

Lecture 4

Why does the colloid osmotic pressure increase throughout the glomerular capillaries whereas it doesn't increase significantly throughout any other systemic capillary?

The cardiac output equals 5 liters per min, 1 liter of them reaches the glomeruli and the remaining 4 litres go to the systemic capillaries. In these 4 liters of blood there are 2 liters of plasma per min which equals 120 liter / hour → 2880 liters/ day.

Only 0.5% (14 liters) is filtered from this huge amount of plasma in the systemic capillaries whereas in the glomerular capillaries, 20% of plasma is filtered from a much less quantity of plasma.

This leads to that the protein concentration (protein mass/ plasma volume) will increase in the glomerular capillaries more than it will do in the systemic capillaries because of the more loss of plasma, also the amount of plasma filtered in the systemic capillaries is very small that it can hardly affect the concentration. So the colloid osmotic pressure will definitely be affected differently in the two areas; increasing at higher degree in the glomerular capillaries.

In addition to that, the relationship between the concentration of the proteins mainly the albumin and the colloid osmotic pressure is not linear, at high concentration of albumin the colloid pressure is more than what's expected by the protein equation that estimates plasma colloid osmotic pressure.

Use of clearance methods to quantify kidney functions:

When a patient comes with renal problem, we should examine if he really has a problem in the kidneys or not and to what extent that problem is. We will make the **kidney function test**; the term that is used clinically is "we will **test** the patient" which means we will do a KFT (kidney function test) for him.

Kidney function test is the measurement of:

- 1- Plasma uria.
- 2- Creatinine.
- 3- Electrolytes (Na, Ca, K, Cl, etc).

The rates at which these different substances are "cleared" from the plasma provide a useful way of quantitating the effectiveness with which the kidneys excrete various substances. By definition, the renal clearance of a substance is the volume of plasma that is completely cleared of the substance by the kidneys per unit time.

IF you know that 650 ml of plasma is entering the kidney in 1 minute then you know RPF = 650 ml/min. And you are given a lab result that says the concentration of substance X in plasma is 1 mg/ml. THEN you can conclude that there is 650 mg of (X) entering the kidney each minute, Now if (X) is removed

COMPLETELY via the kidney to the URINE and NOTHING is reabsorbed Back to the renal vein, here we say the clearance is 100%, that is, all the 650 ml of plasma are cleared. (From the previous lecture)

We can use the **clearance** to correlate it with the **Renal Plasma Flow** which will correlate with **Glomerular Filtrate Rate** which gives us an image about the Kidney Health Status.

❖ Creatinine clearance:

The creatinine is added to the circulation from the muscles, each day we add 2 grams whatever its concentration in plasma was. So every day the kidney should remove 2 grams of creatinine in order to make the concentration of it stable in the plasma. Creatinine is removed from the plasma mainly by one root which is the **filtration** in the glomeruli in the kidney.

Let's assume that its normal concentration in blood is 1mg/dl. As long as the filtration = 125 ml/min, we can guarantee that 2 grams are filtered. If the filtration = 65 ml/ min, we can guarantee 1 gram is filtered, but this is not enough. In order to increase the filtered quantity if the filtration is decreased by half, we find that creatinine concentration is double its normal (2mg/dl) so that the 2 grams that should go out are gone.

This helps us when we measure creatinine concentration in blood to know how much approximately the filtration is; if creatinine concentration is normal (1 mg/dl for example) we know that the filtration is normal. And if the creatinine is 4 times its normal concentration (4 mg/dl) we can guess that the filtration is reduced by 1/4. **We can conclude that creatinine in plasma reflects and is the mirror image of the filtration rate since it's mainly removed only by the kidneys.**

The clearance of creatinine is used to assess the **GFR** by this equation:

$$\text{Creatinine clearance} = \frac{\text{Concentration in the urine}}{\text{Concentration in the plasma}} * \text{Urine output}$$

(True GFR or tGFR)

In order to calculate the **true** GFR, we must find the urine output but this needs 24 hours of urine collection and this can be difficult in old patients and young children. So instead of the collecting urine we use the **estimation of GFR**. The estimation of GFR is more than 95% accurate.

