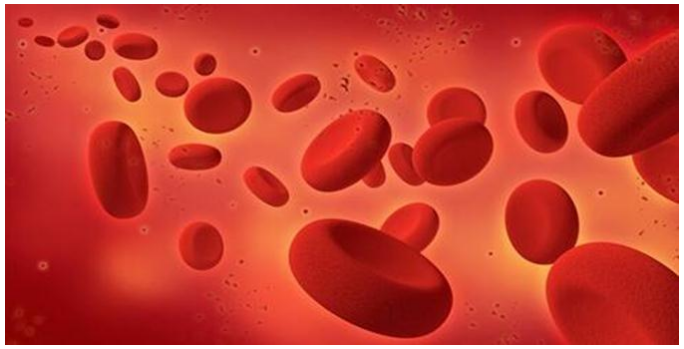
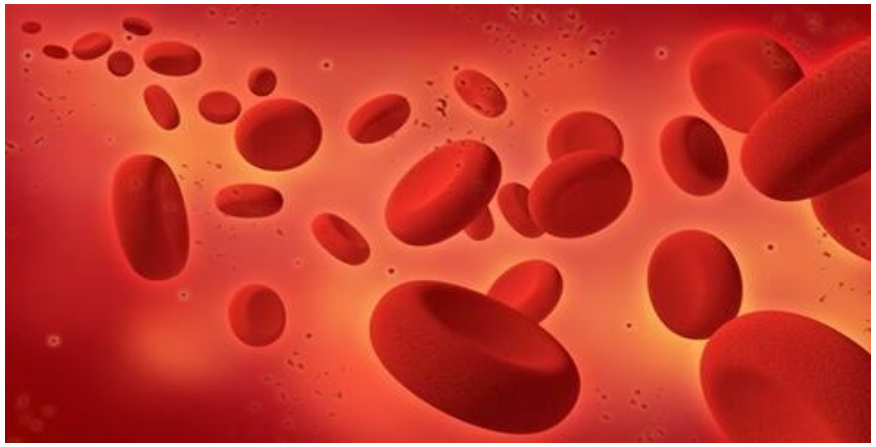


**Practical Biochemistry**
**2nd Class , 1st Semester Department of Dentistry**

# Blood Clinical Biochemistry

**Dr. Ahlam Said**

# Blood



## Introduction:

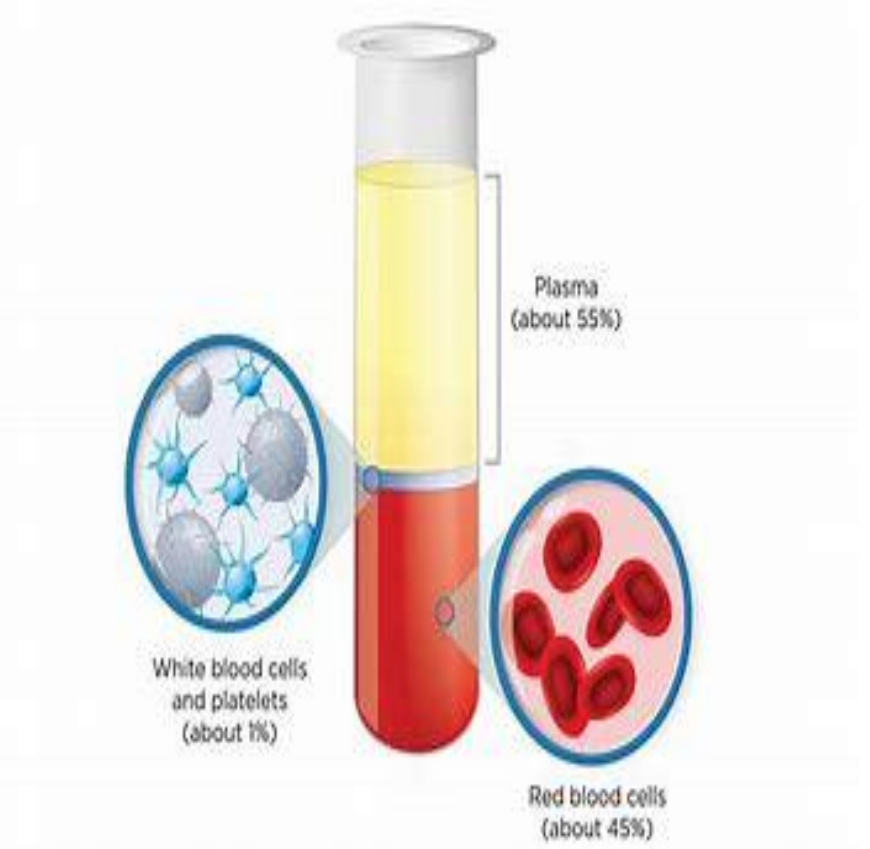
- **Blood** : is the red fluid constituent of the body that flows through the vascular channels ( artery, vein and capilary). The total volume of blood in a 70Kg adult is about 5.5 liters of 8% of the body weight.

