YU - Medicine Passion Academic Team

The Urogenital System

Sheet# 5 - Physiology

Lec. Title : Concentration & Dilution of Urine

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QUESTIONS ASKED TO THE DOCTORS EMAIL

- Sodium in **isovolume** changes: **does not change**
  Hematocrit in isovolume changes: **changes** (because rbc size is constant)
  Sodium in **iso-osmolarity** changes: **changes**
  Hematocrit in iso-osmolarity: **does not change** (due to modification on rbc size)
- Some patients **do not respond to calcium treatments** and need to be given **magnesium** treatments. Magnesium helps with the **production of parathyroid hormone**. The reabsorption of magnesium is **very different** from other solutes as it takes place in the thick ascending loop of Henle. The **filtration** rate of magnesium is **80%** and **reabsorption is 95%.** Reabsorption in the thick ascending limb **changes** based on the **calcium** level.
- If calcium level **increases** and we don't want magnesium reabsorption to increase, we **decrease reabsorption and increase its excretion**.
  .If calcium level **decreases**, we **increase parathyroid hormone** synthesis, so we **need more magnesium** = **increase reabsorption** and **decrease its excretion.**

Concentration and dilution of urine

المحاضرة تبدأ من صفحة298 من كتاب كوستانزو النسخة السادسة

دعواتكم، كلّ الحبّ...

- Body fluid osmolarity is maintained at a value of about **290 mOsm/L** (for simplicity, 300 mOsm/L) by processes called **osmoregulation.**

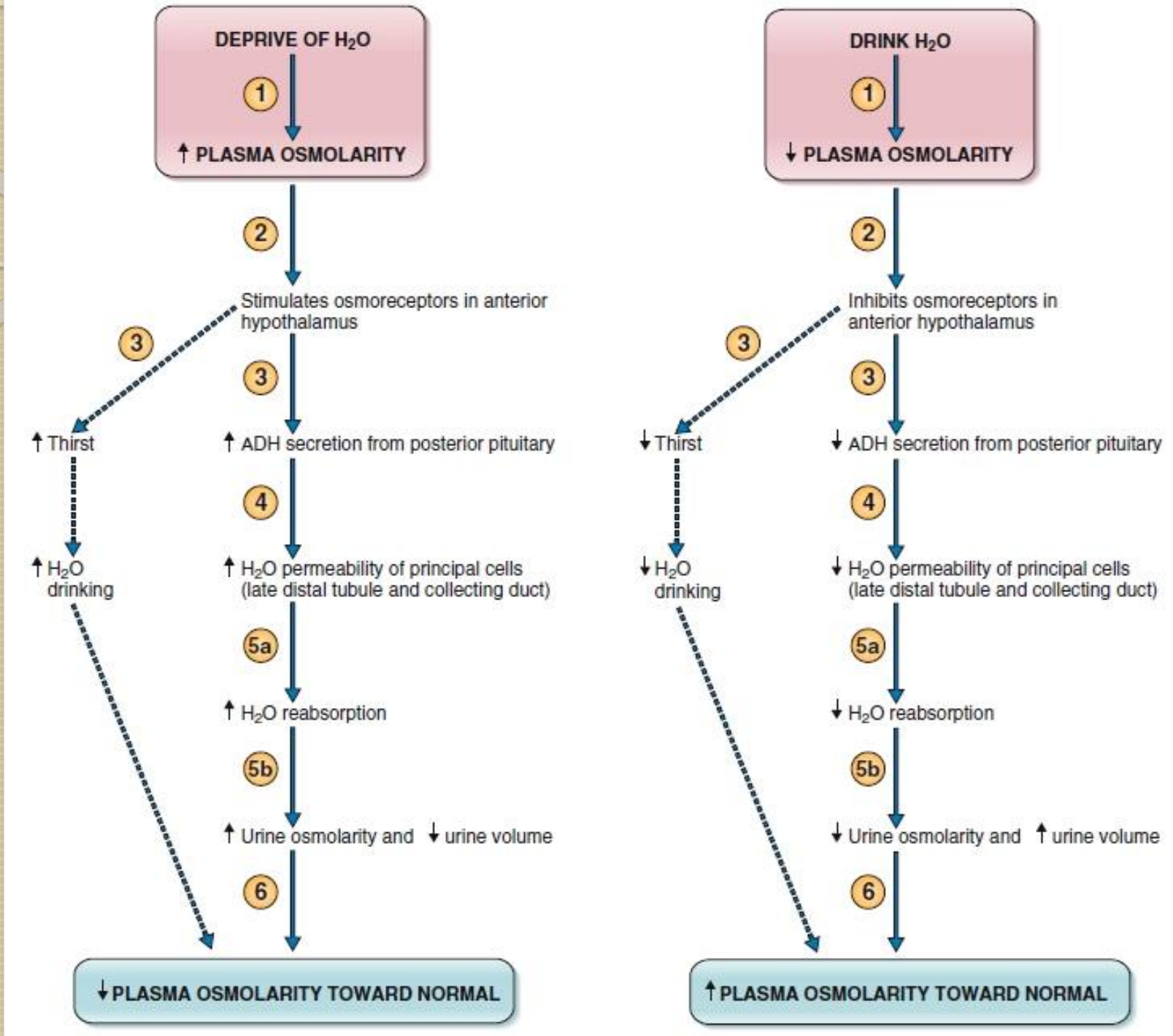
- Control of water balance is exerted by **hormones** at the level of the late distal tubule and collecting duct.

- When urine osmolarity is equal to blood osmolarity,it is called **isosmotic** urine.
- When urine osmolarity is higher than blood osmolarity, it is called **hyperosmotic** urine.
- When urine osmolarity is lower than blood osmolarity, it is called **hyposmotic** urine.

Regulation of plasma osmolarity

- Is accomplished by varying the amount of water excreted relative to the amount of solute excreted (i.e., by varying urine osmolarity).