But in some cases of end stage renal failure, the filtration may become very small (GFR is almost zero) yet we may find some creatinine in the urine that comes from the secretion. We can't use the estimation equations here and we should collect the patient's urine in order not to overestimate the GFR.

There are **many equations** for GFR estimation; we are interested in one equation for **adults** and another one for **children**.

1- The Cockcroft and Gault equation that's used for adults:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \text{ if Female}]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

- Females have less muscle mass than males that the values for women are 85% of the predicted so **For females we multiply by (.85).**
- Please note that we put the **ideal body mass (IBM)** = height – 100, and not the mass.
Why **ideal** body mass not the true body mass (weight)? Because ideal body mass is the accurate indicator for muscle mass without including the fat in the calculations.
- So to estimate GFR by this equation we only need a blood sample to get creatinine concentration in plasma. American Unit of creatinine is mg/dl (milligram per deciliter). Europe use micromole unit. To convert from the gram to molar concentration, **the conversion factor between the two is (88.4)**. So a patient of Creatinine conc. = 1 mg/dl = 88.4 micromole. (molecular weight of the creatinine is 114 g/mol)
- **The normal creatine plasma level = 0.7 – 1.4 mg/dl**
The range here is **very wide** because the creatinine concentration mainly depends on the **muscle mass**; in thin people the muscle mass is low so the creatine concentration should be **lower** than 1.2. Thin females may come with creatinine concentration = .7 and this is normal. If thin females have 1.4 mg/dl, that's not normal. A male body builder with creatinine concentration = 1.6 is normal and doesn't indicate any kidney problem.
- Remember that gradual loss of renal function with age is a normal process (1ml/min each year after the age of 40y).
- If a patient starts with creatinine concentration equal 0.7 and after a period of time he comes again with 1.4 this means that the GFR decreases to the half.
If I want to give a patient a drug that is nephrotoxic that decrease the GFR such as NSAID so we should first estimate the creatinine so we need only a blood sample in order to know the concentration of the creatinine in the plasma .If the creatinine concentration was 0.7 then after the administration of the drug it become 0.8 then you should reconsider the whole situation although the concentration is still normal.
Another example:
The Plasma concentration of creatinine at beginning of treatment was 0.5 mg/dl Then after six weeks when you did the second test it was 1.0 mg/dl What Does this mean? Notice that 1.0 mg/dl is within the normal range but in this patient the raise in creatinine concentration (Double) means that the GFR has been reduced to (HALF) , so half of the nephron in this patient had Stopped Functioning although it's within the normal range.

2- In children we use another equation which is the Schwartz equation:

Schwartz equation

$K \times \text{height in cm} / \text{creatinine in plasma (milligram per deciliter)}$

K is constant

K = 0.33 in premature infants

K = 0.45 in term infant to 1 year (0-1 year)

K = 0.55 in children to 13 year (1-13)

K = 0.65 for male children older than 13 year (>13 for males)

- The creatinine concentration in children ranges between 0.2 – 0.8 rather than 0.7 -1.4 since they have lower muscle mass.
- Example: for 6-y old child:
 $k = 0.55$, $h = 110\text{cm}$, $\text{Pcr} = 0.33\text{mg/dl}$
 $\text{eGFR} = (0.55 \times 110) / 0.33 = 183\text{ml/min}/1.73\text{m}^2$ (body surface area).
- There are a lot of equations but those are enough for now. So usually when a patient come to us we make a kidney function test, the most important thing in the kidney function test is the urea and the creatinine along with the electrolytes (Na, K, Cl).
- During kidney impairment both urea and creatinine increase. Since the creatinine is removed from our body mainly by the glomerular filtration, in the case of impairment the glomerular filtration (decreases for example) the creatinine concentration in the plasma immediately increases (no other root for creatinine to get out).
The urea may increase in the plasma from **non renal causes** like dehydration, GI bleeding. If any patient comes with high urea and normal creatinine, usually he doesn't have kidney problem but if the creatinine was high this indicates the presence of kidney disease.
So, the most sensitive kidney function test is creatinine function test.
- Don't forget: once GFR decreases to the half the creatine increases twice because at the end of the day we should remove the same amount of creatine that has been released from the muscle (2 grams).
- Normal ranges of creatinine are not to be memorized.