- **Blood** is one of the most common specimens studied in biochemical laboratories in search of blood disorders, metabolic disorders and infection. Blood is actually the delivery medium for dissolved gases, nutrients, hormones, and more.

# COMPOSITION OF BLOOD

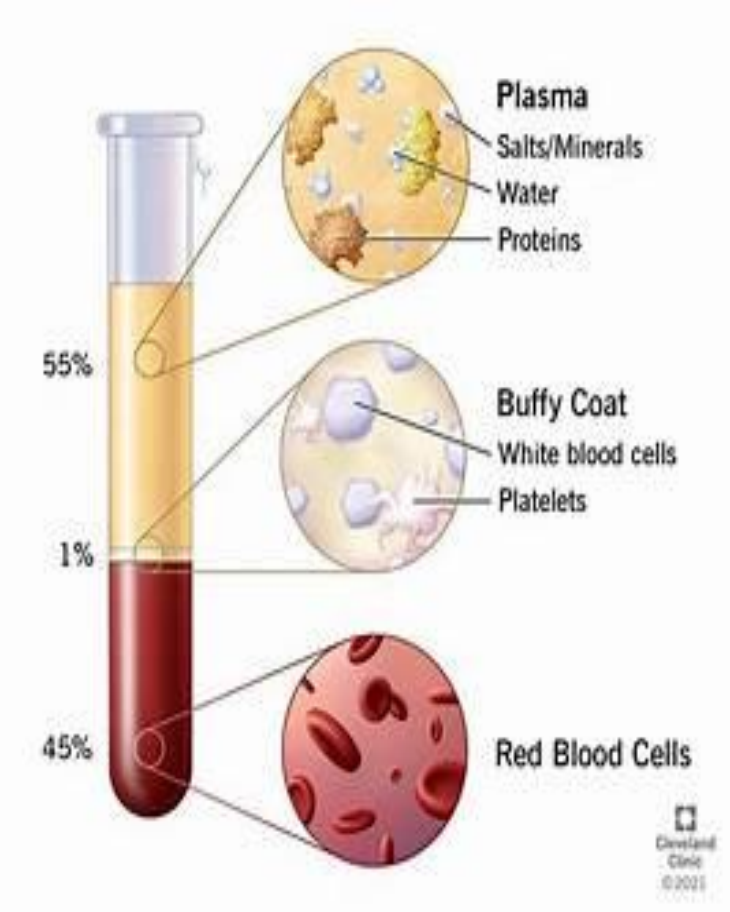
Blood has **four major** components:

**1-Plasma**:consists **55%** of total blood volume, plasma is the clear yellow fluid consisting of a solub protein called fibrinogen. Plasma combines water, sugar, fat, protein, and salt that make up blood's liquid component. Plasma's primary function is to carry blood cells and nutrients, waste products, antibodies, clotting proteins, chemical messengers such as hormones, and blood proteins also known as plasma proteins assist in regulating the body's fluid balance.

