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Clinical Implications of Renal Physiology

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Summary

- Besides maintaining a stable acid base, electrolyte, and fluid status of the body, kidneys also have an important endocrine role in producing and secreting 1,25-dihydroxycholecalciferol (calcitriol), renin, and erythropoietin
- More than 98% of water in the filtered urine is reabsorbed in the tubules; 90–95% of water is reabsorbed as it follows sodium (Na^+), which is avidly reabsorbed by the Na^+-deficient epithelial cells, except in the collecting duct (CD), where 5–10% of water is reabsorbed (independent of Na+) under the direct influence of vasopressin or antidiuretic hormone (ADH)
- The tubular epithelial cells constantly lose three Na^+ and gain two potassium (K^+) ions from the basolateral membrane (due to the Na+-K+-ATPase), which keeps these cells deficient in Na^+
- The countercurrent mechanism, which is dependent on the impermeability of the thick ascending limb (TAL) to water, leads to the creation of an increasing osmotic gradient from the cortex to the deeper medulla, which in turn enables ADH to reabsorb water in the CD
- Aldosterone helps in Na^+ reabsorption in the CD and also leads (directly) to K^+ and (indirectly) to hydrogen (H^+) secretion into urine
- All diuretics act by inhibiting tubular reabsorption of Na^+
- Formation of urine begins in the glomerular capillaries where the filtrate has to cross the three filtration layers: endothelium, glomerular basement membrane (GBM) and the foot processes of the podocytes; all these three layers are negatively charged and hence repel anionic proteins like albumin
- Nephrotic syndrome, which is marked by abnormally increased filtration of plasma proteins in the urine, may be due to widening of the pores in the three filtration layers, but is almost always associated with loss of negative charges in these layers
- Nephritis, which is due to glomerular inflammation, is characterized by haematuria with red blood cell (RBC) casts and dysmorphic RBCs, some degree of oliguria, hypertension, and reduction in glomerular filtration rate
- Goodpasture's disease, which is characterized by antibodies against subtype of type IV collagen, can lead to nephritis and haemoptysis, as this particular collagen is found predominantly in the GBM and alveolar membranes of the lungs
- Familial hypocalciuric hypercalcaemia, which manifests with hypercalcaemia, characteristically low urinary calcium and normal to high serum parathyroid hormone (PTH) level, is due to mutation in the calcium-sensing receptor (found in the kidney and parathyroid gland) leading to abnormally increased renal

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reabsorption of calcium and inappropriate secretion of PTH
- Distal renal tubular acidosis (type 1 RTA) is due to an inability of the distal tubules to excrete H^+, whilst proximal renal tubular acidosis (type 2 RTA) is due to an inability of the proximal tubules to reabsorb bicarbonate (HCO_3^-)
- Metabolic acidosis leads to hyperkalaemia and vice versa

The kidneys are paired retroperitoneal structures that are normally located between the transverse processes of the T12–L3 vertebrae, with the left kidney typically somewhat more superior in position than the right. Each kidney has an outer cortex and an inner medulla which protrudes into the pelvis. The pelvis is practically the funnel-shaped dilated upper end of the ureter.

The kidney maintains a stable acid base, electrolyte, and fluid status inside the body by selective elimination or retention of water, electrolytes, and other solutes (Table 1.1). It does so by three mechanisms:

1. Filtration of blood in the glomerulus to form an ultrafiltrate (water with low molecular weight solutes) which then enters the tubule.
2. Selective reabsorption of water, electrolytes, and solutes from the tubules into the interstitium and peritubular capillaries.
3. Selective secretion from the peritubular capillaries across the tubular epithelium into the tubular fluid.

Besides these mechanisms, the kidneys also play an active endocrine role by the production and secretion of:

- *1,25-dihydroxycholecalciferol*: cholecalciferol is derived from 7-dehydrocholesterol in the skin on exposure to the ultraviolet rays in sunlight. Cholecalciferol then undergoes two subsequent hydroxylations by 25-hydroxylase in the liver and 1-hydroxylase in the proximal tubules of the kidney to yield 1,25-dihydroxycholecalciferol (calcitriol). Calcitriol is the active form of vitamin D, without which calcium cannot be absorbed from the intestine.
- *Renin*: discussed later under juxtaglomerular apparatus.
- *Erythropoietin (EPO)*: specialized interstitial cells in the inner cortex and outer medulla of the kidney produce and secrete EPO, which stimulates red blood cell (RBC) production in the bone marrow.

Table 1.1 Urine to plasma ratios of some physiologically important body substances

Substance	Urine to plasma ratio
Glucose	0
Sodium	0.6
Urea	60
Creatinine	150

The kidney consists of nephrons with the supporting interstitium, collecting ducts (CDs), and the renal microvasculature. The nephron consists of a glomerulus and a twisted tubule which drains into the CD. The tubule consists of a proximal and a distal tubule connected by Henle's loop [1]. Each kidney has approximately one million nephrons and we cannot develop new nephrons after birth.

Cortex

This is the outer layer of the kidney and all the glomeruli are located here (Figure 1.1). The tubules of the superficial and midcortical nephrons are situated entirely within the cortex. The juxtamedullary nephrons (in the deeper regions of the cortex and nearer the medulla) have longer tubules and their loop of Henle goes down into the medulla and helps in the countercurrent mechanism, as discussed later.

Medulla

The renal medulla contains the loops of Henle of the juxtamedullary nephrons, vasa recta (peritubular capillaries surrounding the long loops of the juxtamedullary nephrons), and the CDs. The medulla consists of 7–10 conical subdivisions called pyramids, whose broad base faces the cortex, and the apical papilla points into the minor calyx. After traversing through the pyramid, the CDs open at the papilla and drain the urine into the minor calyx. Two or three minor calyces converge to form a major calyx, through

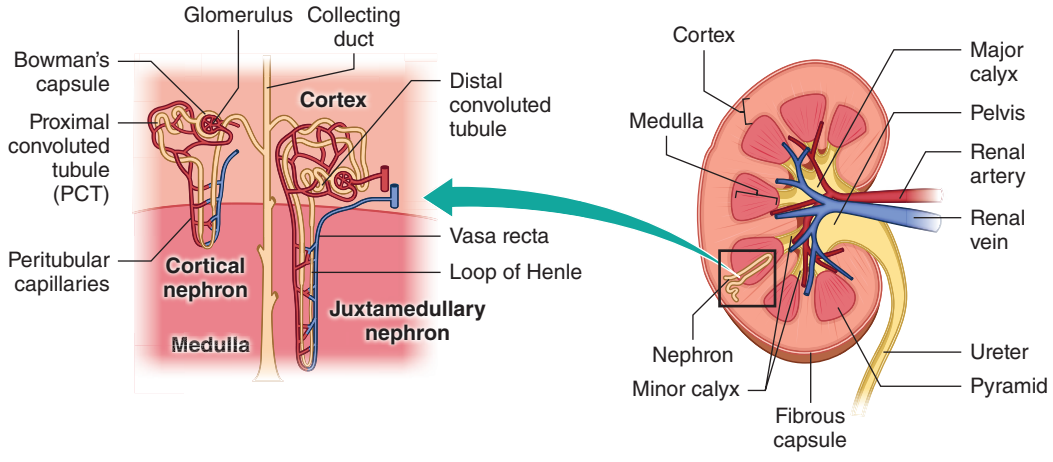

Figure 1.1 The renal cortex, medulla (with the pyramids), and minor and major calyces with their relation to the ureter and renal vasculature.

which urine continues into the renal pelvis, which is the funnel-shaped dilated proximal end of the ureter. There are usually two or three major calyces in each kidney.

Renal Vasculature

The kidneys receive 1.2–1.3 l of blood per minute (about 25% of the cardiac output), making them highly vascular organs. After originating from the aorta, the renal artery enters the renal sinus and divides into the interlobar arteries, which extend towards the cortex in the spaces between the medullary pyramids. At the junction between the cortex and medulla, the interlobar arteries divide and pass over into the arcuate arteries. The arcuate arteries give rise to the interlobular arteries, which rise radially through the cortex. It is interesting to note that none of these arteries penetrates the medulla.

Afferent arterioles arise from the interlobular arteries and supply the glomerular tufts. The glomerular tufts are drained by the efferent arterioles, which then form the peritubular plexus and are of two types: cortical and juxtamedullary. The shorter cortical efferent arterioles arise from the superficial and midcortical nephrons and supply the cortex. The longer juxtamedullary efferent arterioles, which arise from the deeper nephrons, represent the sole blood supply to the medulla and are termed vasa recta. Whilst the descending vasa recta supply blood to the medulla, the ascending vasa recta drain it.