❖ Clearance of Para-aminohippuric acid:

This is used to measure the renal plasma flow in order to measure the renal blood flow. (In the recent lecture).

❖ Glucose clearance:

It's the volume of plasma cleaned from glucose each min = volume of plasma that provides glucose for excretion per min. If the glucose in the urine is zero then the clearance is zero since none of the plasma has been cleaned from glucose.

Same law is applied here:

Clearance of glucose = (the concentration of glucose in the urine / the concentration of glucose in the plasma) * the urine output .

Since the concentration of glucose in the urine equals zero, the clearance of glucose should equal zero.

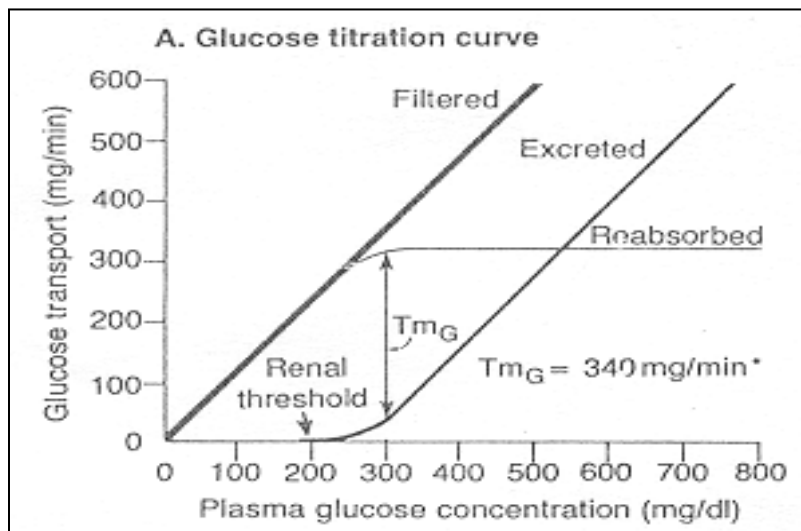
Glucose is small molecule so it's freely filtered and it's reabsorbed by the secondary active transport along with Na. **We have two types of transporters: Na glucose luminal transporter 1 and Na glucose luminal transporter 2**

1- Type one: has high affinity, low capacity.

2- Type two: has modest affinity but high capacity.

- **Glucose titration curve:**

please have a look on this figure before reading:



As glucose concentration increases in the plasma, more of it will be delivered to the glomeruli and the **filtered load** increases. The filtered load of glucose is how much glucose is filtered in the bowmen space per min. This is a **linear** relationship because filtration is an entirely **passive** process.

And as glucose concentration increases in tubular fluid, it is reabsorbed more but since glucose **reabsorption** is coupled to sodium transport by means of **secondary active transport**, there is a **limit** to the rate at which glucose can be transported. This limit is referred to as **transport maximum, T_m**. (The two glucose transporters have saturation phenomena; they transport glucose according to their T max)

Tubular transport maximum (T_m) can be defined as the maximum capacity of the proximal tubular cells to transport glucose from the lumen to renal interstitial fluid and back into the blood, it averages about **320 mg/dl or 375 mg/min**.

Because the kidney can't reabsorb the entire filtered glucose if the glucose in the plasma exceeds 320, any additional glucose filtered is going to be **excreted**.

So the amount of glucose which enters should go back through: either the renal vein only or the renal vein and urine; it's not going to be metabolized inside the kidney.

But at T max of a transporter, it will have **saturation** but this doesn't mean that all the transporters have the same T_{max} or are occupied at one concentration. This introduces another concept which is **renal threshold for glucosuria** which means the concentration of glucose in plasma at which glucose starts to appear in urine, and it equals **180 mg/dl** in venous blood and equals **200 mg/dl** in arterial blood.

Theoretically, threshold and T_{max} should match, but practically they don't:

The area on the reabsorption curve which is rounded deviation is called **splay**. And it represents the T max.

Also, we see rounded area at the beginning of the excretion curve and it's also called **splay**, as well. The beginning is the renal threshold.

To measure T max we must supply suprasaturated concentration. What does this mean?