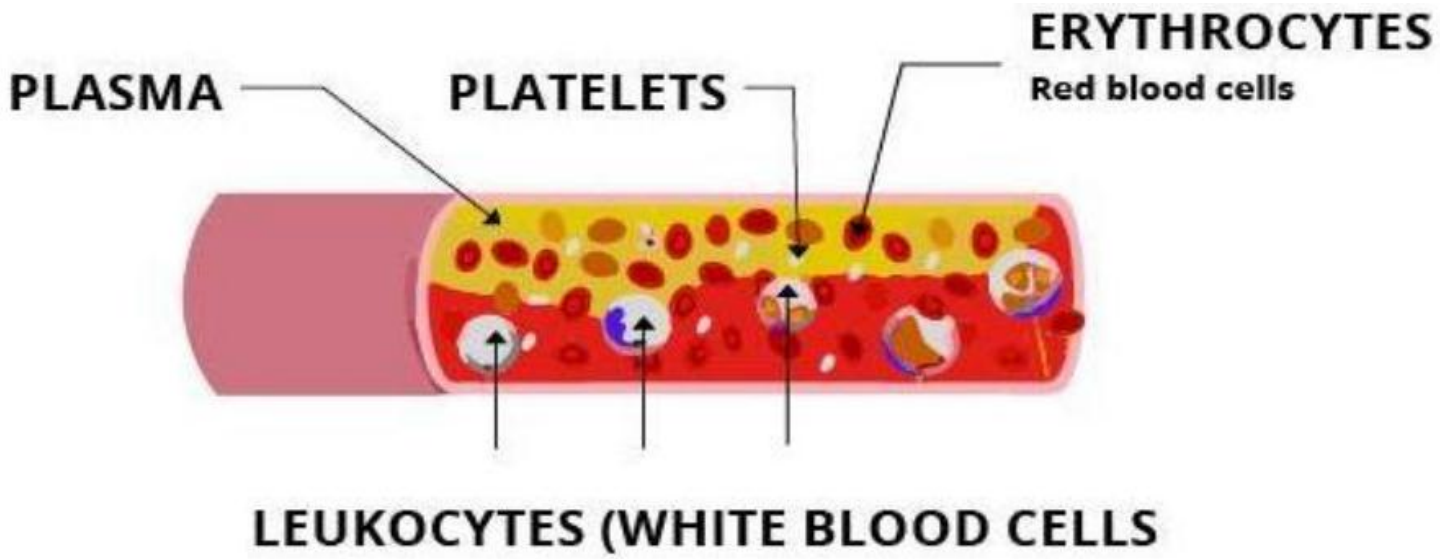


**2-Red blood cells**: The hematocrit is the volume of erythrocytes in a
blood sample. Hematocrit values vary by gender; men's values range from 44 to 45 per cent of blood volume, while women's values range from 39 to 44 per cent of blood volume. Blood appears red because of
the considerable quantity of red blood cells, which acquire their colour from haemoglobin.

**3-White blood cells (WBCs):** White blood cells, often known as leukocytes, account for less than 1% of total blood volume and play an important role in illness and fighting infection. The number of white blood cells in one ml of blood is typically between 3,700 and 10,500.



**4-Platelets:** Platelets, also known as thrombocytes, work with clotting proteins to prevent or minimize bleeding. Platelets should range between 150,000 and 400,000 per microliter of blood. Red blood cells, white blood cells, and platelets are produced in the bone marrow and then enter circulation



# Blood coagulation (clotting)

- Coagulation is the process by which blood forms clots. Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and protein (coagulation factor) component.
- Coagulation begins almost intantly after an injury to the blood vessel has damaged the endothelium lining the vessel.

## Blood sample

- There are three biochemical sample (whole blood, serum and plasma). the selection of sample depends on the parameter to be estimated. Anticoagulants are used when either whole blood or plasma is required.