Glomerulus and Filtration across it

The glomerulus is the invagination of a tuft of capillaries into the dilated proximal end of the nephron called the Bowman's capsule (Figure 1.2). Supplied by the afferent and drained by the efferent arteriole, the glomerular capillary bunch is attached to the mesangium on the inner side and covered by the glomerular basement membrane (GBM) on the outer side. In a way, the GBM forms the skeleton of the glomeruli, with the podocytes (visceral epithelial cells with long foot processes) on the outer side and the capillaries and mesangium on the inner side. Thus, blood within the glomerular capillary is separated from the urinary space by endothelium, GBM, and podocyte.

Formation of urine begins with the filtration of blood from the glomerular capillaries. For this to happen blood must cross three layers:

- *Endothelium*: these highly fenestrated cells have 50–100 nm pores with a highly electronegative luminal surface.
- *GBM*: with podocytes externally and endothelium/mesangium internally, its main constituents are collagen IV, laminin, and heparin, all of which are negatively charged.
- *Visceral epithelium (podocytes)*: a big cell body floating in the urinary space with long primary processes which affix on the glomerular capillaries by foot processes, with filtration slits sized 30–40 nm

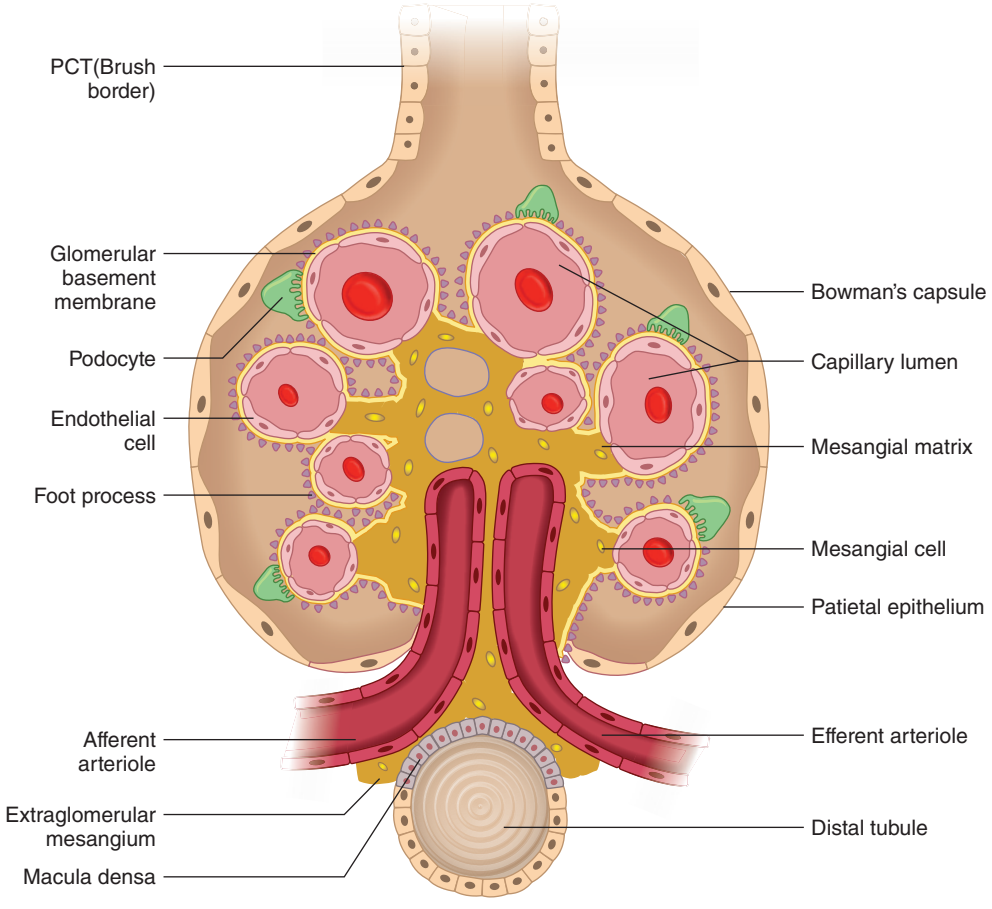

Figure 1.2 Parts of glomerulus and the juxtaglomerular apparatus (JGA). PCT, proximal convoluted tubule.

in between. These slits are bridged by the slit diaphragms, which are themselves penetrated by small pores sized about 8 nm. The luminal surfaces of both the podocyte foot processes and the slit diaphragms are rich in negatively charged proteins such as podocalyxin in the former and nephrin in the latter.

Whilst water can freely cross these three layers, the filtration of macromolecules depends on both size and charge. Molecules bigger than 8 nm are normally completely restricted by the GBM. Albumin with a diameter of 7.2 nm would have been effectively filtered were it not for its negative charge.

Clinical Notes

Common Glomerular Pathologies

- Nephrotic syndrome is characterized by proteinuria >3.5 g/24 hours, hypoalbuminaemia, generalized oedema, and hyperlipidaemia. Nephrotic syndrome is due to abnormally increased filtration of plasma proteins across the glomerular capillary. This may be due to an increase in the size of the pores mentioned earlier, but there is always an associated loss of negative charges across the filtration barrier. Proteinuria leads to hypoalbuminaemia, with a resultant decrease in plasma oncotic pressure in turn leading to oedema. In an effort to compensate for the low oncotic pressure, the liver starts to make excessive lipids, leading to hyperlipidaemia.

- Nephritis is characterized by haematuria with RBC casts and dysmorphic RBCs, some degree of oliguria, hypertension, and reduction in glomerular filtration rate (GFR). Nephritis is due to glomerular inflammation leading to leakage of RBCs across the filtration barrier. As the RBCs traverse the inflamed filtration barrier and travel down the tubules, they develop cytoplasmic blebs on their surface and are seen as dysmorphic RBCs under the microscope. Whilst travelling through the thick ascending loop of Henle, the RBCs get struck in the physiologically secreted tubular Tamm–Horsfall protein, leading to the formation of RBC casts. Thus, the presence of dysmorphic RBCs and RBC casts in a patient with haematuria helps to differentiate nephritis as the pathology rather than ureteric or bladder pathologies.
- Type IV collagen molecules are composed of three alpha chains that form triple-helical structures through specific interactions of C-terminal non-collagenous domains. GBM and alveolar capillary basement membrane collagen consist of alpha3, -4, and -5 chains, unlike other basement membranes with alpha1 (two of them) and alpha2 chains. Two disease processes pathogenetically caused by defects primarily in the GBM are:
  a. Goodpasture's disease, characterized by anti-GBM antibody against the alpha3 chain of the type IV collagen present in the GBM and alveolar membranes in the lungs [2]. Patients present with nephritis and haemoptysis.
  b. Alport syndrome, which results from mutations in genes encoding the alpha3, -4, and -5 chains of type IV collagen (80% are due to mutations in the alpha5 chain) [3]. It is the commonest inherited form of nephritis and is often associated with sensorineural deafness and ocular abnormalities due to the presence of similar alpha3, -4, and -5 chains in the type IV collagen in the basement membranes of the lens and cochlea.

Tubules

With a GFR of 125 ml/min, about 180 l of urine is formed at the glomerular level each day. Thankfully, under physiological conditions almost 98–99% of this filtered urine is reabsorbed by the tubules. More than 90% of this tubular water reabsorption is a passive process and water just follows the sodium (Na^+) being reabsorbed. Only in the CD is water absorbed actively and independently of Na^+ under the action of vasopressin or anti-diuretic hormone (ADH).

It is thus obvious that most of the water reabsorption in the tubules is dependent on the reabsorption of Na^+. So, what makes the tubules take up Na^+ so avidly?

Why do the Tubules Reabsorb Na^+ and Water?

The tubular epithelial cells, irrespective of which segment of the tubule they belong to, have a basolateral Na^+-K^+-ATPase that continuously pumps out three Na^+ ions and brings in two potassium (K^+) ions, thus making the cells permanently Na^+ deficient. Thus, the fact that the tubular epithelial cells are always deficient in Na^+ helps in the Na^+ reabsorption from the tubules. In the same vein, the fact that the tubular cells are rich in K^+ helps them to secrete K^+ into the tubular lumen.

