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** Note: the curve discussed in this page "[TF]/[P] curve" is found in the slides, so please refer to them.**

INULIN characteristics :
1 – filtered 100 % . 2-Not secreted. 3-Not reabsorbed
so;
* If it is not secreted and not reabsorbed, its amount remains unchanged throughout the nephron.
* Its concentration remains constant in the tubular fluid (TF) if there is no water re-absorption. If water is reabsorbed its concentration will be increased in the TF.
2 – INULIN is used as a marker for measuring GFR since it is only filtered; In addition, inulin is used to study the segmental function of the nephron.
3- The **[TF]**/ **[P]** of inulin is:

* [1] inside the Bowman's capsule & at the beginning of the proximal tubules

* [3] at the end of the proximal tubules, this ratio jumps to 3; since 66.7% (2/3) of water and none of the inulin is reabsorbed, resulting at the end in the same amount of inulin in only 1/3 of total water volume filtered at the glomerulus.

* Within the loop of Henle: the curve of the [TF]/[P] differs, at the 1st half of the loop –descending segment-, water is actually reabsorbed, thus, the ratio keeps rising … however, at the 2nd half of the loop –ascending segment- , water is NOT reabsorbed even in the presence of ADH, thus, the curve stops rising.

* Within the distal tubule & collecting tubule, the ratio goes back rising due to further re-absorption of water.

*Note here, that the **flow of inulin (mg/min)** inside the nephron & the collecting tubule is **the SAME** –constant flow-, however, the **concentration of inulin** throughout the nephron & the collecting tubule **INCREASES**.

CREATININE: Its clearance is a good estimation of GFR.

The curve of [TF]/[P] ratio of creatinine is slightly above that of inulin; due to the fact that a slight amount of creatinine is secreted from the proximal tubular cells in addition to the already filtered amount of creatinine in the glomerulus.

Creatinine in urine comes from 2 sources: *90 % filtered & NOT reabsorbed and *10 % secreted
Due to the latter source, creatinine clearance will overestimate GFR by 10 %, still, clinically we measure GFR by using creatinine; because 10% of it will be bound to plasma proteins; so, they will cancel each other.

NOTE: The ratio of a substance's concentration in the tubular fluid [TF] to its levels in the plasma [P] changes along the course of the tubular system depending on how it is handled/ cleared across each segment.

For example, The **[TF]** / **[P]** for sodium remains 1 throughout the proximal tubule since Na+ and water are reabsorbed "in the same proportion ", remember that 66.7% of filtered water and also 66.7 % of filtered Na+ are reabsorbed in the proximal tubule… now, how did we know the amount of water –percentage- that has been reabsorbed within the proximal tubule –which is 66.7%- ? by using inulin, how? As we said previously that the [TF]/[P] of inulin measured at the end of the proximal

tubule was (3) & from this, we concluded that 2/3 of water have been reabsorbed & that's why the ratio tripled.

Back to one of the most important uses of inulin, which is to study the segmental function of the nephron (how each segment handles/ clears a certain substance (X), in other words, what happens to substance (X) –changes in its concentration, re-absorption, secretion, etc- as it passes through each segment)… How is that done?

1) By taking 2 samples via micropuncture, one from the beginning & the other from the end of the segment that we want to study its function & changes of (X) that occur within it, lets say the proximal tubule for example
2) These 2 samples will be analyzed; i.e. we measure the concentration of inulin as well as the concentration of substance (X) –which can be Na+, K+, Cl-, urea, glucose, a.a …- from the 2 samples
3) We calculate clearance on a small scale here (clearance throughout one segment between 2 points) … so, clearance here will be as follows:
   C (inulin throughout the prox. Tubule) = [TF] (inulin)/[P] (inulin) * V

   Where:
   #C: Clearance
   #[TF] (inulin): conc. of inulin at the end of the proximal tubule
   #[P] (inulin): conc. of inulin at the beginning of the proximal tubule (inside the Bowman'scapsule) = conc. of inulin in the plasma
   #V: fluid flow rate

   C (X throughout the prox. Tubule) = [TF] (X)/[P] (X) * V … same equation but regarding another substance other than inulin.

