

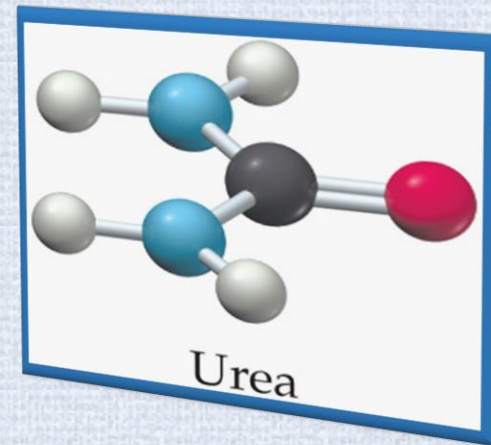


Al-Mustaqbal University College Pharmacy Department – Third Class



Practical Biochemistry

Estimation of Urea



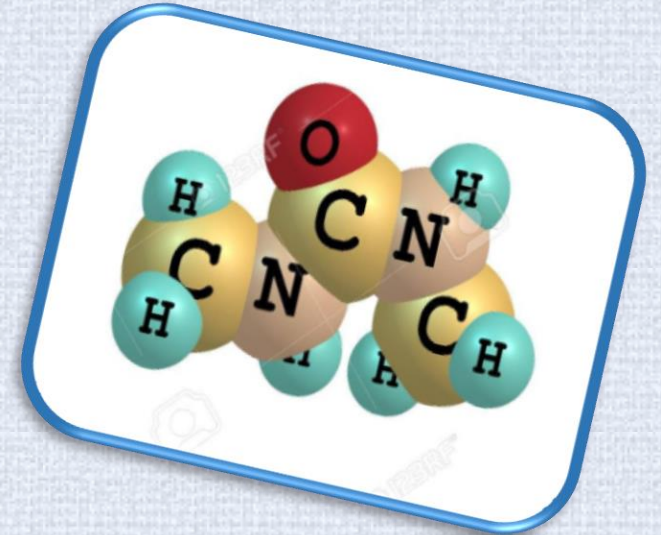
Test	Result	Unit
Direct bilirubin		
AST		
ALT		
ALP		
BUN	60.2 H	mg/dL
Creatinine	4.53 H	mg/dL
Uric acid		mg/dL
Cholesterol		mg/dL
Triglyceride		mg/dL

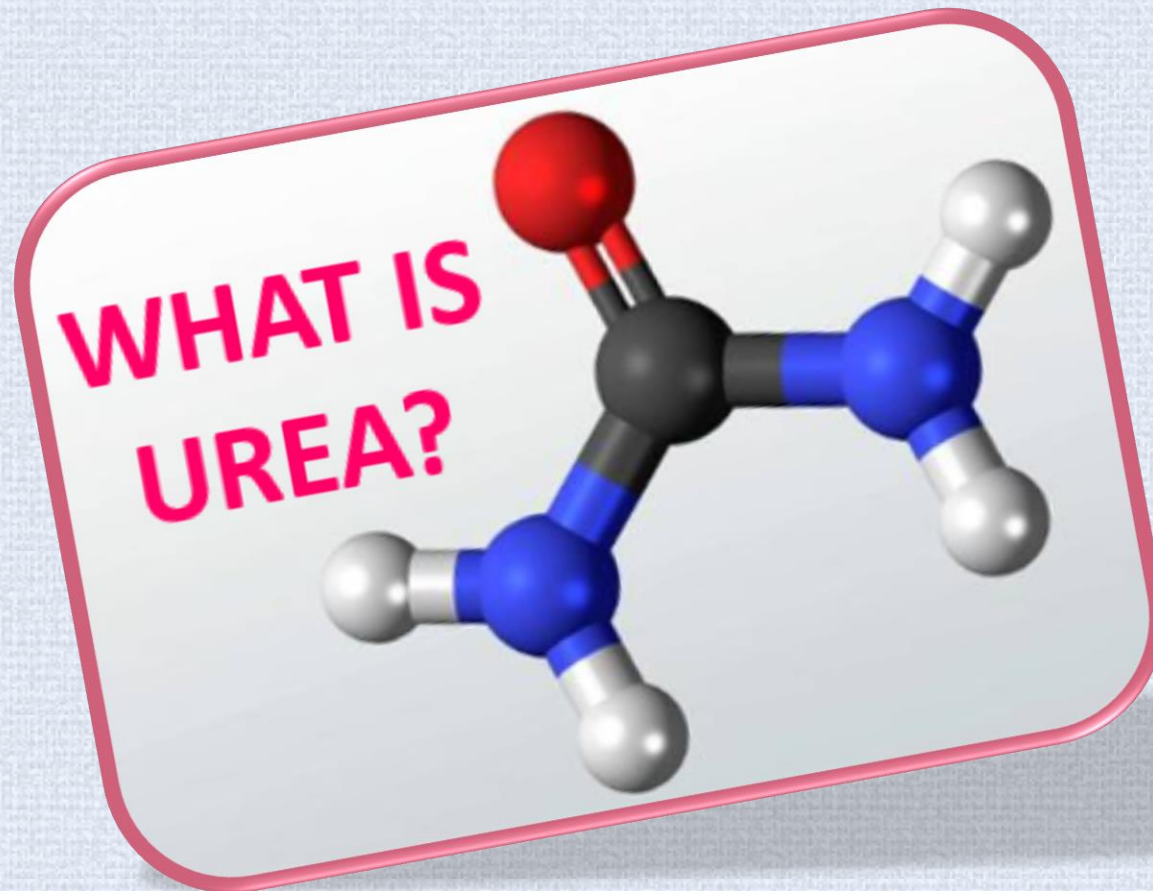
Fourth Lec.

