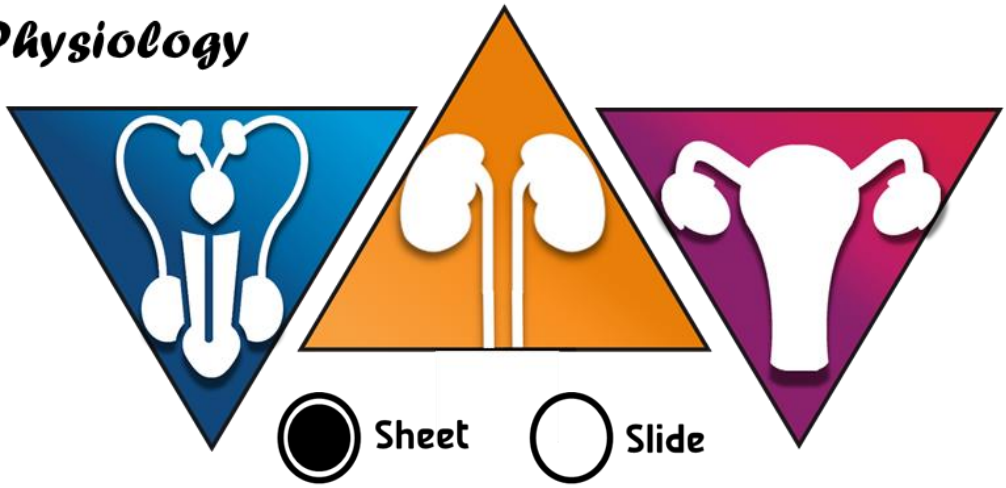
Urogenital system

Physiology



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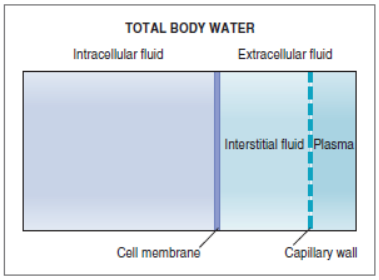
In this lecture we are going to study the tubular reabsorption of Na+.

- We know that the body must maintain its **homeostasis** by keeping its internal environment balanced and under control, and sodium is not an exception, the body must keep sodium within certain pre-set limits by balancing the intake and the excretion processes. (so, Na+ intake = Na+ output).

  The daily intake of Na+ is varying depending on many factors (location, culture, age …etc), but let's say that it is equal to 155 mMol/day (or 4 g). Of these 155 mMol, 150 mMol will be renally excreted, and the other 5 mMol will be excreted via other mechanisms (such as GIT secretions or sweating).

- It is very important to study sodium, as we always give patients fluids which must have a specific characteristic (**such as osmolarity**). Let's revise the body fluids and the definition of osmolarity:



  Body fluids are distributed between two major fluid compartments: fluid within the cells, or intracellular fluid (ICF), and fluid surrounding the cells, or extracellular fluid (ECF). The ECF compartment is further subdivided into plasma (which is the fluid portion of blood) and interstitial fluid (which is the fluid in the spaces between cells).

  These compartments **differ** in their components; such as their proportions of ions (Na+, Ca+2, K+…etc), sugars, and proteins (plasma, for example, has more proteins than the interstitial fluid).

  On the other hand, they are similar in their **osmolarities.** And osmolarity can be defined as: the concentration of a solution expressed as the total **number** of solute particles per litre. Now how do these compartments have the same osmolarities although they differ in their components?

  Normally, the osmolarities of the ECF and ICF are the same because the total concentration of ions and proteins inside the cells is equal to the total concentration of ions and proteins in the fluid surrounding the

cells. Even though particles in the ECF and ICF differ, their concentrations are normally identical, and the **number (not the nature)** of the unequally distributed particles per volume determines the **fluid's osmolarity** (as we said).

And because the osmolarities of the ECF and ICF are normally equal, no net movement of H2O usually occurs between compartments (although the water can move freely between them). Therefore, cell volume normally remains constant.