1. **Response to water deprivation:** water decreases in ECF, so, **osmolarity increases**.
2. **Response to water intake:** water dilutes ECF so **osmolarity decreases**.


DEPRIVE OF H_2O
1
↑ PLASMA OSMOLARITY
2
Stimulates osmoreceptors in anterior hypothalamus
3
3
↑ Thirst
↑ ADH secretion from posterior pituitary
4
↑ H_2O drinking
↑ H_2O permeability of principal cells (late distal tubule and collecting duct)
5a
↑ H_2O reabsorption
5b
↑ Urine osmolarity and ↓ urine volume
6
↓ PLASMA OSMOLARITY TOWARD NORMAL
DRINK H_2O
1
↓ PLASMA OSMOLARITY
2
Inhibits osmoreceptors in anterior hypothalamus
3
3
↓ Thirst
↓ ADH secretion from posterior pituitary
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5a
↓ H_2O reabsorption
5b
↓ Urine osmolarity and ↑ urine volume
6
↑ PLASMA OSMOLARITY TOWARD NORMAL

Response to Water Deprivation

1. Water is continuously lost from the body in sweat and in water vapor from the mouth and nose . If this is not replaced by drinking, **plasma osmolarity increases**.
2. The increase in osmolarity stimulates **osmoreceptors** in the anterior hypothalamus, which are exquisitely sensitive .
3. That has two effects. It stimulates **thirst,** which drives drinking. It also stimulates **secretion of ADH** from the posterior pituitary gland.
4. ADH **circulates** in the blood to the kidneys, where it produces an **increase in water permeability** of the **principal** cells of the **late distal** tubule and **collecting duct**.
5. results in **increased water reabsorption** in the late distal tubule and collecting ducts. As more water is reabsorbed, **urine osmolarity increases** and **urine volume decreases.**
6. plasma osmolarity is **decreased**, back toward the normal value. This example of **negative feedback**, in which the (increased plasma osmolarity) causes a set of feedback responses (secretion of ADH and increased water reabsorption) that restore plasma osmolarity to its normal value.

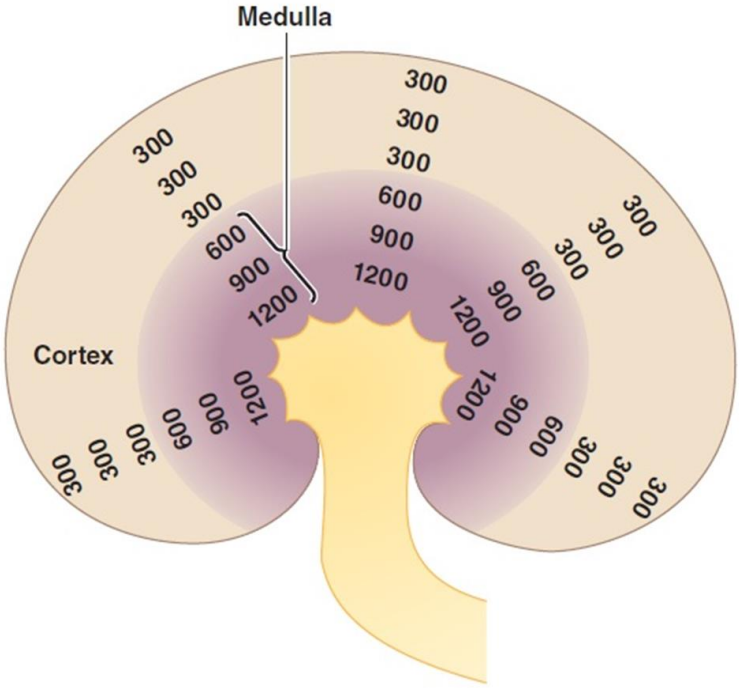
- Response to water intake is the opposite to what we discussed, pleas for more details, read the book page 299

- **ADH direct action:** works on **V2 receptors** in distal tubule. Those receptors are G coupled and stimulate cyclic AMP. **Increases aquaporin 2 insertion**.

- **ADH indirect action: increases urea cycling** (will be discussed)

Corticopapillary osmotic gradient

- It is a gradient of osmolarity in the ***interstitial fluid*** of the kidney from the cortex to the papilla.
- The osmolarity of the cortex is approximately **300 mOsm**/L, similar to the osmolarity of other body fluids. **Moving from the cortex to** the outer medulla, inner medulla, and papilla, the **interstitial fluid** osmolarity **progressively increases**. At the **tip of the papilla**, the osmolarity can be as **high as 1200 mOsm/L.**



Corticopapillary osmotic gradient in the renal medulla (values are in mOsm)

