DETERMINATION OF SERUM UREA

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Advanced Clinical Biochemistry I

Fall Semester

Week Three

15/10/2024

Objectives

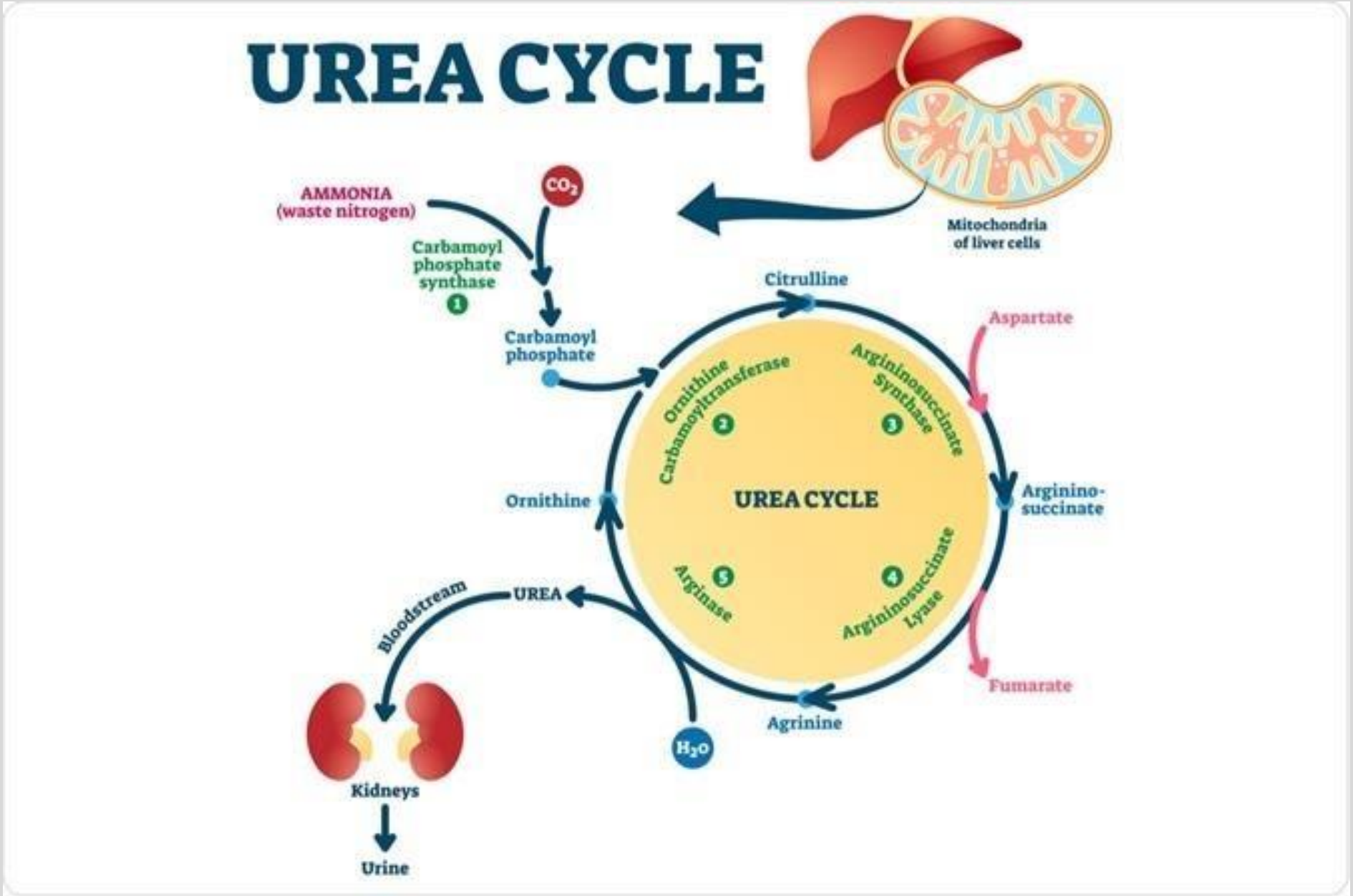
- At the end of this lab session, **students should understand:**
- The importance of Urea test in the diagnosis of kidney disease
- The principles behind the urea test
- The step-by-step procedures for urea test
- The clinical significance of urea tests