Before we continue, we need to review some **TERMINOLOGY:**

- **Molarity (Mol/L):** is the number of moles per liter which equals = # of moles/volume
- **Osmolarity (Osmol/L):** is the total number of all osmotically active solutes per liter, which equals =molarity *number of particles [example: 1 mole/L of NaCl equals 2 Osmol/L because 1 mole of NaCl means 1 mole of Na and 1 of Cl so its 2 Osmoles]
- **Equivalents (Eq)=**number of univalent counter ions needed to react with each molecule of substance(example: 1 mole of Ca++= 2eq because you need 2 moles of Cl- to react with it)

  Since Na+ has a univalent charge these terms will be used interchangeably throughout this sheet

Ex: Assuming that the membrane is only permeable for water, a 2 g of which of the following particles would have the strongest tendency to attract water? Na+, albumin, or glucose.

The tendency to attract water is determined by the osmolarity (osmolarity↑ then the tendency to attract water↑).

As we said Osmolarity equals the molarity multiplied by the number of particles in solution. So:
Osmolarity= #of moles/ L * # of particles
Osmolarity = (mass/MW)/L * # of particles

- **One mole of any molecule = 6.022×10^{23} particles**

From the box to the right we can notice that the osmolarity is directly proportional to the mass and inversely proportional to the MW. So, the particle with the lowest MW will have the higher tendency to attract water, and this particle is Na+ followed by glucose, and the least is being albumin. (if you don't trust the answer, you can use the equation in the box).

The plasma osmolarity ranges **between 285 and 310 mOsm/L.** so, if we need to give the patient fluids, you must prepare an isotonic solution to avoid the imbalance (which causes either dehydration or edema).

- Now how much NaCl is needed to prepare the normal saline (isotonic solution with osmolarity of 285 mOsm/L? Figures are approximate

Keep in mind the NaCl (MW=58) will be dissociated into 2 particles!

Osmolarity/ L = (mass/MW) * # of particles
to calculate it in milliosmoles per liter (mOsm/L):

$$\text{mOsmol/L} = \frac{\text{Weight of substance (g/L)}}{\text{Molecular weight (g)}} \times \text{Number of species} \times 1000$$

X=58*285 /2000 = 9g/L
By using this equation, we find that the needed mass of the NaCl is 9 g/L = 0.9 g/100 ml
From this the 0.9% N/S (normal saline) is named!

- We can do the same, to calculate the needed mass of glucose (MW=180) to prepare an isotonic solution with osmolarity of 285 mOsm/L? **The needed mass will be 5 (g/100 ml) dextrose/ water solution** notice that the # of particles here is 1. (X= 180*285/1000 = 50g/L = 5g/dl = 5% O/W).

Now we are going to start our discussion about sodium and its tubular reabsorption.

Sodium is very important as we said, and its concentration must be maintained, but why sodium?

1. **It Contributes to the osmolarity of the plasma and controls fluid volumes**

In the plasma there are cations and anions, both contribute to the osmolarity (in addition to other particles like sugars and proteins). We have the same number of cations and anions. Sodium and its attendant anions, being by far the most abundant solutes in the ECF in terms of numbers of particles, account for the vast majority of the ECF osmotic activity. But Na+ is not the only cation; we also have K+ , Ca++, etc. If Na+ was the only cation, we can know the osmolarity by multiplying its concentration by 2; for example, the concentration of Na+ is 140 mMol/L → so the osmolarity=140 * 2=280. But because it is not the only cation we multiply its concentration by 2.1. **So, we can predict the osmolarity using Na+ concentration**.

