

**King Saud university**

**College of pharmacy**

**Pharmacology department**

**Practical Biochemistry**

**224 PHL**



# 224 PHL

# Practical Biochemistry

# Part(II)

Lab No. (8)

## Enzymes

**Definition:** Enzymes are highly specific biologic catalysts that greatly speed up the rate of a chemical reaction occurring in living cells. Enzymes are found in low concentration in body fluids.

The enzyme activity is expressed in the international unit (I.U).

**Definition of (IU):** The amount of an enzyme that will convert one micro-mole of substrate per minute in an assay system.

### Techniques for determination of enzyme activity:

#### 1. Two- Point Assay

**a-** A sample is incubated with substrate for a fixed time.

**b-** Stop the reaction.

**c-** Measure the amount of product formed or substrate used.

**N.B.** Must follows zero order reaction.

#### 2. Kinetic or Rate Reaction Assay

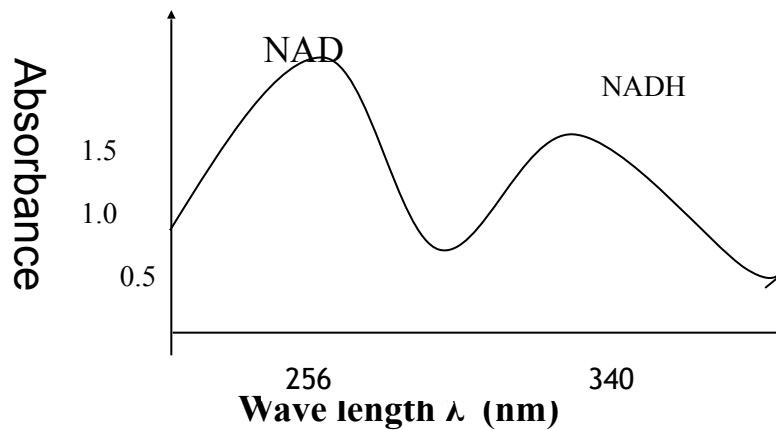
Changes are measured at short time intervals or continuously monitored.

- Co-enzyme NAD or NADP is used in this type of reaction.

- Using chromogenic substrate.

**Example:** In determination of lactate dehydrogenase (LDH) activity:





- The extinction at 256 nm will increase due to the formation of NAD and it will decrease at 340 nm due to the consumption of NADH.

### 3. **Radio- Immuno- Assay (RIA)**

it measures the concentration of enzyme but not the catalytic activity.

## **Classification of Enzymes:**

### 1. **Oxidoreductases:**

There is a hydrogen donor and a hydrogen acceptor.

**a. Aerobic oxidases:** use  $O_2$  as H-acceptor forming  $H_2O$  **e.g.** Tyrosinase.

**b. Aerobic dehydrogenases:** use  $O_2$  as H-acceptor forming  $H_2O_2$  **e.g.** Glucose oxidase.

**c. Anaerobic dehydrogenases:** use co-enzyme as H-acceptor **e.g.** LDH

**2. Transferases:** Transfer a group from one organic compound to another ( $CH_3$ ,  $NH_2$ , Phosphate etc...).

e.g. Aminotransferases, Kinases, Transketolases.

2. **Hydrolases:** hydrolyse the substrate e.g. Enzymes acting on :

- Ester bond (lipase, cholinesterase).
- Peptide bond (pepsin, trypsin).
- C-N bond (urease).

4. **Lyases:** Remove groups without hydrolysis leaving a double bond e.g. Decarboxylases.

5. **Isomerases:** convert one pair of isomers into another e.g. Racemases.

6. **Ligases = Synthetases:** linking two molecules together coupled with the breakdown of phosphate bond.

#### **factors affecting enzymatic reaction:**

1. Substrate concentration.
2. Enzyme concentration.
3. Product concentration.
4. PH.
5. Temperature.
6. Activators and Co-enzymes.
7. Inhibitors.
8. Specificity of enzymes.