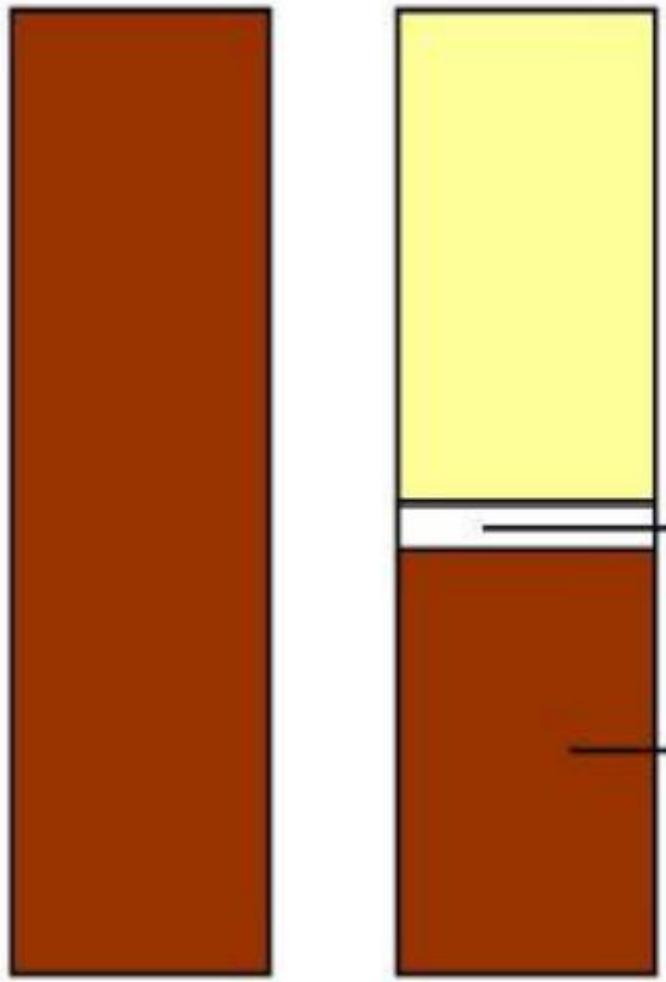
1. *Whole blood* (usually mixed with an anticoagulant) is used for the estimation of hemoglobin, carboxyhemoglobin, pH, glucose, urea, non-protein nitrogen, pyruvate, lactate, ammonia etc. (Note : for glucose determination, plasma is prefered in recent years).

2. **Plasma,** obtained by centrifuging the whole blood collected with an anticoagulant, is employed for the parameters—fibrinogen, glucose, bicarbonate, chloride, ascorbic acid etc.

3. **Serum** is the supernatant fluid that can be collected after centrifuging the clotted blood. It is the most frequently used specimen in the clinical biochemistry laboratory. The parameters estimated in serum include proteins (albumin/globulins), creatinine, bilirubin, cholesterol, uric acid, electrolytes (Na+, K+, Cl-), enzymes (ALT, AST, LDH, CK, ALP, ACP, amylase, lipase) and vitamins.

All biochemical tests come under chemical pathology. These are performed on any kind of body fluid, but mostly on serum or plasma. **Serum** is the yellow watery part of blood that is left after blood has been allowed to clot and all blood cells have been removed. This is most easily done by centrifugation which packs the denser blood cells and platelets to the bottom of the centrifuge tube, leaving the liquid serum fraction resting above the packed cells. **Plasma** is essentially the same as serum, but is obtained by centrifuging the blood *without* clotting. Plasma therefore contains all of the clotting factors, including fibrinogen.

# WHOLE BLOOD AND AFTER CENTRIFUGATION



Plasma is fluid component of blood.
Contains proteins, sugars, vitamins, minerals, lipids, lipoproteins and clotting factors.
95% of plasma is water