4) Calculate the ratio of clearance of substance (X) to clearance of inulin
   C (X throughout the prox. tubule)/ C (inulin throughout the prox. Tubule)
   Note that the flow rate (V) is the same for both clearance calculations, so, in measuring the ratio, the 2 (V)s cancel each other … i.e. you won't need the (V) value if you're willing to measure the ratio.
5) Do the conclusion:
   If **C(x)/ C (inulin) = 1**, it means that the substance is handled exactly like inulin in the studied segment, i.e. not reabsorbed nor secreted.
   If **C(x)/ C (inulin) = 2**, it means that the same amount of (X) that is filtered was also secreted to the tubule.
   If **C(x)/ C (inulin) = 0.3**, it means that 0.7 of (X) was reabsorbed & 0.3 only remained in the tubule.

PAH characteristics:
1 – Filtered. 2 –Secreted. 3- NOT reabsorbed.
4 – PAH is used as a marker for measuring renal plasma flow "RPF".
5- If administered in low amounts, it'll be completely cleared from plasma & excreted by the kidneys, however, if the amount exceeds certain limit (80mg/min), this will make the kidneys unable to clear the whole plasma from it (PAH) & will underestimate RPF.
As we go distally in the tubular fluid, the concentration of PAH will increase because it is secreted to the lumen of the tubule and water is reabsorbed. PAH concentration at the end of the collecting

duct will be 585 times more than its concentration inside the Bowman's caspsule… from where did we get this number? (585)?
This number came from the following:
The conc. of PAH in blood is 1 mg/ml… conc. of PAH in urine –at the end of the collecting duct- is 650 mg/ml … UOP (Urinary OutPut) = 1 ml/min … we conclude that RPF = 650ml/min
** But this isn't the case truly; because 10% of the renal blood goes to nourish the kidney structures (capsule, medulla, calices, pelvis, etc…) & this portion of the blood never reaches afferent arterioles & thus, doesn't participate in renal function**
So… 90% of 650ml/min is the effective RPF (eRPF) which = the clearance of PAH = 585ml/min.
The 100% is the true/ total RPF (tRPF).

- Regarding glucose and amino-acids; before they reach the END of proximal tubule, their conc. becomes zero; because they're totally reabsorbed … we know this through micropuncture technique discussed previously.

SODIUM HOMEOSTASIS: How the kidney handles Sodium:

Filtered load of sodium = GFR *Plasma conc. Of Na+ = 180 L/day * 140mEq/L=25200 mEq/day

We know that the excretion rate of Na+/ day = 150 mEq/day (that is how much Na+ is in urine passed/ day)

So, the excretion rate of Na+ will be less than 1% of the filtered load of it; excretion rate /filtered load= 150/25200= 0.6% → which is less than 1%... In other words, more than 99% of the filtered load of Na+ will be reabsorbed.

General rule: Sodium balance is achieved when the input of Na+ (Na+ intake) = the output of Na+: Sodium intake is about 155 mmol/ day in the average diet. Logically, the daily output would be 155 mmol/ day as well.

• The kidney accounts for 150 mmol of this output. Hence, <u>the kidney is a major organ in sodium homeostasis.</u>
5 mmol/ day is excreted by other routes "sweat ….."

Clearance of a substance= ([u]/ [P]) * V … Apply this equation to calculate clearance of Na+: such that:
[U] = concentration of Na+ in urine = amount of Na+ (150 mmol/ day) / volume of urine (1.5 L/ day)= 100 mmol/L = 100 mEq/ L.

[P] = concentration of Na+ in the plasma = 140 mEq/L
V = flow of urine = 1 ml/ min

So, clearance of Na+ = [u]/ [p] * V = (100/ 140) * 1 = 0.7 ml / min, <u>which is less than 1 ml/ min … this is to emphasize on the previously mentioned note; that most of Na+ filtered is reabsorbed.</u>

Note: In case of a patient having chronic hypertension, his diet must be restricted to a certain extent from Na+ intake, furthermore, this patient might have nephropathy, and so by reducing Na+ intake, we are relieving the kidneys to some extent; because most of the kidneys' work is to reabsorb Na+.

