Lecture No. 4 1/4/2015

The composition of the ultrafiltrate, tubular function and the Micropuncture Technique

---

Ultrafiltrate is plasma-protein
How do we know that?
By using the micropuncture technique (taking a sample from Bowman's capsule *in vivo* and analyze it)

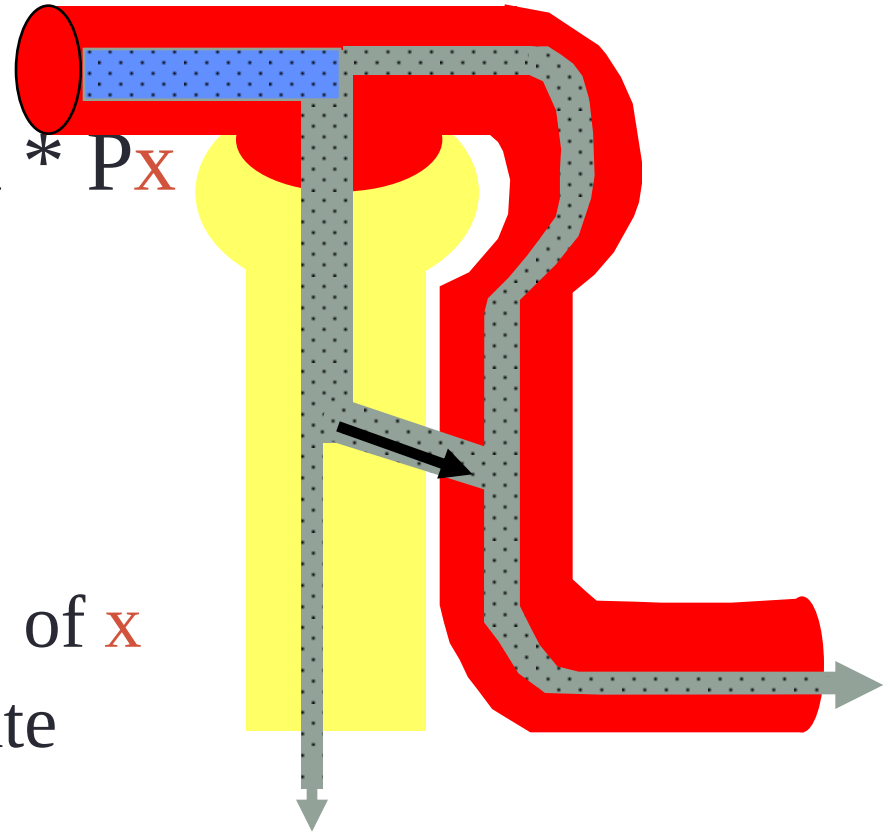
Calculation of Tubular Reabsorption: Glucose, amino acids and others

(when Excret s < Filt s)

Reabsorption = Filtration -Excretion

Filtered load of x = GFR * Px

(Px = Plasma conc. of x)



Excret x = Ux * V

Ux = Urine conc. of x

V = urine flow rate

Example: Given the following data, calculate the rate of Na^+ filtration, excretion, and reabsorption

GFR =100 ml/min (0.1 L/min)

P_{Na} = 140 mEq/L

urine flow = 1 ml/min (.001 L/min)

urine Na conc = 100 mEq/L

Filtration Na = GFR x P_{Na}

= 0.1 L/min x 140 mEq/L = 14 mEq/min

Excretion Na =Urine flow rate x Urine [Na]

=.001 L/min x 100 mEq/L

= 0.1 mEq/min

*** Examples of Modification of the Ultrafiltrate:**
In the proximal tubule, 100 % of filtered glucose is reabsorbed by a carrier mediated transport mechanism secondary active transport.

- In the apical luminal side of the cell we have two Na^+- Glucose luminal transporters: SGLT 1&2
SGLT 1 : high affinity, low capacity.
SGLT 2 : modest affinity, high capacity.

*** Here, Na+ is transported down its gradient, but glucose is actively transported against its gradient.**
BUT, if we don't have Na+, No glucose will be absorbed.

