

Lab 7: The Urinary System

Protocol slides
PCB 3702L
FIU



Lab 7 Protocol objectives

1. Conduct a urinalysis to determine whether unknown simulated urine samples contain glucose or protein.
2. Identify the kidney structures and functions while performing the dissection of a pig's kidney.
3. Explain the filtrate pathway from the nephron until it reaches the outside of the body.

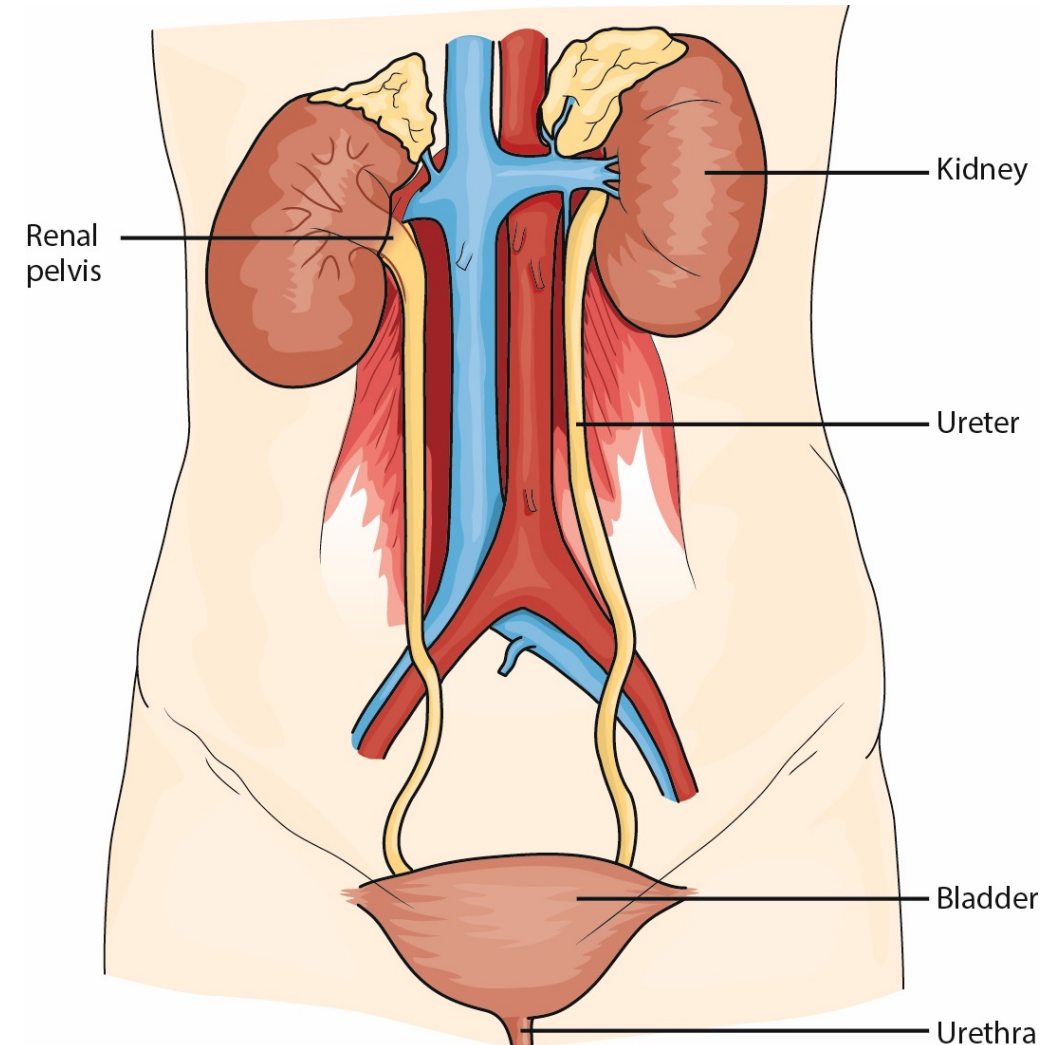
Introduction to the Urinary System

Functions

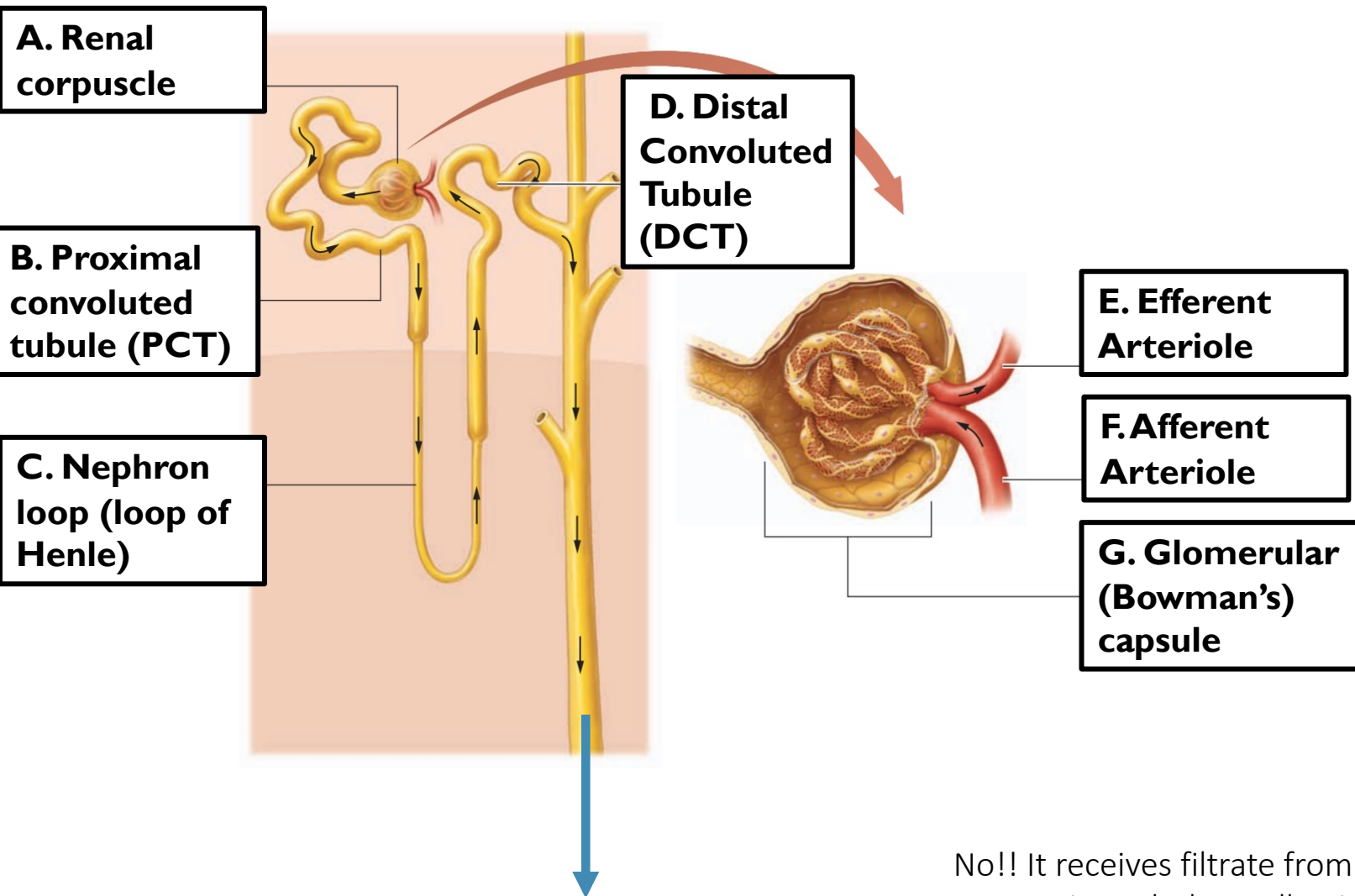
- Main function is to regulate the composition of plasma by eliminating excess water, wastes, solutes, and maintaining those that are needed.
- Also regulates blood ionic composition, blood pH, blood volume, blood pressure, blood osmolarity, and the production of hormones.

Anatomy

- **Kidneys - main organ of the urinary system**
 - Right kidney is 2-8cm lower than the left kidney because of the large liver superior to it
 - Functions include excreting wastes in the urine
- **Ureters:** transport urine from the kidneys to the urinary bladder
- **Urinary Bladder:** stores urine and expels it into the urethra
- **Urethra:** discharges urine from the body



Nephron Anatomy

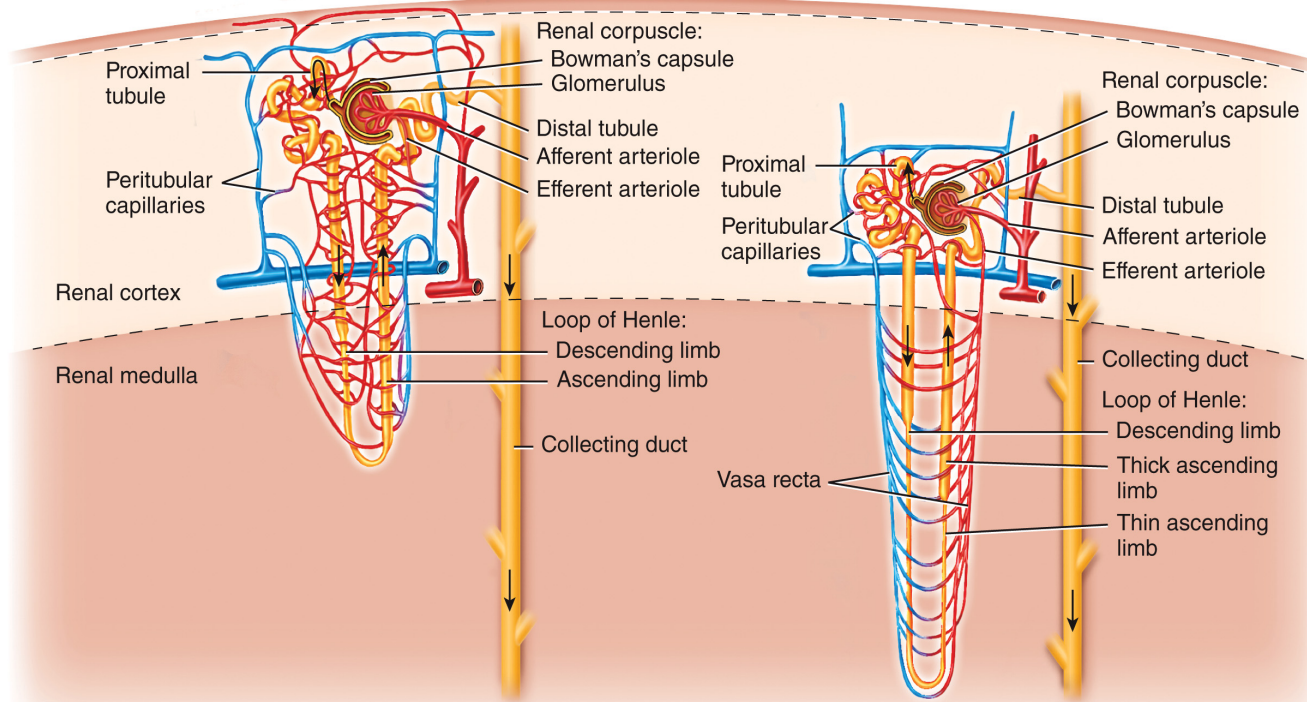
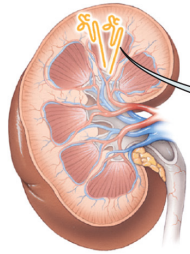


Is the collecting duct part of the nephron?

The Nephron

- The nephron is the functional unit of the kidney!
- The nephron consists of two parts: the renal corpuscle and the renal tubule.
 - **Renal Corpuscle:** includes the Bowman's capsule (glomerular capsule) and the glomerulus.
 - **Renal Tubule:** includes the proximal convoluted tubule, nephron loop (Loop of Henle), and the distal convoluted tubule.

No!! It receives filtrate from several nephrons!!! Together the connecting tubules, collecting ducts, and papillary ducts form the collecting system.



