CREATININE ESTIMATION
AND
CREATININE CLEARANCE TEST
OBJECTIVES

1. To estimate creatinine in serum and urine.
2. To calculate creatinine clearance test value.
3. To assess renal function, i.e. to assess the glomerular filtration rate.
What’s Cratine and Creatinine...?

- Creatine and creatinine are not the same substance!
- Creatine is found in the muscles......
- **Creatinine** is a break-down product (a waste product) of **creatine phosphate and creatine** in **muscles**, and is usually produced at a fairly constant rate by the body (depending on muscle mass).
1. The creatine is an amino acid that does not found in proteins.

2. Creatine is a nitrogenous organic acid.

Structure of Creatine
Structure of Creatine Phosphate

Creatine\(\sim\)phosphate
Formation of Creatinine from Creatine Phosphate

\[
\text{Creatinine} \quad \xrightarrow{\text{P}_i + \text{H}_2\text{O}} \quad \text{Creatine}\sim\text{phosphate}
\]
What’s the Relationship between Creatine and Creatinephosphate?

- Creatine and creatine phosphate exist in a reversible equilibrium in skeletal muscle.
- In skeletal muscle, approximately one-fourth of creatine exists as free creatine and three-fourth exists as creatine phosphate.
The Reversible Equilibrium between Creatine and Phosphocreatine
Creatine Phosphate is a Compound with High-Energy Phosphate Bond

- **Creatine can be charged with energy:** The enzyme creatine kinase (CK) which transfers the high-energy phosphate group from ATP to creatine to make creatine phosphate, a compound with high-energy phosphate bond.
The Reversible Equilibrium between Creatine and Phosphocreatine

Creatine Phosphate-ATP interaction

Phosphocreatine

Creatine

ADP

ATP

Creatine Kinase
Formation of Creatinine

Creatine + ATP \[\xrightarrow{\text{Creatine Kinase}}\] Phosphocreatine + ADP

Spontaneous, non-enzymatic cyclization

Creatine \[\xrightarrow{\text{Spontaneous}}\] Creatinine

Phosphocreatine \[\xrightarrow{\text{Pi}}\] Creatinine
Enzymatic Interconversion of PCr and ATP

Creatine

ATP

Creatine kinase (2-way enzymatic catalyst)

PCr

ADP + H⁺ "acidic"
Spontaneous One-Way Conversion of PCr and Creatine

The rate of formation of creatinine from phosphocreatine is twice the rate of formation of creatinine from free creatine.
Creatine Phosphate is a Source of Energy During Exercise….

- Creatine phosphate, by reversal of the above reaction, plays an important role in recharging ADP to ATP during severe anaerobic exercise.
- The reaction contributes to ATP resynthesis for about 10 to 20 seconds of maximal exercise.
Why Creatine Phosphate is Important for the Body..?

- Creatine phosphate functions as a 'battery' that stores the energy at excess ATP.
- Therefore it has a major importance for energy production in muscles.
We Know that Creatine is Found in the Muscles ....but Where is it Synthesized ?

1. **Endogenous Source**
   - **CREATINE SYNTHESIS IN DIFFERENT TISSUES OF THE BODY**: Creatine is synthesized primarily by the liver, kidneys, and pancreas at a rate of 1 to 2 g/day.

2. **Exogenous Source**: An additional 1 to 2 g/day is obtained in the diet, mainly from fish and meats.
What is the Fate of Creatinine that was Produced from Break Down of Creatine Phosphate during Anaerobic Exercise..?

- **CREATININE EXCRETION**
  - The creatinine is a waste product of creatine phosphate and *it will be excreted* by the kidney in the urine at a rate of 1 to 2 g/day.
Creatinine Metabolism

Approximately 2% of the body's creatine is converted to creatinine every day.

Creatinine is transported through the bloodstream to the kidneys.

The kidneys filter out most of the creatinine and excrete it in the urine.
What Happens to Creatinine Level (in Both Blood and Urine) if the Patient Has Renal Failure....?
Creatinine has been found to be a fairly reliable indicator of kidney function. The amount of creatinine in urine goes down while its level in blood goes up. If the kidneys are damaged or impaired and cannot work normally, serum creatinine level is an important diagnostic tool to assess renal function.
How is Creatinine Used to Monitor Renal Function...?

- **Serum creatinine**: (creatinine level in the blood)
  - In renal failure the kidney will not be able to excrete creatinine in urine leading to an elevation in serum creatinine level.
Levels of Creatinine in the Blood Depends Mainly on Renal Function....but...

- Is there other factors may affect creatinine level in the blood...?
Serum Creatinine may be Affected Partly by…. 

1. **The amount of muscle tissue you have.** Men tend to have higher levels of blood creatinine because they have more *skeletal muscle tissues* than women.

2. **Protein in diet.** Vegetarians have been shown to have lower creatinine levels in blood.
How is Creatinine Used to Monitor Renal Function....?

1. Creatinine Clearance Test:

• Measures *how well creatinine is removed from blood by kidneys* [which reflects glomerular filtration rate (GFR )].
How is Creatinine Used to Monitor Renal Function....?.....cont.