#### 1. **Determination of Serum Lactate Dehydrogenase Activity (LDH)**

**Type:** anaerobic dehydrogenase enzyme.

**Occurrence:** Heart > liver > skeletal muscle > erythrocytes > pancreas

**Principle:**

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**Procedure:**

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**Calculation:**

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**Normal value:**

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**Clinical significance:**

↑ LDH activity indicates:

- a. Myocardial infarction.
- b. High value 20 X normal is seen in pernicious anemia.
- c. Moderate increase in viral hepatitis and skeletal muscle disease.

Lab No. (9)

## Determination of Serum Aminotransferases Activities

### 1. Determination of Serum Glutamate Oxaloacetate Transaminase (GOT) or Aspartate-Amino Transferase (AST) Activity :

Type : Aminotransferases.

Occurrence: Heart > liver > skeletal muscle > kidney > pancreas

#### Principle:

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#### Procedure:

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#### Calculation:

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#### Normal value:

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#### Clinical significance:

**GOT activity increase in :**

1. Myocardial infarction.
2. Hepatobiliary disease.

**2. Determination of Serum Glutamate Pyruvate Transaminase (GPT) or Alanine Amino Transferase (ALT) activity :**

**Type :** Aminotransferases.

**Occurrence:** Liver > Heart > Kidney > skeletal muscle > spleen

**Principle:**

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**Procedure:**

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**Calculation:**

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**Normal value:**

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**Clinical significance:**

**GPT activity increase in:**

- a. Liver damage and toxic hepatitis (high level).
- b. Myocardial infarction.

Lab No. (10)

## Determination of Serum Phosphatases Activity

- **Phosphatases** are enzymes which catalyze the splitting of phosphoric acid from mono-phosphate esters.
- They are hydrolases.

Organic phosphate esters + water  $\longrightarrow$  alcohol + phosphate ion

- **Two types are commonly estimated in the serum :**
  - a. Alkaline phosphatase with maximum activity at pH10.
  - b. Acid phosphatase with maximum activity at pH5.

### 1) Determination of Serum Alkaline Phosphatase (ALP) Activity :

**Occurrence:** in most tissues of the body, mainly in:

- Osteoblasts in bone .
- Bile canaliculi in liver .
- Small intestinal epithelium .
- Proximal tubules of kidney.
- Breasts during lactation .

In all these sites ALP seems to be involved in transport of phosphates across cell membranes.

- **ALP is activated** by  $Mg^{+2}$ ,  $Mn^{+2}$ ,  $Co^{+2}$ .
- **ALP is inactivated** by  $Zn^{+2}$ ,  $Cu^{+2}$ ,  $Hg^{+2}$ , EDTA.



**Principle:**

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**Procedure:**

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**Calculation:**

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**Normal value:**

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**Clinical significance:**

**Increase in ALP occurs mainly in:**

- 1) Bone diseases like Paget's disease (highest level), rickets, hyperparathyroidism, bone cancer.
- 2) Liver diseases like obstructive jaundice, biliary cirrhosis, carcinoma liver abscess.
- 3) Drugs producing cholestasis like androgens, sulfonamides.  
or hepatotoxic drugs like aspirin, gentamycin, cyclophosphamide, halothane .

**Decrease in ALP occurs in:** anemia, scurvy, and cretinism.

## 2-Determination of Serum Acid phosphatase (ACP)Activity :

### Occurrence:

The highest concentration of ACP is found in prostate (prostatic ACP), also in RBCs, leucocytes and platelets (non prostatic ACP).

- ACP has a maximum activity at pH5.6
- A variety of substrates have been used for determination of serum ACP activity . **These include:**
  - a) Nitrophenylphosphate-attacked by phosphatases of **non-prostatic origin**.
  - b) B-Glycerophosphate,  $\alpha$  naphthylphosphate, phenolphthalein monophosphate are **all non specific substrates for both**.
- **Prostatic Acid Phosphatase** is obtained by subtracting the results of the Non-Prostatic Acid Phosphatase assay from the results of the Total Acid Phosphatase assay on the same sample.