(The following explanation is from the slides)

Ex.: if you delivered 800 mg/min glucose (as filtered load), you'll get: 425 mg/min excreted & 375 mg/min reabsorbed (375: T_{max})

If you delivered 600 mg/min: 375 reabsorbed, 225 excreted

BUT if we delivered 400 mg/min: Only 300 will be reabsorbed

Why? Because the affinity of the carriers differs (depending on the conc. of glucose)

Increase in conc. → increase chance of the carrier to catch G.

Decrease in conc. (slightly above T_{max}) → decrease in affinity of the carriers (they reabsorb less than T_{max})

So when we want to measure T_{max}, we use high concentration to get the true value.

Some of the sodium-glucose carriers have low affinity. This means that if you don't have supra-saturated concentrations, you will not guarantee the reabsorption of the entire 320 mg/dL (or 375 mg/min). If

plasma glucose concentration was 320 mg/dL some of the glucose molecules will actually escape and appear in urine. This explains the splay observed in the graph.

So threshold is the tubular load at which transport maximum is exceeded in some nephrons. This is not exactly the same as the transport maximum of the whole kidney because some nephrons have **lower transport max than others**.

To conclude the difference between T max and renal threshold:

T max refers to the point at which increases in concentration do not result in an increase in movement of glucose across the membrane because the transporters are saturated. Some transporters are overwhelmed before other (have lower T max), others have less affinity, or the transporters may be reduced in number, consequently, part of the glucose isn't reabsorbed and remains in the tubular fluid. So at this concentration of glucose the kidneys begin to remove it into the urine, and is called renal threshold.

180mg/dl is very imp and it is a threshold for **glucosuria**, this means if we find a patient with glucose in urine the plasma should be 180 or above since the glucose will appear at the urine when its concentration reaches 180 mg/100ml. In this case, this glucose is due to diabetes and comes to urine from hyperglycemia, so it's called **diabetogenic glucosuria**. Sometimes, there might be glucosuria but blood sugar is 110 mg/dl (normal), this is most probably due to a problem resides in the kidney which may be less number of glucose carriers or maybe the transporters are not exhibiting their proper kinetics so the threshold is less and here any small rise in plasma glucose concentration (ex: after meals) will induce glucosuria. So here it's **nephrogenic glycosuria**.

How can we distinguish between them?

If glucose is found in the urine and I take at the same time blood sample and the blood glucose is normal, definitely the glucose which is found in the urine doesn't come from endocrine problem (not diabetogenic).

Nephrogenic glycosuria is a **benign** condition, its prognosis is good, it's not associated with any other renal disturbances, it's much better **not to tell** the "patient" about it.

Now, does the kidney participate in glucose homeostasis (i.e. when glucose increases does the kidney play a role in lowering it and vice versa)?

No, because the threshold and T max are **away from and much higher than** the normal physiological concentration of glucose. The kidneys cannot increase plasma glucose levels when they decline under physiological conditions (this does not include the process of gluconeogenesis in the kidneys during starvation conditions) and cannot get rid of all the extra glucose when plasma glucose levels elevate considerably to 160 – 170 mg/dl and will still reabsorb the entire amount of glucose filtered.

Normal fasting blood sugar is under 126 (70 – 110 in average) If the glucose increases and the kidneys bring it back to normal through decreasing reabsorption or If the glucose decreases and the kidneys added glucose through increasing reabsorption (the gluconeogenesis from amino acids is not our interest

here), If the kidneys bring the glucose back to the normal in these two tails physiologically then they participate in the homeostasis.

Indeed, if the body undergoes hypoglycemia under physiological condition or the glucose increases, the kidney will do nothing.

To understand this more, here is an example of a substance that the kidneys participate in its homeostasis which is the **phosphate**:

The concentration of it in plasma is around **1 millimolar /L**

T max of phosphate is around **0.1 millimolar/min**

If we consider GFR as 100, the filtered load of the phosphate = 0.1 (actually it's .125 millimolar/min as long as the GFR equals 125) and all of this quantity is going to be reabsorbed, and the transporters will be saturated because T max = 0.1 millimolar.

Now, if the phosphate concentration in plasma becomes 2 millimolar/L, the filtered load is .2 here which is more than T max. The extra phosphate is not going to be reabsorbed and it's going to be excreted. So that is the whole story, the T max is very close to the plasma concentration so once the plasma concentration of phosphate increases a little bit more than 1 millimole, the excess will be excreted bringing the concentration back to normal. So the kidney **does participate in the phosphate homeostasis** by stimulating the excretion, so it regulates the phosphate concentration from one tail at least (phosphate increase) and this is enough.

