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Solute and Water Transport Along the Nephron: Tubular Function

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. What three processes are involved in the production of urine?
2. What is the composition of "normal" urine?
3. What transport mechanisms are responsible for NaCl reabsorption by the nephron? Where are they located along the nephron?
4. How is water reabsorption "coupled" to NaCl reabsorption in the proximal tubule?
5. Why are solutes but not water reabsorbed by the thick ascending limb of Henle's loop?
6. What transport mechanisms are involved in secretion of organic anions and cations? What is the physiological relevance of these transport processes?
7. What is glomerulotubular balance, and what is its physiological importance?
8. What are the major hormones that regulate NaCl and water reabsorption by the kidneys? What is the nephron site of action of each hormone?
9. What is the aldosterone paradox?

The formation of urine involves three basic processes: (1) **ultrafiltration** of plasma by the glomerulus, (2) **reabsorption** of water and solutes from the ultrafiltrate, and (3) **secretion** of selected solutes into tubular fluid. Although an average of 115 to 180 L/day in women and 130 to 200 L/day in men of essentially protein-free fluid is filtered by the human glomeruli each day,[a] less than 1% of the filtered water and sodium chloride (NaCl) and variable amounts of other solutes are typically excreted in urine (Table 34.1). By the processes of reabsorption and secretion, the renal tubules determine the volume and composition of urine (Table 34.2), which in turn allows the kidneys to precisely control the volume, osmolality, composition, and pH of the extracellular and intracellular fluid compartments. Transport proteins in cell membranes of the nephron mediate reabsorption and secretion of solutes and water in the kidneys. Approximately 5% to 10% of all human genes code for transport proteins, and genetic and acquired defects in transport proteins are the cause of many kidney diseases (Table 34.3). In addition, numerous transport proteins are important drug targets. This chapter discusses NaCl and water reabsorption, transport of organic anions and cations, the transport proteins involved in solute and water transport, and some of the factors and hormones that regulate NaCl transport. Details on acid-base transport and on K^+, Ca^{++}, and inorganic phosphate (P_i) transport and their regulation are provided in Chapters 35 through 37.

Solute and Water Reabsorption Along the Nephron

The general principles of solute and water transport across epithelial cells were discussed in Chapter 2.

Quantitatively, reabsorption of NaCl and water represent the major function of nephrons. Approximately 25,000 mEq/day of Na^+ and 179 L/day of water are reabsorbed by the renal tubules (see Table 34.1). In addition, renal transport of many other important solutes is linked either directly or indirectly to reabsorption of Na^+. In the following sections, the NaCl and water transport processes of each nephron segment and their regulation by hormones and other factors are presented.

Proximal Tubule

The proximal tubule reabsorbs approximately 67% of water, Na^+, Cl^-, K^+, and most other solutes filtered by the glomerulus. In addition the proximal tubule reabsorbs virtually all the glucose, proteins and amino acids filtered by the glomerulus, as well as most of the HCO_3^-. The key element in proximal tubule reabsorption is Na^+,K^+-ATPase in the basolateral membrane. Reabsorption of every substance, including water, is linked in some manner to the operation of Na^+,K^+-ATPase.

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[a] Normal glomerular filtration rate (GFR) averages 115 to 180 L/day in women and 130 to 200 L/day in men. Thus the volume of the ultrafiltrate represents a volume that is approximately 10 times that of the extracellular fluid volume (ECFV). For simplicity, we assume throughout the remainder of this section that GFR is 180 L/day.

TABLE 34.1 Filtration, Excretion, and Reabsorption of Water, Electrolytes, and Solutes by the Kidneys

Substance	Measure	Filtered[a]	Excreted	Reabsorbed	% Filtered Load Reabsorbed
Water	L/day	180	1.5	178.5	99.2
Na^+	mEq/day	25,200	150	25,050	99.4
K^+	mEq/day	720	100	620	86.1
Ca^{++}	mEq/day	540	10	530	98.2
HCO_3^-	mEq/day	4320	2	4318	99.9+
Cl^-	mEq/day	18,000	150	17,850	99.2
Glucose	mmol/day	800	0	800	100.0
Urea	g/day	56	28	28	50.0

[a] The filtered amount of any substance is calculated by multiplying the concentration of that substance in the ultrafiltrate by the glomerular filtration rate (GFR); for example, the filtered amount of Na^+ is calculated as $[Na^+]$ultrafiltrate (140 mEq/L) × GFR (180 L/day) = 25,200 mEq/day.

TABLE 34.2 Composition of Urine

Substance	Concentration
Na^+	50–130 mEq/L
K^+	20–70 mEq/L
Ammonium (NH_4^+)	30–50 mEq/L
Ca^{++}	5–12 mEq/L
Mg^{++}	2–18 mEq/L
Cl^-	50–130 mEq/L
Inorganic phosphate (Pi)	20–40 mEq/L
Urea	200–400 mmol/L
Creatinine	6–20 mmol/L
pH	5.0–7.0
Osmolality	500–800 mOsm/kg H_2O
Glucose	0
Amino acids	0
Protein	0
Blood	0
Ketones	0
Leukocytes	0
Bilirubin	0

The composition and volume of urine can vary widely in the healthy state. These values represent average ranges. Normal water excretion typically ranges between 0.5 and 1.5 L/day.

Data from Valtin HV. *Renal Physiology.* 2nd ed. Boston: Little, Brown; 1983.

Na^+ Reabsorption

Na^+ is reabsorbed by different mechanisms in the first and the second halves of the proximal tubule. In the first half of the proximal tubule, Na^+ is reabsorbed primarily with bicarbonate (HCO_3^-) and a number of other solutes (e.g., glucose, amino acids, P_i, lactate). In contrast, in the second half, Na^+ is reabsorbed mainly with Cl^-. This disparity is mediated by differences in the Na^+ transport systems in the first and second halves of the proximal tubule and by differences in the composition of tubular fluid at these sites. In absolute terms the first half of the proximal tubule reabsorbs significantly more Na^+ than the second half.

In the first half of the proximal tubule, Na^+ uptake into the cell is coupled with either H^+ or organic solutes, including glucose (Fig. 34.1). Specific transport proteins mediate entry of Na^+ into the cell across the apical membrane. For example, the Na^+/H^+ antiporter, NHE3 (see Fig. 34.1A), couples entry of Na^+ with extrusion of H^+ from the cell. H^+ secretion results in reabsorption of sodium bicarbonate ($NaHCO_3$) (see Chapter 37). Na^+ also enters proximal tubule cells via several symporter mechanisms, including Na^+/glucose (SGLT2), Na^+/amino acid, Na^+/P_i, and Na^+/lactate (see Fig. 34.1B). The glucose and other organic solutes that enter the cell with Na^+ leave the cell across the basolateral membrane via passive transport mechanisms (e.g., GLUT2, a passive glucose transporter). Any Na^+ that enters the cell across the apical membrane leaves the cell and enters the blood via Na^+,K^+-ATPase. Thus reabsorption of Na^+ in the first half of the proximal tubule is coupled to that of HCO_3^- and a number of organic molecules, and this generates a negative transepithelial voltage across the proximal tubule that provides the driving force for the paracellular reabsorption of Cl^-. Reabsorption of many organic molecules, including glucose and lactate, is so avid they are almost completely removed from the tubular fluid in the first half of the proximal tubule (Fig. 34.2). Reabsorption of $NaHCO_3$ and Na^+–organic solutes across the proximal tubule establishes a transtubular osmotic gradient (i.e., the osmolality of the interstitial fluid bathing the basolateral side of the cells is a few mOsm/L higher than the osmolality of tubule fluid) that provides the driving force for the passive reabsorption of water by osmosis. Because more water than Cl^- is reabsorbed in the first half of the proximal tubule, the $[Cl^-]$ in tubular fluid rises along the length of the proximal tubule (see Fig. 34.2).

TABLE 34.3 **Selected Monogenic Renal Diseases Involving Transport Proteins**

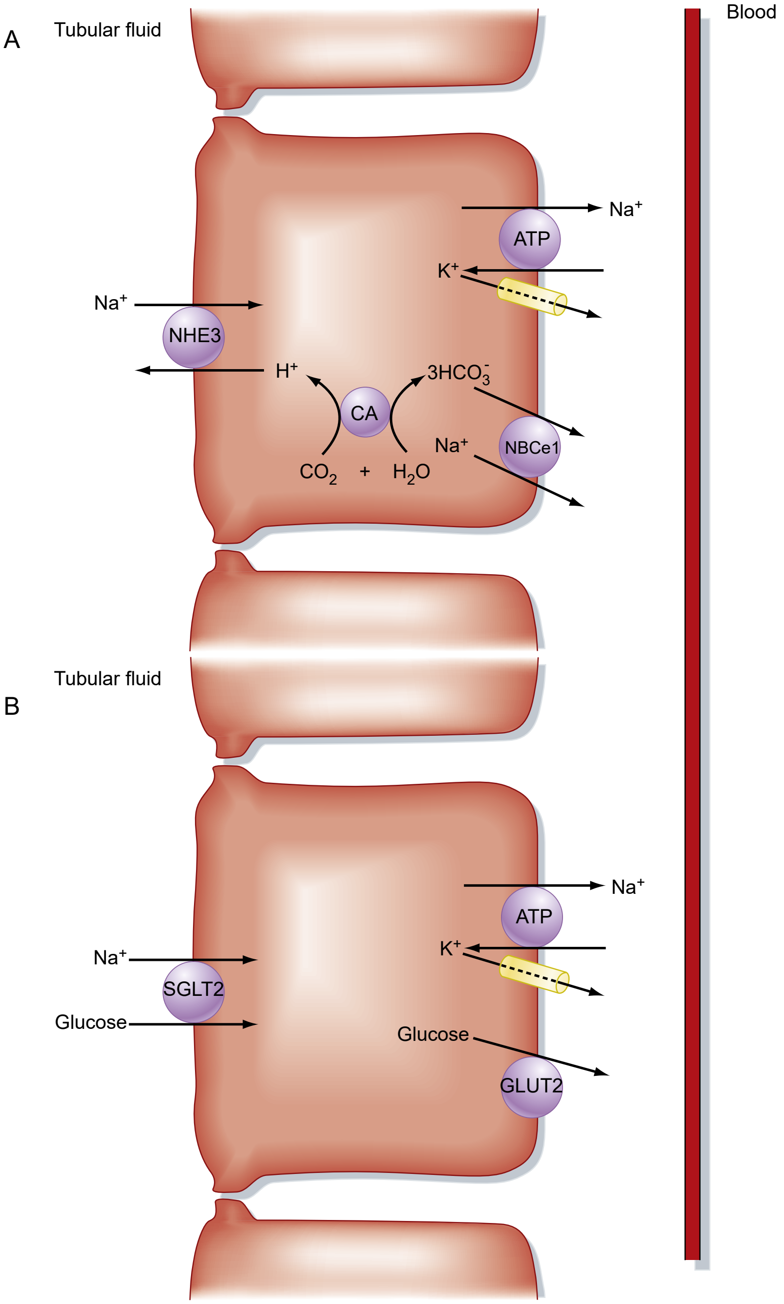
Diseases	Mode of Inheritance	Gene	Transport Protein	Nephron Segment	Phenotype
Cystinuria type I	AR	*SLC3A1, SLC7A9*	Amino acid symporters	Proximal tubule	Increased excretion of basic amino acids, nephrolithiasis (kidney stones)
Proximal renal tubular acidosis (RTA)	AR	*SLC4A4*	Na^+/HCO_3^- symporter	Proximal tubule	Hyperchloremic metabolic acidosis
X-linked nephrolithiasis (Dent's disease)	XLR	*CLCN, OCRL1*	Chloride channel	Distal tubule	Hypercalciuria, nephrolithiasis
Bartter syndrome	AR-type I	*SLC12A1*	$Na^+/K^+/2Cl^-$ symporter	TAL	Hypokalemia, metabolic alkalosis, hyperaldosteronism
	AR-type II	*KCNJ1*	ROMK potassium channel	TAL	Hypokalemia, metabolic alkalosis, hyperaldosteronism
	AR-type III	*CLCNKB*	Chloride channel (basolateral membrane)	TAL	Hypokalemia, metabolic alkalosis, hyperaldosteronism
	AR-type IV	*BSND, CLCNKA CLCNKB*	Subunit of chloride channel, chloride channels	TAL	Hypokalemia, metabolic alkalosis, hyperaldosteronism
Hypomagnesemia-hypercalciuria syndrome	AR	*CLDN16*	Claudin-16, also known as *paracellin 1*	TAL	Hypomagnesemia-hypercalciuria, nephrolithiasis
Gitelman syndrome	AR	*SLC12A3*	Thiazide-sensitive Na^+/Cl^- symporter	Distal tubule	Hypomagnesemia, hypokalemic metabolic alkalosis, hypocalciuria, hypotension
Pseudohypoaldosteronism type I	AR	*SCNN1A, SCNN1B,* and *SCNN1G*	α, β, and γ subunits of ENaC	Collecting duct	Increased excretion of Na^+, hyperkalemia, hypotension
Pseudohypoaldosteronism type II	AD	*MLR*	Mineralocorticoid receptor	Collecting duct	Increased excretion of Na^+ hyperkalemia, hypotension
Liddle syndrome	AD	*SCNN1B, SCNN1G*	β and γ subunits of ENaC	Collecting duct	Decreased excretion of Na^+, hypertension
Nephrogenic diabetes insipidus (NDI) type II	AR/AD	*AQP2*	Aquaporin 2 water channel	Collecting duct	Polyuria, polydipsia, plasma hyperosmolality
Distal renal tubular acidosis	AD/AR	*SLC4A1*	Cl^-/HCO_3^- antiporter	Collecting duct	Metabolic acidosis, hypokalemia, hypercalciuria, nephrolithiasis
Distal renal tubular acidosis	AR	*ATP6N1B*	Subunit of H^+- ATPase	Collecting duct	Metabolic acidosis, hypokalemia, hypercalciuria, nephrolithiasis

