. J Clin Invest 91:2565.

Jaenike JR. The renal functional defect of post-obstructive nephropathy. The effects of bilateral ureteral obstruction in the rat. J Clin Invest 51:2999.

Jamison RL, Buerkert J, Lacy F. 1971. A micropuncture study of collecting tubule function in rats with hereditary diabetes insipidus. J Clin Invest 50:2444.

Jard S, Elands J, Schit A, Barbers C. 1988. Vasopressin and oxytocin receptors: An overview. In Progress in Endocrinology. Edited by H Imura, K Shizume. Amsterdam. Elsevier. 1183.

Jo I, Harria HW, Amendt-Raduege AM, Majewski RR, Hammond TG. 1995. Rat kidney papilla contains abundant synaptobrevin protein that participates n the fusion of antidiuretic hormone-regulated water channel-containing endosomes in vitro. Proc Natl Acad Sci 92:1876.

Jung JS, Preston GM, Smith BL, Guggino W, Agre P. 1994. Molecular structure of the water channel through aquaporin CHIP: the hourglass model. J Biol Chem 269:14648.

Jung JS, Bhat RV, Preston GM, Baraban JM, Agre P. 1994. Molecular characterization of an aquaporin cDNA from brain: a candidate osmoreceptor and regulator of water balance. proc Natl Acd Sci 91:13052.

Kanno K, Sesaki S, Hirata Y, Ishikawa SE, Fusgimi K, Nakanishi S, Bichet DG, Marumo F. 1995. Urinary excretion of aquaporin-2 in patients with diabetes insipidus. N Engl J Med 332:1540.

Kaplowitz PB, Dercole AJ, Robertson GL. 1982. Radioimmunoassay of vasopression in familial central diabetes insipidus. J Pediatr 100:76.

King LS, Agre P. 1996. Pathophysiology of the aquaporin water channels. Annu Rev Physiol 58:619.

Knepper MA, Roch-Ramel F. 1987. Pathways of urea transport in the mammalian kidney. Kidney Int 31:629.

Knepper MA, Star RA. 1990. The vasopressin-regulated urea transporter in renal inner meduallry collecting duct. Am J Physiol 259:F393.

Knepper MA, Han KS, Maeda Y, Ecelbarger C, Digiovani SR, Chou CL, Nielsen S, Wade JB. 1993. Vasopressin-independent regulation of collecting duct water permeability: Long term versus short term factors. In: Vasopressin, edited by D Richter and G Gross. 381.

Krishnamani MRS, Phillips III JA, Copeland JC. 1993. Detection of a novel arginine vasopressin defect by dideoxy fingerprinting. Clin Endocrinol Metab 77:596.

Land H, Schutz G, Schmale H, Richter D. 1982. Nucleotide sequence of cloned cDNA encoding bovine arginine vasopressin-neurophysin II precursor. Nature 295:299.

Land H, Grez M, Ruppert S, Schmale H, Rehbeln M, Richter D, Schutz G. 1983. Deduced amino acid sequence from the bovine oxytocin neurophysin I precursor cDNA. Nature 302:342.

Langley JM, Balfe JW, Selander T, Ray PN, Clarke JTR. 1991. Autosomal recessive inheritance of vasopressin-resistant diabetes insipidus. Am J Med Genetics 38:90.

Lankford SP, Chou CL, Terada Y, Wall SM, Wade JB, Knepper MA. 1991. Regulation of collecting duct water permeability independent of cAMP-mediated AVP response. Am J Physiol 261:F554.

Levinger EL, Excamilla RF. 1955. Hereditary diabetes insipidus: report of 20 cases in seven generations. J Clin Endocr 15:547.

Lolait SJ, O'Caroll AM, McBride OW, Konig M, Morel A, Brownstein MJ. 1992. Cloning and chracterization of the vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. Nature 357:336.

Macey RI. 1984. Transport of water and urea in red blood cells. Am J Physiol 246:C195.

Mannitus A, Levitan H, Beck D, Epstein FH. 1960. On the mechanism of renal concentrating ability in potassium deficiency. J Clin Invest 39:684.

Marples D, Frøkiær J, Dørup J, Knepper MA, Nielsen S. 1996. Hypokalemia-induced downregulation of AQP2 water channel expression in rat kidney medulla and cortex. J Clin Invest 97:1960.

