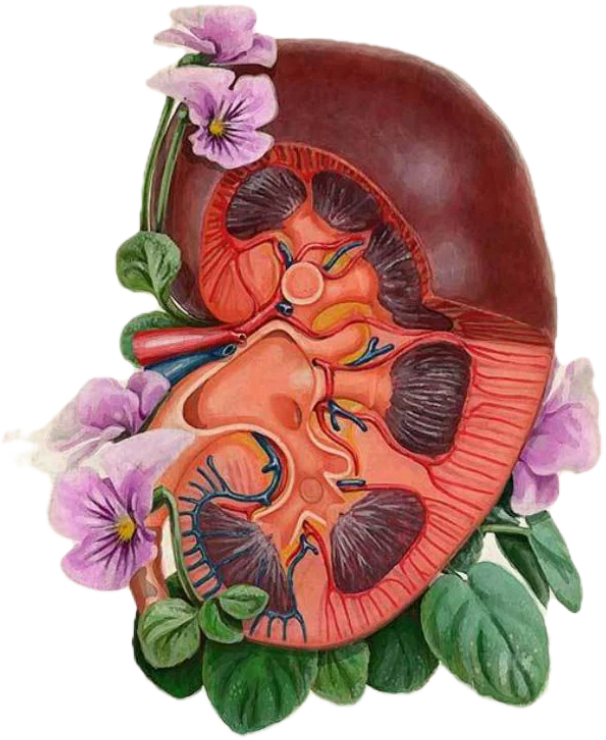
UGS Physiology Sheet 5 – V1



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Micropuncture Technique

How do we study the function of each nephron segment?

We inject inulin to the body and so it enters the kidney.

Then we take samples from different parts of the nephron using micro pipettes (0.25 micrometer diameter).

Basis:

> Since inulin is *not reabsorbed* (nor secreted), any changes in its concentration along the nephron (by measuring different samples we took) are *due to reabsorption of water***.**
>
> Thus, the increase in inulin concentration reflects the amount of water remaining in the nephron, allowing us to calculate water reabsorption.

Example:

> Let's say we took a segment from the nephron and took fluid samples from the beginning (A) and the end (B).
>
> If inulin is **2 times as concentrated** at B than at A; this means the water volume left in the tubule is one half; so, one half of the water volume has been reabsorbed.
>
> If inulin is **3 times as concentrated** at B than at A, then water volume at B is one third of the volume that was at A, so two thirds of water have been reabsorbed.

By sampling different parts of the nephron, we can determine how water is reabsorbed in each segment.

$$Water\ remaining\ fraction\ = \frac{1}{Ratio\ inulin\ (B/A)}$$

> Inulin ratio (B / A) = 2; means water halved. Inulin ratio = 3; means one third (1/3) of water is remaining.

Once we know how water is being reabsorbed, then we can know how any other substance is being modified.

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 the solute, the solute will become more concentrated in the tubule (e.g. creatinine, inulin)
- If water is reabsorbed to a lesser extent than the solute, the solute will become less concentrated in the tubule (e.g. glucose, amino acids)

To know the fraction of a substance left in the tubule:

$$Fraction\ of\ substance\ left\ = \frac{CL\ (substance\ x)}{CL\ (inulin)}$$

$$Fraction\ of\ substance\ left\ = \frac{\frac{B_x}{A_x} \times V}{\frac{B_{inulin}}{A_{inulin}} \times V}$$

In previous lectures, A represented plasma concentration and B represented urine concentration to calculate whole-kidney clearance. Here, A and B represent the start and end of a nephron segment, allowing us to analyze clearance specifically for a segment.

Since we can cancel flow (V) out, we can get this equation (final important one):

$$Fraction\ of\ substance\ left\ = \frac{\frac{B_x}{A_x}}{\frac{B_{inulin}}{A_{inulin}}}$$

- A fraction of < 1 means substance got **reabsorbed**.
- A fraction of > 1 means a substance got **secreted**.
- A fraction of 1, a substance **neither** absorbed nor secreted.

