ANATOMY

☑ Sheet ○ Slide ○ Handout

Number

2

Subject

Structure and function of renal tubules

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Date: 00/00/2016

Price:

- This sheet is written according to the record of section 1
- Some photos in this sheet are taken from Junqueira for a clearer view of the histological section, the doctor's photos are just beside them.

❖ Filtration & Starling forces

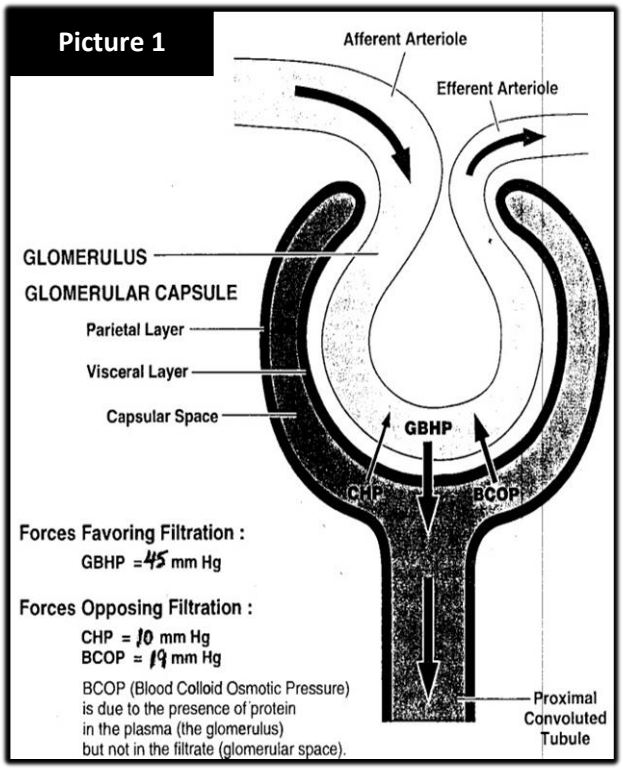
- Formation of urine starts by the process of filtration. Filtration of plasma (without plasma proteins) occurs at the glomerulus and the filtrate accumulate in Bowman's capsule.

- Filtrate then will continue its way along the nephron: Bowman's capsule (the beginning of the nephron) → proximal tubule → loop of Henle → distal tubule → the end of the nephron → collecting duct → papillary duct → minor calyx …

- Filtration is a net of what we call starling forces (*picture 1)*:
    1- <u>Glomerular blood hydrostatic pressure (GBHP)/ blood pressure:</u>
        - This pressure equals 45 mmHg
        - It tends to force fluids out of the capillary

    2- <u>Blood Colloid Osmotic Pressure (BCOP):</u>
        - This pressure equals 19 mmHg but could change across the capillary, it tends to work as an opposing force (i.e., to force fluids to move inward)

    3- <u>Capsular Hydrostatic Pressure (CHP):</u>
        - This is the pressure of fluid inside Bowman's capsule which is also an opposing force. It tends to push fluids inwards and equals 10 mmHg. However, this pressure could increase due to many pathological reasons:



- Kidney stone obstructing the ureter leading to backward accumulation of fluids
- Enlargement of the prostate which constricts the urethra and leads to accumulation of urine in the urinary bladder.

The net filtrating pressure = Glomerular blood hydrostatic pressure – the opposing forces = GBHP- (BCOP + CHP) = 45 – (10 +16) = +16 mmHg (filtration)

- Filtration Fraction:

  - Renal Blood flow (RBF) = ¼ of the cardiac output = 1100 mL/ min.
  - Plasma contributes to 57% of RBF ((the rest 43% is Hct (PCV))
    Renal plasma flow (RPF) = 1100 X 0.57= 625 mL/min
  - About 20% of plasma entering the kidneys is filtered at the renal glomerulus
    Glomerular filtration rate (GFR) = 625 X 0.20 = 125 mL/ min
  - Filtration fraction is the ratio between glomerular filtration rate (GFR) and renal plasma fluid (RPF), and is an indicator of how much plasma entering the kidney is filtered at the renal glomerulus *or how much of plasma entering the kidney passes to the renal tubules.*

Filtration fraction = GFR/ RPF

- Glomerular blood, which is being filtered, enters the glomerulus through afferent arteriole and leaves through efferent. The efferent arteriole is normally narrower than the afferent. Any obstruction of blood flow throughout these vessels will affect GFR and filtration fraction.

  Examples: *(table 1)*

  1- Constriction of the afferent arteriole:

  If the afferent arteriole is constricted, this will decrease volume entering the glomerulus (↓ RPF). Also, hydrostatic pressure is highly dependent on volume so filtration decreases as well (↓ GFR). Notice that decreasing both will not affect the ratio between them (filtration fraction stays the same).

2- Constriction of the efferent arteriole:

If constriction occurred in the efferent arteriole, the flow of plasma will be resisted (↓RPF) and plasma will accumulate inside the glomerulus leading to an increase in volume and hydrostatic pressure, which increases GFR & FF increases in this case. *Remember that the efferent arteriole is normally narrower than the afferent*.

3- If plasma protein amount increases:

This will have no effect on RPF, but obviously will increase colloid osmotic pressure (opposing force) and decrease GFR. As a result, filtration fraction will decrease.

4- If plasma proteins amount decreases:

RPF will not change, however, GFR and filtration fraction will increase due to decreasing of the opposing force.