Corticopapillary osmotic gradient

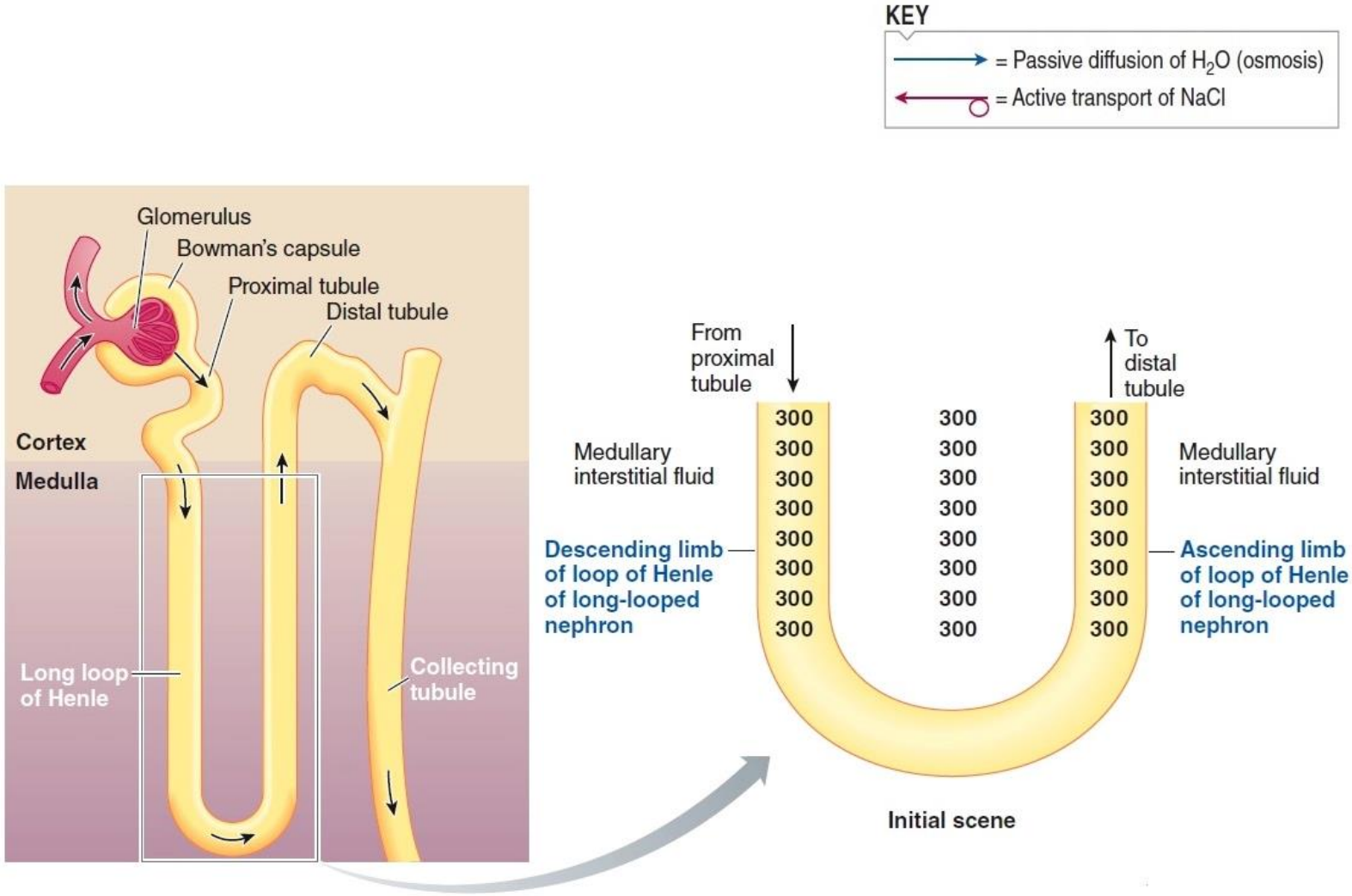
- Corticopapillary osmotic gradient is established by:
  1. **countercurrent multiplication:**
     a function of the **loop of Henle** which **deposits NaCl** in the deeper regions of the kidney
  2. **urea recycling**

     a function of the inner medullary collecting ducts**, which deposits urea.**

First: countercurrent multiplication:

- the loop of Henle is initially shown with no corticopapillary gradient; osmolarity is 300 mOsm/L throughout the loop and in the surrounding interstitial fluid. Countercurrent multiplication will build up a gradient of osmolarity in the interstitial fluid through a repeating **two-step process.** The first step is called the single effect, and the second step is the flow of tubular fluid.
- Look to the next slide

Counter-current multiplication



- Step 1:single effect:

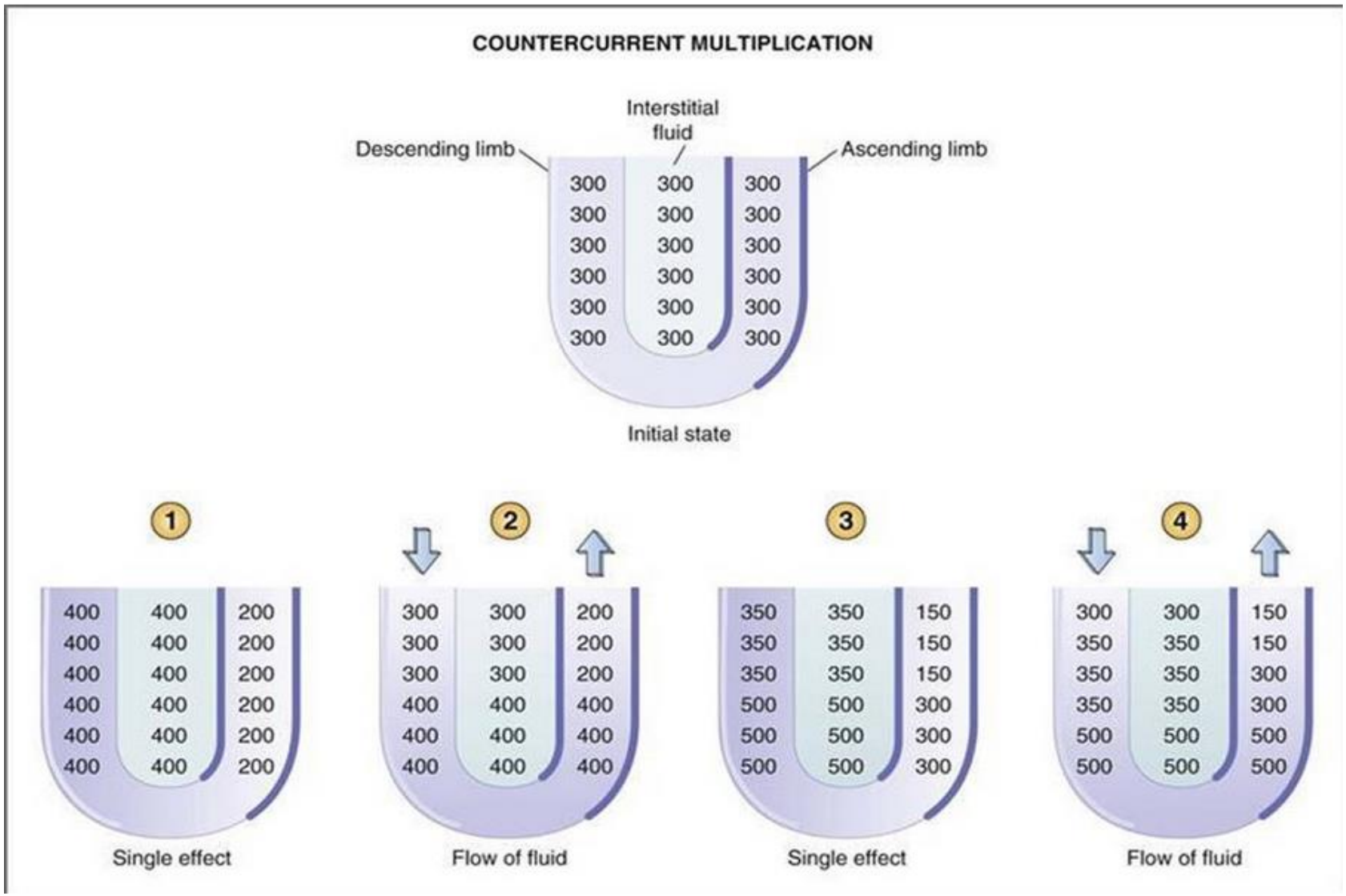
- In the **thick ascending limb,** NaCl is reabsorbed via the **Na+-K+-2Cl– cotransporter (NKCC2 cotransporter).** Because the thick **A**scending limb is **impermeable** to water, water is not reabsorbed along with NaCl
- NaCl enters the interstitial fluid, **increasing** its **osmolarity** to 400 mOsm/L and the fluid in the **A**scending limb is **diluted** to 200 mOsm/L.
- **D**escending limb is **permeable to water**, water flows out of the Descending limb until its osmolarity increases to the level of the adjacent interstitial fluid. (Fluid in the Descending limb **equilibrates with the interstitial fluid**, and its osmolarity also becomes 400 mOsm/L.)
- **ADH** increases the activity of the Na+-K+-2Cl– cotransporter and therefore enhances the single effect

