

Urine Concentration and Dilution; Regulation of Extracellular Fluid Osmolarity and Sodium Concentration

Total Renal Excretion and Excretion Per Nephron in Renal Failure

Normal

75 % loss of
nephrons

Number of nephrons	2,000,000	500,000
Total GFR (ml/min)	125	40
GFR per nephron (nl/min)	62.5	80
Total Urine flow rate (ml/min)	1.5	1.5
Volume excreted per nephron (nl/min)	0.75	3.0

KFT...cont.

One of the most important KFT is to look if the kidney can concentrate urine....this is tubular function...this is not a glomerular test.

- Specific gravity of the urine can tell us if the kidney can concentrate urine or not.
- *High specific gravity means concentrated urine*
- *Low specific gravity means diluted urine*
- This is the last function to retain to normal following acute renal failure

- For scientists, to make diluted urine was somehow easy to understand...the kidneys needs just to actively transport Na^+ and Cl^- out of the collecting duct, leaving water inside and thus making diluted urine = hypoosmolar urine.
- To make concentrated urine was an enigma for 100 years, until the micropuncture technique solved the problem. The reason for that is active water transport is not known.

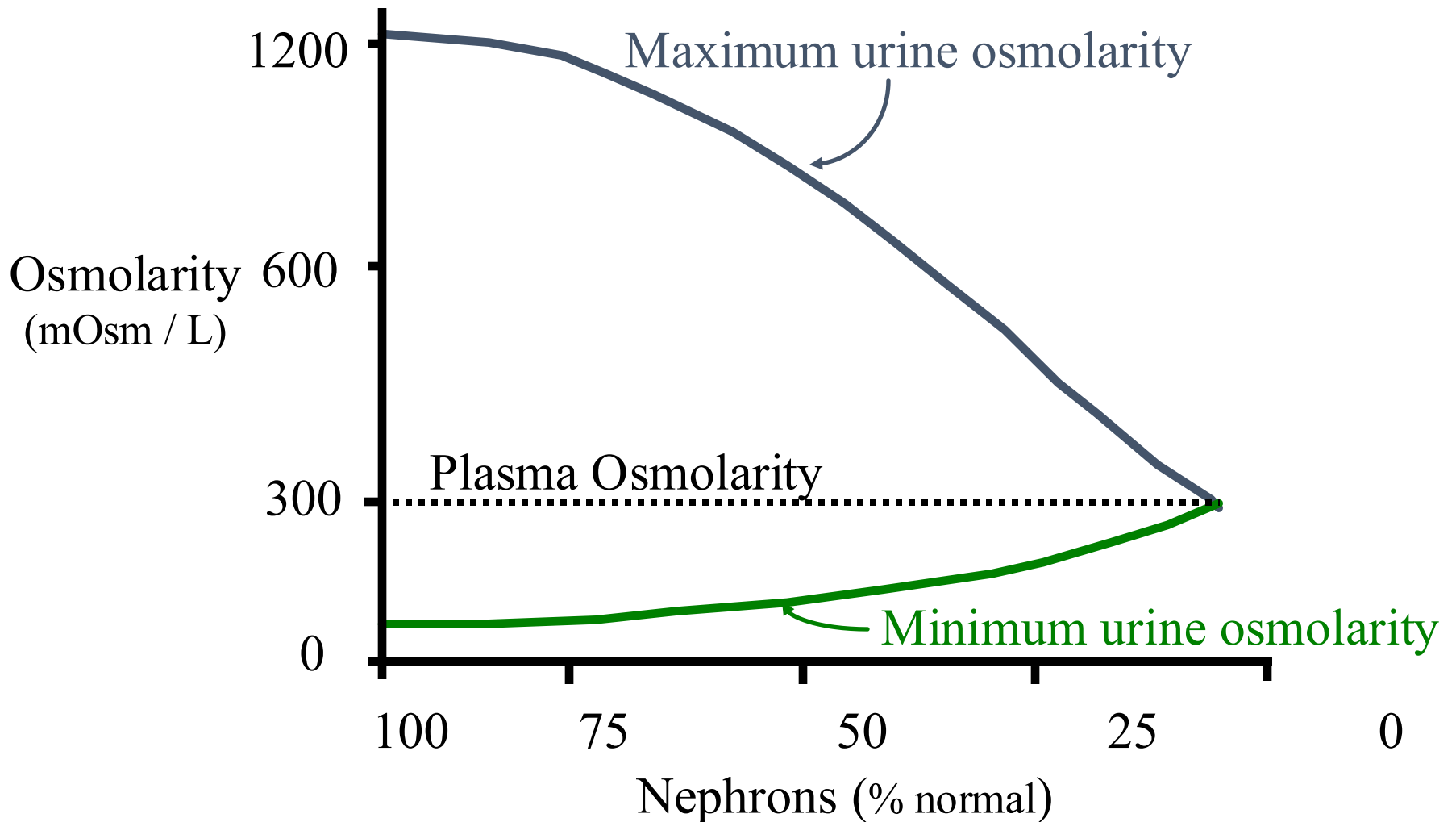
Section 4. Urine Concentration and Dilution

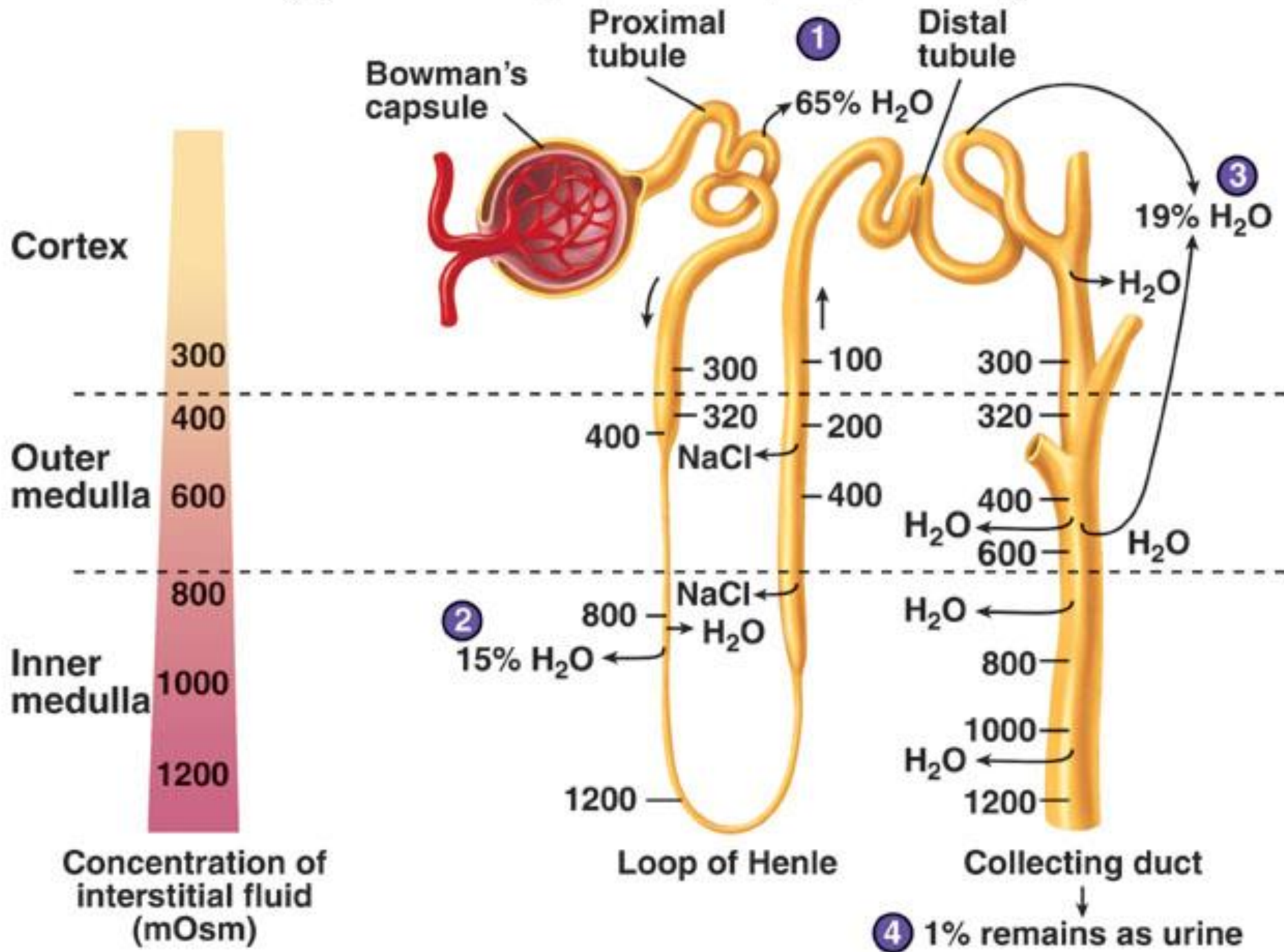
Importance:

When there is excess water in the body and body fluid osmolarity is reduced, the kidney can excrete urine with an osmolarity as low as 30-50 mOsm/liter, a concentration that is only about one sixth the osmolarity of normal extracellular fluid....removing extra ingested water without losing solutes

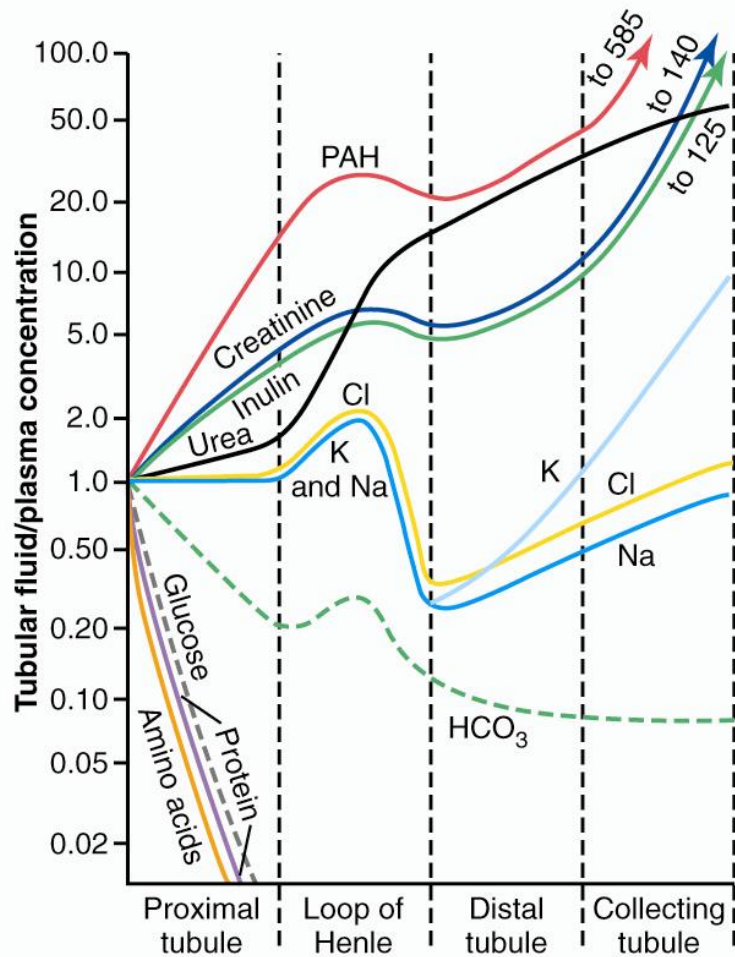
Conversely, when there is a deficient of water and extracellular fluids osmolarity is high, the kidney can excrete urine with a concentration of about 1200 to 1400 mOsm/liter. By doing so we can remove all waste products and at the same time conserve water.

Development of Isosthenuria With Nephron Loss in Chronic Renal Failure (inability to concentrate or dilute the urine)





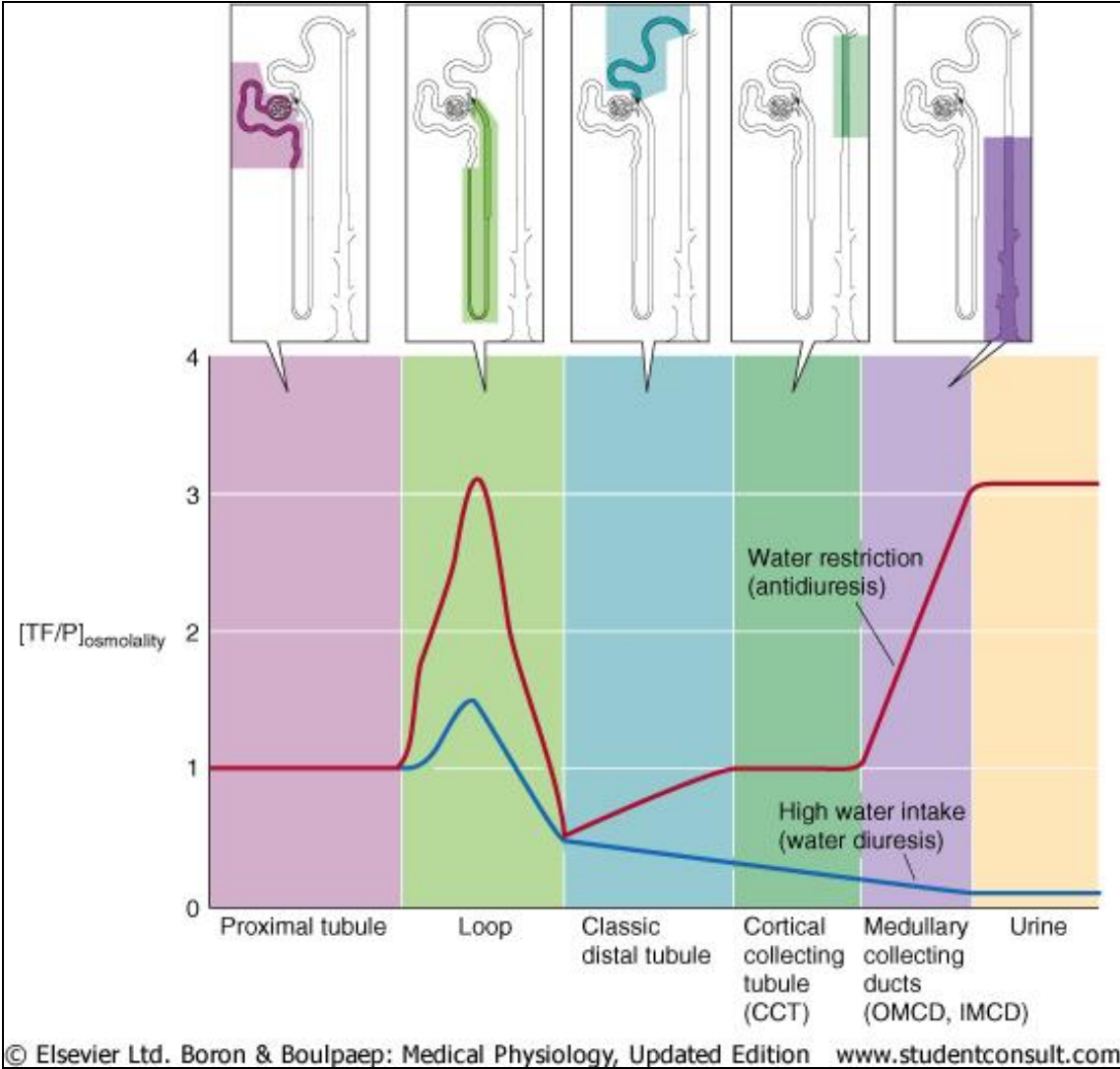
Concentration of Different Substances in Tubular System compared to Plasma Concentration



- If water is reabsorbed to a greater extent than the solute, the solute will become more concentrated in the tubule (e.g., creatinine).
- 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).