5- Constriction of the ureter

RPF will stay the same, but any interruption to the flow of urine will increase capsular hydrostatic pressure (an opposing force) and decrease GFR and filtration fraction. This constriction might occur in the ureter by a kidney stone or in the urethra in the case of prostatic hypertrophy.

"Cases of renal stones and prostatic hypertrophy should never be neglected" – Dr. Faraj

Table 1: Effect of Changes in Starling Forces on RPF, GFR, and the Filtration Fraction

Effect	RPF	GFR	Filtration Fraction (GFR/RPF)
Constriction of afferent arteriole	↓	↓	N.C.
Constriction of efferent arteriole	↓	↑	↑
Increased plasma protein concentration	N.C.	↓	↓
Decreased plasma protein concentration	N.C.	↑	↑
Constriction of the ureter	N.C.	↓	↓

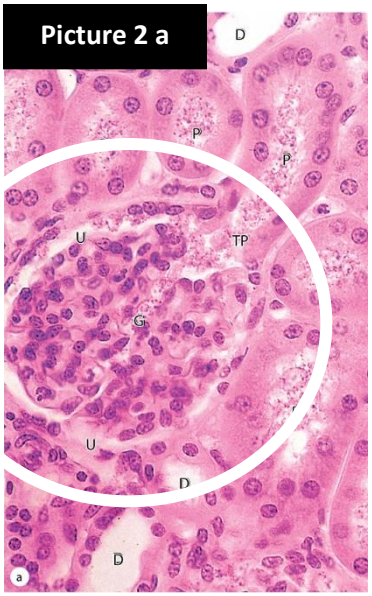
GFR, glomerular filtration rate; N.C., no change; RPF, renal plasma flow.

❖ Histology of the proximal convoluted tubule:

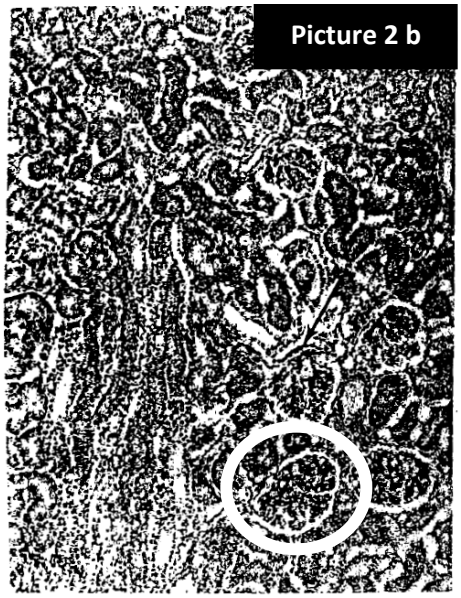
General ideas about a section in the kidney:

- Whenever a section is taken from the kidney, we have to know whether it's in the cortex or the medulla.
- The presence of renal corpuscles indicates that the section is taken from the cortex. *Picture 2(a +b)*
- Most of the tubules seen in renal sections are the proximal convoluted tubules (PCT); this is because each PCT is 15 mm long and the distal is only 8 mm long. The longer the tubule, the higher the chance to be crossed in the section.
- The proximal tubule is wider (60 micrometer) than the distal (30 micrometers). Clinicians don't care about the diameter but researchers do. *Please notice that the proximal tubule as a whole is wider, not the lumen.*

Picture 2 a



Picture 2 b



ig. 13-6. Photomicrograph of the cortex of the kidney, show-g several glomeruli and proximal and distal convoluted bules. Note a macula densa (arrow). (H&E; ×100.)

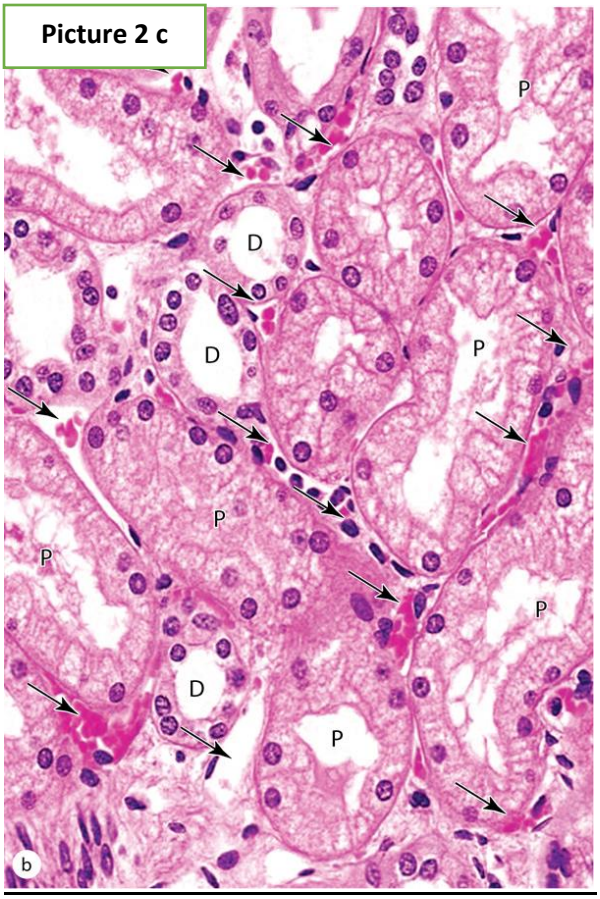
Under the light microscope *(pictures 2(c+d))*:

- Proximal tubule cells range from low columnar to cuboidal.
- They have indistinct (ill-differentiated) cell boundaries.
- Stellate-shaped bounded by a distinct regular **brush border**, brush border is composed of microvilli that serve an important role in reabsorption.
- Cells are **deeply eosinophilic** because they are rich in mitochondria that generates the needed energy for active transport as we will see.