Example: From A to B, Inulin concentration doubled → 50% of water reabsorbed

Now if the salt conc:

- Doubled? – then salt wasn't reabsorbed
- Stayed the same? – salt was reabsorbed at the same ratio as water
- Halved -? Solve on your own.
- Quadrupled -? Solve on your own.

Once we understand how different substances are being modified in the nephron, we can determine the **segmental function of the nephron.**

Water and Sodium Handling

Sodium importance

Why do we care to study sodium? It is very important for several reasons:

- It is important for excitability of cells and depolarization
- It is important for ECF volume. An increase in sodium will cause water to follow causing ECF volume expansion, leading to hypervolemia and hypertension (and vice versa).
- It is important for reabsorption of glucose and amino acids
- It's important for the formation of concentrated urine
- It is responsible for around half of the osmolarity of the ECF.
    - $Blood\ osmolarity \approx 2.1 \times [P_{Na^+}]$; Blood Osmolarity = 300, P_{Na^+} = 140 mOsm.

Sodium balance

We calculated the FL and reabsorption of sodium in sheet 2: around 25,200 mEq/day are filtered with about 99.4% absorption. We have *sodium balance,* so excretion is equal to our daily intake, with both being equal 155 mEq/day; 150 mEq/day of which are excreted through urine and 5 mEq via sweat and GI secretion.

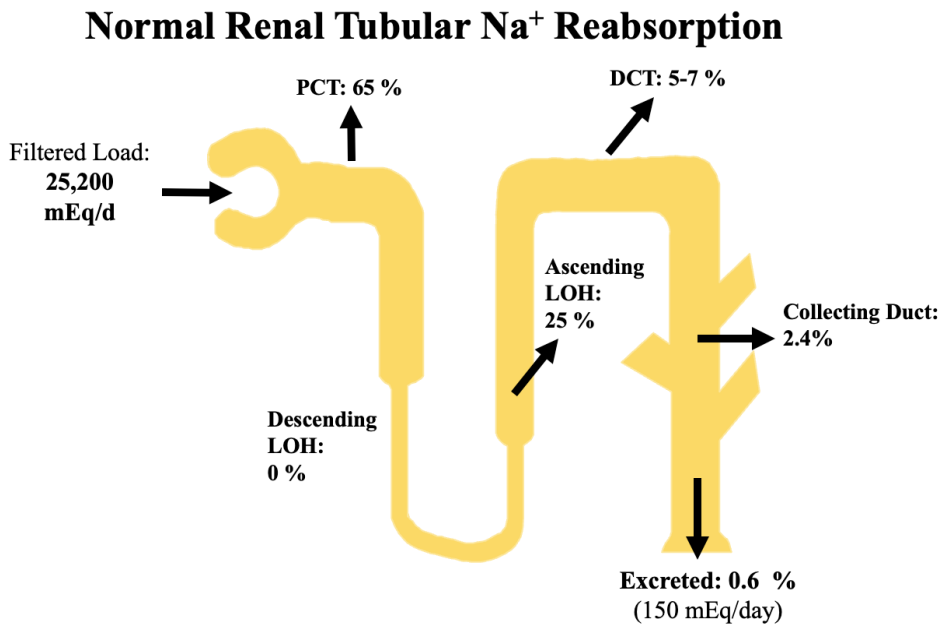
Water is filtered at around 180 L / Day, and we urinate 1.5 L (0.8%). Water reabsorption is largely coupled to (driven by) sodium reabsorption, so they are best studied together.

So how is 99.4% sodium and 99.2% of water reabsorbed along the nephron?

Sodium reabsorption

Sodium is filtered and reabsorbed, and NOT secreted. Its net total is slightly excreted.