(d) Cortical nephron and blood supply

(e) Juxtamedullary nephron and blood supply

Types of Nephrons

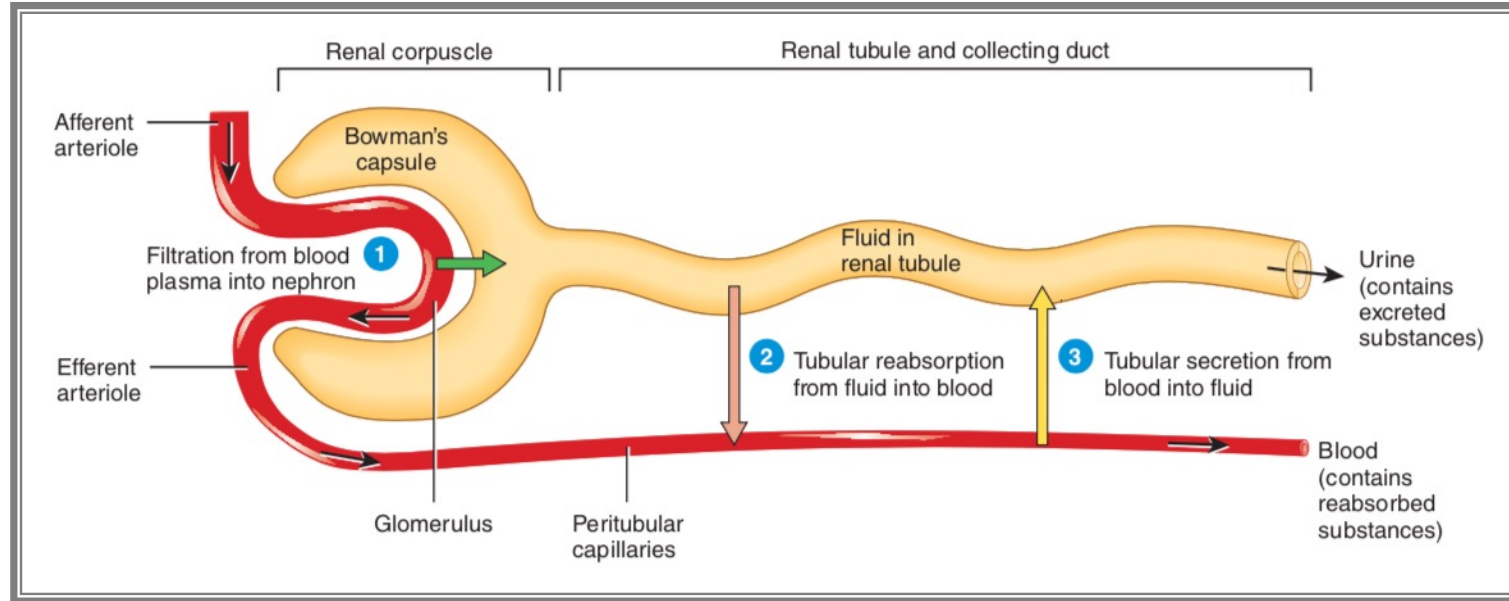
1. Cortical Nephrons

- ~80% of the kidney's nephrons are this type
- Most the nephron is found within the renal cortex, some parts reach the renal medulla
- Have short loops of Henle

2. Juxtamedullary Nephrons

- Remaining ~20% of the kidney's nephrons are this type
- Renal corpuscles lie deep in the cortex, closer to the medulla
- Have long loops of Henle that goes into the deepest regions of the renal medulla

Renal Processes



Glomerular Filtration (Pressure Filtration): Filter's blood before it enters the tubules. Movement is driven by blood pressure

- Water and most solutes in blood plasma move from the glomerulus to Bowman's capsule
- Produces glomerular filtrate

Tubular Reabsorption: Substances in the filtrate are reabsorbed into the bloodstream

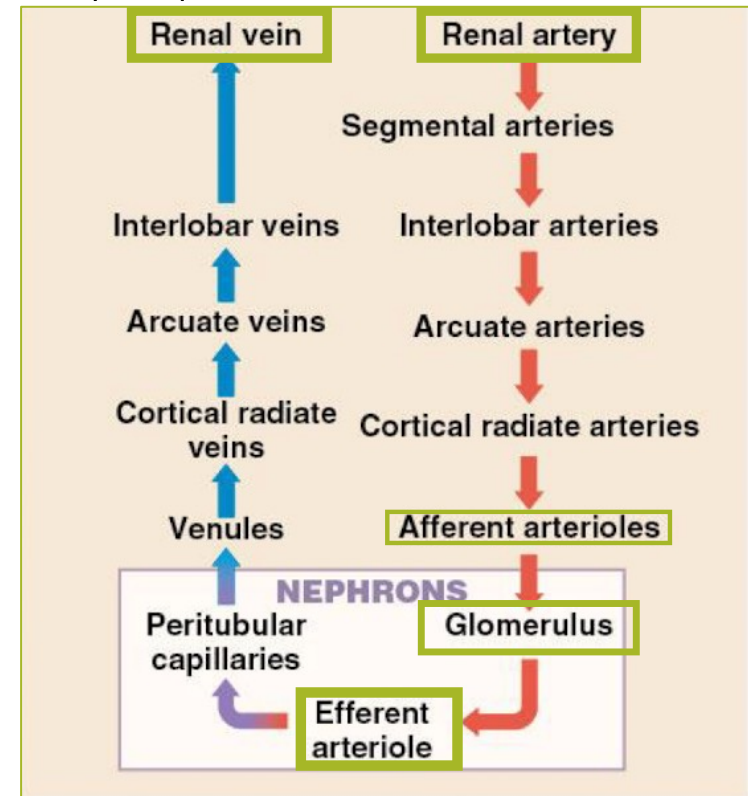
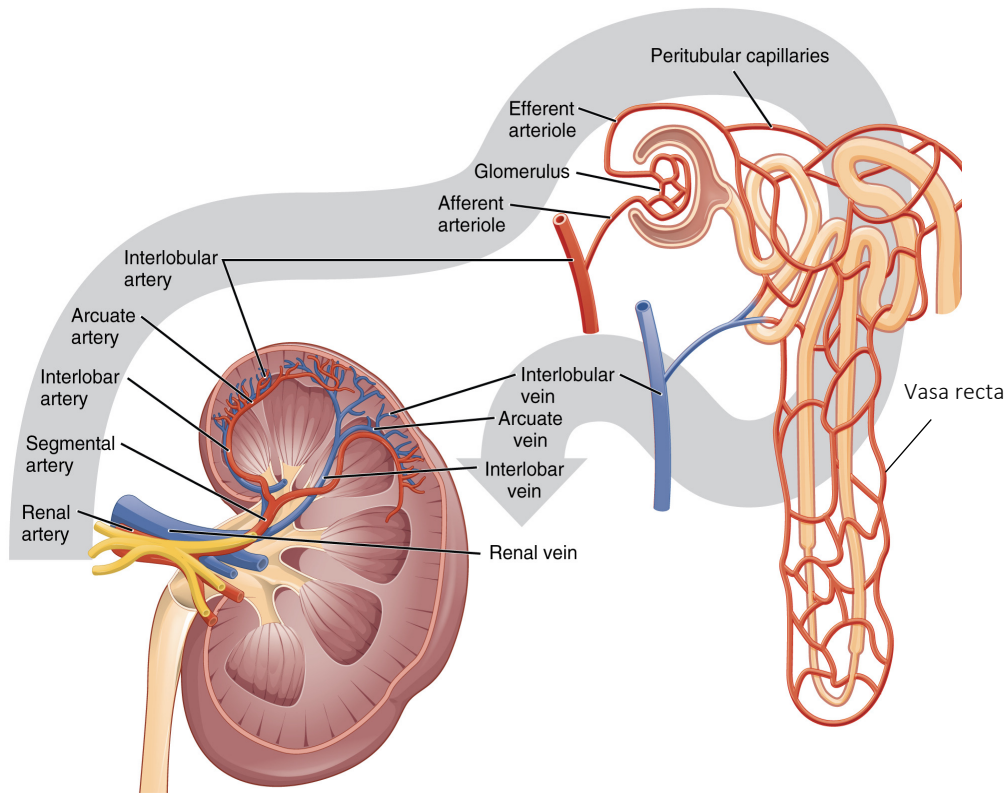
- Starts within the PCT, which is where most reabsorption occurs (~75%)
- 99% of the glomerular filtrate reabsorbed by renal tubules
- 1% excreted as urine

Tubular Secretion: Substances are removed from the blood and secreted into the tubules

- Removes wastes such as urea, uric acid, and creatinine; excess ions such as H^+ , K^+ , and $HClO_3^-$, penicillin, aspirin, and morphine
- Primarily occurs in the proximal and distal tubules and involves active transport mechanisms

Renal Blood Flow

- Blood enters the kidney via the renal artery and exits via the renal vein.
- Blood enters the glomerulus via the afferent arteriole and exits via the efferent arteriole.
- Peritubular capillaries surround the:
 1. The tubular parts of both cortical and juxtamedullary nephrons that are within the renal cortex
 2. Short loops of Henle of cortical nephrons
- Vasa recta surrounds the long loops of Henle of juxtamedullary nephrons.



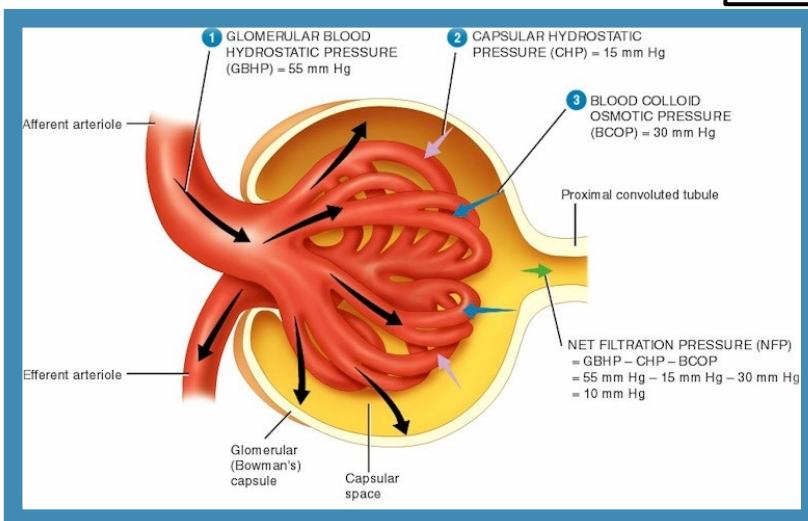
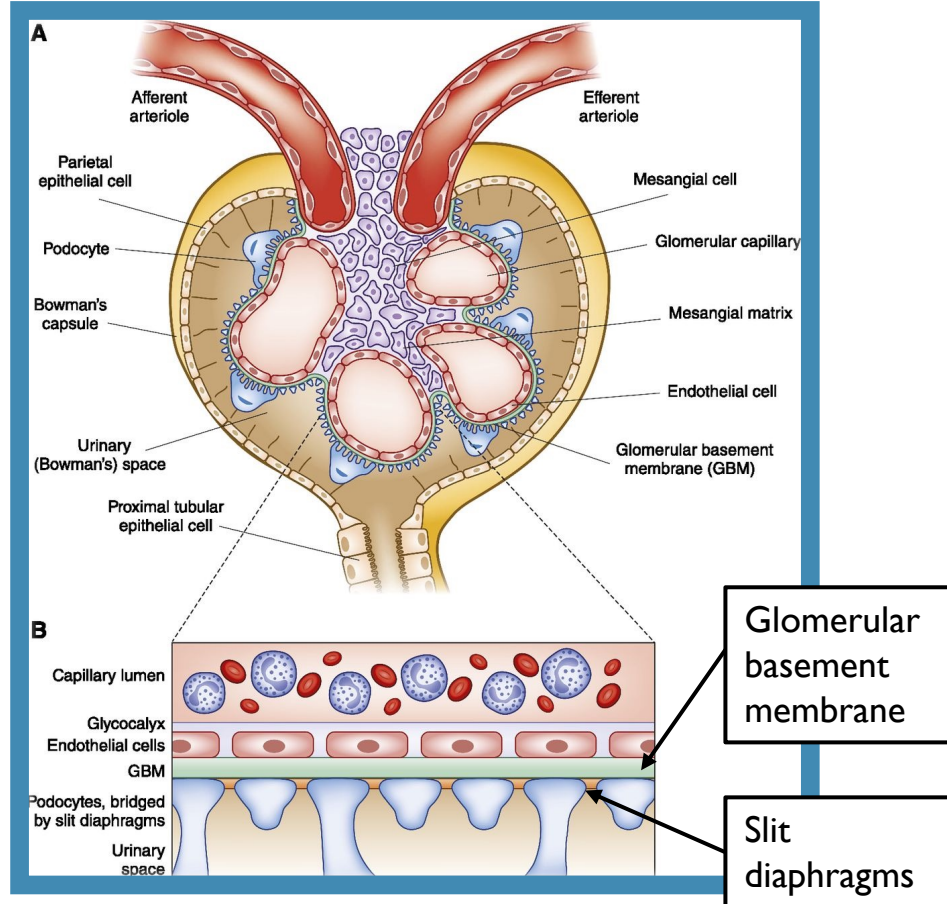
Renal Corpuscle

Bowman's (Glomerular) Capsule:

- Double-walled epithelial cup surrounding the glomerular capillaries.
- Made up of two layers (inner visceral and outer parietal layer) composed of simple squamous (flat) cells.