2. **It controls the extracellular volume**

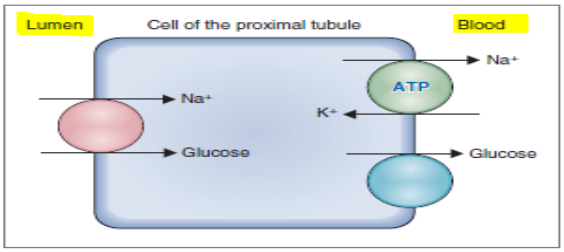
   If its concentration increases (increased intake), more water is retained (from the kidney) which increases the volume causing edema. But if it decreases, less water is retained which causes volume contraction(dehydration). (this will be discussed later)

3. **It is important for the excitable tissue to function**

   Excitability of the cell means; its ability to reverse its membrane potential which can be done by the influx of **sodium**, calcium, or both. Excitable cell is the cell that doesn't perform its function unless it is excited.

4. **It is essential for the kidney to reabsorb useful particles (glucose, amino acids, and other) and git rid of waste products (H+) via the secondary active transport. See the figure.**

   Notice that: **active reabsorption** takes place if any step in the transepithelial transport of a substance requires energy, even if the other steps are passive**.**



   The basolateral Na+–K+ pump actively transports Na+ from the tubular cell into the interstitial fluid (blood side). This process establishes a concentration gradient for passive movement of Na+ from the lumen into the tubular cell at the luminal side. This Na+ gradient is used to **co-transport** the glucose, amino acids, and others against their electrochemical gradient.

   This Na+ gradient is used also to **counter-transport** waste products such as H+ against their electrochemical gradient (from the interstitial fluid with the higher PH toward the lumen with the lower PH(Higher {H+})).

5. **It is targeted by diuretics**

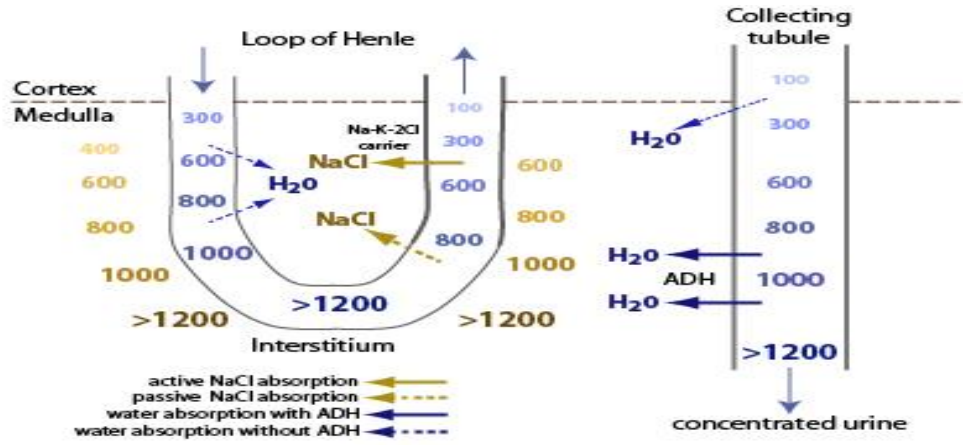
   Diuretics inhibit the reabsorption of **sodium** then increase the excretion of sodium and water. They inhibit Na+ reabsorption → Na+ remains in the lumen of the tubule and keeps water with it → so you get rid of extra Na+ and water.

6. **It has a role in the formation of the corticomedullary osmotic gradient**

   (This topic is in the online lecture by dr Najeeb explained briefly below)

The cells of the thick ascending limb are impermeable to water, clearly an unusual characteristic because virtually all other cell membranes are highly permeable to water. As a consequence of the water impermeability, Na Cl and k (by Na+:K+:2*Cl- carrier) is reabsorbed by the thick ascending limb, but water is not reabsorbed along with it. As a result, the osmolarity in the peritubular interstitium and capillaries increases highly around the ascending limb, and it may reach 1200-1400 mOsmol/L (and it is decreasing as the ascending limb ascends) see the figure below. And this gradient is very important for the process of water reabsorption.