- Step 2: flow of fluids:

- The new fluid that enters the descending limb will have an osmolarity of 300 mOsm/L because it has come from the proximal tubule.
- At the same time, the high-osmolarity fluid in the descending limb (created by the single effect) is pushed down toward the bend of the loop of Henle

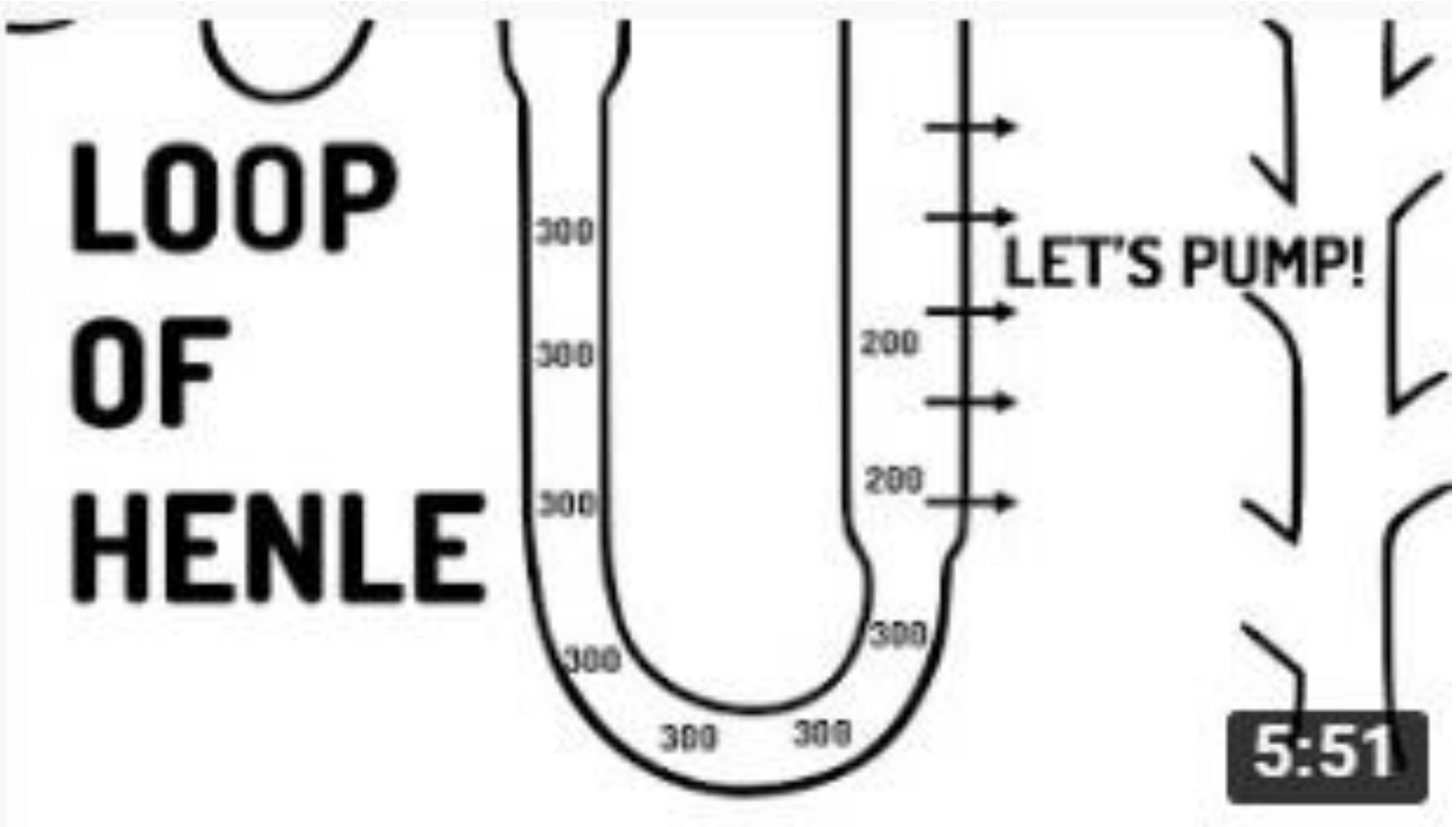
- Look to the next slide

- Both steps are repeated, forming Corticopapillary osmotic gradient
- The size of Corticopapillary osmotic gradient depends on the **length of the loop of Henle**



https://www.youtube.com/watch?v=cYyJF_aSC6o

"Loop of Henle explained!!"