Tubular Function GTC

- Renal threshold for glucose is 180mg/dl. What does this tell you?
- This implies that the kidney is not involved in glucose homeostasis under normal physiological conditions because the threshold is far from the physiological range (70 – 110mg/dl) which must be tightly controlled.
- The kidney does however participate in the homeostasis of other substances. One good example is phosphate. The normal plasma concentration of this anion is 1mmol/l, and the transport maximum for reabsorption is 0.1mmol/min.
- What do these numbers tell us? Let's examine them more closely. Every minute 125ml of plasma is filtered, and a maximum of 0.1mmol of phosphate is reabsorbed. For every 1 liter to be filtered, a maximum of 0.8mmol is reabsorbed, which is very close to the plasma concentration of 1mmol/l. Therefore, regardless of how much phosphate is ingested, the maximum amount to be reabsorbed corresponds to the normal plasma concentration. So the kidney participates in phosphate homeostasis.

*** At the basolateral membrane, glucose is transported by facilitated diffusion.**

Transport through a carrier means that we'll have saturation (Tmax).

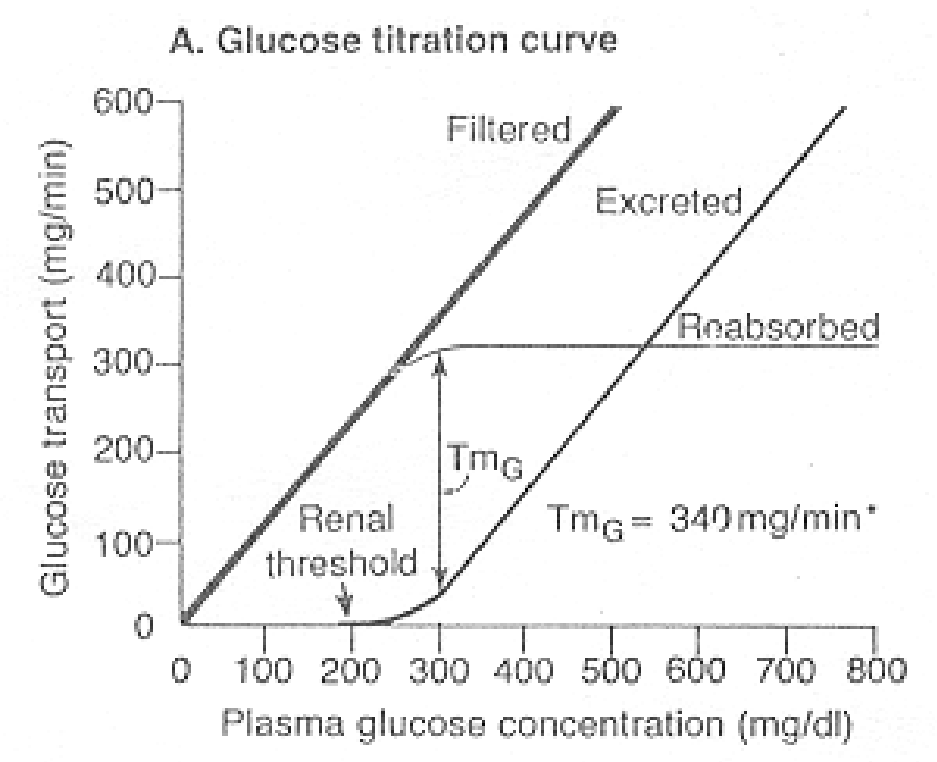
Tmax means if you delivered too much glucose to the proximal tubules (more than the capacity of the carriers), then it won't be reabsorbed completely, instead, it will be excreted in the urine & we'll have Glucosuria.

Glucose MW is small (180), → freely filtered.
- Plasma concentration of glucose is between 70 – 110 mg/dl)

-What is the filtered load of glucose?
It's the amount of glucose filtered per minute.
Filtered load = Plasma conc. * GFR
= 100 mg / dl * 1.25 dl/min.
= 125 mg / min

Now, if we increase the glucose conc. in plasma
- Filtration will increase , (Linear relationship).
- Reabsorption will increase until we reach Tmax

