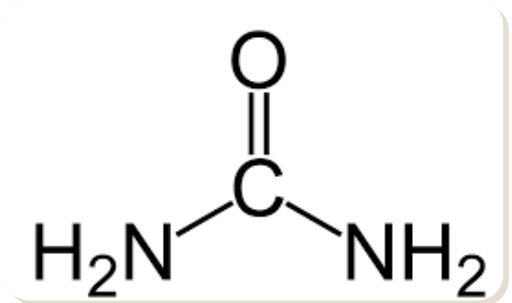


Estimation of Serum Urea

-Urea:

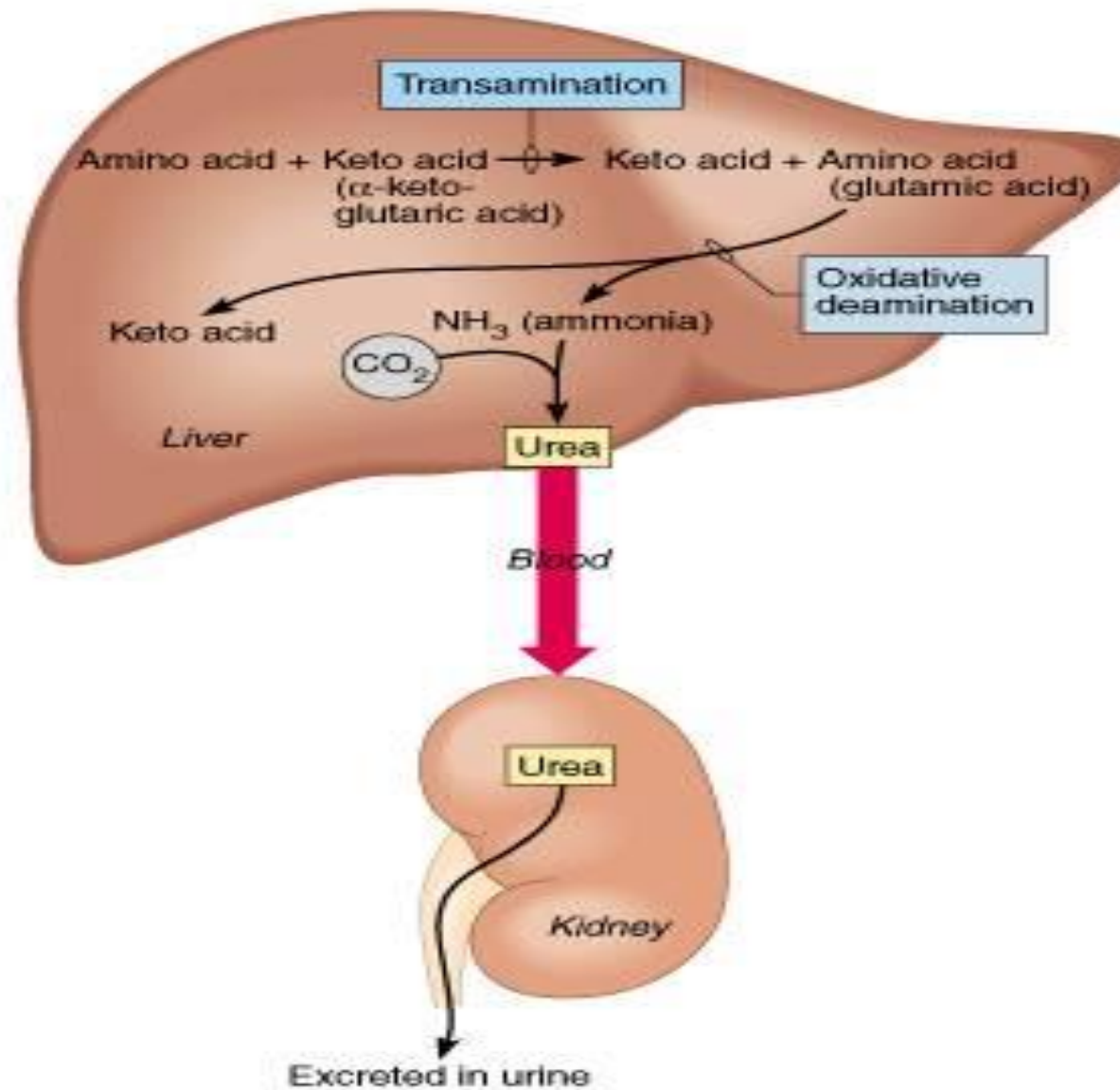
- Urea is the highest non-protein nitrogen compound in the blood.
- Urea is the major excretory product of protein metabolism.
- It is formed by urea cycle in the liver from **free ammonia** generated during **protein catabolism**.
- Since historic assays for urea were based on measurement of nitrogen, the term blood urea nitrogen (BUN) has been used to refer to urea determination.



