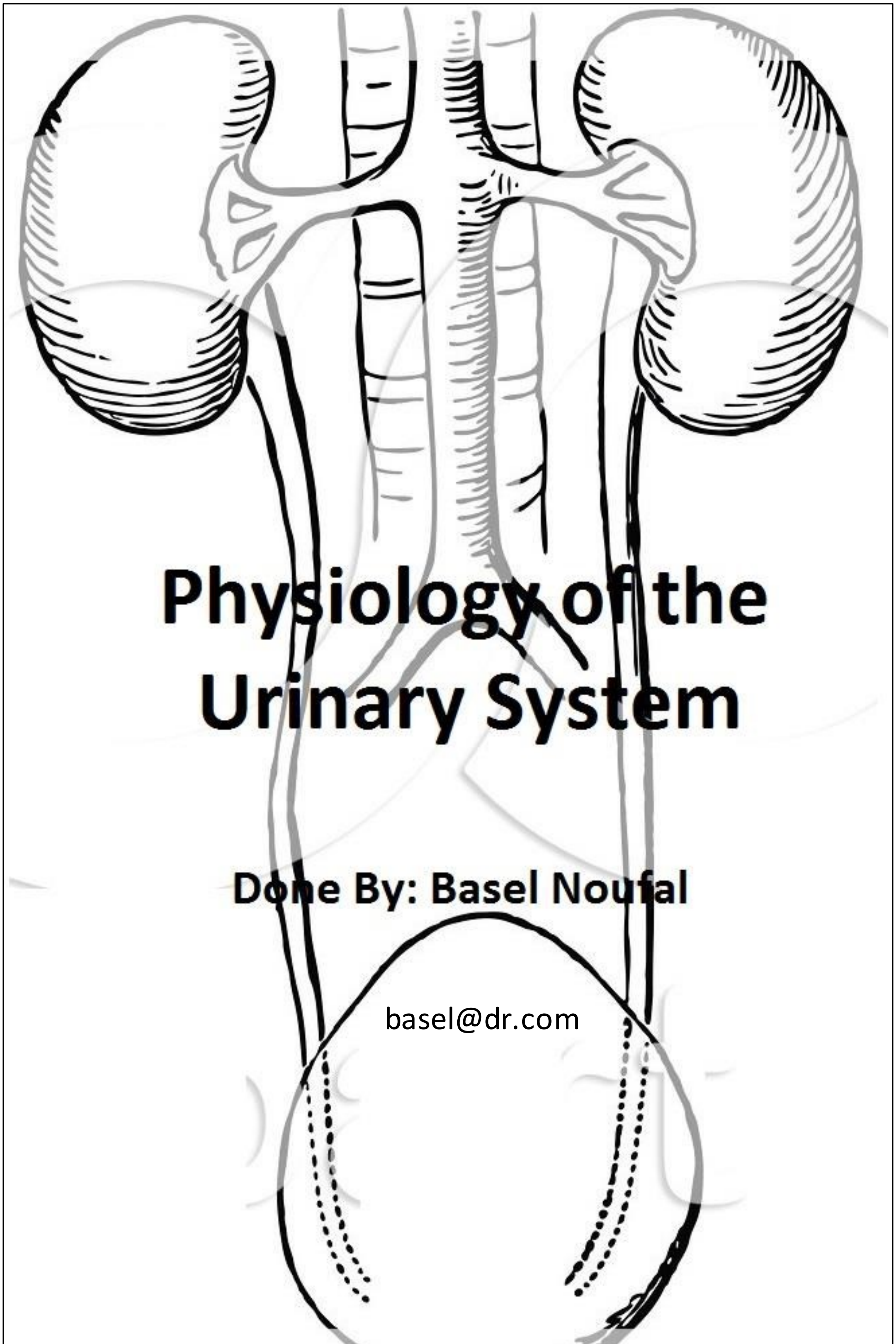

Physiology of the Urinary System

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Corrected and modified by Yanal A. Shafagoj MD. PhD

Textbook of medical physiology, by A.C. Guyton and John E, Hall•

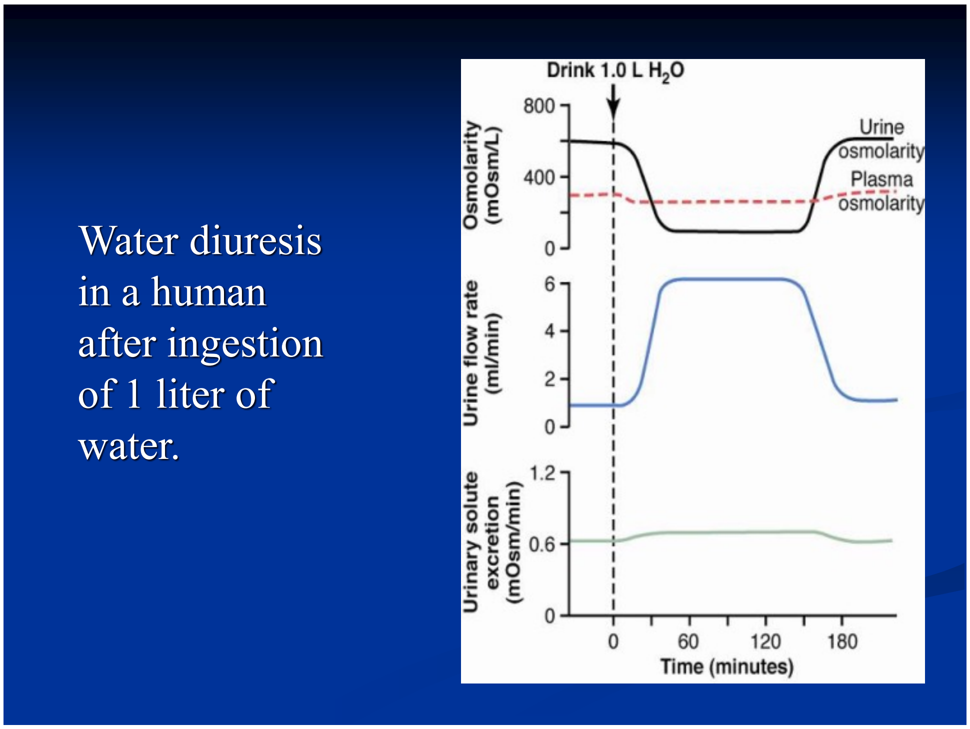
If you prefer to study from textbook, I'II send you the outline

Lecture (7&8)

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

- **General concepts from "Introduction to Physiology…1st year)**
- The osmole (# of particles… 6.023×10^{23}; Avogadro's law).) is the number of moles of solutes that contribute to the osmotic pressure in a solution. The osmolarity of 1 mol/L solution of glucose is the same as the osmolarity of 1 mol/L solution of Na^+, despite the large difference in size between glucose and Na^+. One mole of NaCl contains 2 Osmoles, since NaCl dissociates into Na^+ and Cl^+ in aqueous solution. Thus, one mole of NaCl solution attracts water twice as much as one mole glucose.
- Since most cell membranes are permeable to water, the osmolarity of ICF and ECF (interstitial and plasma) is 285-310 mOsm/L (avg, 300 mOsm/l).
- The molecular weight of NaCl is 58.5 Da. The osmolarity of 1 mol/L of NaCl solution is 2,000 mOsm/L. Thus a solution with an osmolarity of 300 mOsm/L has a concentration of NaCl = 9 g/L, or 0.9 g/100ml (0.9% Normal saline or "N/S" which is an isotonic solution…the same tonicity as plasma).
- The molecular weight of glucose is 180 Da. Thus, a solution with an osmolality of 300 mOsm/L has a concentration of glucose = 50 g/L, or 5% Dextrose water "5% D/W" is again an isotonic solution
    - **Importance of concentration and dilution of urine:**
    - 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 ((specific gravity ~ 1.003)), a concentration that is only about one sixth the osmolarity of normal extracellular fluid….**removing extra ingested water without losing too much solutes**

