

## RENAL WATER AND ION TRANSPORT SYSTEMS

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**T**he teaching of renal physiology is an ever-evolving and fascinating opportunity to learn as well as to teach. Regardless of the particular format of our teaching, a constantly waxing tide of detailed information forces us to be selective. We cannot teach it all. In my own lectures and discussions of renal ion and water transport systems, I try to 1) avoid complex and detailed areas that are not essential to a basic understanding of renal function, 2) highlight the commonalities of transport processes among segments, 3) relate the details of transporters to clinical conditions that are especially interesting, 4) make connections between physiological details and clinical therapy, and 5) stress quantitative problems. Although generally simple, the quantitative understanding is essential to appreciate the role of the kidney in homeostasis and derangements thereof. When I review what I have taught, I try to integrate multiple basic mechanisms in individual segments to produce a picture of “whole kidney” function.

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The teaching of renal physiology is an ever-evolving and fascinating opportunity to learn as well as to teach. Whatever the format in which we teach this subject, be it traditional lectures, problem-based learning, or some combination, an ever-growing body of information forces us to select those details that are most relevant for the medical student and put them in useful and interesting contexts. This focus on the medical student is also valid for our PhD graduate students. There is simply too much information for us to expect them to learn the whole body of information about each organ system, as might have been possible 20 or 30 years ago. On the other hand, our graduate students still need the breadth of a general physiology course to fulfill their roles as both scientists and teachers. Hopefully, the future physiologist will have this breadth of knowledge that will facilitate the integration of molecular and genetic information with physiological and pathophysiological processes; i.e., to be the link from molecule to clinic.

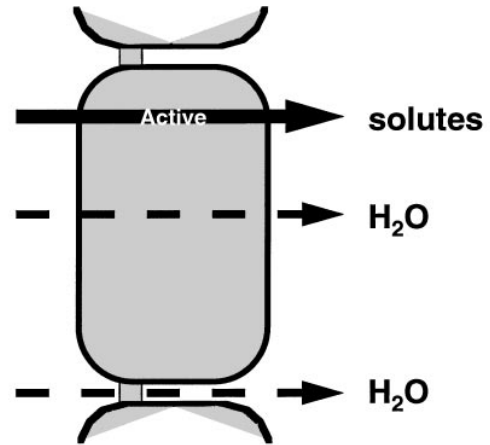
This presentation deals with some of the challenges involved in approaching these ideals when teaching about renal water and ion transport systems in the multiple nephron segments. Here, I summarize my own methods of dealing with the more complex topics, including those that are better taught only superficially. I also present some of my favorite examples that relate discrete renal transporter defects to interesting diseases and the therapeutic interventions for those diseases. It is also my firm belief that most of the key concepts in renal physiology depend on a good quantitative understanding of the processes involved, and for that reason, I also spend some verbiage developing my own approach to improving the students' quantitative understanding. Finally, I provide some examples of basic overviews that integrate the detail of epithelial transport mechanisms into a picture of the “whole kidney” effects of changes in the operation of those transporters.

### WHY TEACH NEPHRON FUNCTION?

Many of our nephrology and pathology colleagues might disagree with the detail with which we present the transepithelial transport processes involved in the function of the nephron. After all, they might argue, the most important and common renal diseases are not disorders of tubular transport. Glomerular immunopathology, specifically glomerulonephritis, is by far the most prevalent cause of chronic and end-stage renal disease. It might seem, therefore, that we should concentrate our teaching on glomerular function and immunology to the exclusion of renal transport processes. The counterpoint to this argument is that an understanding of how the kidney maintains salt and water balance through regulation of its multiple transport processes is essential for understanding fluid and electrolyte disorders and for the rational choice of therapy for those disorders. Fluid and electrolyte disorders are much more common than glomerular disease, and most physicians deal with these disorders daily. As just one of many examples, the most common fluid and electrolyte disorder in the hospital setting is hyponatremia, and 50% of hyponatremic patients die. Also, the concepts learned in understanding renal epithelial transport processes are closely related to similar processes occurring in many other systems, for example, intestinal absorption and secretion, and electrolyte balance in the respiratory system and in the brain.

### AVOID EXPLAINING COMPLEX CONCEPTS WHEN THEY ARE NOT ESSENTIAL

In addressing the more complex topics in renal salt and water transport, the lecturer has to make the decision of whether a full discussion is warranted. All of the topics that we teach involve details and complexities that could require hours of self-study for the student to begin to appreciate. A primary job of the teacher is to decide whether a full discussion of the topic is worth the time that it would take from more important, more clinically applicable, subjects. An example dear to my own heart is the process by which fluid is reabsorbed isosmotically from the proximal tubule. Despite the large volume of information on this subject that my colleagues and I, and many others, have laboriously gathered (2, 4, 18, 28), I feel that the student can appreciate the essence of isosmotic volume absorption according to the simple principles



- Water reabsorption passively by osmosis
- Osmotic water permeability is very high. Only a small osmolality difference is needed.
- Active solute reabsorption makes the lumen slightly dilute
- Preferentially reabsorbed solutes with high reflection coefficients render the interstitium "effectively hypertonic."

FIG. 1.