Osmolality of fluid along nephron

- Red = water restriction
- Blue = high water intake
- Initial concentration of tubular fluid at loop of Henle, then finally at collecting ducts.



Changes in osmolarity of the tubular fluid

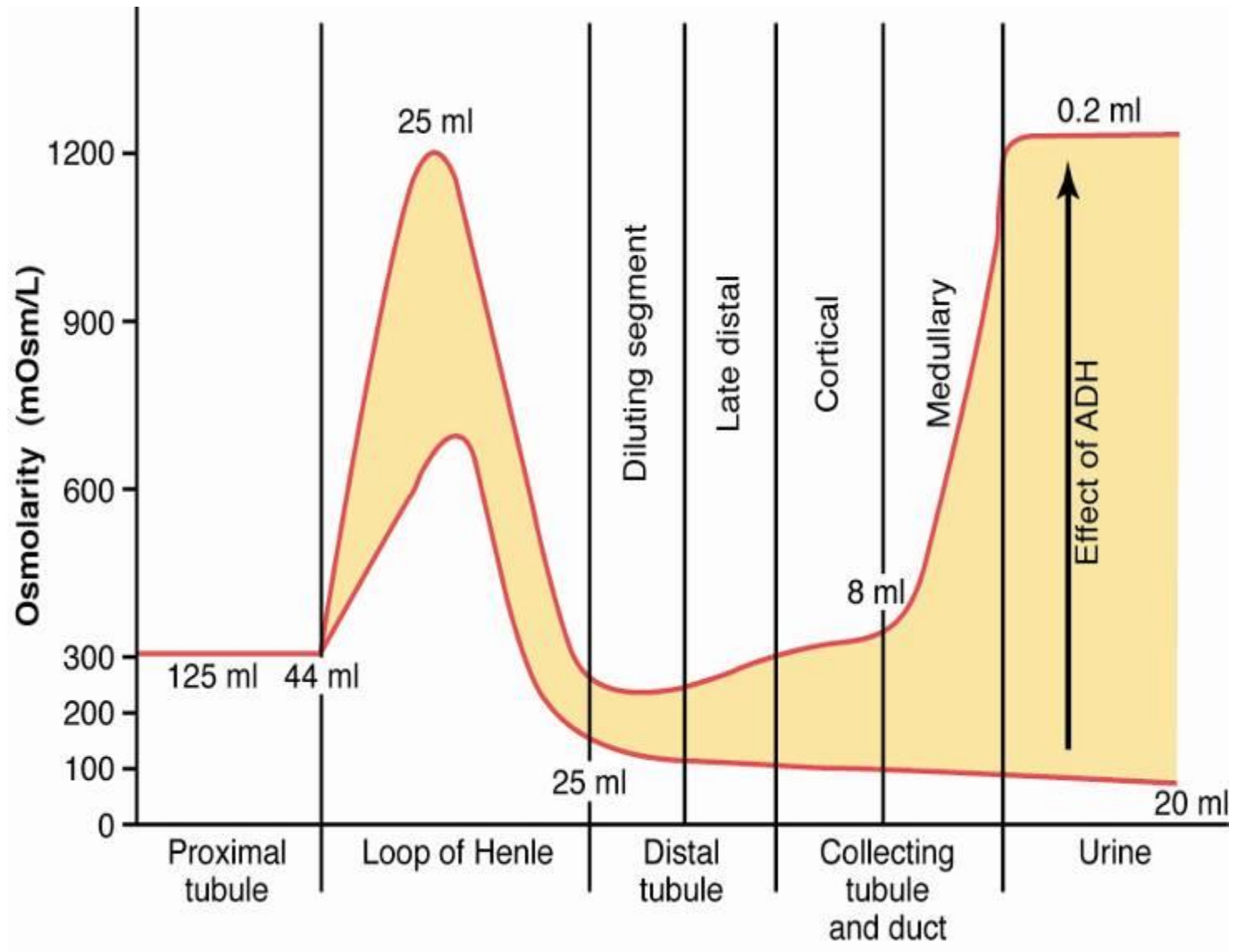
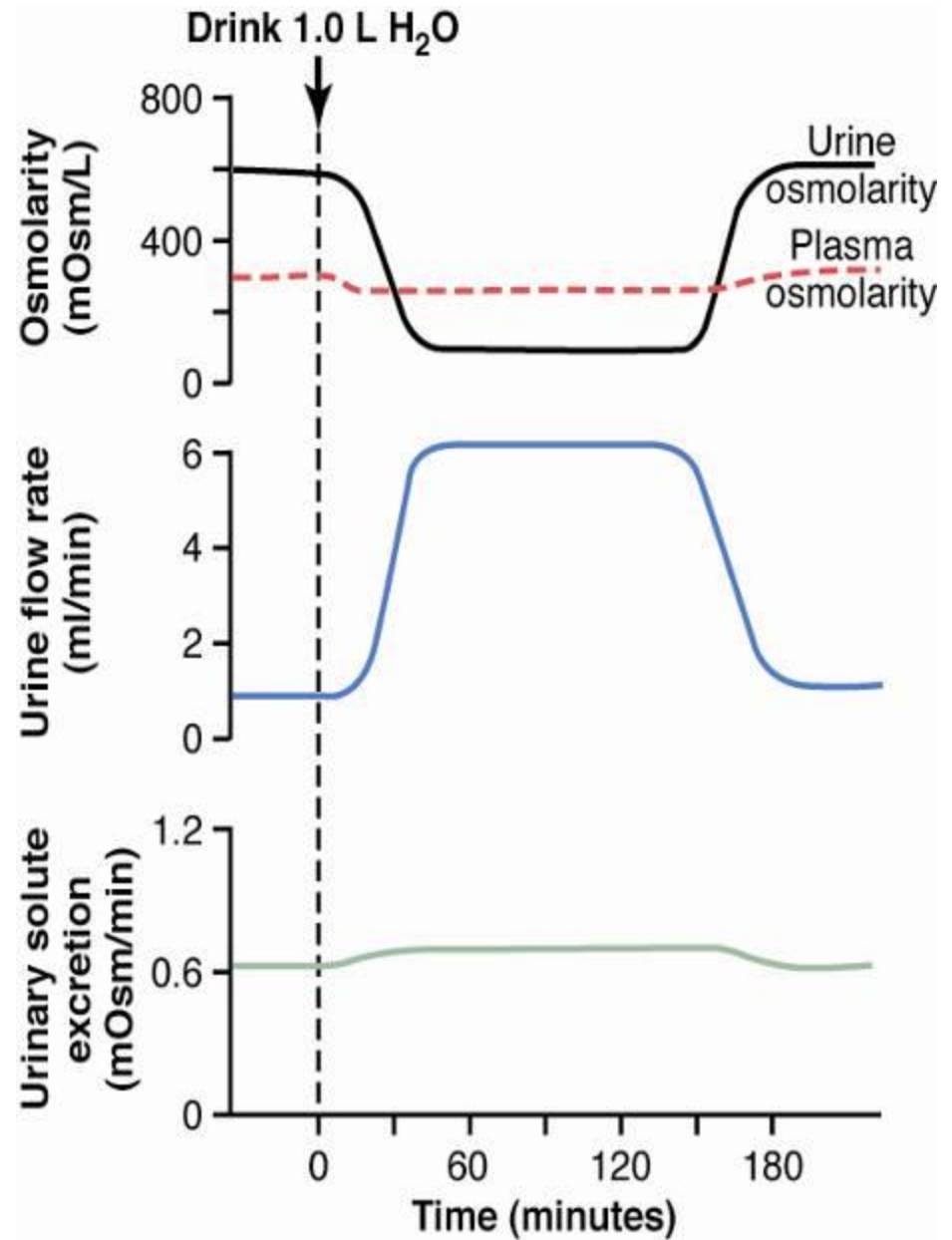


Figure 28-8

Water diuresis
in a human
after ingestion
of 1 liter of
water.



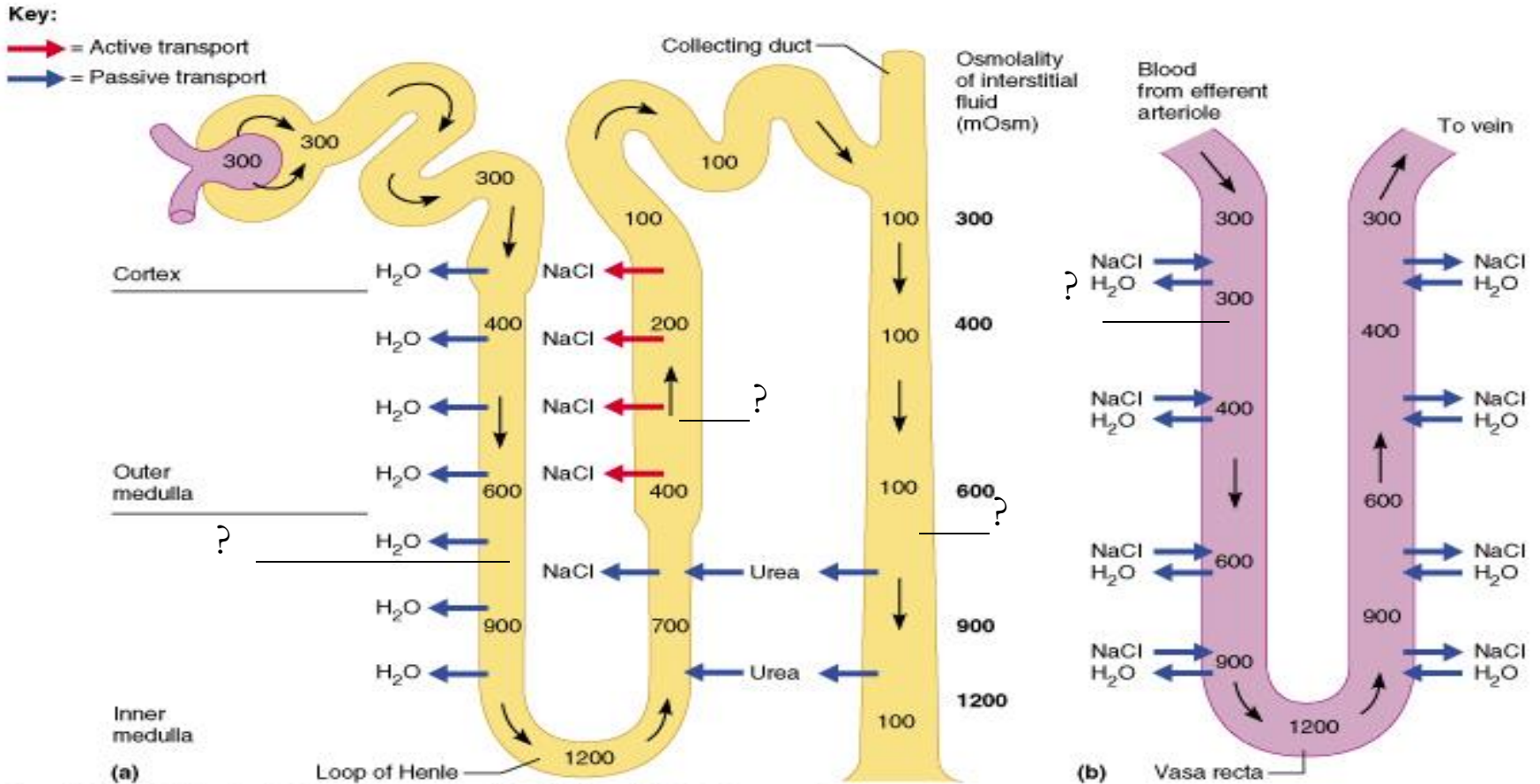
The counter current mechanism through which the kidneys excrete a concentrated urine as a collaborative work of the followings: sodium chloride and urea cycle, posterior pituitary, ADH, hypothalamus, collecting duct, osmosis, interstitial fluid, vasa recta, diffusion, and loop of Henle

Concentration and Dilution of the Urine

- Maximal urine concentration
= 1200 - 1400 mOsm / L
(specific gravity ~ 1.030)
- Minimal urine concentration
= 30-50 mOsm / L
(specific gravity ~ 1.003)

The Counter Current Mechanism

Compare to the Nephron and recall parts



Summary of Tubule Characteristics

Tubule Segment	Active NaCl Transport	Permeability		
		H ₂ O	NaCl	Urea
Proximal	++	+++	+	+
Thin Desc.	0	+++	+	+
Thin Ascen.	0	0	+	+
Thick Ascen.	+++	0	0	0
Distal	+	+ADH	0	0
Cortical Coll.	+	+ADH	0	0
Inner Medullary Coll.	+	+ADH	0	+++

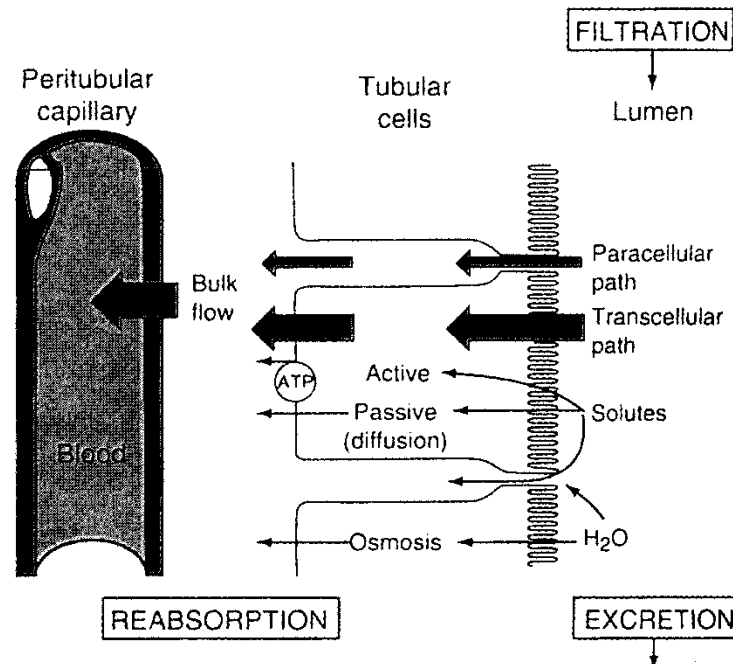
Summary of water reabsorption and osmolarity in different parts of the tubule

- Proximal Tubule: 65 % reabsorption, isosmotic
- Desc. loop: 15 % reabsorption, osmolarity increases
- Asc. loop: 0 % reabsorption, osmolarity decreases
- Early distal: 0 % reabsorption, osmolarity decreases
- Late distal and coll. tubules: ADH dependent water reabsorption and tubular osmolarity
- Medullary coll. ducts: ADH dependent water reabsorption and tubular osmolarity