Water is absorbed osmotically along with solutes, with Na^+ being the predominant solute (Table 1.2; Figure 1.3). The two exceptions are in the thick ascending limb (TAL) of the loop of Henle, which is impermeable to water, and the CD, where water is reabsorbed by the action of vasopressin, as discussed later.

Proximal Convoluted Tubule

The bulk of tubular reabsorption occurs in the proximal convoluted tubule (PCT), with 65–70% of the total Na^+ being reabsorbed here and about the same amount of water being reabsorbed passively. The PCT is also responsible for reabsorption of the bulk of phosphate (PO^{3-}_4), bicarbonate (HCO_3–), potassium (K^+), calcium (Ca^+) and almost all glucose, amino acids, uric acid, and low molecular weight proteins such as microglobulins and the light chain fraction of immunoglobulins.

$NaHCO_3$ is filtered in the glomeruli and dissociates in the lumen of the PCT into Na^+ and HCO_3^-. Na^+ is

Table 1.2 Absorption of various ions and water across different segments of the renal tubules

Ion or substance	PCT	Loop of Henle	DCT	CD
Na^+	65–70%	30–35%	5%	1–2%
H_2O	65–70%	25%	5%	5–10%
K^+	45–50%	40–45%		Secreted by principal cells
Ca^+	70%	20%	10–15%	
Mg^+	15–25%	60–70%	5–10%	
PO^{3-}_4	70–80%	Insignificant	Insignificant	Insignificant
HCO_3^-	85–90%			Secreted by beta- intercalated cells

Ca^+: calcium; CD: collecting duct; DCT: distal convoluted tubule; HCO_3^-: bicarbonate; H_2O: water; K^+: potassium; Mg^+: magnesium; Na^+: sodium; PCT: proximal convoluted tubule; PO^{3-}_4: phosphate.

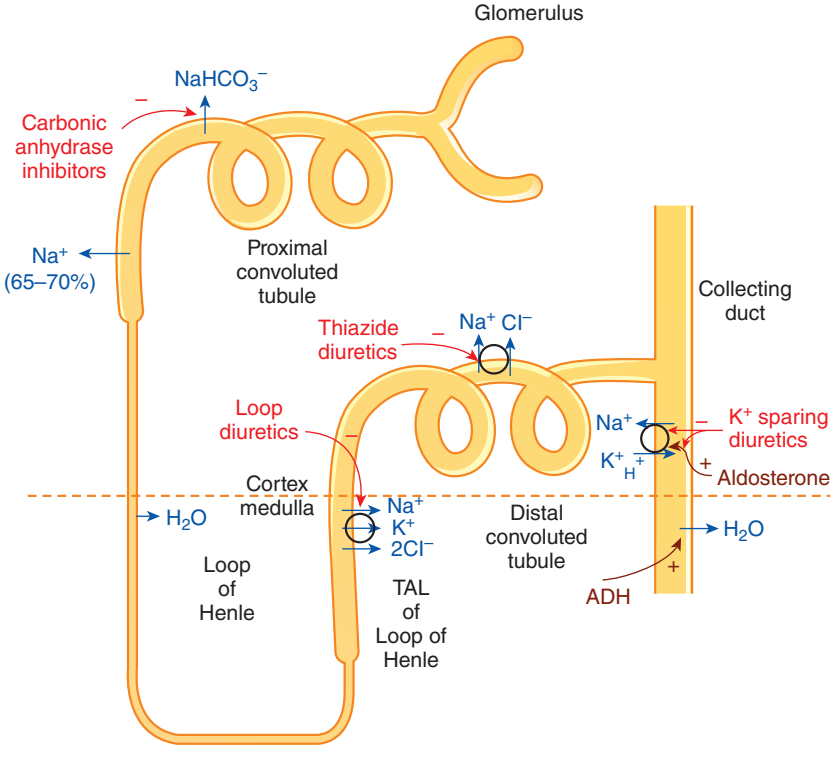

Figure 1.3 Diagram showing the movement of sodium (Na^+), chloride (Cl^-), potassium (K^+), and water (H_2O) across various portions of the renal tubule, with the site of action of different diuretics highlighted. ADH: anti-diuretic hormone; $NaHCO_3^-$: sodium bicarbonate; TAL: thick ascending limb.

reabsorbed by the Na^+-hungry tubular epithelial, as already discussed. The HCO_3^- remaining (from the original $NaHCO_3$) in the lumen combines with hydrogen (H^+) by the action of the tubular carbonic anhydrase (CA) to form carbonic acid (H_2CO_3), which then splits into carbon dioxide (CO_2) and water (H_2O). CO_2 enters the tubular cell and now the reaction goes in the reverse direction, with CO_2 combining with H_2O

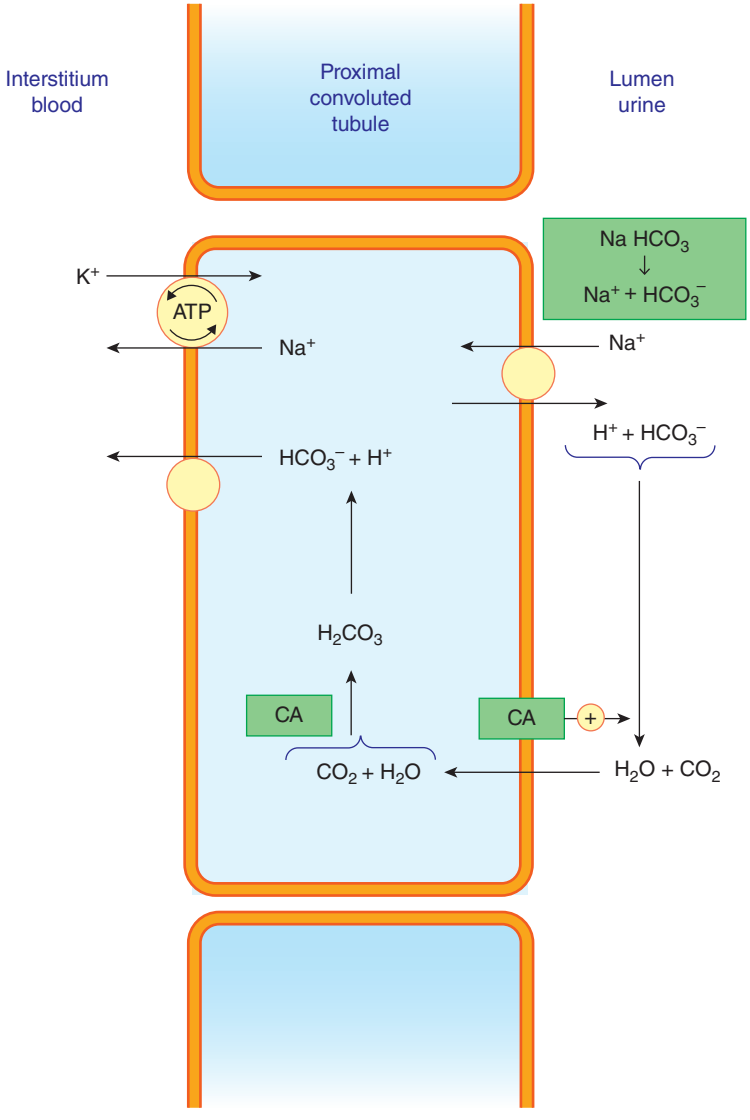

Figure 1.4 Action of carbonic anhydrase (CA) in reclaiming bicarbonate (HCO_3^-) in the proximal convoluted tubule (PCT): sodium bicarbonate ($NaHCO_3$) dissociates in the lumen of the PCT into sodium (Na^+) and HCO_3^-; Na^+ is reabsorbed by the tubular cells in exchange for hydrogen (H^+), whilst the HCO_3^- combines with the H^+ to form carbonic acid (H_2CO_3) under the influence of tubular CA. H_2CO_3 then splits into carbon dioxide (CO_2) and water (H_2O), followed by the CO_2 diffusing into the tubular cells and regenerating H_2CO_3 after combining with H_2O. Aided by the intracellular CA, this H_2CO_3 then splits to form H^+ and HCO_3^-. This H^+ is secreted into the PCT lumen in exchange for Na^+, whilst the HCO_3^- diffuses into the peritubular capillaries. ATP, adenosine triphosphate.

to form H_2CO_3, which is then split to form H^+ and HCO_3^- by the intracellular form of CA. This H^+ is then secreted into the PCT lumen in exchange for Na^+ by the Na^+–H^+ exchange transport, whilst the HCO_3^- diffuses into blood in the peritubular capillaries (Figure 1.4). Thus, for each H^+ secreted into the tubule, the body gains an HCO_3^-.

