DETERMINATION OF SERUM CREATININE

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Advanced Clinical Biochemistry I

Fall Semester

Week Four

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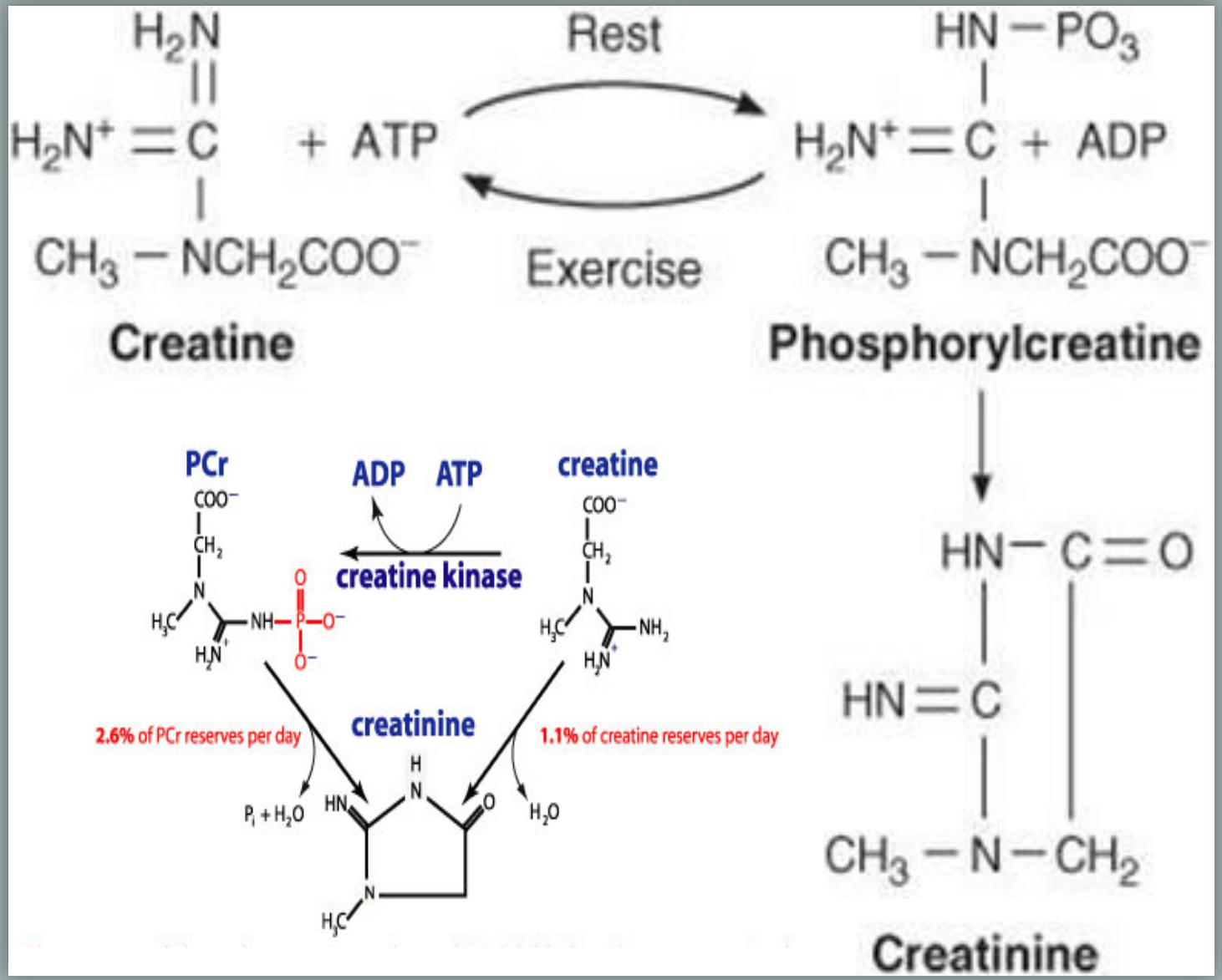
Objectives

- At the end of this lab session, **students should understand:**
- Generation of creatinine in the muscles
- The clinical significance of creatinine tests
- The principles behind the creatinine test
- The step-by-step procedures of the test

Introduction

- Interconversion of p-creatine and creatine is a feature of metabolic processes of muscle contraction.
- Creatine and p-creatine partially convert to creatinine.
- The amount of creatinine produced per day does vary from day to day.