- Conversely, when there is a deficiency in water supply and extracellular fluids osmolarity is high, the kidney can excrete urine with a concentration of about 1200 to 1400 mOsm/liter ((specific gravity ~ 1.030)). By doing so we can remove all waste products and at the same time conserve water.
- Look at the below graph when a person ingest one lit of water in short period of time (after breaking fast during the coming Ramadan)

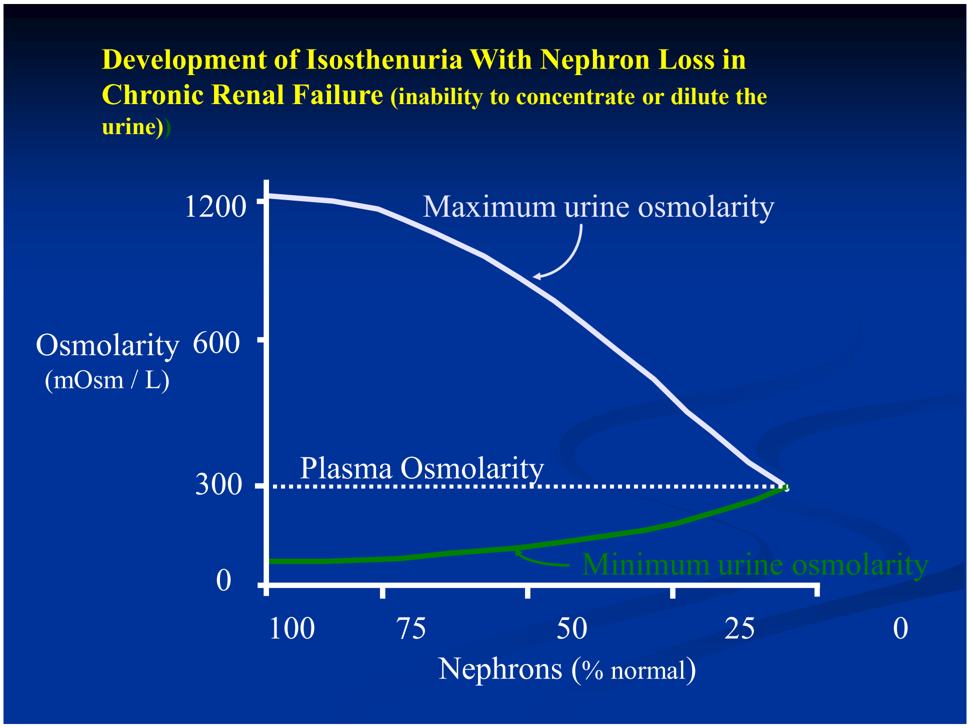


- **<u>Historical point of view:</u>**
- For scientists, to make diluted urine was somehow easy to understand….the kidneys needs just to actively transport Na+ and Cl- out of the collecting duct, leaving water inside and thus making diluted urine = hypoosmolar urine. We know that active Na+ and Cl- do exist.
- However, 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. H20 cannot actively transported out of the collecting duct, leaving Na+ and Cl- inside and thus making concentrated urine = hyperosmolar urine.
- **Observation**
- Only those species who have long loop of Henle can concentrate urine.

Clinical point:

When GFR drops to 20-50%. The earliest sings is **isosthenuria** or polyuria with isotonic urine.

<u>In End stage Renal Failure:</u> osmolarity of urine and S.G of becomes like glomerular filtrate which is 300 mOsm/l and 1:010 respectively. Hence, kidney neither can concentrate nor dilute urine = this is known as isosthenuria. The graph below shows the effect of **Nephron Loss and the ability of the kidney** to concentrate or dilute urine.

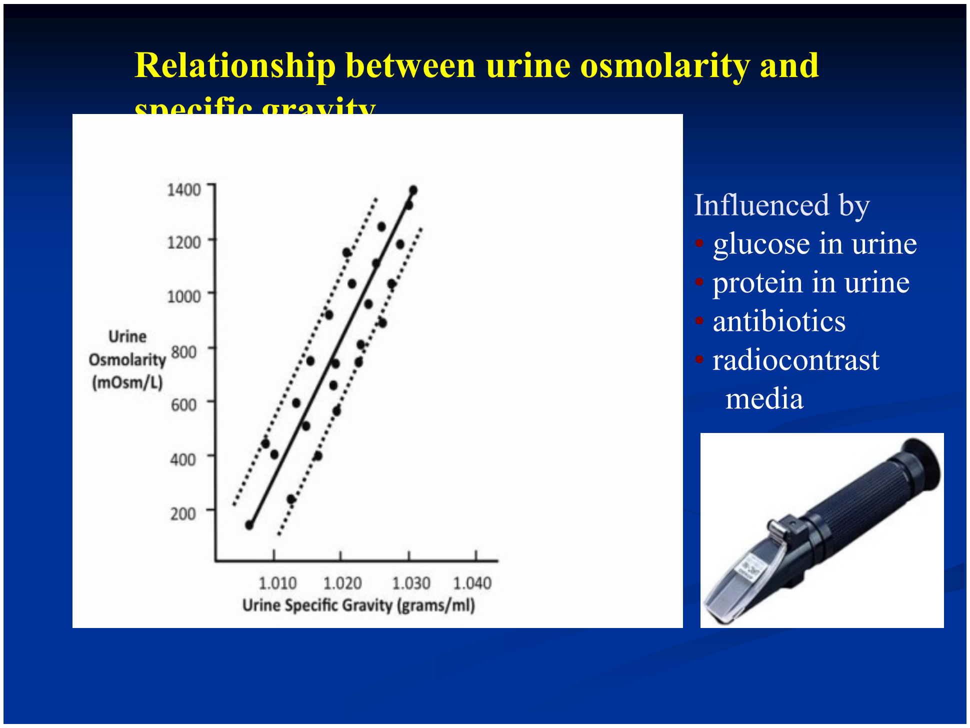


- Following acute kidney injury, the ability of the kidney to concentrate urine is the last kidney function test to return to normal. This means if S.G is more than 1.025, then the kikney has regain all its function (back to normal).
- This is tubular function (not glomerular KFT) it give very valuable important information regarding the kidney
-

- **<u>The concept of "minimum daily obligatory volume of urine (MDOVU)</u>" and Oliguria**
- Before we dig deep in this topic, let us answer the following simple question: Why human needs to drink water? Why other species (dessert mouse) can survive without drinking water?
- The answer depends on the maximum concentrating ability of our kidney. Our kidney can make concentrated urine up to 1400 mOsm/l. Some dessert animals can concentrate their urine up to 10,000 mOsm/L. This allows them to survive without drinking water.