Mathai JC. Muri S. Smith BL. Preston GM, Mohandas N, Agre P. Functional analysis of aquaporin -1 deicient red cells: the Colton null phenotype. J Biol Chem 271:1309.

MaDougal WS. Wright FS. Defect in proximal and distal sodium transport in post-obstructive diuresis. Kidney int 2:304.

McLeod JF, Kovacs I, Gaskill MB, Rittib S, Bradley GS, Robertson GL. 1991. Familial neurohypophyseal diabetes insipidus associated with a signal peptide mutation. J Clin Endocrinol Metab 77:599A.

Miller WL, Eberhardt NL. 1983. Structure and evolution of the growth hormone gene family. Endocrine Rev 4:97.

Mohr E, Bahnsen U, Keissling C, Richter D. 1988. Expression of the vasopressin and oxytocin genes in rats occurs in mutually exclusive sets of hypothalamic neurons. FEBS Lett 242:144.

Mohr E, Richter D. 1990. Regulation of vasopressin gene expression. In Recent Advances in Basoc and Clinical Neuroendocrinology. Edited by FF Casanueva and G Dieguez. Amsterdam. Elsevier Science Publishers 95.

Monn E, Osnes JB, Øye I. 1976. Basal and hormone-induced urinary cAMP in children with renal disorders. Acta Pediatr Scand 65:739.

Morgan T, Sakai F, Berliner RW. 1968. In vitro permeability of medullary collecting ducts to water and urea. Am J Physiol 214:574.

Moses AM, Miller JL, Levine MA. 1988. Two distinct pathophysiological mechanisms in congenital nephrogenic diabetes insipidus. J Clin Endocrinol Metab 66:1259.

Muggleton-Harris AL, Festing MFW, Hall M. 1987. A gene location for the inheritance of the cataract frase mouse congenital

cataract. Genet Res 49:235.

Nagasaki H, Ito M, Yuasa H, Saito H, Fukase M, Hamada K, Ishikawa E, Katakami H, Oiso Y. 1995. Two novel mutations in the coding region for neurophysin II associated with familial central diabetes insipidus. J Clin Endocrinol Metabolism 80:1352.

Nielsen S, Digiovanni SR, Christensen EI, Knepper MA, Harris HW. 1993. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. Proc Natl Acad Sci 90:11663.

Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. 1995. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membranes. Proc Natl Acad Sci 92:1013.

Nielsen S, Marples D, Mohtashimi M, Dalby NO, Trimble W, Knepper MA. 1995. Expression of VAMP-2-like protein in kidney collecting duct intracellular vesicles: colocalization with AQP2 water channels.J Clin Invest 96:1834.

Nielsen S, Agre P. 1995. The aquaporin family of water channels in kidney. Kidney Int 48:1057.

Nielsen S, Marples D, Frøklær J, Knepper M, Agre P. 1996. The aquaporin family water channels in kidney: an update on physiology and pathophysiology of aquaporin-2. Kidney Int 49:1718.

Ohzeki T, Igarashi T, Okamoto A, 1984. Familial cases of congenital nephrogenic diabetes type II: remarkable increment of urinary cAMP in response to antidiuretic hormone. J Pediatr 104:593.

Oksche A, Möller A, Dickson J, Rosendahl W, Rascher W, Bichet DG, Rosenthal W. 1996. Two novel mutations in the aquaporin 2 and the vasopressin V2 receptor genes in patients with congenital nephrogenic diabetes insipidus. Hum Genet 98:587.

Ollet SHR, Bourque CW. 1993. Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. Nature 364:341.

Paganelli CV, Solomon AK. 1957. The rate of exchange of tritiated water across the human red cell membrane. J Gen Physiol 41:259.

Pan Y, Metzeuberg A Das S, Jing B, Gitschier J. 1992. Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. Nature Genetics 2:103.

Pender CB, Frase C. 1953. Dominant inheritance of diabetes insipidus: a family study. Pediatr 11:246.

Preston GM, Agre P. 1991. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kD: member of an ancient chnnel family. Proc Natl Acd Sci 66:11110.

Preston GM, Carroll TP, Guggino WB, Agre P. 1992. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 265:385.

Preston GM, Smith BL, Zeidel ML, Mould JJ, Agre P. 1994. Mutations in Aquqporin-1 in phenotypically normal humans without functional CHIP water channels. Science 265:1585.