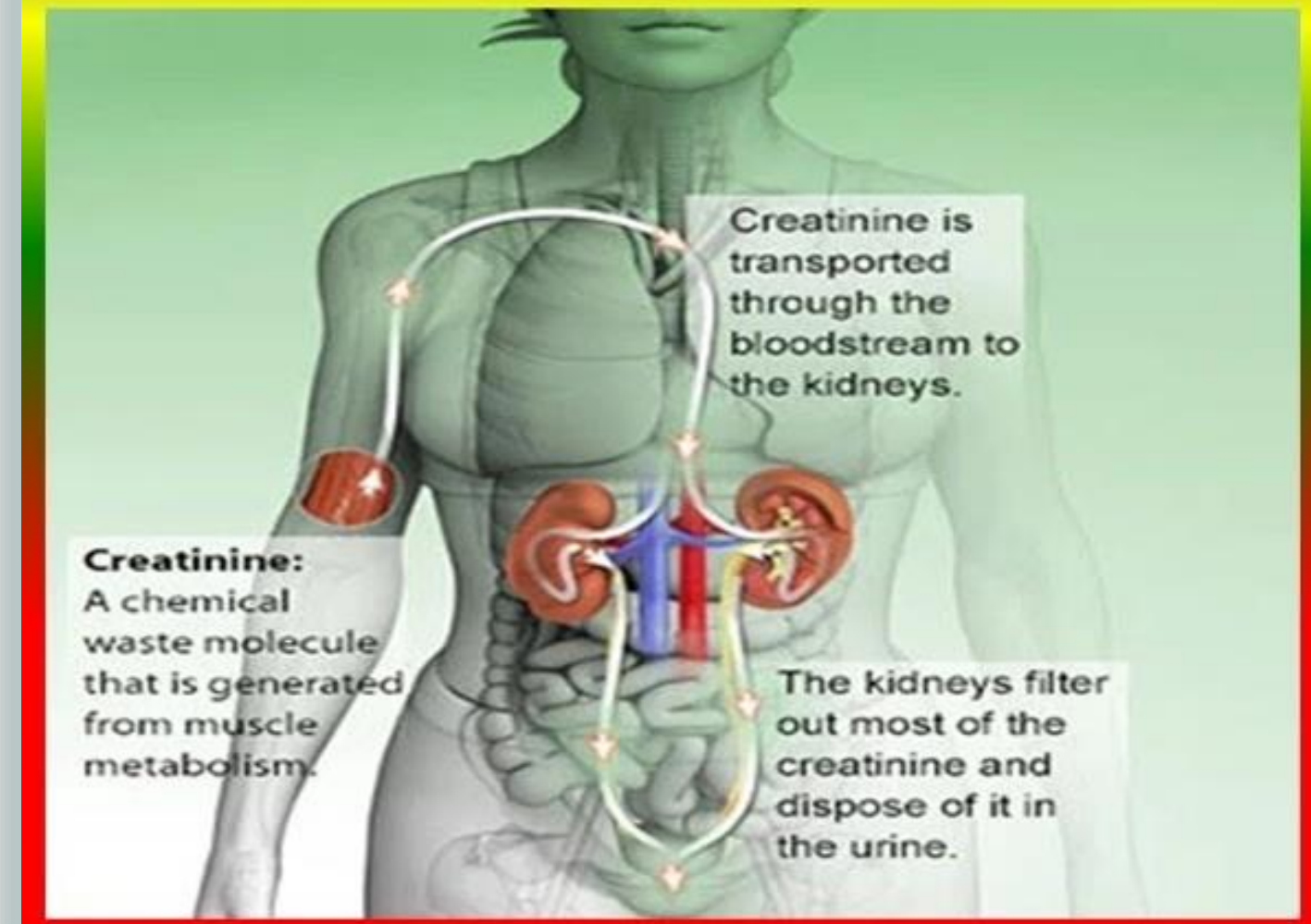
Generation of creatinine



Clinical Significance of the test

- It is a fundamental tool for diagnosing kidney-related disorders and monitoring the overall health of the kidneys. These include:
  - **Evaluation of Kidney Function**
  - **Glomerular Filtration Rate (GFR) Estimation**
  - **Monitoring Chronic Kidney Disease (CKD)**
  - **Assessment of Acute Kidney Injury (AKI)**
  - **Detection of Kidney Damage from Diabetes and Hypertension**
  - **Muscle Mass and Kidney Function Relationship**
  - **Guiding Drug Dosing**
  - **Screening for Urological Obstructions such as kidney stones or tumors**

Creatinine


Creatinine is transported through the bloodstream to the kidneys.
Creatinine:
A chemical waste molecule that is generated from muscle metabolism.
The kidneys filter out most of the creatinine and dispose of it in the urine.

Reagents

- **R1 Creatinine (corrosive)**
  - Disodium Phosphate 6.4 mmol/L
  - Sodium hydroxide 150 mmol/L

- **R2: (safe to handle)**
  - Sodium dodecyl sulfate 0.75 mmol/L
  - Picric acid 4.0 mmol/L pH 4.0.

- **R3: (safe to handle)**
  - Standard 177 µmol/L (2 mg/dL)

Preparation of working reagent

- Mix R1 and R2 in equal volume
- Shake the content gently before use

Principle

- Colorimetric reaction of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pre-treatment step.

- This reaction has been improved (specificity, speed, and adaptability) by the development of an initial-rate method.

Specimen Collection and Handling

- Serum or heparinized plasma.
- Urines: Collect at a precise timed intervals (4, 12 or 24 h).
- Dilute 1+19 in demineralized water before determination.
- Creatinine is stable for 24 h at 2-8°C

Preparation for working reagent & Procedure

PROCEDURE

Manual method

Let stand reagent and specimens at room temperature.