There are over 300 different solute transporter genes that form the so-called SLC (solute carrier) family of genes.

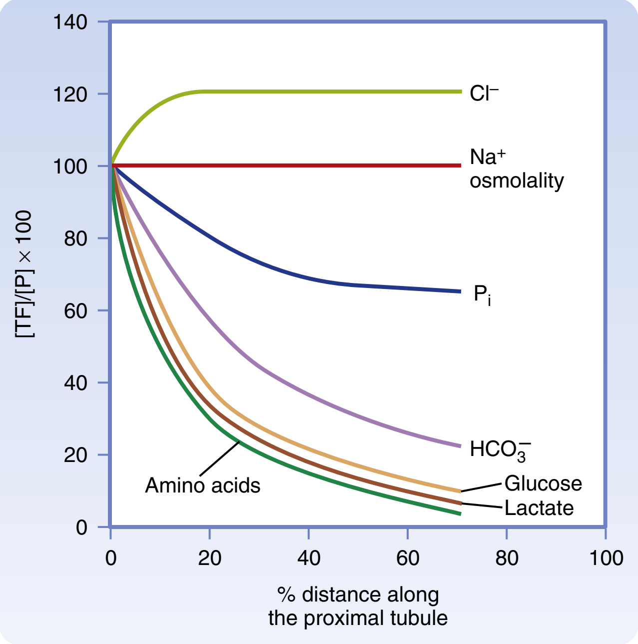
AD, Autosomal dominant; *AR*, autosomal recessive; *ENaC*, epithelial Na^+ channel; *TAL*, thick ascending limb of Henle's loop; *XLR*, X-linked recessive.

Modified from Nachman RH, Glassock RJ. *NephSAP*. 2010;9(3).

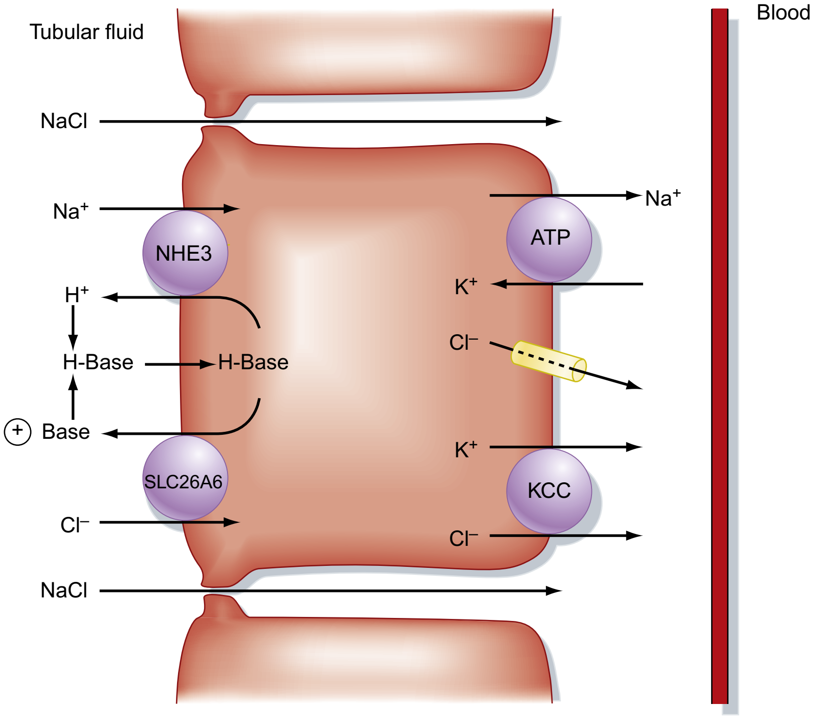
In the second half of the proximal tubule, Na^+ reabsorption is largely accompanied by Cl^- reabsorption via both transcellular and paracellular pathways (Fig. 34.3). Na^+ is primarily reabsorbed with Cl^- rather than organic solutes or HCO_3^- as the accompanying anion because the Na^+ transport mechanisms in the second half of the proximal tubule differ from those in the first half, and because the tubular fluid that enters the second half contains very little glucose or amino acids. In addition the high $[Cl^-]$ (140 mEq/L) in tubule fluid, which is due to preferential reabsorption of Na^+ with HCO_3^- and organic solutes in the first half of the proximal tubule, facilitates reabsorption of Cl^- with Na^+.



• **Fig. 34.1** Na^+ transport processes in the first half of the proximal tubule. These transport mechanisms are present in all cells in the first half of the proximal tubule but are separated into different cells to simplify the discussion. **A**, Operation of the Na^+/H^+ antiporter *(NHE3)* in the apical membrane and the Na^+,K^+-ATPase and HCO_3^- transporters, including the Na^+/HCO_3^- symporter (*NBCe1*; see also Chapter 37) in the basolateral membrane, mediates reabsorption of $NaHCO_3$. Carbon dioxide and water combine inside the cells to form H^+ and HCO_3^- in a reaction facilitated by the enzyme carbonic anhydrase *(CA)*. **B**, Operation of the Na^+/glucose symporter *(SGLT2)* in the apical membrane, in conjunction with Na^+,K^+-ATPase and the glucose transporter *(GLUT2)* in the basolateral membrane, mediates Na^+-glucose reabsorption. Inactivating mutations in the *GLUT2* gene lead to decreased glucose reabsorption in the proximal tubule and glucosuria (i.e., glucose in the urine). Though not shown, Na^+ reabsorption is also coupled with other solutes, including amino acids, P_i, and lactate. Reabsorption of these solutes is mediated by the Na^+/amino acid, Na^+/P_i, and Na^+/lactate symporters, respectively, located in the apical membrane and the Na^+,K^+-ATPase, amino acid, P_i, and lactate transporters, respectively, located in the basolateral membrane. Three classes of amino acid transporters have been identified in the proximal tubule: two that transport Na^+ in conjunction with either acidic or basic amino acids and one that does not require Na^+ and transports basic amino acids.



• **Fig. 34.2** Concentration of solutes in tubule fluid as a function of length along the proximal tubule. *[TF]* is the concentration of the substance in tubular fluid; *[P]* is the concentration of the substance in plasma. Values above 100 indicate that relatively less of the solute than water is reabsorbed, and values below 100 indicate that relatively more of the substance than water is reabsorbed.



• **Fig. 34.3** Na⁺ transport processes in the second half of the proximal tubule. Na⁺ and Cl⁻ enter the cell across the apical membrane through the operation of parallel Na⁺/H⁺*(NHE3)* and Cl⁻-base (e.g., formate, oxalate, and bicarbonate) antiporters *(SLC26A6)*. More than one Cl⁻-base antiporter is involved in this process, but only one is depicted. The secreted H⁺ and base combine in the tubular fluid to form an H-base complex that can recycle across the plasma membrane. Accumulation of the H-base complex in tubular fluid establishes an H-base concentration gradient that favors H-base recycling across the apical plasma membrane into the cell. Inside the cell, H⁺ and the base dissociate and recycle back across the apical plasma membrane. The net result is uptake of NaCl across the apical membrane. The base may be hydroxide ions (OH⁻), formate (HCO_2^-), oxalate, HCO_3^-, or sulfate. The positive transepithelial voltage in the lumen, indicated by the *plus sign* inside the circle in the tubular lumen, is generated by diffusion of Cl⁻ (lumen to blood) across the tight junction. The high [Cl⁻] of tubular fluid provides the driving force for diffusion of Cl⁻. Some glucose is also reabsorbed in the second half of the proximal tubule (not shown) by a mechanism similar to that described in the first half of the proximal tubule, except that the Na⁺/glucose symporter (*SGLT1* gene) transports 2Na⁺ with one glucose and has higher affinity and lower capacity than the Na⁺/glucose symporter in the first part of the proximal tubule, depicted in Fig. 34.1. In addition, glucose exits the cell across the basolateral membrane via GLUT1 rather than via GLUT2 as in the first part of the proximal tubule (not shown). *KCC*, KCl symporter.

IN THE CLINIC

Fanconi syndrome, a renal disease that is either hereditary or acquired, results from an impaired ability of the proximal tubule to reabsorb HCO_3^-, P_i, amino acids, glucose, and low-molecular-weight proteins. Because other downstream nephron segments cannot reabsorb these solutes and protein, Fanconi syndrome results in increased urinary excretion of HCO_3^-, amino acids, glucose, P_i, and low-molecular-weight proteins.

