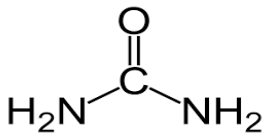
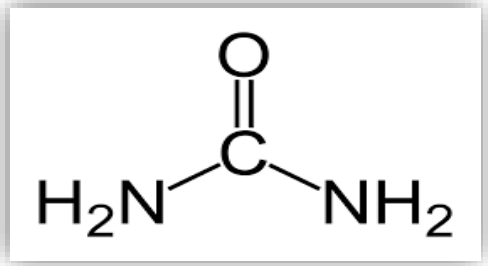
Estimation of Serum Urea

BCH 472



Urea:

- Urea is the highest non-protein nitrogen compound in the blood.
- Urea is the major excretory product of protein metabolism.
- It is formed in the liver from free ammonia generated during protein catabolism.
- The most important catabolic pathway for **eliminating excess nitrogen** in the human body.
- 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.



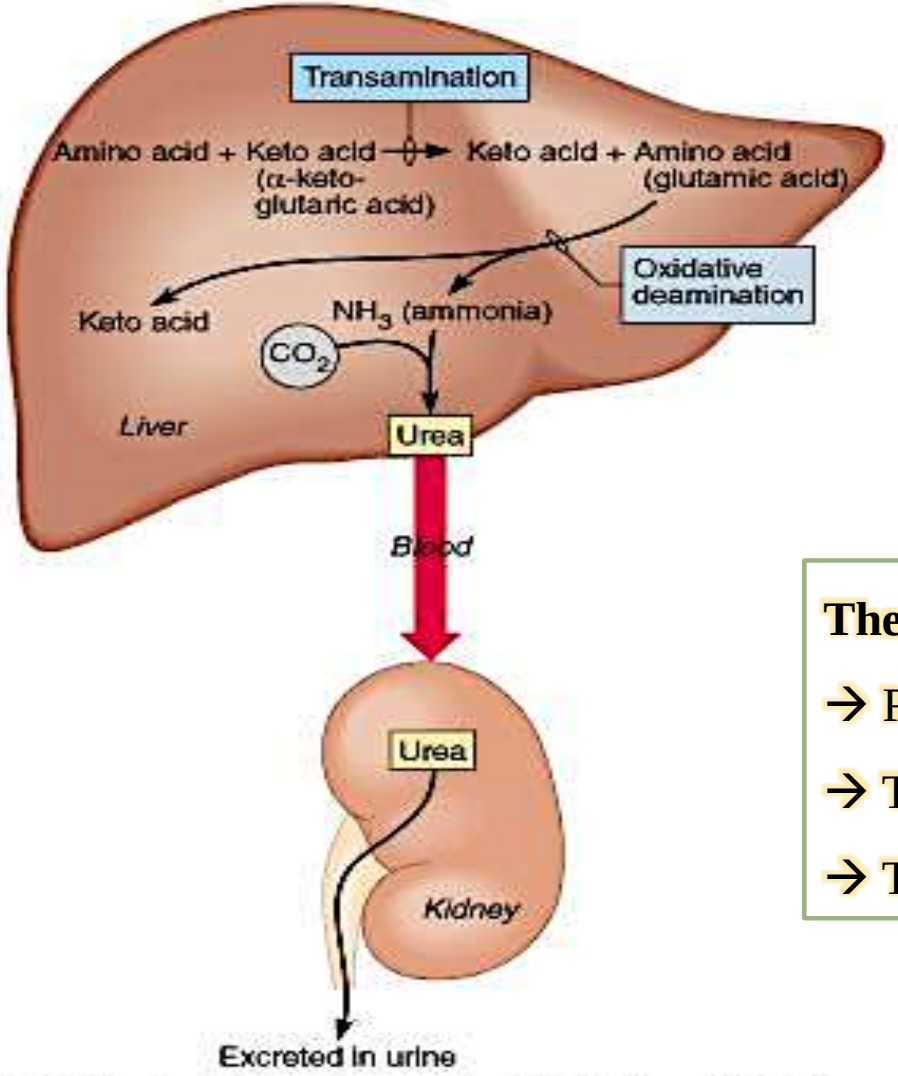
Urea synthesis :

- 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,

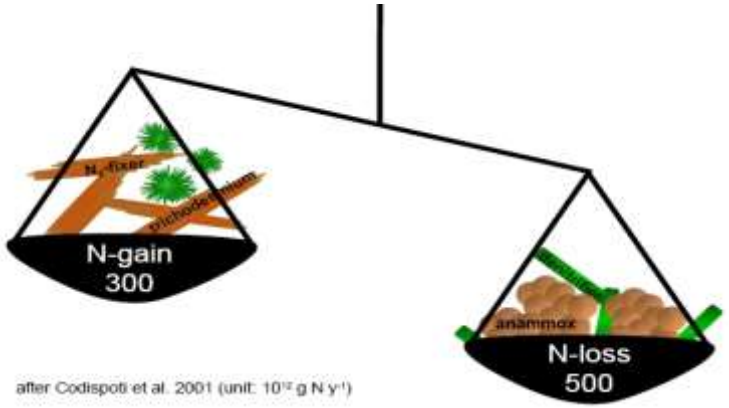
→ The protein content in diet, and

→ The rate of protein catabolism

Clinical Application:

Measurement of urea used in :

- Evaluate renal function
- To assess hydration status
- To determine nitrogen balance
- Check a person's protein balance
- To aid in the diagnosis of renal diseases
- To verify adequacy of dialysis



after Codispoti et al. 2001 (unit: 10^{12} g N y^{-1})

Urea Concentration- ***plasma:***

- 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 .
- But its diagnostic value improves **with serum creatinine values**.

	Type	Cause	Note
High urea (azotemia)	*Pre-renal*	• Cognitive heart failure • Dehydration • High protein diet • Increased protein catabolism	Cognitive heart failure → causes 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 (Starvation, anorexia) • Liver disease • Pregnancy	During pregnancy, the glomerular filtration rate increases by 50%

Urea Concentration- *Urine*:

- 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 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

Experiments

- Estimation of Blood Urea

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

2. 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).

The amount of the urea in the sample is proportionally related to the decreased absorbance at 340 nm as a result of NADH oxidation to NAD^+.

Method:

	Std	Test
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 60 seconds, read and record the absorbance A2 and determine ΔA

Calculations of the Results :

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

** There are two nitrogen atoms in each urea molecule, hence the concentration of std. nitrogen conc. is almost half std. urea conc.*

Discussion:

Comment on the level of Urea in serum .

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

Questions:

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.