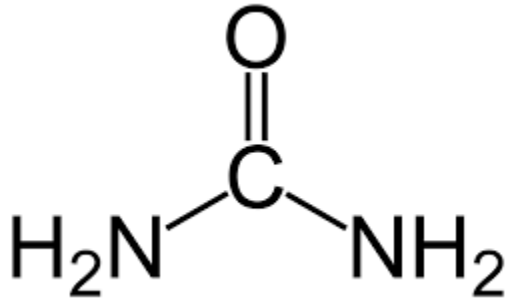
Estimation of Serum Urea

-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:

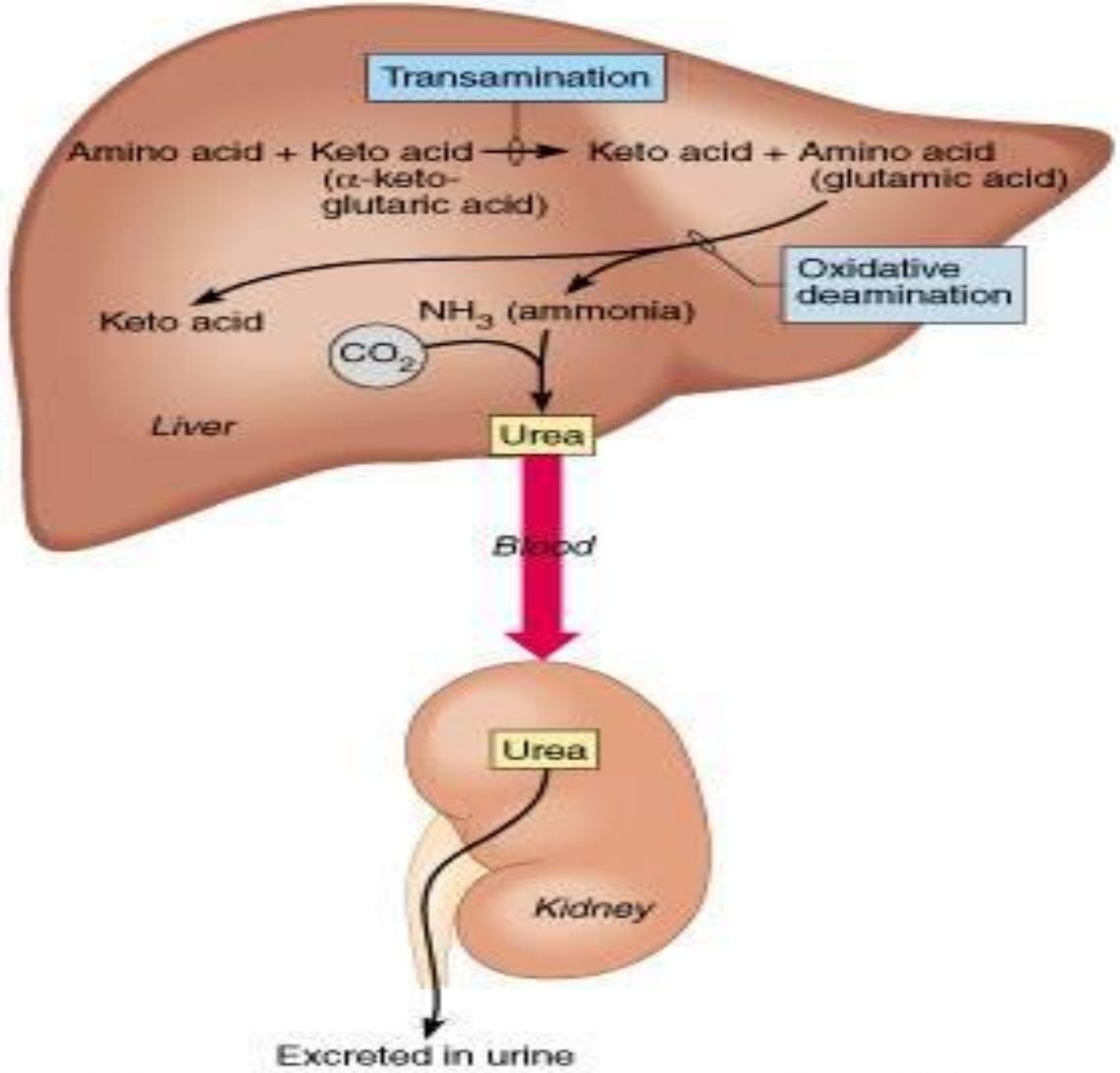
- Protein metabolism produces amino acids that can be oxidized.
- This result in the release of ammonia which is converted to urea (via urea cycle) and excreted as a waste product.
- Following synthesis in the liver, urea is carried out in the blood to the kidney which is readily filtered from the plasma 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.).
- **The concentration of urea in the plasma is determined by:**