-Urea synthesis:

- **Protein metabolism** produces amino acids that can be oxidized.
- This results in the release of **ammonia** which is **converted to urea** (via urea cycle) and excreted as a waste product.
- Following synthesis in the **liver**, urea is carried out in the **blood to the kidney** which is readily **filtered from the plasma by glomerulus**.
- **Most of the urea** in the glomerular filtrate excreted in the urine, and **some urea is reabsorbed** through the renal tubules.
- The amount reabsorbed **depends on urine flow rate and extent of hydration** (the amount of urea reabsorbed increases with dehydration).

-Urea synthesis:



- Clinical Application:

- **Measurement of urea used in :**
- Evaluate renal function.
- To assess hydration status.
- To determine nitrogen balance.
- To aid in the diagnosis of renal diseases.
- Check a person's protein balance.

1-Plasma urea Concentration:

- Measurement of **Blood Urea Nitrogen (BUN)** alone is **less useful in diagnosing kidney diseases** because it's blood level is influenced by **dietary protein and hepatic function**.

	Type	Cause	Note
High urea (High urea concentration in plasma is called azotemia)	Pre-renal	<ul style="list-style-type: none">• Cognitive heart failure.• <u>Dehydration</u>.• High protein diet.• Increased protein catabolism.	Caused by reduced renal blood flow, less blood is delivered to kidney, then less urea is filtered.
	Renal	<ul style="list-style-type: none">• Renal failure .	
	Post-renal	<ul style="list-style-type: none">• Urinary tract obstruction.	
Low urea		<ul style="list-style-type: none">• Low protein intake.• Liver disease.• Pregnancy.	

Practical Part

-Objective:

- Estimation of Blood urea nitrogen (BUN).

-Principle (of the kit used):

- **The Reagent used contains:** Urease, Glutamate Dehydrogenase, NADH, α -ketoglutaric acid, buffers and stabilizers .

1. **Reaction one:** Urea is hydrolysed in the presence of urease enzyme and water to yield ammonia and carbon dioxide.



2. **Second reaction:** The ammonia reacts with α -ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and nicotinamide adenine dinucleotide (NAD).



-Reference Value:

SPECIMEN	UREA
Serum/Plasma	10-50 mg/dL

-Materials:

- BUN-ZYME Reagent: UREASE, GLDH, NADH, α -KETOGLOUTARIC ACID , buffers and stabilizers.
- BUN-ZYME Standard solution 25 mg/dl (nitrogen = 53.57 mg/dl).
- BUN-ZYME Serum sample.

-Method:

	Standard	Serum
Reconstituted Reagent	3ml	3ml
Pre-warm at 37°C for 2 min. and add:		
Standard	0.025/25μl	-
Serum	-	0.025/25μl

- After exactly 30 seconds . read and record absorbance A1 against distilled water at 340 nm.
- At exactly another 60 seconds after A1, read and record the absorbance A2 and determine ΔA (A1-A2).

-Calculations of the Results :

- Concentration of urea in serum sample:

- Standard concentration= 53.57 mg/dl

$$\text{- Urea (mg/dL)} = \frac{\Delta A (\text{Sample})}{\Delta A (\text{Standard})} \times 53.57$$