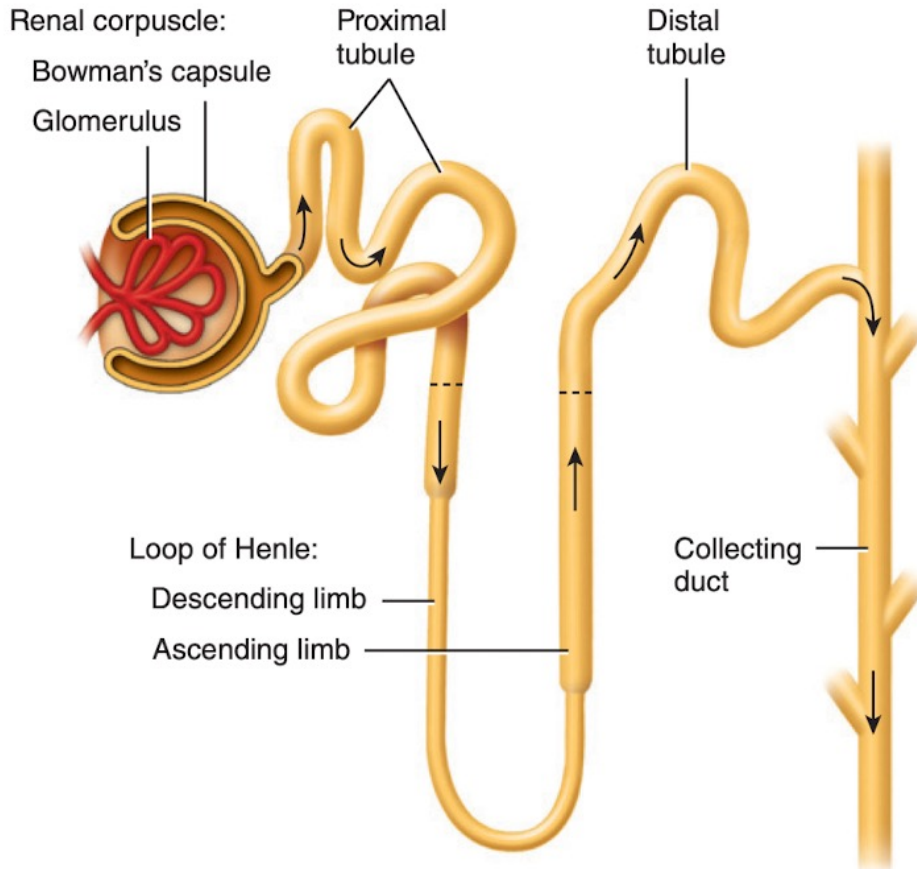
Glomerulus: capillary network

- This is the site of blood filtration
- The glomerular membrane is **semipermeable**, its mesh like shape makes it selective
 - Permits passage of water and solutes including ions, glucose, amino acids, creatinine, and uric acid
 - **DOES NOT** allow passage of cells (like RBCs) and proteins unless there is damage to the glomerulus
- **Glomerular Blood Hydrostatic Pressure (GBHP):** force exerted from the pressure of the blood vessels. Makes filtration possible by pushing filtrate out of the capillaries and into the renal tubules.



Renal Tubules

- Consists of the PCT, loop of Henle, and DCT



(c) Nephron and collecting duct

Proximal Convoluted Tubule: Receives filtrate from the renal corpuscle

- Reabsorption:
 - Largest amount of solute & water reabsorption occurs here
 - 65% of water and sodium (Na^+) from the filtrate is reabsorbed
 - Glucose, amino acids, vitamins, and ions such as Cl^- , K^+ , Ca^{2+} , HCO_3^- should be reabsorbed at this point
- Secretion: Hydronium ions (H_3O^+)
- Brush border of microvilli along their apical membranes which increase the surface area for reabsorption and secretion

Loop of Henle (Nephron Loop): Includes the descending and ascending limbs

- Descending Limb:** Permeable to water and impermeable to ions
 - 15% of water reabsorbed here
 - NO transport of ions
- Ascending Limb:** Permeable to ions and impermeable to water
 - Na^+ , K^+ , Cl^- ions are reabsorbed here
- Countercurrent Multiplier:** Osmolarity of filtrate gets larger as it descends (filtrate gets more concentrated with solutes)

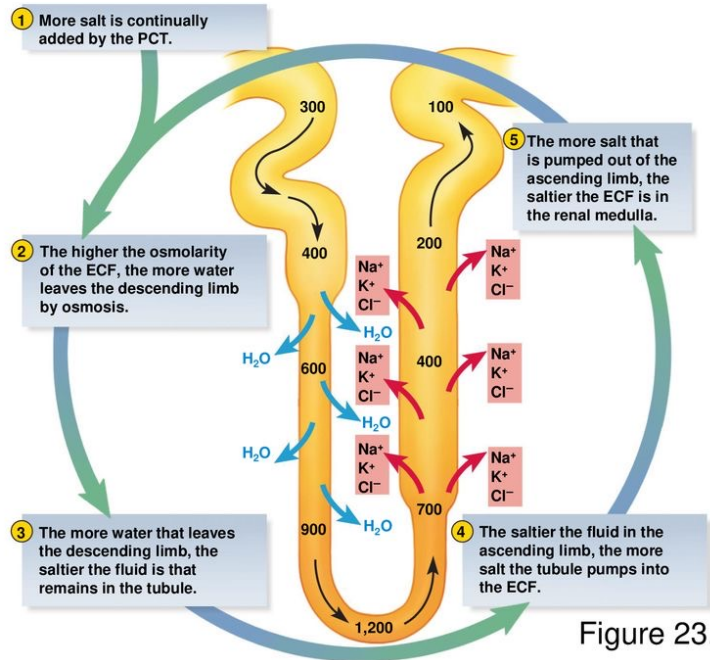
Distal Convoluted Tubule: Region between the loop of Henle & collecting duct

- Partially responsible for the regulation of potassium, sodium, calcium, and pH
- Reabsorption: Water, sodium (Na^+), chloride (Cl^-)
- Secretion: potassium (K^+) and hydrogen (H^+)

Countercurrent Mechanisms

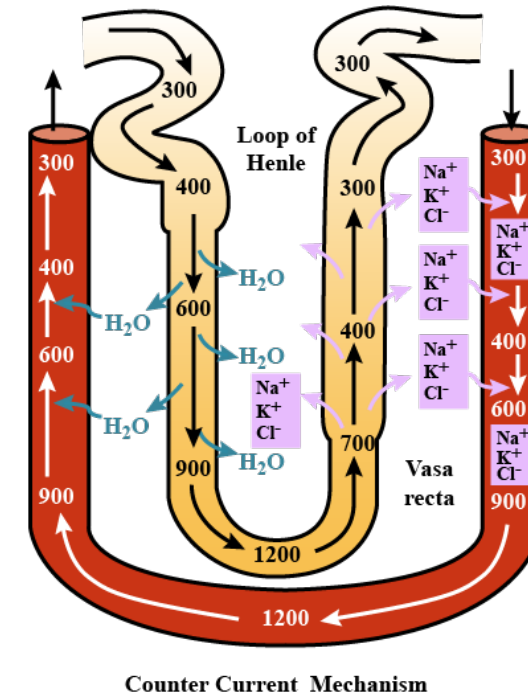
Countercurrent Multiplier

Loop of Henle



Countercurrent Exchanger

Vasa Recta



Renal Balance

- The kidneys filter 180 liters of plasma per day, with most of the filtrate being reabsorbed
- Renal imbalances can lead to the appearance of certain substances not usually found in urine
- **Glucosuria:** Appearance of glucose in the urine
 - Causes: diabetes, high carbohydrate meals, stress
- **Hematuria:** Presence of erythrocytes (red blood cells) in the urine
 - Causes: Bleeding somewhere in the urinary tract due to kidney stones, kidney damage, etc
 - If there is a high number of RBCs being broken down in circulation, the kidneys will excrete chains of hemoglobin. This will give urine a dark brown to reddish color.
- **Ketonuria:** Excessive number of ketones in the urine
 - Causes: Starvation or low carbohydrate diets
 - When carbohydrates are low in the body, the body starts to catabolize fats into fatty acids and glycerol. Fatty acids are then converted into ketones in the liver. This increase in ketones in the body leads to the appearance of ketones in the urine.
- **pH:** The healthiest urine pH readings hover around 7.0, usually fluctuating between 6.5 and 7.5
 - A higher urine pH reading would indicate that your system is more alkaline, while a lower urine pH indicates acid residues in your body.
 - Acidic urine can contribute to the formation of stones of uric acid in the kidneys, ureters, or bladder.
 - A diet high in citrus, vegetables, or dairy can increase urine pH. pH is regulated by bicarbonate and H^+ ions.

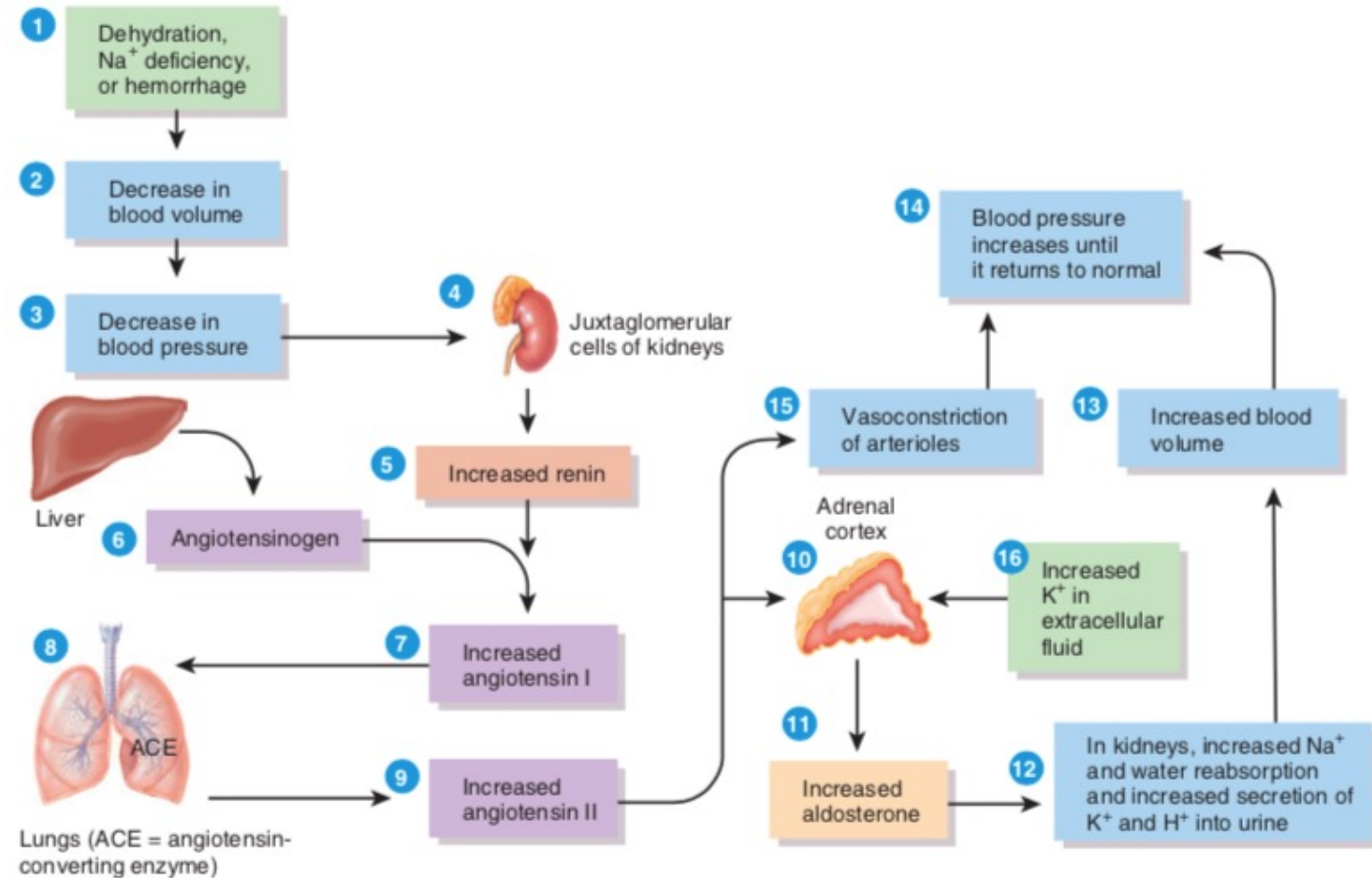


Renin-Angiotensin-Aldosterone System (RAAS)