- Let me give you two examples:
- In complete bed ridden patient, a minimum of 700 mOsm\day must be removed from our body through the kidney (such as urea, creatinine, Na, Cl-, K+, acid buffers etc.).
- Normal person under normal daily activity, this minimum amount goes up to 1000/d mOsm.
- **Conclusion**: MDOVU is 500 ml in a complete bed rest patient (700/1400*1000 ml) or 714 ml (1000/1400*1000ml) in normal person under normal daily activity and normal diet. Therfore, the average osmolarity of human urine is about 650mOsm/L; thus, the urine, is considered hypertonic (compared to the osmolarity of the plasma which averages 300 mOsm/L).
- Below this volume is considered " oliguria" and is sign of kidney injury: MDOVU at different ages:
- In Infants 1 ml/kg/h
- In Children 0.5 ml/kg/h
- In Adults 0.3 ml/kg/h
- **Regardless of the age, in general MDOVU is equal to 300ml\m^{2}BSA\day …below this volume is oliguria…this number you need to remember**
- **Using urine specific gravity (S.G) as index for urine osmolarity**
- Osmolarity of any solution (including urine) is determined by the number of particles in that solution (regardless of their size…wither it is large as protein or small as Na+). In contrast, the urine specific gravity takes a mass in consideration. It is a measure of the weight of the solution compared to that of an equal volume of distilled water, is determined by the size of particles in the solution ((mass/mass or weight of urine/weight of water)).
- Osmolarity is measured using osmometer. Since osmometer is not available in most medical centers and hospitals, we instead use S.G of the urine and translate the results to osmolarity
- How osmometer works?: Each 1 Osmole of a solution depresses the freezing point by -1.86 degrees. Thus, in case of aqueous solutions, such as plasma:
- Freezing temperature = osmolarity x 1.86.

- If the freezing point of a solution is -0.5 degrees, then the osmolarity = 0.5/1.86 *1000 mOsm/l = 269 mOsm/L.
- S.G is not always a mirror image of osmolarity. SG is increased (without an increase in osmolarity) when urine contains appreciable quantities of larger particles in the urine, such as: WBC, RBC, protein, glucose, epithelial cells, casts, contrast dye… → increased SG (but not osmolarity)→ misleading results (falsely suggesting a concentrated urine), despite a low urine osmolarity. Conclusion: to transfer S.G to osmolarity, urine must not contain those particles.
- In most cases, the urine specific gravity varies in a relatively predictable way with the osmolarity, with the specific gravity rising by .001 for every 35 to 40 mosmol/kg increase in osmolarity. Thus, a urine osmolarity of 280 mosmol/kg (which is isosmotic to plasma) is usually associated with a specific gravity of 1.008 or 1.009.
- **Convert SG to osmolarity:**
- Example SG = 1.025
- Take the last two digits (25 in this example) multiply by 40.
- 25 * 40 = 1000 mOsm/L
- If SG = 1.010 at all time (± H_2O) → 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 (compared to plasma)
- 1.010 isotonic (isosthenuria) (As in intrarenal AKI)
- 1.040 concentrated…healthy kidney
- Now look at the graph below (S.G vs. osmolarity)
- The protein-free plasma specific gravity is 1.010. Isosthenuria refers to urine whose osmolarity equals 1.010; hyposthenuria and hypersthenuria refer to urine whose osmolarity is lower or higher than 1.010, respectively.



- <u>**How to perform the test**</u>
- It is a simple test detecting concentrating ability of the kidney
- Ask the patient to remain NPO (Nil per os, Latin for "nothing by mouth-English": to withhold oral intake of food and fluids) starting from midnight. At 8:00 a.m., take the first spot urine sample, then after half an hour take a second urine sample, then after another half an hour a third sample.
- Now you have 3 urine samples. One of them should be > 1000 mOsm/L → (healthy kidney).
- When you do urine routine and microscopy test for your patient (urine R&M), I advise you to ask the lab to measure the S.G of urine, some labs don't do it

•Changes of the osmolarity of the tubular fluid across the nephron. The counter current mechanism

- Countercurrent is easy to understand; fluid flows **down** the descending limb and **up** the ascending limb.

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…all these mechanisms are working if urine SG>1.025. Therefore, high S.G means the following:

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

Countercurrent: Ascending and descending currents.

Multiplication: Multiplies osmolarity of interstitium by the so called single effect, which is reabsorption of Na-K-2Cl from TALH without water.

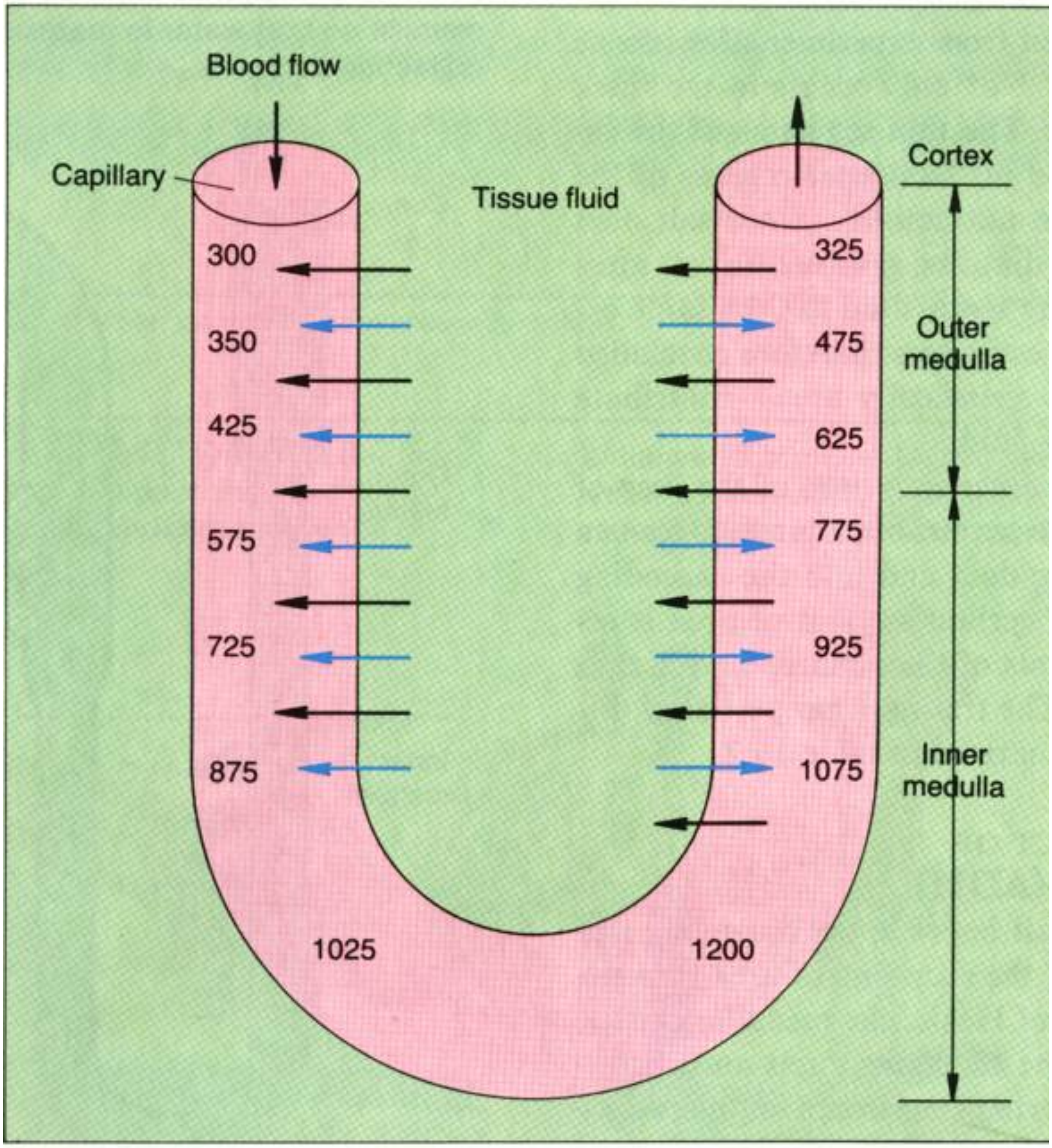
- The **critical** characteristics of the loops which make them countercurrent **multipliers** are:

1. The ascending limb of the loop of Henle actively co-transports Na+ and Cl- ions out of the tubule lumen into the interstitium. The ascending limb is impermeable to H2O.