The collecting duct can adjust water reabsorption to produce urine as hypotonic (dilute) as 50 mOsm/L or as hypertonic (concentrated) as 1,400 mOsm/L, depending on the body's need for water conservation or removal. In a state of hydration, ADH is not secreted and the collecting duct reabsorbs salt without reabsorbing water (it will be water impermeable like the ascending loop of Henle); the water remains to be excreted in the dilute urine. In a state of dehydration, ADH is secreted, the collecting duct reabsorbs water (by inserting a special type of water channels), and the urine is more concentrated. The CD can do this because it passes through an osmotic gradient in the medulla from 300 mOsm/L near the cortex to 1,200 mOsm/L near the papilla. This gradient is produced by a **countercurrent multiplier** of the nephron loop, which concentrates ions in the lower medulla.



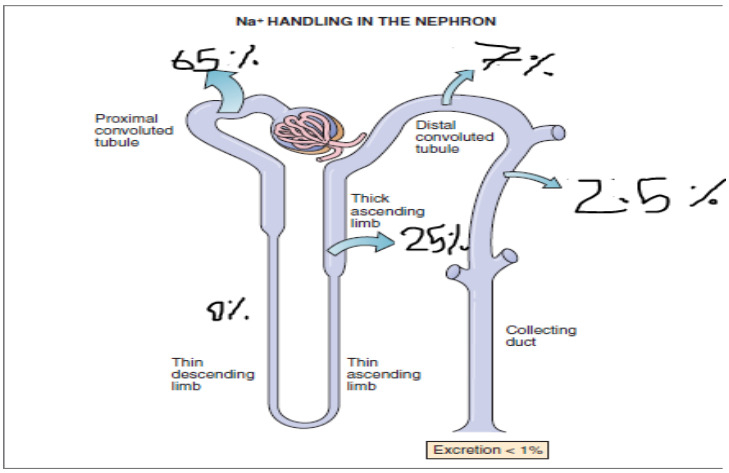
It is important for those with renal impairment to

1- **reduce the Na+ intake, as the majority of renal ATP consumption is to reabsorb Na+.**
2- **reduce their protein intake due to GFR impairment(proteins increases urea which is a waste product that the impaired kidney cannot handle).**
3- **maintain their blood pressure tightly, as hypertension causes scleroses in the basement membranes of the kidney.**

Now, we finished the introduction of this lecture, and it is time to talk about the renal function in handling sodium.

All plasma constituents except the plasma proteins are indiscriminately **filtered** together through the glomerular capillaries.

The kidneys are responsible for maintaining a normal body Na+ content. On a daily basis, the kidneys must ensure that Na+ excretion exactly equals Na+ intake, a matching process called **Na+ balance**. For example, to remain in Na+ balance, a person who ingests 150 mEq of Na+ daily must excrete exactly 150 mEq of Na+ daily. What happens? See the figure below.



1- Na+ is **freely filtered** across glomerular capillaries and subsequently reabsorbed throughout the nephron.
2- By far, the bulk of the Na+ reabsorption occurs in the **proximal convoluted tubule**, where two-thirds (or 65%) of the filtered load is reabsorbed. In the proximal tubule, water reabsorption is always linked

to Na+ reabsorption and the mechanism is described as isosmotic. (will be proved later)

