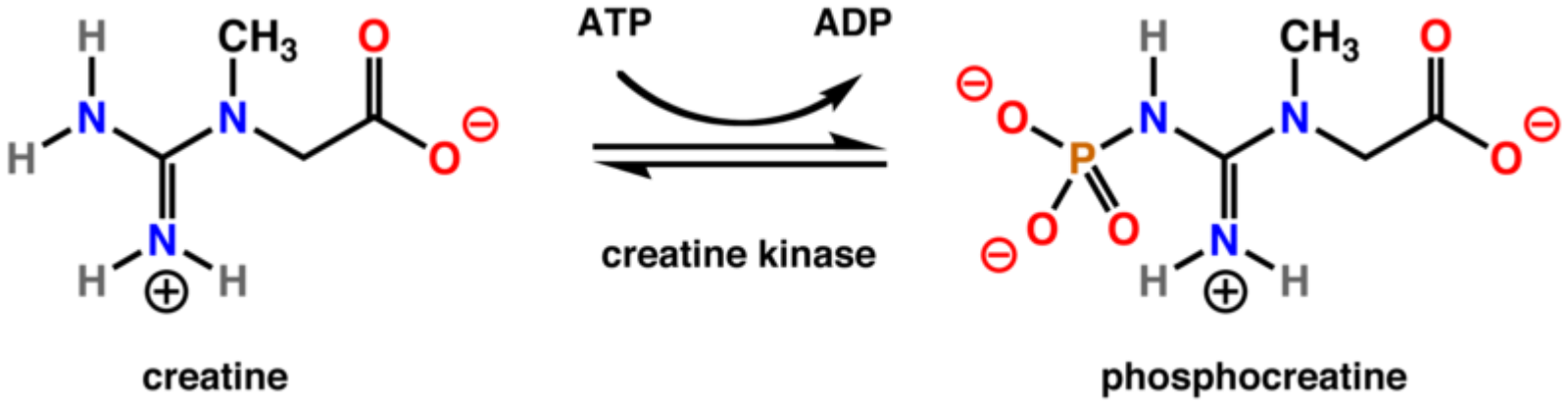
Experiment 3

Creatinine estimation and creatinine clearance tests

Introduction:

- Creatinine is derived from " creatine" which is synthesized in the liver and kidney , it moves through the circulation and is taken up entirely by muscles.
- In the muscles it is converted to creatine phosphate which becomes the source of a high energy phosphate bond for the immediate reformation of ATP. (ATP is needed continuously for muscle contraction)

ATP + creatine ⟶ creatine phosphate + ADP



➢Creatinine is a bi product of creatine metabolism and it is excreted in urine , it is totally endogenous and related to muscle mass and so it remains the same from day to day.

Normal values:

In serum: 0.1 – 1.2 **mg/dl**

In urine: 1 – 1.8 **g/day**

High levels of serum creatinine indicates kidney failure since it is excreted by the glomeruli, neither excreted or reabsorbed by the tubules so it is considered parallel to glomelular filtration rate (GFR)

What Abnormal Results Mean

may be due to any of the following:

- High meat diet
- Kidney problems, such as damage to the tubule cells
- Kidney failure
- Too little blood flow to the kidneys, damage to filtering units
- Kidney infection (pyelonephritis)
- Muscle breakdown (rhabdomyolysis), or loss of muscle tissue (myasthenia gravis)
- Urinary tract obstruction
- 2- Starvation and fever
- 3- Diabetes mellitus
- 4- Hyperthyrodism

Creatinine clearance:

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 any substance in urine

V= volume of urine(ml/min).

P= concentration of the same substane in plasma

*Creatinine Clearance also calculated by:

$C = U \times V_{ml} \times 1.73 / P \times 1440 \times A$ (A is surface area)

There are 3 major clearance tests:

1- Creatinine clearance.

2- Urea clearance

3- Inulin clearance

**Creatinine clearance is preferred because it is a normal constituent of blood and no infusion is needed unlike inulin. Moreover it is not reabsorbed by the tubules as in the case of urea.