Assist. Lec. ZAINAB GHALEB

Out Line

- ✓ **Introduction**
- ✓ **Formation of Urea (Urea Cycle).**
- ✓ **Blood Urea Nitrogen (BUN) Test.**
- ✓ **Normal Value**
- ✓ **Clinical significance.**
- ✓ **Determination of Urea in Serum.**



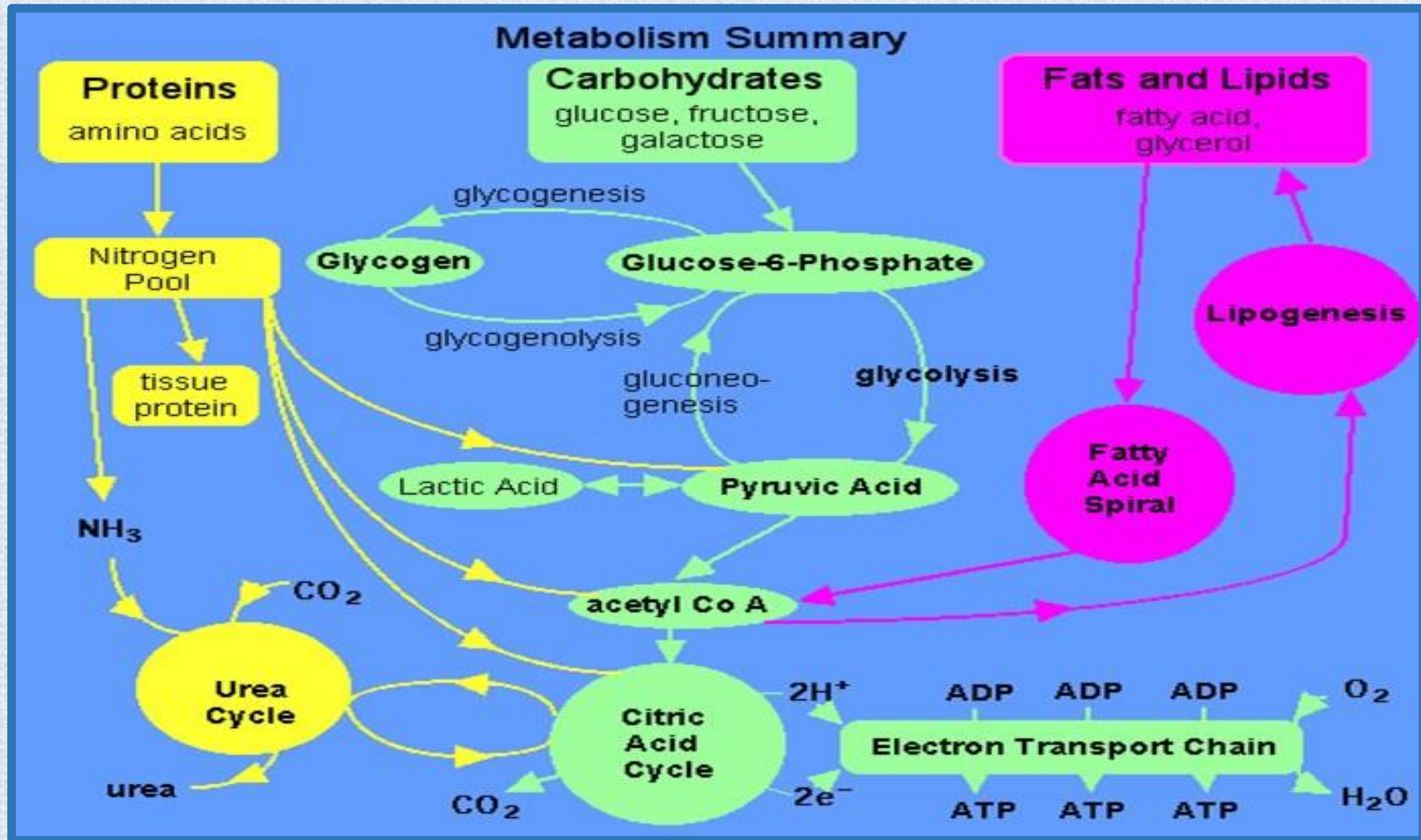


Introduction

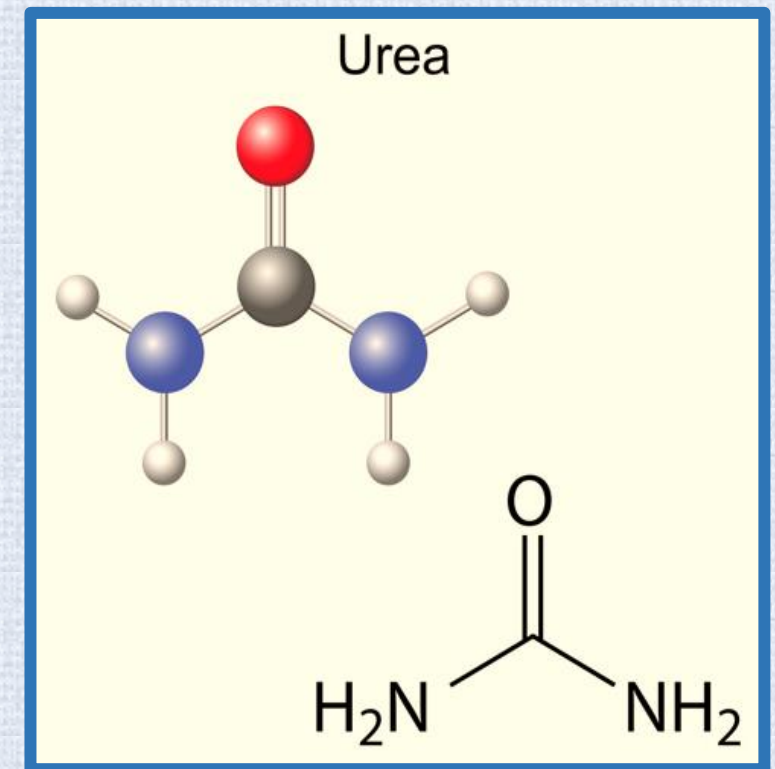
- ❑ The determination of **Nonprotein Nitrogenous** substances in the **Blood** has been used to monitor **Renal Function**.
- ❑ The **NPN** fraction comprises about **15 compounds**. The majority of these compounds arise from the **Catabolism** of **Proteins** and **Nucleic acids**.
- ❑ The **NPN** compound present in **highest concentration** in the blood is **Urea**.