**Isosmotic volume reabsorption in proximal tubule.** Distribution of osmotically driven water flow between transcellular and paracellular pathways (junctional complexes and lateral intercellular spaces) remains controversial. This issue may be addressed experimentally in the near future because of development of a mouse strain with a knockout of aquaporin-1 (AQP1; Ref. 14).

outlined in Fig. 1. The important points to stress are as follows (18). 1) Water movement across the epithelium and all regions of the nephron occurs in response to an osmotic gradient, whether that gradient is localized to a small region such as the intracellular spaces or between two compartments such as the tubular lumen and the interstitial fluid. 2) The essential ingredient for (nearly) isosmotic volume absorption is the extremely high osmotic water permeability of the proximal tubule. Because of this high water permeability, only a very small osmolality difference, one that was not easily measured in early studies, is needed to drive the observed rates of volume reabsorption. 3) The active transport of solutes makes the lumen slightly dilute and the interstitial fluid slightly hypertonic. 4) The actively reabsorbed solutes such as glucose, amino acids, and bicarbonate have higher reflection coefficients than NaCl; thus the preferential reabsorption of these solutes results in the develop-

ment of a larger “effective” osmolality difference across the epithelium. Students should already be familiar with the concept of a reflection coefficient, but even if they are not, they should be able to intuitively grasp the fact that solutes of lower permeability (and thus higher reflection coefficient) will make a greater contribution to the true osmolality of interstitium compared with the fluid remaining in the tubule lumen, which becomes increasingly composed of NaCl, which replaces the preferentially reabsorbed solutes.

Another area that I find too demanding in time for the student to understand in detail is the mechanism of countercurrent multiplication, especially passive countercurrent multiplication in the inner medulla. Certainly, the postulation and experimental verification of the countercurrent multiplication system was one of the most intellectually gratifying achievements of renal physiology research. For that reason, I believe many of us have given it undue emphasis in our practical teaching. If the students want to know these details, I refer them to an appropriate text. However, at least in the lecture setting, I stress the essential ingredients for the development of medullary hypertonicity and the clinically important events that would interfere with the urinary concentrating and diluting ability. Obviously, the crucial element is active NaCl reabsorption by the thick ascending limb of the loop of Henle via the  $\text{Na}^+\text{-K}^+\text{-2Cl}$  cotransporter (BSC1). The students can well appreciate that this active solute reabsorption, which occurs in a segment that is water impermeable, should concentrate the medullary interstitium. They can also readily appreciate that the countercurrent arrangement and low blood flow rate through the vasa recta curtail the “washout” of the hypertonicity that would occur in most other tissues. The second component is the addition of urea to the tubular fluid in the thin limbs of the loop of Henle, which increases the amount of urea that is delivered to the thick ascending limb of the loop of Henle. Urea is then trapped in the tubular lumen, because all nephron segments from the thick ascending limb through the outer medullary collecting duct are impermeable to urea. However, because of the presence of a urea transporter (UT1) in the most distal portion of the inner medullary collecting duct, urea can diffuse passively across this epithelium. When the urine urea concentration is very high, as in antidiuresis, urea diffuses from the inner medullary collecting duct to

the medullary interstitium, where it contributes to the medullary hypertonicity and enhances water reabsorption from the descending limb of the loop of Henle. These points encompass the essential ingredients of the countercurrent multiplication system, and using these points, the teacher can examine mechanisms by which the medullary osmolality gradient can be disturbed. One can discuss, for example, the effects of changes in medullary blood flow, saline loading, loop diuretics, low-protein diets, and water diuresis on the medullary concentration gradient.

### **SIMPLIFY THE DETAILS OF EPITHELIAL TRANSPORT AND HIGHLIGHT THE COMMONALITIES AMONG SEGMENTS**

One of the most important characteristics of the nephron is its ability to dissociate salt and water transport in various segments. Some nephron segments, including the thin and thick ascending limbs of the loop of Henle and the collecting duct in the absence of vasopressin (AVP), are very water impermeable. In these water-impermeable segments, solute reabsorption results in dilution of the tubular fluid. In contrast, most cell membranes, including those of the proximal tubule and thin descending limb of the loop of Henle, are very water permeable under all circumstances. This high water permeability is a consequence of the presence of a water channel that has come to be referred to as aquaporin-1 (AQP1). I find the students interested to know that this water channel is found in many cells, including red blood cells, and that it contributes to the very high water permeability of the proximal tubule and the descending limb of the loop of Henle, where it is present in both luminal and basolateral membranes (Fig. 2).

Perhaps even more interesting in terms of water permeability is the collecting duct. The luminal membrane of the principal cells in this segment can be regulated from nearly water impermeable, in the absence of AVP, to quite water permeable in the presence of the hormone. The intrinsically low water permeability of these cells is increased by the insertion of another aquaporin (AQP2; see Fig. 2) into the luminal membrane. AQP2 is stored in vesicles in the cytoplasm of the principal cells of the collecting duct and the connecting tubule arcades. Basolateral membranes of the principal cells, which remain water permeable even in the absence of AVP, contain yet

**The water channels - Aquaporins**

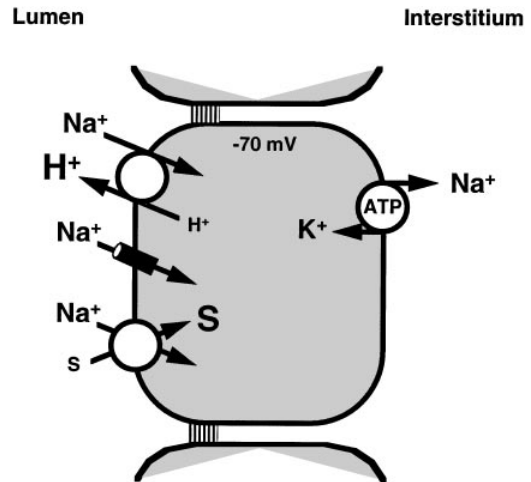
Aquaporin	Location	Effect of Mutations
<b>AQP1</b> (CHIP28)	protein cloned from red cells; found in many tissues, especially in the basolateral and luminal membranes of proximal tubule and DLH	functional knockout in humans; transgenic mouse model with low proximal tubule water permeability
<b>AQP2</b> (WCH-CD)	luminal membrane and intracellular vesicles in principal cells of collecting duct & connecting tubule arcades	AVP → insertion in luminal membrane and ↑ expression; mutation → X-linked nephrogenic DI
<b>AQP3</b>	basolateral membrane of collecting duct principal cells	increased expression with AVP
<b>AQP4</b>	basolateral membrane of collecting duct principal cells	knockout mice have 4-fold lower IMCD water permeability

**FIG. 2.**

**Characteristics of primary aquaporin water channels found in kidney. CHIP28 and WCH-CD are alternate names that were previously used for AQP1 and AQP2, respectively. AVP, vasopressin; DLH, descending limb of the loop of Henle; IMCD, inner medullary collecting duct; DI, diabetes insipidus. Right arrow indicates production. See text for fuller explanations.**

two other aquaporins: AQP3 and AQP4. At this point, it is instructive to point out that mutations in human AQP2 that result in nephrogenic diabetes insipidus have been identified and that there may be as yet unidentified abnormalities in AQP3 or AQP4 that could also interfere with the ability to concentrate the urine. (For references to aquaporins, see Refs. 1, 7, 12, 14, and 23.)