### a) **Determination of Serum Total Acid phosphatase Activity**

#### Principle:

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#### Procedure:

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**Calculation:**

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**Normal value:**

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b) Determination of Serum Non-Prostatic Acid phosphatase Activity

**Principle:**

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**Procedure:**

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**Calculation:**

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**Normal value:**

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c) **Determination of Serum Prostatic Acid phosphatase Activity**

**Calculation:**

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**Normal value:**

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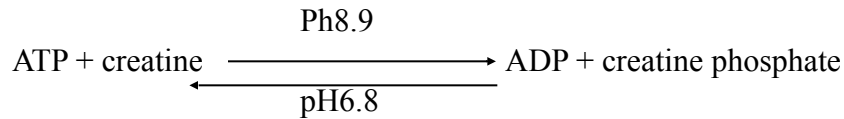
**Clinical Significance:**

Prostatic ACP is used mostly to detect prostatic carcinoma when it may reach very high level. In benign hypertrophy of prostate ACP is normal.

**Lab No. (11)**

**Determination of Serum Creatine Kinase (CK) Activity**

- Creatine kinase is a phosphotransferase enzyme which catalyses reactions responsible for formation of ATP in tissues.



When muscle contraction occurs, ATP is hydrolysed to ADP to produce energy for contraction process. An important step in regeneration of ATP is the reaction with creatine phosphate.

- The creatine kinase is activated by  $\text{Mg}^{+2}$

**Tissue Distribution:**

CK is widely distributed in skeletal muscle, brain and cardiac muscle.

**Sample Collection:**

- 1) Serum sample is used for CK assay.
- 2) Anti-coagulants inhibit enzyme activity.
- 3) CK is light sensitive and loses significant amounts of activity when exposed to light for long periods.

**Principle**

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**Procedure**

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**Calculation**

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**Normal value:**

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**Clinical Significance:**

**CK levels increase in a variety of disorders in which cardiac or skeletal muscle tissue is affected.**

- Myocardial infarction.
- Muscular dystrophy.
- Polymyositis.
- Malignant hyperthermia.
- In strenuous physical exertion and IM injection there is a transient elevation of CK.
- Malignant neoplasms of brain.
- Brain infarction.

## Determination of Serum $\alpha$ -Amylase Activity

- Amylase is an enzyme secreted by the salivary glands of the oral cavity and is known as salivary amylase or ptyalin.
- It is also secreted by the pancreas and is known as pancreatic amylase or amylopsin.

### Function of $\alpha$ -Amylase :

- Amylase catalyses the hydrolysis of starch, splitting the 1-4glycosidic linkages.
- This hydrolysis occurs randomly along the polysaccharide chain with the production of maltriose and dextrin.
- The carbohydrate digestion by amylase begins in the mouth and continues briefly in the stomach until the pH drops too low .
- It is completed in the small intestine by the action of amylase secreted by the pancreas.

### Determination of Serum $\alpha$ Amylase Activity by Enzymatic Colorimetric Method :

#### Principle:

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#### Procedure:

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**Calculation:**

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**Normal Value:**

**Clinical Significance:**

- **Very high serum amylase is seen in :**
  - a) Acute pancreatitis.
  - b) Obstruction of pancreatic duct by stone tumor .
  
- **Moderate increase is seen in :**
  - a) Acute peritonitis.
  - b) Perforated peptic ulcer.
  - c) Mesenteric thrombosis.
  - d) Small intestinal obstruction.



**Lab No. (12)**

## **Urine Analysis**

Urine analysis is performed to detect abnormal constituents that indicate a pathological state.

### **General characteristics of urine:**

- 1. Volume:** normally 1.5 – 2 L / Day.
- 2. Color:** urochrome (amber yellow).
- 3. Transparency:** Clear transparent.
- 4. Odor:** faint aromatic odor due to the presence of volatile organic acid.
- 5. PH :** slightly acidic 5.5 – 6.5.

### **Physiological and normal constituents of urine:**

Normally the urine is composed of 99% water and 1% solids.

Solids are:

- a. organic substances: urea, uric acid, creatine, creatinine, amino acids, lactic acid , vitamins, pigments, enzymes.
- b. inorganic substances:  $\text{NH}_4$ ,  $\text{SO}_4$ ,  $\text{Ca}^{+2}$ ,  $\text{Cl}^-$ ,  $\text{PO}_4$ ,  $\text{CO}_3$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{NO}_3$ , Fe, F, silicate.