Picture 2 d

Fig. 13-17. Photomicrograph showing many proximal convoluted tubules cut in oblique and cross sections. Note that each tubule is lined with cuboidal epithelium and the cytoplasm stains strongly with eosin because of the many mitochondria (not shown). The nuclei are centrally placed, and the luminal cell surfaces have indistinct brush borders formed of microvilli. Three distal convoluted tubules are also present (D). Note that the cytoplasm of the cuboidal cells lining the distal convoluted tubules stains lighter with eosin. (H&E; ×400.)

Picture 2 c



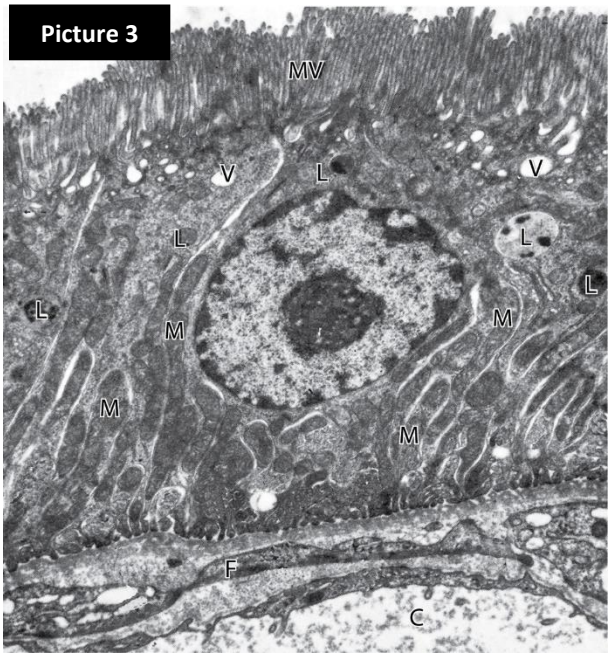
Under the electron microscope *(picture 3)*:

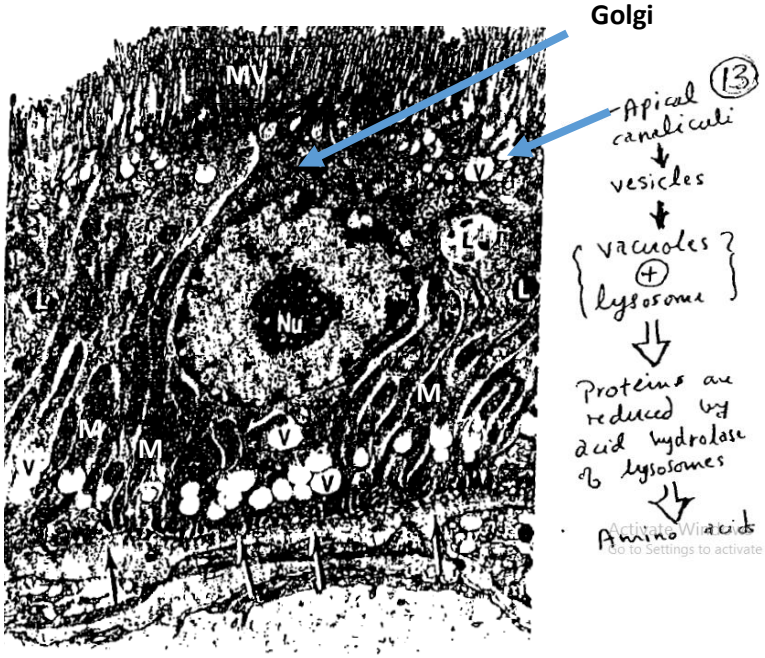
- At the basal membrane, a huge amount of mitochondria *(M)* work to produce energy.
- At the apical membrane of the cells (membrane toward the lumen of the cell), we can see the brush border. This makes sense as microvilli *(MV)* are important for reabsorption.
- Between the bases of the microvilli, there are small clefts called **Apical Canaliculi**. The canaliculi form vesicles *(V)* which condense to form vacuoles, vacuoles then fuse with lysosomes to allow hydrolysis of its content. But why do we need hydrolysis in the urinary tract?

  Suppose that some proteins passed to the filtrate, they will pass through the apical canaliculi. These canaliculi will form a vesicle and follow the mentioned steps to be hydrolyzed into amino acids. Amino acids then go back to the blood because normally no protein should be found in urine.

  Note: The complex formed of (1) apical canaliculi, (2) vesicles and (3) vacuoles is called the endocytic complex.

Picture 3





❖ Functions of the proximal convoluted tubule:

- Renal tubular epithelial cells can transport solutes and water from one side of the tubule to other. This allows them to function in both reabsorption (movement of substances from tubular fluid → blood), or secretion (blood → tubular fluid).

- Some substances are excreted in the urine but not filtered in the glomerulus, these are added to the filtrate via **secretion**.

- Notice the apical membrane facing the lumen, and the basolateral membrane which contains high numbers of mitochondria. *(picture 4)*

- Between cells there are tight junctions which are not tight enough to prevent the passage of water and many other substances *(actually tight is not an accurate description for these junctions)*; which means that fluid can pass from the lumen toward the blood through them!

- From now on, the filtrate in the lumen of renal tubules is called **tubular fluid**.