The mechanism of transcellular Na^+ reabsorption in the second half of the proximal tubule is shown in Fig. 34.3. Na^+ enters the cell across the luminal membrane primarily via the parallel operation of a Na^+/H^+ antiporter (NHE3) and one or more Cl^--base antiporters (e.g., SLC26A6). Because the secreted H^+ and base combine in the tubular fluid and reenter the cell, operation of the Na^+/H^+ and Cl^--base antiporters is equivalent to uptake of NaCl from tubular fluid into the cell. Na^+ leaves the cell via Na^+,K^+-ATPase, and Cl^- leaves the cell and enters the blood via a K^+/Cl^- symporter (KCC) and a Cl^- channel in the basolateral membrane.

Some NaCl is also reabsorbed across the second half of the proximal tubule via a **paracellular route.** Paracellular NaCl reabsorption occurs because the rise in $[Cl^-]$ in tubule fluid in the first half of the proximal tubule creates a $[Cl^-]$ gradient (140 mEq/L in the tubule lumen and 105 mEq/L in the interstitium). This concentration gradient favors diffusion of Cl^- from the tubular lumen across the tight junctions into the lateral intercellular space. Movement of the negatively charged Cl^- results in the tubular fluid becoming positively charged relative to blood. This positive transepithelial voltage causes diffusion of positively charged Na^+ out of the tubular fluid across the tight junction into blood. Thus in the second half of the proximal tubule, some Na^+ and Cl^- are reabsorbed across the tight junctions via passive diffusion.

In summary, reabsorption of Na^+ and Cl^- in the proximal tubule occurs via both paracellular and transcellular pathways. Approximately 67% of the NaCl filtered each day by the glomerulus is reabsorbed in the proximal tubule. Of this amount, two-thirds move across the transcellular pathway, whereas the remaining one-third moves across the paracellular pathway (Table 34.4).

Water Reabsorption

The proximal tubule reabsorbs 67% of the filtered water (Table 34.5). The driving force for water reabsorption is a transtubular osmotic gradient established by reabsorption of solute (e.g., NaCl, Na^+-glucose). Reabsorption of Na^+ along with organic solutes, HCO_3^-, and Cl^- from tubular fluid into the lateral intercellular spaces reduces the osmolality of the tubular fluid and increases the osmolality of the lateral intercellular space. The osmotic gradient across the proximal tubule established by these transport processes is only a few mOsm/L (Fig. 34.4). Because the proximal tubule is highly

TABLE 34.4 NaCl Transport Along the Nephron

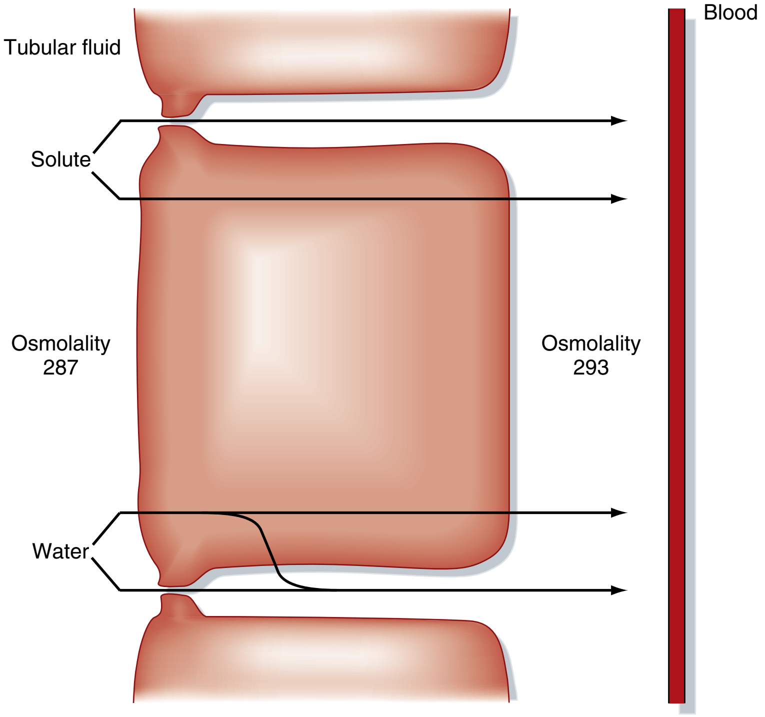
Segment	% Filtered NaCl Reabsorbed	Mechanism of Na^+ Entry Across Apical Membrane	Major Regulatory Hormones
Proximal tubule	67	Na^+/H^+ antiporter (NHE_3), Na^+ symporter with amino acids and organic solutes, paracellular	Angiotensin II Norepinephrine Epinephrine Dopamine
Loop of Henle	25	1 Na^+/1K^+/2Cl^- symporter	Aldosterone Angiotensin II
Distal tubule	≈5	NaCl symporter	Aldosterone Angiotensin II
Late distal tubule and collecting duct	≈3	ENaC	Aldosterone, ANP, BNP, urodilatin, uroguanylin, guanylin, angiotensin II

ANP, Atrial natriuretic peptide; *BNP*, brain natriuretic peptide, *ENaC*, epithelial Na^+ channel.

TABLE 34.5 Water Transport Along the Nephron

Segment	% Filtrate Reabsorbed	Mechanism of Water Reabsorption	Hormones That Regulate Water Permeability
Proximal tubule	67	Passive	None
Loop of Henle	15	Descending thin limb only; passive	None
Distal tubule	0	No water reabsorption	None
Late distal tubule and collecting duct	≈8–17	Passive	AVP, ANP[a], BNP[a]

[a] Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) inhibit vasopressin (AVP)-stimulated water permeability.



• **Fig. 34.4** Routes of reabsorption of water and solute across the proximal tubule. Transport of solutes, including Na^+, Cl^-, and organic solutes, into the lateral intercellular space increases the osmolality of this compartment, which establishes the driving force for osmotic reabsorption of water across the proximal tubule. This occurs because some Na^+,K^+-ATPase and some transporters of organic solutes, HCO_3^-, and Cl^- are located on the lateral cell membranes and deposit these solutes between cells. Furthermore, some NaCl also enters the lateral intercellular space via diffusion across the tight junction (i.e., paracellular pathway). An important consequence of osmotic water flow across the transcellular and paracellular pathways in the proximal tubule is that some solutes, especially K^+ and Ca^{++}, are entrained in the reabsorbed fluid and thereby reabsorbed by the process of solvent drag.

permeable to water, primarily owing to expression of aquaporin water channels (AQP1) in the apical and basolateral membranes, water is reabsorbed across cells by osmosis. In addition the tight junctions in the proximal tubule are also water permeable, so some water is also reabsorbed across the paracellular pathway between proximal tubular cells. Accumulation of fluid and solutes within the lateral intercellular space increases hydrostatic pressure in this compartment. The increased hydrostatic pressure forces fluid and solutes into the capillaries.[b] Thus water reabsorption follows solute reabsorption in the proximal tubule. The reabsorbed fluid is slightly hyperosmotic relative to plasma. However, this difference in osmolality is so small; it is commonly said that proximal tubule reabsorption is isosmotic (i.e., ≈67% of both the filtered solute and water are reabsorbed). Indeed, there is little difference in the osmolality of tubular fluid at the start and end of the proximal tubule. An important consequence of osmotic water flow across the proximal tubule is that some solutes, especially K^+ and Ca^{++}, are entrained in the reabsorbed fluid and thereby reabsorbed by the process of solvent drag (see Fig. 34.4). Reabsorption of virtually all organic solutes, Cl^- and other ions, and water is coupled to Na^+ reabsorption. Therefore changes in Na^+ reabsorption influence reabsorption of water and other solutes by the proximal tubule. This point will be discussed later, notably in Chapter 35, and is especially relevant during volume depletion when increased Na^+ reabsorption by the proximal tubule is accompanied by a parallel increase in HCO_3^- reabsorption, which can contribute to metabolic alkalosis (i.e., volume contraction alkalosis).

Protein Reabsorption

Proteins filtered by the glomerulus are reabsorbed in the proximal tubule. As mentioned previously, peptide hormones, small proteins, and small amounts of larger proteins such as albumin are filtered by the glomerulus. Overall, only a small percentage of proteins cross the glomerulus and enter Bowman's space (i.e., the concentration of proteins in the glomerular ultrafiltrate is only ≈ 40 mg/L). However, the total amount of protein filtered per day is significant because the glomerular filtration rate (GFR) is so high:

Equation 34.1

$$\begin{aligned}\text{Filtered protein} &= \text{GFR} \times [\text{Protein}] \text{ in the ultrafiltrate}\\ \text{Filtered protein} &= 180 \text{ L/day} \times 40 \text{ mg/L}\\ &= 7200 \text{ mg/day, or } 7.2 \text{ g/day}\end{aligned}$$

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[b] In addition, protein oncotic pressure in the peritubular capillaries (π_{pc}) is elevated because of the process of glomerular filtration (see Chapter 33). The elevated π_{pc} facilitates uptake of fluid and solute into the capillary.

Filtered proteins are reabsorbed in the proximal tubule by endocytosis either as intact proteins or after being partially degraded by enzymes on the surface of proximal tubule cells. Once the proteins and peptides are inside the cell, enzymes digest them into their constituent amino acids, which then leave the cell across the basolateral membrane by transport proteins and are returned to the blood. Normally this mechanism reabsorbs virtually all the proteins filtered, and hence the urine is essentially protein free. However, because the mechanism is easily saturated, an increase in filtered proteins can result in **proteinuria** (appearance of protein in urine). Disruption of the glomerular filtration barrier to proteins increases the filtration of proteins and results in proteinuria, which is frequently seen with kidney disease.

Secretion of Organic Anions and Organic Cations

Cells of the proximal tubule also secrete organic anions and organic cations into the tubule fluid. Secretion of organic anions and cations by the proximal tubule plays a key role in regulating the plasma levels of xenobiotics (e.g., a variety of antibiotics, diuretics, statins, antivirals, antineoplastics, immunosuppressants, neurotransmitters, and nonsteroidal anti-inflammatory drugs [NSAIDs]) and toxic compounds derived from endogenous and exogenous sources. Many of the organic anions and cations (Boxes 34.1 and 34.2) secreted by the proximal tubule are end products of metabolism that circulate in plasma. Many of these organic compounds are bound to plasma proteins and thus are not readily filtered. Therefore only a small fraction of these potentially toxic substances are eliminated from the body by excretion resulting from filtration alone. Thus secretion of organic anions and cations, including many toxins from the peritubular capillary into the tubular fluid, promotes elimination of these compounds from plasma entering the kidneys. Hence these substances are removed from plasma by both filtration and secretion. It is important to note that when kidney function is reduced by disease, urinary excretion of organic anions and cations is severely reduced, which can lead to increased plasma levels of xenobiotics and potentially toxic accumulation of organic anions and cations.

• BOX 34.1 Some Organic Anions Secreted by Proximal Tubule

Endogenous Anions

cAMP, cGMP
Bile salts
Hippurates
Oxalate
Prostaglandins: PGE_2, $PGF_{2\alpha}$
Urate
Vitamins: ascorbate, folate

Drugs

Acetazolamide
Acyclovir
Amoxicillin
Captopril
Chlorothiazide
Furosemide
Losartan
Penicillin
Probenecid
Salicylate (aspirin)
Hydrochlorothiazide
Simvastatin
Bumetanide
Nonsteroidal anti-inflammatory drugs (NSAIDs): indomethacin

cAMP, Cyclic adenosine monophosphate; *cGMP*, cyclic guanosine monophosphate.