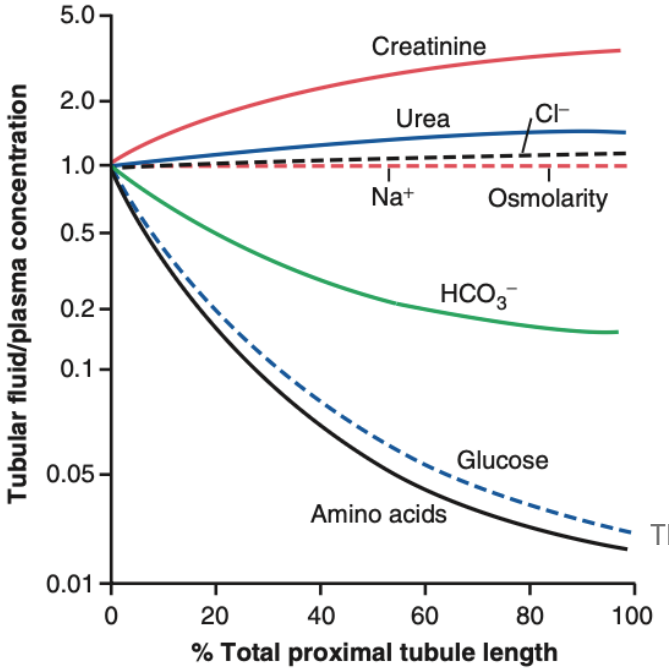
Segment	***% of Na+ reabsorbed***	***% of water reabsorbed***
Proximal convoluted tubule (PCT)	65%	65%
Descending loop of Henle (LOH)	-	15%
Ascending loop of Henle (LOH)	25%	-
Distal tubule (DCT)	*7%*	10%
Collecting duct	2.4%	*9%*
Excreted	0.6%	*0.8%*



1- Proximal convoluted tubule (PCT)

Sodium concentration stayed constant in the PCT, meaning it got reabsorbed in the same proportion as water - 65%. Same applies to Cl^-.

Glucose and amino acids are totally reabsorbed in the PCT, and if they don't, they will appear in urine as there are no transporters for them in later segments. Their transport mechanisms will be discussed later.



They should reach 0

2- Descending LOH

Solutes are not reabsorbed in this segment, but water is (15%); which makes the tubular fluid hyperosmolar (as high as 1200 mOsm).

3- Ascending LOH

Ascending LOH is the only human membrane not permeable to water even in the presence of ADH.

Water is *not* reabsorbed in this segment, but sodium is (25%); Fluid leaving this segment can have an osmolarity as low as 100 mOsm.

This step is special because it has a ***single effect***. Usually when sodium is excreted water follows (double effect), however since the membrane is impermeable to water this step has solute movement only. This creates:

- a very dilute tubular fluid
- a hyperosmolar inner medullary interstitium (as high as 1200 mOsm);

4- Distal convoluted tubule (DCT)

Although this segment has only 7% of sodium reabsorption, it is regulated by *aldosterone*, and its range can vary between 6 – 8%; which is a huge range considering that we only excrete around 0.6%. More on regulation later.

5- Collecting duct

Final 2.4% of sodium are reabsorbed.

Urea can actually be reabsorbed here (in sheet 4 p.4); also contributes to the hyperosmolar inner medullary interstitium.

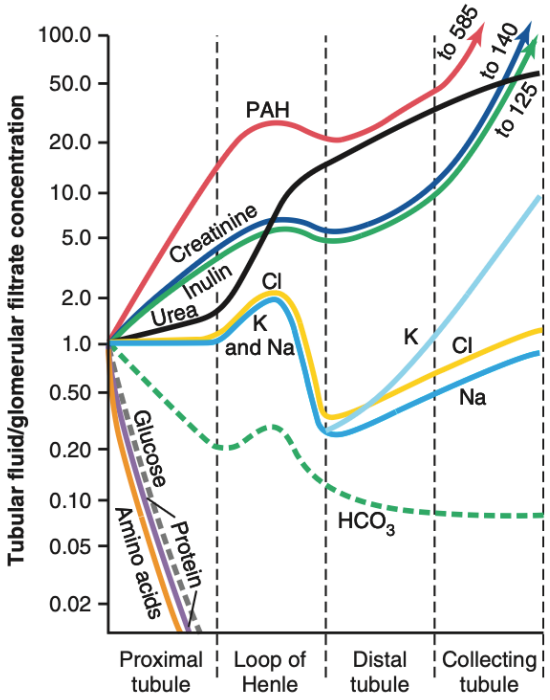
In the collecting duct tubular fluid arrives with an osmolarity of around 100 mOsm.