Reabsorption of Na^+ in the PCT facilitates the reabsorption of glucose, amino acids, phosphate, and uric acid.

Clinical Notes

Disorders of the Proximal Renal Tubules

- Proximal renal tubular acidosis (type 2 RTA) is due to a defect in the reabsorption of HCO_3^- leading to urinary loss of HCO_3^- and the resultant metabolic acidosis. As already explained, the reabsorption of HCO_3^- in this segment is linked to the coupled exchange of H^+ and Na^+ (Figure 1.4). As Na^+ reabsorption here is linked to the reabsorption of glucose, amino acids, phosphate, and uric acid, the patient may present with glycosuria, hypophosphataemia, and hypouricaemia (due to urinary losses), a condition that is termed Fanconi syndrome. Urine pH is usually less than 5.5 despite bicarbonaturia, as the intact distal tubules excrete excess H^+ as a compensatory mechanism for the acidosis.
- Type 2 RTA is discussed further in the clinical notes following the section on the distal convoluted tubule and collecting duct.
- Acezolamide, which is a CA inhibitor often used by ophthalmologists for the treatment of glaucoma, can also lead to a type 2 RTA-like picture, with full-blown Fanconi syndrome at times. The anti-epileptic drug topiramate and anti-HIV medication tenofovir, by their CA inhibitor activity, can also lead to proximal RTA and Fanconi syndrome [4].
- PCT is responsible for the reabsorption of the easily filtered light chain fragments of immunoglobulins. In myeloma, the excess of light chains is toxic to the PCT and may lead to proximal RTA with or without Fanconi syndrome [5].
- In prerenal AKI, serum urea increases disproportionately to creatinine due to its enhanced PCT reabsorption that follows the enhanced transport of Na^+ and water. Elevated serum urea/creatinine may also be seen in upper gastrointestinal haemorrhage, since the upper intestinal proteolytic enzymes break down haemoglobin to amino acids, which are then reabsorbed by the body and lead to the generation of urea.

Clinical Notes

Disorders of the Loop of Henle

- *Bartter syndrome*: an autosomal recessive syndrome that characteristically presents with hypokalaemia and metabolic alkalosis is described later in the chapter with Gitelman syndrome (following the section on the distal convoluted tubule and collecting duct).
- *Familial hypocalciuric hypercalcaemia (FHH)*: this benign autosomal dominant condition is caused by inactivating mutations in the gene for the CaSR.

  CaSR is predominantly found in the parathyroid gland and the kidneys. In the parathyroid gland, it decreases the secretion of PTH in response to serum Ca^+. Under normal conditions, the TAL CaSR, on being activated by Ca^+, downregulates the production of Ca^+-carrying transcellular proteins Claudrin 16 and 19 by forming Claudrin 14, which has an inhibitory effect on the two [7]. The mutated CaSR in FHH is unable to inhibit Claudrin 16 and 19, leading to uninhibited Ca^+ reabsorption and hence hypocalciuria and hypercalcaemia, and at the same time a non-suppressible serum PTH level. It is easy to confuse this condition with primary hyperparathyroidism unless one looks at the urinary Ca^+, which is characteristically low. A spot urine Ca^+/creatinine ratio of <1/100 is helpful in differentiating the hypercalcemia with inappropriately high serum PTH levels seen in FHH from primary hyperparathyroidism (where the urinary Ca+ would be high).

About 40–50% of the filtered urea is reabsorbed in the PCT. As urea is reabsorbed in this section along with Na+, in conditions of volume contraction when the PCT reabsorbs more Na^+, the net reabsorption of urea is also significantly increased. Thus, a disproportionate rise in blood urea concentration in comparison to creatinine might be a marker of a prerenal cause of acute kidney injury (AKI).

Loop of Henle

The loop of Henle consists of the thick and thin descending limbs and the thick ascending limb (TAL), with a thin ascending limb seen only in long looped nephrons.

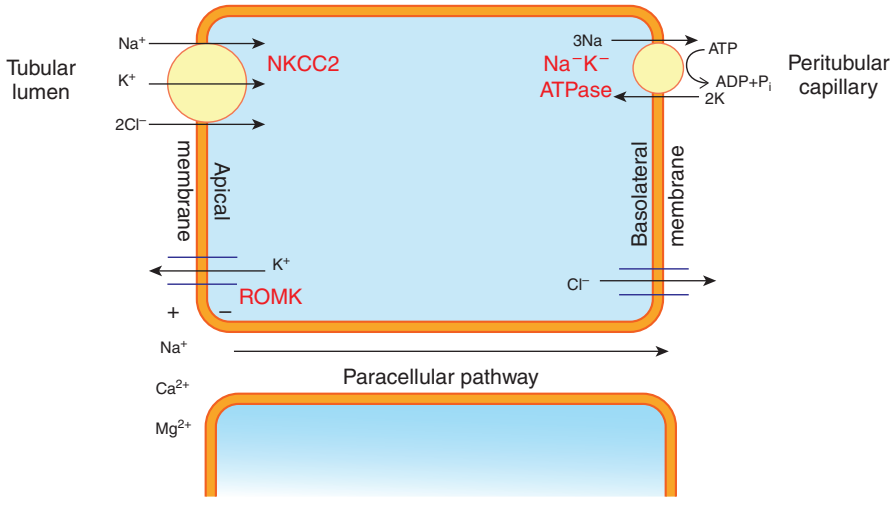

Figure 1.5 Transport mechanisms in the thick ascending limb of the loop of Henle: The basolateral (peritubular) membrane due to the action of Na+-K+-ATPase pumps three sodium (Na^+) out of, and two potassium (K^+) into, the cell. This creates a low intracellular Na^+ concentration, which allows the cell to absorb Na^+ from the urinary lumen by the electroneutral Na^+-K^+-$2Cl^-$ (NKCC2). The cell is rich in K^+ and so much of the reabsorbed K^+ recycles back into the lumen through the renal outer medullary potassium channel (ROMK) on the apical/luminal membrane. This movement of one cationic K^+ into the lumen plus the flux of one reabsorbed Na^+ (by Na-K-ATPase pump) and two chloride (Cl^-) out of the cell and into the peritubular capillary (via Cl^- channels) causes the lumen to become more positively charged compared with the cell and peritubular capillaries/interstitium. The resultant electrical gradient promotes passive reabsorption of cations calcium (Ca^+) and magnesium (Mg^+) via the paracellular pathway. The Cl^- channels require interaction with a small protein called barttin to function normally. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pi, inorganic phosphate.

The loop of Henle is responsible for the reabsorption of 30–35% of the luminal Na^+ but only 25% of the water, as the TAL is resistant to the movement of water across it.

The frusemide-sensitive and electroneutral Na^+-K^+-$2Cl^-$ cotransporter reabsorbs one Na^+ and K^+ each along with two Cl^- ions from the TAL lumen to the tubular epithelial cell (Figure 1.5). High intracellular K^+ concentration (due to the basolateral Na-K-ATPase pumping out three Na^+ and bringing in two K^+ ions constantly) causes the reabsorbed K^+ to be secreted back into the lumen via the renal outer medullary potassium (ROMK) channel. The Cl^- is absorbed into blood by the basolateral Cl^- channel (ClC-Kb), which has barttin as a crucial accessory protein. Movement of one positive charge (K^+) into the lumen and net of one negative charge into the peritubular capillary (1 Na^+ and 2 Cl^-) causes the lumen to be more positive compared to the tubular peritubular capillary and interstitium. This electrical gradient promotes the passive reabsorption of cations Ca^+ and Mg^+ via the paracellular cleft between the cells. Membrane proteins Claudrin 16 and 19 help in this paracellular reabsorption of Ca^+ and Mg^+ and are themselves tightly regulated by the calcium-sensing receptor (CaSR) (Figure 1.6) [6].