3- **The descending limb doesn't reabsorb Na+ at all** (0%), but water is reabsorbed here, and this results in an increasing osmolarity of the tubular fluid as the water is being reabsorbed along this limb.
4- **The thick ascending limb of the loop of Henle** reabsorbs 25% (along with 2*Cl-, and K+ reabsorption by **Na+:K+:2*Cl- carrier**, it is electroneutral) of the filtered load of Na+. In contrast to the proximal tubule, where water reabsorption is linked to Na+ reabsorption, the thick ascending limb is impermeable to water.
   The **single effect phenomena of the countercurrent multiplier** happen at this level. Na cl k are reabsorbed out of the ascending limb and deposited in interstitial fluid, and water remains behind in the ascending limb. So, we end up with **hyper**osmolar interstitium and **hypo**osmolar filtrate
5- **The early distal convoluted tubule** reabsorbs approximately 7% of the filtered load, and, like the thick ascending limb, it is impermeable to water. **The late distal convoluted tubule** and **collecting ducts** reabsorb the final 2.5% of the filtered load and are responsible for the fine-tuning of Na+ reabsorption, which ultimately ensures Na+ balance. Not surprisingly, then, the late distal convoluted tubule and collecting duct are the sites of action of the Na+-regulating hormone **aldosterone.**
6- **Approximately, 0.6 % of Na+ will be excreted**, how to calculate this percent?
   With an average Na+ intake of 150 mEq/day, to maintain Na+ balance, excretion should be 150 mEq/day, which is less than 1% of the filtered load ($FL=GFR*P_{Na}+$). (If GFR is 180 L/day and plasma Na+ concentration is 140 mEq/L, then the filtered load of Na+ is 25,200 mEq/day. Excretion of 150 mEq/day, therefore, is 0.6% of the filtered load [150 mEq/day divided by 25,200 mEq/day]. You can easily calculate the absorbed load to be 99.4% [25,050(25,200-150) divided by 25,200].

Now, How could the scientists determine the proportion of reabsorption in each segment?!

Along the nephron, the clearance of Na+ can be easily calculated as follows:

$C_{Na^+} = (U_{Na^+}/P_{Na^+}) * V$

$=(100/140) * 1 = 0.7$ ml/min

CNa+:. Clearance of sodium

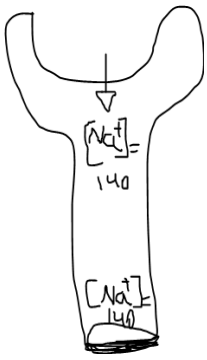
UNa+: concentration of sodium in urine =(150 $_{mEq/day}$)/ 1.5 L (urine output per day)= 100 mEq/L

PNa+: concentration of sodium in plasma which is equal to its concentration in bowman capsule=140 mEq

V: urine flow rate

Now how to calculate the **Segmental Clearance** (the proportion of reabsorption in each segment)

Let's take the proximal tubule as an example:



by using the micropuncture technique, by which a micropipette (25 µm) is inserted in different parts of the nephron, we take two samples the 1st one from the bowman's space and the 2nd one from the end of proximal tubules and analyze the concentration of sodium in both samples we would find that they have same concentration, How?!

- There could be neither secretion nor reabsorption, but it is not the case here!
- What happens is that, sodium and water are reabsorbed to the same proportion through the proximal tubules then the concentration of the sodium still the same!

Clearance of Na^+ across the proximal tubule:

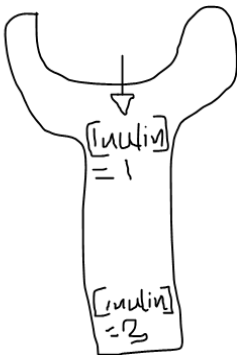
$$C\,(Na^+) = \frac{Na^+ \text{ concentration in } B}{Na^+ \text{ concentration in } A} X \text{ flow rate} = \frac{TF(Na^+)}{P(Na^+)} X\, V$$

- Concentration of Na+ at A = concentration of Na+ in plasma =P(Na+)
- TF (Na+): concentration of Na+ in the tubular fluid
- V is the flow rate in this particular segment

So, we find it to be 140/140=1!

Now, we measure the proportion of water reabsorption to know the proportion of sodium reabsorption in the proximal tubules, how can we do that?!

We Inject the patient with inulin (it is important to remember its characteristics, neither reabsorbed nor secreted) then we take two; samples 1st in the bowman's space and the 2nd at the end of the proximal tubule. After the analysis of these two samples we calculate the clearance as below:



Clearance of inulin across the proximal tubule:

$$C\ (inulin) = \frac{inulin\ concentration\ in\ B}{inulin\ concentration\ in\ A} X\ flow\ rate = \frac{TF(inulin)}{P(inulin)}\ X\ V$$

Clearance of inulin= [inulin at the end of proximal tubule] / [Inulin in the bowman's space-plasma] =3/1=3 (the concentration is tripled, so we expect the water to be reduced by 2 thirds) .
So, 65% of water is reabsorbed, and 65% of Na is reabsorbed (to keep the same concentration they are handled similarly as we said).