The collecting duct can then form urine that is

- 100 mOsm: by leaving it as it is
- <100 mOsm: by actively transporting solutes out of the collecting duct.
- as high as 1200 mOsm: water is reabsorbed under *ADH* stimulation through osmosis, so osmolarity equilibrates (with the hyperosmolar inner medulla).

Doctor: Notice that in the diagram there are mistakes:

- In the loop of Henle (ascending or second half) PAH, creatinine and inulin should have constant concentration. (They are not reabsorbed and water is not secreted).
- Bicarbonate should also reach near 0 by the end of the collecting tubule.



Lastly, we urinate:

- 0.6% sodium (150 mOsm), 1.5 L of water; 100 mOsm/L of sodium.
- 585 mg/min of PAH, 140 mg/min creatinine, 125 mg/min inulin.

$$CL_{Na} = \frac{U}{P} \times V = \frac{100}{140} \times 1 = < 1\%$$

Summary: Tubular fluid osmolarity (mOsm):

300	1200	100	Variable (≤1200)
Plasma to end PCT	End of descending LOH	End of ascending LOH	End of collecting duct / Urine

Mechanism of Reabsorption

Reabsorption occurs from the tubular lumen → interstitial space → blood vessels.

The movement from *interstitial space* → *blood vessels* occurs via **bulk flow**.

Movement from lumen to interstitium occurs by two pathways:

- Paracellular: between cells (through tight junctions)
- Transcellular: through the cells

Paracellular transport can occur because tight junctions are "leaky" in the PCT, in later segments they become tighter and this mode of transport decreases.

Sodium reabsorption

1- PCT

Sodium reabsorption is unique because it involves two modes of transportation: passive and active.

Passive transport is paracellular through diffusion.

Active transport is Transceullular;

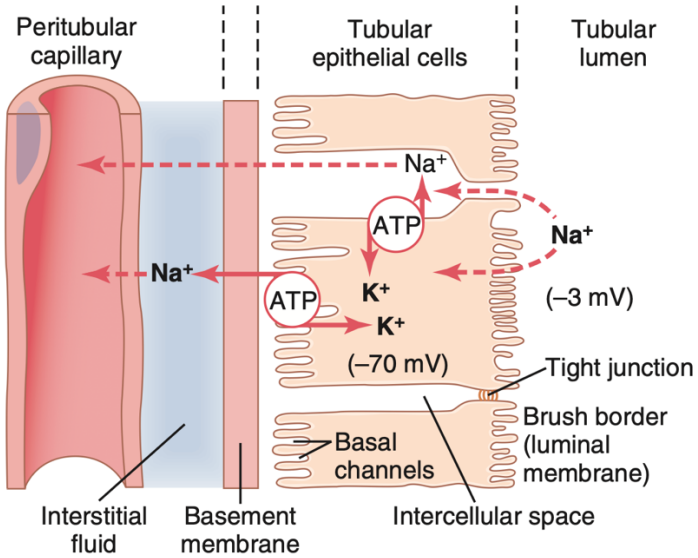
the sodium-potassium pump creates a low intracellular sodium concentration. The low intracellular sodium concentration cause sodium ions to diffuse from the tubular lumen **(140mOsm)** into the cell **(14 mOsm**). Memorize numbers.

This process is active even though sodium enters the cell passively, because the low concentration gradient inside the cell was created using energy (actively).

For any process if a single step is active then the entire process is active even if some individual steps are passive.