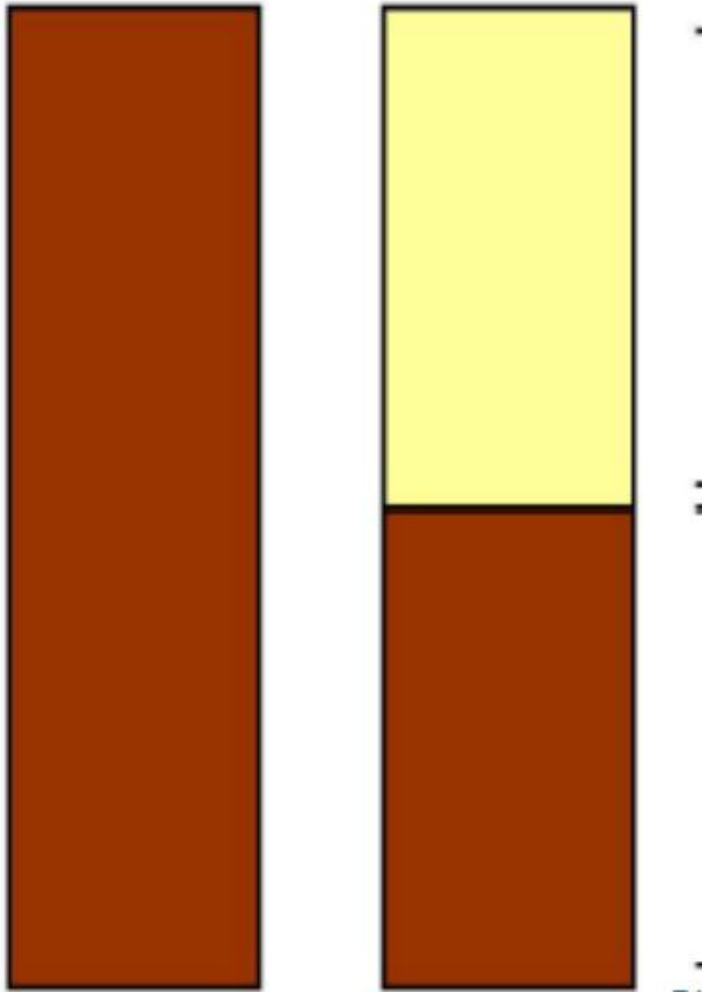
Plasma

White Blood cells (WBC) & Platelets

Red Blood cells (RBC)

Cellular Components

If blood is collected and allowed to stand it will clot. Formation of an insoluble fibrin clot. If blood is then centrifuged the fluid portion is known as SERUM



**Serum**

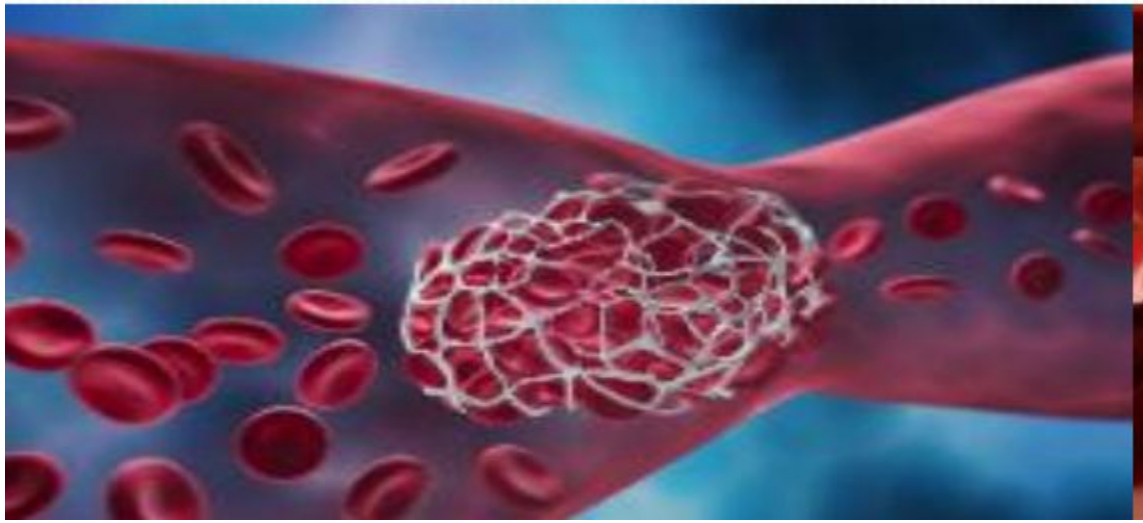
Plasma is fluid component of blood. Comprises ~55% of total volume of whole blood. Contains proteins, sugars, vitamins, minerals, lipids, lipoproteins
No clotting factors