In discussing the details of transport processes, it is also useful to generalize about transepithelial transport before dealing with the details of individual systems. For example, when I introduce transport in the proximal tubule, as shown in Fig. 3, I first point out the aspects that are common to all regions of the nephron. 1) The epithelium is a single cell layer, and cells are joined by junctional complexes near their apical border. This topology provides two pathways by which solutes can traverse the epithelium, either through the cell or through the paracellular pathway, i.e., the junctional complexes and lateral intercellular spaces. 2) All epithelial cells in the nephron have the Na<sup>+</sup>-K<sup>+</sup>-ATPase that is common to virtually every cell in the body. This Na<sup>+</sup>-K<sup>+</sup>-ATPase is responsible for maintaining a low intracellular Na<sup>+</sup> concentration and for developing the ion gradients that lead to a negative



**FIG. 3.**

**All nephron segments have common transport properties: a single layer of cells connected by junctional complexes near their luminal aspects, basolateral Na<sup>+</sup>-K<sup>+</sup>-ATPase, and passive Na<sup>+</sup> entry across the luminal entry that may be dissipative or coupled to active antiport or cotransport of other solutes (S). It is primarily the details of the luminal entry process that differ among nephron segments.**

intracellular voltage. However, most epithelia, including all the nephron segments, are unique because this transporter is localized only to the basolateral membrane. 3) In all nephron segments, the Na<sup>+</sup>-K<sup>+</sup>-ATPase develops an Na<sup>+</sup> electrochemical potential gradient across the opposite membrane, i.e., the luminal membrane; thus there is a driving force for the passive entry of Na<sup>+</sup> from the lumen into the epithelial cell. This entry may occur through an electrogenic Na<sup>+</sup> channel, such as the amiloride-sensitive Na<sup>+</sup> channel in the collecting duct, in which case the potential energy embodied in the Na<sup>+</sup> electrochemical potential gradient is dissipated. However, if the movement of Na<sup>+</sup> is coupled to the movement of another solute, for example, by an antiporter, such as the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE3) in the proximal tubule, or by a cotransporter, such as the Na<sup>+</sup>-glucose symporter, then the energy available in the Na<sup>+</sup> electrochemical potential gradient can be used to perform useful work. That useful work is the active transport of the coupled solutes against their own electrochemical potential gradients. It should be emphasized to the student that this general plan for the transport of Na<sup>+</sup> holds true throughout the nephron. That is, the Na<sup>+</sup> electrochemical potential gradient is maintained by

the basolateral  $\text{Na}^+\text{-K}^+\text{-ATPase}$ , and this potential energy gradient provides the driving force for passive entry of  $\text{Na}^+$  into the cell across the luminal membrane either alone or in combination with secondary active transport of other solutes. It is only the details of the transporters, usually those on the luminal membrane, that vary from one nephron segment to another. I try constantly to refer back to this overall scheme to avoid the students becoming belabored by the details of specific transporters in each nephron segment.

**RELATE TRANSPORTER DETAILS TO CLINICAL CONDITIONS THAT ARE ESPECIALLY INTERESTING**

In my experience, medical students are more willing to address the details of an epithelial transport process that are associated with clinically relevant defects. Graduate students are now acutely aware of the direct associations between gene mutations and functional defects and are also receptive to these practical applications of basic science. Thus, whenever possible, I try to relate the details of the operation of a membrane transporter to a particular clinical situation. A perfect example is a comparison of the two  $\text{Na}^+$ -glucose cotransporters that are found in the kidney: SGLT1 and SGLT2 (Fig. 4). (The number associated with the acronyms of these two transporters presents the order in which they were cloned and sequenced. This can be somewhat confusing, because the SGLT1 protein cotransports two  $\text{Na}^+$  in association with one glucose, whereas the SGLT2 has a 1:1 stoichiometry.) In the kidney, SGLT2 is the primary transporter and is found in the proximal convoluted tubule, whereas the SGLT1 cotransporter is found in the proximal straight tubule. Because of the higher stoichiometry of SGLT1, the transporter can develop a concentration gradient (lumen to cell) of up to 1:10,000, whereas SGLT2 develops only a 1:100 gradient. Thus it appears that SGLT2 serves to reabsorb the bulk of the glucose filtered in the kidney, whereas SGLT1 is able to reabsorb the last bit of sugar against a high transepithelial concentration gradient (3, 19, 22).

Where is the clinical interest here? The answer is that genetic defects occur in both transporters in humans that result in completely different phenotypes as illustrated in Fig. 4. A single base pair mutation in SGLT1, which causes a loss of function, produces the



**SGLT1**

**SGLT2**

- Cloned from rabbit intestine, but also present in the proximal straight tubule
- Stoichiometry: luminal glucose concentration can be reduced to < 1/10,000 of that in the cell
- Specificity: glucose and galactose
- **Glucose-galactose malabsorption syndrome** Autosomal recessive trait (1 bp mutation) severe diarrhea and dehydration.
- This mutation has little effect on renal glucose reabsorption.
- Cloned from human kidney; has not been detected in the intestine
- Stoichiometry: luminal glucose concentration can be reduced to ~ 1/100 of that in the cell
- Specificity: glucose only
- **Familial renal glucosuria:** Restricted to the kidney; relatively benign
- Produces a reduced plasma glucose threshold or a decreased  $T_m$ . Probably multiple mutant forms in the population.