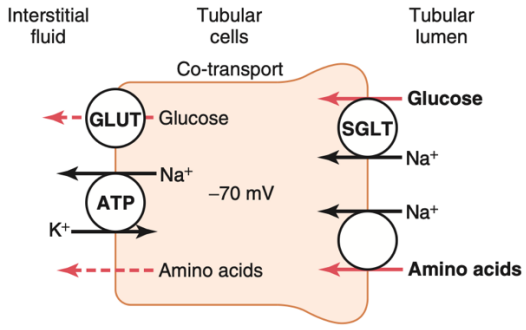
Sodium reabsorption is not Tm-limited, so it is not described as Tm dependent like other actively transported substances. This is because Na^+ transport involves multiple mechanisms along the nephron, making a single Tm difficult to define.

Instead, sodium reabsorption is considered **time-gradient dependent**. The main limiting factors under physiological conditions for transportation rate are tubular flow rate (time available for reabsorption) and the electrochemical gradient driving Na^+ transport.



Secondary reabsorption in the PCT

In cotransport (both substances are transported in the same direction), sodium always moves down its concentration gradient while the other substance moves against its gradient. Amino acids and glucose depend on sodium for their reabsorption.



Amino acids are reabsorbed in the PCT by secondary active transport by multiple carriers. Acidic, basic, neutral groups each has multiple carriers. An important one clinically is the cysteine carrier, because congenital absence of *cysteine transporter* cause cysteine urea which predisposes the patient to kidney stones as cysteine forms the nucleus of stones because it is insoluble. It should be cystine (cysteine dimer), but the doctor wrote cysteine.

Glucose is reabsorbed in the PCT via secondary active transport along with sodium. Sodium is going down its concentration gradient (140 to 14) through a sodium-glucose cotransporter (**SGLT-2**) on its apical border that transports glucose.

SGLT – 2 is high in capacity and low in affinity (dominant in the kidney, drug target)

SGLT – 1 is low in capacity and high in affinity.

2- Loop of Henle

The descending LOH doesn't have sodium transport, the ascending however does. The Ascending LOH has a (Na^+-K^+-$2Cl^-$) cotransporter, transporting salt from lumen to interstitium. Notice the net charge is 0.

3- DCT

The end of ascending LOH to early DCT has sodium chloride channels which transport 1 sodium and 1 chloride.

The late DCT has sodium potassium pumps (3 Na+ out and 2 K+ in per cycle), this pump is unique in that it causes reabsorption of sodium but *secretion* of potassium to the tubule.

4- Collecting duct

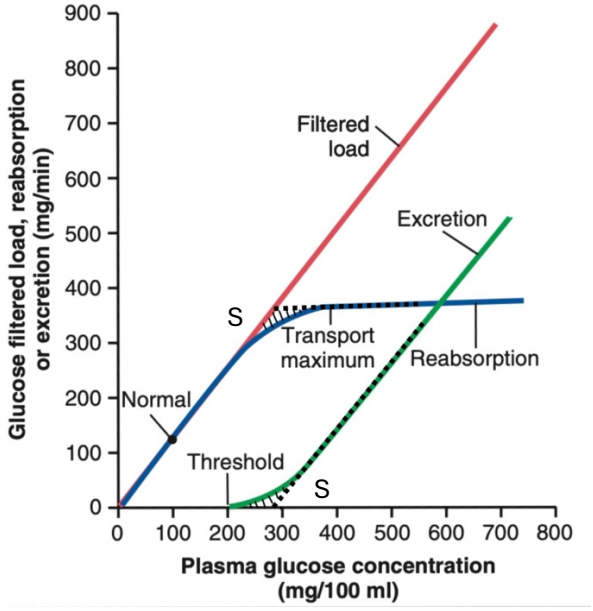
This is where the regulated step for water reabsorption is. ADH Regulates the insertion of water channels (*aquaporin*) in the collecting duct wall, which allows water (and osmolarity) to equilibrate between inner medullary interstitium and urine through osmosis.