Water reabsorption - 1

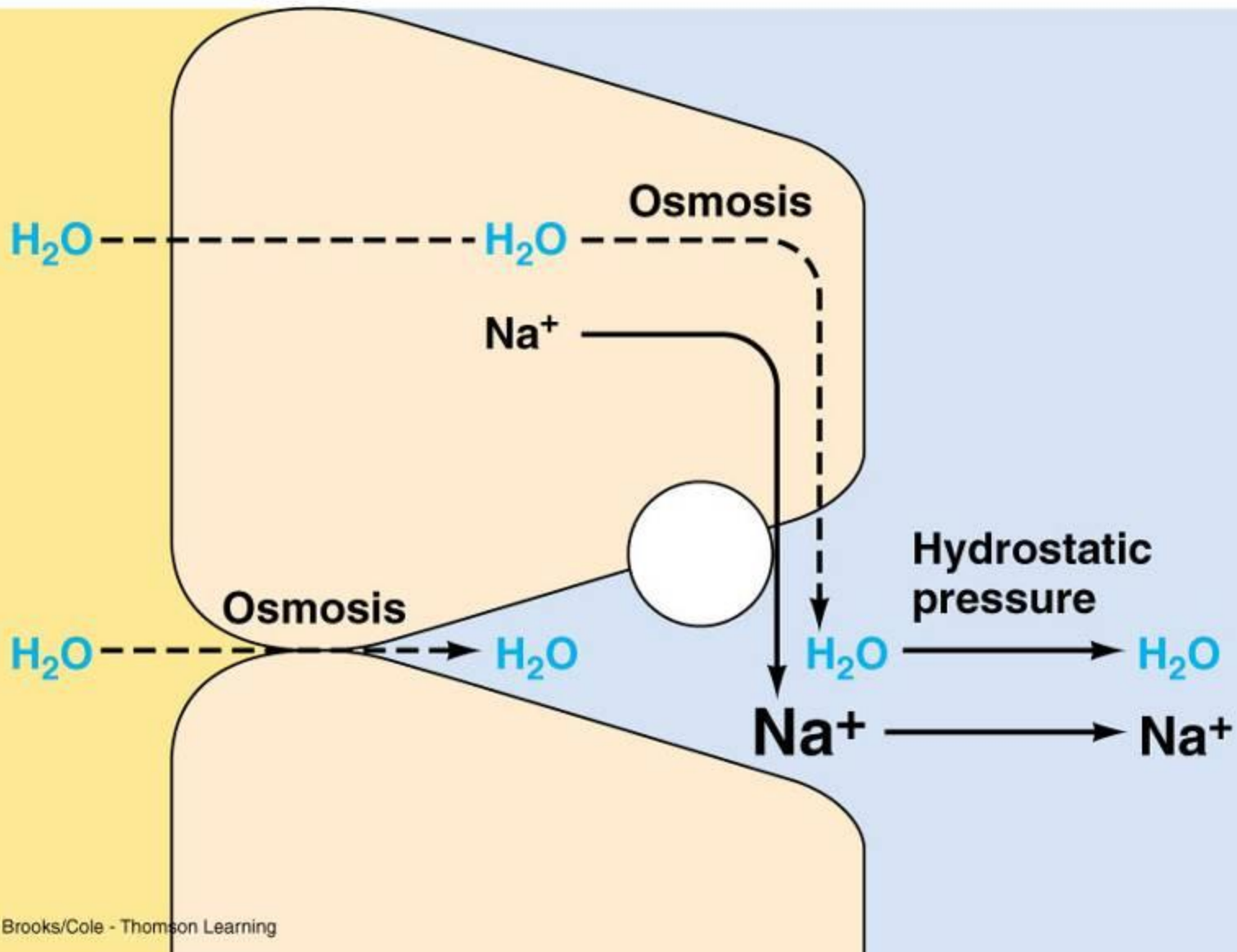
Obligatory water reabsorption:

- Using sodium and other solutes.
- Water follows solute to the interstitial fluid (transcellular and paracellular pathway).
- Largely influenced by sodium reabsorption



Obligatory water reabsorption

Lumen **Proximal tubular cell** **Interstitial fluid**

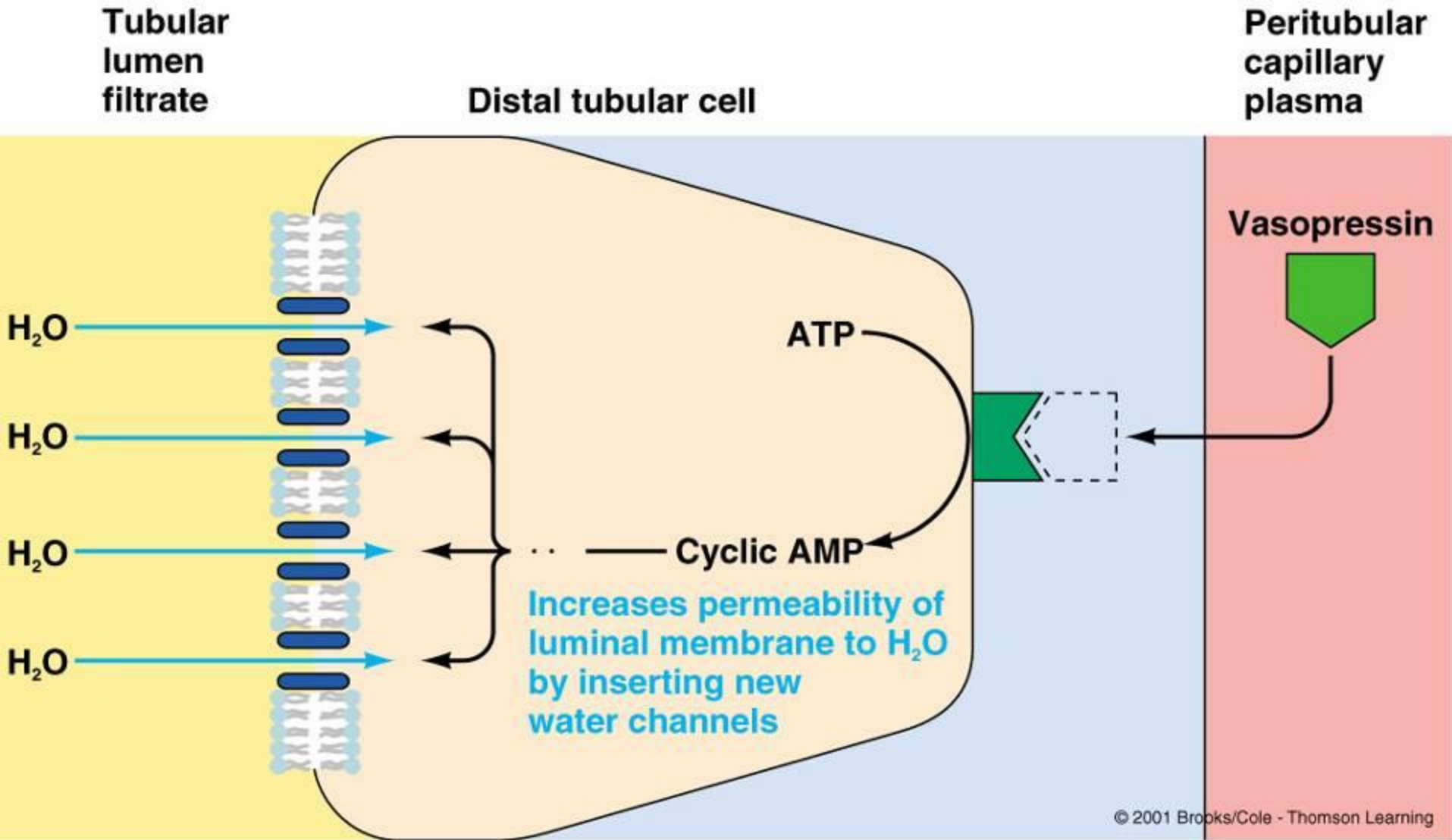


Water reabsorption - 2

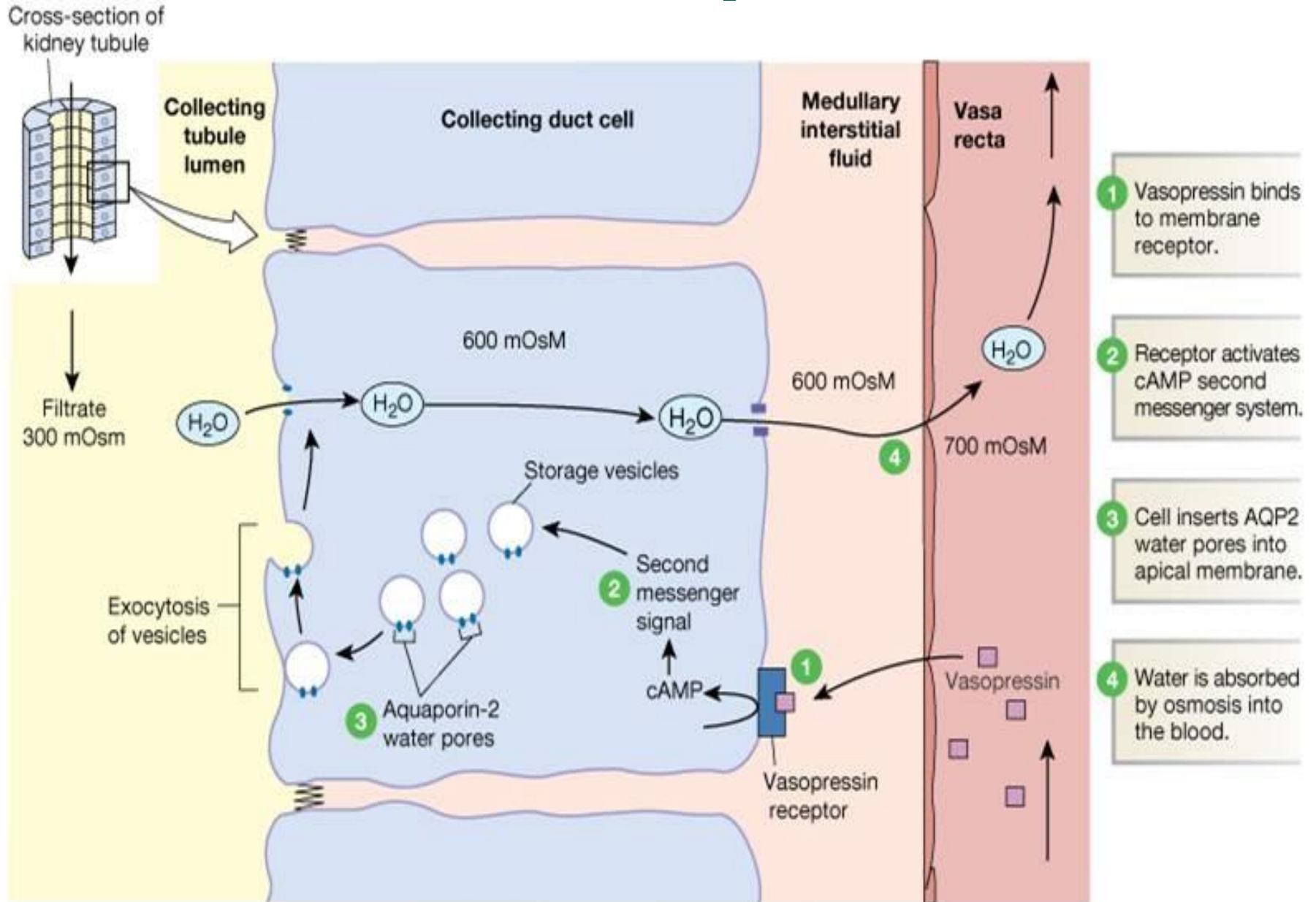
Facultative (selective) water reabsorption:

- Occurs mostly in collecting ducts
- Through the water channels
- Regulated by the ADH

Facultative water reabsorption



Formation of Water Pores: Mechanism of Vasopressin Action



Countercurrent Multiplication Mechanism:

Countercurrent: Ascending and descending currents.

Multiplication : Multiplies osmolarity of interstitium.

This mechanism is related to tubules contributed by:

1. **Single effect:** $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ transporter
Only absorbs Na^+ , K^+ , Cl^- without H_2O
2. Urea recycling
3. Slow flow of blood in Vasa Recta.

Countercurrent exchange system:

Vasa recta with its exchange activities, related to blood vessels not tubules.

So, with hypertension, vasodilatation, loop diuretics, low protein diet → Interstitium will lose its maximum hyperosmolarity,
→ *urine will be less concentrated.*

The Counter Current Mechanism

We will start with the ascending limb of the loop of Henle (ALLH). This portion of the nephron reabsorbs NaCl into the interstitium of the medulla. The medulla then becomes very hyperosmotic. 700 mOs out of the 1200 mOsm is due to NaCl and the rest is due to urea.

Countercurrent Multiplier

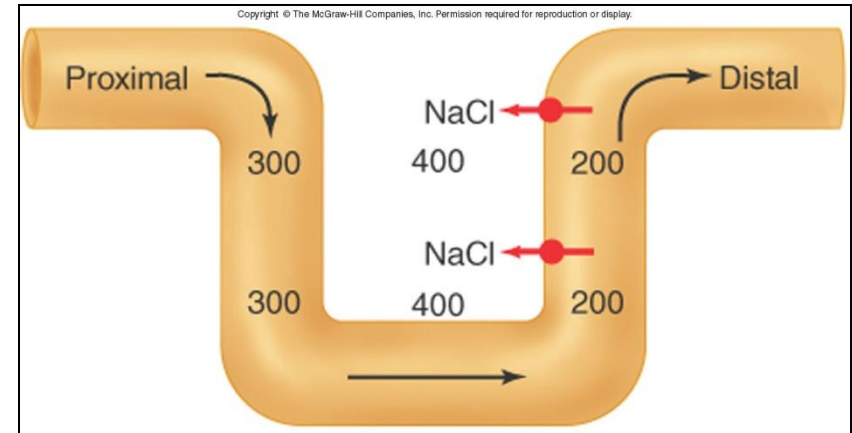
- Countercurrent is easy, fluid flows **down** the descending limb and **up** the ascending limb.

- The **critical** characteristics of the loops which make them countercurrent **multipliers** are:

- 1. The ascending limb of the loop of Henle **actively** co-transport Na^+ and Cl^- ions **out of the tubule lumen** into the interstitium. The ascending limb is **impermeable** to H_2O .

- 2. The descending limb is **freely permeable** to H_2O but relatively **impermeable** to NaCl .

H_2O that moves out of tubule into interstitium is removed by blood vessels called vasa recta – thus gradients maintained and H_2O returned to circulation.



Net Effects of Countercurrent Multiplier

1. More solute than water is added to the renal medulla.
i.e solutes are “trapped” in the renal medulla
2. Fluid in the ascending loop is diluted
3. Most of the water reabsorption occurs in the cortex
(i.e. in the proximal tubule and in the distal convoluted tubule) rather than in the medulla
4. Horizontal gradient of solute concentration established by the active pumping of NaCl is “multiplied” by countercurrent flow of fluid.

The Counter Current Mechanism

As salt (NaCl) leaves the ascending limb of the loop of Henle ALLH, the osmolarity of the Tubular Fluid decreases from 1,200 to 100 milliosmole/l. This happens because the ALLH is impermeable to water. The net effect of this activity is to remove salt from the kidney filtrate and transfer it into the medulla where it can be saved for use by the body.

The Counter Current Mechanism

The accumulated salt in the interstitium of the medulla acts as an osmotic force which can be used to “draw” and conserve water from other parts of the nephron: the descending limb of the Loop of Henle (DLLH) and the collecting duct. The DLLH is a thin passive segment that is permeable to water, but, impermeable to salt.