• BOX 34.2 Some Organic Cations Secreted by Proximal Tubule

Endogenous

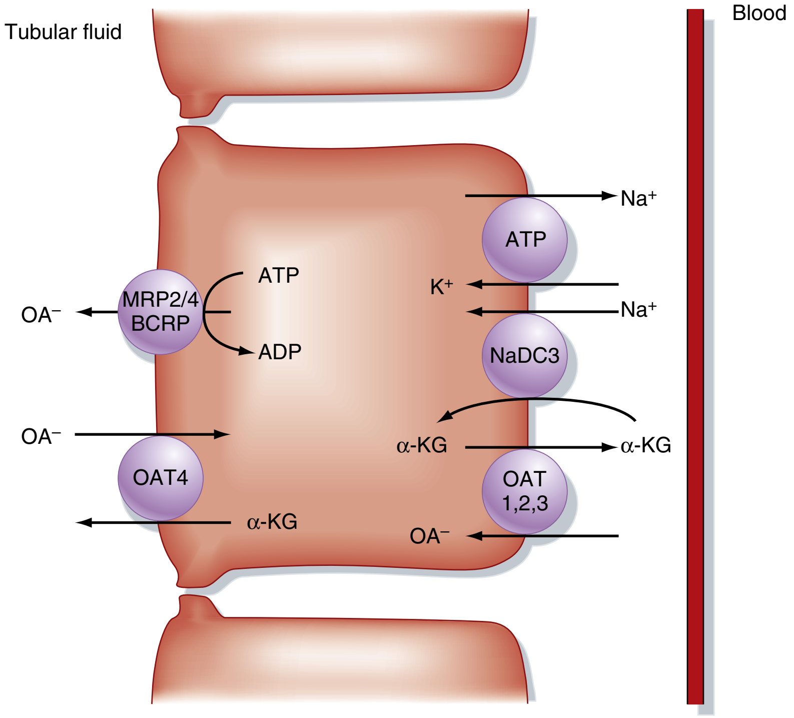
Creatinine
Dopamine
Epinephrine
Norepinephrine

Drugs

Atropine
Isoproterenol
Cimetidine
Morphine
Quinine
Amiloride
Procainamide

AT THE CELLULAR LEVEL

Water channels called **aquaporins (AQPs)** mediate transcellular reabsorption of water across many nephron segments. To date, 13 aquaporins have been identified. The AQP family is divided into two groups based on their permeability characteristics. One group (aquaporins) is permeable to water (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, AQP8, AQP11, and AQP12). The other group (aquaglyceroporins) is permeable to water and small solutes, especially glycerol (AQP3, AQP7, AQP9, AQP10). Aquaporins form tetramers in the plasma membrane of cells, with each subunit forming a water channel. In the kidneys, AQP1 is expressed in the apical and basolateral membranes of the proximal tubule and in portions of the descending thin limb of Henle's loop. The importance of AQP1 in renal water reabsorption is underscored by studies in which the *AQP1* gene was "knocked out" in mice. These mice exhibit increased urine output (polyuria) and reduced ability to concentrate urine. In addition the osmotic water permeability of the proximal tubule is fivefold less in mice lacking APQ1 than in normal mice. AQP7 and AQP8 are also expressed in the proximal tubule. AQP2 is expressed in the apical plasma membrane of principal cells in the collecting duct, and its abundance in the membrane is regulated by arginine vasopressin (AVP) (see Chapter 35). AQP3 and AQP4 are expressed in the basolateral membrane of principal cells in the collecting duct, and mice deficient in these AQPs (i.e., AQP3 and AQP4 knockout mice) have defects in the ability to concentrate urine (see Chapter 35).



• **Fig. 34.5** Secretion of organic anion *(OA⁻)* across the proximal tubule. OA⁻s enter the cell across the basolateral membrane by one of three OA⁻/α-ketoglutarate *(α-KG)* antiporter mechanisms (organic anion transporters, *OAT1, OAT2, OAT3*). Uptake of α-KG into the cell against its chemical concentration gradient is driven by movement of Na^+ into the cell via the Na^+-dicarboxylate transporter *(NaDC3)*. The $[Na^+]$ inside the cell is low because of the Na^+,K^+-ATPase in the basolateral membrane, which transports Na^+ out of the cell in exchange for K^+. The α-KG recycles across the basolateral membrane on the *OATs* in exchange for OA⁻. OA⁻s leave the cell across the apical membrane by multidrug drug resistance proteins *(MRP2 and 4)*, and by breast cancer resistance protein *(BCRP)*, which require *ATP*. *OAT4* in the apical membrane reabsorbs urate, an organic anion.

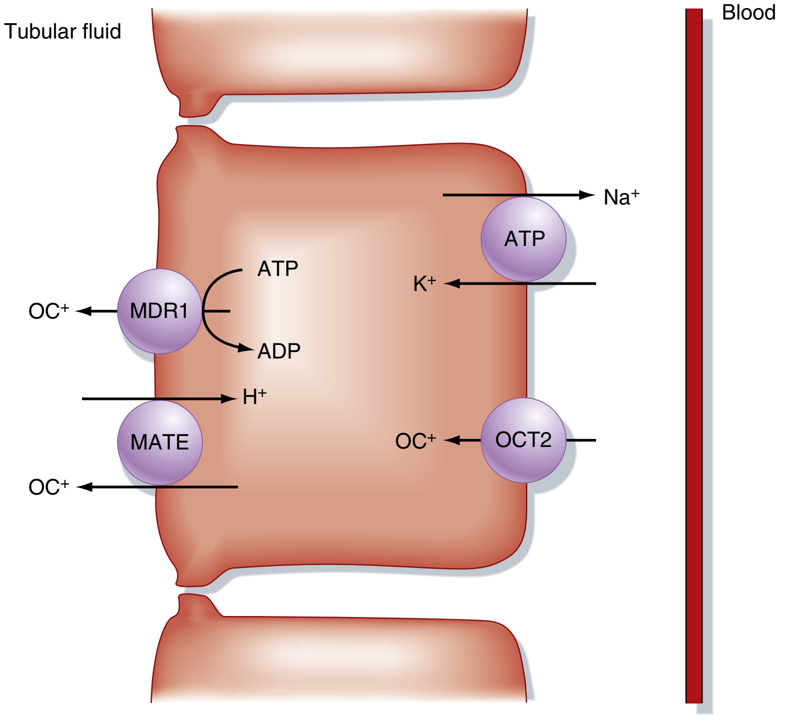
AT THE CELLULAR LEVEL

The endocytosis of proteins by the proximal tubule is mediated by apical membrane proteins that specifically bind proteins and peptides in tubule fluid. These receptors, called **multiligand endocytic receptors**, can bind a wide range of peptides and proteins and thereby mediate their endocytosis. **Megalin** and **cubilin** mediate protein and peptide endocytosis in the proximal tubule. Both are glycoproteins, with megalin being a member of the low-density lipoprotein receptor gene family.

Fig. 34.5 illustrates the mechanisms of organic anion (OA⁻) transport across the proximal tubule. These secretory pathways have maximum transport rates, low specificity (i.e., they transport many OA⁻s), and are responsible for secretion of the OA⁻s listed in Box 34.1. OA⁻s are taken up into the cell across the basolateral membrane against their chemical gradient in exchange for α-ketoglutarate (α-KG) via several OA⁻/α-KG antiporters, including OAT1, OAT2, and OAT3. α-KG accumulates inside the cells via metabolism of glutamate and by a Na^+/α-KG symporter (i.e., the Na^+/dicarboxylate transporter [NaDC3]) also present in the basolateral membrane. Thus uptake of OA⁻ into the cell against an electrochemical gradient is coupled to the exit of α-KG out of the cell, down its chemical gradient generated by the Na^+/α-KG symporter mechanism. The exit

IN THE CLINIC

Urinalysis is an important and routine tool for detection of kidney disease. A thorough analysis of urine includes macroscopic, microscopic, and biochemical assessments. This is performed by visual assessment of the urine, microscopic examination of urinary sediment, and biochemical evaluation of urinary composition using dipstick reagent strips. The dipstick test is both inexpensive and fast (i.e., <5 minutes) and tests urine for both pH and the presence of many substances (e.g., bilirubin, blood, glucose, ketones, protein). It is normal to find trace amounts of protein in urine, particularly concentrated urine. Urinary proteins are derived from two principal sources: (1) filtration exceeding the reabsorptive capacity of the proximal tubule and (2) synthesis and secretion of **Tamm-Horsfall glycoprotein** by the thick ascending limb of Henle's loop. Because the mechanism for protein reabsorption is "upstream" of the thick ascending limb (i.e., in the proximal tubule), the secreted Tamm-Horsfall glycoprotein appears in urine. Proteinuria in greater than trace amounts is often indicative of renal disease.



• **Fig. 34.6** Organic cation *(OC^+)* secretion across the proximal tubule. OC^+s enter the cell across the basolateral membrane primarily by *OCT2*. Uptake of OC^+s into the cell against their chemical concentration gradient is driven by the cell-negative potential difference. OC^+s leave the cell across the apical membrane in exchange with H^+ by electrically neutral multidrug and toxin transporters (MATE1 and MATE2-K) and by multidrug resistance protein *(MDR1)*, which requires *ATP*.

of OA^-s across the luminal membrane into the tubular fluid is mediated by multidrug resistance proteins 2 and 4 (MRP2/4) and breast cancer resistance protein 1 (BCRP), which require adenosine triphosphate (ATP) for their operation. Recent studies reveal that OAT4 mediates reabsorption of the organic anion urate, the end product of purine catabolism, by the proximal tubule (see Fig. 34.5).

Fig. 34.6 illustrates the mechanism of organic cation (OC^+) transport across the proximal tubule. Organic cations, including xenobiotics such as the antidiabetic agent metformin, the antiviral agent lamivudine, and the anticancer drug oxaliplatin, and many important monoamine neurotransmitters including dopamine, epinephrine, histamine, and norepinephrine are secreted by the proximal tubule. Organic cations are taken up into the cell across the basolateral membrane, primarily by the organic cation transporter 2 (OCT2). Uptake of organic cations is driven by the magnitude of the cell-negative potential difference across the basolateral membrane. Organic cation transport across the luminal membrane into the tubular fluid, which is the rate-limiting step in secretion, is mediated primarily by electroneutral multidrug and toxin extrusion transporters (MATEs) and MDR1 (also known as *P-glycoprotein*), which requires ATP for its operation. These transport mechanisms are nonspecific, and several organic cations usually compete for secretion via a given transport pathway.