- The blood capillary around the proximal tubule is part of the peritubular capillaries. Remember that blood in the peritubular capillaries is rich in proteins because many

of the plasma fluid has already leaked to the filtrate in the glomerulus. This increase in colloid osmotic pressure helps in reabsorption of water.

- Substances have two pathways to be reabsorbed:
    1. Paracellular pathway: through the tight junctions (between cells), it is important here to notice the **lateral intercellular space** *(picture 4)*.
    2. Transcellular pathway: across cell membranes (through the cell).

- The basolateral membrane has a lot of Na^+/ K^+ pumps, this pump transports 3 Na^+ ions outside the cell, and 2 K^+ ions to inside of the cell. This active transporter needs ATP to work which explains the presence of mitochondria at the basolateral membrane.

- The most important function for the proximal tubule or even the kidney is reabsorption of Na^+ (99.9% of Na^+ is reabsorbed in the kidney & 65% of Na^+ reabsorption occurs at the level of PCT). We care about Na^+ the most due to the fact that fluids follow Na^+ by osmosis, so if Na^+ was regulated other body fluid volumes will be balanced.

- Na^+/ K^+ pump actually is the key factor in reabsorption of Na^+ because it builds the electrochemical gradient *(gradient in both charge and concentration)* needed for this process by decreasing concentration of Na^+ intracellularly, allowing its passive diffusion at the apical membrane from the tubular fluid to the tubular cells through a transporter protein.

- Mechanisms of transporting Na^+:

    1- Passive diffusion: Na^+ diffuses passively by a transport protein along its gradient (tubular fluid → tubular cells at the apical membrane). *Picture 4/ point 1*

    - Note that this is not simple diffusion, simple diffusion doesn't require a transporter.

    2- Primary active transport: Na^+ is transported against its gradient by Na^+/ K^+ pump (Tubular cells → lateral intercellular space & peritubular capillaries at the basolateral membrane). *Picture 4/ point 2*

    - These 2 mechanisms are the 2-step process of Na+ reabsorption

    3- Secondary active transport: This occurs at the apical membrane when Na^+ diffuses downhill (across its gradient) and the potential energy produced by its movement downhill is utilized in transporting another substance uphill (against its own gradient) through the same specific transporter. Here we can differentiate between two types of secondary active transport:

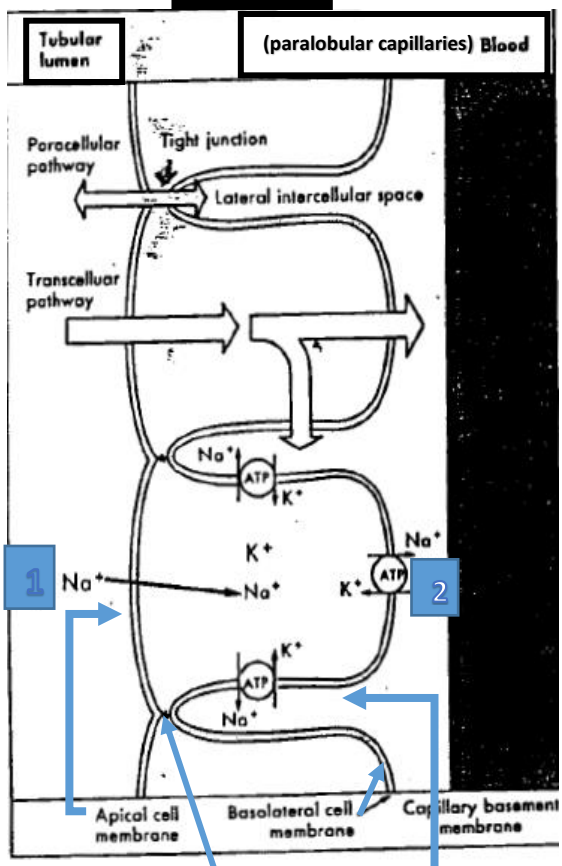
a- Co-transport/ symport transport: Na^+ and another substance in the same direction (tubular fluid → tubular cell) and by the same transporter. The other substance can be glucose, amino acids, lactate, phosphate ... etc. *picture 5/ point 1*

b- Anti-cotransport/ antiport/ counter transport: Na^+ and H^+ move in opposite directions, which means that Na^+ moves to the cell from the tubular fluid, but H^+ is excreted to the tubular fluid. This explains how the kidney functions in acid/ base balance. *See picture 5/ point 2*

Note:

Any living cell accumulates CO_2 and H_2O as products of metabolism, these form carbonic acid under the effect of carbonic anhydrase enzyme (CA enzyme). Carbonic acid easily dissociates to form H^+ and HCO_3^-. H^+ is secreted in urine, whereas bicarbonate ion is reabsorbed. So for every H^+ secreted, one HCO_3^- is reabsorbed to the blood. *Picture 5/ point 2*