Countercurrent Mechanism

A major function of the loop of Henle is to create an interstitial osmotic gradient that increases from the renal cortex (290 mOsm/kg) to the tip of the medullary pyramid (1200 mOsm/kg) (Figure 1.7). This countercurrent mechanism is fundamentally dependent on the impermeability of the TAL to water. The reabsorption of Na^+ (and its passage to the interstitium by the basolateral Na^+-K^+-ATPase pump) in the absence of water in the ascending limb has two consequences:

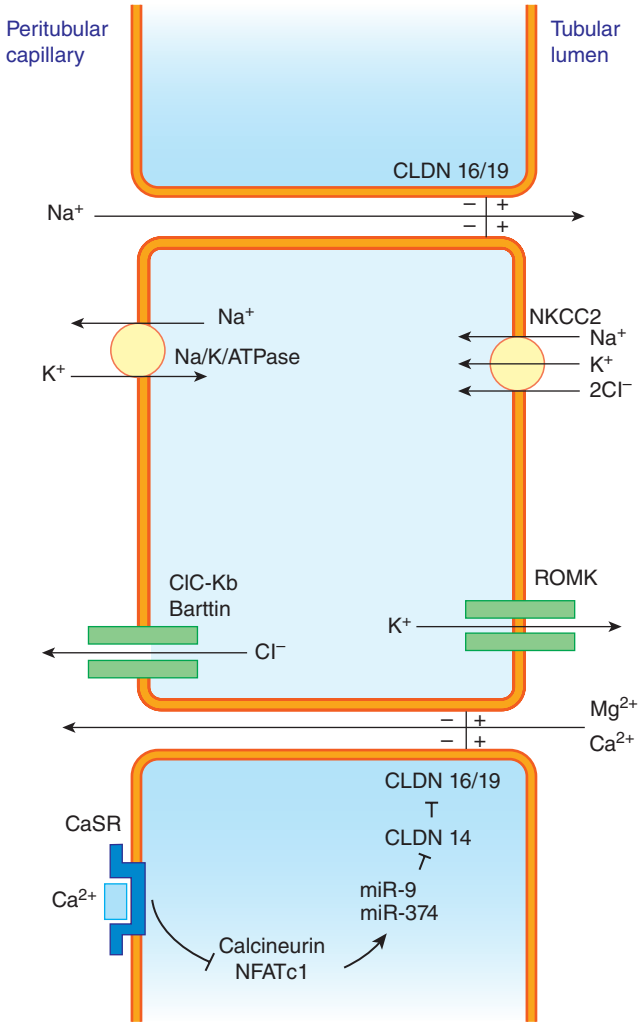

Figure 1.6 Absorption of calcium (Ca^+) and magnesium (Mg^+) through the paracellular pathway in the thick ascending limb of the loop of Henle. Sodium (Na^+) is reabsorbed via the apical Na^+-K^+-$2Cl^-$ cotransporter and pumped out basolaterally by the Na^+-K^+-ATPase; chloride (Cl^-) exits via the Cl^- channel (ClC-Kb); and potassium (K^+) is secreted into the tubular lumen by the renal outer medullary potassium channel (ROMK), thereby generating a lumen-positive transtubular electric potential. Ca^+ and Mg^+ are reabsorbed passively across the paracellular pathway because of this lumen-positive electrical potential. Claudrin 16 and 19 are the two chief transport proteins which help to carry the Ca^+ and Mg^+ across the paracellular cleft. Blood Ca^+ levels regulate this process by stimulating the Ca^+-sensing receptor (CaSR), which then upregulates Claudin-14 (CLDN14), a protein that normally binds to and inhibits Claudin 16 and -19. CaSR controls the production of Claudrin 14 by the calcineurin-NFATc1-microRNA pathway. NFAT: nuclear factor of activated T cells.

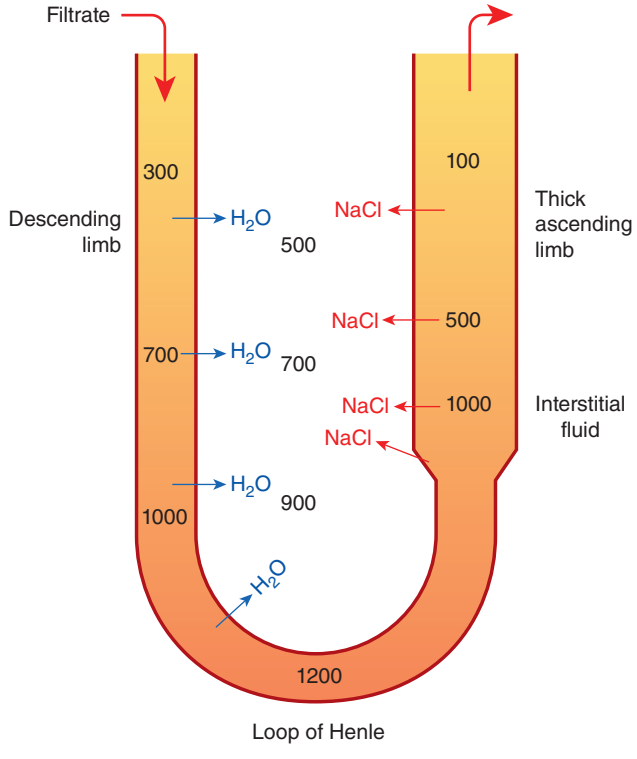

Figure 1.7 Countercurrent mechanism: reabsorption of sodium (Na^+) with relative resistance to the reabsorption of water by the thick ascending limb of the loop of Henle leads to generation of the interstitial osmotic gradient; the gradient is higher for the longer loops of the juxtamedullary nephrons, which travel deeper into the medulla. H_2O, water; NaCl, sodium chloride.

1. A higher concentration of Na^+ in the interstitium leads to higher interstitial osmotic pressure, with the highest osmotic pressure developed in tips of medullary pyramids where the longer loops of Henle reach.
2. The tubular fluid leaving the TAL is hypotonic because of excess water in comparison to Na^+.

The creation of this gradient helps in the reabsorption of water in the medullary CD under the influence of vasopressin, with water moving from the hypo-osmolar tubular environment to the hyper-osmolar medullary interstitium.

Clinical Notes

Can Frusemide Lead to Hyponatraemia?

Frusemide acts by interrupting the Na^+ uptake in the TAL by the Na^+-K^+ -$2Cl^-$ transporter. This interferes with the countercurrent mechanism and the generation of the medullary interstitial osmotic gradient. As discussed, without this gradient, ADH cannot reabsorb water in the medullary CD. Thus, frusemide leads to the excretion of a dilute urine which would prevent the development of hyponatraemia.

Distal Convoluted Tubule and Collecting Duct

The distal convoluted tubule (DCT) is responsible for the reabsorption of 5% of Na^+ by the thiazide-sensitive sodium chloride (NaCl) cotransporter. Between 10% and 15% of the Ca^+ is also reabsorbed in this segment in an active process, unlike the more passive process in the proximal parts (70% of Ca^+ is co-transported passively with Na^+ in the PCT). Ca^+ enters tubular cells via the transient receptor potential vanilloid 5 (TRPV5) channel, and 5–10% of the total Mg^+ reabsorption also occurs in this segment by the transient receptor potential melastatin 6 (TRPM6) and TRPM7 channels.

The CD is responsible for reabsorption of up to 2% of the Na^+ and water, but this can vary depending on the needs of the body. The two chief types of cells found in the CD are:

- Principal cells, which are responsible for Na^+ reabsorption and K^+ secretion (under the influence of aldosterone) and water reabsorption (under the influence of ADH).

- Intercalated cells, which are responsible for the secretion of H^+ (by alpha-intercalated cell) or HCO_3^- (by beta-intercalated cell).

ADH

ADH is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and travels down the pituitary stalk to be stored in the posterior pituitary. Secreted in response to decreased intravascular volume or increased plasma osmotic pressure as in dehydration, the main physiological effects of ADH are retention of water by the kidneys and vasoconstriction.

There are three types of ADH receptors: V1A, V1B, and V2. V1A receptors cause vasoconstriction by increasing vascular smooth muscle intracellular Ca^+ concentration, whilst V1B receptors lead to adrenocorticotropic hormone (ACTH) release by the pituitary gland. V2 receptors are found on the principal cells in the CD and via the cyclic adenosine monophosphate (cAMP) pathway lead to movement and fusion of the aquaporin 2 (AQP2) water channels to the luminal membrane (Figure 1.8). The medullary osmotic gradient established due to the selective reabsorption of Na^+ in the TAL helps in the water being reabsorbed in the CD.