**TABLE 11-1 CLINICALLY SIGNIFICANT
NONPROTEIN NITROGEN COMPOUNDS**

COMPOUND	APPROXIMATE PLASMA CONCENTRATION (% OF TOTAL NPN)	APPROXIMATE URINE CONCENTRATION (% OF EXCRETED NITROGEN)
Urea	45-50	86.0
Amino acids	25	—
Uric acid	10	1.7
Creatinine	5	4.5
Creatine	1-2	—
Ammonia	0.2	2.8



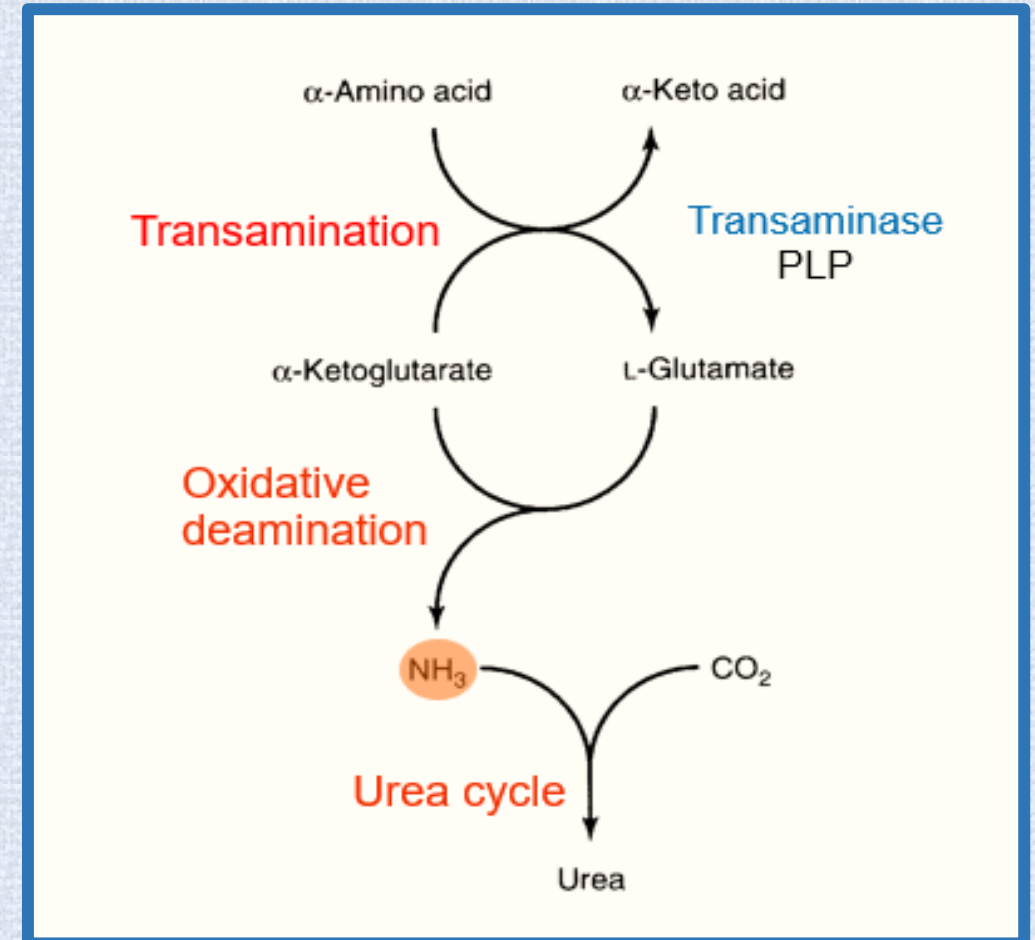
- ❑ **Urea** is the Major Excretory Product of **Protein** Metabolism (**Amino acid** metabolism) in **Human** and **Mammals**.
- ❑ **Urea** is a **Nitrogenous Compound** containing a **One Carbonyl Group** attached to **Two Amine Groups**
- ❑ **Protein** metabolism produces **Amino acids** that can be **Oxidized** to produce **Energy** or stored, these processes release **Nitrogen** as **NH₃**.