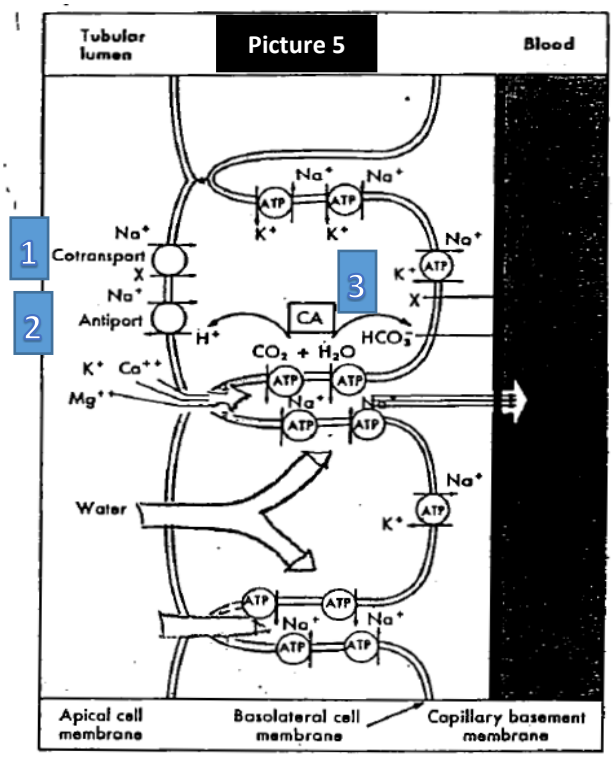
Picture 4



Tight junctions

Lateral intercellular space

Picture 5



- So, the proximal tubule is important for reabsorption of many substances:
    1. Two thirds of water, Na^+, Cl^- and K^+.
    2. All of glucose and amino acids (there shouldn't be any glucose or amino acids in urine)
- Because plasma (without proteins) has diffused freely in the glomerulus, the filtrate there is an isotonic solution. Now after the reabsorption of many substances in the proximal tubule, what happened to the osmolarity of the tubular fluid? Due to reabsorption of Na^+ and water in the same proportion (67% of both), the tubular fluid in the proximal tubule is still an **isosmotic solution** until it enters the loop of Henle.

- Reabsorption of water occurs through paracellular transport (through tight junctions) across the proximal tubule due to accumulation of Na^+ and other substances in the lateral intercellular space. Water will follow solutes by osmosis and fluid will accumulate there leading to an increase in hydrostatic pressure. This increase helps in driving water to capillaries. Also, water diffuses via the transcellular pathway by osmosis. *Picture 6*

Picture 6

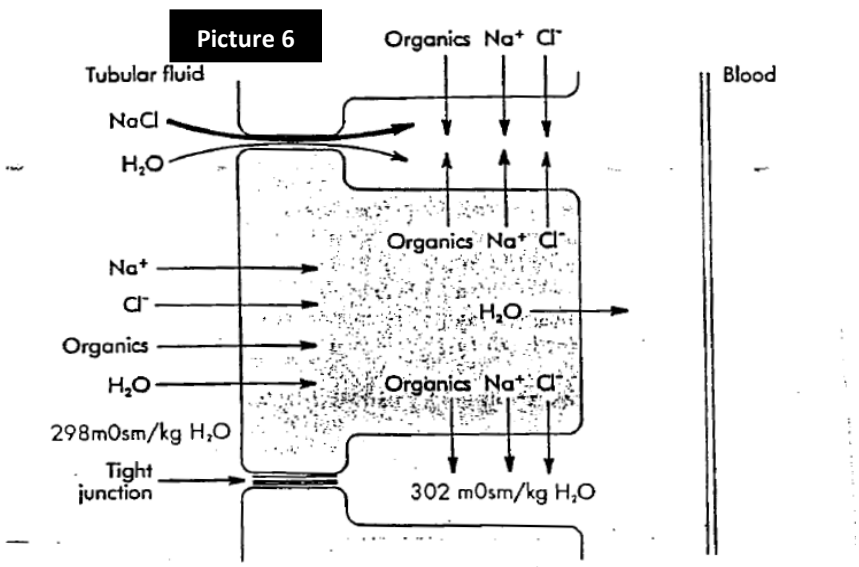


FIGURE 36-5 Routes of water reabsorption across the proximal tubule. Transport of Na^+, Cl^-, and organic solutes into the lateral intercellular space increases the osmolality of this compartment, which establishes the driving force for osmotic water reabsorption across the proximal tubule. An important consequence of osmotic water flow across the proximal tubule is that some solutes, especially K^+, Ca^{++}, and Mg^{++}, are entrained in the reabsorbed fluid and are thereby reabsorbed by the process of solvent drag.

- We divide the proximal tubule into 2 halves:

  1- During the first phase (half):

Na^+	Reabsorbed	Tubular fluid → cell Passive diffusion	Cell → blood Na^+/ K^+ ATPase
Glucose Amino Acids Phosphate	Reabsorbed	Tubular fluid → cell Secondary active transport (co-transport)	Cell → blood Down their gradient through a specific transporter.
H^+	Secreted	Source of H^+ is from dissociation of carbonic acid inside the cell	Cell → tubular fluid Secondary active transport (counter-transport)
HCO_3^-	Reabsorbed	Source of bicarbonate is from dissociation of carbonic acid inside the cell	Cell → blood Down its gradient