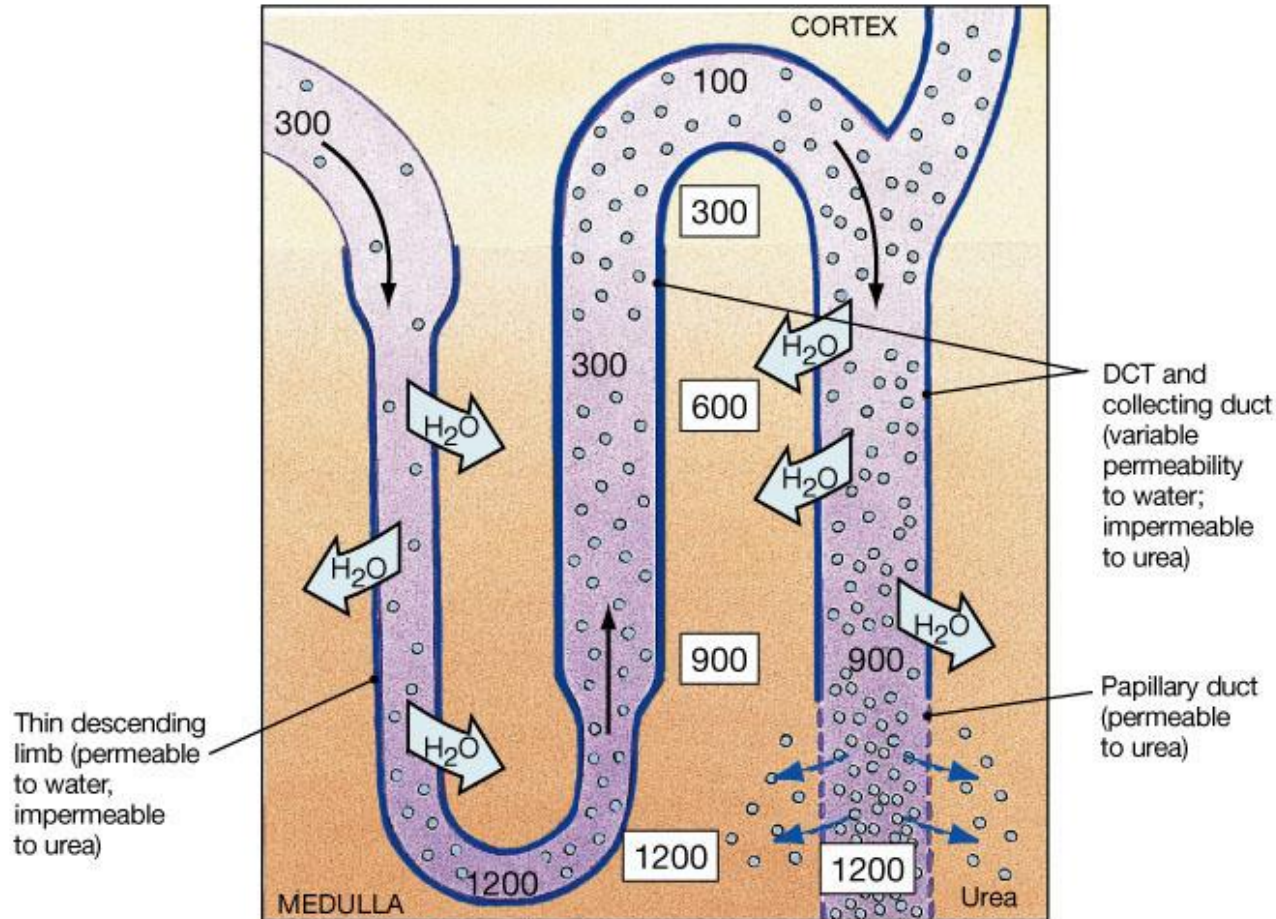
The Counter Current Mechanism

As the DCT gives up water to the medullary interstitium, the osmolarity of the fluid changes from 300 to 1,200 mOsm/L. The net effect of this process is to conserve water for the body. Thus, the loop of Henle actively transfers salt back into the kidney which can be used to save water osmotically. A remarkable process!

The Counter Current Mechanism

The hyperosmotic interstitium of the medulla will also “pull” and conserve water from the collecting duct, but, on a variable basis depending on the availability of ADH. As water moves from the collecting duct, urea will follow. Thus, as water is conserved at this level, a certain amount of urea is also conserved. The urea contributes to the high osmolarity of the medulla

Countercurrent Multiplication and Concentration of Urine



(c) The permeability characteristics of both the loop and the collecting duct tend to concentrate urea in the tubular fluid and in the medulla. The loop of Henle, DCT, and collecting duct are impermeable to urea. As water reabsorption occurs, the urea concentration rises. The papillary ducts' permeability to urea accounts for roughly one-third of the solutes in the deepest portions of the medulla.

The Counter Current Mechanism

The availability of Antidiurectic Hormone (ADH) is determined by dehydration and thirst. Under these conditions, the **hypothalamus makes** extra **ADH** and **stores it** in the **posterior pituitary** where it can be released. The increased release of ADH causes the “water pores” of the collecting duct to open and allow water to move from the urine to the medulla.

ADH is produced mainly by the supraoptic nuclei (85%), and to a lesser extent by paraventricular nuclei (15%).

Osmoreceptors will send impulses to posterior pituitary to secrete ADH.

In the kidney, ADH binds to its receptors on basolateral membrane, introducing H₂O pores at apical site (AQP2).

AQP 3 and AQP 4 don't respond to ADH

The Role of ADH

- There is a high osmolarity of the renal medullary interstitial fluid, which provides the osmotic gradient necessary for water reabsorption to occur.
- Whether the water actually leaves the collecting duct (by osmosis) is determined by the hormone ADH (anti-diuretic hormone)
- Osmoreceptors in the hypothalamus detect the low levels of water (high osmolarity), so the hypothalamus sends an impulse to the pituitary gland which releases ADH into the bloodstream.
- ADH makes the wall of the collecting duct more permeable to water.
- Therefore, when ADH is present more water is reabsorbed and less is excreted.

Figure 26.15 The Effects of ADH on the DCT and Collecting Ducts

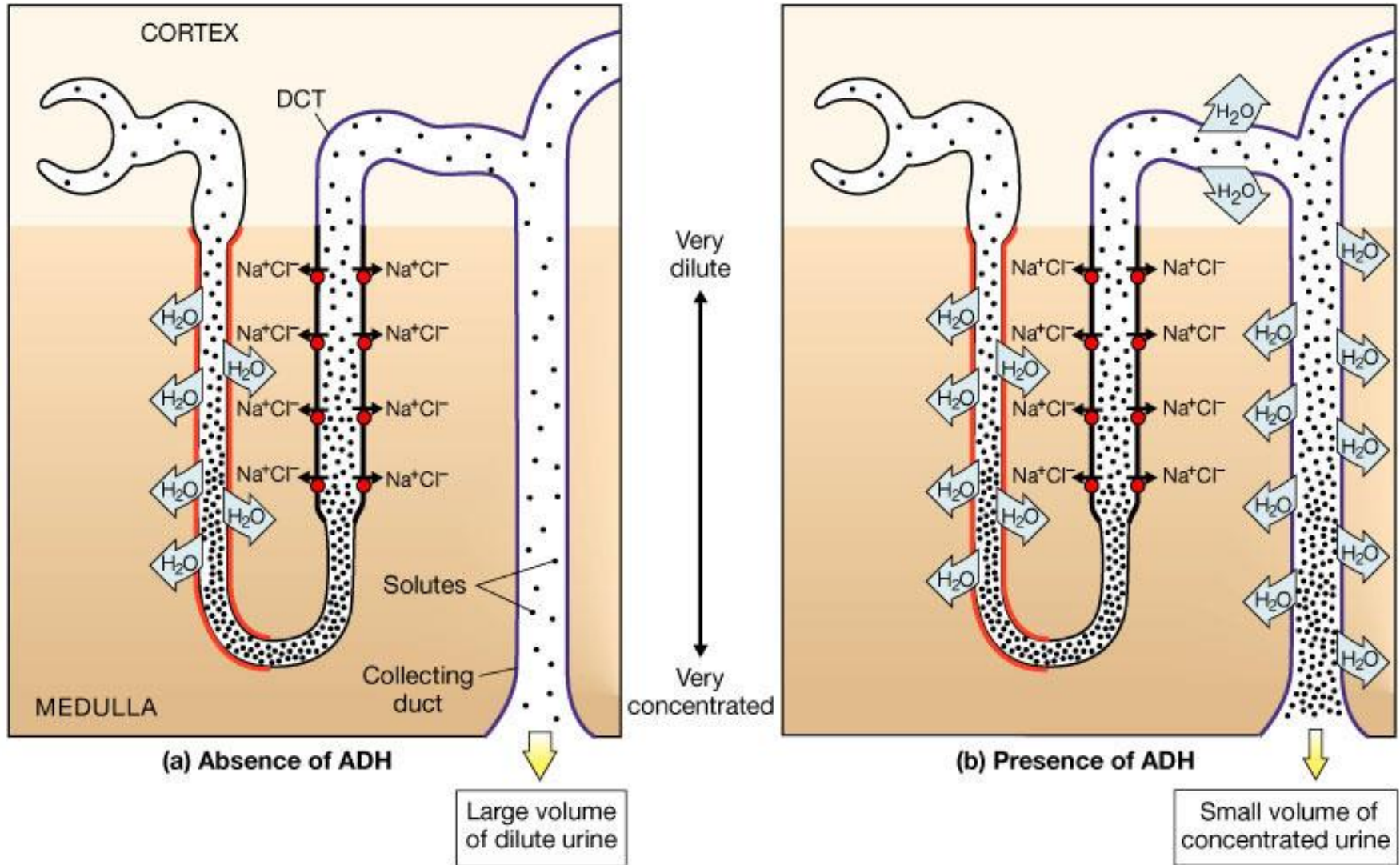


Figure 26.15a, b

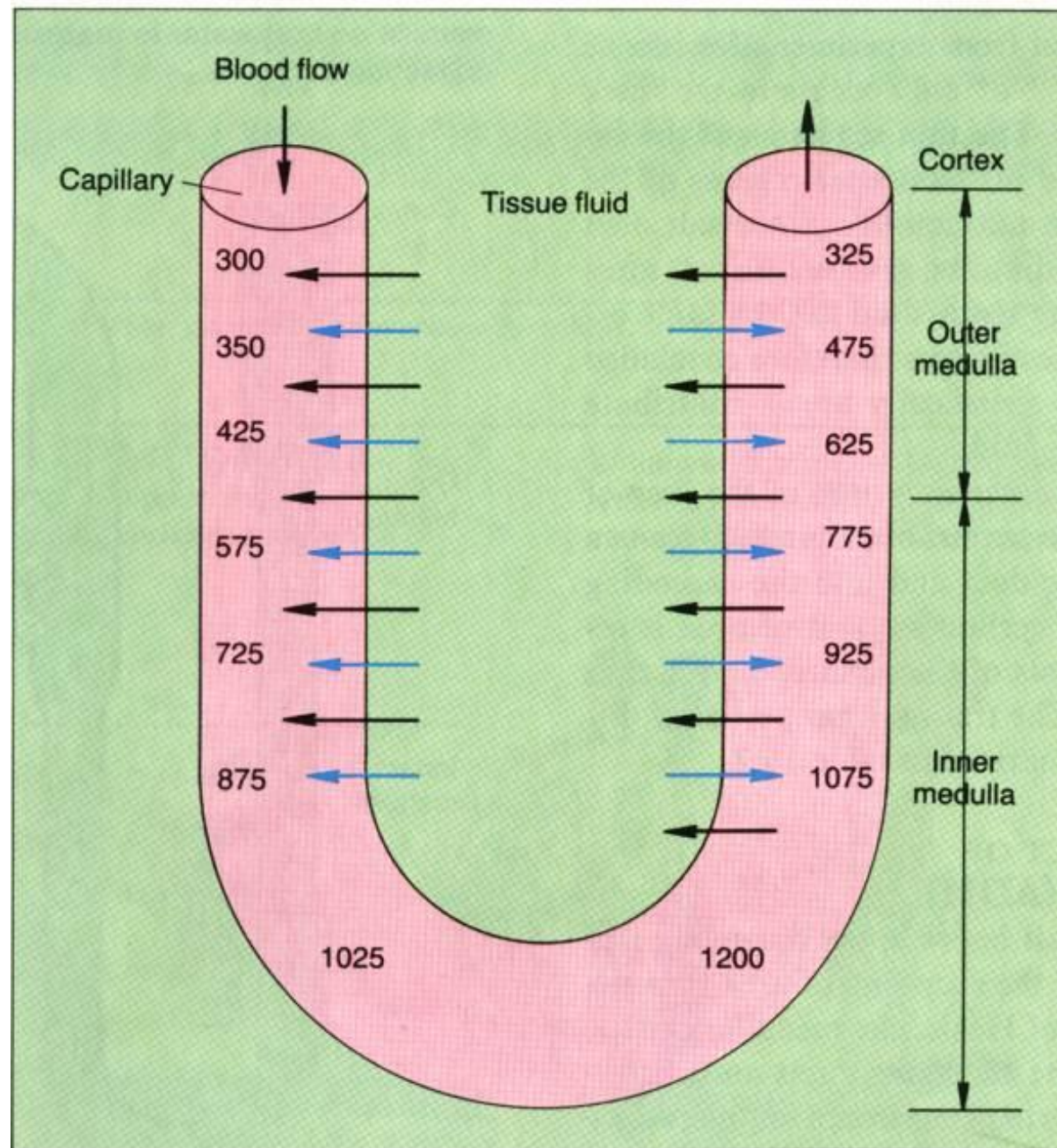
The Counter Current Mechanism

As water leaves the collecting duct, the urine becomes progressively more concentrated. The osmolarity of the collecting duct fluid will increase from about 150-300 to 1,200 mOsm/l under these conditions. If ADH is not present, water is not conserved and is lost as part of a dilute urine (100 mOsm/l).

The Counter Current Mechanism

The vasa recta is made up of a group of capillary like vessels and is freely permeable to salt and water. The vessels of the vasa recta roughly flow counter to the loop of Henle and acts as a counter current exchanger. As blood flows through the vasa recta it picks up water and leaves behind salt. Thus, the vasa recta returns conserved water back to the body and leaves the salt which maintains the hyperosmotic medulla.

The vasa recta trap salt and urea within the interstitial fluid but transport water out of the renal medulla



Black arrows = diffusion of NaCl and urea

Blue arrows = movement of water by osmosis

The Vasa Recta Preserve Hyperosmolarity of Renal Medulla

- The vasa recta serve as countercurrent exchangers
- Vasa recta blood flow is low (only 1-2 % of total renal blood flow)

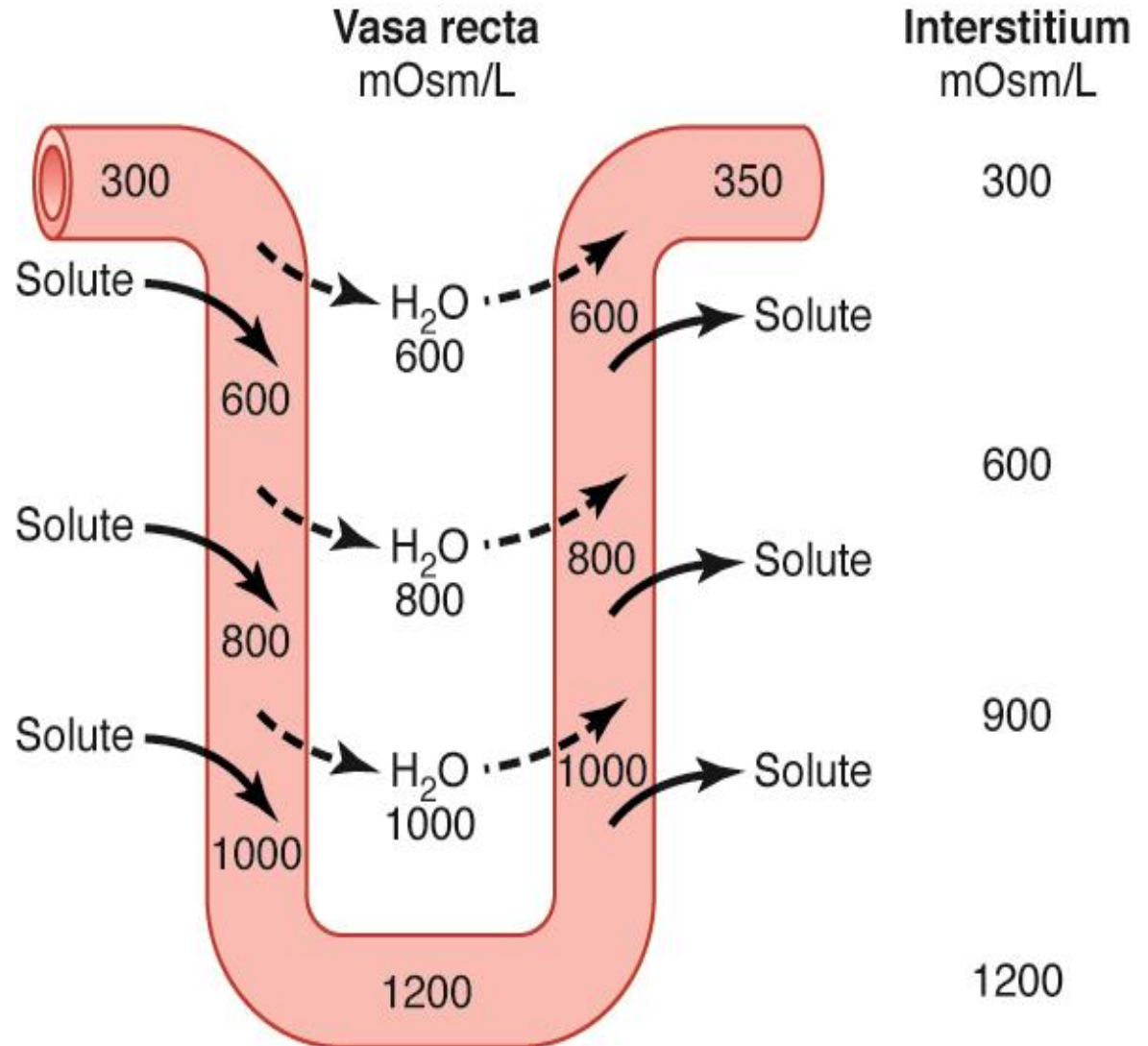


Figure 28-7

Countercurrent multiplier system in the loop of Henle.