Introduction

- Ammonia can be produced by the breakdown of amino acids, or by the gut bacteria in humans.
- If the level of ammonia in the blood becomes too high, then it becomes toxic to the brain.
- The urea cycle removes ammonia from the blood and makes urea, which is eventually excreted as urine.
- This cycle is carried out by the cells of the liver.

Urea Cycle

- **Carbamoyl phosphate** is formed from NH_3 and bicarbonate, by carbamoyl phosphate synthetase (CPS) in the mitochondria.
- Ornithine transcarbamoylase (OTC) condenses carbamoyl phosphate and ornithine to form **citrulline** and then transported to the cytosol
- Argininosuccinate synthetase (AS) condenses citrulline and aspartate to form **argininosuccinate in the cytosol.**
- Argininosuccinate is broken down into **arginine** and fumarate by argininosuccinate lyase (AL).
- Arginine is broken down into **urea** and ornithine **by arginase.**
- **Ornithine translocase** transports ornithine into the mitochondria.


UREA CYCLE
Mitochondria of liver cells
AMMONIA (waste nitrogen)
CO_2
Carbamoyl phosphate synthase
1
Carbamoyl phosphate
Citrulline
Aspartate
Ornithine Carbamoyltransferase
2
Argininosuccinate synthase
3
Ornithine
UREA CYCLE
Arginino-succinate
Arginase
5
Argininosuccinate Lyase
4
Fumarate
Agrinine
H_2O
UREA
Bloodstream
Kidneys
Urine

Clinical Significance

- Elevated levels of urea can appear in blood (Uremia) in diets with excess protein.

- Renal disease, heart disease, gastrointestinal hemorrhage, dehydration, or renal obstruction could be developed due to defect in the cycle

- Clinical diagnosis should not be made on a single test result.

Principle

- Urea in the sample is hydrolyzed enzymatically into ammonium (NH_4+) and CO_2.

- Ammonium ions react with salicylate and hypochlorite (NaClO), in the presence of the catalyst nitroprusside, to form a blur-green indophenol as follows:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH_3 + \text{Salicylate} + NaClO \xrightarrow{\text{Nitroprusside}} \text{2,2 dicarboxyIndophenol}$$

(blue-green complex) absorb at 600 nm

Reagents

- R1: Salicylate
- R2: (Enzymes) Urease
- R3: Alkaline reagent
- R4: Standard (40mg/dL or 6.66 mmol/L)

Procedure

Pipette into the tubes	Blank	Standard	Assay/Test
$R_W = R_1 + R_2$ (µL)	1000	1000	1000
dH_2O (µL)	5		
Standard (R_4) (µL)		5	
Serum (µL)			5
Mix the reagents and wait for 4 minutes at room temperature (T_R)			
R3 (Alkaline) (µL)	1000	1000	1000
Mix and allow to stand for 5 minutes at T_R. Read the absorbance at 600 nm against blank. The color of the reaction stays only for 2 minutes hence OD should be taken immediately.			

Calculation of result

- Urea Conc. (mg/dl) = $\frac{A_{sample}}{A_{standard}}$ x 40 (std conc.)

- Conversion factor: mg/dl x 0.1665 = mmol/L

- Reference Value:

  - Serum: 15 – 45 mg/dL (2.49 – 7.49 mmol/L)

Second Practical Report

- Refer to practical note 1 and write a step-by-step practical report for today's experiment.
- The report should comprise all the components outlined in the standard report format and should not be more than five (5) or less than three (3) pages.
- The submission date is Tuesday 22nd October 2024 during practical session


Comments/
Questions