Why does the kidney play a role in phosphate homeostasis but not in glucose? Coz T max of phosphate is close to the physiological level of plasma concentration unlike glucose.

❖ clearance of Amino acid :

They are freely filtered because their average molecular weight = 110 (much less than 70000).

It's reabsorbed in the early proximal tubule by **secondary active transport** along with sodium (so the sodium has a lot of functions; It helps in the reabsorption of glucose and amino acid).

We have different types of carriers for amino acids:

We have carriers for **neutral ,acidic , basic** aminoacid and sometimes we have **special** carriers for special amino acid like **cystine** for example. (Cystine has its own carrier, if we don't have cystine carrier, we will end up with **Cystinuria**. Cystinuria can be a nucleus for stone formation so it's a serious disease of kidney damage.)

Clearance of amino acids is zero, they are entirely reabsorbed from the proximal tubule.

Na homeostasis:

The sodium intake should **equal** the sodium output and this is called **sodium balance**. If output is **less** than the input this is **positive balance**, if the output is **more** than the input this is **negative balance**.

Why the sodium homeostasis is important?

1-More Na means more water (extracellular expansion →hypervolemia, edema, brain and pulmonary edema, and hypertension)

2-Na means excitability of the membrane and the depolarization.

3-We said that the kidney reabsorb the glucose, the amino acid in the co transport mechanism with Na.

4-Also Na is counter transported in potassium exchange.

5-Also it's counter transported with the H ion so it plays a role in the acid – base balance.

6-Sodium is very imp in making the interstitium of the kidney hyperosmolar so the kidney is able to form concentrated urine.

7-Na is also a target for most of the diuretics.

The average intake of sodium per day in Jordanian people= 4 grams, 4 grams = 155 millimole per day

These 155 millimoles have many routes to go out of the body: GI secretions, during summer we have like 5- 10 millimoles from the sweat glands, and **about 150 millimoles** (or less in summer) go out by the **kidneys**.

How are the 150 millimoles of sodium excreted in the urine (renal sodium)?

The filtered load of sodium per day:

GFR per day = 180 liter /day, each liter contains 140-145 millimoles of Na. So the filtered load is about 25200 milliequivalent per day. (The doctor assumed the number is 25000 millimoles so the reabsorption equals 24850)

So 99.4 % is reabsorbed (25050 millimoles), 0.6 % is excreted (150 millimoles).

The question comes; where is the sodium reabsorbed in the nephron? how can only 150 millimoles get out from all the 25200 millimoles?

The answer comes in a lecture later, but now we'll study how they knew it by using the micropuncture technique.

Micro puncture technique

This technique is used in the kidney in order to know the function of each part (the segmental function) of the nephron.

The renal micropuncture technique allows direct access to study nephrons **in vivo**.

Why in vivo?

Because the kidney has a very special environment (a miracle of unique renal interstitium); here is its explanation:

In our body we have three compartments: intracellular, interstitium, intravascular. These have different concentrations of proteins, anions, Na, potassium, Ca. For example Na = 140 mEq/L extracellular and 14 intracellular (10X difference), K = 4 extracellular and 150 intracellular (30-40X difference), and Ca = 2 millimoles extracellular and 10 nanomole intracellular (there is 100000X difference in the concentration between intracellular and extracellular compartment, this number is very imp.)

Those numbers are very imp, you should memorize them

The point is that despite these differences in the three compartments, they have only one thing in common; the **osmolarity**. It is 300 millimolar everywhere. This is because the biological membrane is permeable to water with few exceptions in the kidney. Since the water freely moves, it can bring the osmolarity the same in these three compartments.

But the story is different when it comes to the renal interstitium. Although no membranes are there, very delicate mechanisms throughout the segments of the nephron -will be introduced in a lecture later- lead to **different osmolarities!** The osmolarity in the renal medulla equals 1400 and as we move away from the medulla it will become 1399, 1398 and in the cortex it's 300. The interstitium is without barriers and without membranes, yet osmotic gradient is perfectly created!