**FIG. 4.**

**Secondary active transport of glucose by  $\text{Na}^+$ -glucose cotransporters SGLT1 and SGLT2. Although these 2 transporters are quite similar, and both are expressed in proximal tubule, subtle differences in molecular details of their operation explain profound phenotypic differences produced by loss-of-function mutations. G, glucose;  $T_m$ , maximum renal reabsorption rate, or transport maximum.**

glucose-galactose malabsorption syndrome that is characterized by severe diarrhea and dehydration, but it has little effect on the renal reabsorption of glucose. There are multiple mutant forms of SGLT2 in the human population that are associated with different forms of familial renal glucosuria. However, this condition is relatively benign, and its effects are restricted to the kidney because SGLT2 is not found in the intestine. This example provides students with an excellent example of how relatively small differences in quite similar transporters can lead to quite different disease phenotypes (for details, see Refs. 9, 10, 15, and 29).

Another condition that integrates genetics, molecular biology, physiology, and disease in a most elegant collage is Liddle's syndrome. Liddle's syndrome is a rare autosomal dominant disease that was first described by Grant Liddle et al. (13) in 1963. It is characterized by severe hypertension, often leading to stroke even at a young age. It is also frequently associated with hypokalemia and metabolic alkalosis. Thus, in most aspects, Liddle's syndrome looks very

similar to primary aldosteronism, i.e., the overproduction of aldosterone by an adrenal carcinoma or diffuse renal hyperplasia. However, as determined by bioassays, which at the time of Liddle's report were available in his laboratory and a few others, it was determined that plasma renin and aldosterone levels were both immeasurable, and the condition was not ameliorated by the aldosterone receptor antagonist spironolactone. Liddle and his co-workers could find no hormonal explanation for the abnormality, and, on the basis of the physiological information of that time, they correctly concluded in 1963 that the defect probably represented an unregulated absorption of  $\text{Na}^+$  by the distal nephron. Their suggested treatment, which is the administration of triamterene or amiloride in combination with a low- $\text{Na}^+$  diet, remains the treatment of choice. As shown in Fig. 5, since 1963, we have learned that the target of amiloride and triamterene action is the electrogenic  $\text{Na}^+$  channel found in the luminal membrane of the principal cell of the collecting duct. This channel, referred to as the epithelial  $\text{Na}^+$  channel (ENaC), consists of three subunits labeled  $\alpha$ ,  $\beta$ , and  $\gamma$ , which were originally cloned and sequenced by Cecilia Canessa in Bernard Rossier's laboratory just four years ago (5). Once the genetic sequence for these channel subunits was known and their position on human chromosomes ascertained, it was possible to examine whether there was a mutation associated with one of the subunits that results in Liddle's syndrome. Fortunately, the Liddle's kindred,

which resides in Southern Alabama, is relatively extensive, and immortalized lymphocytes obtained from family members were used for DNA analysis. It was found that Liddle's syndrome was associated with a mutation in the  $\beta$ -ENaC sequence that resulted in an early truncation in the COOH-terminal region (20). This truncation results in an overactive  $\text{Na}^+$  channel that is not regulated normally and can explain the excessive  $\text{Na}^+$  reabsorption seen in Liddle's patients. Since these original findings, several similar kindreds have been identified with other mutations in the COOH-terminal regions of the  $\beta$ - and  $\gamma$ -subunits (24). It is also interesting that the congenital form of pseudo $hypo$ aldosteronism is associated with a mutation in the internal region of the  $\alpha$ -ENaC subunit that results in a loss-of-function mutation rather than the gain-of-function observed in Liddle's syndrome (6).

Students are fascinated with this kind of information because it so beautifully transfers information from the basic physiology of the kidney, which allowed Grant Liddle to interpret the syndrome he observed, to the final resolution of the clinical problem by the application of molecular biology and physiology. This example can be used to demonstrate the reasons for the other manifestations of the Liddle's syndrome. As shown in Fig. 5, if the  $\text{Na}^+$  channel is unregulated and "wide open," the high  $\text{Na}^+$  conductance of the luminal membrane would depolarize it, just as the activation of  $\text{Na}^+$  channels in nerve or muscle membrane results in depolarization of those cells. The result of this depolarization is a more favorable  $\text{K}^+$  electrochemical potential gradient that drives the passive movement of  $\text{K}^+$  out of the principal cells into the lumen, thus enhancing  $\text{K}^+$  excretion and leading to the hypokalemia that is observed in at least some of the patients. The associated alkalosis may also be a result of an increased lumen-negative voltage that favors  $\text{H}^+$  secretion.

It is also important to point out that these syndromes illustrate the importance of  $\text{Na}^+$  balance in maintaining normal blood pressure and volumes of body fluid compartments. About 25,000 meq of  $\text{Na}^+$  are filtered per day by the glomeruli in two adult human kidneys, but less than 2,000 meq/day are reabsorbed in the distal tubule and collecting duct, with the vast majority being reabsorbed in the proximal tubule and the ascending limb of the loop of Henle. Aldosterone

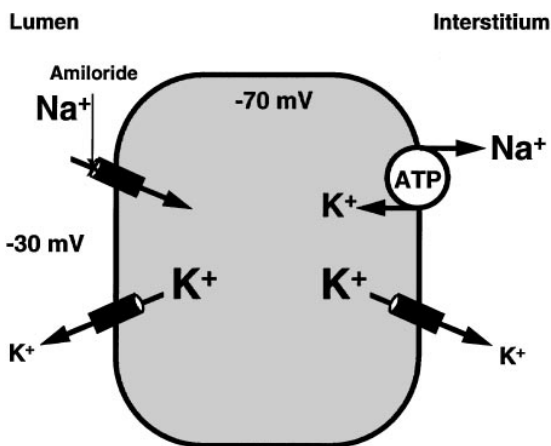


FIG. 5.