IN THE CLINIC

Because many organic anions compete for the same secretory pathways, elevated plasma levels of one transported anion often inhibit secretion of the others. For example, infusing *p*-aminohippuric acid (PAH) can reduce secretion of penicillin by the proximal tubule. Because the kidneys are responsible for eliminating penicillin, infusion of PAH into individuals receiving penicillin reduces penicillin excretion and thereby extends its biological half-life. In World War II, when penicillin was in short supply, hippurates were given with penicillin to extend its therapeutic effect. Similar competition is observed for organic cation secretion by the proximal tubule, and elevated plasma levels of one transported cation species can inhibit secretion of the other competing cations. For example, the histamine H_2 antagonist cimetidine used to treat gastric ulcers is secreted via organic cation transport mechanisms in the proximal tubule. If cimetidine is given to patients receiving procainamide (an organic cation used to treat cardiac arrhythmias), procainamide and cimetidine secretion is reduced by direct competition for a common secretory pathway. As a consequence, coadministration of cationic drugs competing for the same pathway can increase the plasma concentration of both drugs to levels much higher than those observed when the drugs are given alone. This effect can lead to drug toxicity.

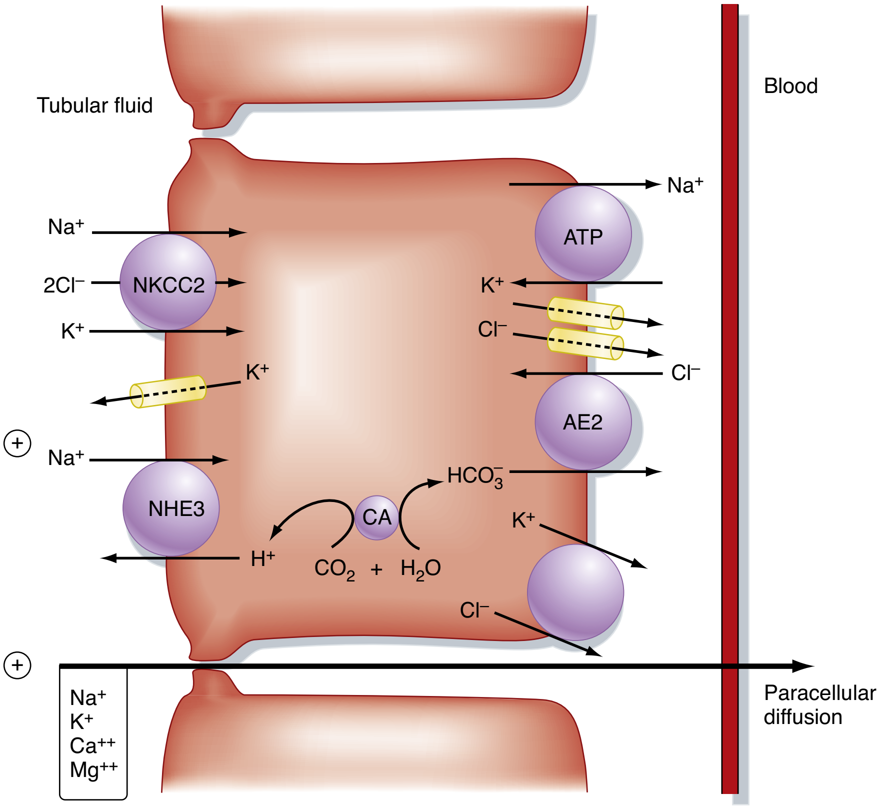
Henle's Loop

Henle's loop reabsorbs approximately 25% of the filtered NaCl and 15% of the filtered water. Reabsorption of NaCl in the loop of Henle occurs in both the thin and thick ascending limbs, whereas the descending thin limb does not reabsorb NaCl. In contrast, water reabsorption mediated by AQP1 water channels is exclusively restricted to the descending thin limb, whereas the ascending limb is impermeable to water. In addition, divalent cations (e.g., Ca^{++} and Mg^{++}) and HCO_3^- are also reabsorbed in the loop of Henle (see Chapters 36 and 37 for more details).

The thin ascending limb reabsorbs NaCl by a passive mechanism. Reabsorption of water, but not NaCl, in the descending thin limb increases [NaCl] in the tubule fluid entering the ascending thin limb. As the NaCl-rich fluid moves toward the cortex, NaCl diffuses out of the tubule lumen across the ascending thin limb and into the medullary interstitial fluid, down a concentration gradient directed from the tubule fluid to the interstitium (see Chapter 35 for details).

The key element in reabsorption of solute by the thick ascending limb is Na^+,K^+-ATPase in the basolateral membrane (Fig. 34.7). As with reabsorption in the proximal tubule, reabsorption of every solute by the thick ascending limb is linked to Na^+,K^+-ATPase activity. This transporter maintains a low intracellular [Na^+], which provides a favorable chemical gradient for the movement of Na^+ from tubular fluid into the cell. This movement of Na^+ across the apical membrane into the cell is mediated by the $1Na^+/1K^+/2Cl^-$ symporter (NKCC2), which couples the movement of $1Na^+$ with $1K^+$ and $2Cl^-$. Using the potential energy released by the downhill movement of Na^+ and Cl^-, this symporter drives the uphill movement of K^+ into the cell. K^+ channels (ROMK and Maxi-K) in the apical plasma membrane play an important role in reabsorption of NaCl by the thick ascending limb. These K^+ channels allow the K^+ transported into the cell via the $1Na^+/1K^+/2Cl^-$ symporter to recycle back into tubule fluid. Because the [K^+] in tubule fluid is relatively low, K^+ recycling is required for continued operation of the $1Na^+/1K^+/2Cl^-$ symporter. A Na^+/H^+ antiporter (NHE3) in the apical cell membrane also mediates Na^+ reabsorption as well as H^+ secretion (HCO_3^- reabsorption) in the thick ascending limb (see also Chapter 37). The operation of the Na^+/H^+ antiporter in the apical membrane results in cellular uptake of Na^+ in exchange for H^+. The production of H^+ inside cells generates HCO_3^-, which exits the cell across the basolateral membrane via a Cl^-/HCO_3^- antiporter (AE2). Na^+ leaves the cell across the basolateral membrane via the Na^+,K^+-ATPase, whereas K^+ and Cl^- leave the cell via separate pathways in the basolateral membrane (i.e., K^+ and Cl^- channels and the K^+/Cl^- symporter).

The voltage across the thick ascending limb is important for reabsorption of several cations. The tubular fluid is



• **Fig. 34.7** Transport mechanisms for NaCl reabsorption in the thick ascending limb of the loop of Henle. The positive voltage in the lumen plays a major role in driving the passive paracellular reabsorption of cations. Because the apical membrane is conductive primarily to K^+, the apical membrane voltage is more negative than the basolateral membrane voltage, which is conductive to K^+ and Cl^-, thereby resulting in a lumen positive transepithelial potential. Mutations in the apical membrane K^+ channel (ROMK), the apical membrane $1Na^+/1K^+/2Cl^-$ symporter *(NKCC2)*, or the basolateral Cl^- channel *(ClCNKB)* cause Bartter syndrome (see the clinical box on Bartter syndrome). *CA*, Carbonic anhydrase.

positively charged relative to blood because of the unique location of transport proteins in the apical and basolateral membranes. Two points are important: (1) increased NaCl transport by the thick ascending limb increases the magnitude of the positive voltage in the lumen and (2) this voltage is an important driving force for reabsorption of several cations, including Na^+, K^+, Mg^{++}, and Ca^{++}, across the paracellular pathway (see Fig. 34.7). The importance of the paracellular pathway to solute reabsorption is underscored by the observation that inactivating mutations of the tight junction protein claudin-16 reduce reabsorption of Mg^{++} and Ca^{++} by the ascending thick limb, even in the presence of a lumen positive transepithelial voltage.

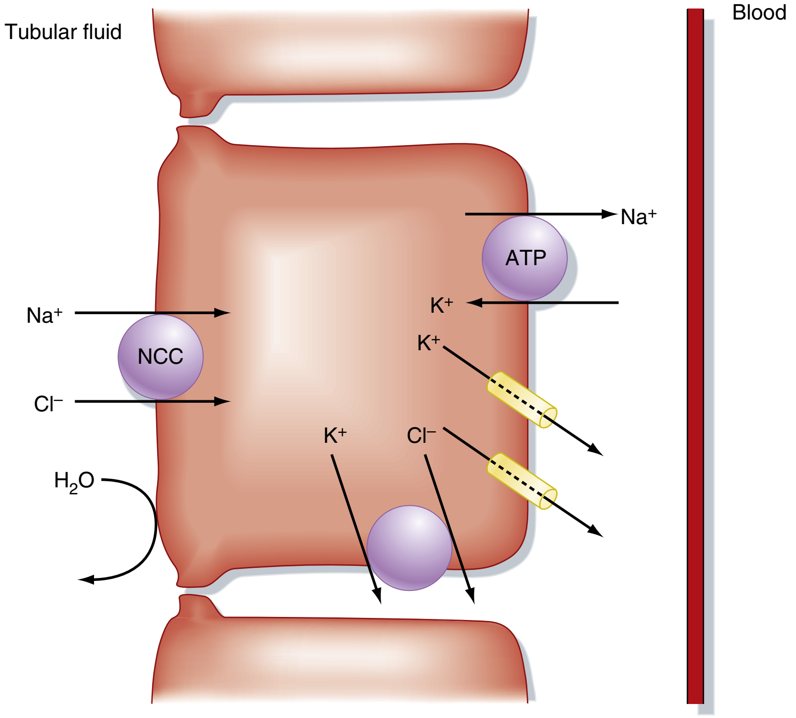
In summary, NaCl reabsorption across the thick ascending limb occurs via transcellular and paracellular pathways. Fifty percent of NaCl reabsorption is transcellular, and 50% is paracellular. Because the thick ascending limb does not reabsorb water, owing to a lack of water channels (i.e., AQPs), reabsorption of NaCl and other solutes reduces the osmolality of tubular fluid to less than 150 mOsm/kg H_2O. Thus because the thick ascending limb of Henle's loop produces a fluid that is dilute relative to plasma, this segment and the adjacent distal tubule (as discussed next) are often collectively referred to as the **"diluting segments."**

Distal Tubule and Collecting Duct

The distal tubule and collecting duct reabsorb approximately 8% of the filtered NaCl, secrete variable amounts of K^+ and H^+, and reabsorb a variable amount of water (≈8%–17%). The initial segment of the distal tubule (early distal tubule) reabsorbs Na^+, Cl^-, and Ca^{++} and is impermeable to water (Fig. 34.8). Entry of NaCl into the cell across the apical membrane is mediated by a Na^+/Cl^- symporter, NCC (see Fig. 34.8). Na^+ leaves the cell via the action of Na^+,K^+-ATPase, and Cl^- leaves the cell via diffusion through Cl^- channels and a K^+/Cl^- symporter (KCC4). Thus dilution of tubular fluid begins in the thick ascending limb and continues in the early segment of the distal tubule.

AT THE CELLULAR LEVEL

As described in Chapter 2, epithelial cells are joined at their apical surfaces by tight junctions (zonula occludens). A number of proteins have now been identified as components of the tight junction, including proteins that span the membrane of one cell and link to the extracellular portion of the same molecule in the adjacent cell (e.g., occludin and claudins), as well as cytoplasmic linker proteins (e.g., ZO-1, ZO-2, and ZO-3) that link the membrane-spanning proteins to the cytoskeleton of the cell. Of these junctional proteins, claudins appear to be major determinants of the permeability characteristics of tight junctions. For example, claudin-16 and claudin-19 are critical determinants of divalent cation permeability of the tight junctions in the thick ascending limb of Henle's loop. Mutations in human claudin-16 and claudin-19 cause familial hypomagnesemia (i.e., low plasma $[Mg^{++}]$) with hypercalciuria (i.e., increased Ca^{++} in the urine) and nephrocalcinosis (i.e., calcification of the kidney). Claudin-2 is permeable to water and may be responsible for paracellular water reabsorption across the proximal tubule. Claudin-4 controls the permeability of the tight junction to Na^+, whereas claudin-15 determines whether a tight junction is permeable to cations or anions. Thus the permeability characteristics of the tight junctions in different nephron segments are determined at least in part by the specific claudins expressed by the cells in that segment.