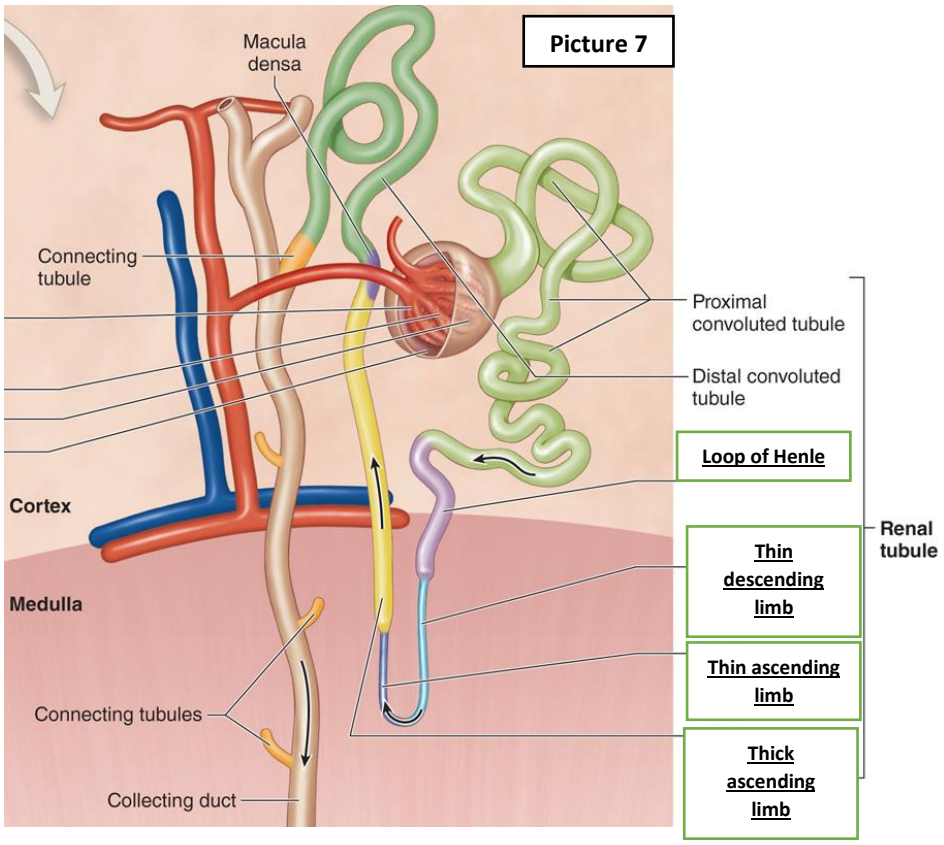
  2- During the second half:

- The second phase of proximal tubular reabsorption involves reabsorption on Na^+ and Cl^- in the second half of the proximal tubule. This occurs because Na^+ is reabsorbed with bicarbonate as the primary accompanying anion in the proximal half, leaving behind a solution that becomes enriched in Cl^-. The rise in Cl^- concentration in the tubular fluid creates a gradient that favors the diffusion of chloride from the tubular lumen across the tight junctions and into the lateral intercellular space. Movement of the negatively charged chloride ions attracts the positively charged sodium ions. Thus, in the second half of the proximal tubule some Na^+ and Cl^- are reabsorbed across the tight junctions (paracellularly) by passive diffusion.

- Na^+ and Cl^- reabsorption by the second half of the proximal tubule also occurs by a transcellular route. The pathway of Na^+ and Cl^- transport across the apical membrane is unknown.

Na^+	Reabsorbed	Tubular fluid → cell Passive diffusion	Cell → blood Na^+/ K^+ ATPase
Cl^-	Reabsorbed	Tubular fluid → cell Passive diffusion	Most probably paracellularly but could be transcellularly

❖ Loop of Henle:



- Tubular fluid enters loop of Henle as an isotonic solution.
- Tubular fluid in the loop of Henle is surrounded by hypertonic media (medulla)
- The loop is composed of three limbs: thin descending, thin ascending & thick ascending. (*Picture 7*)
- The three limbs can be grouped according to their permeability to water:

(1) Thin descending limb: highly permeable to water

Equilibrium occurs there via **reabsorption of water** from the isosmotic tubular fluid (300 mOsm) to the hypertonic surrounding/ interstitium (600 mOsm). We took water and left solutes behind).

(2) The thin ascending limb, thick ascending limb and the beginning of distal tubule: all impermeable to water

Here, osmotic equilibrium occurs by reabsorption of solutes (Na^+, Cl^-) instead of water.

- Thin ascending limb:

  -Tubular fluid enters as hypertonic tubular fluid (600mOsm).

  - Interstitium around it starts as isosmotic (300mOsm), but after movement of Na^+ from tubular fluid to interstitium there would be an increase in its osmolarity.

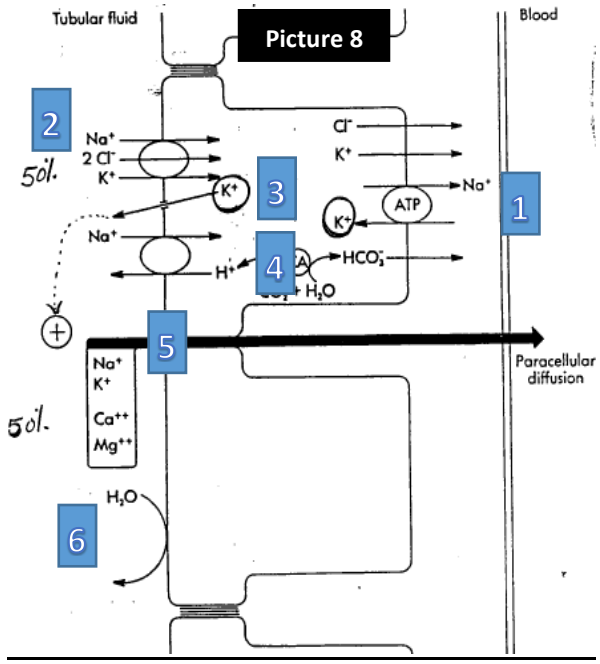
  - Water cannot follow Na^+ and fluid becomes **hypotonic**, so we call this segment **"Medullary diluting segment"**.