Transport processes in principal cell of collecting duct. A lumen-negative voltage of  $-30$  mV (compared with interstitium) is taken as an average, but it can vary with relative luminal  $\text{Na}^+$  and  $\text{K}^+$  conductances.

regulates the reabsorption of ~500 meq of the 2,000 meq reabsorbed by the distal tubule and collecting duct. Thus, in the total absence of aldosterone, only ~500 meq/day are excreted, and at very high aldosterone levels, Na<sup>+</sup> excretion can be reduced nearly to zero. The important point to stress is that aldosterone is operating on only a very small fraction of the filtered Na<sup>+</sup> load, but this regulation of Na<sup>+</sup> excretion in the range of 0–1% of the filtered load is extremely critical. The retention of only 150 meq of Na<sup>+</sup> (with accompanying monovalent anions) requires the retention of 1 liter of water to make it isotonic. Given the fine nature of this regulation, it is remarkable that disturbances, such as those produced by defects in ENaC, can lead to either salt wasting in the case of the α-subunit mutation or Na<sup>+</sup> retention with severe hypertension in the case of the β- and γ-subunit mutations.

There are additional excellent examples of transporter or enzyme mutations that have interesting clinical correlations, some of which are described in Fig. 6. In the case of apparent mineralocorticoid excess, the underlying facts are that glucocorticoids such as cortisol

have reasonable affinity for both the mineralocorticoid and glucocorticoid (type I and type II) receptors in aldosterone responsive tissues. However, because of the presence of an enzyme, 11β-hydroxysteroid dehydrogenase (11β-OH HSD), cortisol is degraded to the inactive cortisone. This allows aldosterone, which is usually present in much lower concentration in the plasma than glucocorticoids, to react with mineralocorticoid and, to some extent, glucocorticoid receptors in aldosterone target tissues such as the principal cell of the cortical collecting duct. The 11β-HSD can be inhibited by certain compounds and drugs such as glycyrrhetic acid (in licorice) and gossypol, which leads to a syndrome that is very similar to Liddle's syndrome or apparent hyperaldosteronism (11, 25, 26). The syndrome is also caused by mutations in the human 11β-HSD gene (16, 27).

Bartter's syndrome (Fig. 6) illustrates the importance of specific transporters in the thick ascending limb of the loop of Henle that result in the development of a lumen-positive voltage and thus favor Ca<sup>2+</sup> reabsorption. Mutations in BSC1 as well as mutations in the luminal K<sup>+</sup> channel (ROMK) or in the basolateral Cl<sup>-</sup> channel have been linked to Bartter's syndrome. All these defects lead to decreased salt reabsorption in the thick ascending limb of the loop of Henle, and thus to a concentrating defect with increased Na<sup>+</sup> delivery to the collecting duct. The latter leads to salt wasting with an elevation of renin and aldosterone concentrations. Na<sup>+</sup> reabsorption by the collecting duct is increased by the aldosterone. In addition, the increased Na<sup>+</sup> delivery as well as the aldosterone produce inappropriate levels of K<sup>+</sup> and H<sup>+</sup> secretion, leading to hypokalemia and metabolic alkalosis. These effects are accompanied by a relative failure to absorb Ca<sup>2+</sup> and hypercalciuria. Gitelman's syndrome is a similar condition with all of the characteristics of Bartter's syndrome but with *hypo*- rather than hypercalciuria and hypomagnesemia. This syndrome results from a loss-of-function mutation in the thiazide-sensitive NaCl cotransporter (TSC1), which is found in the distal convoluted tubule. By examining abnormalities in transporters or enzymes associated with syndromes such as these, the teacher can provide superb examples of the importance of physiological detail in the context of clinical understanding (for references to Bartter's and Gitelman's syndromes, see Ref. 8, 17, and 21).

**Examples of correlations between molecular defects and disease**

Condition	Manifestations	Cause
Liddle's syndromes and similar familial hypertension	early onset severe hypertension with hypokalemia and alkalosis variably; low plasma aldosterone and renin; ameliorated by amiloride or triamterene and a low-salt diet	Gain of function mutations in C-terminal region of β or γ ENaC resulting in truncation or missense
apparent mineralocorticoid excess	Similar to Liddle's syndrome above	Loss of function of 11β-OH HSD due to mutation or drugs
pseudohypoaldosteronism, type 1	salt wasting, hyperkalemic acidosis, hypotension	loss of function mutation in N-terminal region of α-ENaC
Bartter's syndrome	salt wasting, elevated renin & aldosterone, hypokalemia, metabolic alkalosis, mental & growth retardation, hypercalciuria	loss of function mutations in the Na/K/2Cl cotransporter (BSC1); or the ROMK or Cl <sup>-</sup> channels
Gitelman's syndrome	as in Bartter's syndrome, but with hypo- rather than hypercalciuria & hypomagnesemia	loss of function mutation in the thiazide sensitive NaCl cotransporter (TSC1)

**FIG. 6.**

**Associations between defects at molecular level and pathophysiology of disease states they produce. 11β-OH HSD, 11β hydroxysteroid dehydrogenase; ENaC, epithelial Na<sup>+</sup> channel; ROMK, inwardly rectifying K<sup>+</sup> channel found in luminal membrane of thick ascending limb of loop of Henle.**