Glycosuria

Glucose reabsorption in the proximal convoluted tubule is limited by a transport maximum (Tm), since the SGLT carriers can become saturated (around 375 mg/min).
Under normal conditions P glucose is 70-110 mg/dL.

When viewing the glucose titration curve, the relationship between plasma glucose and transport shows a **splay** (S), meaning T max is not reached abruptly but with a gradual rounding of the curve, because glucose carriers saturate progressively rather than all at once.

If the filtered load exactly equals the T_m (≈ 375 mg/min), not all glucose can be reabsorbed, so some appears in the urine. Therefore, the actual T_m is only reached when the filtered load exceeds this value. Glucose excretion (glycosuria) thus begins before T_m is reached, at the renal ***threshold***, which is approximately 180 mg/dL.



Glycosuria severity increases with plasma glucose levels and may be graded (+1 to +4), with severe cases (+4) seen in uncontrolled diabetes (plasma glucose >500 mg/dL).

Nephrogenic glycosuria is mild glycosuria (+1) with normal blood glucose and normal HbA1c, caused by a defect or reduced capacity of proximal tubular glucose transporters (decreased Tm). This lowers the renal threshold for glucose, so glucose appears in urine at lower plasma levels and may occur after meals even when plasma glucose is within or only slightly above the normal range (140-150 mg/dL). It is benign and not associated with other abnormalities, so better not to tell the patient about it. This is debatable.

Note:

FL in some graphs can be written in equivalent plasma concentration values and units, since we can assume GFR is constant (and glucose is freely filtered).

e.g: normal values for FL can either be 125 mg/min, or 100 mg/dL, since at 100 mg/dL plasma concnetration (and normal GFR - 1.25 mg/dL) FL is 125 mg/min.

This is because we measure plasma concentration so seeing its values in graphs can have more meaning.

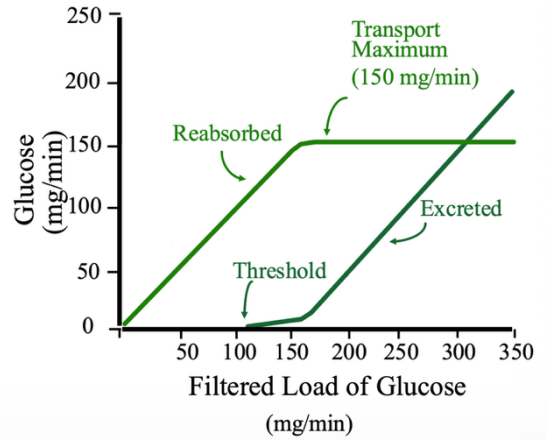
To convert between these two value modes multiple or divide by the GFR.

Glucose plasma concentration values are generally either written as mg/100 mL or mg/dL, so for GFR use 1.25 (mg/dL , mg/100 mL)

In lec MCQ

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?
(The numbers do not make since)

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



Sodium Homeostasis

We have 3 factors:

1st factor GFR

Increase in GFR increases FL and thus the amount of excretion available. Blood pressure increase causes pressure diuresis and natriuresis (sheet 4), through a slight increase in GFR, along with some other mechanisms.

2nd factor Aldosterone

The regulated step of sodium reabsorption is the late DCT where *aldosterone* acts to stimulate the sodium potassium pump. Aldosterone's action is to reabsorb sodium and excreted potassium.

3rd factor ANH (Atrial Natriuretic Hormone)

ANH inhibits sodium reabsorption and RAAS. Almost the only mechanism that actively decreases plasma sodium. Released from the right atrium in response to stretch (caused by hypervolemia), so it acts to decrease plasma volume through natriuresis and can be considered as an endogenous diuretic.

Diuretics

A) Osmotic diuresis

Osmotic diuretics increase water excretion through osmotic effect in the tubular lumen.