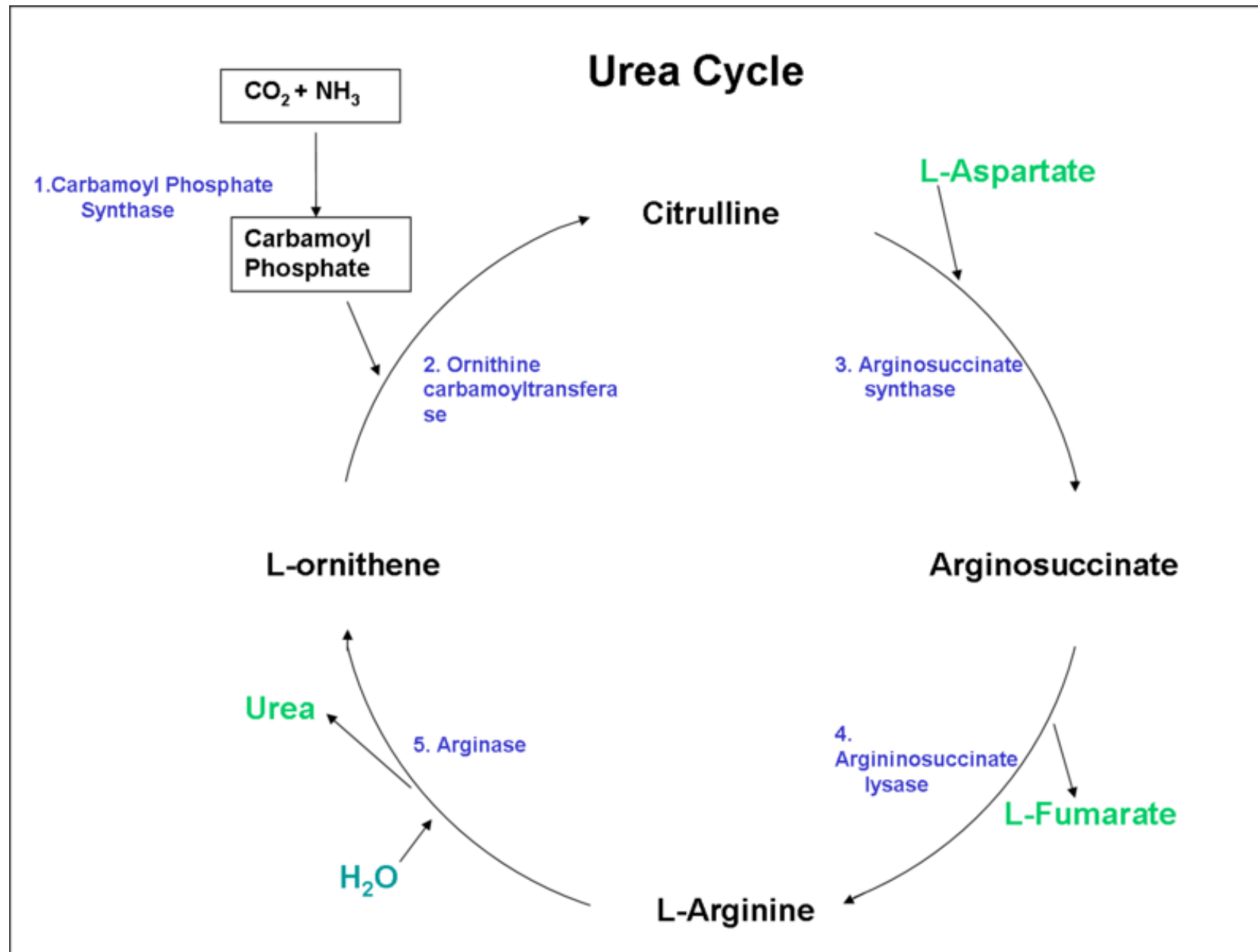
-Discussion:

- Comment on the level of Urea in serum .

Estimation Of Arginase Activity In Liver Extract

- Introduction:

- ❖ Ammonia product of oxidative deamination of amino acids.
- ❖ It is toxic in even small amount and it must be removed from the body.
- ❖ Arginase is one of the important enzymes in urea cycle which is the major disposal form of amino groups derived from amino acids.
- ❖ Urea cycle catalyzed by a set of enzymes (Five enzymes) present in the liver ,and then is transported in the blood to the kidneys for excretion.



- Principle:

- The arginase enzyme catalyzes the fifth reaction in the urea cycle, the enzyme is present **exclusively in the liver**.
- Arginase catalyzes the hydrolytic cleavage of the guanidine group of Arginine to regenerate ornithine and urea.



- Arginase is present exclusively in liver (Cytoplasm).
- Two isozymes of this Enzyme exist , **First ; Arginase I (In cytoplasm)** for functions of urea cycle, **Second; Arginase II** to regulate the arginine/ornithine concentration in the cell (**In mitochondria**).
- Arginase requires a two-molecules metal of Co^{2+} and Mn^{2+} for it's activation while ornithine and lysine are potent inhibitors.

- The activity of the enzyme is determined by measuring the amount of urea produced, urea is reacted with the reagent isonitrosopropiophenone and heated in boiling water, leading to the production of a red color compound which is measured by spectrophotometry at 520nm.

- Question:

- What are the causes of high blood ammonia level?**