• **Fig. 34.8** Transport mechanism for NaCl reabsorption in the early segment of the distal tubule. This segment is impermeable to water. Mutations in the apical membrane NaCl symporter *(NCC)* cause Gitelman syndrome.

AT THE CELLULAR LEVEL

Bartter syndrome is a set of autosomal recessive genetic diseases characterized by hypokalemia, metabolic alkalosis, and hyperaldosteronism (see Table 34.3). Inactivating mutations in the gene coding for the $1Na^+/1K^+/2Cl^-$ symporter (NKCC2), the apical K^+ channel (ROMK), or the basolateral Cl^- channel (ClCNKB) decrease both NaCl reabsorption and K^+ reabsorption by the ascending thick limb, which in turn causes hypokalemia (i.e., low plasma $[K^+]$) and a decrease in ECFV. The fall in ECFV stimulates aldosterone secretion, which in turn stimulates NaCl reabsorption and H^+ secretion by the distal tubule and collecting duct.

The last segment of the distal tubule (late distal tubule) and the collecting duct are composed of three cell types: **principal cells** and two types of **intercalated cells**. As illustrated in Fig. 34.9, principal cells reabsorb NaCl and water and secrete K^+. Both Na^+ reabsorption and K^+ secretion by these cells depend on the activity of Na^+,K^+-ATPase in the basolateral membrane. By maintaining a low intracellular $[Na^+]$, the Na^+,K^+-ATPase provides a favorable chemical gradient for movement of Na^+ from tubular fluid into the cell. Because Na^+ enters the cell across the apical membrane via diffusion through epithelial Na^+-selective channels (ENaCs), the negative voltage inside the cell facilitates entry of Na^+, which then exits the cell and enters the blood via the basolateral membrane Na^+,K^+-ATPase. Reabsorption of Na^+ generates a negative luminal voltage across the late distal tubule and collecting duct, which provides the driving force for paracellular reabsorption of Cl^-. Intercalated cells secrete either H^+ or HCO_3^- and play important roles in acid-base homeostasis (see Chapter 37). The α-intercalated cell (see Fig. 34.9, *center*) secretes H^+ and reabsorbs both HCO_3^- and K^+ and is thus important in regulating acid-base balance (see Chapter 37) and K^+ balance (see Chapter 36). α-Intercalated cells reabsorb K^+ by the operation of an H^+,K^+-ATPase (HKA) located in the apical plasma membrane. In contrast, β-intercalated cells (see Fig. 34.9, *bottom*) secrete HCO_3^- and reabsorb both H^+ and Cl^-. Chloride enters the β-intercalated cell across the apical membrane via a Cl^-/HCO_3^- antiporter (pendrin, SLC26A4) and leaves the cell across the basolateral membrane via a Cl^- channel. β-Intercalated cells also reabsorb NaCl. This process involves the tandem operation of pendrin and an apical membrane $Na^+/HCO_3^-/2Cl^-$ antiporter (NDCBE). This mechanism of NaCl is inhibited by thiazide diuretics (see Chapter 37 for more details). A variable amount of water is reabsorbed across principal cells in the late distal tubule and collecting duct. Water reabsorption in these segments is mediated by the AVP-regulated AQP2 water channel located in the apical plasma membrane and by AQP3 and AQP4 located in the basolateral membrane of principal cells. In the presence of AVP, water is reabsorbed. By contrast, in the absence of AVP the late distal tubule and collecting duct reabsorb little water (see Chapter 35).

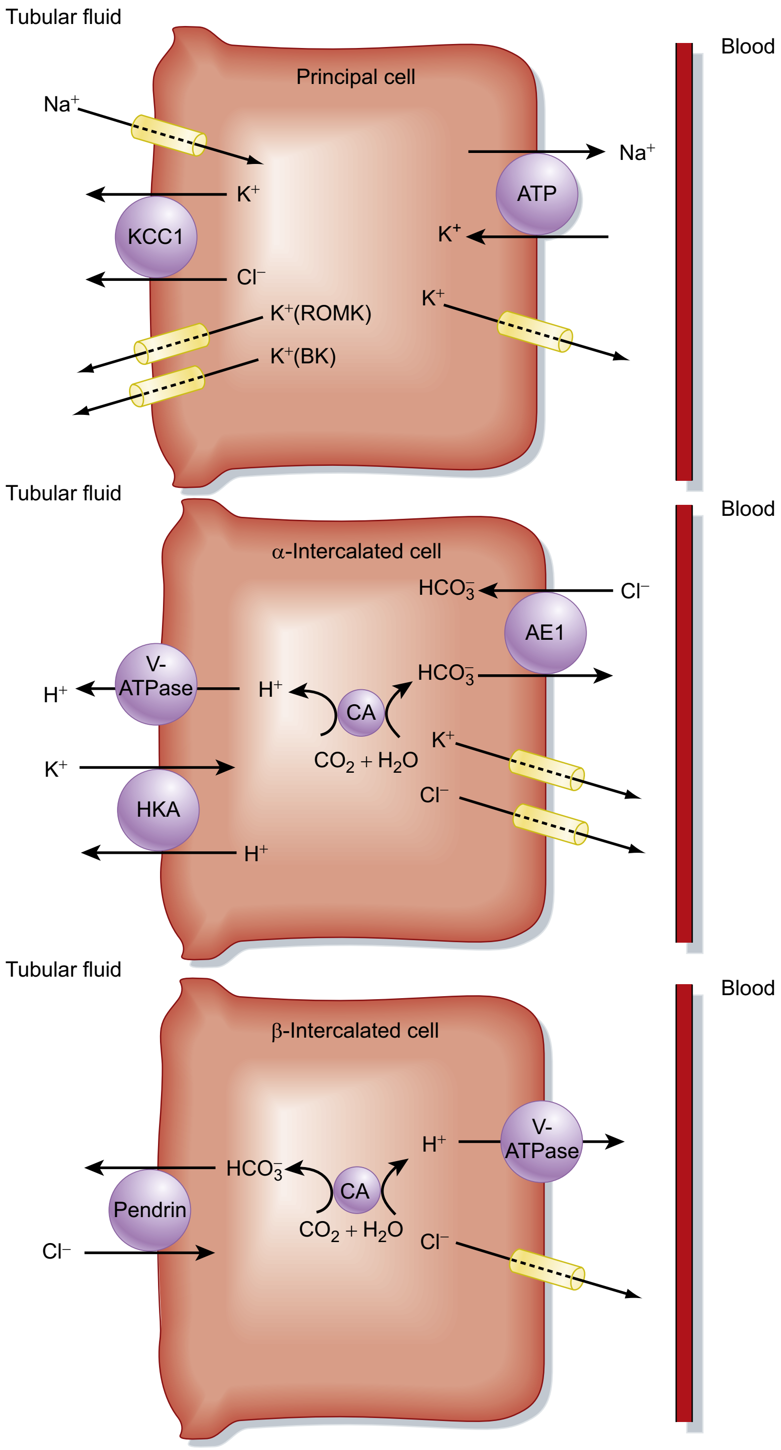
K^+ is secreted from blood into tubular fluid by principal cells in two steps (see Fig. 34.9, *top*). First, uptake of K^+ across the basolateral membrane is mediated by the Na^+,K^+-ATPase. Second, K^+ leaves the cell via passive diffusion. Because $[K^+]$ inside principal cells is high (≈150 mEq/L) and $[K^+]$ in tubular fluid is low (≈10 mEq/L), K^+ diffuses down its concentration gradient through apical cell membrane K^+ channels (ROMK and BK) into tubular fluid. Although the negative potential inside these cells favors intracellular K^+ retention, the electrochemical gradient across the apical membrane promotes secretion of K^+ from the cell into tubular fluid (see Chapter 36). In contrast, K^+ reabsorption by α cells is mediated by an H^+,K^+-ATPase (HKA) located in the apical cell membrane (see Fig. 34.9, *center*). As a consequence, these distal nephron segments possess the ability to both secrete and reabsorb K^+ via independently regulated mechanisms, which contrasts with the general tendency to reabsorb Na^+ along most nephron segments.

Regulation of NaCl and Water Reabsorption

Quantitatively, angiotensin II, aldosterone, catecholamines, natriuretic peptides, and uroguanylin are the most important hormones regulating NaCl reabsorption and thereby urinary NaCl excretion (Table 34.6). However, other hormones (including dopamine and adrenomedullin), Starling forces, and the phenomenon of glomerulotubular balance also influence NaCl reabsorption. AVP is the only major hormone that directly regulates the amount of water excreted by the kidneys.

Angiotensin II has a potent stimulatory effect on the isosmotic reabsorption of NaCl and water in the proximal tubule. It also stimulates reabsorption of Na^+ in the thick ascending limb of Henle's loop, as well as the late distal tubule and collecting duct. A decrease in ECFV activates the renin-angiotensin-aldosterone system (see Chapter 35 for more details), thereby increasing the plasma concentration of angiotensin II.

Aldosterone is synthesized by the glomerulosa cells of the adrenal cortex and stimulates reabsorption of NaCl by the thick ascending limb of Henle's loop, the late distal tubule, and the collecting duct. Most of aldosterone's effect on NaCl reabsorption reflects its action on the late distal tubule and collecting duct (collectively referred to as the ***aldosterone-sensitive distal nephron*** **(ASDN)** [see Chapters 35 and 37]). Aldosterone enhances reabsorption of NaCl across principal cells in these segments by four mechanisms: (1) increasing the amount of Na^+,K^+-ATPase in the basolateral membrane; (2) increasing expression of the sodium channel (ENaC) in the apical cell membrane; (3) elevating Sgk1 (***s***erum ***g***lucocorticoid-stimulated ***k***inase; see the molecular box) levels, which also increases the expression of ENaC in the apical cell membrane; and (4) stimulating CAP1 (***c***hannel-***a***ctivating ***p***rotease, also called *prostatin*), a serine protease that directly activates ENaCs by proteolysis. Taken together, these actions increase Na^+ uptake across the apical cell membrane and facilitate Na^+ exit from the cell interior into blood. The increase in reabsorption of Na^+ generates a negative transepithelial luminal voltage across the late distal tubule and the collecting duct. This negative voltage in the lumen provides the electrochemical driving force for reabsorption of Cl^- across the tight junctions. Aldosterone also stimulates secretion of K^+ by



• **Fig. 34.9** Transport pathways in principal cells, α-intercalated cells, and β-intercalated cells of the late segment of the distal tubule and collecting duct. *CA*, Carbonic anhydrase. Principal cells reabsorb Na^+ and secrete K^+. K^+ is secreted by two types of K^+ channels (ROMK and BK) and by a K^+/Cl^- symporter *(KCC1)*. α-Intercalated cells secrete H^+ and reabsorb HCO_3^- and K^+, and β-intercalated cells secrete HCO_3^- and reabsorb H^+ and Cl^-.