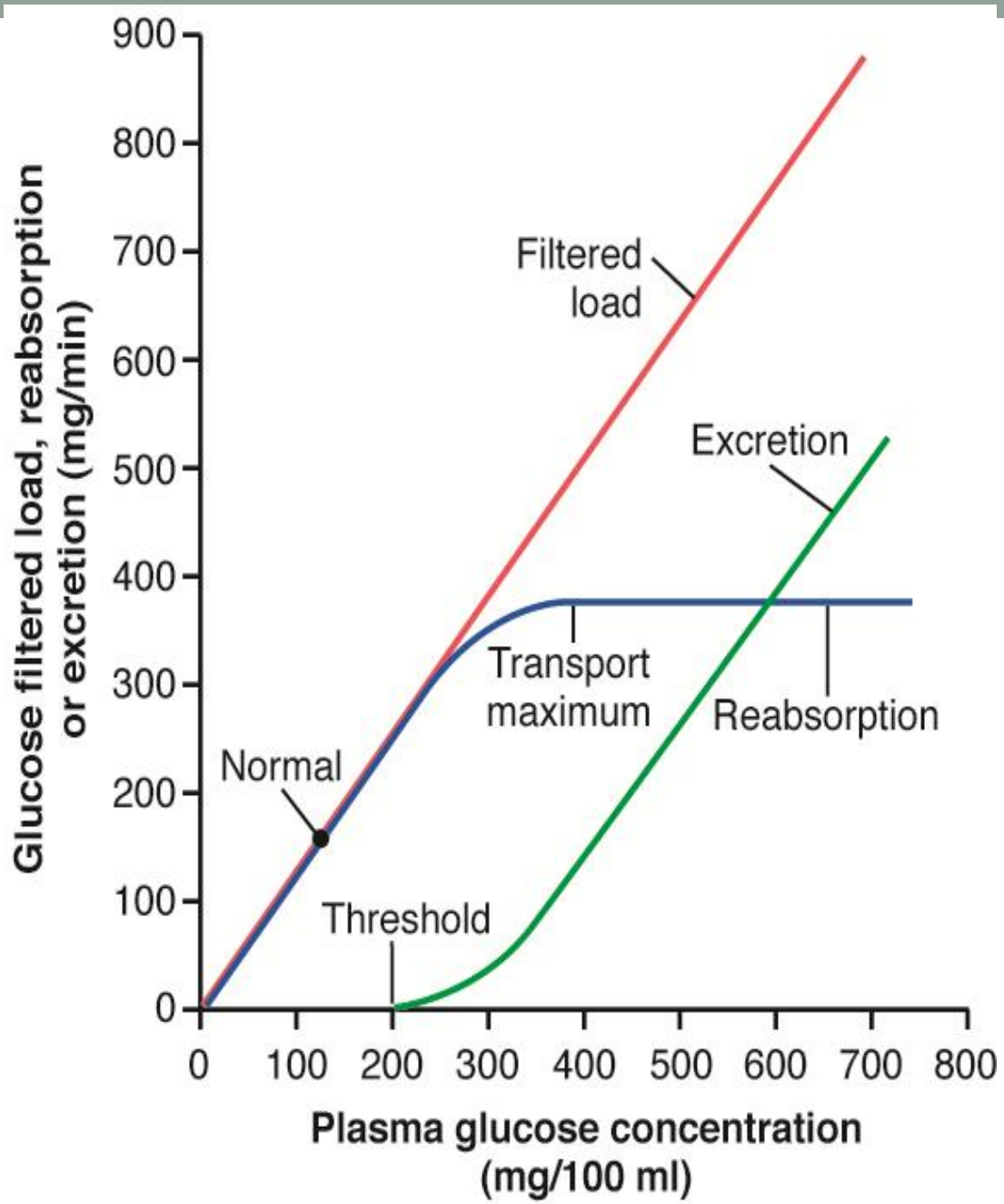
*** Tmax for glucose is 320 mg/dl, or 375 mg/min (filtered load)**



This curve is called: Glucose Titration Curve GTC.

Until plasma concentration of glucose is 180 mg/dl, we have no excretion ⟶ 100% of glucose is reabsorbed.

Glucose Transport Maximum



Threshold **:**

The conc. of glucose in plasma at which glucose starts to appear in urine = (180 mg/dl) venous blood or 200 mg/dl art.bld

Theoretically, Threshold & Tmax should match, but practically they do not.

So, we have *Splay*:

deviation of the threshold from Tmax

<u>OR</u>: appearance of glucose in urine before Tmax is reached

increasing [Glu. plasma] > 320 mg/dl

⟶ Fraction of glucose will be reabsorbed, the other fraction will be excreted.

⟶ More increase in Glu. conc. ⟶ increase in excretion, because reabsorption becomes constant.

To measure Tmax, we must supply suprasaturated concentrations.

What does this mean?!?