## Some Pathological Constituents of Urine :

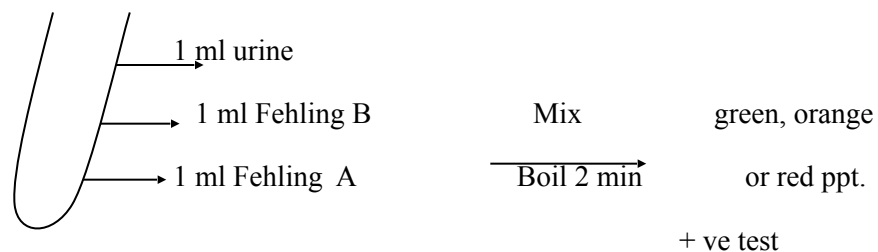
The following parameters are normally not present in urine:

1. **Glucose:** if serum glucose level exceeds the renal glucose threshold (180 mg/dl), it appears in urine (**Glucosurea**), as in diabetes mellitus.
2. **Protein:** the presence of protein in urine (**Proteinurea or Albuminurea**) can be seen in patients with glomerulonephritis.
3. **Blood:** blood in urine (Hematuria or Hemoglobinuria) could be seen in patients with bilharziasis or hemolytic anemia.
4. **Bile salts:** can be seen in patients with Jaundice.
5. **Ketone bodies or Acetone:** could appear in urine in late stages of diabetes mellitus.

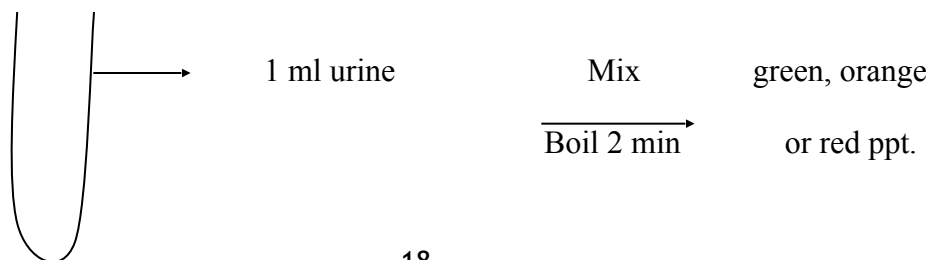
## Tests For Abnormal Constituents of Urine :

### Test For Glucose:

1-Fehling test :



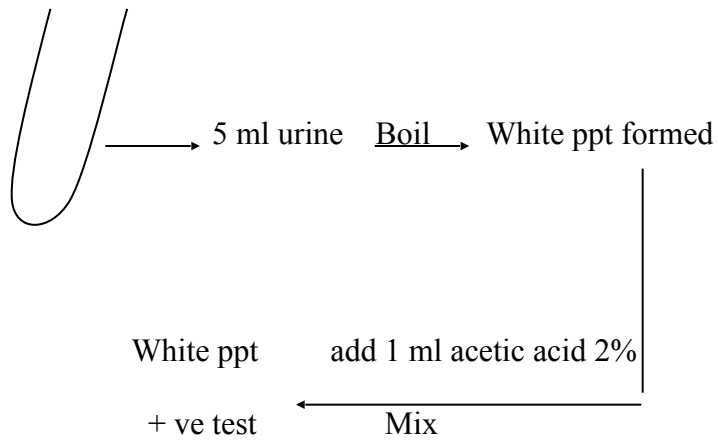
2-Benedict test:



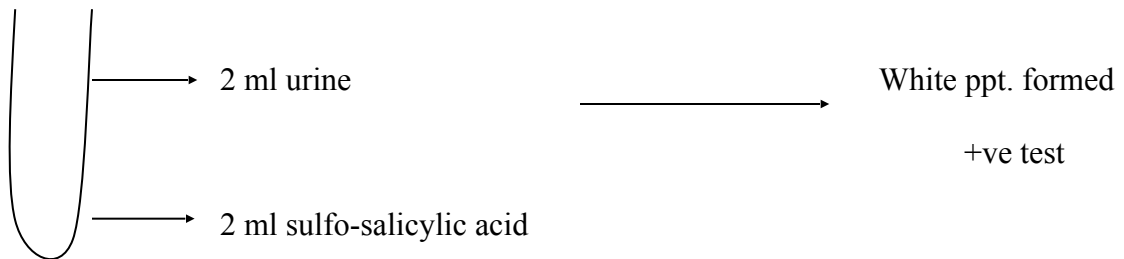
—————→ 5 ml benedict reagent + ve test

**Test For Proteins:**

1-Heat coagulation test:



2-Sulfo-salicylic acid test:



**Test For Bile Salts :**

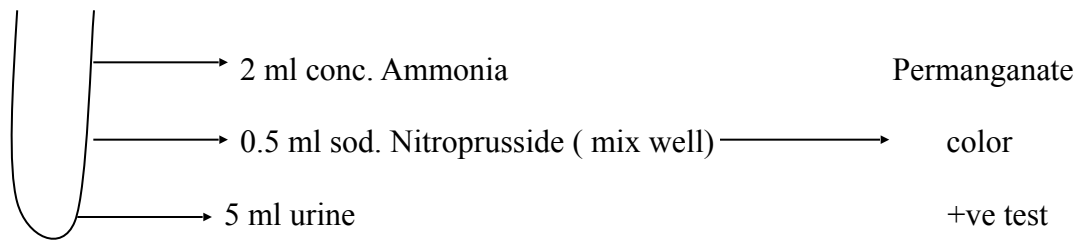
a small beaker take 10 ml urine then sprinkle some sulfur powder over it.

If sulfur powder floats —————→ - ve test (no bile salts)

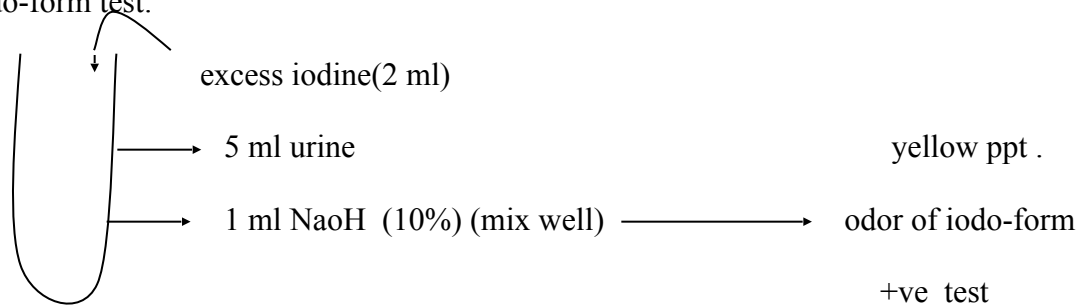
If sulfur powder sinks to the bottom of the beaker → +ve test

**Test For Acetone:**

a. Nitroprusside test:



b. Iodo-form test:



## Urine Analysis

Test	Observation	Comment
<p><b><u>Glucose</u></b>  <b>1) Fehling Test:</b>            1ml urine + 1ml Fehling A+1ml Fehling B. Mix Boil well.</p> <p><b>2) Benedict Test:</b>            1ml urine + 5ml Benedict reagent. Mix. Boil well.</p>		
<p><b><u>Proteins</u></b>  <b>1) Heat Coagulation Test:</b>            5ml urine. Boil well. Add 1ml acetic acid.</p> <p><b>2) Sulfo salicylic Acid Test:</b>            2ml urine+ 2ml sulfosalicylicacid. Mix.</p>		
<p><b><u>Bile Salts</u></b>            5ml urine in a small beaker sprinkle sulphur powder.</p>		
<p><b><u>Ketone Bodies</u></b>  <b>1) Nitroprusside Test:</b>            5ml urine + 0.5ml Na Nitroprusside. Mix well.            +2ml conc. Ammonia. Mix.</p> <p><b>1) Iodo form Test:</b>            5ml urine + 1ml NaOH (10%).            Mix. Add excess iodine soln. (about 2 ml)</p>		