ASDN. Aldosterone secretion is increased by hyperkalemia and by hypovolemia (i.e., reduced ECFV via increased angiotensin II following activation of the renin-angiotensin system [RAS]; see Chapter 35 for more details). Aldosterone secretion is decreased by hypokalemia and natriuretic peptides (discussed in more detail next). Through its stimulation of NaCl reabsorption in the collecting duct, aldosterone also indirectly increases water reabsorption by this nephron segment.

The aldosterone paradox. As noted earlier, aldosterone stimulates both NaCl reabsorption and K^+ secretion by the collecting duct. Although both a reduction in the ECFV (i.e., hypovolemia, see Chapter 35) and hyperkalemia (see

TABLE 34.6 **Hormones That Regulate NaCl and Water Reabsorption**

Hormone[a]	Major Stimulus	Nephron Site of Action	Effect on Transport
Angiotensin II	↑Renin	PT, TAL, DT/CD	↑NaCl and H_2O reabsorption
Aldosterone	↑Angiotensin II, ↑$[K^+]_p$	TAL, DT/CD	↑NaCl and H_2O reabsorption[b]
ANP, BNP, urodilatin	↑ECFV	CD	↓H_2O and NaCl reabsorption
Uroguanylin, guanylin	Oral ingestion of NaCl	PT, CD	↓H_2O and NaCl reabsorption
Sympathetic nerves	↓ECFV	PT, TAL, DT/CD	↑NaCl and H_2O reabsorption[b]
Dopamine	↑ECFV	PT	↓H_2O and NaCl reabsorption
AVP	↑P_{osm}, ↓ECFV	DT/CD	↑H_2O reabsorption

[a] All these hormones act within minutes, except aldosterone, which exerts its action on reabsorption of NaCl with a delay of 1 hour. Aldosterone achieves its maximal effect after a few days.

[b] The effect on reabsorption of H_2O does not include the TAL.

ANP, Atrial natriuretic peptide; *BNP*, brain natriuretic peptide, *BP*, blood pressure; *CD*, collecting duct; *DT*, distal tubule; *ECFV*, extracellular fluid volume; $[K^+]_p$, plasma K⁺ concentration; P_{osm}, plasma osmolality; *PT*, proximal tubule; *TAL*, thick ascending limb.

TABLE 34.7 **Aldosterone Paradox**

Condition	Aldosterone	AII	Sodium Reabsorption Upstream of the CD	ENaC activity	ROMK	Urine Sodium	Urine Potassium
⇓ ECFV	⇑	⇑	⇑	⇑	⇓	⇓	NC
Hyperkalemia	⇑	NC	⇓	⇑	⇑	NC	⇑

CD, Collecting duct; *ENaC*, epithelial Na^+-selective channel.

Chapter 36) increase aldosterone levels, the physiological response of the kidneys differs in these two conditions. In the setting of ECFV depletion, NaCl excretion by the kidneys is reduced to restore ECFV, without an accompanying change in K^+ excretion. By contrast, during hyperkalemia, K^+ excretion by the kidneys is increased to normalize plasma $[K^+]$, albeit without an accompanying change in NaCl excretion. This phenomenon—the apparent independent effects of aldosterone on urinary Na^+ and K^+ excretion—is called the **aldosterone paradox.** The paradox can be explained by the observation that ECFV depletion increases aldosterone release via activation of the renin-angiotensis system (RAS), whereas hyperkalemia directly stimulates adrenal release of aldosterone without a requirement for RAS activation. As such, aldosterone increases in both conditions, whereas angiotensin II levels increase only during ECFV depletion and not during hyperkalemia. It is the differential regulation of transport processes by aldosterone and angiotensin II that accounts for this paradox. The integrated physiological response to a reduction in ECFV is depicted in Table 34.7. During hypovolemia, angiotensin II stimulates NaCl reabsorption by the proximal tubule and early distal tubule. Aldosterone stimulates ENaC-mediated Na^+ reabsorption in principal cells of the collecting duct. In parallel, angiotensin II inhibits K^+ secretion via ROMK, thereby preventing increased K^+ excretion despite elevated aldosterone levels, which would be expected to promote K^+ secretion. Angiotensin II stimulation of proximal tubule NaCl and water reabsorption also reduces delivery of NaCl and fluid to the collecting duct, which also suppresses K^+ secretion in this segment (see Chapter 36 for more details). The corresponding integrated physiological response to hyperkalemia is depicted in Table 34.6. During hyperkalemia, aldosterone stimulates ROMK-mediated K^+ secretion by principal cells in the collecting duct, which increases urinary K^+ excretion. Urine Na^+ excretion is unchanged because, in the absence of elevated angiotensis II, Na^+ reabsorption by NCC in the early distal tubule is reduced, an effect that opposes the effect of aldosterone to stimulate ENaC in the collecting duct.

Atrial natriuretic peptide (ANP) and **brain natriuretic peptide (BNP)** inhibit NaCl and water reabsorption. Secretion of ANP by the cardiac atria and BNP by the cardiac ventricles are both stimulated by increased ECFV and increased myocardial wall pressure. ANP and BNP reduce blood pressure by decreasing total peripheral resistance and enhancing urinary excretion of both NaCl and water, primarily by increasing renal blood flow (RBF) and GFR. These natriuretic peptides vasodilate the afferent arterioles and vasoconstrict the efferent arterioles, which increases GFR and thus filtration of NaCl, thereby increasing NaCl excretion (see later discussion of glomerulotubular balance for the mechanism). In addition the increase in RBF decreases the concentration of NaCl in the medullary interstitium, which in turn reduces passive NaCl reabsorption by the thin ascending limb of Henle's loop (see earlier discussion for details on NaCl reabsorption by this segment). ANP and BNP also inhibit NaCl reabsorption by the medullary portion of the collecting duct and inhibit AVP-stimulated water reabsorption across the collecting duct. Moreover, ANP and BNP also reduce secretion of AVP from the posterior pituitary. These actions of ANP and BNP are mediated by the activation of membrane-bound guanylyl cyclase receptors, which increases intracellular levels of the second messenger cyclic guanine monophosphate (cGMP). ANP is a more profound natriuretic and diuretic agent than BNP.

Urodilatin and ANP are encoded by the same gene and have similar amino acid sequences. Urodilatin is a 32–amino acid hormone that differs from ANP by the addition of four amino acids to the amino terminus. Urodilatin is secreted by the distal tubule and collecting duct and is not present in the systemic circulation; thus urodilatin influences only the function of the kidneys. Secretion of urodilatin is stimulated by a rise in blood pressure and an increase in ECFV. It inhibits NaCl and water reabsorption across the medullary portion of the collecting duct. Urodilatin is a more potent natriuretic and diuretic hormone than ANP because some of the ANP that enters the kidneys in blood is degraded by a neutral endopeptidase that has no corresponding effect on urodilatin.

AT THE CELLULAR LEVEL

Sgk1 (***s***erum ***g***lucocorticoid-stimulated ***k***inase), a serine/threonine kinase, plays an important role in maintaining NaCl and K^+ homeostasis by regulating excretion of both NaCl and K^+ by the kidneys. Studies in Sgk1 knockout mice reveal that this kinase is required for animals to survive severe NaCl restriction and K^+ loading. NaCl restriction and K^+ loading enhance plasma [aldosterone], which rapidly (in minutes) increases Sgk1 protein expression and phosphorylation. Phosphorylated Sgk1 enhances ENaC-mediated Na^+ reabsorption in the collecting duct, primarily by increasing the number of ENaCs in the apical plasma membrane of principal cells and also by increasing the number of Na^+,K^+-ATPase pumps in the basolateral membrane. Phosphorylated Sgk1 inhibits Nedd4-2, a ubiquitin ligase that monoubiquitinylates ENaC subunits, thereby targeting them for endocytic removal from the plasma membrane and subsequent destruction in lysosomes. Inhibition of Nedd4-2 by Sgk1 reduces the monoubiquitinylation of ENaC, thereby reducing endocytosis and increasing the number of channels in the membrane. Sgk1 induces the translocation of K^+ channels (ROMK) from an intracellular pool to the plasma membrane, and thereby enhances ROMK-mediated K^+ secretion by principal cells. These effects of Sgk1 precede the aldosterone-stimulated increase in ENaC, ROMK, and Na^+,K^+-ATPase abundance, which leads to a delayed (>4 hours) secondary increase in NaCl and K^+ transport by the collecting duct. Activating polymorphisms in Sgk1 cause an increase in blood pressure, presumably by enhancing NaCl reabsorption by the collecting duct, which increases the ECFV and thereby blood pressure.

IN THE CLINIC

Liddle syndrome is a rare genetic disorder characterized by an increase in blood pressure (i.e., hypertension) secondary to an increase in ECFV. Liddle syndrome is caused by activating mutations in either the β or γ subunit of the epithelial Na^+ channel (ENaC). These mutations increase the number of Na^+ channels in the apical cell membrane of principal cells and thereby the amount of Na^+ reabsorbed. In Liddle syndrome, the rate of renal Na^+ reabsorption is inappropriately high, which leads to an increase in ECFV and hypertension. There are two different forms of **pseudohypoaldosteronism (PHA)** (i.e., the kidneys reabsorb NaCl as they do when aldosterone levels are low; however, in PHA, aldosterone levels are elevated). The autosomal recessive form is caused by inactivating mutations in the α, β, or γ subunit of ENaC. The cause of the autosomal dominant form is an inactivating mutation in the mineralocorticoid receptor. Pseudohypoaldosteronism is characterized by an increase in Na^+ excretion, a reduction in ECFV, hyperkalemia, and hypotension. Some individuals with expanded ECFV and elevated blood pressure are treated with drugs that inhibit **angiotensin-converting enzyme (ACE)** (e.g., Captopril, Enalapril, Lisinopril) and thereby lower fluid volume and blood pressure. Inhibition of ACE blocks degradation of angiotensin I to angiotensin II and thereby lowers plasma angiotensin II levels. The decline in plasma angiotensin II concentration has three effects. First, NaCl and water reabsorption by the nephron (especially the proximal tubule) falls. Second, aldosterone secretion decreases, thus reducing NaCl reabsorption in the thick ascending limb, distal tubule, and collecting duct. Third, because angiotensin is a potent vasoconstrictor, a reduction in its concentration permits the systemic arterioles to dilate and thereby lower arterial blood pressure. ACE also degrades the vasodilator hormone bradykinin; thus ACE inhibitors increase the concentration of bradykinin, a vasodilatory hormone. ACE inhibitors decrease ECFV and the arterial blood pressure by promoting renal NaCl and water excretion and by reducing total peripheral resistance.

Uroguanylin and **guanylin** are produced by neuroendocrine cells in the intestine in response to oral ingestion of NaCl. These hormones enter the circulation and inhibit NaCl and water reabsorption by the kidneys via activation of membrane-bound guanylyl cyclase receptors, which increase intracellular [cGMP]. The involvement of these gut-derived hormones helps explain why the natriuretic response of the kidneys to an oral NaCl load is more pronounced than when delivered intravenously.