Working Reagent (R1+R2)	1000 µL
Specimen (Note 3)	100 µL

Mix well. Perform kinetic tests at 37°C (verify constant temperature). After 30 seconds read absorbance A1 and exactly 120 sec after read absorbance A2 at 490 nm (490-510) against distilled water. Test tube by tube with water, calibrator, controls and then assays as specimen

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other applications proposal are available on request.
3- Specimen: serum, plasma, urines diluted (1-19) in demineralised water and water (zero point).

Result

CALCULATION (6)

Serum or plasma

$$\text{Result} = \frac{(A2 - A1)\ \text{Assay} - (A2 - A1)\ \text{Blank}}{(A2 - A1)\ \text{Standard} - (A2 - A1)\ \text{Blank}} \times \text{Standard Concentration}$$

Urines diluted with 1+19: Multiply the above result by dilution factor 20

GFR (by creatinine clearance determination):

Using 24 h urine and serum creatinine

$$\text{Corrected Creatinine Clearance (mL/min)} = \frac{\text{UCr} \times \text{V} \times 1.73}{\text{SCr} \times \text{BSA}}$$

UCr = Urine Creatinine in mg/dL or µmol/L
SCr = Serum Creatinine in mg/dL or µmol/L
V = Urine volume excreted in mL/min (24 h urine volume/1440)
BSA = Body Surface Area in m^2

OR

Using only serum creatinine (by Cockcroft and Gault formula)

$$\text{Creatinine Clearance} = \frac{140 - \text{age in years} \times 2.12 \times \text{weight in Kg} \times \text{K}}{\text{Serum Creatinine (µmol/L)} \times \text{BSA } (m^2)}$$

K = 1.00 for men or K = 0.85 for women

Reference Values

Serum or Plasma	µmol/ L	mg/dL
Male	80 115	0.9 – 1.3
Female	53 - 97	0.6 – 1.1
Urine	**µmol/ kg / 24/ h**	**mg/ kg / 24/ h**
Male	124 – 230	14 - 26
Female	97 - 177	11 – 20

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 29th October 2024 during practical session


Comments/
Questions