جامعة الملك سعود
King Saud University
1957

ESTIMATION OF SERUM UREA

BCH 472 [Practical]

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.

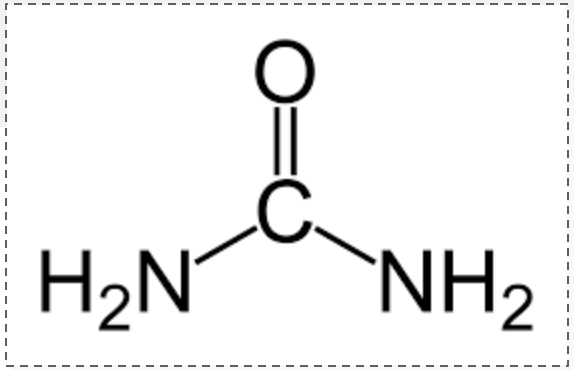

Figure 1. Chemical structure of urea

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).

Dehydration → ↓ Urine Flow → Concentrated Urine ↑ Urea reabsorption

The concentration of urea in the plasma is determined by:

1. Renal and liver function
2. The protein content in diet
3. The rate of protein catabolism

Urea synthesis

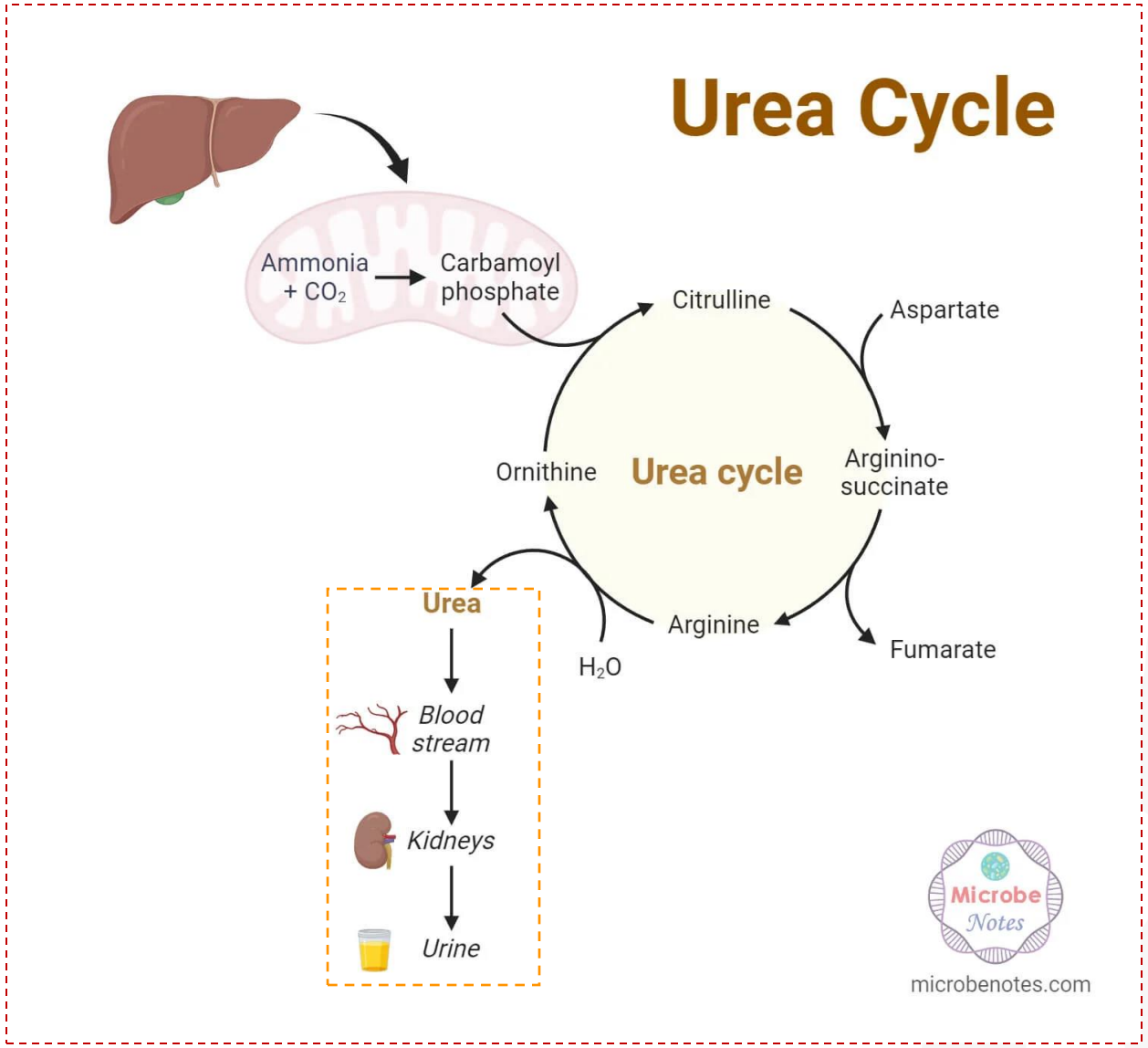

Figure 2. Urea cycle

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

Plasma urea Concentration

1. 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*?).
2. But its diagnostic value improves with **serum creatinine values**.

	Type	Cause	Note
High urea (High urea concentration in plasma is called **uremia**) (***Uremia vs 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	

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.

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

💡 Urea nitrogen VS urea ?

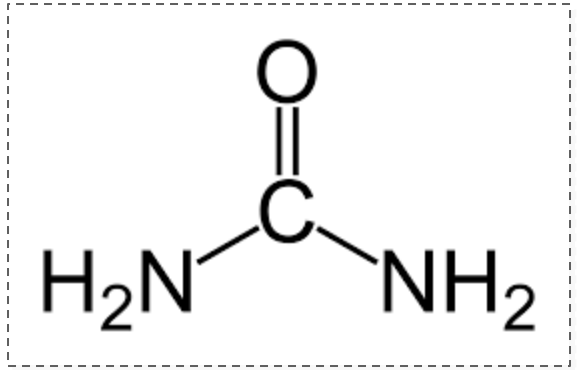

Figure 3. Chemical structure of urea

Practical Part

Objectives:

- Estimation of blood urea nitrogen (BUN).

Principle (*of the used kit*):

The Reagent used contains: Urease, Glutamate Dehydrogenase, NADH, 2-oxoglutarate, buffers and stabilizers .

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

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

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

$$\mathbf{2NH_3} + \text{2-oxoglutarate} + \mathbf{NADH + H^+} \xrightarrow{GLDH} \text{Glutamate} + \mathbf{NAD^+} + H_2O$$

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.

Materials:

Stanbio urea nitrogen (BUN) liquid-UV procedure

Method:

	Standard	Serum
Working reagent	1ml	1ml
Pre-warm at 37°C for 3 min. and add:		
Standard	0.01/10µl	-
Serum	-	0.01/10µl

1. After exactly **30 seconds,** read and record absorbance $\mathbf{A_1}$ against distilled water at **340 nm.**
2. 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:

$$\textbf{-Serum BUN (mg/dL)} = \frac{\Delta A\ (\text{Sample})}{\Delta A\ (\text{Standard})} \times \text{Std. Conc. (30mg/dL)}$$

-Serum urea (mg/dL) = BUN x 2.14

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

- Urea nitrogen urine test: MedlinePlus Medical Encyclopedia. (2025, Feb 13). Retrieved from https://medlineplus.gov/ency/article/003605.htm