1. Creatinine Clearance Test

- A *Creatinine Clearance Test* gives better information than a blood creatinine test on how well your kidneys are working (as serum createnine may be affected by muscle bulk).
The Glomerular Filtration Rate

- GFR is the rate at which blood is filtered through all of the glomeruli (the plural of glomerulus)
  
  or

- GFR is the measure of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time.

GFR is often used to determine renal function
WHAT IS THE GLOMERULUS?
Glomerulus Structure
1. A glomerulus is one of the small units in the kidney made up of capillaries and nerve fibers or it is a capillary tuft surrounded by Bowman's capsule in nephrons of the kidney.
2. It is the structure where blood filtration takes place.
Structure and Function of Glomerulus

Glomerular Filtration Rate

Diagram showing the blood flow through the glomerulus, including afferent and efferent arterioles, Bowman's capsule, and the proximal tubule, leading to the filtrate.
How is Creatinine Used to Monitor Renal Function? ……cont.

• In renal failure, the filtering (GFR) of the kidney is deficient, *blood levels of some substances will rise and urine levels will decrease.*

• **Creatinine Clearance.** Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the GFR.
How to do Creatinine Clearance Test?

The Principle Protocol of Creatinine Clearance Test:

- A creatinine clearance test is done on both a blood sample and on a sample of urine collected over 24 hours (24-hour urine samples).
Are there any substances used to assess GFR other than creatinine?
Different Clearance Tests Used to Assess Renal Function

Kidney function tests (Clearance tests):

is a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning.

There are 3 major clearance tests:

2. Urea clearance test.
3. Inulin clearance.
What’s urea ....? 

- Urea is a: 
  1. Waste product of protein; it is created by protein catabolism 
  2. Excreted in the urine.
• Urea clearance test is a clinical test for renal function determined by the clearance of urea from the plasma by the kidney each minute.
Urea clearance test in order to determine the amount of urea that is filtered, or cleared, by the kidneys into the urine we require the following:

1. A blood sample to measure the amount of urea in the bloodstream
2. Two urine specimens, collected one hour apart.
3- Inulin Clearance Test

What’s Inulin?

- **Inulin** is the most accurate substance, after creatinine, to assess renal function because:
  1. it is a small, inert polysaccharide molecule that readily passes through the glomeruli into the urine.
  2. It is not reabsorbed by the renal tubules.
Creatinine clearance is the preferred, most common procedure used to assess renal function.
The Preference of Creatinine Clearance Test in Evaluating Kidney Function .....cont.

- Creatinine clearance is preferred because:

1. It is a normal constituent of blood and no infusion is needed unlike inulin.

2. Moreover it is not reabsorbed by the tubules as in the case of urea.
Glomerular Filtration Rate
Clearance Tests

- One of the most important kidney function tests, it is; the amount of plasma (in ml) that is cleared from a certain substance by the kidneys per minute.

Clearance = UV/P

- U = concentration of the substance in urine.
- P = concentration of the same substance in plasma
- V = volume of urine (ml/min)
High Levels of Creatine

- High Levels of Creatine in blood and urine are caused by:
  1. Break down of muscle cells.
  2. Starvation and fever.
  3. Diabetes mellitus.
  4. Hyperthyroidism.
Principle

- **Determination of Creatinine**

  - The methods most widely used today are based on the *Jaffe reaction*. This reaction occurs between creatinine and the picrate ion formed in alkaline medium (sodium picrate); a red-orange solution develops.
Principle.....cont.

Creatinine + Picric acid → Yellow color

NaOH (Alkaline medium)

Creatinine Picrate

Orange color
Measured colorimetrically at 520 nm
Materials

1. Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)
2. Creatinine working standard for serum (3mg/dl): dilute 10 ml of stock and increase the volume up to 500 ml with water.
3. Creatinine working standard for urine (0.75 mg/dl): dilute 50 ml of stock and bring the volume up to 200 ml with water.
4. Serum and urine samples.
5. NaOH (2.5 M).
6. Picric acid.
## Method

### 1. Serum Creatinine:

<table>
<thead>
<tr>
<th></th>
<th>Standard (A,B)</th>
<th>Test (C,D)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Standard for serum</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Picric acid</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
</tr>
</tbody>
</table>

1. Mix well
2. Add 0.4 ml of 2.5 M NaOH
3. Allow to stand for 20 minutes
4. Read the absorbance against the blank at 520 nm
## Method ......cont.

2) **Urinary Creatinine**

<table>
<thead>
<tr>
<th>Component</th>
<th>Standard (A,B)</th>
<th>Test (C,D)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>-</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>2 ml</td>
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</tbody>
</table>
Method ......cont.

2) Urinary Creatinine
5- mix well
6- Add 0.4 ml of 2.5 M NaOH
7- Allow to stand for 20 minutes
8- Read the absorbance against the blank at 520 nm
Calculations

- **Serum creatinine** = \( \text{Abs of test} \times \text{concentration of standard} \)
- **Urinary creatinine** = \( \text{Abs of test} \times \text{concentration of standard} \)
- **Creatinine clearance** = \( \frac{\text{Urinary creatinine}}{\text{Volume of urine ml/min/1.73 m}^2} \times \frac{\text{Serum creatinine}}{1.73 m^2} \)

- **Normal creatinine clearance:** 100-130 ml/min/1.73m².