Catecholamines stimulate reabsorption of NaCl. Catecholamines released from the sympathetic nerves (norepinephrine) and the adrenal medulla (epinephrine) stimulate reabsorption of NaCl and water by the proximal tubule, thick ascending limb of the loop of Henle, distal tubule, and collecting duct. Although sympathetic nerves are not active when ECFV is normal, when ECFV declines (e.g., after hemorrhage), sympathetic nerve activity rises and dramatically stimulates reabsorption of NaCl and water by these four nephron segments.

Dopamine, a catecholamine, is released from dopaminergic nerves in the kidneys and is also synthesized by cells of the proximal tubule. The action of dopamine is opposite to that of norepinephrine and epinephrine. Secretion of dopamine is stimulated by an increase in ECFV, and its secretion directly inhibits reabsorption of NaCl and water in the proximal tubule.

Adrenomedullin is a 52–amino acid peptide hormone that is produced by a variety of organs, including the kidneys. Adrenomedullin induces a marked diuresis and natriuresis, and its secretion is stimulated by congestive heart failure and hypertension. The major effect of adrenomedullin on the kidneys is to increase GFR and RBF and thereby indirectly stimulate excretion of NaCl and water (see earlier discussion about ANP and BNP).

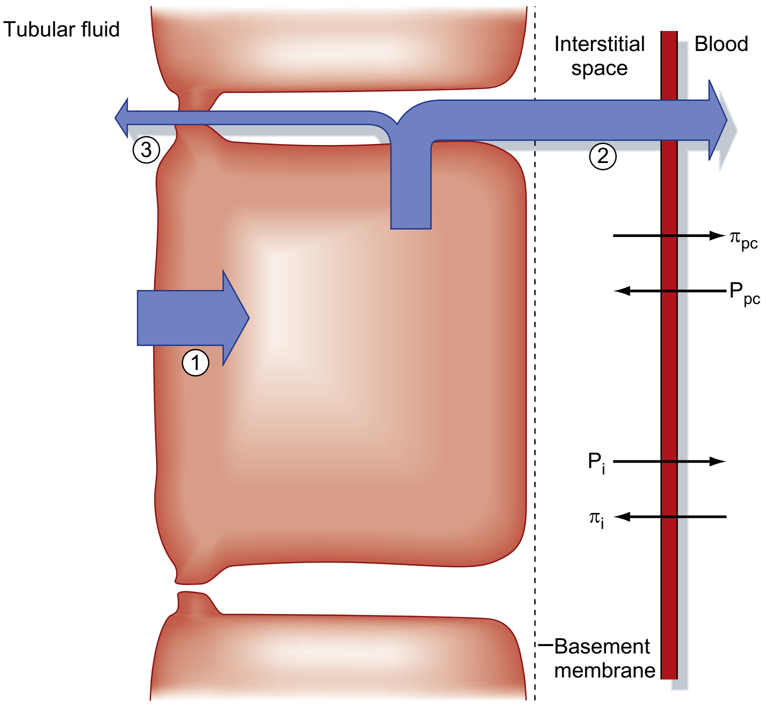
Arginine vasopressin (AVP) is the most important hormone that regulates the reabsorption of water in the kidneys (see Chapter 35). This hormone is secreted by the posterior pituitary gland in response to an increase in plasma osmolality (1% or more) or a decrease in ECFV (>5%–10% from steady-state). AVP increases the permeability of the collecting duct to water. It increases reabsorption of water by the collecting duct because of the osmotic gradient that exists across the wall of the collecting duct (see Chapter 35). AVP has little effect on urinary NaCl excretion.

Starling forces regulate reabsorption of NaCl and water across the proximal tubule. As previously described, Na^+, Cl^-, HCO_3^-, amino acids, glucose, and water are transported into the intercellular space of the proximal tubule. Starling forces between this space and the peritubular capillaries facilitate movement of the reabsorbed fluid into the capillaries. Starling forces across the wall of peritubular capillaries consist of hydrostatic pressure in the peritubular capillary (P_{pc}) and lateral intercellular space (P_i) and oncotic pressure in the peritubular capillary (π_{pc}) and lateral intercellular space (π_i). Thus reabsorption of water as a result of transport of Na^+ from tubular fluid into the lateral intercellular space is modified by the Starling forces. Accordingly:

Equation 34.2

$$J = K_f\left[\left(P_i - P_{pc}\right) + \sigma\left(\pi_{pc} - \pi_i\right)\right]$$

where J is flow (positive numbers indicate flow from the intercellular space into blood). Starling forces that favor movement from the interstitium into the peritubular capillaries are π_{pc} and P_i (Fig. 34.10). The opposing Starling forces are π_i and P_{pc}. Normally the sum of the Starling forces favors movement of solute and water from the interstitial space into the capillary. However, some of the solutes and fluid that enter the lateral intercellular space leak back into



• Fig. 34.10 Starling forces modify proximal tubule solute and water reabsorption. *(1)* Solute and water are reabsorbed across the apical membrane. This solute and water then cross the lateral cell membrane. Some solute and water reenters the tubule fluid *(3)*, and the remainder enters the interstitial space and then flows into the capillary *(2)*. The width of the arrows is directly proportional to the amount of solute and water moving by pathways 1 to 3. Starling forces across the capillary wall determine the amount of fluid flowing through pathway 2 versus pathway 3. Transport mechanisms in the apical cell membranes determine the amount of solute and water entering the cell (pathway 1). P_i, Interstitial hydrostatic pressure; P_{pc}, peritubular capillary hydrostatic pressure; π_i, interstitial fluid oncotic pressure; π_{pc}, peritubular capillary oncotic pressure. Thin arrows across the capillary wall indicate the direction of water movement in response to each force.

the proximal tubular fluid. Starling forces do not affect transport by the loop of Henle, distal tubule, and collecting duct because these segments are less permeable to water than the proximal tubule.

A number of factors can alter the Starling forces across the peritubular capillaries surrounding the proximal tubule. For example, dilation of the efferent arteriole increases P_{pc}, whereas constriction of the efferent arteriole decreases it. An increase in P_{pc} inhibits solute and water reabsorption by increasing back-leak of NaCl and water across the tight junction, whereas a decrease stimulates reabsorption by decreasing back-leak across the tight junction.

Peritubular capillary oncotic pressure (π_{pc}) is partially determined by the rate of formation of the glomerular ultrafiltrate. For example, if one assumes a constant plasma flow in the afferent arteriole, the plasma proteins become less concentrated in the plasma that enters the efferent arteriole and peritubular capillary as less ultrafiltrate is formed (i.e., as GFR decreases). Hence, π_{pc} decreases. Thus π_{pc} is directly related to the **filtration fraction** (FF = GFR/renal plasma flow [RPF]). A fall in the FF resulting from a decrease in GFR, at constant RPF, decreases π_{pc}. This in turn increases the backflow of NaCl and water from the lateral intercellular space into tubular fluid and thereby decreases net reabsorption of solute and water across the proximal tubule. An increase in FF has the opposite effect.

The importance of Starling forces in regulating solute and water reabsorption by the proximal tubule is underscored by the phenomenon of **glomerulotubular (G-T) balance.** Spontaneous changes in GFR markedly alter the filtered amount of Na^+ (filtered Na^+ = GFR × $[Na^+]$ in the filtered fluid). Without rapid adjustments in Na^+ reabsorption to counter the changes in filtration of Na^+, urinary excretion of Na^+ would fluctuate widely and disturb the Na^+ balance of the body and thus alter ECFV and blood pressure (see Chapter 35 for more details). However, spontaneous changes in GFR do not alter Na^+ excretion in urine or Na^+ balance when ECFV is normal because of the phenomenon of G-T balance. When body Na^+ balance is normal (i.e., ECFV is normal), *G-T balance* refers to the fact that reabsorption of Na^+ and water increases in proportion to the increase in GFR and filtered amount of Na^+. Thus a constant fraction of the filtered Na^+ and water is reabsorbed from the proximal tubule despite variations in GFR. The net result of G-T balance is to reduce the impact of changes in GFR on the amount of Na^+ and water excreted in urine when ECFV is normal.

Two mechanisms are responsible for G-T balance. One is related to the oncotic and hydrostatic pressure differences between the peritubular capillaries and the lateral intercellular space (i.e., Starling forces). For example, an increase in the GFR (at constant RPF) raises the protein concentration in glomerular capillary plasma above normal. This protein-rich plasma leaves the glomerular capillaries, flows through the efferent arterioles, and enters the peritubular capillaries. The increased π_{pc} augments the movement of solute and fluid from the lateral intercellular space into the peritubular capillaries. This action increases net solute and water reabsorption by the proximal tubule.

The second mechanism responsible for G-T balance is initiated by an increase in the filtered amount of glucose and amino acids. As discussed earlier, reabsorption of Na^+ in the first half of the proximal tubule is coupled to that of glucose and amino acids. The rate of Na^+ reabsorption therefore partially depends on the filtered amount of glucose and amino acids. As the GFR and filtered amount of glucose and amino acids increase, reabsorption of Na^+ and water also rises.

In addition to G-T balance, another mechanism minimizes changes in the filtered amount of Na^+. As discussed in Chapter 33, an increase in GFR (and thus in the amount of Na^+ filtered by the glomerulus) activates the *tubuloglomerular feedback mechanism.* This action returns the GFR and filtration of Na^+ to normal values. Thus spontaneous changes in GFR (e.g., caused by changes in posture and blood pressure) increase the amount of Na^+ filtered for only a few minutes. The mechanisms that underlie G-T balance maintain urinary Na^+ excretion constant and thereby maintain Na^+ homeostasis (and ECFV and blood pressure) until the GFR returns to normal.

Key Points

1. The four major segments of the nephron (proximal tubule, Henle's loop, distal tubule, and collecting duct) determine the composition and volume of urine by the processes of selective reabsorption of solutes and water and secretion of some solutes.
2. Tubular reabsorption of substances filtered by the glomerulus allows the kidneys to retain substances that are essential and regulate their levels in plasma by altering the degree to which they are reabsorbed. Reabsorption of Na^+, Cl^-, other anions, and organic anions and cations together with water constitutes the major function of the nephron. Approximately 25,200 mEq of Na^+ and 179 L of water are reabsorbed each day. Proximal tubule cells reabsorb 67% of the glomerular ultrafiltrate, and cells of Henle's loop reabsorb about 25% of the NaCl that was filtered and about 15% of the water that was filtered. The distal segments of the nephron (distal tubule and collecting duct system) have a more limited reabsorptive capacity. However, although the proximal tubule reabsorbs the largest fraction of the filtered solutes and water (i.e., 67%), final adjustments in the composition and volume of urine and most of the regulation by hormones and other factors occur primarily in the distal tubule and collecting duct.
3. Secretion of substances from the blood into tubular fluid is a means for excreting various byproducts of metabolism,

and it also serves to eliminate exogenous organic anions and cations (e.g., drugs) and toxins from the body. Many organic anions and cations are bound to plasma proteins and are therefore unavailable for filtration. Thus secretion is their major route of excretion in urine.

4. Various hormones (including angiotensin II, aldosterone, AVP, ANP, BNP, urodilatin, uroguanylin, guanylin, and dopamine), sympathetic nerves, and Starling forces regulate reabsorption of NaCl by the kidneys. AVP is the major hormone that regulates water reabsorption.