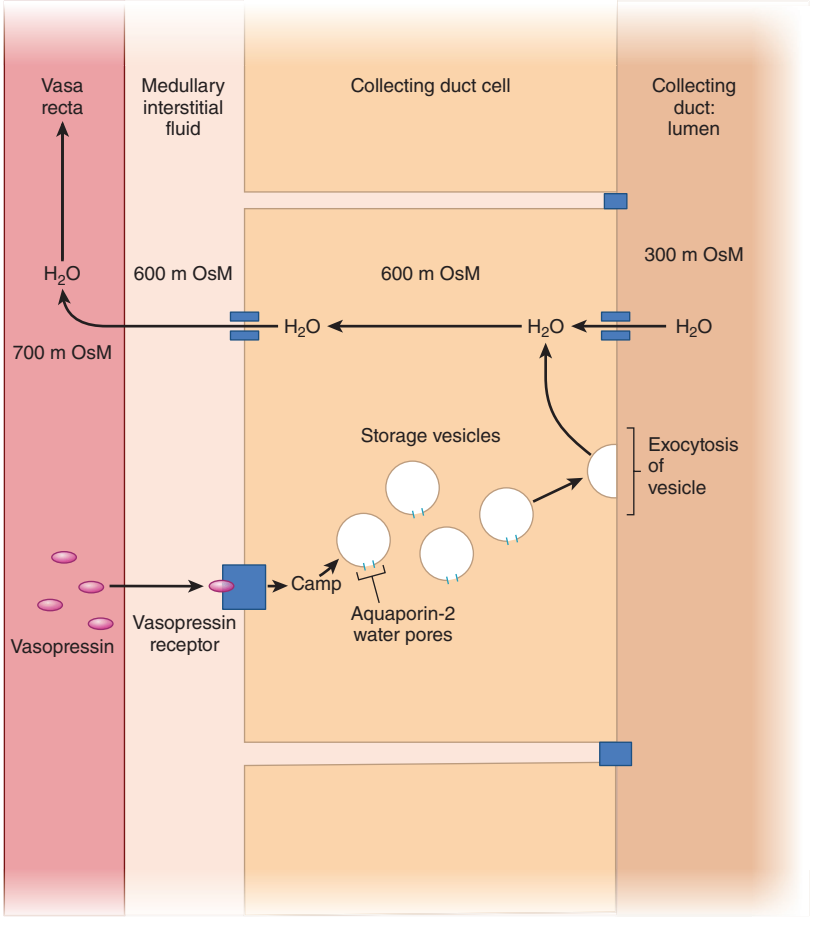

Figure 1.8 Anti-diuretic hormone (vasopressin), acting through cyclic adenosine monophosphate (cAMP), increases the total number of aquaporin-2 molecules in the principal cells of the collecting duct; aquaporin-2 aids in water reabsorption in this part of the renal tubule. H_2O: water.

Aldosterone

Aldosterone is secreted by the zona glomerulosa of adrenal cortex and helps in Na^+ reabsorption and K^+ and H^+ excretion by the distal portions of the tubules. On binding to the mineralocorticoid receptor, aldosterone upregulates and activates the basolateral Na^+-K^+-ATPase in the principal cells, causing lower intracellular Na^+, and at the same time upregulates epithelial sodium channels (ENaC), increasing apical membrane permeability for Na^+. The resultant intracellular movement of Na^+ causes luminal negativity, leading in turn to secretion of the positively charged K^+. The Na^+ and K^+ movement is not a strict one-to-one exchange and more Na^+ is reabsorbed than K^+ is lost. So, the same luminal negativity leads to H^+ secretion by the neighbouring alpha-intercalated cells. The potassium-sparing diuretics (amiloride and triamterene) act by directly inhibiting ENaC channels; spironolactone acts by competing with aldosterone for binding to the mineralocorticoid receptor (Figure 1.9).

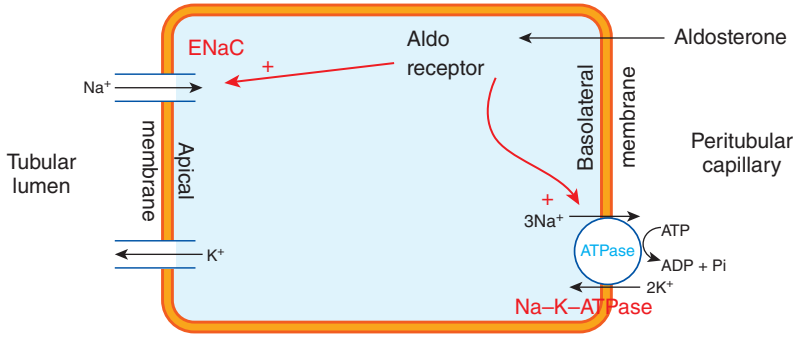

Figure 1.9 Ion transport in collecting tubule principal cells. Aldosterone, after combining with the cytosolic mineralocorticoid receptor (aldo-receptor), leads to enhanced sodium (Na^+) reabsorption and potassium (K^+) secretion by increasing both the number of open Na^+ channels and the number of Na^+-K^+-ATPase pumps. Whilst spironolactone competes with aldosterone for the aldosterone receptor, amiloride and triamterene act by directly inhibiting the epithelial Na^+ channel. ENaC: epithelial sodium channel.

Clinical Notes

Diuretics, the Distal Tubules, Vasopressin and Aldosterone

- The unifying mechanistic principle of action of all diuretics is to prevent tubular Na^+ reabsorption. Both thiazide and loop diuretics can lead to hypokalaemia and metabolic alkalosis by the following mechanisms:
  a. With more Na^+ flowing to the distal parts of the nephron, the tubular cells try to reclaim the excess Na^+, resulting in loss of positive charges from the lumen. The tubular cells try to compensate by secretion of the positively charged K^+ (by principal cells) and H^+ (by alpha-intercalated cell) into the lumen.
  b. Intravascular volume contraction because of diuretics causes activation of the renin-angiotensin-aldosterone (RAS), which contributes to the urinary K^+ and H^+ losses due to the effect of aldosterone.
- Potassium-sparing diuretics can lead to hyperkalaemia and metabolic acidosis. Spironolactone competes with aldosterone for binding to the mineralocorticoid receptor, whilst amiloride and triamterene act by directly inhibiting the ENaC. The resultant effect is inhibition of K^+ and H^+ secretion.
- Effects of diuretics on Ca^+: Let us recapitulate that the paracellular reabsorption of Ca^+ in the loop of Henle is dependent on the positive electrical gradient in the lumen (Figures 1.6). Loop diuretics by inhibiting the Na^+-K^+-$2Cl^-$ transporter inhibit generation of this gradient and hence prevent reabsorption of Ca^+ in this segment of the tubule. On the other hand, thiazide diuretics increase proximal Na^+ and water reabsorption due to volume depletion, leading in turn to increased passive proximal calcium reabsorption. Thus, whilst frusemide helps to excrete Ca^+ in the urine, thiazide diuretics decrease urinary Ca^+ excretion and lead to hypercalcaemia.

- Lithium causes nephrogenic diabetes insipidus by causing decreased expression of AQP 2 genes.
- Primary hyperaldosteronism should always be suspected in a patient with hypokalaemia, metabolic alkalosis, and hypertension (more than half of patients are normokalaemic). This is discussed in more detail in Chapter 3.
- Bartter and Gitelman syndromes, both autosomal recessive disorders, are characterized by hypokalaemia, metabolic alkalosis, and low to normal blood pressure, with hypomagnesaemia being an additional distinctive feature of Gitelman syndrome, which tends to present in adults and is generally the milder of the two. The tubular defects in sodium chloride reabsorption in Bartter and Gitelman syndromes are almost identical to those seen with chronic ingestion of loop and thiazide diuretics, respectively [8, 9, 10]. Additional down-regulation of the Mg^+-reabsorbing TRPM6 channel in Gitelman syndrome causes Mg^+ wasting and the resultant characteristic hypomagnesaemia. These disorders are discussed in more detail in Chapter 3.
- Distal renal tubular acidosis (type 1 RTA) is due to an inability of the distal tubules to excrete H^+, leading to metabolic acidosis. The kidney characteristically cannot lower the urinary pH to less than 5.5 due to a deficiency of H in the urine, in contrast to type 2 RTA where the urinary pH is usually greater than 5.5 (Table 1.3) [11].