2. Urea recycling

3. Slow flow of blood in Vasa Recta.

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. Vasa recta is the countercurrent exchange system:

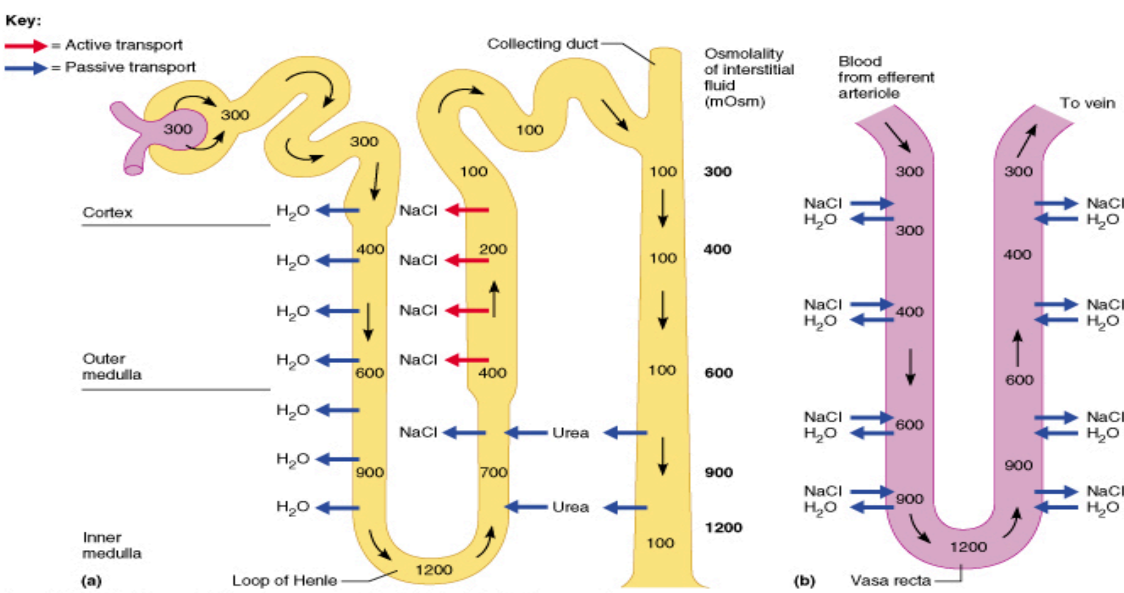


Black arrows = diffusion of NaCl and urea
Blue arrows = movement of water by osmosis

Vasa recta blood flow is low (only 1-2 % of total renal blood flow). So, with hypertension, vasodilatation (increase blood flow in vasa recta) , loop diuretics, low protein diet → Interstitium will lose its maximum hyperosmolarity → urine will be less concentrated.

Summary of Tubule Characteristics

Tubule Segment	Active NaCl Transport	Permeability H_2O	Permeability NaCl	Permeability 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	+++



- **Proximal tubule**
  - As fluid flows through the proximal tubule, solutes and water are reabsorbed in equal proportions (65%), so little change in TF osmolarity occurs; thus, the proximal tubule fluid remains isosmotic to the plasma, with an osmolarity of about 300 mOsm/L.

- 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

- **<u>Loop of Henle</u>**
- **Descending Limb of the Loop of Henle (DLLH)**
    - As fluid passes down the descending loop of Henle, water is reabsorbed by osmosis (15%) and the tubular fluid reaches equilibrium with the surrounding interstitial fluid of the renal medulla, which is very hypertonic—about two to four times the osmolarity of the original glomerular filtrate (1200-1400 mOsm/l). Therefore, the tubular fluid becomes progressively more concentrated as it flows into the inner medulla.
- **Ascending Limb of the Loop of Henle (ALLH).**

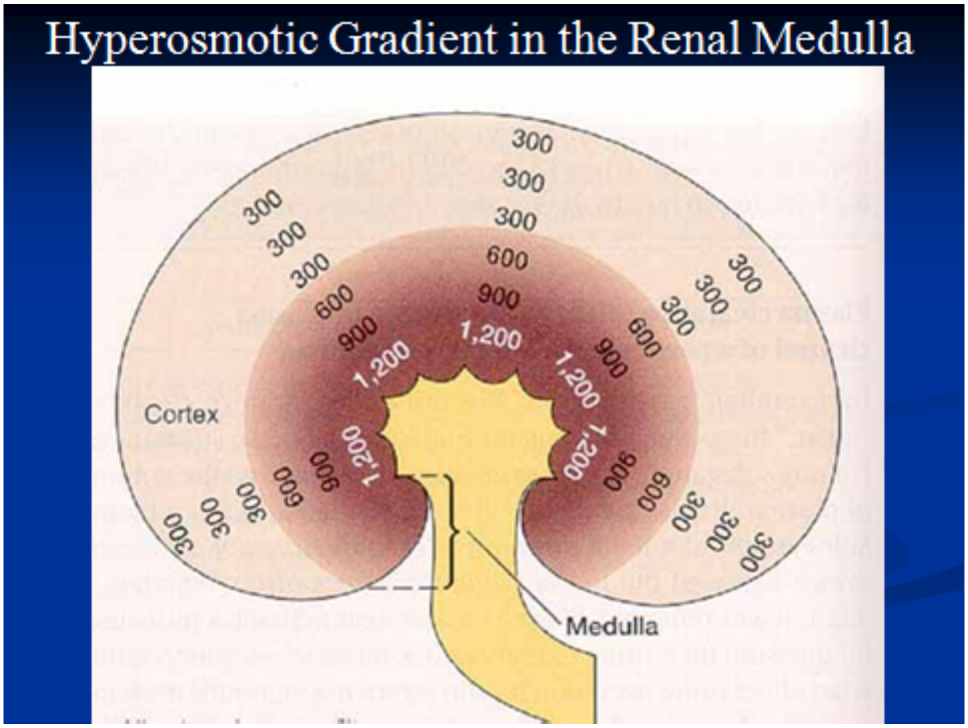
- This portion of the nephron reabsorbs NaCl actively into the interstitium of the medulla. The medulla then becomes very hyperosmotic. 700 mOsm of the medullary interstitium (out of the 1200 mOsm) is due to NaCl and the rest is due to urea. ALLH is impermeable to water. The net effect of this activity is to remove salt from the kidney filtrate and transfer it into the medulla where it can be saved for water reabsorption later on. As we will see later, 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. As described earlier, the DLLH is a thin passive segment that is permeable to water, but, impermeable to salt. 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