SGLT-2 inhibitors (Dapagliflozin) increase glucose in the tubule which pulls water with it (also sodium is not reabsorbed which contributes to the diuretic effect). This is indicated for:

1- T2DM to decrease blood sugar levels
2- Congestive Heart Failure: diuretic decreases blood volume which decreases preload (venous return) and afterload (arterial blood pressure) which decreases load on the heart

Also, **mannitol** (a sugar that is filtered and not reabsorbed) is an osmotic diuretic.

B) Loop diuretics

These drugs such as **Furosemide**, are some of the strongest diuretics, as they work on the ascending LOH which contributes 25% to total sodium excretion. They block Na^+-K^+-2Cl cotransporter.

As we said earlier people taking these drugs are not able to form a concentrated inner medulla, so they will not be able to form concentrated urine.

C) Thiazides

Thiazide diuretics work on the late ascending LOH to early DCT, specifically on NaCl cotransporter. These diuretics are of intermediate strength and over time, their diuretic effect decreases and they become more vasodilatory.

D) Potassium sparing diuretics

(Some of) These drugs antagonize aldosterone receptors and thus inhibit the action of aldosterone; an example of such is **spironolactone**.

The late DCT to early collecting duct is their site of action. Spironolactone is an aldosterone receptor antagonists that inhibits the stimulatory effects of aldosterone on sodium reabsorption and potassium secretion (sodium-potassium pump).

These drugs are special in that they do not cause hypokalemia; however, they are considered weaker because they act after most sodium has already been reabsorbed.

Therapeutic considerations

We may give these drugs acutely (such as in pulmonary edema) or we chronically such as for patients with high blood pressure.

For blood pressure patients we don't prefer to give loop diuretics as they are too strong, so instead we give thiazides (usually hydrochlorothiazide).

However, loop diuretics and thiazides can cause **hypokalemia** – thus called "potassium wasting diuretics", so we advise patients to eat foods high in potassium (bananas, dates) and monitor their potassium levels continuously in case they develop hypokalemia, where the patient is either prescribed potassium supplements or switched to potassium-sparing diuretics.

For liver cirrhosis patients, their aldosterone is elevated so first line management is spironolactone.

Diuretics summary

Indirect: Mannitol (osmotic diuretic)

PCT – SGLT-2: Dapagliflozin (osmotic diuresis); also used for DM patients

Ascending LOH – Na-K-2Cl: furosemide (loop diuretic)

Early DCT – NaCl cotransporter: Hydrochlorothiazide (thiazide)

Late DCT – Na/K pump: spironolactone (potassium-sparing)

Notes from the doctor:

- ECF osmolarity is 300 mOsm,140 mOsm of which are from sodium (almost equal amount is from anions, there is also neutral compounds like glucose which contribute).
- When studying nephron (tubular) modification in physiology we care about the segment that is under regulation from the body; as physiology is concerned with homeostasis and regulation.
- ANH was named ANF (factor) because they didn't know what it was, then named ANP (peptide) when they found out it was a peptide, then named ANH (hormone) when they found out it travelled the blood and fulfilled criteria of a hormone.
- Remember ADH is synthesized in the hypothalamus and stored in the post pituitary along oxytocin. Both are 9 a.a peptide hormones sharing 7 a.a, 1 difference is that ADH has arginine.
- Thiazides are so widespread that most blood pressure drugs have extra thiazide to the original drug (indicated by a "+" next to the drug's name; usually hydrochlorothiazide; 12.5 mg or sometimes 25 mg).
- Commercial names for drugs mentioned: Lasix - furosemide, Aldactone – spironolactone, Forxega – Dapagliflozin.

Feedback form

Changes:

- P.7: Written Na mOsm / L more clearly; required for CL Na calculation.
- P.7: fixed grey summary; ascending and descending LOH where switched
- P.12: deleted "Concentrated urine formation criteria"; explained in sheet 7
- P.14: Added "Potassium wasting diuretics"
- P.15: deleted notes from writer
- Other minor edits