Table 1.3 Differences between types 1 and 2 renal tubular acidosis (RTA)

RTA1 (distal RTA)	RTA2 (proximal RTA)
Inability to secrete H^+	Inability to reabsorb HCO_3^-
Urine pH >5.5 (absence of H^+ in urine)	Urine pH <5.5 (compensatory rise in DCT secretion of excess H^+)
Associations: Sjögren's syndrome, systemic lupus erythematosus, primary biliary cirrhosis, autoimmune hepatitis	Associations: myeloma, Wilson's disease, tenofovir, acetazolamide, gentamicin
No Fanconi syndrome	Fanconi syndrome may be seen: glycosuria, phosphaturia, uric aciduria, and aminoaciduria (low serum phosphate and urate concentrations)
Proximal tubules reabsorb all alkali including citrate, which normally keeps Ca^+ in urine soluble, leading to increased predisposition to nephrolithiasis and nephrocalcinosis	No renal stones
Treat with alkali and K^+ replacement	Same treatment, but needs bigger doses of alkali as glomeruli lose the administered alkali and tubules cannot reabsorb it due to the nature of the defect

DCT: distal convoluted tubule; H^+: hydrogen; HCO_3^-: bicarbonate; K^+: potassium.

Glomeruli and Tubule Balance each other

An increase or decrease in glomerular filtration is balanced by increased or decreased tubular reabsorption. Similarly, an increase in tubular flow leads to a decrease in glomerular filtration and vice versa.

Glomerulotubular Balance

Increased filtration of protein-free fluid across the glomeruli leads to the blood flowing to efferent and peritubular arterioles being more concentrated. The resultant increased oncotic pressure in the peritubular capillaries aids in enhanced reabsorption of water across the tubular epithelium.

Tubuloglomerular Feedback

As more urine flows though tubules it causes decreased glomerular filtration and vice versa via macula densa constricting or dilating the afferent arterioles. The mechanism is explained in the next section.

Juxtaglomerular Apparatus

The juxtaglomerular apparatus (JGA) comprises the modified tubular epithelial cells called macula densa, the terminal portion of afferent arteriole with its renin-producing modified smooth muscle cells (granular or juxtaglomerular cells) and the extraglomerular mesangial cells.

At the point where the afferent arterioles enter the glomerulus and the efferent arteriole leaves it, the tubule touches the arterioles of the glomerulus from which it arose. At this location, which marks the ending of the TAL and the beginning of the DCT, the tubular epithelium is modified into narrowly packed cells with large nuclei called the macula densa. Macula densa sense the Na^+ and Cl^- levels in the tubular fluid and in the case of low flow, as seen in hypovolaemia or renal artery stenosis, relay this information via the extraglomerular mesangial cells to the granular cells. Granular cells are modified smooth muscle cells in the terminal portion of the afferent arterioles that contain and secrete renin when stimulated by the macula densa.

Renin splits the 32-amino acid polypeptide angiotensinogen formed in liver to yield the physiologically inactive decapeptide angiotensin1. As this angiotensin1 circulates through the lungs, angiotensin-converting enzyme (ACE) splits two more amino acids off it to form the octapeptide angiotensin II. Apart from being a potent vasoconstrictor, angiotensin also leads to aldosterone release (Figure 1.10).

Note that angiotensin II constricts efferent more than afferent arterioles in the kidney, thus effectively increasing filtration pressure and glomerular filtration.

In the case of high tubular flow and raised luminal Na^+ and Cl^-, the macula densa cells secrete adenosine triphosphate (ATP) into their surroundings. This ATP is converted into adenosine, which binds to the surrounding extraglomerular mesangial cells and raises their intracellular Ca^+ levels. This Ca^+ signal is then propagated via gap junctions to adjacent cells, including the granular cells of the JGA and the vascular smooth muscle cells of the afferent arteriole, resulting in a decrease in renin release and vasoconstriction. Both these changes decrease the GFR [12].

Clinical Notes

Angiotensin-Converting Enzyme Inhibitors (ACE-I) and Angiotensin Receptor Blockers (ARB)

In renal artery stenosis, diabetic nephropathy or chronic kidney disease, the intrarenal perfusion pressure is already reduced and GFR is maintained in part by an angiotensin II-induced increase in resistance at the efferent arteriole [13, 14]. Blocking this response with an ACE-I or ARB will sequentially relax the efferent arteriole, lower intraglomerular pressure and reduce the GFR (and as a result decrease proteinuria). Renal function should be checked at five to seven days when an ACE-I or ARB is begun and the drug should be discontinued if serum creatinine concentration increases more than 30% above the baseline value.

Hydrogen Ion and the Kidney

As discussed previously, in the PCT H^+ is secreted in exchange for Na^+ and an HCO_3^- is regenerated with the aid of CA. In the more distal tubule and CD, H^+ is secreted by the type A intercalated cell via the luminal H^+-ATPase. Aldosterone indirectly helps this H^+ secretion by enhancing Na^+ reabsorption in neighbouring principal cells. Na^+ absorption generates a lumen-negative potential that drives both K^+ secretion by principal cells as well as H^+ by the alpha-intercalated cells [15]. H^+-K^+-ATPase pumps, which secrete H^+ and reabsorb K^+, are also present in the luminal membrane of alpha-intercalated cells. Whilst they are not normally a major player in K^+ homeostasis, the number and activity of these pumps are increased by K^+ depletion (Figure 1.11).

H^+ in the urine needs buffers, otherwise urine would quickly become saturated with it. The chief buffer that helps to carry the secreted H^+ in urine is ammonia, which becomes converted to ammonium after accepting the H^+:

$$NH_3 + H^+ \leftrightarrow NH_4^+$$

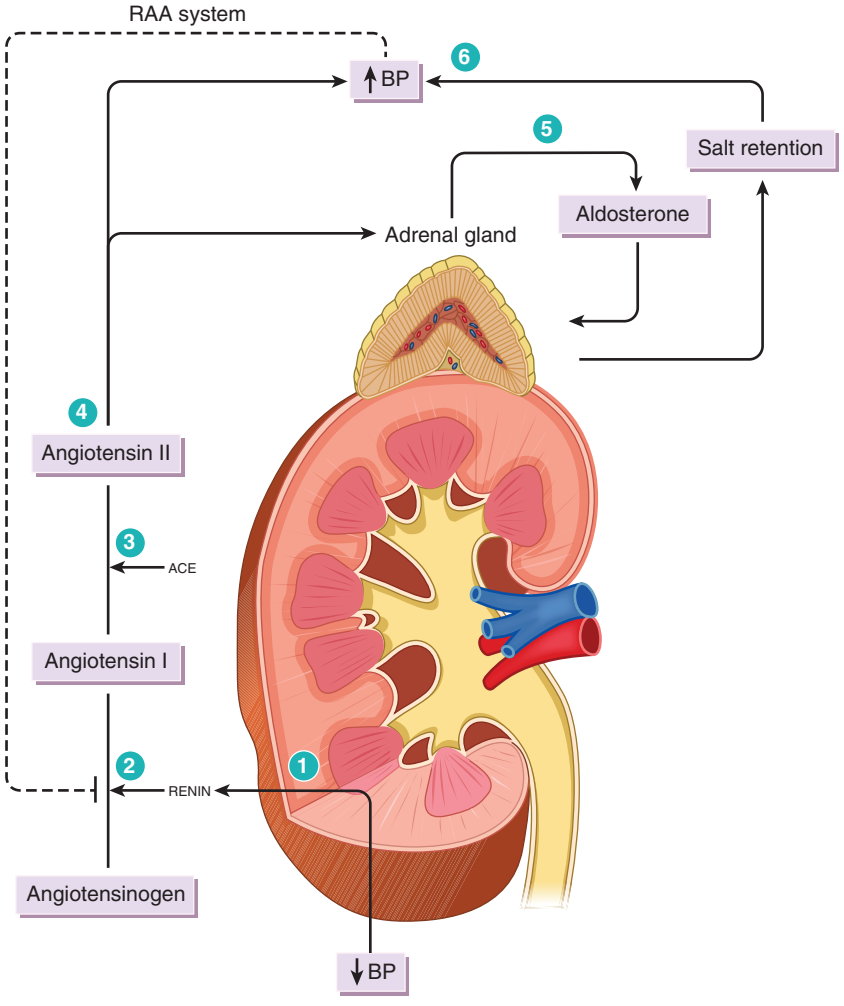

Figure 1.10 Relation between blood pressure and renin–angiotensin–aldosterone (RAA) system. Low tubular flow due to hypovolaemia/hypotension leads to renin secretion (1), which breaks angiotensinogen produced by the liver to yield angiotensin-1 (2); angiotensin-1 is cleaved by angiotensin-converting enzyme (ACE) in the lungs to yield angiotensin-2 (3, 4); angiotensin-2 causes vasoconstriction as well as aldosterone secretion by the adrenal gland (5); the combined effects of the vasoconstriction and salt retention by aldosterone help to raise the blood pressure (6).