Distal and Collecting Tubules

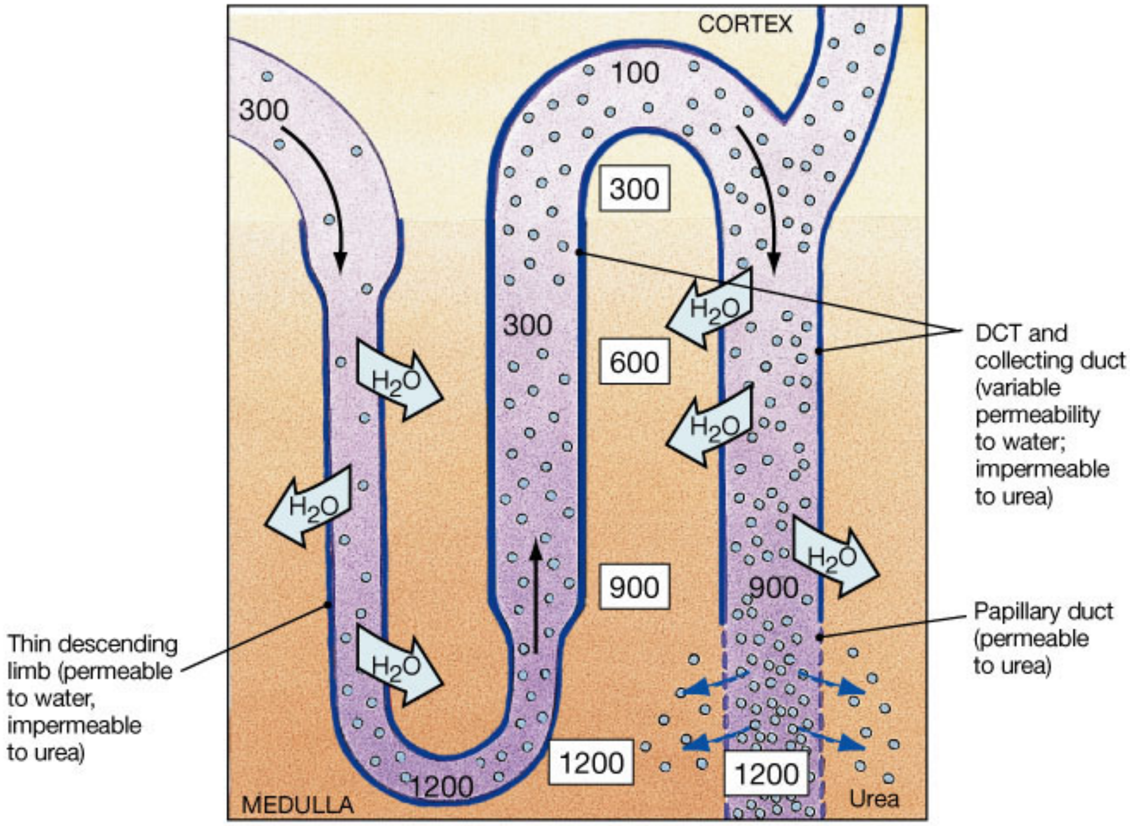
- Additional reabsorption of Na^+ causes the tubular fluid to become diluted, decreasing its osmolarity to as low as 50 mOsm/L

Collecting Ducts

- The osmotic gradient alone cannot cause reabsorption of water from these segments; ADH is also necessary. ADH dependent water reabsorption. It can increase urine osmolarity up to 1200 to 1400 mOsm/L.



- The hyperosmotic interstitium of the medulla will “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 (500 out of 1200 mOsm). As water leaves the collecting duct, the urine becomes progressively more concentrated. The osmolarity of the collecting duct fluid will increase from 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 (50-100 mOsm/l).
- ADH increases permeability of medullary collecting tubule to urea by activating urea transporters (UT-1)



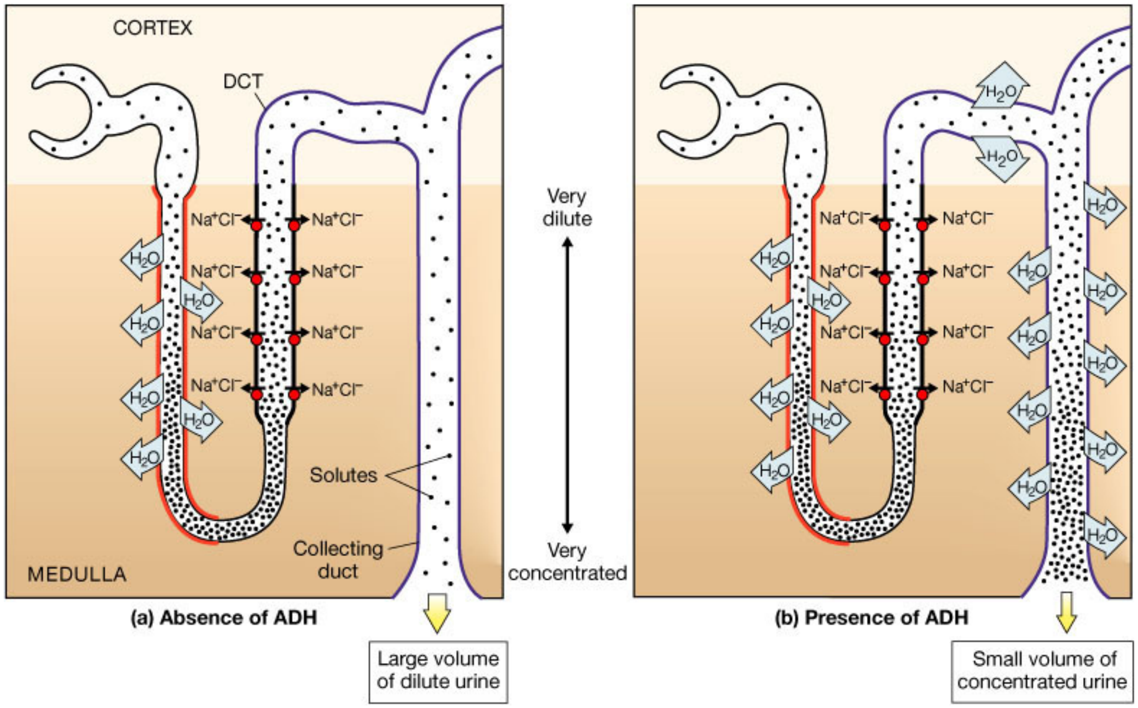
(c) The permeability characteristics of both the loop and the collecting duct tend to concentrate urea in the tubular fluid and in the medulla. The loop of Henle, DCT, and collecting duct are impermeable to urea. As water reabsorption occurs, the urea concentration rises. The papillary ducts' permeability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

The 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 TF to the medulla

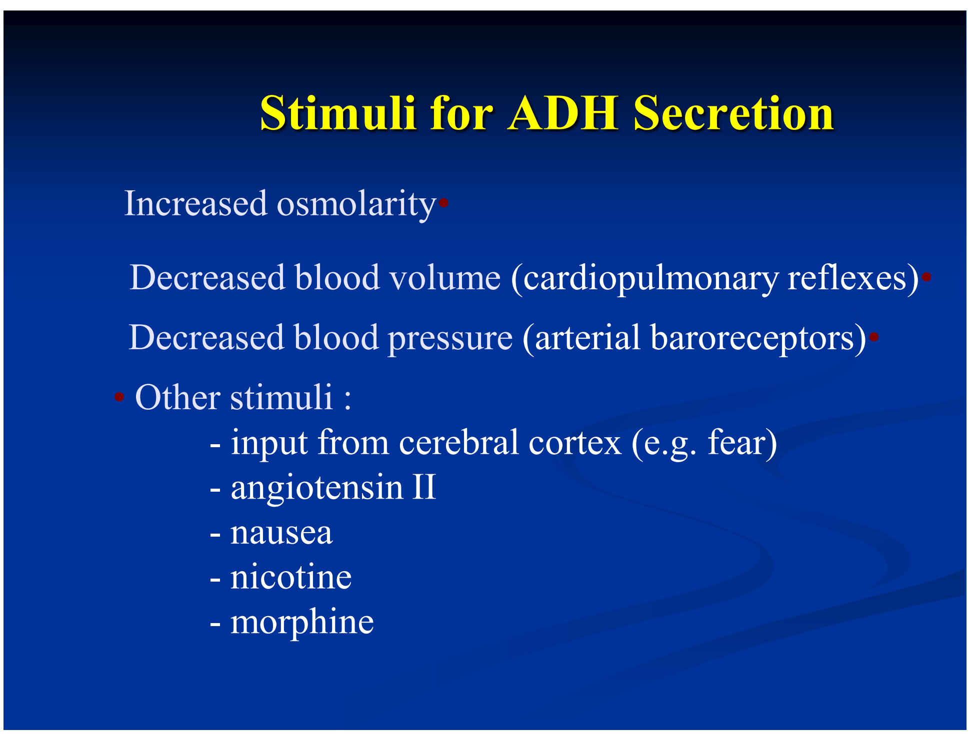
Mechanism of action of Antidiurectic Hormone ADH (vasopressin)