□ **NH₃** produced from **Oxidative Deamination** reaction, is **Toxic** compound **even in small amount** and must be **removed** from the **body**.

□ The amount of **Nitrogen** ingested is **balanced** by the **Excretion** of an **equivalent** amount of **Nitrogen**.

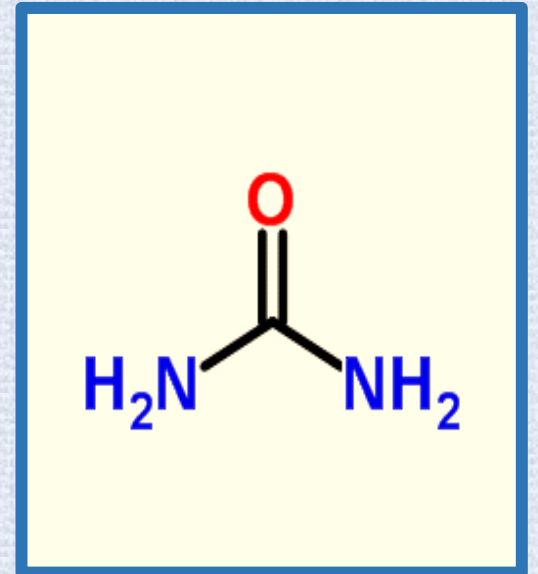
□ **Urea Cycle** is the **Conversion** reactions of **NH₃** into **Urea**.



- ❑ Concentration of **Urea** in the **Blood** mainly balanced between **Urea Formation** from **Protein** Catabolism and **Urea Excretion** from the Body.

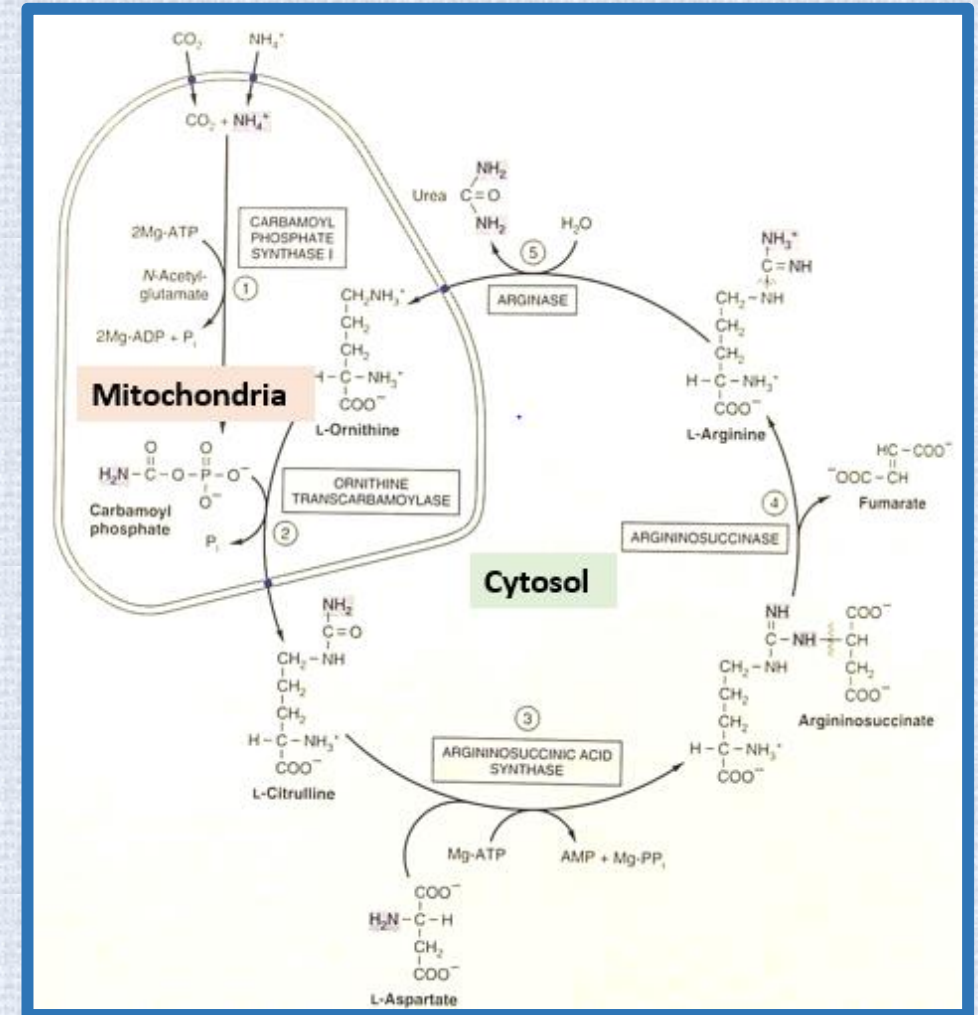
Why NH₃ convert to Urea?