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

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 Tmax) →decrease in affinity of the carriers (they reabsorb less than Tmax)**

When we want to measure Tmax, we use high conc. to get the true value.

Transport Maximum

Some substances have a maximum rate of tubular transport due to saturation of carriers, limited ATP, etc

- Transport Maximum: Once the transport maximum is reached for all nephrons, further increases in tubular load are not reabsorbed and are excreted.

- 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's than others.

Examples: glucose, amino acids, phosphate, sulphate •

KFT

urine analysis ;

* urine is very informative and easy to deal with.
* We can test:

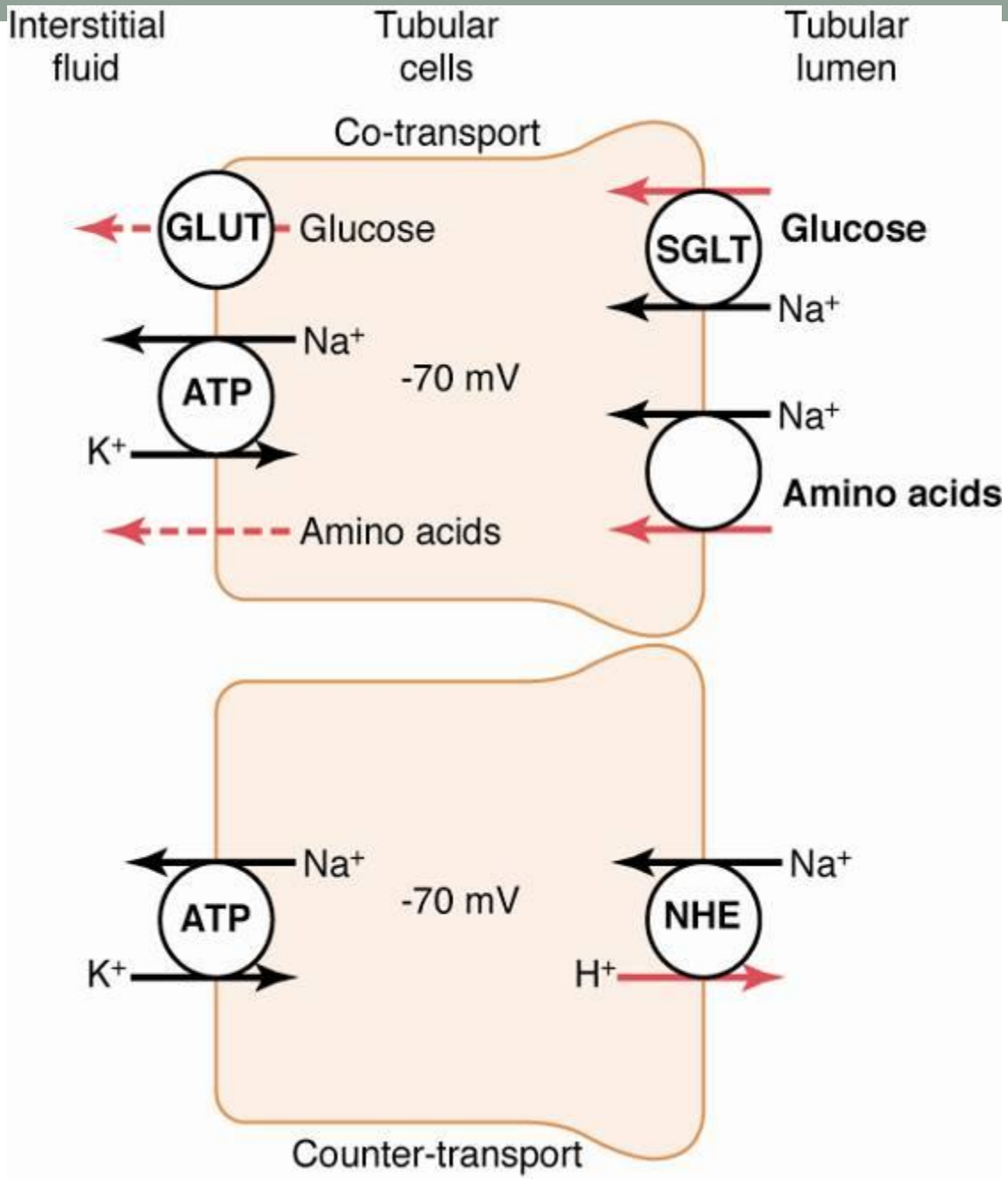
1. Volume of urine (24 h Urine collection)
2. Presence or absence of Glucose, proteins, RBCs, WBC, etc