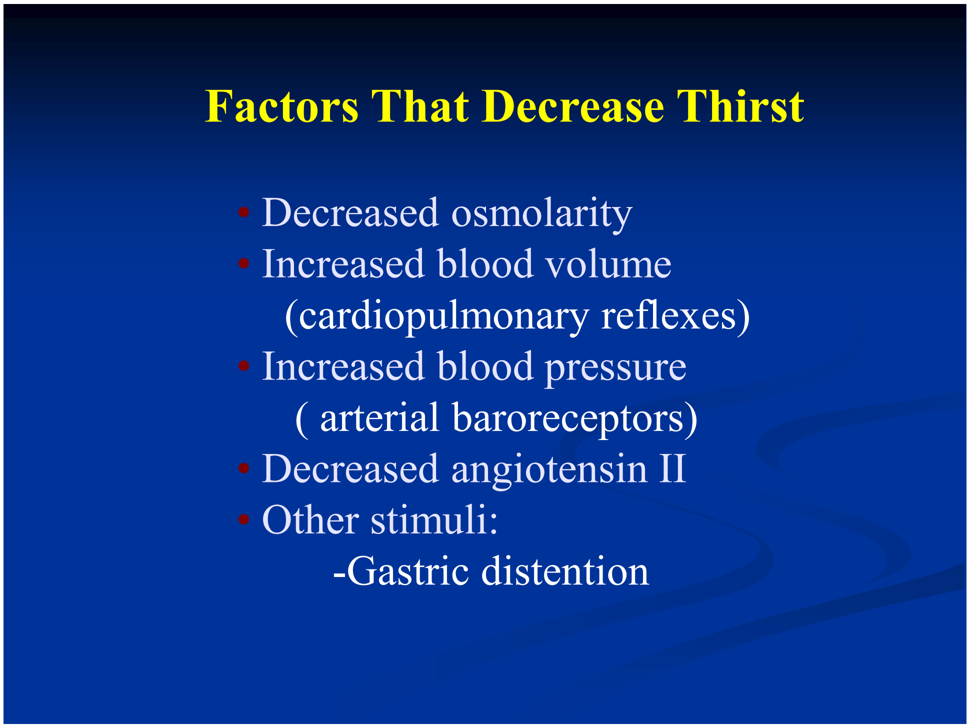
ADH is produced mainly by the supraoptic nuclei (85%), and to a lesser extent by paraventricular nuclei (15%) in the hypothalamus.

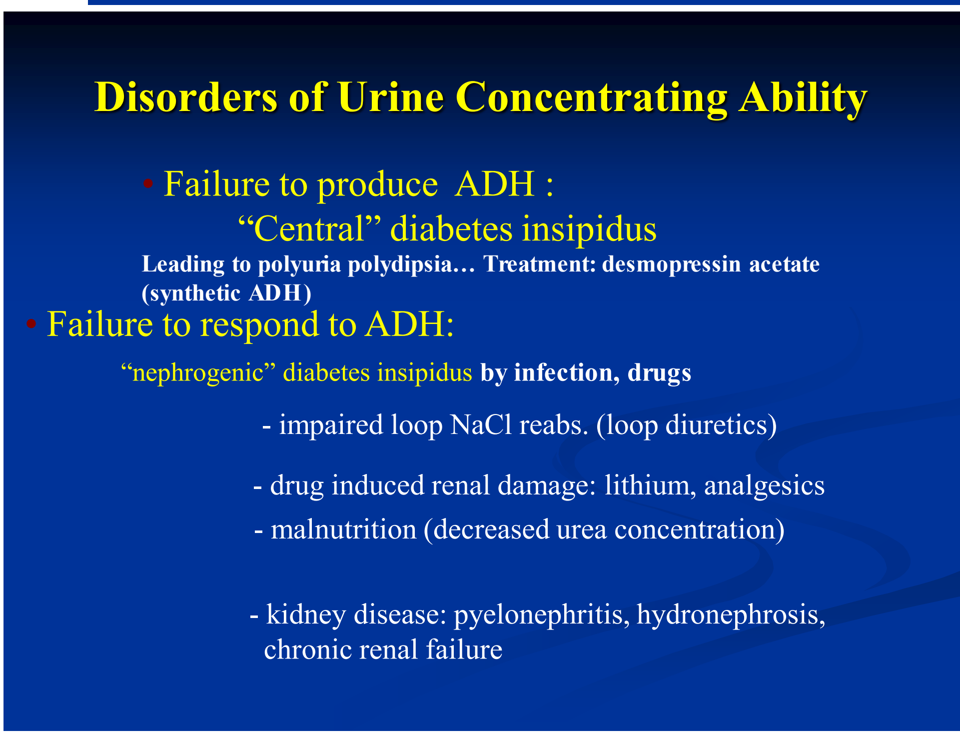
Osmoreceptors will send impulses to supraoptic neurons forcing them to make more ADH, which in turn it is transported by their axon and stored in the nerve terminal in the posterior pituitary. The availability of (ADH) is determined by dehydration and thirst.



In the kidney, ADH binds to its receptors on basolateral membrane, introducing H2O pores at apical site (AQP2). AQP3+4 don't respond to ADH







- **This high osmolarity of the medullary interstitial fluid is maintained by three complex mechanisms:**

1. The thick ascending loop of Henle continuously pumps Na^+ and Cl- into the surrounding interstitium without permeating water to be reabsorbed by osmosis (even in the presence of large amounts of ADH). This process is called the single

effect (it is single because it involves Na+ reabsorption alone without water following it), and when combined with flow of fluids from the descending to the ascending loops of Henle, it is called countercurrent multiplication and accounts for about 700 of the 1200 mOsm/L.

2. The medullary collecting ducts facilitate reabsorption of urea which is found in high concentrations in these ducts. This is even more activated in the presence of ADH. The reabsorption of urea into the interstitium is passive and accounts for about 500 of the 1200 mOsm/L.

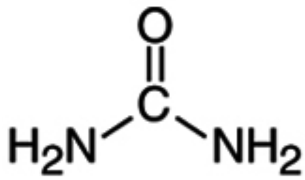
- **Note** that 50% of the filtered load of urea is reabsorbed by the proximal epithelial cells but in the ascending loop of Henle, the distal tubules and the cortical collecting tubules, little urea is reabsorbed because these segments are impermeable to urea.
- **Note**: urea reabsorbed from the medullary collecting ducts is secreted again into the ascending loop of Henle, and so on.