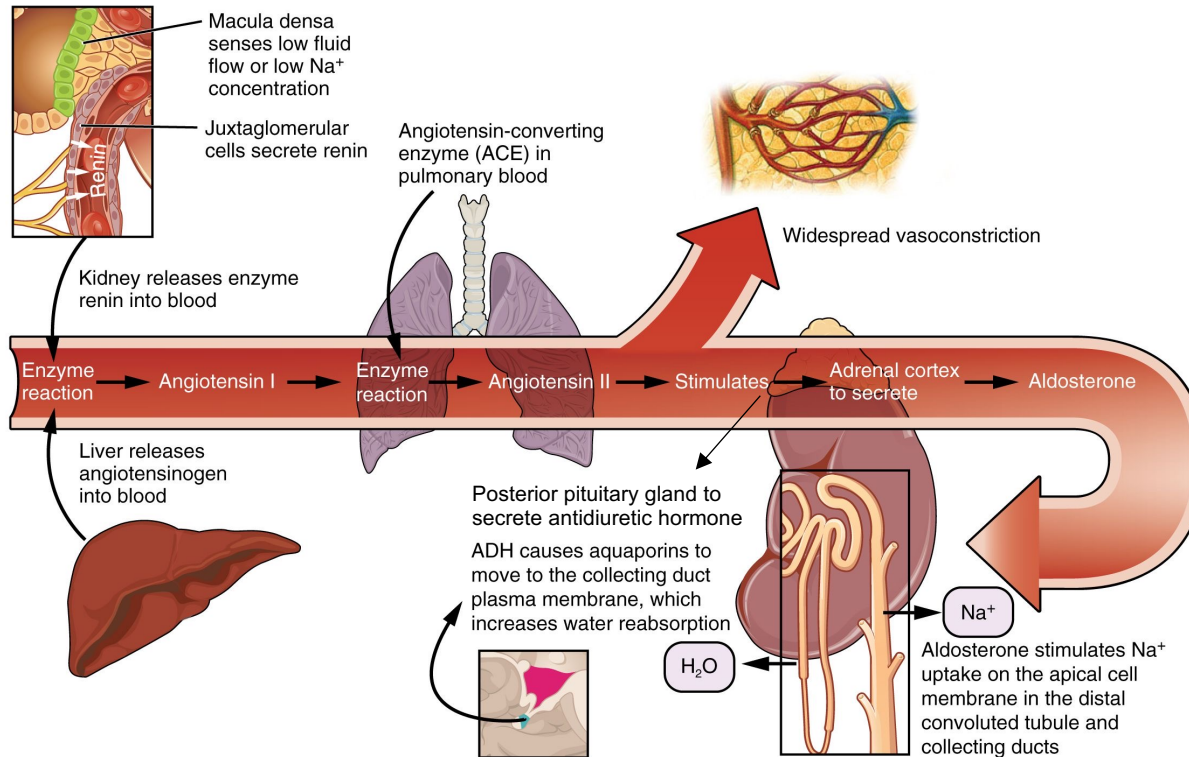
Juxtaglomerular Apparatus (JGA):

- Juxtaglomerular cells of the afferent arteriole
- Macula Densa cells of the DCT
- Plays an important role in regulation blood pressure.

- 1) ↓BP in the renal corpuscle
- 2) Juxtaglomerular cells release the enzyme renin into the bloodstream
- 3) **Renin** converts angiotensinogen (plasma protein produced in the liver) to angiotensin I
- 4) Angiotensin I circulates the body and arrives at the capillaries of the lungs
- 5) Angiotensin Converting Enzyme (ACE) present in these capillaries converts angiotensin I into angiotensin II



SUMMARY



Renin-Angiotensin-Aldosterone Pathway

Angiotensin II has three major effects:

- causes **vasoconstriction**
- stimulates release of **antidiuretic hormone (ADH)** from the **posterior pituitary gland**
 - ADH is produced in the hypothalamus and stored/released by the posterior pituitary gland
 - ADH promotes water reabsorption by inserting aquaporins into DCT and collecting ducts
- stimulates release of **aldosterone** from the **adrenal cortex**
 - aldosterone stimulates Na^+ reabsorption within the DCT and collecting ducts which consequently allows more water to be reabsorbed as well

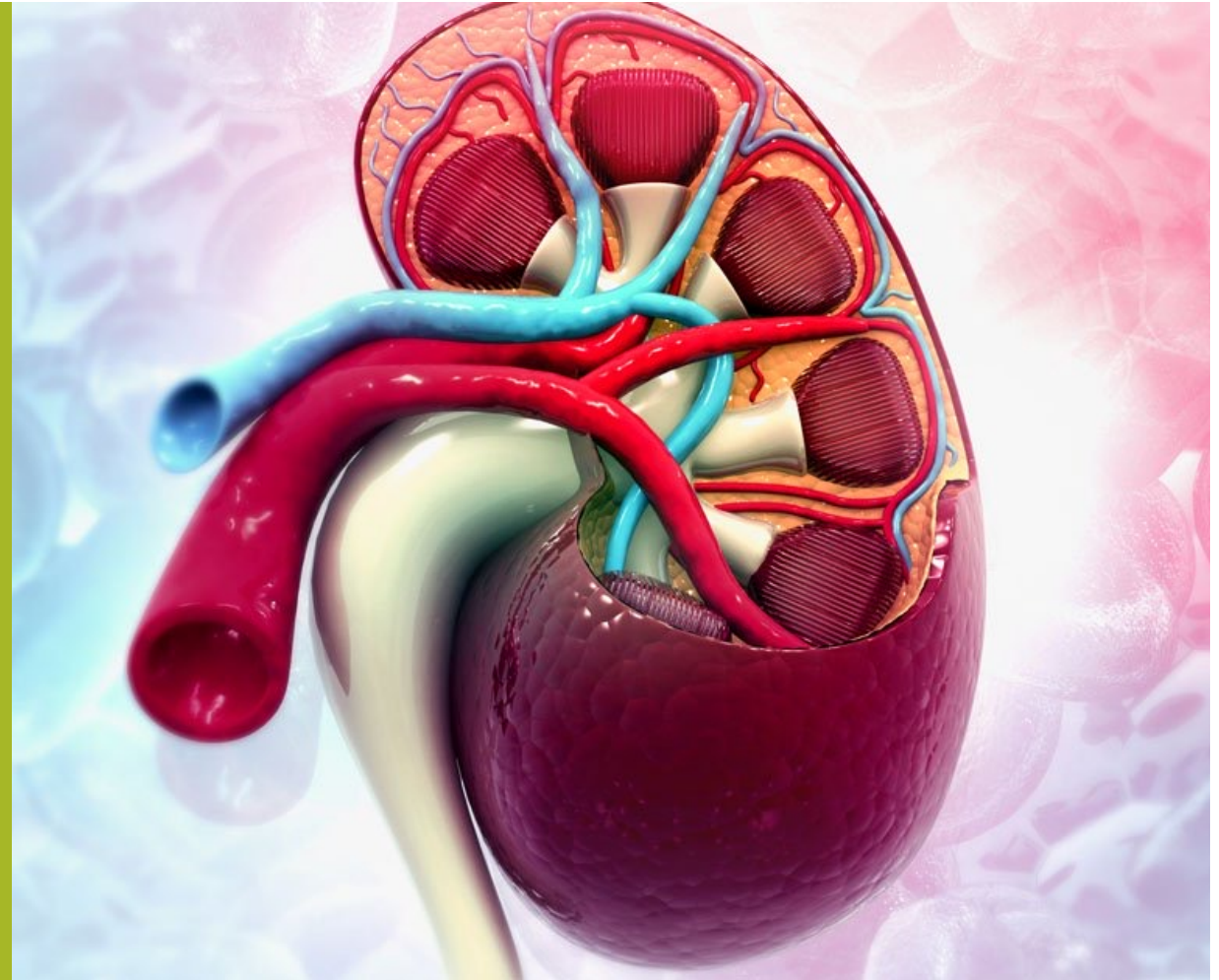
Overall effect: **Increase blood pressure**
(due to vasoconstriction and increased blood volume)

Question

A common medication for treatment of high blood pressure are ACE inhibitors. The inhibition of ACE results in dilated blood vessels and a low blood pressure. Why would the inhibition of ACE have these effects on patients?

Inhibition of ACE prevents the formation of _____ ?

- angiotensin II

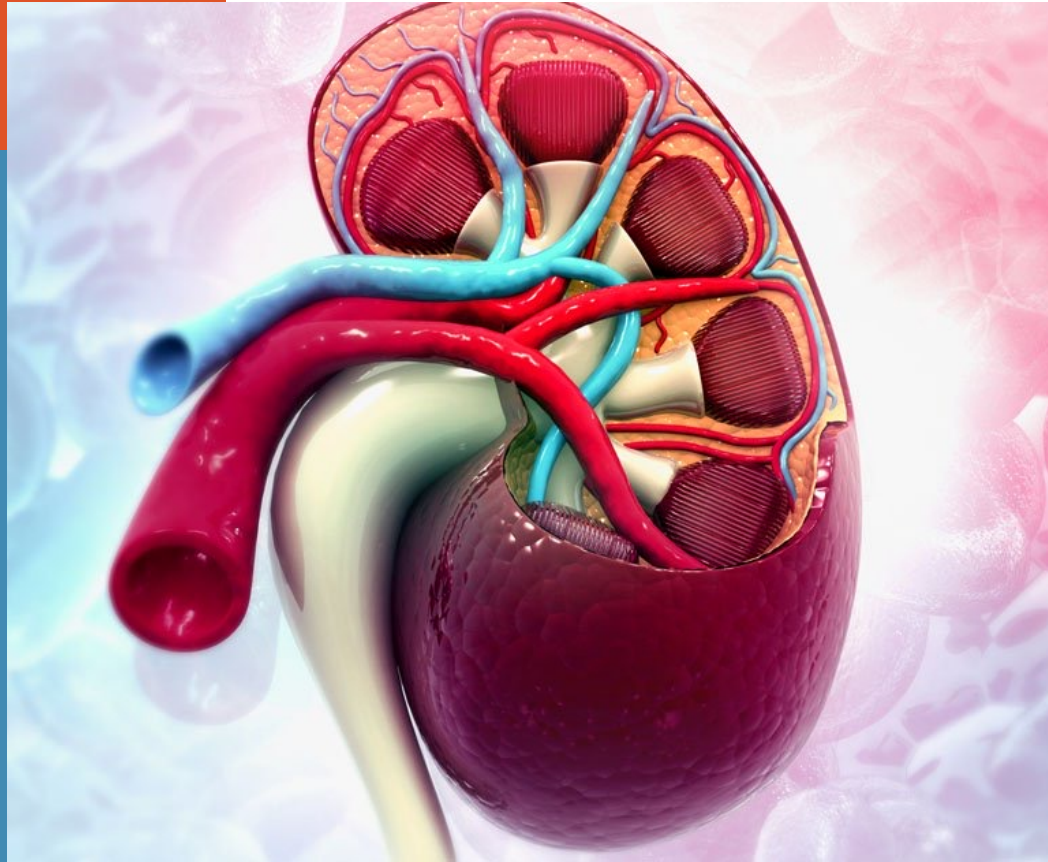


Aldosterone & ADH

Did you know that alcohol inhibits the production of ADH?