Second:Urea recycling

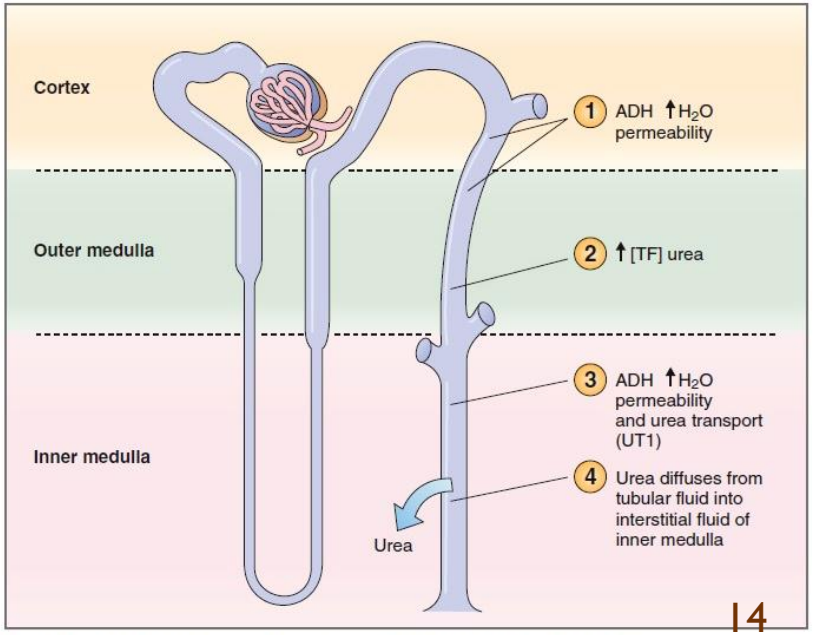
- Urea recycling from the inner medullary collecting ducts
- Steps from the Fig. :

1. In the **cortical and outer medullary collecting ducts,** ADH increases water permeability, but it **does not increase urea permeability**. As a result urea remains behind in the tubular fluid.
2. This causes the **urea** concentration **of tubular fluid** to **increase**.
3. In the **inner medullary collecting ducts,** ADH increases water permeability *and* it **increases the transporter** for facilitated diffusion of **urea**, **UT1**
4. a large concentration gradient has been created for urea in step 1.

urea diffuses down its concentration gradient into the interstitial fluid (due to step 2).

Urea that would have otherwise been excreted is recycled into the inner medulla, where it is added to the corticopapillary osmotic gradient.

* urea recycling also depends on **ADH**
* the corticopapillary osmotic gradient is larger when ADH levels are high



Mechanism of urea recycling from inner medullary collecting ducts.

Vasa recta

- The vasa recta are capillaries that serve the medulla and papilla of the kidney.
- The vasa recta participate in **countercurrent exchange (**we talked about countercurrent multiplication , the exchange is differ).
- Countercurrent **exchange** is a **purely passive process** that helps ***maintain the* gradient**. (countercurrent multiplication is an active process that *establishes* the Corticopapillary osmotic gradient.)
- They are freely permeable to small solutes and water. Blood flow through the vasa recta is slow, and solutes and water can move in and out, allowing for efficient countercurrent exchange
- Blood entering the **d**escending limb has an **osmolarity of 300 mOsm**/L. As this blood flows down the descending limb, it is exposed to interstitial fluid with **increasingly higher osmolarity** (the corticopapillary osmotic gradient). Because the vasa recta are capillaries, small solutes such as NaCl and urea **diffuse into** the **d**escending limb & **water diffuses out**, allowing blood in the to **equilibrate** osmotically with the surrounding interstitial fluid. At **the bend of the vasa recta**, the blood has an osmolarity equal to that of interstitial fluid at **the tip of the papilla**, 1200 mOsm/L.

- In the **a**scending limb, the **opposite events occur**. As blood flows up the **a**scending limb, it is exposed to interstitial fluid with **decreasing osmolarity**. **Small solutes diffuse ou**t of the **a**scending limb and **water diffuses in**, and the blood in the ascending limb of the vasa recta equilibrates with the surrounding interstitial fluid

- blood **leaving** the vasa recta has an osmolarity of **325 mOsm**/L, which is **slightly higher** than the osmolarity of the **original** blood that entered it.

- That's because Some of the **solute** from the corticopapillary osmotic gradient was **picked up** and will **be carried back** to the systemic circulation.

- With time, this process could **dissipate** the Corticopapillary osmotic gradient. The gradient **normally does not** dissipate, why?

because the mechanisms of countercurrent multiplication and urea recycling **continuously replace any solute** that is carried away by blood flow.