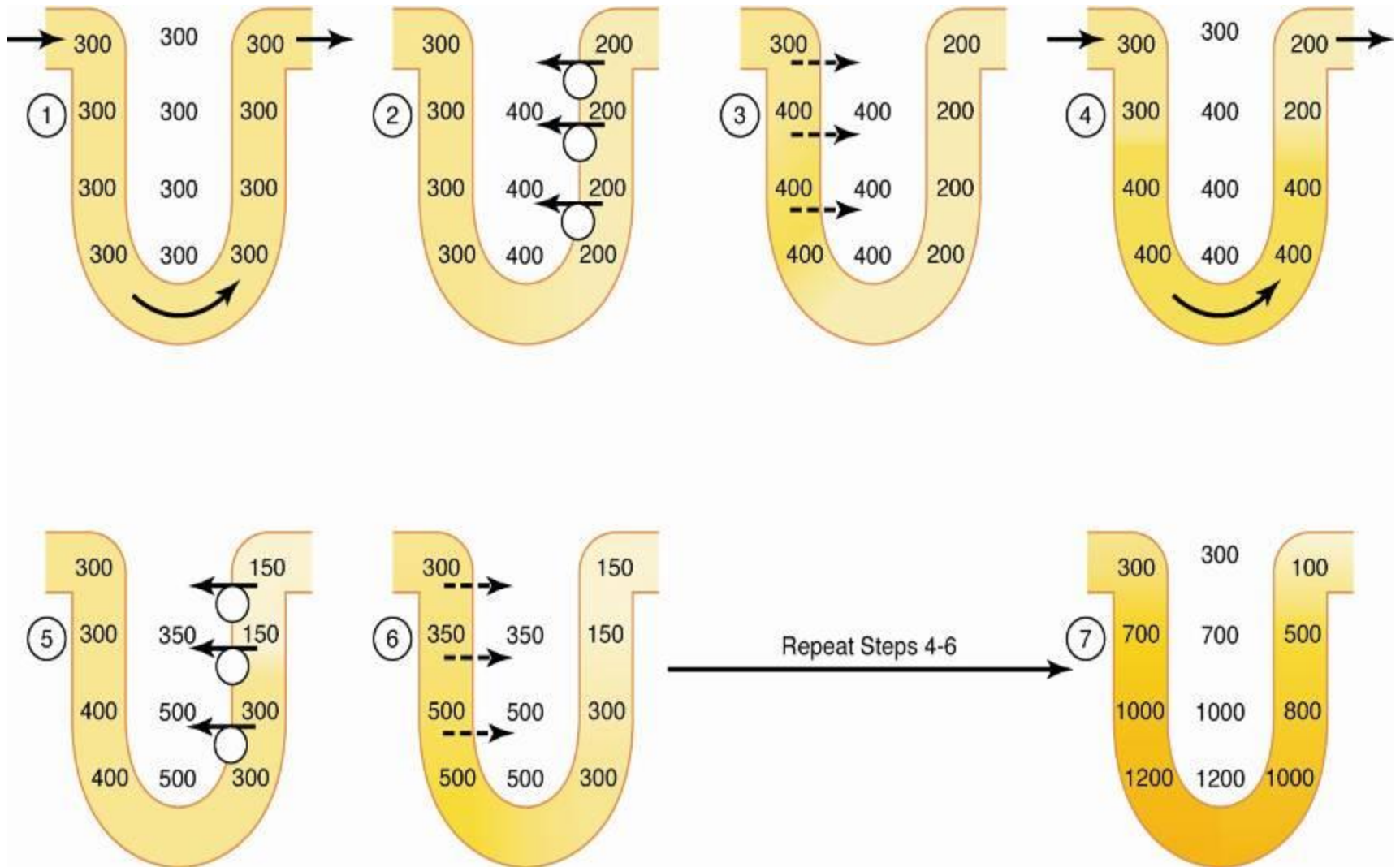
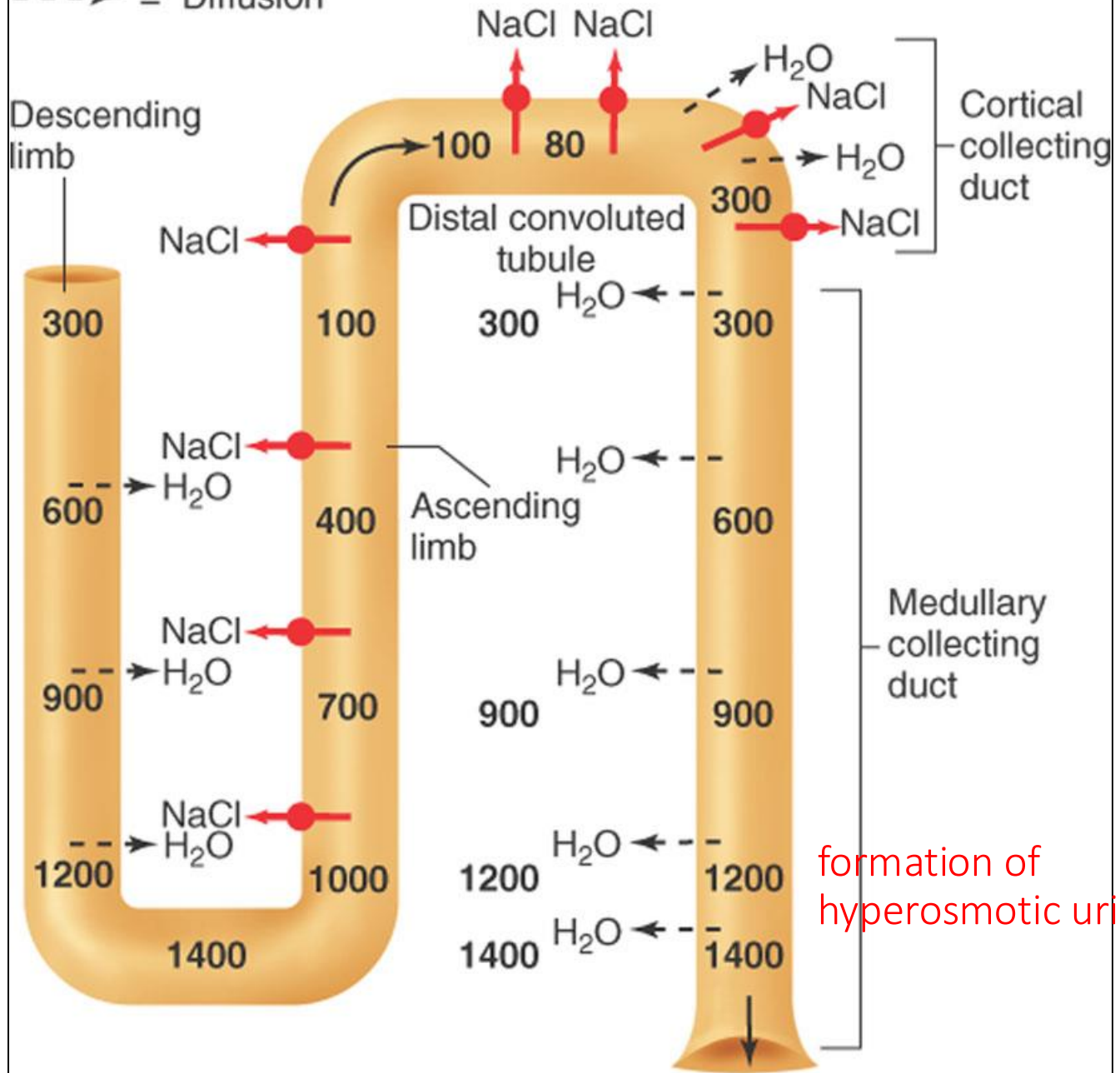


Figure 28-4

—●— = Active transport

- - -> = Diffusion



formation of hyperosmotic urine

Role of urea in concentrating urine

- Urea very useful in concentrating urine.
- High protein diet = more urea = more concentrated urine.
- Kidneys filter, reabsorb and secrete urea.
- Urea excretion rises with increasing urinary flow.

• **Urea** : $(\text{NH}_2)_2\text{C}=\text{O}$ MW 60:

• **Urea**: Its concentration 15-40 mg/dl (2.5-6.5 mmol/L).

• **In complete renal shutdown it rises by about 5 mmol/L per day.:**

• Is the end metabolite of protein.

• Small molecular weight: filtered freely

• Filtered load /day = $180 \text{ L} * 4.5 \text{ mMol} = 700 - 800 \text{ m mol/day}$

→ 50% is excreted, 50% is reabsorbed in proximal tubule passively.

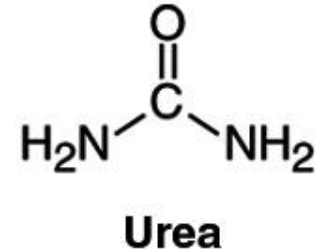
- What is the clearance of urea, if 50% of filtered is excreted?

50% of GFR (180 L/day) → 90L/day

50% will is reabsorbed in proximal. 60% is secreted in thin DLH and thin ALH. 70% is reabsorbed in IMCD.

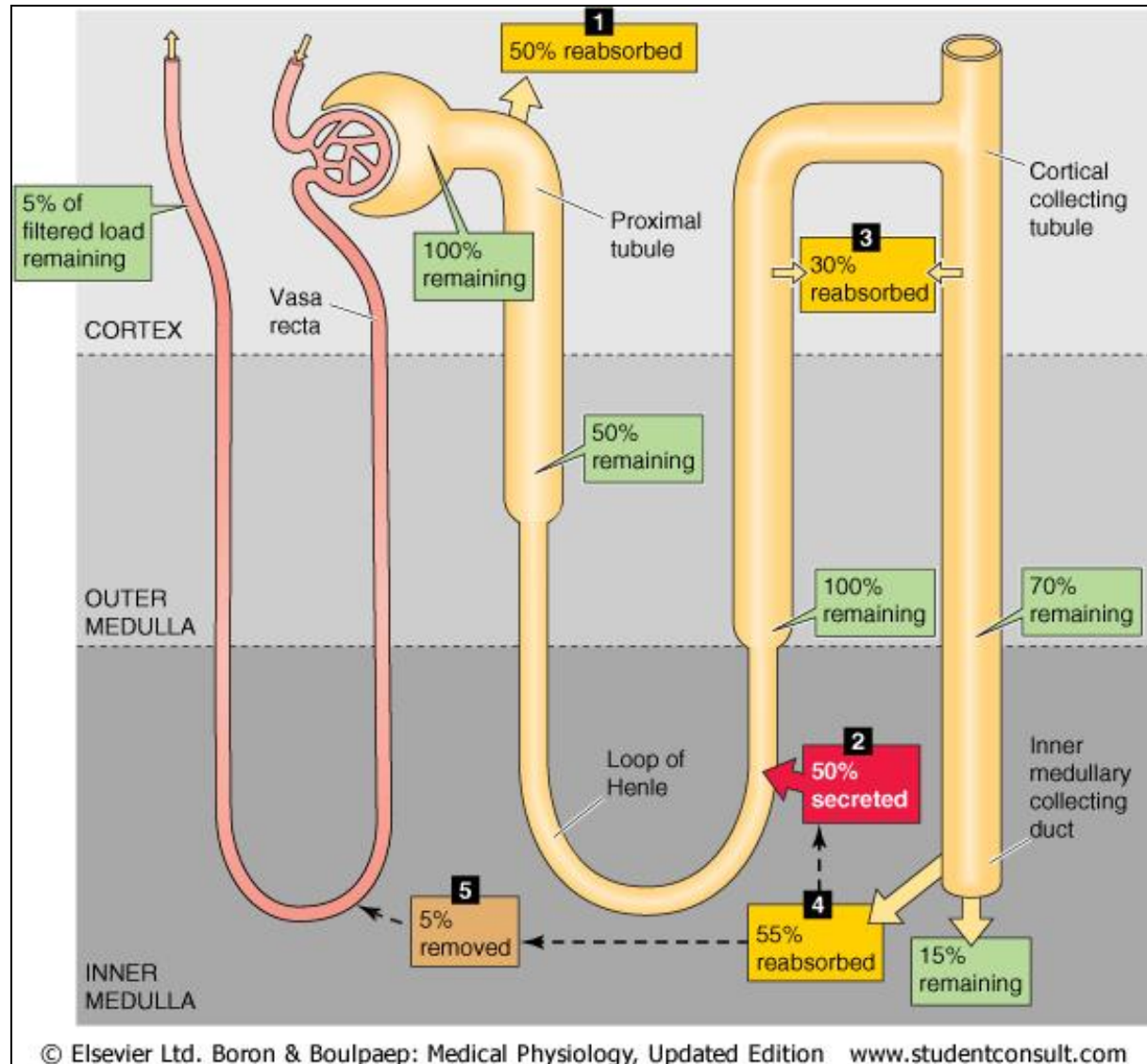
TAL is impermeable to urea, even in the presence of ADH.

Reaching to inner medullary collecting duct which is permeable to urea, especially with the help of ADH.