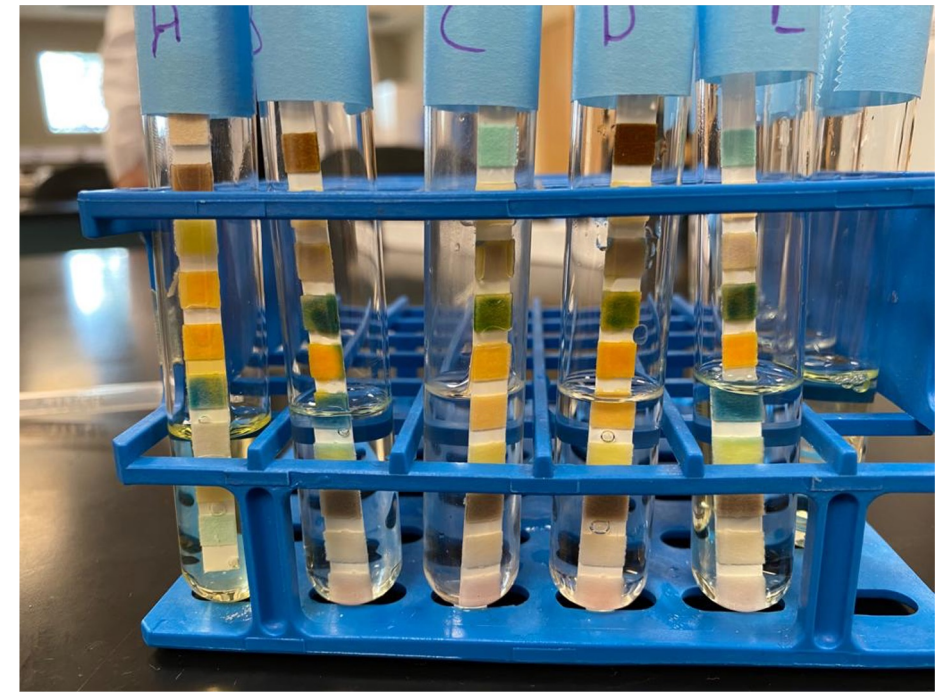
- This explains why you pee a lot when you drink!!!

	Aldosterone	ADH
What is the source of the hormone?	➤ Adrenal Cortex	➤ Produced in the hypothalamus; stored/released from the posterior pituitary gland
What triggers its release?	➤ Increased angiotensin II levels, increased plasma K ⁺ levels	➤ Increased osmolarity of the extracellular fluid or decreased blood volume.
What is the target of the hormone?	➤ Distal Tubule & Collecting Duct	➤ Late distal tubule and collecting duct (via insertion of water-channel proteins called aquaporins)
What are the biological effects of the hormone?	➤ Increases secretion of K ⁺ and reabsorption of Na ⁺ . Increased reabsorption of sodium promotes reabsorption of water (as long as ADH is present), which increases blood volume and blood pressure	➤ Increased reabsorption of water, which decreases osmolarity of body fluids and increases blood volume and blood pressure.



Protocol

Tests	Results / Resultats / Resultados / Ergebnisse				
Leukocytes/Leucocytes Leucocitos/Leukozyten					
	neg.	trace	+70	++125	+++500 WBC/ μ L
Nitrite/Nitritos Nitrit					
	neg.	trace	pos.		
Urobilinogen/Urobilinogène Urobilinógeno					
	0.1 \leftarrow Normal \rightarrow 1(16)		2(33)	4(66)	8(131) mg/dl (μ mol/L)
Protein/Protéines Proteínas					
	neg.	trace	+30(0.3)	++100(1.0) +++300(3.0) ++++1000(10)	mg/dl (g/L)
pH					
	5	6	6.5	7	7.5 8 8.5
Blood/Sang Sangre/Blut					
	neg.	Hemolysis trace	+25	++80	+++200 Non Hemolysis+10 ++80 RBC/ μ L
S.G/Densité Densidad/Spec. Gew.					
	1.000	1.005	1.010	1.015	1.020 1.025 1.030
Ketones/Cétones Cetonas/Ketonkörper					
	neg.	\pm 5(0.5)	+15(1.5)	++40(3.9) +++80(8) ++++160(16)	mg/dl (mmol/L)
Bilirubin/Bilirubine Bilirrubina					
	neg.		+	++	+++
Glucose/Glucosa					
	neg.	\pm 100(5.5)	+250(14)	++500(28) +++1000(55) ++++2000(111)	mg/dl (mmol/L)



Urinalysis – pH Test

Purpose: Identify the pH levels of various urine samples using indicator strips that change color based on pH

Healthy urine pH levels around 7.0, usually fluctuating between 6.5 and 7.5 (typically neutral or slightly acidic)

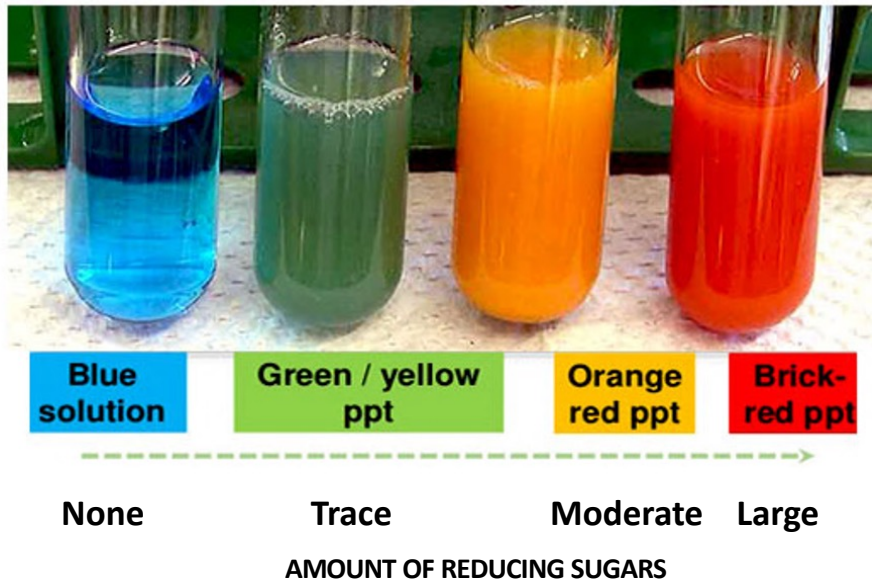
- The kidneys regulate acid-base balance by secreting hydrogen ions and reabsorbing bicarbonate ions

What pH level contributes to the formation of kidney stones?

- Acidic pH (anything below 6.5)

Urinalysis – Glucose Test

Benedict's Reagent

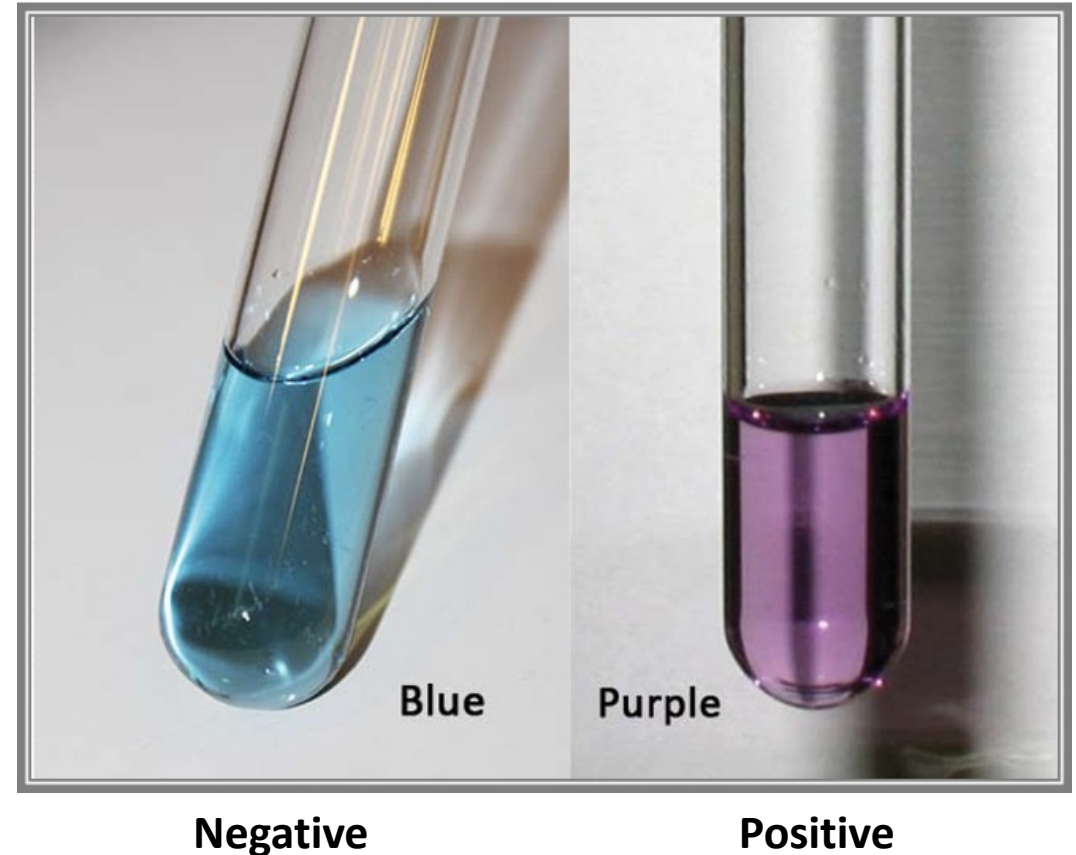


- **Purpose:** To identify the presence of reducing sugars such as glucose in urine using Benedict's reagent
- Initially the urine sample with Benedict's reagent is clear blue
- After being heated for 2-5 minutes, the urine samples change color if glucose is present.
 - Color corresponds to the concentration of reducing sugar (glucose) present
 - **blue** = none ---->> **red** = large
- What structure of the nephron is not working properly if there is glucosuria (glucose in the urine)?
 - Proximal convoluted tubule
- What causes glucosuria?
 - Diabetes or high sugar diets

Urinalysis – Protein Test

- **Purpose:** To identify the presence of protein in urine using Biuret's reagent
- Initially the urine sample with Biuret's reagent is clear blue. If proteins are present, the urine sample changes color to purple.
- Biuret's reagent detects peptide bonds between amino acids
 - **Negative** result is shown as **blue** and clear
 - **Positive** result is shown as **purple/violet** and clear
- What structure of the nephron is not working properly if there is proteinuria (protein in the urine)?
 - Glomerulus
- What causes proteinuria?
 - High protein diets

Biuret's Reagent



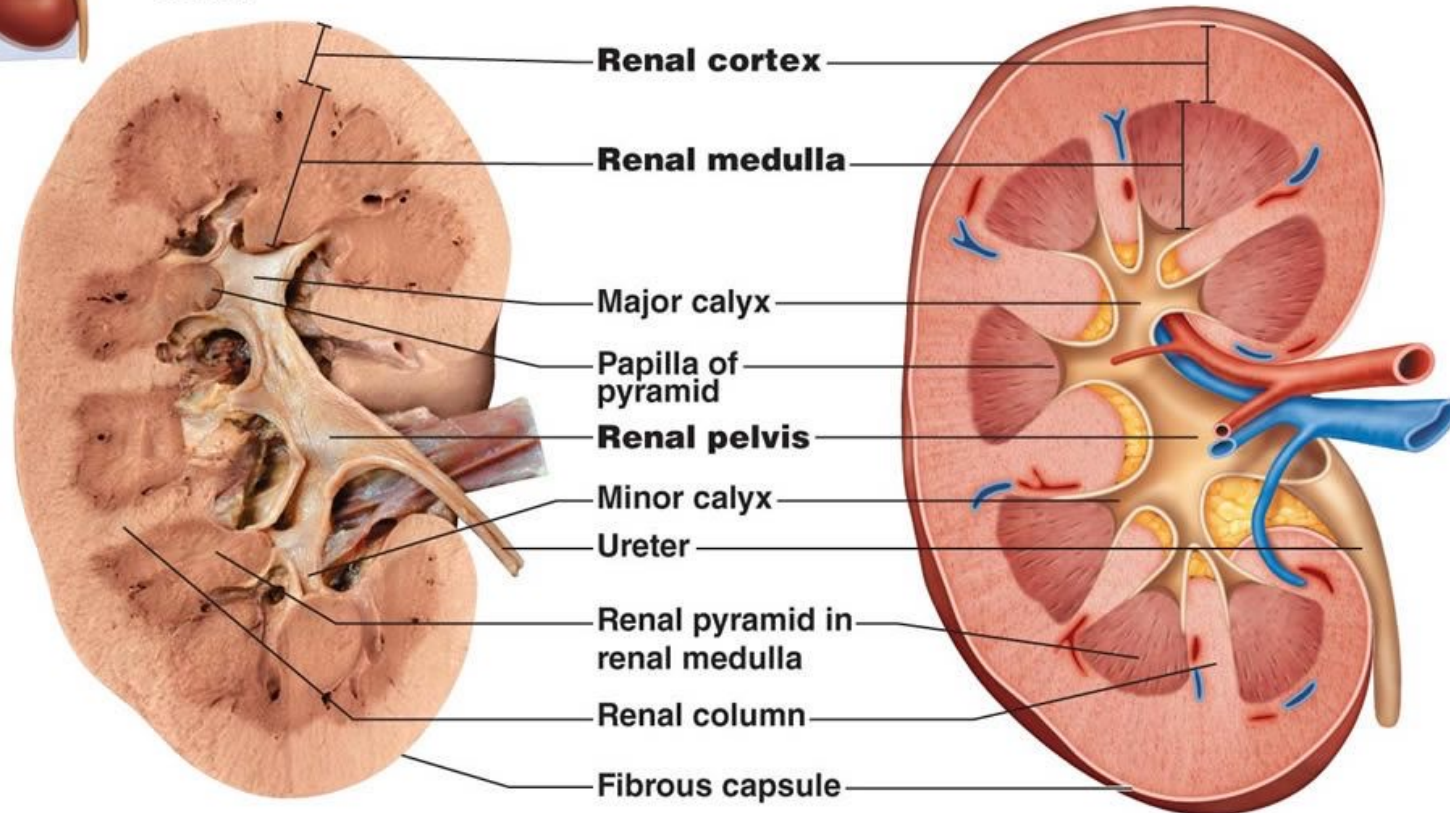
Pig Kidney Dissection

Renal Hilum:

- Where renal arteries enter the kidney
- Where the renal veins and ureters exit the kidney



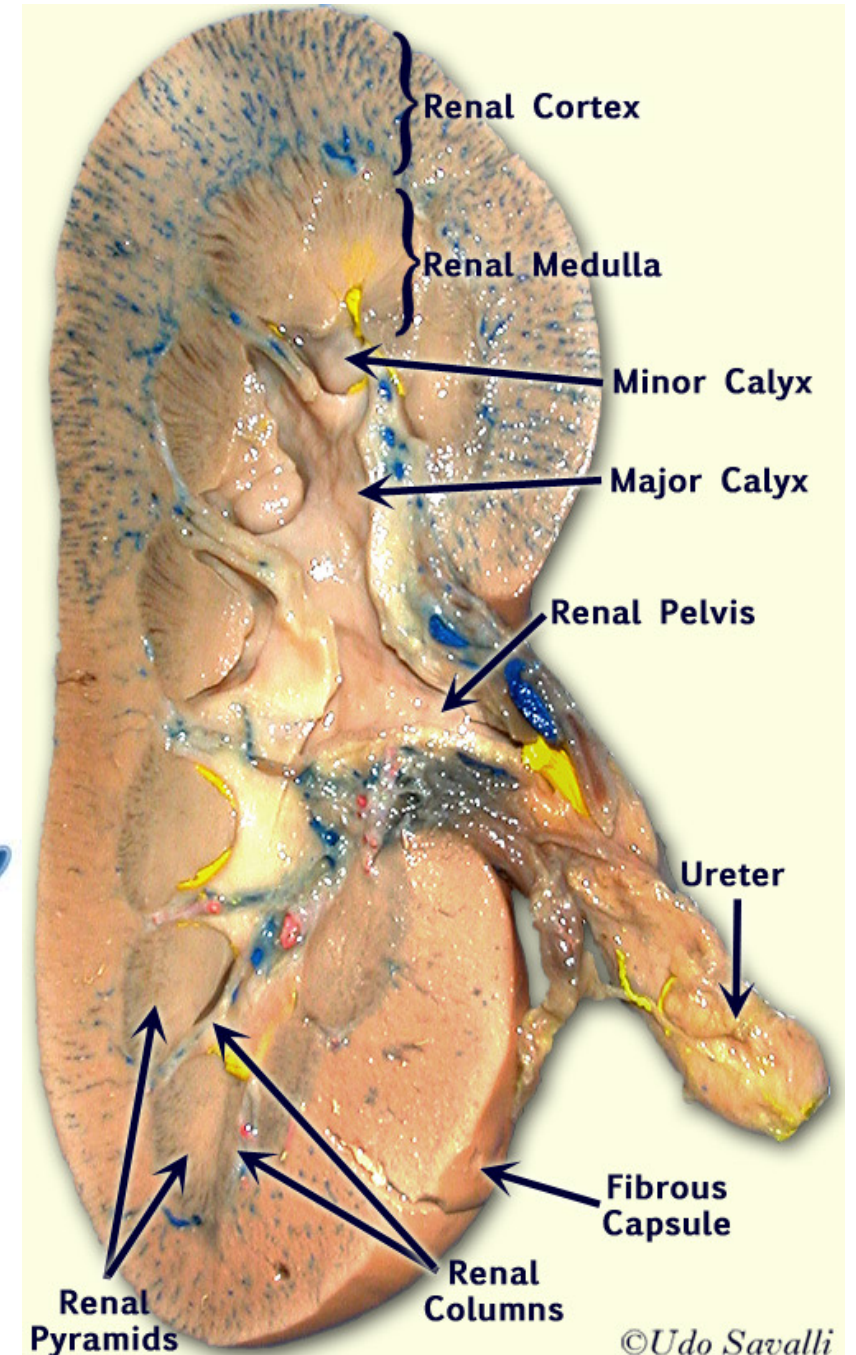
Renal hilum



(a) Photograph of right kidney, frontal section

(b) Diagrammatic view

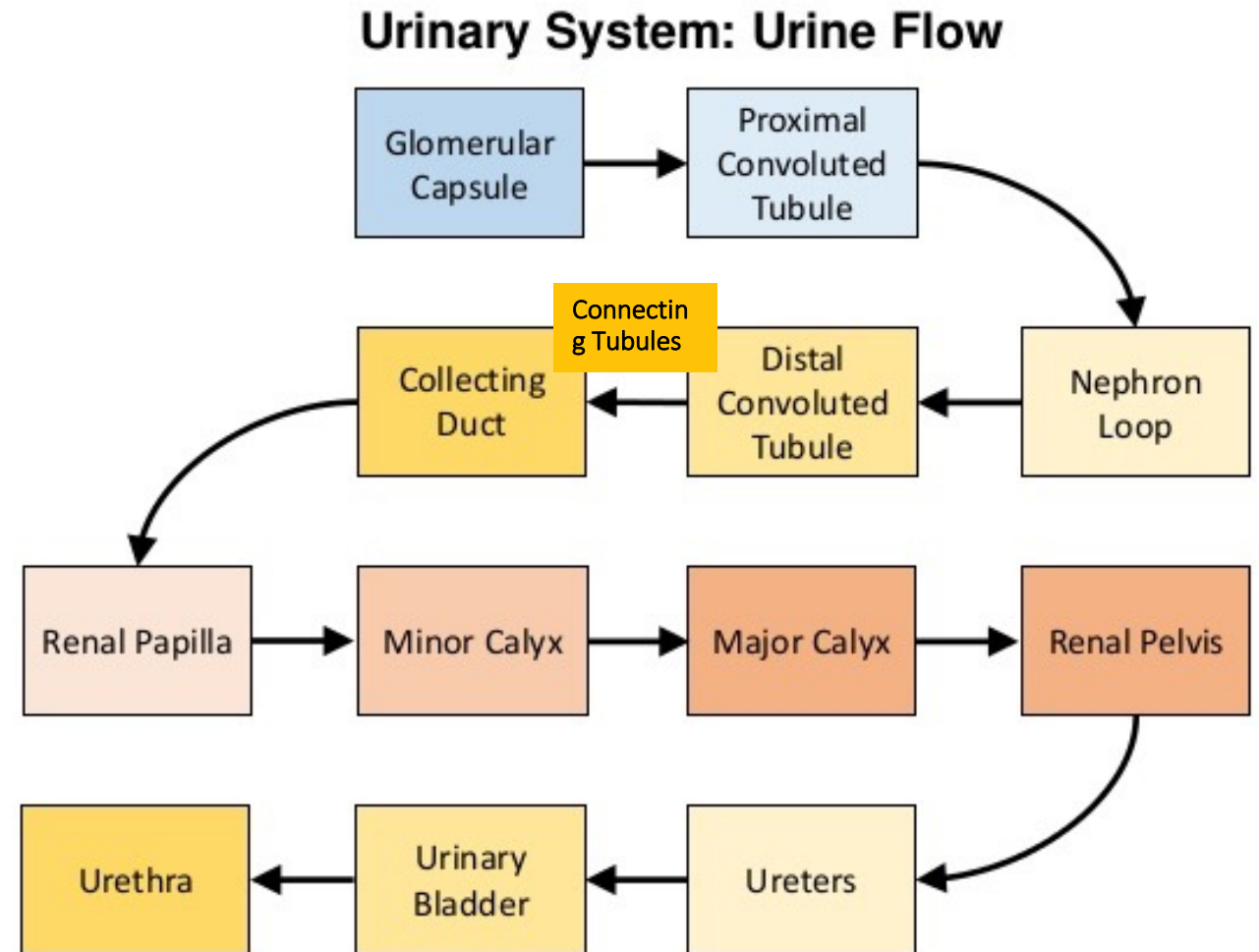
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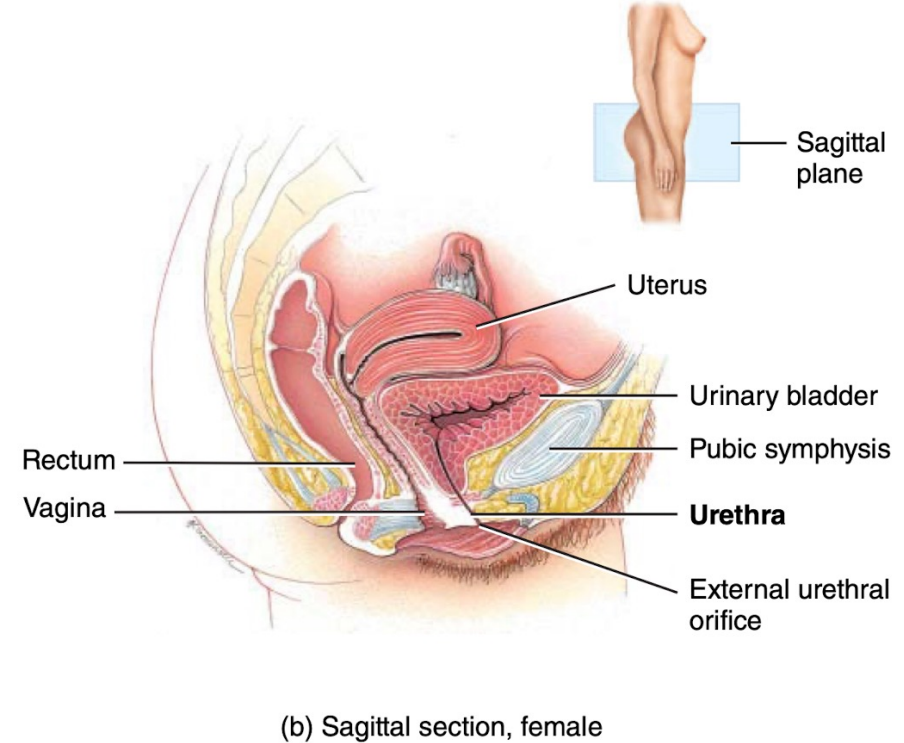
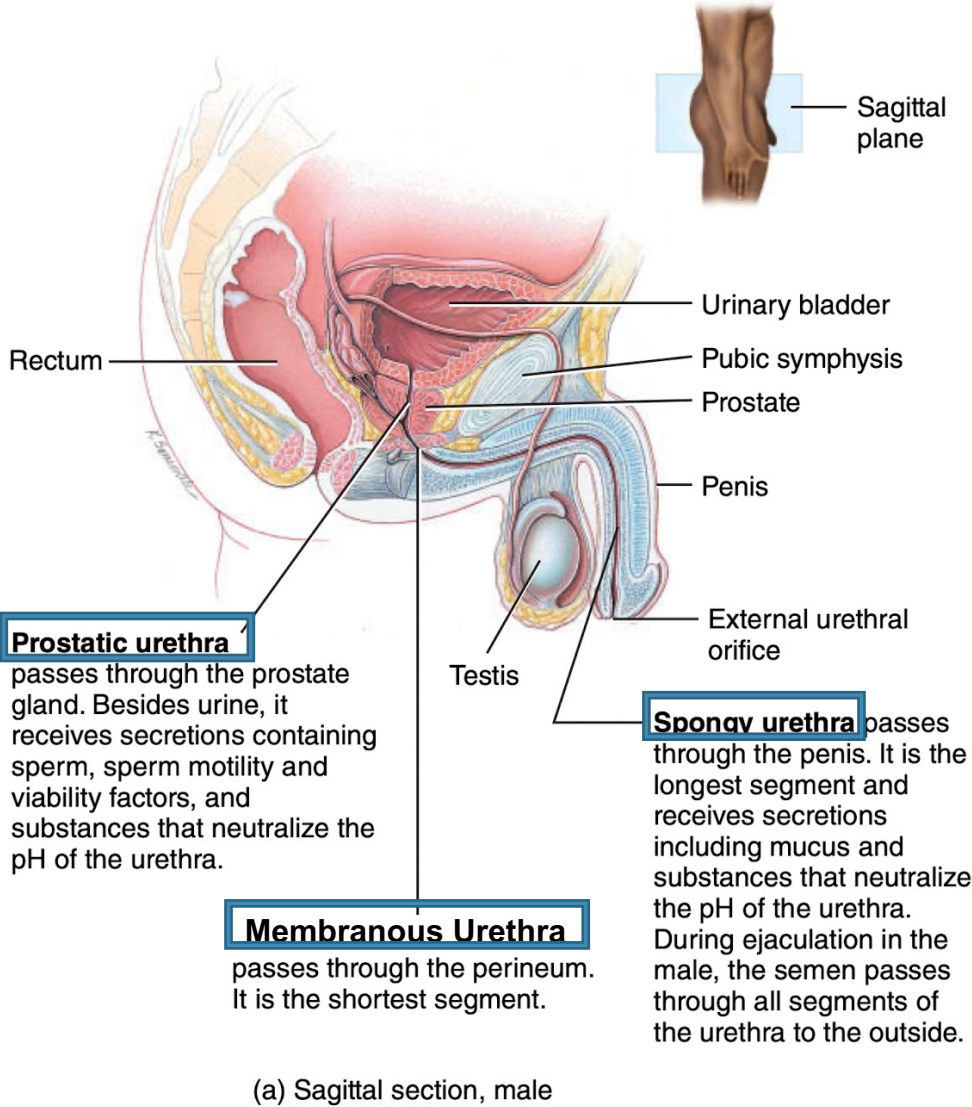


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Flow of Urine

Once at the Minor Calyx, filtrate can no longer be modified and is now referred to as urine.