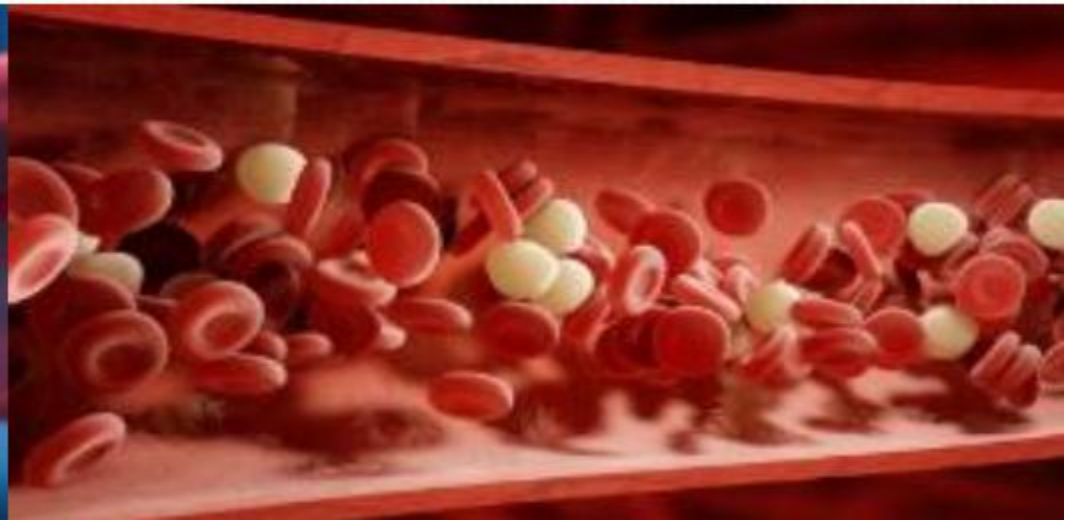
## Blood Clot

-comprised of clotting factors (Fibrin, platelets etc)
-RBCs

# Anticoagulants

**Anticoagulants** definition : are compounds worke to prevent coagulation (clotting) of blood .





# Types of anticoagulants

**Heparin** (inhibits the conversion prothrmobin to thrombin) is the most widely used anticoagulant for clinical chemical analysis. Heparin is an ideal anticoagulant, since it does not cause any change in blood composition. However, other anticoagulants are prefered to heparin, due to the cost factor.

**Ethylene diamine tetra acetic acid (EDTA)** is a chelating agent, and is particularly useful for haematological examination because it preserves cellular components of the BLOOD. It chelates with calcium and blocks coagulation. EDTA is employed to collect blood for haematological examinations It may affect some of the clinical chemistry tests.

**Sodium fluoride** is usually used as a preservative for blood glucose by inhibiting the enzyme systems involved in the glycolysis. Without an antiglycolytic agent, the blood glucose concentration decreases about 10 mg/dl per hour and false results may be obtained. Fluoride is also anticoagulant. It

**Citrate** is widely used for coagulation studies.

**Oxalate** inhibits blood coagulation by forming insoluble complexes with calcium ions. Potassium oxalate may be used at a concentration of 1 -2 mg/ml blood. At concentration of > 3 mg/ml, oxalate may cause haemolysis.

**Potassium or sodium oxalate** : These compounds precipitate calcium and inhibit blood coagulation. Being more soluble, potassium oxalate (5-10 mg per 5 ml blood) is preferred.

**Potassium oxalate and sodium fluoride :** These anticoagulants are employed for collecting blood to estimate glucose. Further sodium fluoride inhibits glycolysis and preserves blood glucose concentration.

**Ammonium oxalate and potassium oxalate :** A mixture of these two compounds in the ratio 3 : 2 is used for blood collection to carry out certain hematological tests.

# Collection Tubes

**The most widely used tubes for blood collection are evacuated tubes (Vacutainers)**

1. Negative pressure facilitates collection
2. Easy to use
3. Sterile
4. Universally used colour-coded rubber stoppers to denote tube type.
5. Tubes can contain various anticoagulants for the collection of whole blood or plasma.
6. Tubes can have additives for specific tests (glucose, metals).