Antidiuretic hormone (ADH)

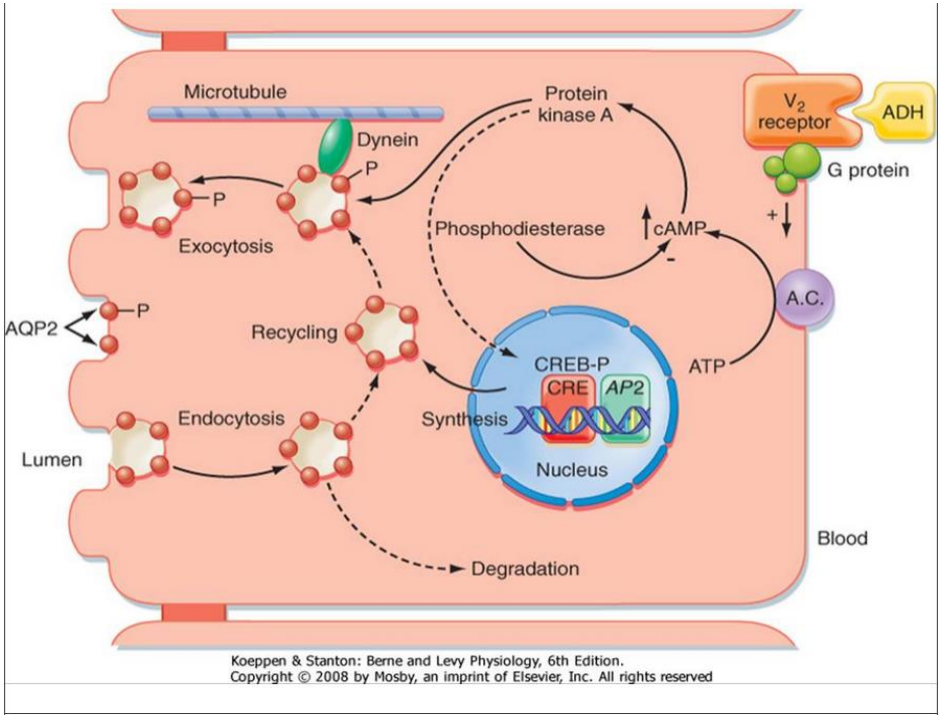
- ADH has three actions on the renal tubule:
  1. It **increases the water permeability** of the **principal cells** of the **late** distal tubule and **collecting** ducts.
  2. It **increases the activity of the Na^+-K^+-$2Cl^-$ cotransporter** of the **thick ascending** limb, thereby enhancing countercurrent **multiplication** and the **size** of the corticopapillary osmotic gradient.
  3. It **increases urea permeability** in the **inner medullary** collecting ducts, enhancing urea recycling and the size of the corticopapillary osmotic gradient.

- principal cells is the best known and physiologically is the most important.
- In the absence of ADH, the principal cells are impermeable to water.
- In the presence of ADH, **water channels,** or **aquaporins,** are inserted in the luminal membrane of the principal cells, making them permeable to water.

- Mecanism of the action of ADH:

1. When circulating levels of ADH are high, ADH is delivered to the principal cells via the peritubular capillary blood. **V_2 receptors** for ADH, present in the basolateral membrane, are coupled to **adenylyl cyclase** via a stimulatory G protein (G_s).
2. When ADH binds to the receptors, adenylyl cyclase is activated and catalyzes the conversion of ATP to **cAMP.**
3. cAMP activates **protein kinase A.** Activated protein kinase A then causes **phosphorylation** of intracellular structures. The identity of these structures is uncertain, although possibilities include microtubules and microfilaments, which are involved in intracellular shuttling mechanisms.
4. After the phosphorylation step, vesicles containing water channels are shuttled to and inserted into the luminal membrane of the principal cell, thus increasing its water permeability. The specific water channel that is controlled by ADH is **aquaporin 2 (AQP2).** Using freeze-fracture electron microscopy, the water channels in the luminal membrane can be visualized in clusters called **intramembranous particles.**

- The Fig. is taken from berne & levy ,page 628 (at cellular level)& 629
- ADH activated V2 receptors which are G protein coupled receptors activates adenyl cyclase forms cyclic amp
- activates protein kinase which changes transcription factors. Then synthesis of new aquaporin 2
- aquaporin 2 may be already transcribed and ready to go in vesicles. So then, go to cell membrane and are released by exocytosis > they are expressed on the cell membrane
- ADH either causes aquaporin transcription or helps with already existing aquaporins exocytosis and insertion in lumenal cell membrane.



A.C., adenylyl cyclase; *AP2*, aquaporin-2 gene; AQP2, aquaporin-2; CRE, cAMP response element; CREB-P, phosphorylated cAMP response element-binding protein; -P, phosphorylated proteins.

- **VERY IMPORTANT QUESTION IN EXAM!:**
- In the thick ascending limb, ADH does not influence water reabsorption. **Why**?
- Due to water impermeability. ADH does NOT insert aquaporin. It only affects NKCC2 (**Na^+-K^+-$2Cl^-$ cotransporter)**. That's why water leaves **passively** in descending limb. **It cannot leave the ascending limb.**

Production of concentrated urine

- Is also called **hyperosmotic urine,** in which urine osmolarity's h**igher than** blood osmolarity.
- Is produced when circulating ADH levels are high (e.g., **water deprivation, volume depletion, SIADH).**
- Before the mechanism is described, Note that:

- the initial glomerular filtrate has the same osmolarity as the blood, 300 mOsm/L
- The heavily outlined portion of the thick ascending limb and early distal tubule indicates that these segments are impermeable to water