Urea recycling

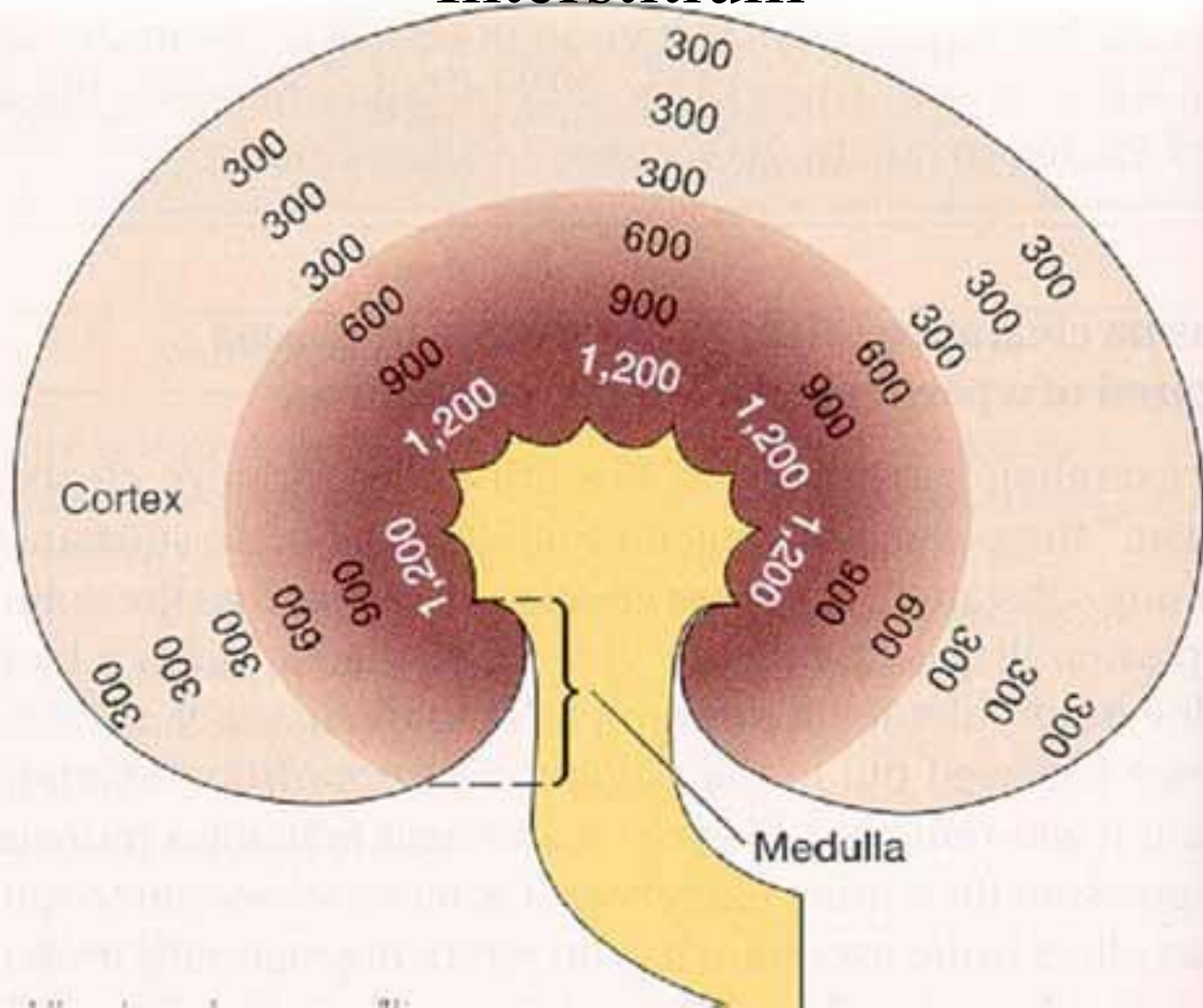
- Urea toxic at high levels, but can be useful in small amounts.
- Urea recycling causes build-up of high [urea] in inner medulla.
- This helps create the osmotic gradient at loop of Henle so H₂O can be reabsorbed.

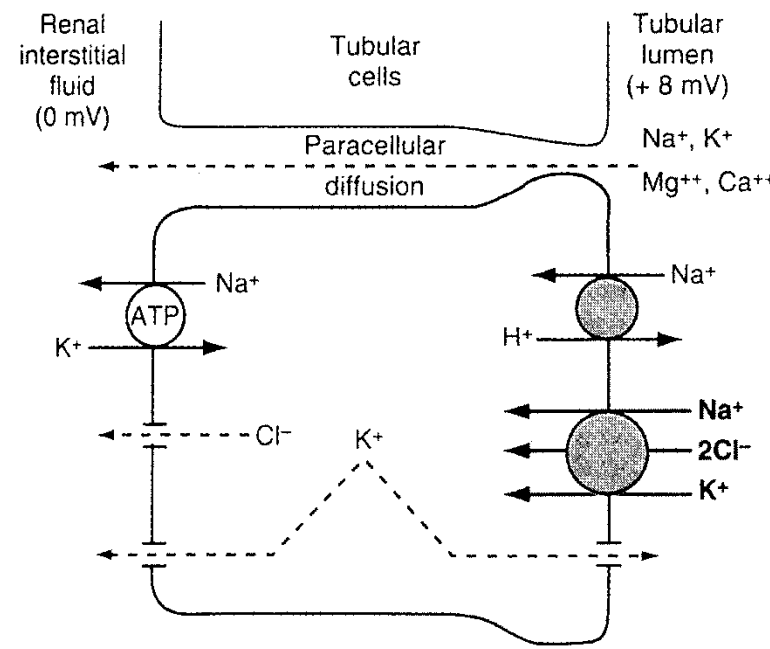
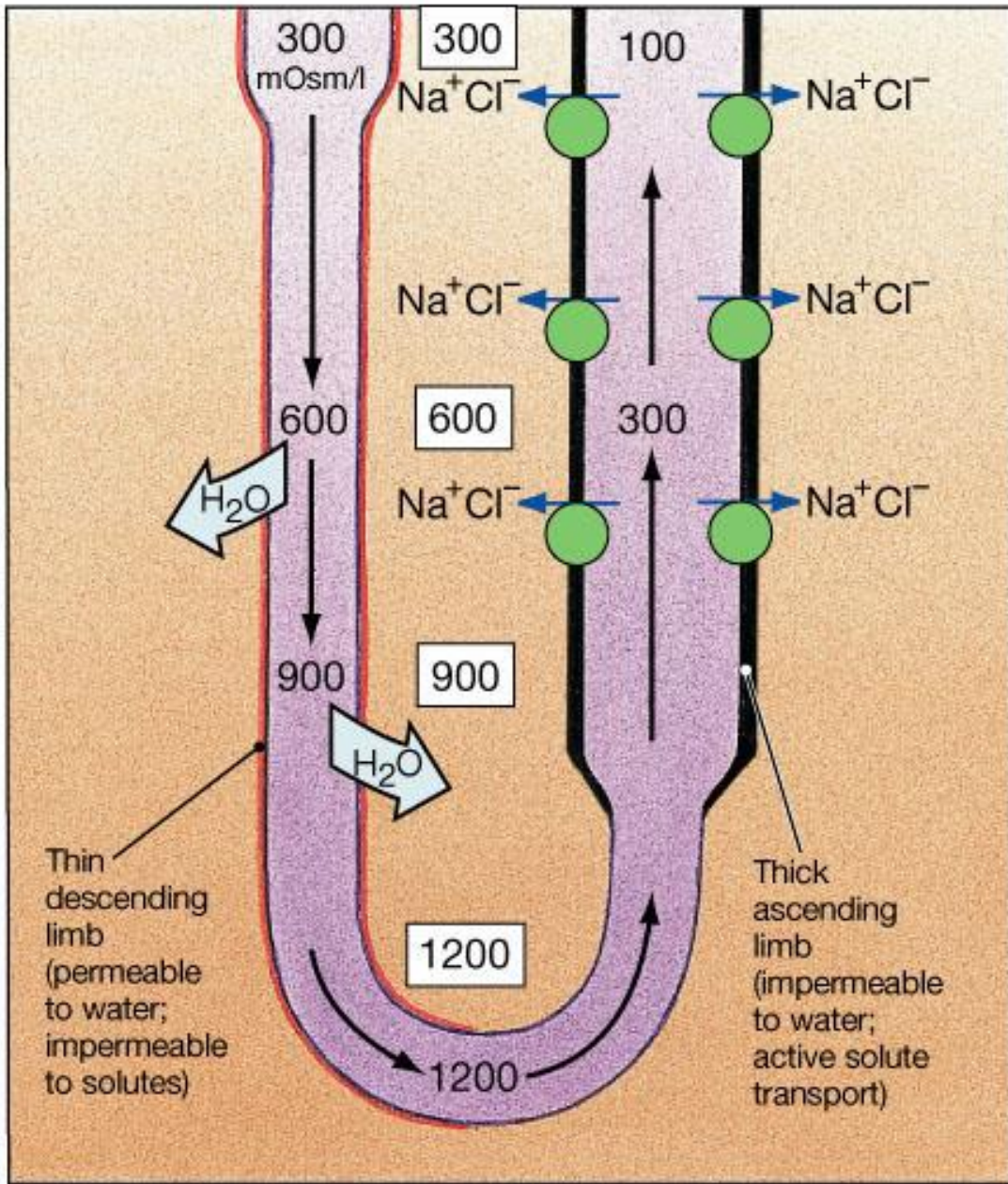


In summary: The basic requirements for forming a concentrated or diluted urine

- (1) the controlled secretion of antidiuretic hormone (ADH), which regulates the permeability of the distal tubules and collecting ducts to water;
- (2) a high osmolarity of the renal medullary interstitial fluid, which provides the osmotic gradient necessary for water reabsorption to occur in the presence of high level of ADH.

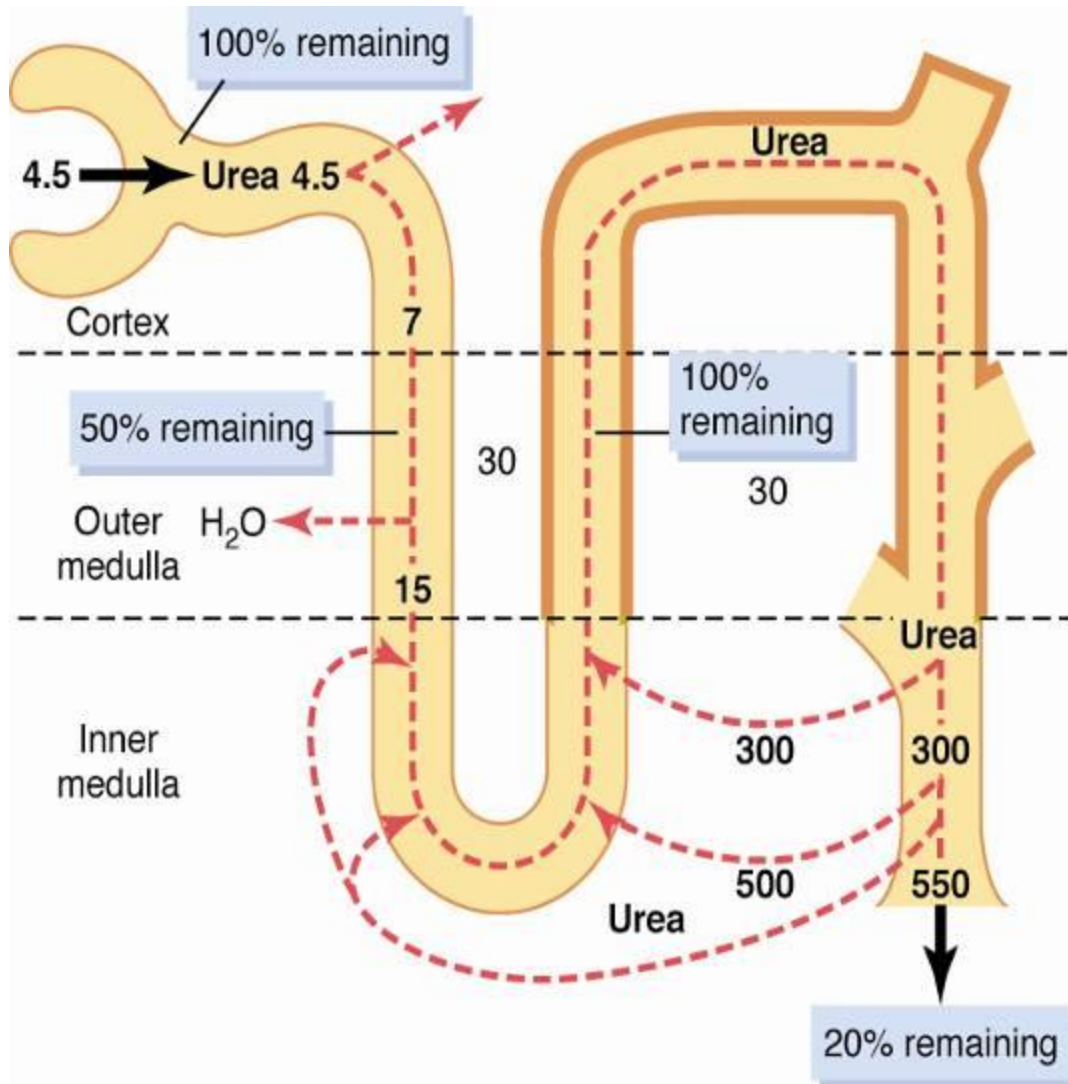
Hyperosmotic Gradient in the Renal Medulla Interstitium





(b) Active transport of NaCl along the ascending thick limb results in the movement of water from the descending limb.

Recirculation of urea absorbed from medullary collecting duct into interstitial fluid.



Urea Recirculation

- Urea is passively reabsorbed in proximal tubule (~ 50% of filtered load is reabsorbed)

In the presence of ADH, water is reabsorbed in distal and collecting tubules, concentrating urea in these parts of the nephron

- The inner medullary collecting tubule is highly permeable to urea, which diffuses into the medullary interstitium
- ADH increases urea permeability of medullary collecting tubule by activating urea transporters (UT-1)

“Free” Water Clearance (C_{H_2O}) (rate of solute-free water excretion)

$$C_{H_2O} = V - \frac{U_{osm} \times V}{P_{osm}}$$

where:

U_{osm} = urine osmolarity

V = urine flow rate

P = plasma osmolarity

If: $U_{osm} < P_{osm}$, $C_{H_2O} = +$

If: $U_{osm} > P_{osm}$, $C_{H_2O} = -$

Question

Given the following data, calculate “ free water” clearance :

urine flow rate = 6.0 ml/min

urine osmolarity = 150 mOsm /L

plasma osmolarity = 300 mOsm / L

Is free water clearance in this example positive or negative ?