3. The blood vessels which supply medullary cells (vasa recta) carry only 2-3% of the renal blood flow. This prevents the loss of the hypertonicity of the medullary interstitial fluid, though does not add to the osmolarity of the interstitial fluid. With vasodilation, more blood flow in vasa recta → washing away NaCl and urea → less concentrated interstitium. Can you imagine if vasa recta leaves the medulla without coming back to the cortex?. Answer: Vasa recta will prevent dissipation of osmolarity, but doesn't make it…it is U-shape, so it comes back to the cortex, otherwise if it is straight, it will remove solutes from the medulla and disrupt hyperosmolar interstitium.

- 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.

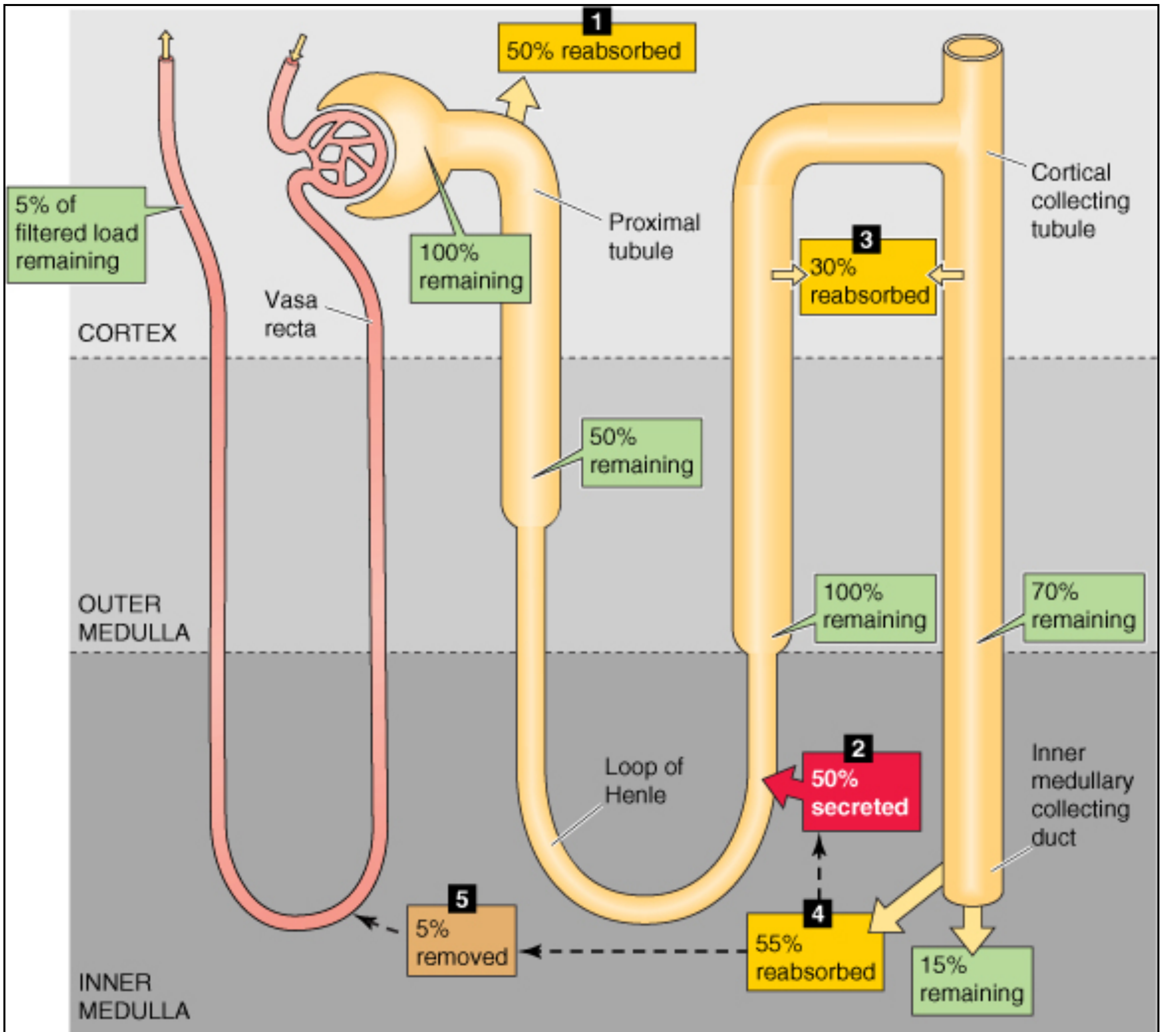
The role of urea in the ability of the kidney to make concentrated urine:

- Urea very useful in concentrating urine.
- High protein diet = more urea = more concentrated urine. Is the end metabolite of proteins?
- Kidneys filter, reabsorb and secrete urea. Therefore, it is not used as a molecular marker. Plasma creatinine is more specific for kidney function. Plus plasma urea might rise in nonrenal causes (Dehydration, GIT bleeding, burns etc.)
- Urea excretion rises with increasing urinary flow.