Two ammonia are derived from the breakdown of glutamine in the PCT cells by the enzyme glutaminase. This ammonia is secreted and subsequently reabsorbed by the TAL cells by the Na^+ K^+2Cl^- channels as the K^+ is substituted by ammonia. The ammonia then enters the medullary interstitium and is secreted into the CD, where it helps to buffer H^+. Hypokalaemia induces renal ammonia production by increasing the cellular uptake of glutamine and increasing the activity of glutaminase (this facilitates the excretion of H^+ by making more ammonia available for buffering it in the tubular lumen).

Potassium and Acid Base Balance

In metabolic acidosis, excess H^+ enters cells to be buffered and the intracellular electroneutrality is maintained by intracellular K^+ moving out, leading to a raised plasma K^+ concentration while the reverse happens in metabolic alkalosis (refer figure 7.2 in chapter 7 - Chronic Kidney Disease). We need to remember that metabolic acidosis and hyperkalemia

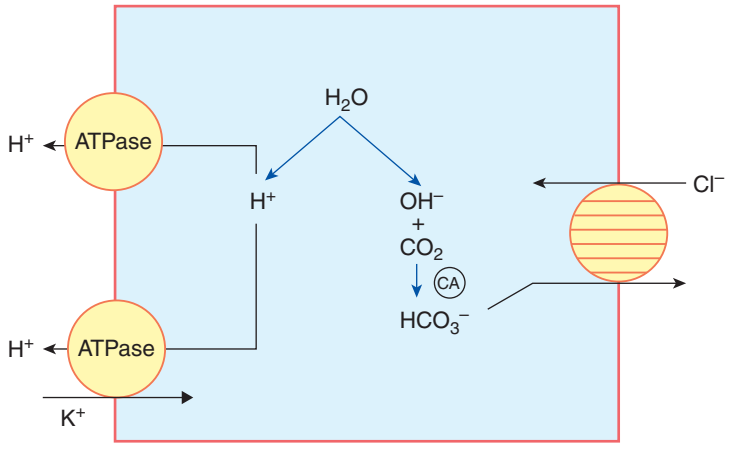

Figure 1.11 Mechanism of hydrogen (H^+) secretion and bicarbonate (HCO_3^-) and potassium (K^+) reabsorption in the type A intercalated cell. Water within the cell dissociates into hydrogen (H^+) and hydroxide (OH^-) ions. Whilst H^+ is secreted into the lumen by the H+-ATPase pumps, the OH^- ions in the cell combine with carbon dioxide (CO_2) to form HCO_3^- in a reaction catalysed by carbonic anhydrase (CA). The cellular HCO_3^- enters the peritubular capillaries in exchange for extracellular chloride (Cl^-) via Cl^--HCO_3 exchangers. In addition, H^+-K^+-ATPase pumps, which secrete H^+ and reabsorb K^+, are also present in the luminal membrane of the type A intercalated cell. The number and activity of these pumps are increased by K^+ depletion.

often go together while metabolic alkalosis is often associated with hypokalemia.

Hyperkalaemia potentiates metabolic acidosis by three mechanisms:

- Excess K^+ enters the cell and in exchange H^+ comes out of the cell.
- K^+ competes with H^+ for secretion by the CD.
- Hyperkalaemia, by decreasing renal ammonia production, inhibits H^+ excretion in urine (ammonia is the chief buffer for urinary H^+).

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Questions and Answers

Questions

1. In the proximal convoluted tubule, sodium is reabsorbed in exchange for:
   a. Hydrogen
   b. Magnesium
   c. Calcium
   d. Glucose, amino acids, and phosphate
   e. Potassium and chloride

2. Fanconi syndrome should be suspected in:
   a. Hyperuricaemia
   b. Hyperphosphataemia
   c. Hypophosphataemia
   d. Hyperkalaemia
   e. Alkalosis

3. Which part of the renal tubule is impermeable to water?
   a. Proximal convoluted tubule
   b. Distal convoluted tubule
   c. Cortical duct
   d. Descending limb of loop of Henle
   e. Ascending limb of loop of Henle

4. The following electrolyte profile may be associated with both thiazide and loop diuretics
   a. Hyperkalaemia with metabolic alkalosis
   b. Hypokalaemia with metabolic alkalosis
   c. Hyperkalaemia with metabolic acidosis
   d. Hypokalaemia with metabolic acidosis
   e. Hyperkalaemia

5. The correct statement regarding macula densa is
   a. The macula densa senses the total amount of NaCl in tubular urine
   b. If the delivery of NaCl is lower than normal, then macula densa signals special cells in the afferent arteriole to release renin
   c. Renin release has no effect on aldosterone production
   d. a and b are correct
   e. All of them are correct

6. The effect of aldosterone on renal H^+ secretion is:
   a. It has no effect
   b. Increases secretion
   c. Decreases secretion
   d. Can do both at different parts of the tubule
   e. Reabsorb H^+

7. Primary hyperaldosteronism is characterized by:
   a. Hypokalaemia
   b. Hyperkalaemia
   c. Metabolic alkalosis
   d. Metabolic acidosis
   e. a and c

Answers

1. a. In the PCT, the reabsorption of Na^+ is coupled to the secretion of H^+. Glucose, amino acids, phosphate, and uric acid are co-transported with the Na^+. Ca^+ and Mg^+ are also reabsorbed here though the bulk of Mg^+ reabsorption occurs in the TAL.
2. c. Type 2 RTA is due to a proximal tubular defect in the reabsorption of HCO_3^- leading to urinary loss of HCO_3^- and metabolic acidosis with hypokalaemia. The absorption of HCO_3^- in this segment is linked to the coupled exchange of H^+ and Na^+. As this reabsorption of Na^+ is linked to the reabsorption of glucose, amino acids, phosphate, and uric acid, the patient may present with glycosuria, hypophosphataemia, and hypouricaemia (due to urinary losses) and this condition is termed as Fanconi syndrome.
3. e. The countercurrent mechanism is fundamentally dependent on the impermeability of the ascending limb of loop of Henle to water. The reabsorption of Na^+ by the Na^+-K^+-$2Cl^-$ transporter in the absence of water reabsorption in longer loops of the juxtamedullary nephrons leads to establishment of the medullary osmotic gradient. It is worth remembering that without this gradient water reabsorption in the medullary collecting duct under the influence of ADH would not be possible.
4. b. All diuretics act by inhibiting the tubular reabsorption of Na^+. In case of loop and thiazide diuretics, with more Na^+ flowing to the distal nephron, the tubular cells reclaim some of the excess Na^+ leading to decrease in luminal positivity. The tubular cells try to compensate for this by secretion of the positively charged K^+ and H^+ into the lumen. Also, intravascular volume contraction because of diuretics cause activation of the renin-angiotensin-aldosterone system (RAAS), which in turn leads to urinary K^+ and H^+ losses under the influence of aldosterone.
5. d. Macula densa (modified tubular epithelial cells at the ending of TAL and beginning of DCT) senses Na^+ and Cl^- level in the tubular fluid and in case of low levels, stimulates granular cells, which are modified smooth muscle cells in the afferent arteriole to secrete renin. Renin breaks down hepatic angiotensinogen to yield angiotensin I, which is then cleaved further by the angiotensin-converting enzyme in the lung to yield angiotensin II. Angiotensin II apart from being a very potent vasoconstrictor also stimulates aldosterone release by the adrenal gland.
6. b. Aldosterone aids in the reabsorption of Na^+ and secretion of K^+ and H^+ in the collecting duct. Aldosterone upregulates the basolateral Na^+-K^+-ATPase (in principal cell) causing lower intracellular Na^+ and at the same time upregulates epithelial sodium channels (ENaC), thus increasing apical membrane permeability for Na^+. The movement of Na^+ from the lumen to the epithelial cells leads to luminal negativity which triggers K^+ secretion by the epithelial cells to correct the potential difference. However, the Na^+ to K^+ exchange is not strictly a one-to-one phenomenon and more Na^+ is reabsorbed than K^+ is secreted. H^+ gets secreted by the neighbouring alpha-intercalated cells to make up for this difference.
7. e. Primary hyperaldosteronism should always be suspected in the hypertensive patient with hypokalaemia and metabolic alkalosis (although more than half the patients are normokalemic) who is not on thiazide or loop diuretics. Aldosterone leads to reabsorption of Na^+ in the CD and the resulting tubular negativity aids in the excretion of K^+ and H^+.