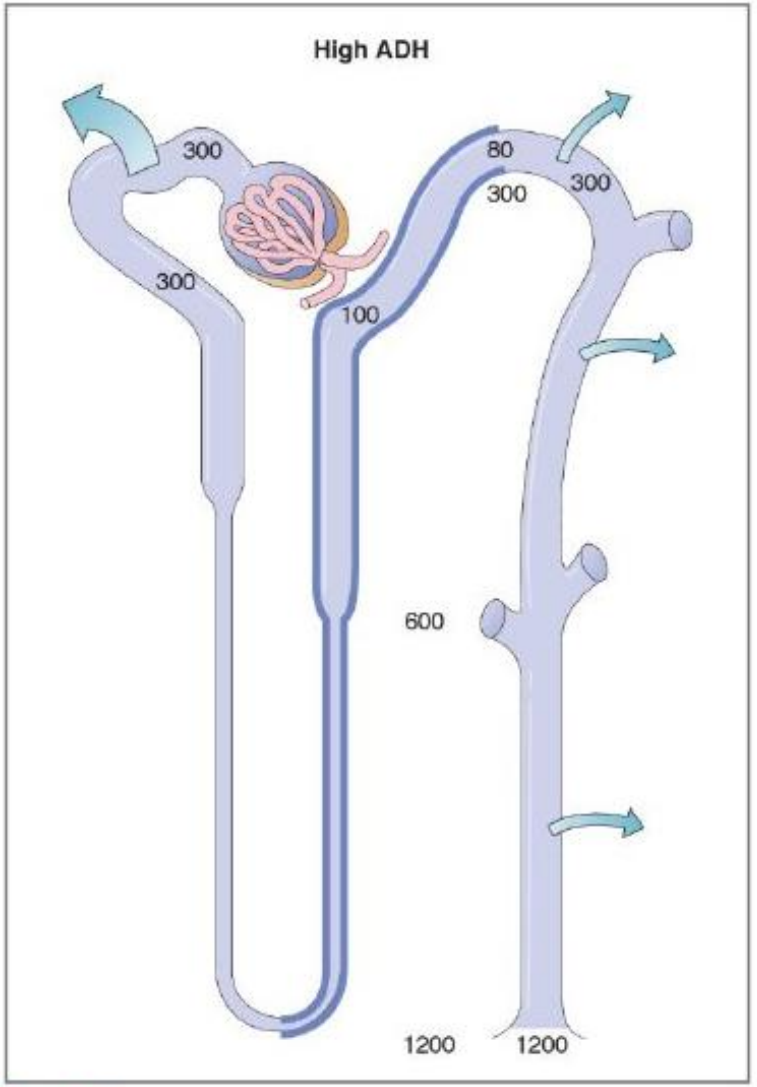


Fig. 6.42 Mechanisms for production of hyperosmotic (concentrated) urine in the presence of antidiuretic hormone *(ADH)*. *Arrows* show location of water reabsorption; *heavy outline* shows water-impermeable portions of the nephron; numbers are osmolarity of tubular fluid or interstitial fluid.

Proximal tubule – high ADH

- The osmolarity of the glomerular filtrate is identical to that of plasma (300 mOsm/L)…because water and small solutes are freely filtered
- Two-thirds of the filtered H2O is reabsorbed **isosmotically (with Na^+, Cl^-, $HCO3^-$, glucose,** amino acids, and so forth) in the proximal tubule…means:

- The **osmolarity remains at 300 mOsm/**L along the entire proximal convoluted tubule, even though a significant volume of water is reabsorbed

- **TF/P_{osm} = 1.0** throughout the proximal tubule because H2O is reabsorbed isosmotically with solute.

Thick ascending limb of the loop of Henle —high ADH

- Reabsorbs NaCl by the **Na^+–K^+–$2Cl^-$ cotransporter.**
- It's **impermeable to H_2O.** Therefore, H_2O is not reabsorbed with NaCl, and the **tubular fluid becomes dilute**.(Is called the **diluting segment).**
- The fluid that leaves the thick ascending limb has an osmolarity of **100 mOsm/**L and **TF/P_{osm} < 1.0** as a result of the dilution process.

Early distal tubule - ADH

- Is called the **cortical diluting segment. (**cortical because the distal tubule is located in the cortex)
- Like the thick ascending limb, the early distal tubule reabsorbs NaCl but is **impermeable to water.** Consequently, tubular fluid **is further diluted, as low as 80 mOsm/L.**

Late distal tubule – high ADH

- **ADH increases the H_2O permeability of the principal cells** of the late distal tubule.
- H_2O is reabsorbed from the tubule until the osmolarity of distal tubular fluid **equals that of the surrounding interstitial** fluid in the renal cortex (300 mOsm/L).
- **TF/P_{osm} = 1.0** at the end of the distal tubule because osmotic **equilibration occurs in the presence of ADH**.

Collecting ducts - high ADH

- Is the same for the late distal, As tubular fluid flows down the collecting ducts, it is exposed to interstitial fluid with **increasingly higher osmolarity (i**.e., the corticopapillary osmotic gradient**).**
- H_2O is reabsorbed from the collecting ducts until the osmolarity of tubular fluid equals that of the surrounding interstitial fluid.
- The osmolarity of the final urine **equals** that at **the bend** of the loop of Henle and the **tip of the papilla (1200 mOsm/L).**
- **TF/P_{osm} > 1.0** because osmotic equilibration occurs with the corticopapillary gradient in the presence of ADH.

SIADH: syndrome of inappropriate ADH:

- hyperosmotic urine is produced *inappropriately*
- In SIADH, ADH is secreted **autonomously (**without an osmotic stimulus),abnormally high owing to either excessive secretion from the posterior pituitary following head injury or secretion of ADH from abnormal sites such as **lung tumors.**
- high levels of ADH increase water reabsorption by the late distal tubule and collecting ducts, making the urine hyperosmotic and diluting the plasma osmolarity
- Treated by **demeclocycline,** which inhibits the ADH action

Production of dilute urine

- Is called **hyposmotic urine,** in which urine osmolarity < blood osmolarity.
- Is produced when circulating levels of ADH are low (e.g., **water intake, central diabetes insipidus)** or when ADH is ineffective **(nephrogenic diabetes insipidus).**
- Corticopapillary osmotic gradient Is **smaller** than in the presence of ADH because ADH stimulates both countercurrent multiplication and urea recycling