Answer

$$\begin{aligned} \text{CH}_2\text{O} &= V - \frac{U_{\text{osm}} \times V}{P_{\text{osm}}} \\ &= 6.0 - \frac{(150 \times 6)}{300} \\ &= 6.0 - 3.0 \\ &= + 3.0 \text{ ml / min (positive)} \end{aligned}$$

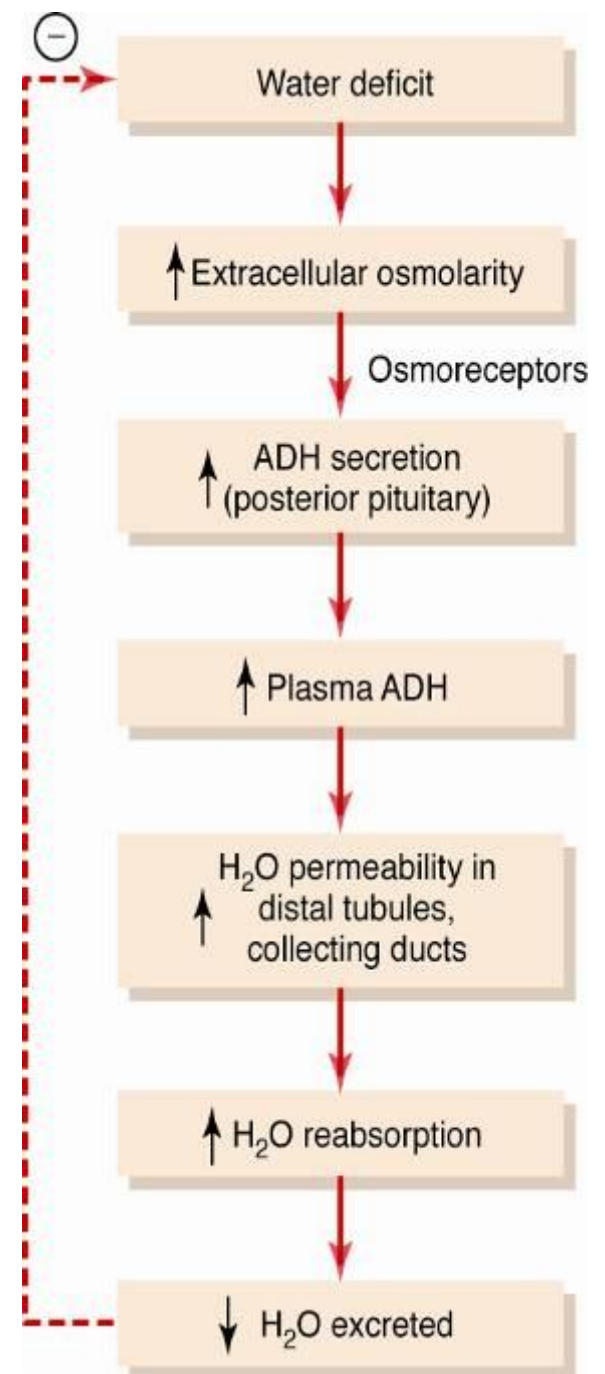
Control of Extracellular Osmolarity (NaCl Concentration)

- ADH
 - Thirst
- ADH -Thirst Osmoreceptor System

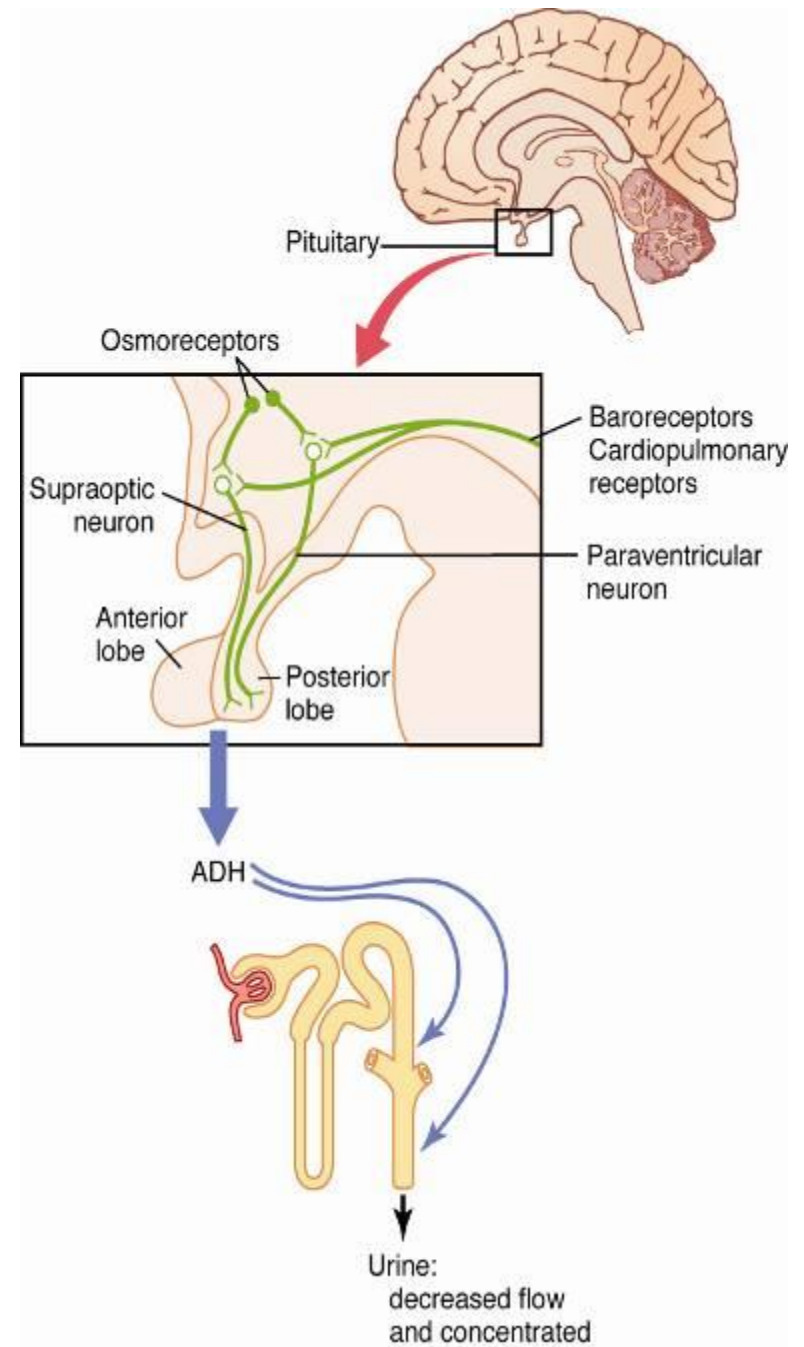
Mechanism:

increased extracellular osmolarity (NaCl)
stimulates ADH release, which increases
H₂O reabsorption, and stimulates thirst
(intake of water)

Osmoreceptor –
antidiuretic hormone
(ADH) feedback
mechanism for regulating
extracellular
fluid osmolarity.



ADH synthesis in the magnocellular neurons of hypothalamus, release by the posterior pituitary, and action on the kidneys



Stimuli for ADH Secretion

- Increased osmolarity
- Decreased blood volume (cardiopulmonary reflexes)
- Decreased blood pressure (arterial baroreceptors)
- Other stimuli :
 - input from cerebral cortex (e.g. fear)
 - angiotensin II
 - nausea
 - nicotine
 - morphine

The effect of increased plasma osmolarity or decreased blood volume.

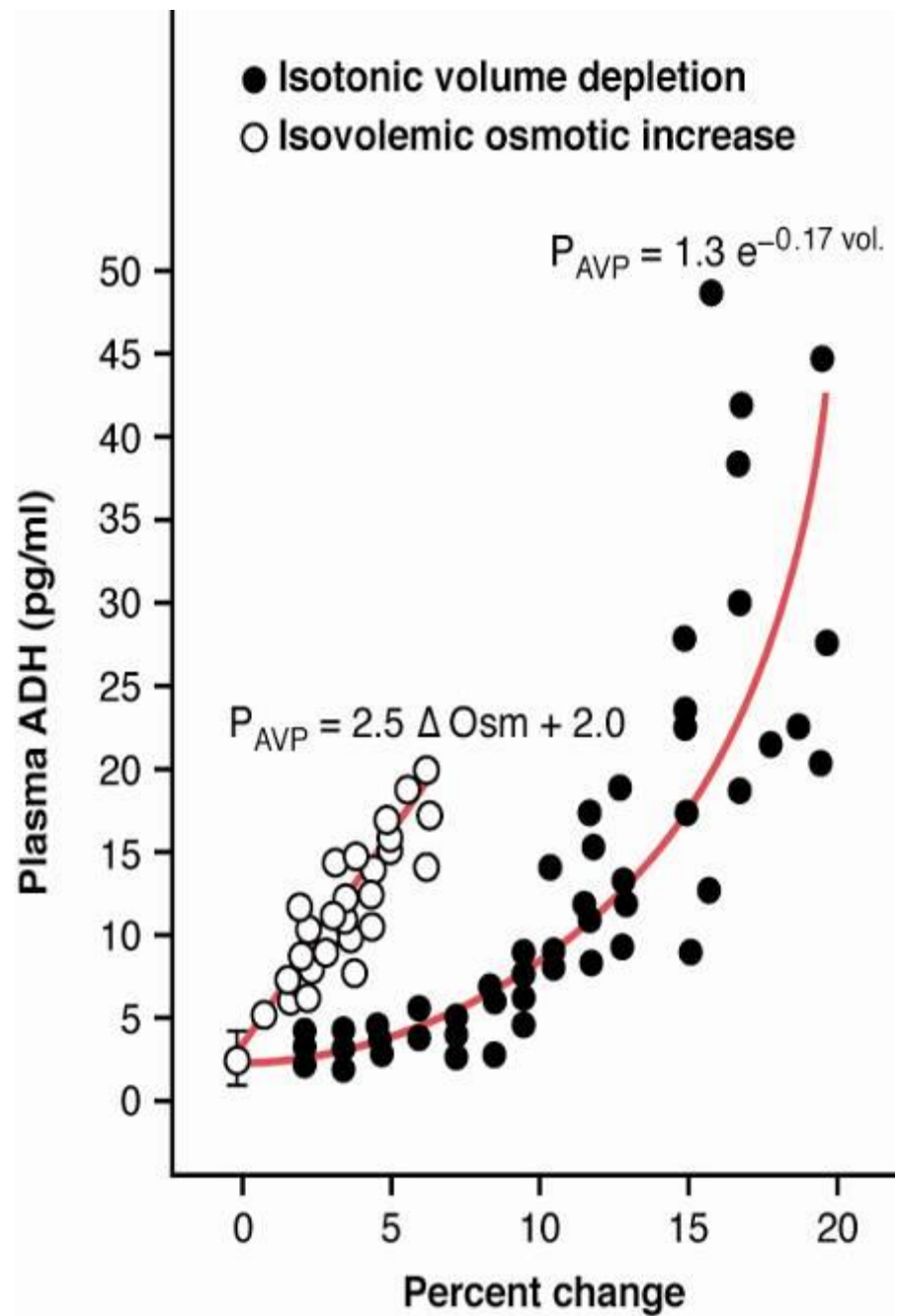


Figure 28-11

Factors That Decrease ADH Secretion

- Decreased osmolarity
- Increased blood volume (cardiopulmonary reflexes)
- Increased blood pressure (arterial baroreceptors)
- Other factors :
 - alcohol
 - clonidine (antihypertensive drug)
 - haloperidol (antipsychotic, Tourette's)

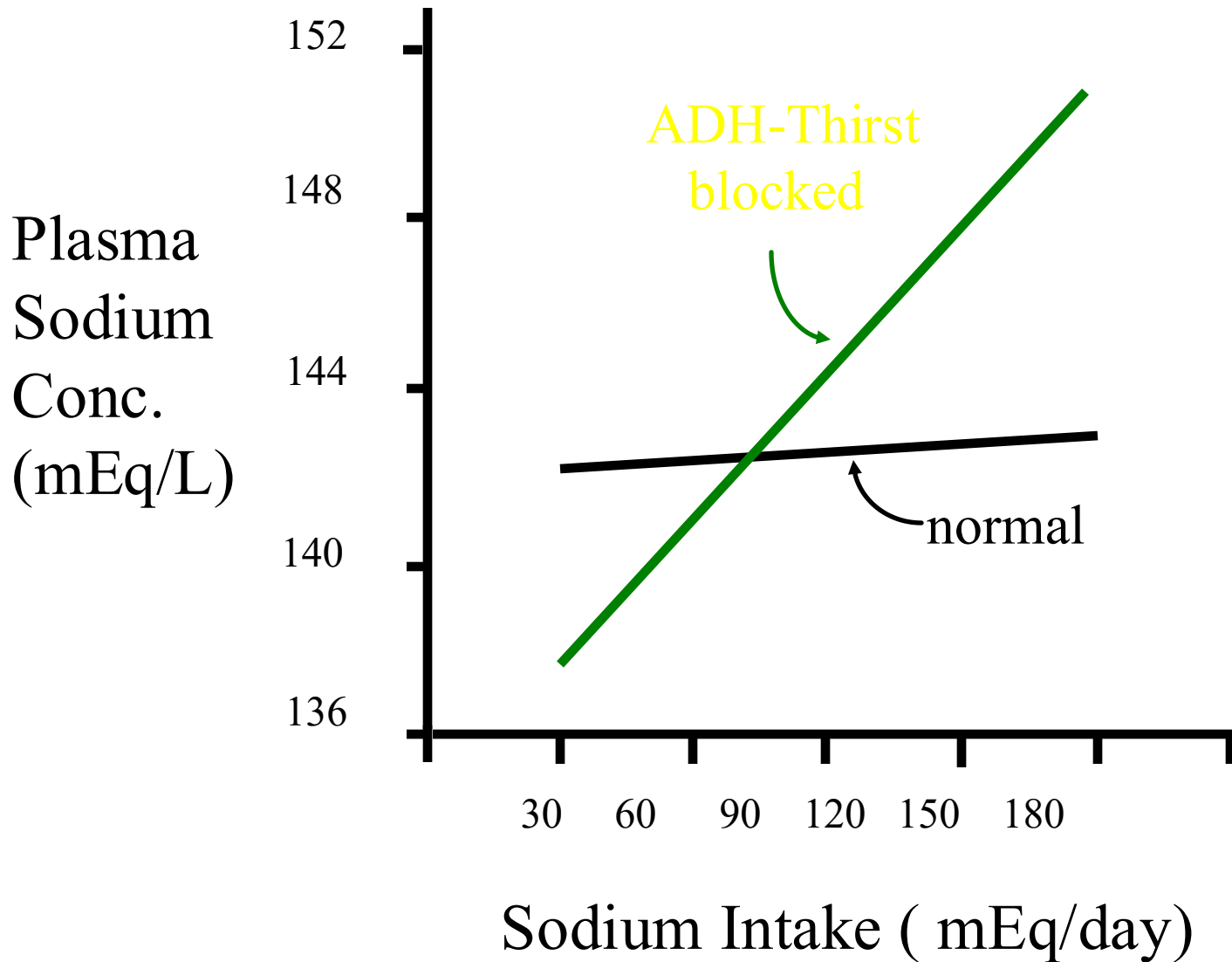
Stimuli for Thirst

- Increased osmolarity
- Decreased blood volume
(cardiopulmonary reflexes)
- Decreased blood pressure
(arterial baroreceptors)
- Increased angiotensin II
- Other stimuli:
 - dryness of mouth