- Thick ascending limb:

  - Most important function is to concentrate solutes around the loop of Henle (**Single Effect**)

  - In order to concentrate solutes around the loop of Henle, we have to create a gradient to allow their movement, this gradient involves the following:

Picture 8



1- K^+ gradient:

- The key element in solute reabsorption by the thick ascending limb is the Na^+/ K^+- ATPase in the basolateral membrane. Like reabsorption in the proximal tubule, the reabsorption of every solute by the thick ascending limb is linked to the Na^+/K^+ ATPase pump. Operation of the pump maintains a low cell [Na^+]. *Picture 8/ point 1*
- This low [Na^+] provides a favorable chemical gradient from the tubular fluid into the cell. The movement of Na^+ across the apical membrane

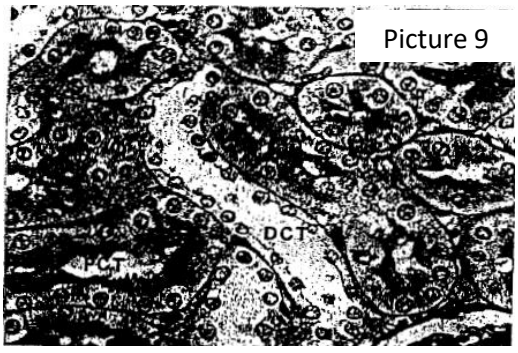
into the cell is mediated by the $1Na^+/2Cl^-/1K^+$ symporter, which couples the movement of 1 Na^+ with 2 Cl^- and 1 K^+. This symport protein uses the potential energy released by the downhill movement of Na^+ and Cl^- to drive the uphill movement of K^+ into the cell. *Picture 8/ point 2*

- Notice that we are driving K^+ to enter the cell through the symport protein & Na^+/K^+ ATPase. This leads to increase in intracellular K^+ concentration, so it exits to the tubular fluid and concentrates there through a channel on the apical membrane. *Picture 8/ point 3*

2- H^+ gradient:

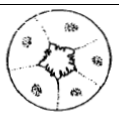
- H^+ is transported uphill through Na^+/ H^+ counterport and for each H^+ secreted one bicarbonate is absorbed. *Picture 8/ point 4*
- As a result, the voltage across the thick ascending limb is positive in the tubular fluid relative to the blood, which is relatively negative, because of the unique location of transport proteins in the apical and basolateral membranes. The important points to recognize are that increased salt transport by the thick ascending limb increases the magnitude of the positive voltage in the lumen, and this voltage is an important driving force for the reabsorption of several cations, including Na^+, K^+, Ca^{++} and Mg^{++} across the Paracellular pathway. (5% of Na^+ is reabsorbed paracellularly). *Picture 8/ point 5*
- Because the thick ascending limb is very impermeable to water, reabsorption of NaCl and other solutes reduces the osmolality of tubular fluid to less than 150 mOsm/kg H_2O → Hyposomotic. *Picture 8/ point 6*
- Shifting of solutes makes the interstitium hyperosmotic and helps the thin descending segment to reabsorb water. This is what we call "**single effect** of the thick ascending limb"

❖ Distal convoluted tubule (DCT):



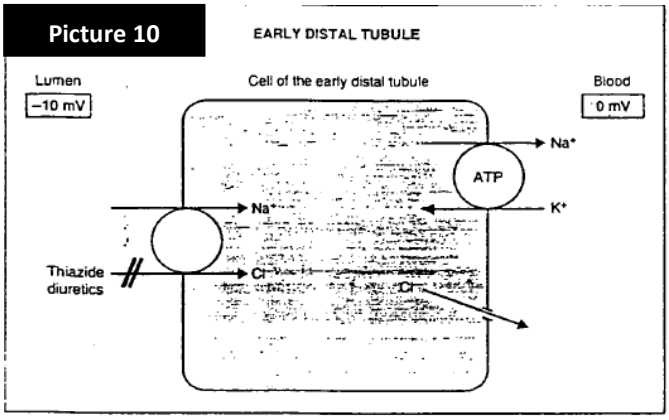
Picture 9

- Wider lumen than proximal convoluted tubule (PCT). However, DCT has a smaller diameter.
- Cells are shorter than that of the PCT
- Microvilli are there but are not continuous (**No brush border**)

 Proximal Convoluted Tubule	 Distal Convoluted Tubule
Diameter: 60 micrometers	Diameter: 30 micrometers
Cells are longer	Cells are shorter
Brush border at the apical surface	No brush border
Net result: narrower lumen	Net result: wider lumen

- DCT Consists of 3 parts:

1. <u>Early DCT:</u>



Cortical diluting segment

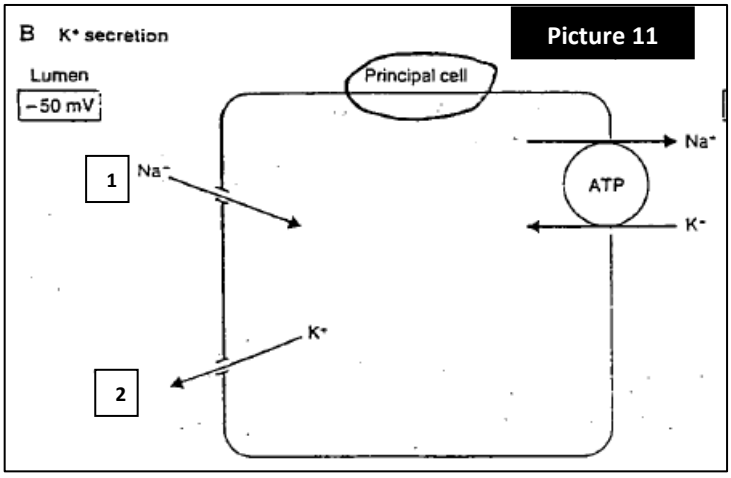
- This part is a continuation of the thick ascending limb of Henle and has the same histological, structural & functional properties.
- This part is impermeable to water, so solutes are reabsorbed in order to achieve osmotic equilibrium. Reabsorption of Na^+ is followed by Cl^- by the same mechanism; Na^+/ K^+ ATPase creates the gradient to allow Na^+ diffusion passively followed by the negatively charged Cl^-. *Picture 10*
- Solutes were reabsorbed leaving water behind, which means that the tubular fluid is being diluted!
- This will further decrease the osmolarity of the tubular fluid and we call the early DCT the **<u>cortical diluting segment.</u>**

2. Intermediate DCT (Macula Densa):

- This segment has closely packed columnar cells, nuclei are packed closely to each other.
- It's a part of the juxtaglomerular apparatus (J-G apparatus) which plays a role in regulating body fluids and Na^+. It is said that macula densa special receptors sense the concentration of Na^+ and Cl^- to indirectly estimate blood pressure and volume. If there was a drop in the concentration of Na^+, it sends signals to juxtaglomerular cells in the afferent arteriole to secrete renin. Renin converts angiotensinogen to angiotensin-I, which is further converted to angiotensin-II by the converting enzyme in the lungs. Angiotensin-II is important in stimulating the secretion of aldosterone (a vasoconstrictor) from the suprarenal cortex. It was thought that aldosterone is the strongest vasoconstrictor, but nowadays they think it is angiotensin-II.

3. Late DCT:

- It resembles the collecting duct structurally and functionally.
- Late distal convoluting tubule and the collecting duct have two important types of cells:
  - Principle cells
  - Intercalated cells

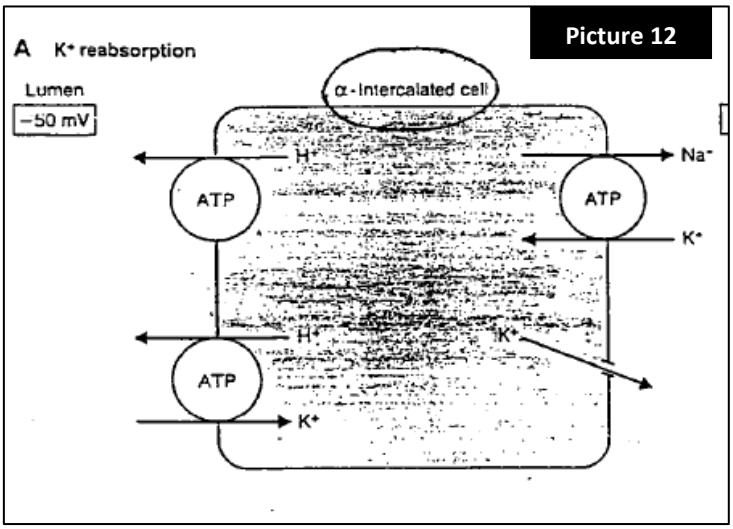


Picture 11

Priciple cells

- They have two functions *(picture 11)*:

1. Reabsorption of Na^+ under the effect of aldosterone.
2. K^+ secretion



Intercalated cells

- They are dark in color *(picture 12)*
- They have two functions:

1. Reabsorption of K^+
2. H^+ secretion

❖ The collecting Duct:

- It starts in the cortex, enters the medulla till reaching the apex of the pyramid.
- At the apex of the pyramid, collecting ducts reach the papillary duct → minor calyx, so fluid that reaches the collecting duct is actually urine.

❖ To recap the story of the tubular fluid:

1. Passive diffusion in the glomerulus→ isotonic filtrate in Bowman's capsule.
2. Reabsorption of Na^+ and water in the same proportion in the proximal convoluted tubule → isotonic tubular fluid.
3. Reabsorption of water in the thin descending segment of Henle → gradual decrease in osmolarity of the tubular fluid in comparison to the hypertonic interstitium (600)
4. Reabsorption of Na^+ and Cl^- leaving water inside the thin ascending limb of Henle (**medullary diluting segment**) → tubular fluid is becoming hypotonic gradually.
5. Further reabsorption of Na^+ and Cl^- in the thick ascending limb of Henle → further hypotonic.

6. Early distal convoluted tubule; (**cortical diluting segment**) → further hypotonic
7. Intermediate distal convoluted tubule → part of the J-G apparatus
8. Late distal convoluted tubule, where we have principle and intercalated cells.
9. Collecting duct → urine.

❖ The effect of some drugs (diuretics):

1- Spironolactone (Aldactone):

- This drug is a strong diuretic.
- It is an aldosterone antagonist.
- Excretes Na^+ and reabsorbs K^+, so it may lead to hyperkalemia which may cause abnormal heart rate, cardiac arrest and death.

2- Furosemide (Lasix)

- This drug is a loop diuretic
- If the patient had sever edema which wasn't relieved by Lasix alone, we give him aldactone with it.

"حد يتكرَّم ياخد الغياب"