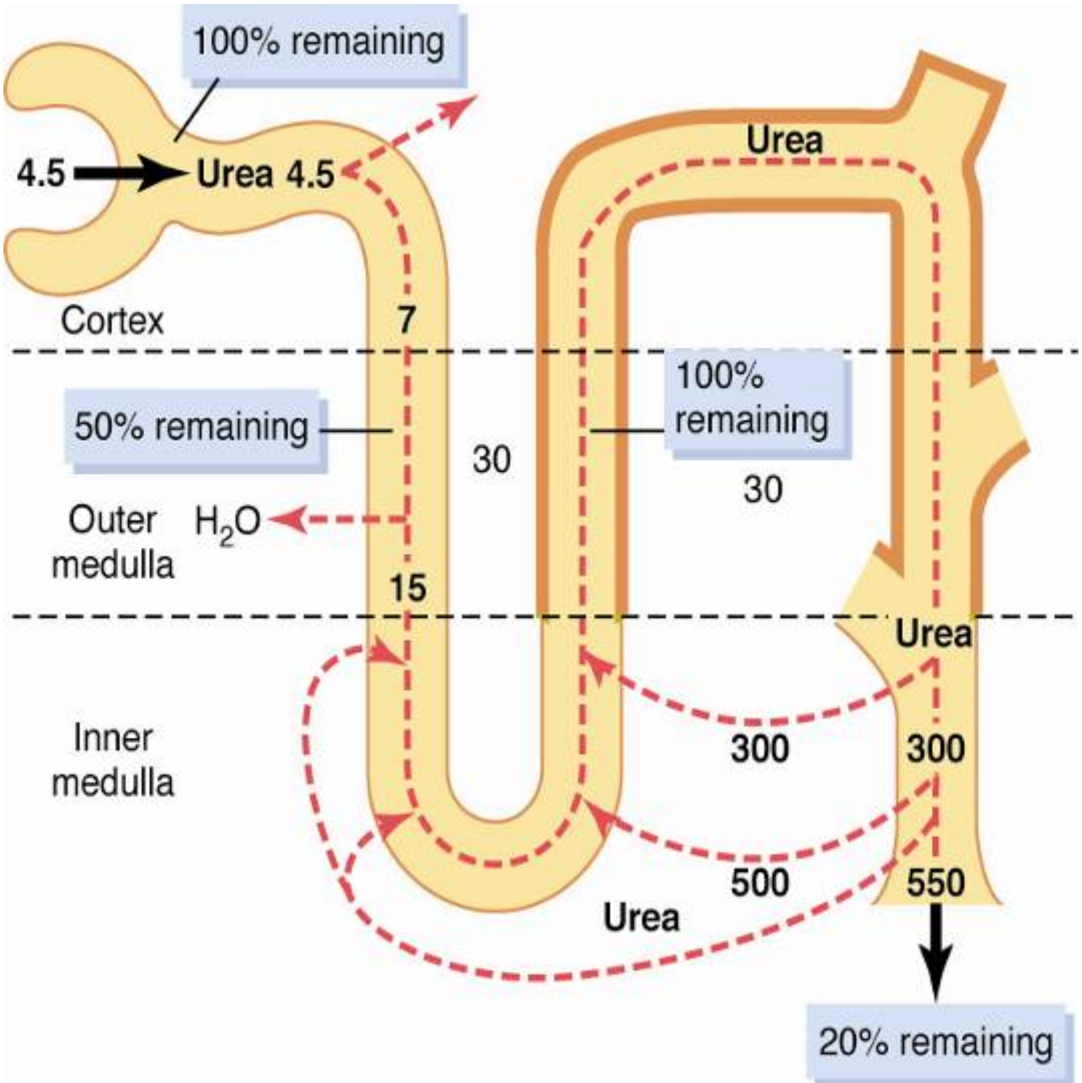


- **Urea**
- Urea MW 60 → small molecular weight→ filtered freely
- Urea: Its concentration 15-40 mg/dl (2.5-6.5 mmol/L).
- In complete renal shutdown it rises by about 5 mmol/L per day.
- Filtered load /day = 180 L * 4.5 mMol =700 – 800 m mol/day
- → 50% is excreted, 50% is reabsorbed in proximal tubule passively.
- What is the clearance of urea, if 50% of filtered amount is excreted?
- 50% of GFR (180 L/day) → 90L/day (65 ml/min)
- 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.

Summary of urea cycle



1
50% reabsorbed
5% of
filtered load
remaining
100%
remaining
Proximal
tubule
Vasa
recta
3
30%
reabsorbed
Cortical
collecting
tubule
CORTEX
50%
remaining
OUTER
MEDULLA
100%
remaining
70%
remaining
2
50%
secreted
Loop of
Henle
Inner
medullary
collecting
duct
5
5%
removed
4
55%
reabsorbed
15%
remaining
INNER
MEDULLA



❖Factors than can depress the maximum concentrating ability of the kidney:

- Low protein diet (as in vegetarians) which decreases urea formation.
- Increased blood pressure or RBF, which increases medullary blood flow in the vasa recta, thereby washing out the solutes contributing to the hyperosmolarity of the interstitium.
- Diuretics, which decrease NaCl reabsorption losing the ability to concentrate the urine.

Few questions (13) related to this lecture. No answers are provided. Try to answer them.

1. Based on MW and concentration only; which one has more osmotic effect: 2 grams of albumin/dl (MW 70KD) or 3 grams of globulin/dl (MW 140KD): I leave this question

A. albumin
B. Globulin
C. both has equal osmotic effect
D. osmotic pressure has nothing to do with MW.
E. cannot be predicted from the above data

2. In a normal human who has a diuresis due to drinking a large volume of water; all the following statements are true EXCEPT:
45, 29
18 A. the osmolarity of the urine is less than 300 mosmol per liter.
30 B. the renal venous blood has a higher osmolarity than the renal arterial blood.
69 C. creatinine clearance may be 50% above that in the nondiuretic situation.
24 D. vigorous (severe) muscular exercise will inhibit the diuresis.
11 E. plasma antidiuretic hormone (ADH) levels are decreased.

3. We must excrete, at least half liter of urine/day? The reason behind that is:
61, 44
7 A. half liter is the minimum daily water intake.
93 B. the ability of the kidney to make concentrated urine is limited to 1200-1400 mOsm/liter.
27 C. GFR is equal to 125 ml/min.
7 D. the proximal tubule cannot reabsorb this extra half liter.
19 E. due our body metabolism, half liter of water is produced daily.

4. Choose the WRONG statement. The cells of the distal convoluted tubule
A. Reabsorb some of the water filtered by the glomeruli
B. Secrete hydrogen ions into the tubular lumen
C. Form NH_4^+ ions
D. Reabsorb sodium in exchange for hydrogen or potassium ions
E. Determine the final composition of urine.

5. Loop diuretics which inhibits NaCl reabsorption in thick ascending limb will
0.64, 0.48
8 A. decrease osmolality of tubular fluid leaving the thick ascending limb.
20 B. increase maximum urine osmolality.
3 C. increase glucose clearance.
90 D. increase NaCl clearance.
20 E. decrease K^+ excretion.

6. In normal individual under normal diet and normal physical activity; compared to plasma, urine has (↑: higher ↓lower →equal):
0.62, 0.37

	2. [K+]	3. pH	4. [Urea]	5. SG
8 A.	6. ↑	7. ↑	8. ↑	9. ↑
18	10. ↓	11. ↓	12. ↓	13. ↓

B.				
12 C.	14. →	15. ↑	16. ↑	17. ↑
87 D.	18. ↑	19. ↓	20. ↑	21. ↑
16 E.	22. ↓	23. →	24. ↑	25. ↑

7. Which of the following does NOT contribute to the formation of maximally concentrated urine?
26. 27 0.72 0.43 165 7 42 6 10 A
165 A. active NaCl transport in the proximal convoluted tubule
7 B. active NaCl transport in the thick ascending limb of the loop of Henle
42 C. impermeability of the thick ascending limb of the loop of Henle to water
6 D. high water permeability of the collecting duct due to presence of ADH
10 E. presence of urea in the inner medullary interstitium

8. Minimum obligatory urine output in a 6 y old child (20 kg body weight) is equal to:
27. 0.82 ,0.34 4 77 6 7 0
4 A. 1 ml/min
77 B. 300 ml/m2 body surface area/day
6 C. 500 ml/day
7 D. 1 ml/kg/h
0 E. There is no minimum obligatory urine output in children

9. In the presence of high concentration of ADH most of the filtered H2O is reabsorbed at:
28. 0.31 , 0.21 29 0 1 9 55
29 A. proximal tubule
0 B. thick ascending limb
1 C. early distal tubule
9 D. late distal tubule
55 E. collecting tubules and ducts

10. Concerning urinary concentration, one of the following statements is CORRECT:

82 A. the thick loop of Henle generates most of the osmotic gradient needed for reabsorption of water in the collecting duct8
8 B. the tubular urine that reaches the collecting duct is generally hypertonic with respect to plasma
1 C. in the absence of ADH, urine is more concentrated.
1 D. osmolarity of urine is not affected by food intake
13 E. if we drink sea water our urine osmolarity might reach as high as 2000 mOsm/l

11. Regarding minimum daily obligatory volume of urine, all the following are true EXCEPT: We have two answers. This question was deleted
27 A. is more if a person eats protein-rich diet.
5 B. in general it is equal to 300 ml/m2 body surface area/Day

6 C. roughly speaking it is around 20 ml/h in an adult.
2 D. is increased if a person is under diuretic therapy.
49 E. is depressed if blood flow to vasa recta is increased.

12. Concerning normal renal function in diluting and concentrating urine, which of the following is TRUE?

4 A. the maximum concentration of urine that the human kidney can make is enhanced by taking diuretics.
17 B. urine osmolarity can be estimated from urine [Na+] * 2.1
7 C. human can survive by drinking sea water instead of fresh water.
54 D. the lowest concentration of urine that the human kidney can produce is still less than the osmolarity of plasma.
22 E. specific gravity depends on the number of the particles present in a given volume of urine.

13. Inhibition of NaCl reabsorption in thick ascending limb will lead to all the following EXCEPT

69,19
6 A. decrease medullary osmolarity
72 B. increase maximum urine osmolarity.
8 C. increase urine output.
13 D. increase NaCl clearance.
5 E. increase NaCl excretion per day