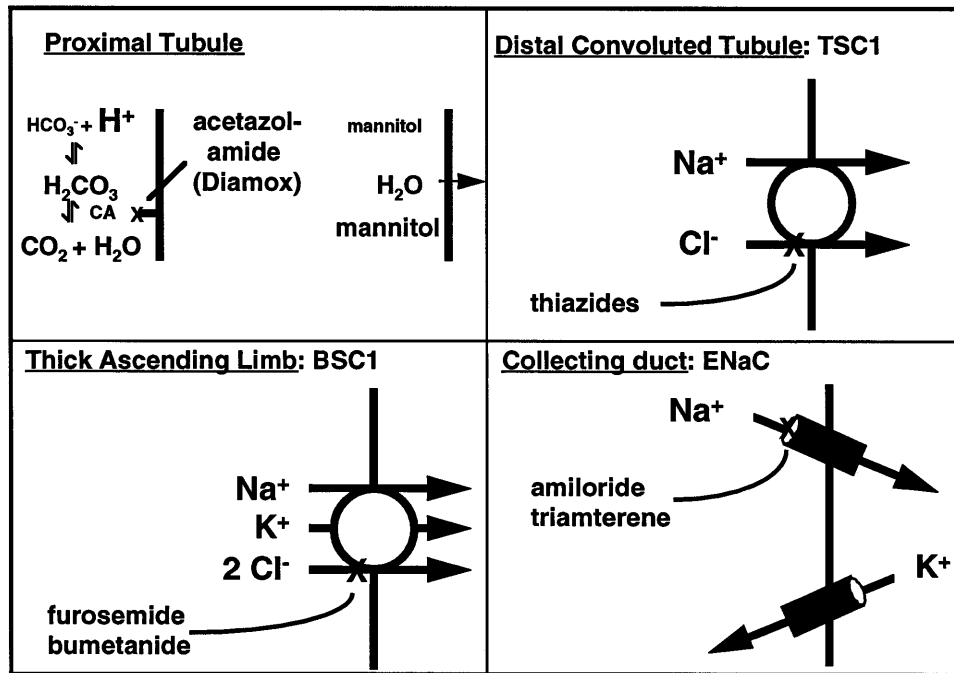


FIG. 7.

Unique transporters and enzymes are targets for diuretic therapy. In proximal tubule, acetazolamide inhibits carbonic anhydrase, decreasing  $\text{HCO}_3^-$  and  $\text{Na}^+$  reabsorption; mannitol acts as an osmotic diuretic. In thick ascending limb, furosemide and bumetanide inhibit  $\text{Na}^+$ - $\text{K}^+$ - $2\text{Cl}^-$  cotransporter (BSC1) by competition for  $\text{Cl}^-$  receptor.  $\text{Cl}^-$  receptor is also the target for inhibitory effect of thiazide diuretics on  $\text{NaCl}$  cotransporter (TSC1) in distal convoluted tubule. In principal cells of collecting duct, amiloride and triamterene block  $\text{Na}^+$  channel (ENaC).

### RELATING PHYSIOLOGICAL DETAILS TO THERAPY

As shown in Fig. 7, the mechanisms of action of drugs such as diuretics can also be used to illustrate physiological processes. The action of acetazolamide (Diamox) can be used to illustrate the mechanism of bicarbonate reabsorption in the proximal tubule. The diuretic and natriuretic effects of a failure to reabsorb bicarbonate can also be related to the similar effects of mannitol operating as an osmotic diuretic. It is also known that furosemide and similar drugs such as bumetanide interfere with the chloride recognition site on BSC1, which results in inhibition of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  reabsorption by the thick ascending limb of the loop of Henle, leading to natriuresis and diuresis. Again, the teacher should stress the effects on increased salt and water delivery to the distal nephron in promoting  $\text{K}^+$  secretion, resulting in hypokalemia as well as hypercalciuria.

In the distal convoluted tubule, the thiazide diuretics interfere with the chloride recognition site on TSC1. Thus the diuretic inhibits  $\text{NaCl}$  reabsorption in the distal convoluted tubule and increases its delivery to the collecting duct with the same consequences on  $\text{K}^+$  secretion. However, because the thiazides act only in the cortex, where the distal convoluted tubule is located, they interfere primarily with the ability of the kidney to dilute the urine. In contrast, furosemide, by inhibiting  $\text{NaCl}$  reabsorption in the medulla, interferes with both the concentrating and diluting abilities of the kidney. I have already discussed the fourth panel in Fig. 7, which is the action of amiloride and triamterene to block the  $\text{Na}^+$  channel in the principal cell of the collecting duct.

### QUANTITATIVE PROBLEMS ARE ESSENTIAL

Despite the fact that our students generally have a good background in the physical sciences, they are



often slow to transfer basic concepts from the physical sciences to biology. For example, students frequently confuse the concentration of a solute with the amount of the solute in a fluid compartment. Students should be led to recognize that a concentration is defined by an amount of the solute in a given volume. Similarly, they need to grasp the concept that mass flow, which is the amount of material flowing per unit of time at some point along the nephron (for example, the rate of filtration or excretion, or the rate of  $\text{Na}^+$  flow at the end of the proximal tubule), is equal to the product of the concentration and the flow rate, shown with the use of some sample units as

$$\text{Mass flow (mmol/min)} = \text{concentration (mmol/l)} \\ \times \text{flow (l/min)}$$

Students lacking this understanding often do not appreciate why, in mannitol diuresis, more  $\text{Na}^+$  is excreted (a natriuresis) despite the fact that the  $\text{Na}^+$  concentration of the tubular fluid in the late proximal tubule is less than that in the plasma and thus less than normal. As simple as the concept may seem, the teacher must be sure that the students appreciate that the mass flow of  $\text{Na}^+$  at this point in the nephron is greater because of the highly increased flow rate despite the lower  $\text{Na}^+$  concentration.