- **If we have Glucosuria, it may be:**

1. **Diabetogenic → because of diabetes**
2. **Nephrogenic → the number of glucose carriers in this person is less than normal → less threshold → any small rise in the plasma glucose conc. (ex: after meals) will induce glucosuria.**

Nephrogenic Glucosuria is benign, not associated with other renal problems, & will not cause any problem later on
SO, WE DO NOT TELL THE PATIENT, that he has glucosuria.
If you find patient with glucosuria, do a blood glucose level test, if normal, then it IS nephrogenic, don't tell your patient.

Mechanisms of secondary active transport.



Segmental Physiology of the nephron

Glucose **is reabsorbed by 2° active transport in the Proximal Tubules.**

•How do we know?

•By micropuncture technique:

~~We tak~~e a sample from the late proximal tubule, we find Zero glucose, so 100% of glucose is reabsorbed in the proximal tubules.

The same thing applies to the *amino acids* which are 100% reabsorbed in proximal tubules, through different carriers:

*** one for neutral amino acid**

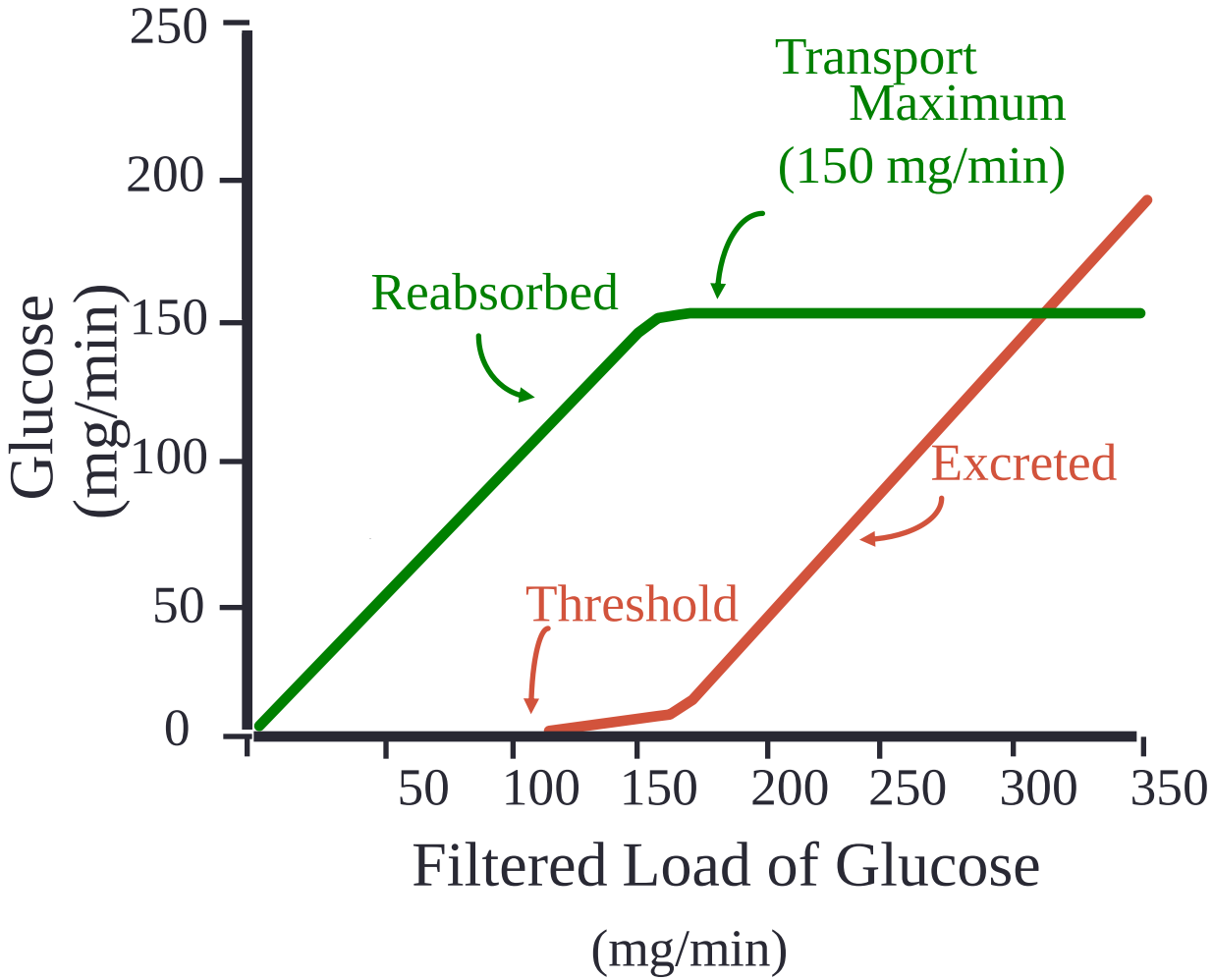
*** one for acidic amino acids**

*** one for alkaline amino acids**

*** And there are special carriers for special amino acids like Cysteine**

Q: A uninephrectomized patient with uncontrolled diabetes has a GFR of 90 ml/min, a plasma glucose of 200 mg% (2mg/ml), and a transport max (Tm) shown in the figure. What is the glucose excretion for this patient? Assuming no Splay and threshold =Tmax