# Serum Separator Tube (SST)

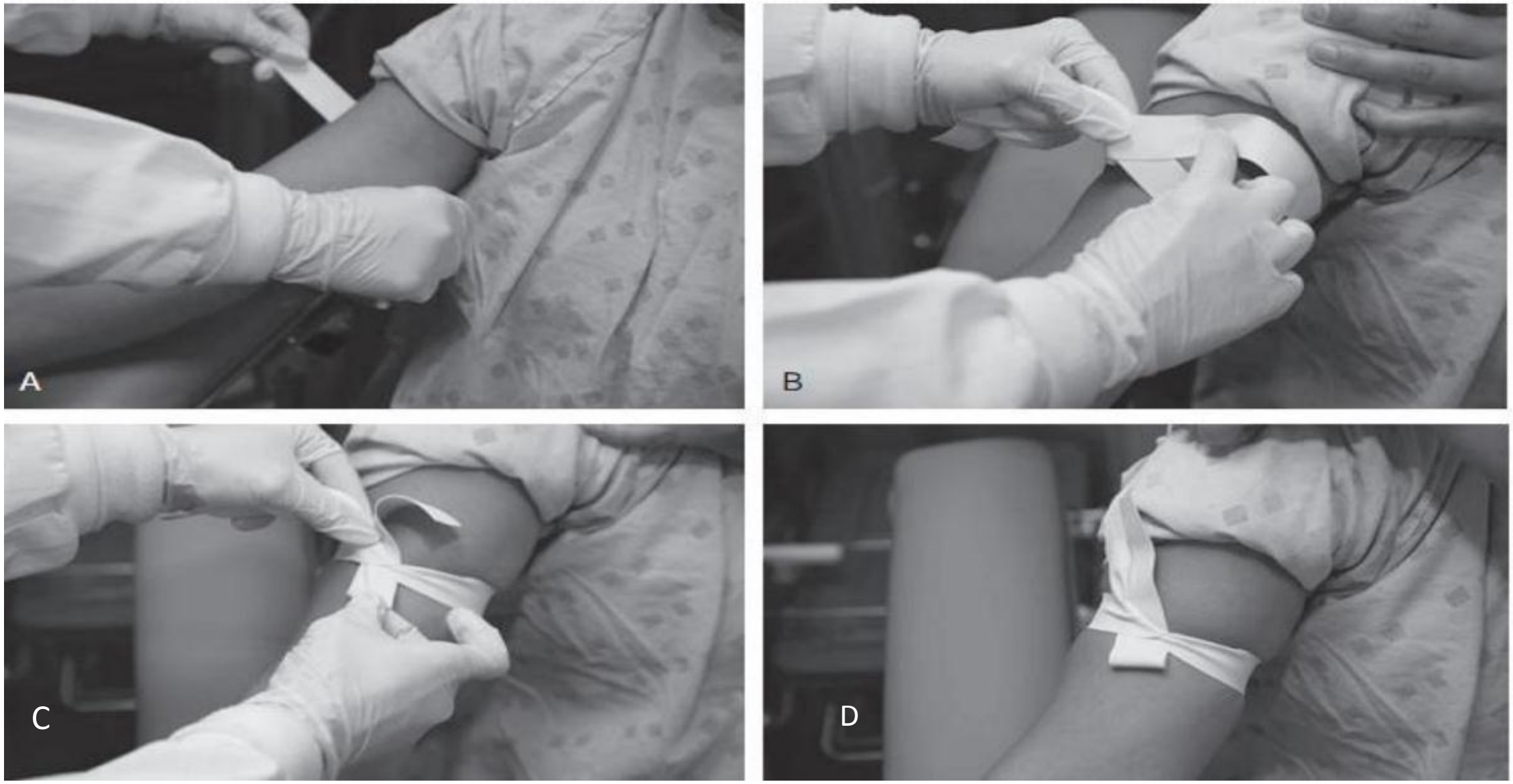
# What are vacutainer tubes?