Reeves WB, Andreoli TE. Nephrogenic diabetes insipidus. In The Metabolic Basis of Inherited Disease. Edited by CR Scriver, Al Beaudet, WAS Sly, D Valle. New York. McGraw-Hill 1985.

Repaske DR, Browning JE. 1994. A de novo mutation in the coding sequence for neurophysin II is associated with onset and transmission of autosomal dominant neurohypophyseal diabetes insipidus. J Clin Endocrinol Metab 79:421.

Richter D. 1988. Molecular events in the expression of the hypothalamic hormones vasopressin and oxytocin and their cognate receptors. Am J Physiol 2255:F207.

Rogers GD. 1919. Joan at Halfway. New York: GH Doran.

Rupert S, Scherer G, Schutz G. 1984. Recent gene conversion involving bovine vasopressin and oxytocin precursor genes suggested by nucleotide sequence. Nature 308:554.

Rutishauser J. Boni-Schetzler M, Boni J, Wichman W, Huisman T, Vallotton MB. Froesh ER. 1996. A novel point mutation in the translation initiation codon of the preprovasopressin gene: cosegregation with morphologic abnormalities and clinical symptoms in autosomal dominant diabetes insipidus. J Clin Endocrinol Metab 81:192.

Rutihauser J, Froesh ER. 1997. Signal peptide and neurophysin II gene mutations in hereditary central diabetes insipidus. News Physiol Sci 12:67.

Preston GM, Agre P. 1991 Isolation of the cDNA for erythrocyte integral membrane protein of 28 kD: member of an ancient hannel family. Proc Natl Acad Sci 88:11110.

Sabolic I, Katsura JM, Verbavatz JM, Brown D. 1995. The AQP2 water channel: Effect of vasopressin treatment, microtubule disruption, and distribution in neonatal rarts. J Memb Biol 143:165.

Saboori AM, Smith BL, Agre P. 1988. Polymorphism in the Mr32,000 Rh protein purified from Rh(D) positive and negative erythrocytes. Proc Natl Acad Sci 85:4042.

Sands JM, Nonoguchi H, Knepper MA. 1987. Vasopressin effect on urea and water transport in inner medullary collecting duct subsegments. Am J Physiol 253:F823.

Schmale H, Heinsohn S, Richter D. 1983. Structural organization of the rat gene for the arginine vasopressin-neurophysin precursor. EMBO J 2:763.

Schmitz E, Mohr E, Richter D. 1991. Rat vasopressin and oxytocin genes are linked by a long interspersed repeated DNA element. DNA Cell Bio 10:81.

Shiels A, Griffin CS. 1993. Aberrant expression of the gene for lens major intrinsic protein in the CAT mouse. Curr Eye Res 12:913.

Shuster VL, Seldin DW. 1993. Water Clearance. In Clinical Disturbances of water metabolism. Edited by DW Seldin and G Giebisch. Raven Press New York, 51.

Seibold A, Brabet P., Rosenthl W, Birnbaumer M. 1992. Structure and chromosomal localization of the human antidiuretic hormone receptor gene. Am J human Genet 51:1078.

Sidel VW, Solomon AK. 1957. Entrance of water into human red cells under an osmotic presure gradient. J Gen Physiol 41:243.

Solomon AK, Chasan B, Dix JA, Lukacovic MF, Toon MR, Verkman AS. 1983. The aqueous pore in the red cell membrane. Ann NY Acad Sci 414:97.

Solomon AK. 1989. Water channels across the red blood cell and other biologic membranes. Methods Enzymol 173:192.

Sonnenberg H, Wilson DR. 1976. The role of the medullary collecting ducts in postobstructive diuresis. J Clin Invest 57:1564.

Smith BL, Agre P. 1991. Erythrocyte Mr 28,000 transmembrane protein exists as a multisubuit oligomer similar to channel proteins. J Biol Chem 266:6407.

Sugawara M, Hashimoto K, Ota Z. 1988. Involvement of prostaglandin E2, cAMP, and vasopressin in lithium-induced polyuria. Am J Physiol 254:R863.

Star RA. 1990. Hyperosmolar states. Am J Med Sci 300:402.

Star RA. 1993. Pathogenesis of diabetes insipidus and other polyuric states. In Clinical Disturbances of Water Metabolism. Edited by DW Seldin & G Giebisch. Raven Press Ltd., New York. 211.