1. 0 mg/min
2. 30 mg/min
3. 60 mg/min
4. 90 mg/min
5. 120 mg/min



Answer: $Filt_{Glu} = (GFR \times P_{Glu}) = (90 \times 2) = 180$ mg/min

$Reabs_{Glu} = T_{max} = 150$ mg/min

$Excret_{Glu} =$ 30 mg/min

GFR = 90 ml/min

P_{Glu} = 2 mg/ml

T_{max} = 150 mg/min

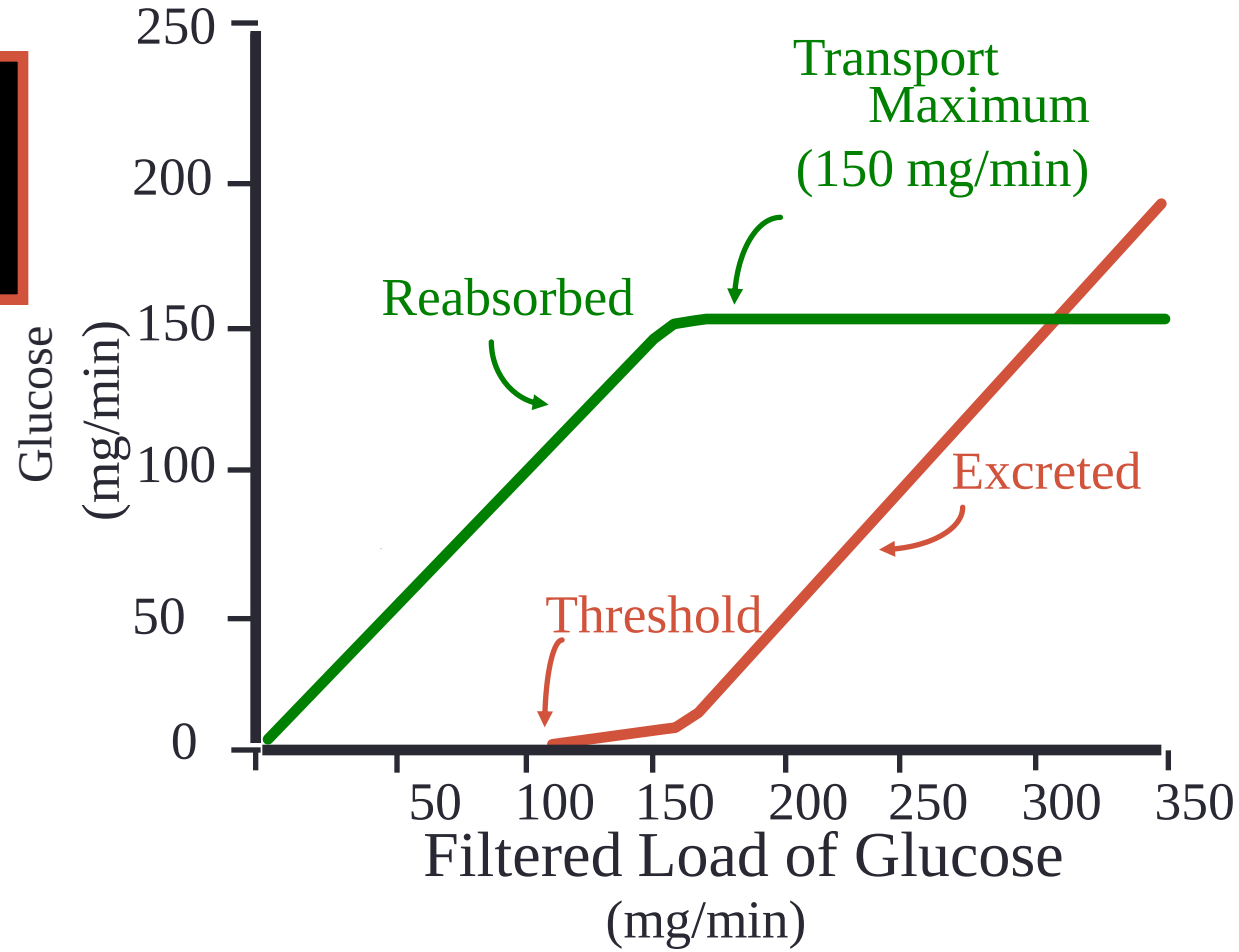
a. 0 mg/min

b. 30 mg/min

c. 60 mg/min

d. 90 mg/min

e. 120 mg/min

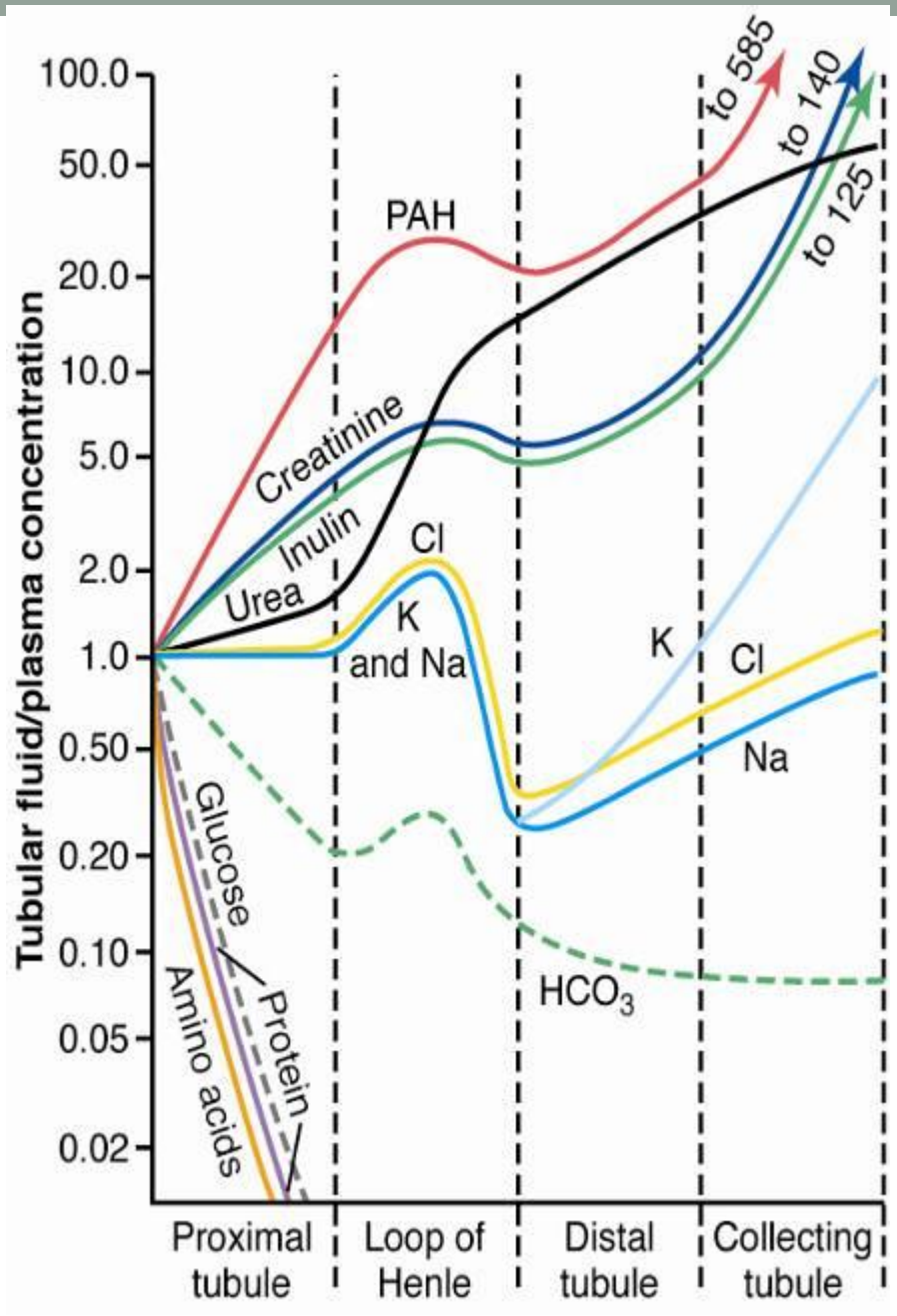


Concentrations of solutes in different parts of the tubule depend on relative reabsorption of the solutes compared to water

- If water is reabsorbed to a greater extent than “X”, then X becomes more concentrated in the tubule (e.g. creatinine, inulin).