Now, to relate the Na+ to inulin we use the following equation:

$$\frac{C_{Na}}{C_{in}} = \frac{\frac{T_{Na} \times V}{P_{Na}}}{\frac{T_{in} \times V}{P_{in}}}$$ Since the flow rate is the same: $$\frac{C_{Na}}{C_{in}} = \frac{\frac{T_{Na}}{P_{Na}}}{\frac{T_{in}}{P_{in}}}$$

If CNa+/Cinulin :

a) =1 → this means that Na , is neither reabsorbed nor secreted i.e. whatever is filtered at A reached point B. However, this is not necessarily true; this substance may be reabsorbed at point and secreted at another point then they cancel each other. Because of this, short segments give more accurate conclusions.
b) >1 → there is secretion of Na at this segment
c) <1 → there was reabsorption

Going back to our example: If we compare the clearance of Na with the clearance of inulin:
Then, Clearance Na / Clearance Inulin = 1/3

What does this mean?! This means that 2/3 of sodium is reabsorbed and only 1/3 of sodium reach this point.

How can we study each segment by in the lab?

We inject a dye from cortical side. If the dye returned from the medulla to the cortex, we know that we are inside the nephron. Then, we inject a drop of oil, wait a bit and then inject another drop of oil. So now I have 1 segment that is isolated between the 2 drops of oil which I can work on. By using this technique, we divided the nephron into segments: early proximal, late proximal, descending, thin and thick ascending, early and late distal, cortical and medullary collecting ducts. And at last, we can apply the equations above to determine the changes that take place within a specific segment.

-The micropuncture technique(micropippate): segmental function of the nephron, discovered by 1924 by Richard brothers, what makes it difficult that it should be done in **vivo not vitro**. also, it can isolate the cortical segments only not the medullary ones, too.
-To study the medullary segments, the scientists find a way through the pelvis of ureter to reach the kidney and take samples to study them.

Notes:

What is the most important part in handling Na+?? We can answer this question from different aspects.
- We can say that the proximal part is the most important because it reabsorbs 65% of filtered Na+ (the highest percentage of reabsorbed Na+).
- We can also say that the distal part is important because it is under control (it can be controlled through aldosterone) which is important for **physiologists**.
- The descending part can be considered important because it is not permeable to Na+.
- For **pharmacologists**, the most important part is the thick ascending part because it can be controlled by drugs. These drugs are diuretics, which can manipulate the sodium and potassium levels. These diuretics can be divided into:

1- Potassium wasting agents:. Such as furosemide(Lasix) and thiazide.
   a- Loop diuretics like Furosemide: works by inhibiting the (Na+: K+: 2*Cl-) carrier of the thick ascending limb of the loop of Henle. This family of diuretics is the strongest one so it is used to treat pulmonary edema (remember the 25% of Na+!).

b- Thiazide: works distally to furosemide by inhibiting another carrier (NaCl), it is widely used as it increases the reabsorption of Ca++, so, it reduces kidney stones.

2- Potassium sparing agents (Aldosterone antagonists); increase potassium in the body by inhibition of aldosterone secretion. So if a patient takes potassium wasting diuretics, he should take a banana with the drug (to compensate for potassium loss), if this does not work we give him potassium supplements, or I can give him aldosterone antagonists.

Why do we care about potassium?

The maintenance of potassium (K+) balance is essential for the normal function of excitable tissues. The K+ concentration gradient across excitable cell membranes determines the resting membrane potential. Extra note: changes in resting membrane potential alter excitability by opening or closing gates on the Na+ channels, which are responsible for the upstroke of the action potential.