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

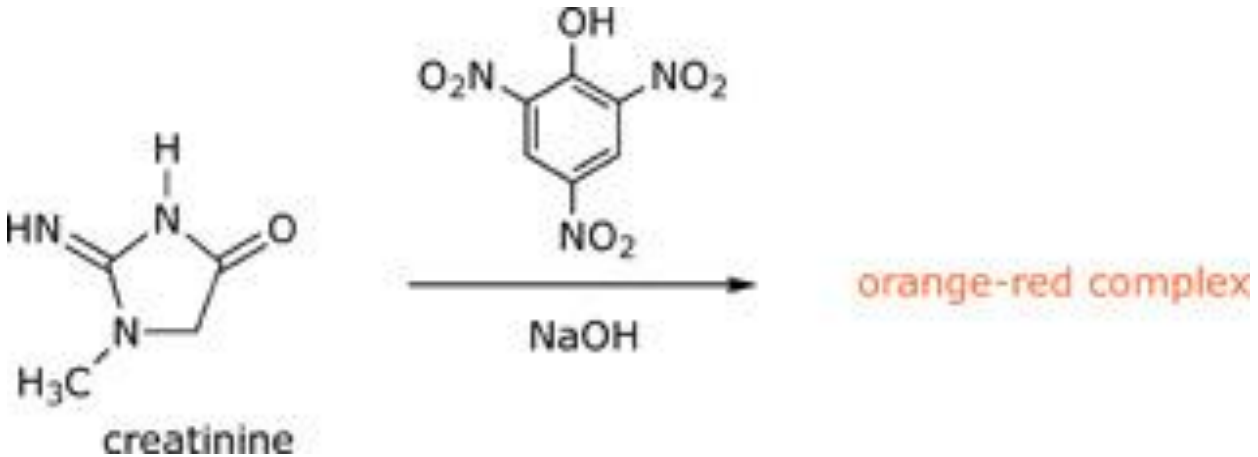
OBJECTIVES:

1- To estimate creatinine in serum and urine.
2- To calculate creatinine clearance value.
3- To diagnose a patient.

Principle (Jaffe's method):

Colorimetric estimation of creatinine using the alkaline picrate method.

Creatinine + picric acid ⟶ Creatinine picrate (orange) has Absorbance at 520nm



METHOD:

-Take one ml of urine and dilute it to 100 ml

Following the table :

(Note: Creatinien working standard (3mg/dl))

Chemical	Standard		Test (serum)		Test (urine)		Blank
	(A)	(B)	(C)	(D)	(E)	(F)	
Serum	-	-	**0.5 ml**	**0.5 ml**	-	-	-
Urine	-	-	-	-	**0.5 ml**	**0.5 ml**	-
Standard	**0.5 ml**	**0.5 ml**	-	-	-	-	-
Water	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	2 ml
Picric acid	6 ml	6 ml	6 ml	6 ml	6 ml	6 ml	6 ml

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

Results:

Tube	Standard		Test (serum)		Test (urine)	
	(A)	(B)	(C)	(D)	(E)	(F)
Absorbance at 520 nm						

Calculations

- **Mean Absorbance of Standard**
- **Mean Absorbance of Serum**
- **Mean Absorbance of Urine**

Serum creatinine = $\frac{\text{Mean Absorbance of serum X concentration of standard}}{\text{Mean Absorbance of Standard}}$ **= mg /dl**

Urinary creatinine= $\frac{\text{Abs of test serum X concentration of standard X D.F}}{\text{Mean Absorbance of Standard}}$ **=mg /dl**

concentration of standard= 3 mg/dl

Dilution factor =100

Creatinine Clearance =(U XV X1.73)/ (P X 1440 X A*)* : *V =1000 ml A=1.58*

ml/min/1.73m²

Normal creatinine clearance= 100-130 ml/min/1.73m²

Thank You