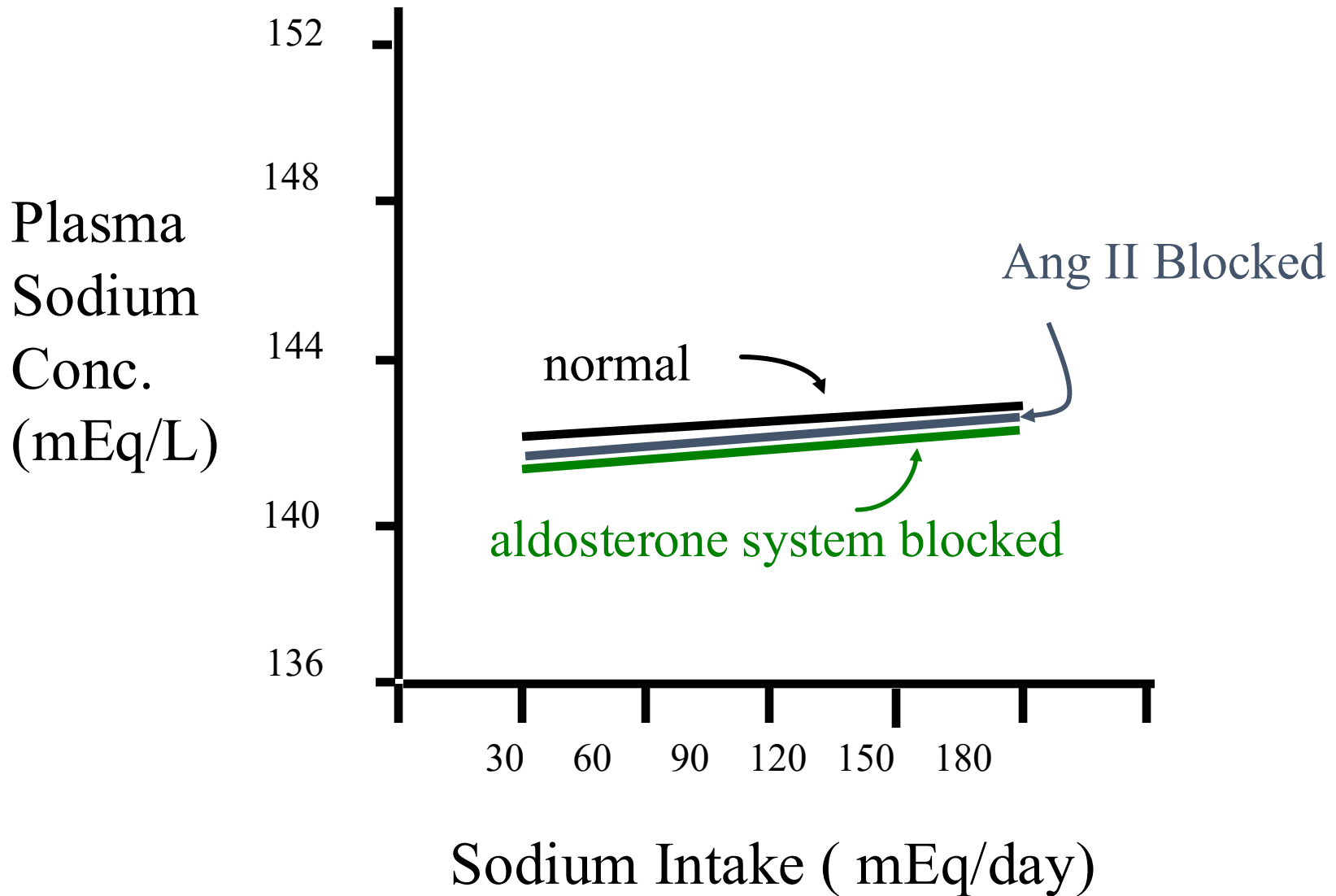
Factors That Decrease Thirst

- Decreased osmolarity
- Increased blood volume
(cardiopulmonary reflexes)
- Increased blood pressure
(arterial baroreceptors)
- Decreased angiotensin II
- Other stimuli:
 - Gastric distention

Effect of Changes in Sodium Intake on Plasma Sodium After Blocking ADH-Thirst System



Effect of Changes in Sodium Intake on Plasma Sodium After Blocking Aldosterone or Ang II System



Regulation of extracellular volume (repeat again):

1. Through ADH osmoreceptors in hypothalamus sense the increase in Na^+ concentration.
2. Thirst receptors.
3. Volume receptor in atrium

ADH regulation :

- Mild increases in plasma osmolarity (2-3 mosmoles) is enough to stimulate ADH.
- Baroreceptors can stimulate or suppress ADH secretion e.g.hypotension stimulates secretion of ADH.
- Volume receptor in low pressure chambers like atrium if these chambers witnessed expansion, this expansion will suppress ADH.
- Volume should decrease 10-20% to stimulate ADH.
- **Osmo-receptors are the most sensitive.**

Disorders of Urine Concentrating Ability

- Failure to produce ADH :

“Central” diabetes insipidus

Leading to polyuria polydipsia... Treatment: desmopressin acetate (synthetic ADH)

- Failure to respond to ADH:

“nephrogenic” diabetes insipidus by infection, drugs

- impaired loop NaCl reabs. (loop diuretics)
- drug induced renal damage: lithium, analgesics
- malnutrition (decreased urea concentration)
- kidney disease: pyelonephritis, hydronephrosis, chronic renal failure

- With vasodilation, more blood flow in vasa recta
→ washing away NaCl and urea → less concentrated interstitium.
Vasa recta will prevent dissipation of osmolarity, but doesn't make it.
- **Loop diuretics:**
prevent NaCl reabsorption → hypoosmolar medullary interstitium
no concentrated urine
→ Water passing out with Na⁺ : high urine output.
- Low protein diet: less urea production, maximum urine concentration is depressed.

In the cortex:

High blood flow, with tubules being permeable to solutes and water. Interstitium osmolarity is 300 m Osm/L

Why we must drink water?

- Normally, we must excrete 600-700 mOsm\day as a minimum amount (Resting...complete bed ridden), or 1000 mOsm under normal daily activity.
- The kidney can make a concentrated urine up to 1400 mOsm/l
- The minimum daily obligatory volume of urine (MDOVU)
- MDOVU is 500 ml at rest (700/1400) or 714 ml (1000/1400) during normal daily activity
- Below this level is oliguria:
 - Infants < 1 ml/kg/h
 - Children < 0.5 ml/kg/h
 - Adults <0.3 ml/kg/h
- In general MDOVU= 300ml\m²\day

Obligatory Urine Volume

The minimum urine volume in which the excreted solute can be dissolved and excreted

Example:

If the max. urine osmolarity is 1200 mOsm/L, and 600 mOsm of solute must be excreted each day to maintain electrolyte balance, the obligatory urine volume is:

$$\frac{600 \text{ mOsm/d}}{1200 \text{ mOsm/L}} = 0.5 \text{ L/day}$$

Make sure to answer this question: How to generates the hyperosmolar interstitium?

By the so called “single effect”. In thick ascending loop of Henle, $\text{Na}^+\text{-K}^+\text{-2Cl}$ transporter carry those ions to the interstitium. But, H_2O reabsorption is not allowed, making interstitium hyperosmolar with 700 mOsm/L from NaCl

- The rest of the hyperosmolarity comes from urea
- Na^+ that is reabsorbed must not be removed from the interstitium...that is why we have low blood flow at the medullary regions. The Vasa Recta, having less than 5% of renal blood flow.

- **One more time: To Make Concentrated Urine we need:**
- **1. Hyperosmolar interstitium around the medullary collecting ducts**
- **2. ADH to permit H₂O permeability.**
- **By the micropuncture technique we measure the interstitium osmolarity in collecting tubules and found it to be hyperosmolar.**

Urea penetrate to interstitium, making it hyperosmolar, contributing by 500 mOsm; more osmolarity in Medulla than cortex, creating cortecomedullary gradient

Inner medullary osmolarity:

700 mOsm + 500 mOsm = 1200 mOsm/L
(by NaCl) (by urea)

Urea recycling:

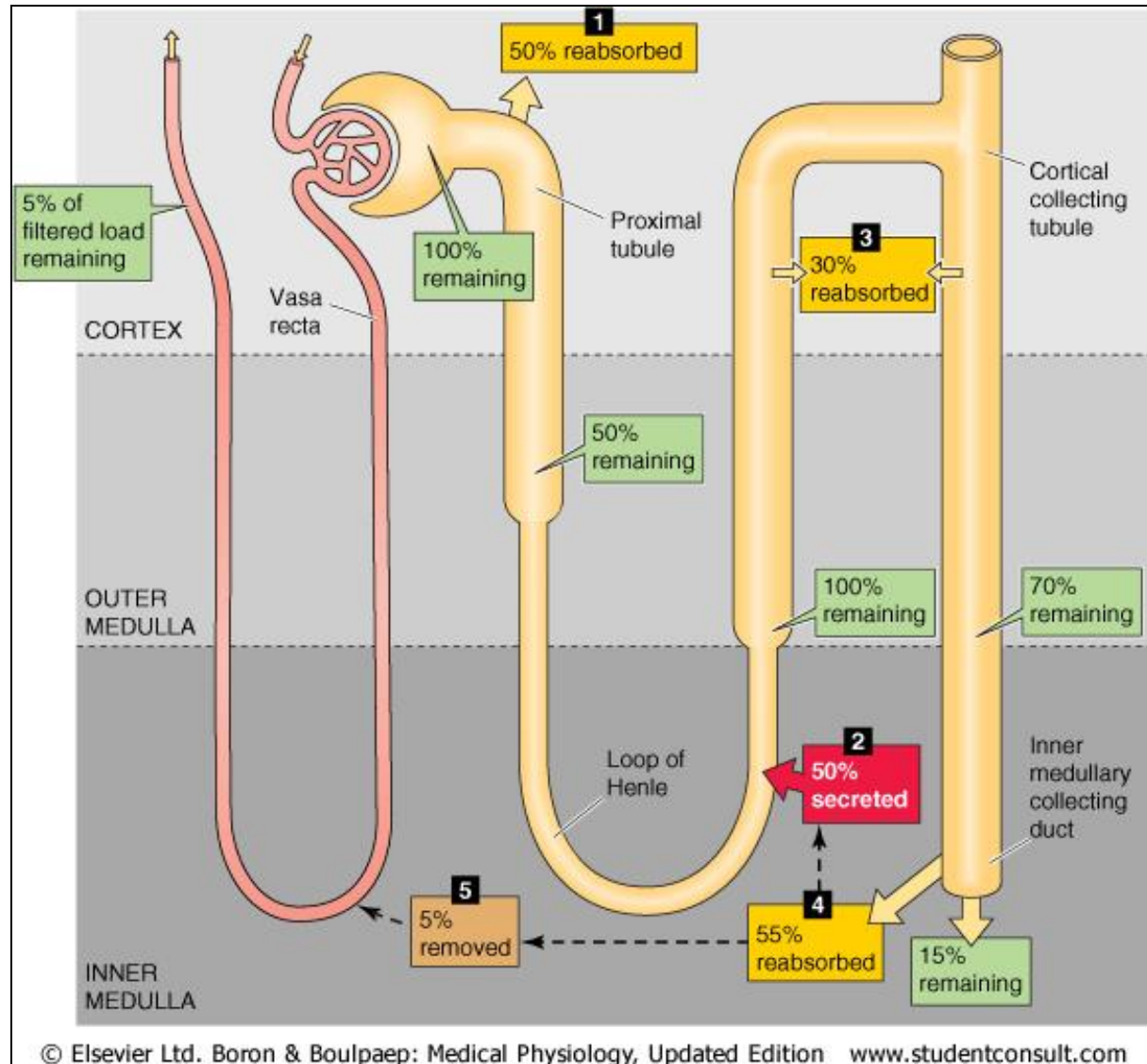
from inner medullary collecting ducts to interstitium of medulla → increased by ADH

So, ADH helps urea to diffuse

→ alters permeability of collecting tubule of cortex and medulla to H₂O

Urea recycling

- Urea toxic at high levels, but can be useful in small amounts.
- Urea recycling causes buildup of high [urea] in inner medulla.
- This helps create the osmotic gradient at loop of Henle so H₂O can be reabsorbed.



Urine can be isotonic, hypotonic, or hypertonic?

Normal urine under normal diet and normal physical exercise is hypertonic (600-700 mOsm/l).

- **1000 mOsm is excreted in the urine/D**
 - **1.5 L/day is the normal urine output.**
 - **$1000 \text{ mOsm} \div 1.5 \text{ L urine per day} = 600 - 700 \text{ Osm/L}$**
- * Compared to plasma = 300 mOsm/L, urine is hypertonic**

- **In bed ridden patients, with minimal activity, a minimum of 600-700 mOsm must be excreted. This amount needs 0.5 liter of urine to carry it. (UOP=0.5 L/D). Therefore, less than 0.5L/D urine is considered “Oliguria”**
- **If Kidneys can concentrate urine→ this indicate:**
 - 1) **hypothalamus with its osmoreceptors are working normally**
 - 2) **posterior pituitary is functioning well**
 - 3) **collecting ducts have working receptors for ADH.**
 - 4) **Thick ascending is reabsorbing NaCl**

- Simple test to detect: concentrating ability of the kidney

Ask the patient to remain NPO starting from midnight (water deprivation)., at 8:00 a.m., take urine sample, then after half an hour, another sample, then after half an hour a third sample
You have 3 samples. One of them should be $> 1000 \text{ mOsm/L}$ → (healthy kidney)

- *How to measure osmolarity?*

- 1 → by osmometer :this instrument is not available in most medical centers
- 2 → by specific gravity (SG).

SG:

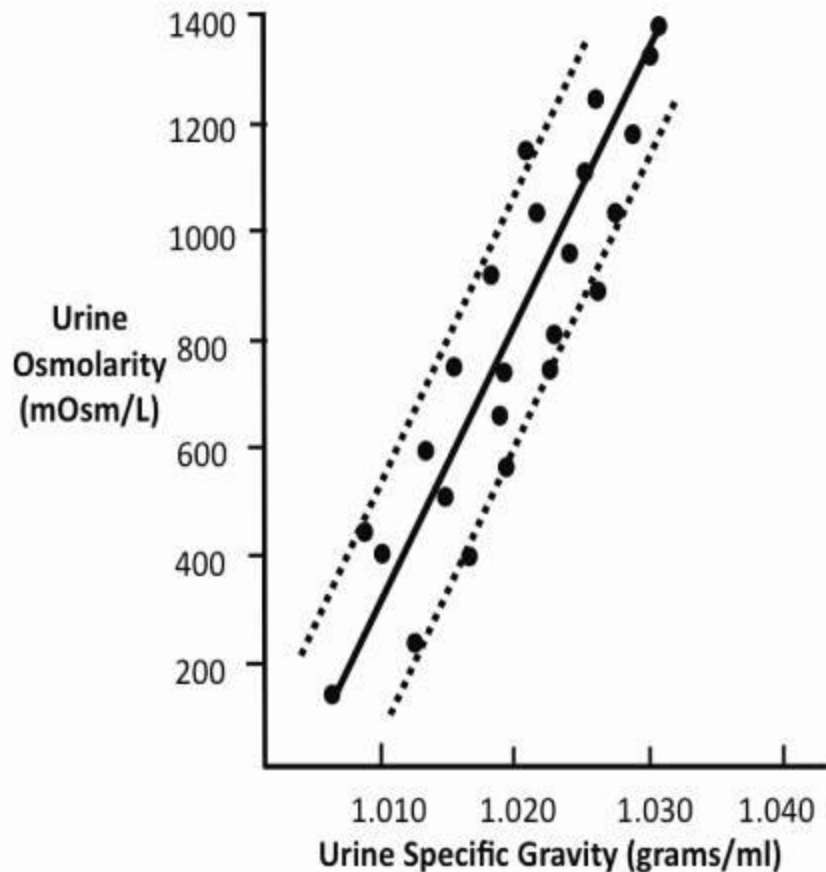
Depends on the mass of the particles present in a given weight of urine

(mass/mass): weight of urine/weight of water

Osmolality depends on the number of particles (number/mass) and is a much better index of true renal concentrating ability

→ if urine has large particles like: WBC, RBC, protein, epithelial cells, casts, ... → increased SG (but not osmolarity)→ misleading results.

Relationship between urine osmolarity and specific gravity



Influenced by

- glucose in urine
- protein in urine
- antibiotics
- radiocontrast media



Osmolarity of the urine varies from as low as 30 mOsm/L to as high as 1400 (30-fold difference).

30 mOsm/L means at least 20 liters of urine per day is needed to remove 600 mOsm/day

Convert SG to osmolarity:

Example **SG = 1.0025**

take the last two digits (25) multiply them by 40.

$25 * 40 = 1000 \text{ mOsm/L}$

If SG = 1.010 at all time ($\pm \text{H}_2\text{O}$) \rightarrow Non functional tubules (Kidney failure)

S.G is a traditional measure of urine concentration (very simple test).

1.000 weight of urine = weight of water (Never)

1.003 diluted (compare to plasma)

1.010 isotonic (isosthenuria)_(As intrarenal ARF)

1.040 concentrated...healthy kidney