- If solute is reabsorbed to a greater extent than H2O then solute becomes less concentrated in the tubule (e.g. glucose, amino acids).

Changes in concentrations of substances in the renal tubules



• The Micropuncture Technique

•When we study the kidney we use the micropuncture technique, we do this *in vivo* , because the interstitium is very unique and we can't offer it *in vitro*.

• We isolate the segment that we want to study by injecting 2 drops of oil, by a pipette that has a diameter of 2 μm & the sample we get is in terms of nano litres.

• We can measure SNGFR (Single Nephrone GFR) by measuring free flow from Bowman's capsule collecting ultrafiltrate for 10 min.

Generally,

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] in Bowman's capsule = [X] in plasma

If we find:

$$\frac{TF_x}{P_x} = 1$$

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, to know how much water is reabsorbed.

So, if $\frac{TF_{In}}{P_{In}} = 3$

This means that two thirds of the water was reabsorbed

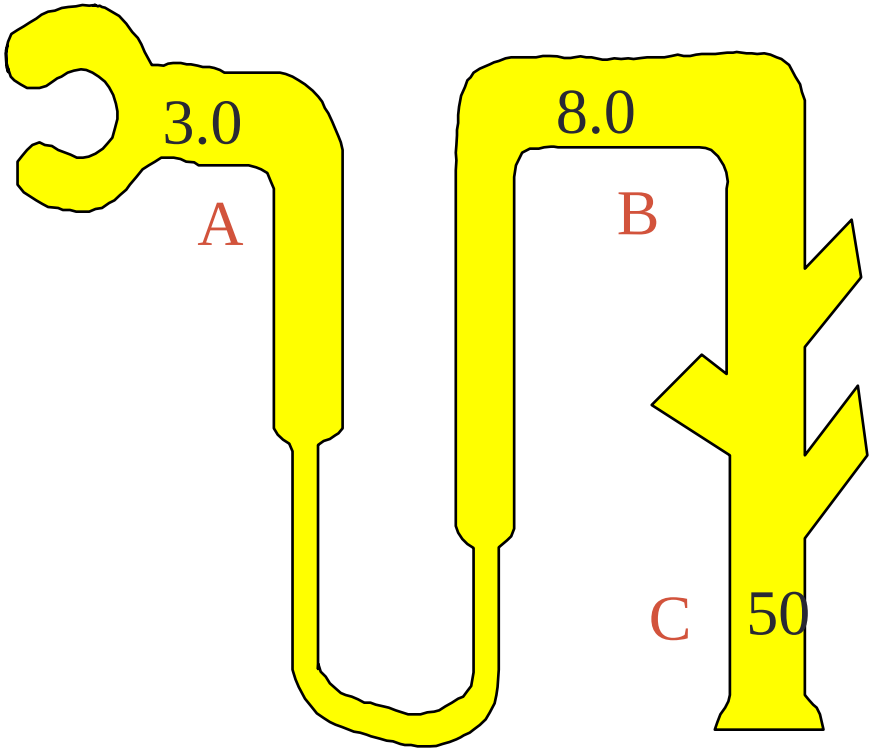
Also 2/3 of substance X must have been reabsorbed too

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 waterhas been reabsorbed up to that point?

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.

$$Cx = \frac{TF_X}{P_X} * V$$

Where

Cx : 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}

* 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.