The concentration of potassium normally inside the cell = 150 mmol/L, and outside the cell = 4 mmol/L, outside the cell concentration should be constant (potassium homeostasis), so if the intake increases, the excretion should increase too. potassium accumulation is very dangerous.

Nernst equation:

$E_m = -61 \log ([K+]_i / [K+]_o)$

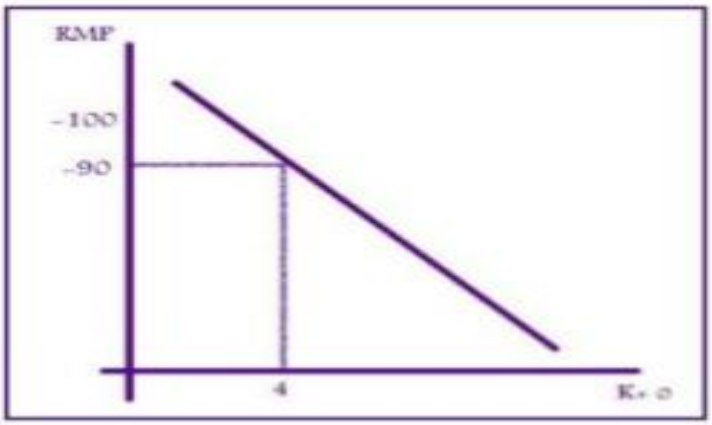
EK+ = -61 log (K+ in / K+ out)

= -61 log (150/4)

= -61 log 35 =

-61 * 1.5 = -90 mV

- From the equation above you can notice that any change in intracellular K+ is not significant, however little change in extracellular K+ can cause a lot of change in Resting Membrane Potential (RMP)



This figure illustrates the relationship between k+ concentration outside the cell and resting membrane potential (Nernst equation).

- When K+ concentration is low, a state of Hyperpolarization is established. This means that excitable tissue will face a difficulty to reach the threshold.
- K+ must be maintained between 3.5 to 5.5 mmol/L extracellularly.
- The most important thing that we, as doctors, are afraid of in renal failure is increase potassium in blood.
- If a patient comes with K+ levels above 7 you go for an ECG, if there are any ECG changes, go for haemodialysis immediately. (potassium causes fatal arrythmias).
- Our body regulates K+ level and prevents its accumulation in the extracellular fluid by secreting insulin after the meal intake that will work on glucose and potassium and pushes potassium inside the cells, instead of being outside the cell which is dangerous as we said, so inside the cell; the concentration will be 150(normally) +3.5(form meal) =153.5 mmol/L which is not a problem because the cell will get rid of this extra potassium toward the blood slowly the most important thing is to keep the concentration of potassium outside the cell within normal range

  <u>It is why we are very careful when we use diuretics or when kidney function is impaired.</u>

The last thing that we will discuss is the "Regulation of Na+ Reabsorption"

As we said, Na+ and its associated anions Cl^- and $HCO3^-$ are the major solutes of ECF. In turn, the amount of Na+ in the ECF determines the ECF volume. Consequently, an increase in the amount of Na+ in the body leads to an increase in ECF volume, blood volume, and blood pressure

Na+ homeostasis is regulated by 3 factors:

1- **GFR**: An increase in Na+ amount in the extracellular fluid will stimulate water intake which causes hypervolemia and elevates the blood pressure. In a consequence to that, GFR increases and thus more Na+ is excreted.

2- **Aldosterone**: aldosterone stimulates Na+ reabsorption in the late distal tubule and the collecting duct through Na+ channels and Na^+-K^+ pump.

Aldosterone act on the distal tubules (on the principle cells there) and does 4 things:

1) It facilitates the formation of proteins (it is steroid which work to increase transcription), so it inserts Na+ and K+ channels on the luminal membrane.
2) It makes Na+/K+ pump (proteins) on the basolateral membrane.
3) It makes the enzymes needed to make ATP for the pump.
4) It helps in making the proteins which facilitate the diffusion of sodium and potassium (for facilitated transport).