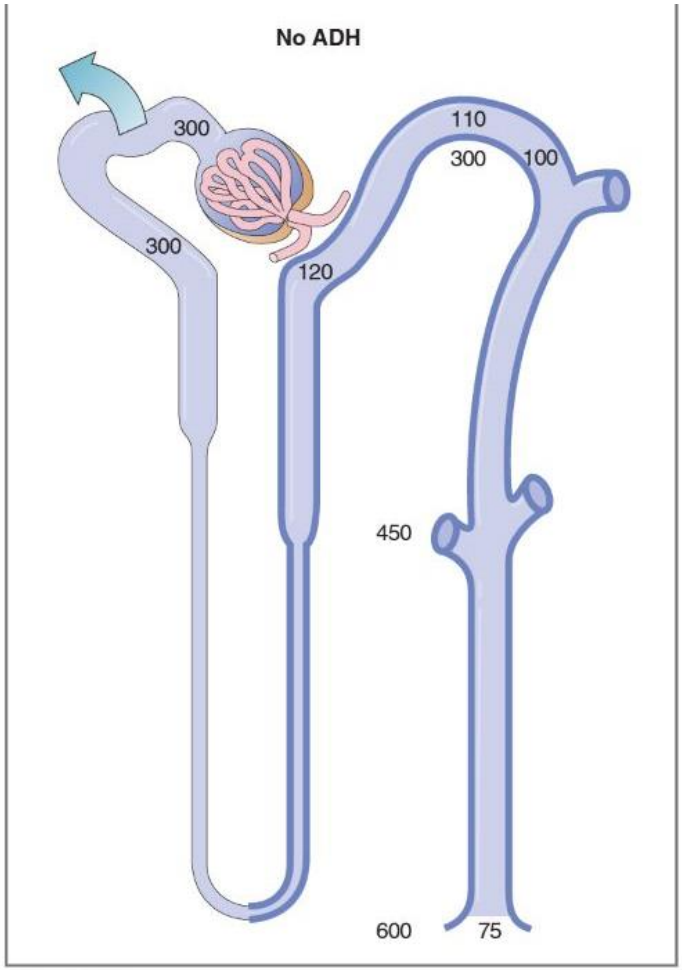


Fig. 6.43 Mechanisms for production of hyposmotic (dilute) urine in the absence of antidiuretic hormone *(ADH).* *Arrow* shows location of water reabsorption; *heavy outline* shows water-impermeable portions of the nephron; numbers are osmolarity of tubular fluid or interstitial fluid.

Proximal tubule—no ADH

- As in the presence of ADH(not affected by ADH), two-thirds of the filtered water is reabsorbed **isosmotically.**
- **$TF/P_{osm} = 1.0$** throughout the proximal tubule.

Thick ascending limb of the loop of Henle -no ADH

- As in the presence of ADH, NaCl is reabsorbed without water, and the tubular fluid becomes dilute (although not quite as dilute as in the presence of ADH).
- **$TF/P_{osm} < 1.0$.**

Early distal tubule—no ADH

- As in the presence of ADH, NaCl is reabsorbed without H_2O and the tubular fluid is further diluted.
- **$TF/P_{osm} < 1.0$.**

Late distal tubules and collecting ducts – no ADH

- In the absence of ADH, the cells of the late distal tubule and collecting ducts are **impermeable to H_2O.**
- Thus, even though the tubular fluid flows through the corticopapillary osmotic gradient, osmotic equilibration does not occur.
- The osmolarity of the final urine will be dilute with an osmolarity as low as 50 mOsm/L.
- **$TF/P_{osm} < 1.0$.**

- What is the difference between central and nephrogenic diabetes?

Central: hypothalamic issue = secretion of ADH is limited

Nephrogenic: kidney issue = ADH level is normal but the receptors in the kidney are abnormal

- Please for more details read the book page 307

Free-water clearance (C_{H2O})

- Is used to **estimate the ability to concentrate or dilute the urine.**
- Free water, or solute-free water, is produced in the diluting segments of the kidney (i.e., thick ascending limb and early distal tubule), where NaCl is reabsorbed and free water is left behind in the tubular fluid.
- In the **absence of ADH,** this solute-free water is excreted and C_{H2O} is positive.
- In the presence of ADH, this solute-free water is not excreted but is reabsorbed by the late distal tubule and collecting ducts and **C_{H2O} is negative.**

Calculation of C_{H2O}

$$C_{H_2O} = \dot{V} - C_{osm}$$

$$= \dot{V} - \frac{[U]_{osm} \times \dot{V}}{[P]_{osm}}$$

where

C_{H_2O} = Free-water clearance (mL/min)

$\dot{V}$ = Urine flow rate (mL/min)

C_{osm} = Clearance of osmoles (mL/min)

$[U]_{osm}$ = Urine osmolarity (mOsm/L)

$[P]_{osm}$ = Plasma osmolarity (mOsm/L)

1. Urine that is isosmotic to plasma (isosthenuric)
   - **C_{H2O} is zero.**
   - is produced during treatment with a loop diuretics, for example.
2. Urine that is hyposmotic to plasma (low ADH)
   - **C_{H2O} is positive.**
   - is produced with high water intake, central diabetes insipidus, or nephrogenic diabetes insipidus.
3. Urine that is hyperosmotic to plasma (high ADH)
   - **C_{H2O} is negative.**
   - is produced in water deprivation or SIADH.

DESCRIPTION OF CASE. A 45-year-old woman is admitted to the hospital following a head injury. She has severe polyuria (producing 1 L of urine every 2 hours) and polydipsia (drinking 3 to 4 glasses of water every hour). During a 24-hour period in the hospital, the woman produces 10 L of urine, containing no glucose. She is placed on overnight water restriction for further evaluation. The following morning, she is weak and confused. Her serum osmolarity is 330 mOsm/L, her serum [Na^+] is 164 mEq/L, and her urine osmolarity is 70 mOsm/L. She is treated with dDAVP by nasal spray. Within 24 hours of initiating the treatment, her serum osmolarity is 295 mOsm/L and her urine osmolarity is 620 mOsm/L.

TREATMENT. The woman is treated with dDAVP, an ADH analogue that activates V2 receptors on the principal cells. When ADH binds to the V2 receptors, adenylyl cyclase is activated, cAMP is generated, and water channels are inserted in the luminal membrane, which restores water permeability of the principal cells. After initiating dDAVP therapy, the woman produces hyperosmotic urine, restoring her serum osmolarity to normal.