Excessive Na+ intake →more FILTERD Na+ → more Na+ reabsorption→ more H2O reabsorption → more ECF volume & blood volume (hypervolemia) → edema & hypertension.
((When Na+ intake↑◊ ↑Na+ filtered ◊ ↑ Na+ reabsorption)).

*how does the body handle Na+:

In proximal tubule we absorb 65% (2/3, it's 66.7 in the slides) of Na+ and water (isosmotic TF=300 m.osm)

In descending loop of henle: Na+ conc. will increase; because it's permeable to water and not permeable to Na+ (hypertonic TF -400 to 600 up to 1200 m.osm-).

So, 0% of Na+ is re-absorbed in the descending loop of Henle.

25% of Na+ is re-absorbed in the ascending loop of Henle.

5% of Na+ is re-absorbed in the distal tubule under the effect of aldosterone.

4.3% of Na+ is re-absorbed in the collecting duct.

(65%+25%+5%+4.3%=99.3% are reabsorbed. → 0.7% will be excreted in urine)

- Na+ is important in the kidneys, because we need Na+ to reabsorb glucose and amino acids from the proximal convoluted tubules as well as to reabsorb water.

*diuretics:

Most of diuretics target sodium, by inhibiting Na+ reabsorbtion & keeping it in the tubular fluid & thus Na+ will drag water with it, making too much urine (diluted) with excretion of too much Na+ … that's how we resolve edema, esp. & most importantly, <u>pulmonary edema</u> (top medical emergency … strong diuretics are required).

We'll mention 4 classes of diuretics:

1. Osmotic diuretics: (K+ sparing)
   osmodiuresis occurs when a substance is not reabsorbed; hence it will be excreted dragging water with it in urine. For ex.: the diabetic patients who have their blood glucose levels higher than 180 mg/dl → they will be exceeding the renal threshold of glucose (180mg/dl), which means that the prox. tubules won't be able to reabsorb those high levels of glucose & thus the tubules will start excreting glucose which in turn will drag water with it and will increase diuresis. That's why diabetic patients have polyuria & polydipsia.
   - Mannitol as an osmo-diuretic is a drug that is not reabsorbed from the nephron, so it will drag water into the tubules and increase diuresis.(we use mannitol for **brain edema**).

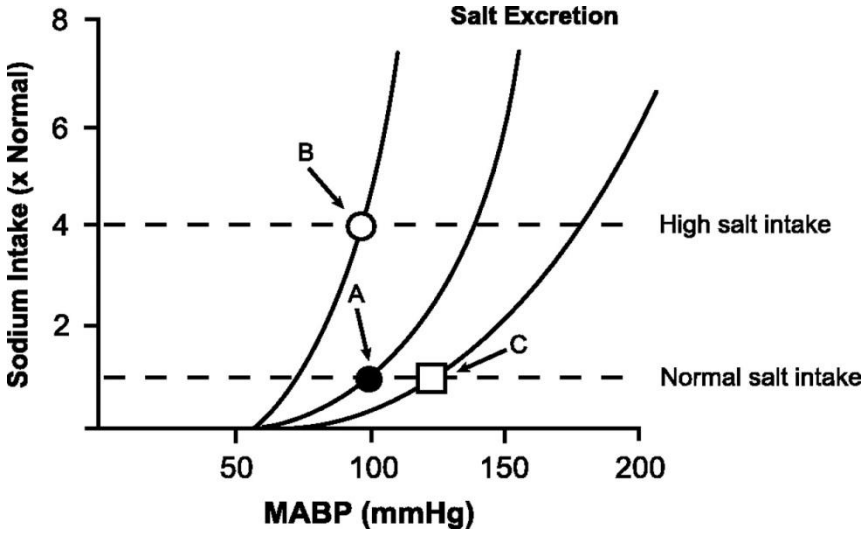
2. Strong diuretics (loop diuretics): for ex.: Lasix "FUROSEMIDE": (K+ wasting)
   - Why are they strong?
     Because they act on 35% of Na+ in the thick ascending limb of loop of Henle, which remain in the tubular fluid (plenty amount)
     (65% of Na+ is reabsorbed in the proximal tubules → 35% remain in the thick ascending limb of loop of Henle & the drug will act by blocking re-absorption of them)

- We use strong diuretics for **edema**; for ex.: <u>pulmonary edema</u> to drain & reduce ECF volume through diuresis.
- Lasix can be "rarely" used in cases of <u>acute hypertension & for a short period of time</u>.
- Mechanism of action: inhibition of Na+/ K+/ 2 Cl- re-absorption in the thick ascending limb of Henle… so, it will decrease K+ re-absorption too.
  ➔ Hypokalemia: due to the fact that these diuretics aren't potassium sparing.