This is just like when you put many color drops on water, and they don't mix although no barriers are between them.

They discovered the osmotic gradient by entering from the pelvis of the ureter, they noted that the osmolarity there in the medulla is very high, and then found that the osmolarity is low in the cortex. So to make a conclusion: micropuncture technique needs in vivo environment that can't be offered in vitro.

The vivo environment was done on rats. It's good to know that applying this technique to study the kidney is really very difficult; the rat kidney weighs only 1-2 grams so it's very small, also the rat is still breathing during the study so the kidney is moving due to the diaphragm movement.

In this technique we try to inject and isolate only one nephron. And in order to isolate one segment in the nephron to study it, we inject two drops of oil; one in the beginning and another in the end of the segment.

A micropipette with small diameter (0.25 micrometer) is injected to the kidney and takes samples from the ultra filtrate and the tubular fluid; then we analyze the samples to know their composition.

We give a drug and see how the drug works on the Na, K, Ca reabsorption to know the function of the segment.

This is not a tool that is used clinically; the data from it is analyzed only to know the function of the nephron, as follows (from the slides):

If we have substance X and we want to study what happens for it as it passes through the proximal tubule (or any other segment)

$[X] \text{ in Bowman's capsule} = [X] \text{ in plasma}$

If we find: $TF[X] = P[X]$ What do we conclude?

Mostly, we conclude that, across this segment, this substance was reabsorbed at **the same proportion as water**. And to be sure we must add inulin (non-absorbed substance) to know how much water is reabsorbed.

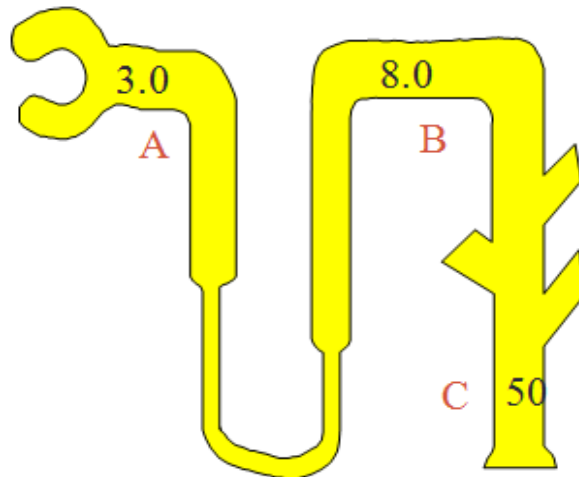
So, if $TF [In] = 3 P [In]$ This means that **two thirds of the water** were reabsorbed, also 2/3 of substance X must have been reabsorbed too.

The figure below shows the concentrations of inulin at different points along the tubule, expressed as the tubular fluid/plasma (TF/P_{inulin}) concentration of inulin. If inulin is not reabsorbed by the tubule, what is the percentage of the filtered water that has been reabsorbed or remains at each point? What percentage of the filtered water has been reabsorbed up to that point? (the figure)

A = $1/3$ (33.33 %) remains
66.67 % reabsorbed

B = $1/8$ (12.5 %) remains
87.5 % reabsorbed

C = $1/50$ (2.0 %) remains
98.0 % reabsorbed



By taking two samples (at the beginning and at the end of the segment) and measuring substance X conc. (using the concept of clearance). Clearance of substance X across that segment is:

$$C[X] = \frac{TF[X]}{P_X} * V$$

Where

C_x : Clearance of X

$T_{[X]}$: Conc. of X in tubular fluid (e.g., late proximal tubule)

$P_{[X]}$: Conc. Of X in plasma (Bowman's capsule)

V : Fluid flow rate

Now, comparing with inulin: C_x / C_{in} (this ration indicates how much X has reaches this point and therefore the remaining is what's reabsorbed.)

* If $\frac{C_x}{C_{In}} = 1$

This means that this substance was handled exactly like inulin: **not reabsorbed, not secreted**.

* If $\frac{C_x}{C_{In}} = 2$

This means that **the same amount** of X that is filtered was also **secreted** to the tubule.

* If $\frac{C_x}{C_{inulin}} = 0.3$

This means that **0.7 of X was reabsorbed & 0.3 only remained** in the tubule.