Similarly, the determinants of concentration need to be appreciated by the student to understand why the plasma  $\text{Na}^+$  concentration is not an indicator of extracellular fluid (ECF) volume. Because  $\text{Na}^+$  and its associated anions make up most of the osmolality of the plasma, the plasma  $\text{Na}^+$  concentration is a good indicator of the plasma osmolality, which can be approximated as

$$P_{\text{osm}} \approx 2 \cdot P_{\text{Na}}$$

or yet more exactly as

$$P_{\text{osm}} \approx 2 \cdot P_{\text{Na}} + \frac{P_{\text{glucose}}}{18} + \frac{\text{BUN}}{2.4}$$

where  $P_{\text{osm}}$ ,  $P_{\text{Na}}$ , and  $P_{\text{glucose}}$  are, respectively, the plasma osmolality,  $\text{Na}^+$  (in mmol/l), and glucose (in mg/dl) concentrations, and BUN is the blood urea nitrogen (in mg/dl). Plasma osmolality is normally

maintained constant by the AVP feedback system, and as a consequence, plasma  $\text{Na}^+$  concentration is also normally kept quite constant. If the plasma  $\text{Na}^+$  concentration is constant, one can then immediately see that ECF volume is proportional to the total body  $\text{Na}^+$  and is not reflected by the plasma  $\text{Na}^+$  concentration. In other words, if  $P_{\text{Na}} \approx \text{constant}$ , then ECF volume is proportional to the amount of  $\text{Na}^+$  in the body by rearrangement of the equation

$$P_{\text{Na}} \approx \frac{\text{total body amount of Na}^+}{\text{ECF volume}} \approx \text{constant}$$

thus

$$\text{ECF volume} \propto \text{total body amount of Na}^+$$

In summary, changes in plasma osmolality are usually indicative of problems in water balance, whereas changes in ECF volume are related to body  $\text{Na}^+$  balance.

Another difficult quantitative concept is that of a “steady state.” Students can intuitively appreciate homeostasis in terms of the maintenance of a constant volume and composition of body fluid compartments, which can be attributed to the fact that daily intake and production of substances are matched by an equivalent daily output of those substances. This matching of output to input and production is a steady state, and when that steady state is poised with ideal (i.e., normal) body fluid volumes and composition, the condition is referred to as homeostasis. Whenever there are disturbances of body solute, water, or acid-base balance, a new steady state is generally achieved fairly quickly, but that steady state represents a nonideal steady state, one not equivalent to homeostasis. I have found it very useful to present these concepts to the student in terms of a very important clinical parameter that is used to assess the progress of renal disease—the plasma creatinine concentration or creatinine clearance.

As shown in Fig. 8, for a steady state of creatinine to exist in the body, the rate of creatinine excretion must be equal to the rate of creatinine production by metabolism. This rate of production is  $\sim 1.8$  g for a typical (70–80 kg) adult human on a Western diet. Neglecting the small amount of secretion, we can

**Even with chronic renal failure a patient is in “creatinine balance”**

$$\begin{aligned} \text{Creatinine Excretion} &= \\ \text{Creatine Production} &\approx 1.8 \text{ g/day} \\ &\approx \text{Creatinine Filtration} = \text{GFR} \cdot P_{Cr} \end{aligned}$$

$$P_{Cr} = \frac{\text{constant (1800 mg/day)}}{\text{GFR}}$$

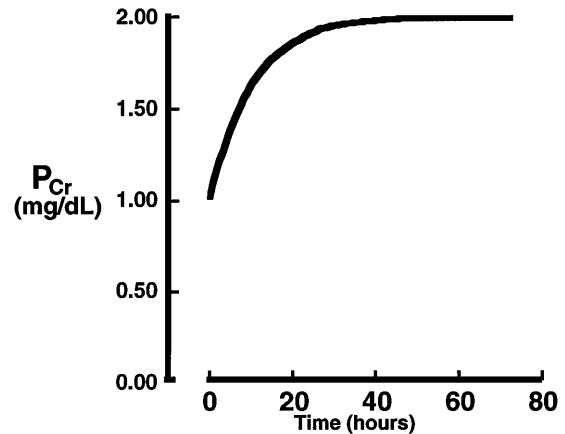
FIG. 8.

Steady-state balance of creatinine in the body persists even in patients with chronic renal failure, but only because the plasma creatinine concentration ( $P_{Cr}$ ) rises to make excretion equal production. This example assumes a daily creatinine production of 1.8 g/day, which is typical for a 70- to 80-kg adult. GFR, glomerular filtration rate.

approximate that the creatinine excretion rate is equal to the creatinine filtration rate, which, in turn, is equal to the product of the glomerular filtration rate (GFR) and the plasma creatinine concentration. This rate of filtration must be equal to the rate of excretion or the rate of production of ~1.8 grams/day. Thus the plasma creatinine concentration is inversely proportional to the GFR as shown by the last equation in Fig. 8. We are all familiar with the graphical form of the last equation in Fig. 8, which shows an asymptotically increasing plasma creatinine concentration with progressive reductions in GFR.

This analysis certainly helps the student to understand why the plasma creatinine concentration is a fairly good indicator of the glomerular filtration rate, provided there are no large fluctuations in diet or metabolic rate. However, I find that most students still have a preconception that, in chronic renal failure, plasma creatinine concentration and BUN are elevated because the kidney is excreting lesser amounts of these by-products of metabolism than are being produced. This assumption seems to be intuitively connected with the idea of kidney failure. However, the student needs to appreciate that a patient with chronic renal failure is actually in creatinine balance, i.e., a steady state with respect to creatinine, but only when the plasma creatinine concentration has increased so that the rate of creatinine filtration is equal to the rate of production.

The question that I challenge the students with is, “How quickly is such a new steady state achieved?” I choose the hypothetical example of removing one kidney and assuming no compensation by the other kidney and no other complications. Most students can quickly determine that the patient will come back into balance when the plasma creatinine concentration has doubled so that at one-half the GFR and double the plasma creatinine concentration, the excretion rate is equal to the normal production rate. How long would it take to attain this new steady state after removal of the one kidney? A rigorous solution of this problem requires compartmental analysis with the solution of a differential equation, the result of which is shown by the plot in Fig. 9. However, I would never attempt to explain it to any student (other than an engineer) in



- For balance to be re-established the creatinine concentration would have to double
- A formal solution requires compartmental analysis and solution of a differential equation
- An approximation of the time required can be appreciated by realizing that the **maximum body creatinine content** would be 10 mg/L x 40 L body water = 400 mg, which is much less than the total daily production of creatinine.