3. Moderately powerful/ potent diuretics: Thiazide: (K+ wasting)
   Why are they moderate in potency?
   Because they work on the distal tubules; i.e. only on the remaining 10% of Na+ in the TF.
   - We use them for **hypertension** NOT for their diuretic effects, but rather because they act as vasodilators after one weak of their administration (within the 1st weak of administration, there's the vasodilator effect as well as the diuretic effect, but after that, diuretic effect vanishes/ stops, but vasodilatation effect is persistent).

4. Weak diuretics: Aldosterone antagonists; like Spironolactone (Aldactone): (K+ sparing)
   We use them for **hypertension when we want to spare potassium**… However, they're weak because they're acting on the (late) distal tubules, i.e. on < 10% of Na+ in TF.

- Three factors affect Na+ homeostasis: GFR, aldosterone, ANP (or ANH)
  1. GFR:



Comments on this kidney function curve:

- Normal Na+ intake is 155 mmol/day, as long as there's Na+ balance (Na+ excretion is 155 mmol/day), then the mean arterial blood pressure is 100 mmHg, however, if the intake is increased (2X or more), there's a danger of having increased blood pressure unless the kidney shifts this curve (the curve that has the "A" point) to the left (the curve that has the "B" point), in this case the arterial blood pressure remains normal around 100 mmHg although there's an increase in Na+ intake, but the output –excretion- here is

increased too & that's how everything is maintained normal … this is what happens in healthy normal functioning kidneys, & the following are the details of this regulation mechanism:

"Pressure-diuresis/ Pressure-natriuresis":

When we increase Na+ intake → increase in water retention → increase ECF volume (including blood volume) → increase in blood pressure → increase GFR→ increase delivery of NaCl to maculadensa cells & thus these cells will sense the increased levels of Na+ in blood → Renin release will be decreased → angiotensin II levels will be decreased too → Na+ re-absorption will decrease→ too much Na+ excretion (diuresis).

- In abnormally functioning kidneys:
  The curve is already shifted to the right (the curve that has the "C" point), so that even with normal regular Na+ intake, there will be rise in BP, that's why patients with such kidneys are always required to have low Na+ intake diet, to guarantee that their BP is within normal… this is what's so called "salt sensitive hypertension", that at any time, if there's a rise in Na+ intake, the patient **can't** shift the curve to the left.

2. Aldosterone:

- Is secreted by the cortex of suprarenal (adrenal) glands, goes to the principal cells –site of action of aldosterone- and via simple diffusion crosses their membranes & goes to the nuclei (because it's a steroid "lipophilic") and it will bind to DNA (at a certain sequence) to increase transcription →mRNA→proteins (Na+ channels, Na+/K+ pumps and enzymes needed for ATP formation).
- What are these proteins needed for?
  In the late distal tubules, Na+ will diffuse passively through Na+ channels from the tubular lumen into the distal tubular cells (principal cells specifically), however, it will exit the principal cells (from the baso-lateral membrane actively through Na+/K+ pump) towards the blood, so aldosterone actually increased Na+ re-absorption through induction of synthesis of those aforementioned proteins… however, aldosterone also increased K+ secretion from these principal cells; since K+ is actually being pumped into the cells through Na+/K+ pump on the baso-lateral membrane, thus, K+ concentration inside these cells is further increasing to become further greater than tubular lumenal K+ concentration; thus, increasing the K+ gradient across the luminal membrane of the cells, leading to a secondary effect of aldosterone (increase K+ secretion).
- All in all, aldosterone does 2 things: increases Na+ re-absorption and increases K+ secretion.
- However, increase in Na+ intake → inhibition of aldosterone → inhibition of Na+ re-absorption in the late distal tubules.
- NOTE: A patient with Conn's disease = hyper-aldosteronism, will have too much aldosterone; too much Na+ reabsorption, too much ECF volume, so this disease is responsible for mild to moderate hypertension and hypokalemia at the same time.
    - "Addison's disease is the opposite of Conn's disease."