- ❑ To **Detoxification** of **NH₃** as Urea is **less Toxic** compound than **Ammonia**.
- ❑ Urea is **Soluble** in **Water**.
- ❑ Urea combine **Two** waste molecules (**CO₂**, **NH₃**) into **One** molecule to excreted from the body.



❑ Urea is formed in the **Liver** by **Urea Cycle** (certain occurs in **Cytosol & Mitochondria**).

❑ Following synthesis in the **Liver**, **Urea** is carried in the **Blood** to the **Kidney**, where it is readily filtered by the **Glomerulus**.



- ❑ Most of the **Urea** is **Excreted** in the **Urine**, although some **Urea** is **Reabsorbed** by the **Renal tubules**.

- ❑ Small quantities of **Urea** (**10% of the total**) are **Excreted** through the **Gastrointestinal tract** and **Skin**.

- ❑ The **Concentration** of **Urea** in the **Plasma** is determined by:
 - ✓ **Renal Function**.
 - ✓ **The Protein content of the Diet**.
 - ✓ **The Rate of Protein Catabolism**.

Blood Urea Nitrogen (BUN) test:

- ❑ Blood Urea Nitrogen **BUN** test is a measure of the amount of **Nitrogen** in the Blood in form of **Urea**.
- ❑ To determine Nitrogen from Urea we use the following formula:
- ❑ **Urea = BUN * 2.14**

Normal Value

- The Normal Value of Urea & BUN is must be between:

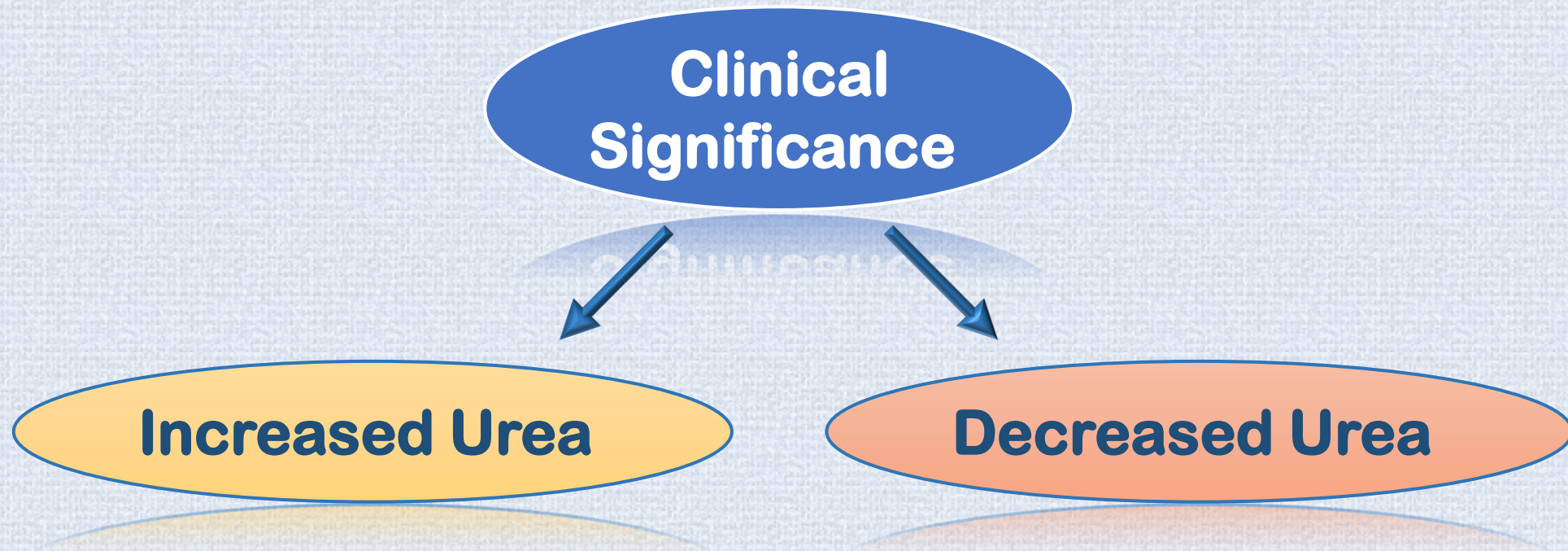
Urea

15 – 45 mg/dl

BUN

7 – 21 mg/dl

- This Value become higher with age.



Increased Urea

- Pathophysiology an **Elevated Concentration of Urea** in the **Blood** is called **Azotemia**.

- ❑ Very **high plasma Urea** Concentration accompanied by **Renal Failure** is called **Uremia**, or the **Uremic Syndrome**.
- ❑ This condition is eventually **fatal** if not treated by **Dialysis** or **Transplantation**.
- ❑ Conditions causing **Increased plasma Urea** are classified according to cause into **three** main categories:
 - ✓ **Prerenal.**
 - ✓ **Renal.**
 - ✓ **Postrenal.**

➤ **Prerenal Azotemia**: is caused by reduced **Renal Blood Flow**. **Less Blood** is delivered to the **Kidney**; consequently, **less Urea** is filtered. **Causative factors include:**

- ✓ **Congestive heart failure.**
- ✓ **Shock.**
- ✓ **Hemorrhage.**
- ✓ **A high-protein diet or increased protein catabolism.**
- ✓ **Dehydration and other factors resulting in a significant decrease in blood volume.**

➤ **Renal Azotemia:** Decreased **Renal Function** causes an **Increase** in **Plasma Urea** concentration as a result of compromised **Urea Excretion**. Renal causes of elevated Urea include:

- ✓ **Acute and Chronic Renal Failure.**
- ✓ **Glomerular Nephritis.**
- ✓ **Tubular Necrosis.**

➤ **Postrenal Azotemia:** can be due to **Obstruction of Urine flow** anywhere in the **Urinary Tract** by:

- ✓ **Renal Calculi.**

- ✓ Tumors of the Bladder or Prostate.
- ✓ Severe Infection.

Decreased Urea

- The major causes of **Decreased Plasma Urea Concentration** include:
 - ✓ Low Protein Intake.
 - ✓ Severe liver disease.
 - ✓ Pregnancy.
 - ✓ Severe Vomiting and Diarrhea.

Principle

- Urea is **Hydrolyzed** by **water** and **Urease** into **Ammonia** and **Carbon Dioxide**.



- The **Ammonia** produced is further acted with **Hypochlorite** and **Salicylate** to form **Green Complex**.
- The **intensity** of **Color** is proportional to the **Urea Concentration** in the **Sample**

Procedure

- In this test (**Urea test**) Wavelength used is **578** nm. Sample used is Serum.

Solutions	Blank	Standard	Sample
Working Reagent	1 ml	1 ml	1 ml
Standard	-	10 μ l	-
Sample	-	-	10 μ l

Mix, incubate for 5 min at RT or for 3 min at 37C. After that add 1000 μ l for each tube, Mix and incubate for 10 min at RT or 5 min at 37C, read the absorbance at 578nm.

Calculations

- The **Urea Concentration** in the **Sample** is calculated by using the following general formula:

$$C_{\text{sample}} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Standard conc.}$$

- The Concentration of the **Standard** is:

80 mg/dl

or

13.3 mmol/l



**THANK
YOU!**

Asst. Lec. ZAINAB GHALEB ABDUL KAREEM