- So when Na+ amount is high, there will not be Aldosterone secretion.

* **Hyperaldosteronism (Conn's Syndrome):** is a disease in which the adrenal gland(s) make too much aldosterone which leads to **hypertension (high blood pressure)** (aldosterone cause **sodium and water retention**) and **low blood potassium levels**.

3- **ANP:** ANP is secreted by the atria in response to an increase in ECF volume and causes vasodilation of afferent arterioles which in turn increases the GFR, and decreased Na+ reabsorption in the late distal tubule and collecting ducts **directly** and by inhibiting aldosterone secretion. These all will increase Na+ excretion and urine output.

Its name changed 3 times, firstly it was called the 3rd factor, as they didn't know exactly what it does. Then they found that it is a peptide, so called ANP. at last, They found out that it is secreted from a place (right atrium) and transported via blood to affect another place (kidney) and due to this fact, they called it a hormone (ANH).

Questions:

1-The following information was obtained in a 20-year-old college student who was participating in a research study in the Clinical Research Unit:

	Concentration in plasma	Concentration in urine
inulin	1mg/ml	150mg/ml
x	2mg/ml	100mg/ml

Assuming that X is freely filtered, which of the following statements is most correct?

A) There is net secretion of X

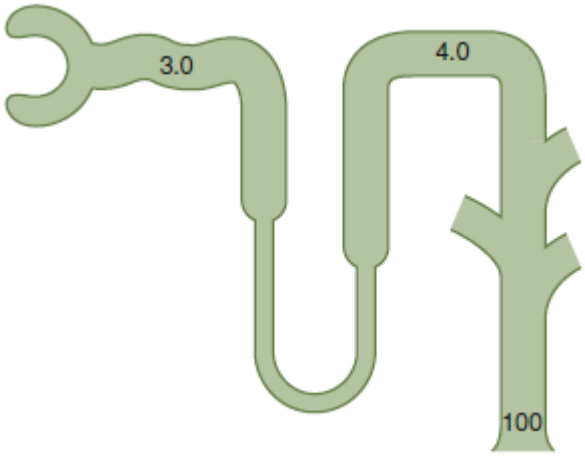
B) There is net reabsorption of X

C) There is both reabsorption and secretion of X

D) The clearance of X could be used to measure the glomerular filtration rate(GFR)

E) The clearance of X is greater than the clearance of inulin

2-



The above figure shows the concentration of inulin at different points along the renal tubule, expressed as the tubular fluid/plasma ratio of inulin concentration. If inulin is not reabsorbed, what is then approximate percentage of the filtered water that has been reabsorbed prior to the distal convoluted
tubule?
A) 25%
B)33%
C) 66%
D) 75%
E) 99%
F) 100%

3- Furosemide (Lasix) is a diuretic that also produces natriuresis. Which of the following is an undesirable side effect of furosemide due to its site of action on the

renal tubule?

A) Edema

B) Hyperkalemia

C) Hypercalcemia

D) Decreased ability to concentrate the urine

E) Heart failure

4- Which of the following would likely lead to hyponatremia?

A) Excessive ADH secretion

B) Restriction of fluid intake

C) Excess aldosterone secretion

D) Administration of 2 liters of 3% NaCl solution

E) Administration of 2 liters of 0.9% NaCl solution

5- Which of the following has similar values for both intracellular and interstitial body fluids?

A) Potassium ion concentration
B) Colloid osmotic pressure
C) Sodium ion concentration
D) Chloride ion concentration
E) Total osmolarity

6- If the cortical collecting tubule tubular fluid inulin concentration is 40 mg/100 ml and plasma concentration of inulin is 2.0 mg/100 ml, what is the approximate percentage of the filtered water that remains in the tubule at that point?

A) 0%
B) 2%
C) 5%
D) 10%
E) 20%
F) 100%

Answers: 1-B 2- D 3- D 4-A 5-E 6-C