FIG. 9.

**Time required for reestablishment of creatinine steady-state balance if GFR were to be instantaneously reduced from 180 to 90 l/day at time 0. The solution of the compartmental model (see *Quantitative problems are essential*) assumes a daily creatinine production rate of 1.8 g/day, and that creatinine is uniformly distributed in a total body water volume of 40 liters.**

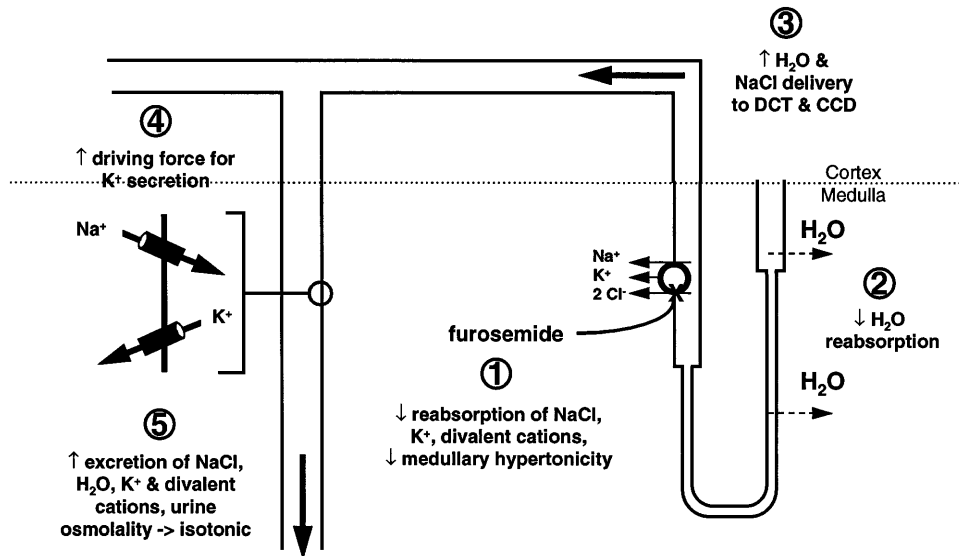


FIG. 10.

Effect of furosemide on salt and water balance and urinary concentrating ability. An example of integrating detailed mechanistic information from all segments of the nephron into an understanding of “whole kidney function” in an important clinical problem, i.e., why do “loop diuretics” tend to cause hypokalemia, divalent cation loss, and diminished urine concentrating ability? DCT, distal convoluted tubule; CCD, cortical collecting duct.

this way. Instead, I attempt to get the student to appreciate the “ball park” rate at which the plasma creatinine concentration would change based on the total body content of creatinine and the rate of its production. As outlined at the bottom of Fig. 9, if one assumes that creatinine is distributed at a concentration of  $\sim 1 \text{ mg/dl}$  uniformly throughout the total body water volume of  $\sim 40$  liters, then the total body content of creatinine would be  $\sim 400 \text{ mg}$ . If the daily rate of creatinine production is  $1,800 \text{ mg/day}$ , the student can then readily appreciate that it will probably take less than one day for the patient to come into a new steady state of creatinine balance, even with this very abrupt decrease in GFR. The important point to be made here is that until end-stage renal disease is reached, the individual with chronic renal failure is really in a steady state with regard to the balance of water and solutes by the kidney. However, this steady state can be achieved only with nonideal or nonhomeostatic fluid volumes and solute concentrations. Again, I find these quantitative details to be of the utmost importance for the student to appreciate what is really meant by salt (or solute) and water balance.

### REVIEW: PUTTING MULTIPLE BASIC MECHANISMS INTO AN INTEGRATED PICTURE OF “WHOLE KIDNEY” FUNCTION

When I review salt and water transport systems in the kidney, I try to provide the student with examples that integrate multiple mechanisms of nephron function and the daily balance of solutes and water by the body. One such example is the effect of the diuretic furosemide, as shown in Fig. 10. Of course, the primary action of furosemide as denoted by *step 1* in Fig. 10 is inhibition of BSC1, which results in decreased reabsorption of  $\text{NaCl}$ ,  $\text{K}^+$ , and divalent cations and also results in a decrease in the medullary hypertonicity. Second, the reduction in medullary hypertonicity decreases osmotic reabsorption of water from the descending limb of the loop of Henle. Third, this decrease in water extraction and salt reabsorption in the loop of Henle results in increased water and salt delivery to the distal convoluted tubule and collecting duct. The presence of an increased  $\text{Na}^+$  concentration enhances the driving force for  $\text{Na}^+$  reabsorption via the amiloride-sensitive  $\text{Na}^+$  channel in the principal

cells of the collecting duct and also depolarizes the luminal membrane so that there is an increase driving force for  $K^+$  secretion. The overall result, shown as *step 5* of Fig. 10, is an increased excretion of NaCl, water,  $K^+$ , and divalent cations, and the urine osmolality approaches isotonicity. The loss of salt and water in turn serves as a stimulus to renin production, leading to increased aldosterone secretion by the adrenal glands, which in turn, enhances *step 4*, i.e., increased reabsorption of  $Na^+$  and secretion of  $K^+$  in the principal cells of the collecting duct. Obviously, exactly the same sequence of events would occur if there were a loss-of-function mutation in BSC1, as occurs in Bartter's syndrome (see above). One can also discuss the changes in reabsorption of  $Ca^{2+}$  and  $Mg^{2+}$  and how the balance of these divalent cations is affected. Using schema such as these, the teacher can review and reinforce many of the important details of the transport processes involved in salt and water reabsorption, while at the same time integrating those processes to develop a comprehensive picture of "whole kidney" physiology and pathophysiology.

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