3. ANP (ANH or ANF): Atrial Natriuretic Peptide/ Hormone/ Factor:

- Secreted in cases of hyper-volemia: hyper-volemia → expansion of the right atrium → leads to secretion of ANP.
- Functions of ANP:

- Inhibition of Na+ re-absorption
- Inhibition of aldosterone
- Vasodilatation in the afferent arterioles to increase GFR & thus increase the formation of urine.
- Antagonizes angiotensin II.

- --> The net effect of all these functions is: too much Na+ excretion dragging plenty of water with it; to decrease blood volume & bring it back to normal.
- NOTE: ANP is the only hormone that <u>inhibits</u> Na+ re-absorption.

Bottom line: the normal kidneys can handle (maintain BP at 100mmHg) an increase in Na intake up to 10 times through the prev. 3 factors.

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* HOW DO WE CONCENTRATE URINE?

Urine can't be modified, neither by adding nor by removing any substance, however, the stage before urine is "tubular fluid" & this can be modified.

In the collecting duct (the last modification in the TF occurs here before the TF becomes urine) we have water channels that open and close; open in the presence of ADH which is secreted from pituitary gland. If these channels are opened, water will provide a chance to equilibrate the concentrations between the tubular fluid and the interstitium; leading to concentration of urine. [[**hyperosmotic interstitium** along with **ADH & its receptors '**TOGETHER**'** are a must to concentrate urine]].

If there is no ADH or ADH receptors are insensitive to ADH →disease called: diabetes insipidus (السكري الكاذب); it's called diabetes; because the patient with D.I will produce symptoms similar to D.M, which are: polyuria (urinating diluted urine very frequently) & polydipsia.

*HOW IS HYPEROSMOLAR MEDIUM CREATED (the dead see :P) ?

The thick ascending limb of loop of henle is permeable to solutes (remember: Na+, K+, 2 Cl- co-transport) and it's impermeable to water, so we made a hyperosmotic medium around the loop of henle. Urea also contributes to the hyperosmolarity of this medium.

NOTE: in the thick ascending limb of loop of henle, there's NO water re-absorption even in the presence of ADH.

- Since Na+ contributes to the creation of the hyper-osmolar interstitum, then Na+ is important in aiding the kidneys to produce more concentrated urine.

NOTE: In a case of acute renal failure (ARF), kidney functions will be back to normal gradually; urea & creatinine levels will be back to normal within the first weak, electrolytes within the second weak, fluid retention also will be back to normal… however, there is one

test that we can do, which if was normal, indicates that everything is back to normal & there's no need to do further tests to check the functional status of the kidneys, which is a test to check the ability of the kidneys to make concentrated urine (making concentrated urine is the last function of the kidneys that goes back to normal after ARF) … how to do it? We have to tell the patient not to drink water for 8 continuous hours (during which, there will be an increase in ADH levels), then we take urine sample from that patient, if the urine was concentrated (above 1000 mosm/L), this means everything is normal, otherwise, there is a persistent underlying pathology or damage (note that 85% of ARF in out-patients *not having underlying disease* is completely reversible, however in in-patients *have underlying disease* with ARF, the mortality rate is > 50%).

Clinical Recommendation: In each hypertension case, you must do 3 tests before starting with the treatment:

- Electrolyte test, to check the levels of different electrolytes in the patients' blood; 2 of the most important electrolytes to focus on "when results are ready" are K+ & Na+.
- Kidney function test, why is it important? To check the health status of the kidneys; because uncontrolled hypertension as well as uncontrolled diabetes mellitus are both the causes of CHRONIC renal failure.
- Urine analysis; to see if there's albumin-uria.

Final Note for you to know: 90% of O2 consumption by the kidneys goes to reabsorb Na+.