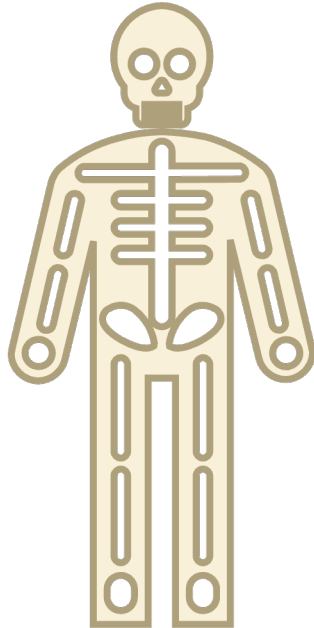
MALES VS. FEMALES

- The urethra is five times longer in males than in females.
- The urethra is divided into three segments in males but is only one short tube in females.
- The urethra is a common duct for the urinary and reproductive systems in males. These two systems are entirely separate in females.

Male vs. Female Urinary Tract

CASE STUDY

Diabetes Mellitus



- Patient: 19-year-old marine
- Signs: Severe weakness, dizziness, and sleepiness.
- First diagnosis: dehydration and treated with IV administration of saline.
- Second follow-up: Unquenchable thirst, polyuria (frequent urination), signs of weight loss (19 pounds), tachypneic, laborious breathing, dehydration, and pale skin. He was also showing severe muscular hypotonia (low muscle tone).
- Treatment: Insulin and saline IV administration. This helped with the recovery.

Laboratory data:

- glucose level **560 mg/dL** (normal: **70 – 114 mg/dL**)
- pH of **7.25** (normal: **7.35 – 7.45**)
- Urine sample shows ***large acetone levels***
- HbA1c (glycated hemoglobin) of **14%** (normal: **4% – 6.2%**)
- Beta-Hydroxybutyrate **20 mM/L** (normal = **0.0 – 0.3 mM/L**)

QUESTION: Why did the patient improve after being given IV saline in his first admission?

- Normal saline contains sodium chloride, so it replaces lost fluid and prevents or corrects some types of electrolyte imbalance.

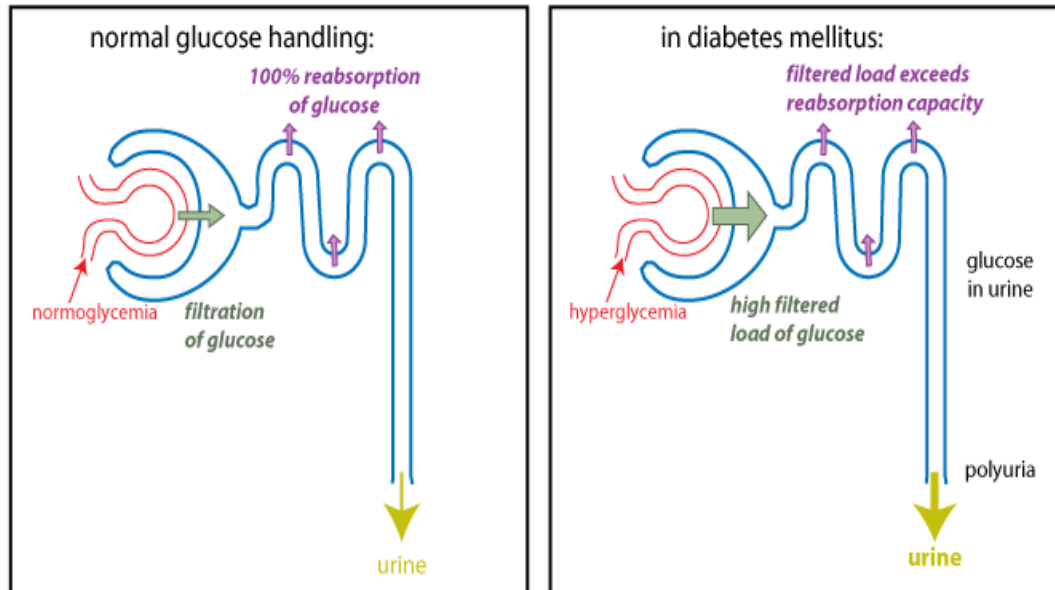
It expanded the extracellular fluid and restored effective blood volume, allowing the patient to return to duty temporarily.

What explains the repeated need to urinate?

Patient was experiencing osmotic diuresis, which is increased urination. Due to the high amount of glucose. Glucose causes additional water to come into the urine, increasing the amount of urine.



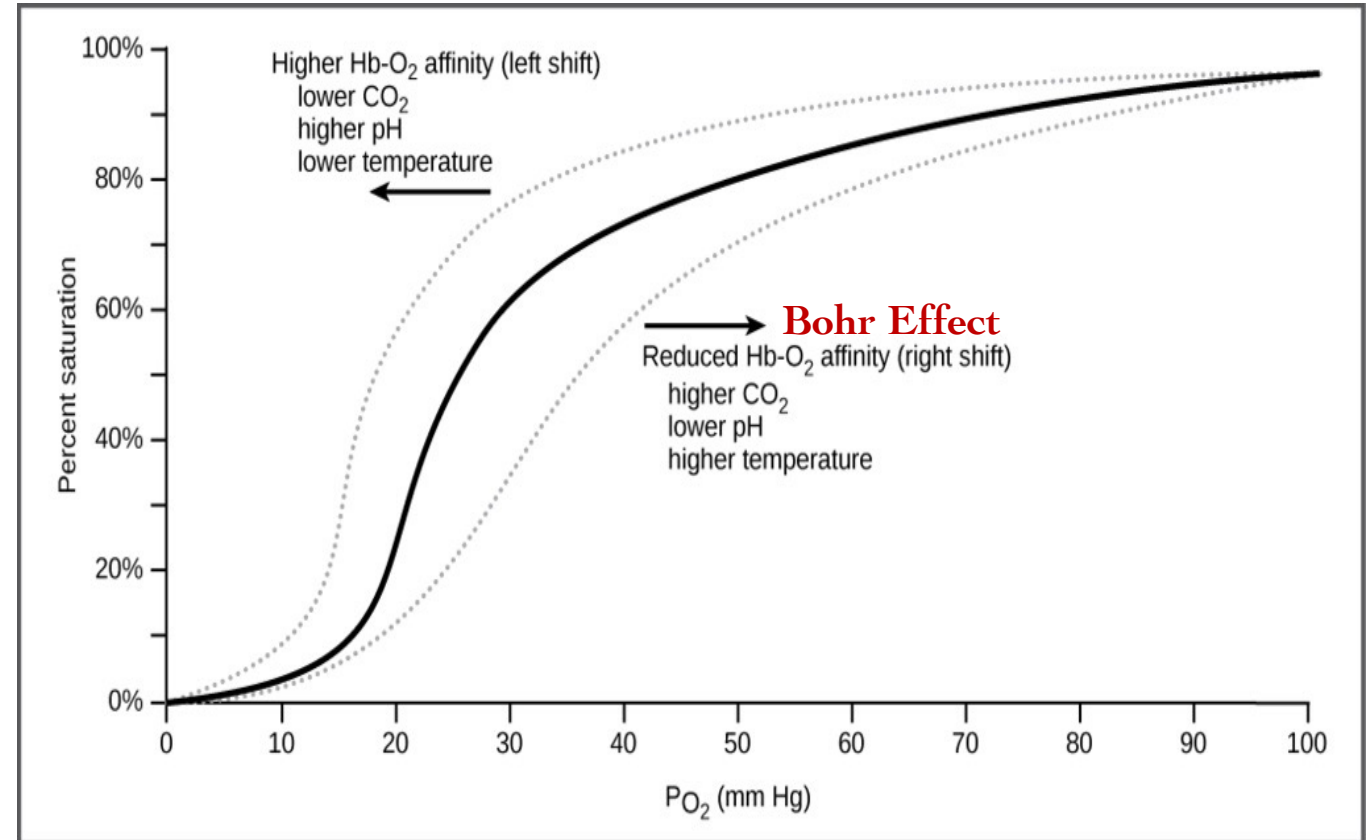
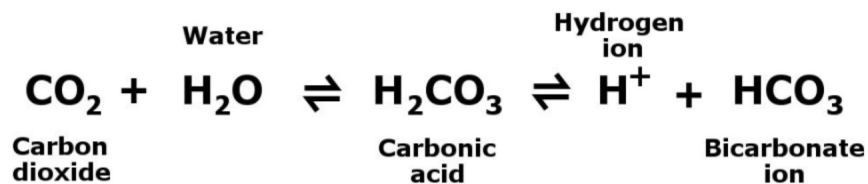
Kidneys & Glucose



- Under normal circumstances, 100% of the glucose that is filtered is reabsorbed. It involves transport proteins that require specific binding.
 - Where in the nephron is glucose reabsorbed?
- In a diabetic that has **hyperglycemia**, the filtered load of glucose can exceed the capacity of the kidney tubules to reabsorb glucose because the transport proteins are saturated. The result is glucose in the urine. **Glucose functions as a solute that draws water into the urine by osmosis.**
- This causes a constant need to drink water to return your body to homeostatic levels and the reason why diabetics urinate so much.

Question: Explain the BOHR EFFECT

- A decrease in the amount of oxygen associated with hemoglobin in response to a lowered blood pH resulting from an increased concentration of carbon dioxide in the blood.