Terris J, Ecelbarger, Nielsen S, Knepper MA. 1996. Long-term regulation of four renal auqporins in rats. Am J Physiol 271:F414.

Tsai ST, Zhang R, Verkman AS. 1991. High channel-mediated water permeability in rabbit erythrocyte characterization in native cells and expression in Xenopus oocytes. Biochemistry 30:2087.

Valtin H. 1966. Sequestration of urea and non-urea solutes in renal tissues of rats with hereditary hypothalamic diabetes insipidus: Effect of vasopressin and dehydration on the countercurrent mechanism. J Clin Invest 45:337.

Valtin H, Edwards BR. 1987. GFR and the concentration of urine in the absence of vasopressin. Berliner-Davidson re-explored. Kidney Int 31:634.

van der Ouweland AMW, Knoop MT, Knoers NVAM. 1992. Colocalization of the gene for nephrogenic diabetes insipidus and vasopressin type 2 receptor gene in the Xq28 region. Genomics 13:1350.

van der Ouweland AMW, Dreesen JFCM, Verdijk M, Knoers NVAM, Monners LAH, Rocchi M, van Oost BA. 1992. Mutations in the vasopressin type 2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus. Nature Genetics 2:99.

van Lieburg AF, Verdijk MAJ, Knoers VVAM, van Essen AJ, Proesmans W, Mallman R, Monnens LAH, van Oost BA, van Os CH, Deen PMT. 1994. Patients with autosomal nephrogenic diabetes insipidus homozygous for mutation in aquaporin 2 water channel gene. Am J Hum Genet 55:648.

van Os CH, Deen PMT, Dempster JA. 1994. Aquaporins:water selective channels in biological membranes, molecular structure and tissue distribution. Biochem Biophy Acta 1197:291.

Verkman AS, Shi LB, Frigeru A, Hasegawa H, Farinas J, Mitra A, Skach W, Brown D, van Hoek AN, Ma T. 1995. Structure and function of kidney water channels. Kidney Int 48:1069.

Wade JB, Stetson DL, Lewis SA. 1918. ADH action: evidence for a membrane shuttle mechanism. Ann NY Acad Sci372:106.

Wall SM, Han JS, Chou CL, Knepper MA. 1992. Kinetics of urea and water permeability activation by vasopressin in rat terminal IMCD. Am J Physiol 262:F989.

Waring AG, Kajdi L, Tappan V. 1945. Congenital defect of water metabolism. Am J Dis Child 69:323, .

Weil A. 1884. Über die hereditäre Form des Diabetes insipidus. Arch Anat 95:70.

Weil A.1908. Über die hereditäre Form des Diabetes insipidus. Dtsch Arch klin Med 93: 180.

Whinery MA, Kunau RT. 1979. Effect of potassium deficiency on papillary plasma flow in the rat. Am J Physiol 237:F226.

Williams RH, Henry C. 1947. Nephrogenic diabetes insipidus: transmitted by females and appearing during infancy in males. Ann Int Med 27:84.

Wilson DR. 1975. Nephron functional heterogeneity in the post-obstructive kidney. Kidney Int 7:19.

Yamaki M, Kusano E, Tetsuka S, Takeda S, Homma S, Murayama N, Asano Y. 1991. Cellular mechanism of lithium-induced nephrogenic diabetes insipidus in rats. Am J Physiol 261:F505.

Yarger WE, Aynedian HS, Band N. 1972. A micropuncture study of postobstructive diuresis in the rat. J Clin Invest 51:625.

Yuasa H, Ito M, Nagasaki H, Oiso Y, Miyamoto SH, Sasaki N, Saito H. 1993. Glu-47 that is essential for neurophysin II to form a salt bridge with arginine vasopressin is deleted in patient with familial central diabetes insioidus. J Clin Endocrinol Metab 77:600.

Zeidel ML, Ambudkar SV, Smith BL, Agre P. 1992. Reconstitution of functional water channels in liposomes contaning purified red cell CHIP28 protein. Biochem 31:7436.

Zhang R, Alper SA, Thorens B, Verkman AS, 1991. Expression of mRNA coding for kidney and red cell water channel in Xenopus oocytes. J Biol Chem 265:15375.

Zimmerman D, Green OC. 1975. Nephrogenic diabetes type II: Defect distal to adenylate cyclase step. Pediat Res 9:381.