➔Renal and liver function,

➔the protein content in diet,

➔and the rate of protein catabolism.

-Urea synthesis:



-Clinical Application:

- Measurement of urea used to in :
- Evaluate renal function.
- To assess hydration status.
- To determine nitrogen balance.
- To aid in the diagnosis of renal diseases.
- To verify adequacy of dialysis .
- 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 (why?).
- But its diagnostic value improves with **serum creatinine values**.

	Type	Cause	Note
High urea (High urea concentration in plasma is called **azotemia**)	Pre-renal	• Cognitive heart failure. • Dehydration. • High protein diet. • Increased protein catabolism.	• Cognitive heart failure ➔ reduced renal blood flow, less blood is delivered to kidney , then less urea is filtered.
	Renal	• Renal failure .	
	Post-renal	• Urinary tract obstruction.	
Low urea		• Low protein intake. • Liver disease. • Pregnancy.	

2-Urine urea Concentration:

- The Urine Urea Nitrogen test (UUN) determines how much urea is in the urine to assess the amount of protein breakdown.
- The test can help determine how well the kidneys are functioning, and if the intake of protein is too high or low.
- Specimen: The urine urea nitrogen test is performed by collecting a **24-hour urine sample.**

	Cause
High urea in urine	• Too much protein in the diet. • Too much protein breakdown in the body.
Low urea in urine	• Malnutrition. • Too little protein in the diet. • Kidney issues.

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 hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide.

$$\underset{\text{Urea}}{NH_2 - CO - NH_2} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

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).

$$\underset{\alpha\text{-KETOGLUTARIC ACID}}{NH_3 + HOOC-(CH_2)_2-CO-COOH} + NADH + H^+ \xrightarrow{\text{GLDH}} \underset{\text{GLUTAMIC ACID}}{HOOC-(CH_2)_2-CH(NH_2)\,COOH} + NAD^+ + H_2O$$

- **The amount of the urea in the sample is proportionally related to the reduced absorbance at 340 nm as a result of NADH oxidation to NAD.**

-Reference Value:

SPECIMEN	UREA NITROGEN	UREA
Serum/Plasma	5-23 mg/dL	10-50 mg/dL
Urine 24 h	9-16g/24h	20-35 g/24 h

-Materials:

- BUN-ZYME Kit.

-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 $\mathbf{A_1}$ against distilled water at **340 nm.**
- At exactly **60 seconds** after A_1, read and record the absorbance $\mathbf{A_2}$ and determine $\Delta\mathbf{A}$ ($\mathbf{A_1}$-$\mathbf{A_2}$).

-Calculations of the Results :

UREA NITROGEN (BUN)	UREA
SERUM OR PLASMA	
Urea Nitrogen (mg/dL) = $\frac{\Delta A\ (Sample)}{\Delta A\ (Standard)}$ x 25	Urea (mg/dL) = $\frac{\Delta A\ (Sample)}{\Delta A\ (Standard)}$ x 53.57

Homework:

- Determine the level of plasma Urea in the following cases and support your answer by some causes :

1- liver disease.
2- Diet high in protein.
3- Dehydration.

-References:

- Clinical Chemistry: Techniques, Principles, Correlations (Bishop, Clinical Chemistry)Mar 31, 2009,by Michael L. Bishop MS MT (ASCP) CLS (NCA) and Edward P. Fody MD
- http://www.nlm.nih.gov/medlineplus/ency/article/003605.htm