Question: Why was dyspnea (labored breathing) his presenting symptom? (Hint: think Bohr effect, metabolic acidosis/respiratory alkalosis)

1. Dyspnea presents when there is an imbalance in blood pH. What was his pH?

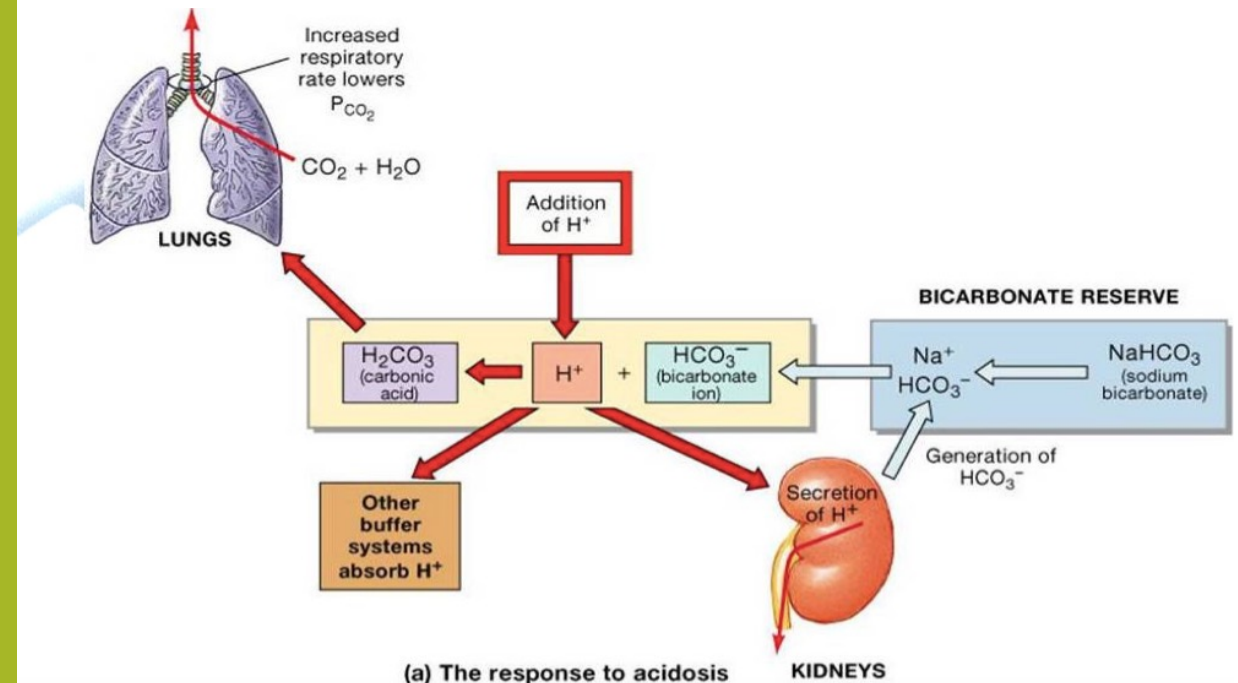
- pH = 7.25 (normal physiological values are between 7.35-7.45)

2. Why was his blood pH low?

- The formation of ketoacids from the breakdown of lipids causes the blood pH to be low. These strong organic acids include Acetoacetate and Beta-Hydroxybutyrate.
- Acidic pH and the low bicarbonate levels are characteristics of metabolic acidosis.

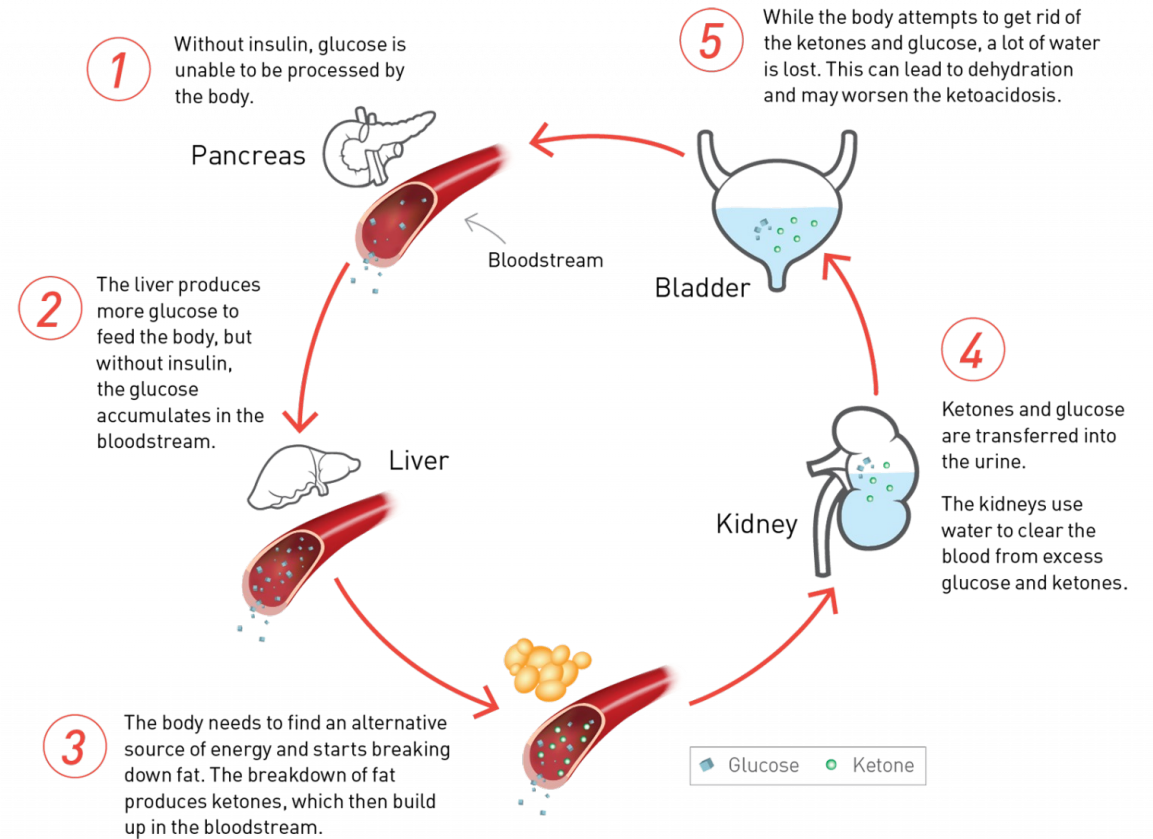
3. How does this lead to labored breathing?

- Chemoreceptors in the medulla oblongata react to the changes in pH by sending signals to the respiratory centers to adjust the ventilation rate to increase or decrease the removal of CO₂.
- Blowing off CO₂ will shift the equilibrium so that bicarbonate ions pick up H⁺ ions to produce CO₂.
- As long as severe metabolic acidosis is present, dyspnea, characterized by deep inspirations and expirations in a rhythmic pattern, called Kussmaul respiration, will appear.



Question: According to the patient's blood sugar level and presence of acetone in the urine, the patient is in.....

- Diabetic Ketoacidosis (DKA)
 - Keto = Ketosis = presence of ketones in the blood
 - Acidosis = low blood pH
 - ALL caused by the inability to use insulin to take in glucose from the food (Diabetes)



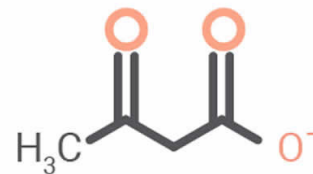
Question: What does your body use as fuel when no insulin is produced?

- We need insulin so that our cells can uptake glucose
- We start metabolizing the storage of lipids (fats) to get energy in the absence of glucose. This will lead to the increased concentration of ketone bodies in the blood (ketosis) and in the urine (ketonuria)
- After the breakdown of fats, proteins will be broken down if needed

TYPES of KETONE BODIES

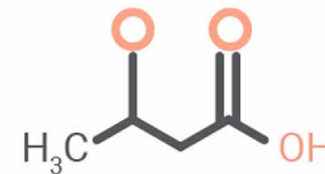
There are three types of ketones produced when the body goes into ketosis:

ACETOACETATE



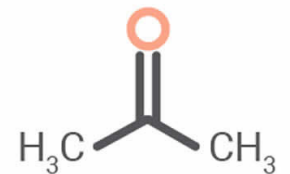
- Created from the breakdown of **fatty acids**.
- Either converted into **BHB** or turned into **acetone**.

BETA-HYDROXYBUTYRIC ACID



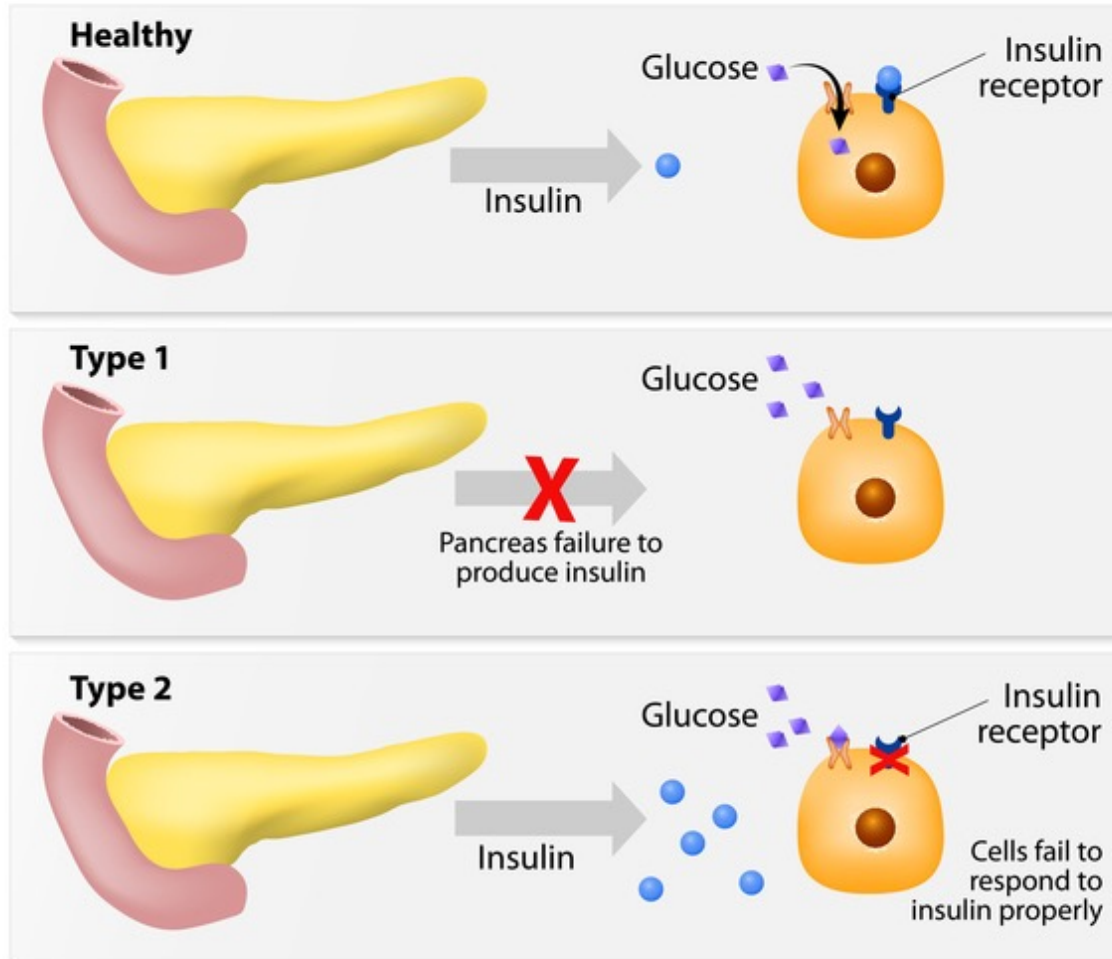
- Formed from acetoacetate.
- Not technically a ketone because of its structure, but we consider it as one within the keto diet.

ACETONE



- Created as a **side product** of acetoacetate.
- Breaks down **quickly**.
- **Is removed** from the body through the waste or the breath

DIABETES MELLITUS



Diabetes Mellitus – Type I vs Type II

Type I:

- Formally known as Insulin-dependent diabetes mellitus/juvenile diabetes
- 5% of all cases of diabetes
- No or very little insulin production; beta cells in the pancreas cannot produce insulin
- Diagnosis usually in young children or teens (usually ages 0-40)
- Requires insulin injections

Type II:

- Non-insulin dependent diabetes mellitus/adult-onset diabetes
- 90% of all cases of diabetes are this type
- Cells do not use insulin well (insulin resistance) or insulin deficiency
- Diagnosis usually in adults over than 40
- Insulin injections may be needed

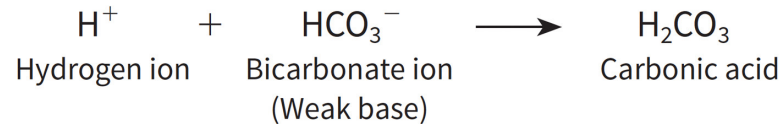
Diabetes is a metabolic disease that causes high blood sugar

Acid-Base Balance

- Major mechanisms for controlling blood pH

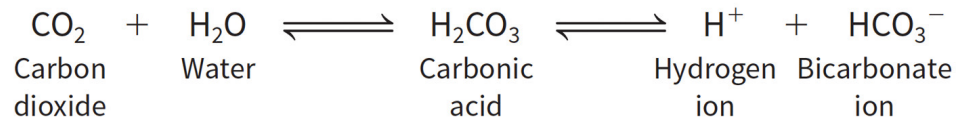
- Buffer systems

- Quickly but temporarily bind to H^+ and increase pH
 - Carbonic acid-bicarbonate buffer system:



- Exhalation of CO_2

- Increase rate and depth of breathing releases excess CO_2 , which reduces carbonic acid levels, increasing pH



- Kidney excretion of H^+

- Slowest mechanism
 - Only way to eliminate acids (other than carbonic acid)
 - Increases pH