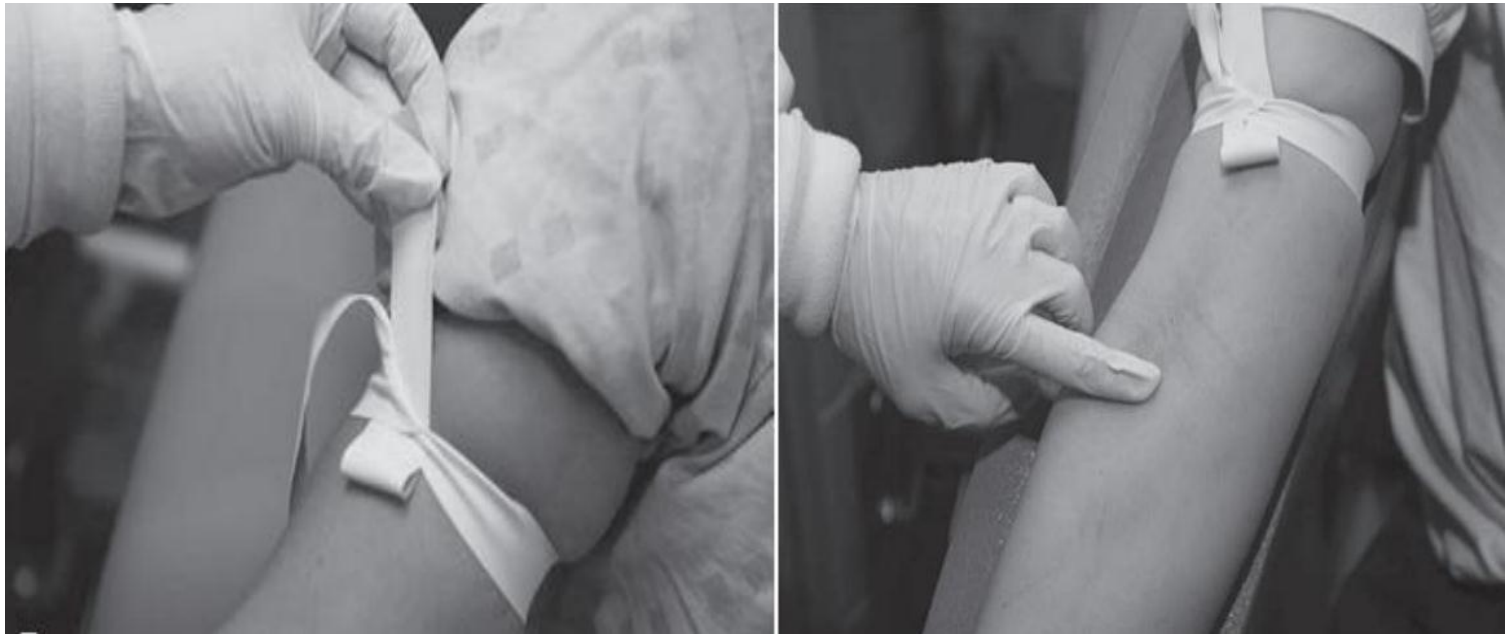
These are the color coded plastic tubes with rubber stopper. They are used to collect blood for various investigation.

| Cap Color | Additive | Tube Material | Tube Size(mm) | Draw Volume(ml) | Clinical Use |
|---|---|---|---|---|---|
|  Red | No Additive | PET, Glass | 2,3,4,5,6,7,8,9(PET)<br>2,3,4,5,6,7,8,9,10(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Biochemistry, Immunology |
| | Clot Activator | PET, Glass | 2,3,4,5,6,7,8,9(PET)<br>2,3,4,5,6,7,8,9,10(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Biochemistry, Immunology |
|  Yellow | Gel & Clot Activator | PET, Glass | 2,3,3.5,4,5,6,7,8,8.5 | 13 x 75<br>13 x 100<br>16 x 100 | Biochemistry, Immunology |
|  Grey | EDTA / Sodium Fluoride | PET, Glass | 2,3,4,5,6,7,8,9(PET)<br>2,3,4,5,6,7,8,9,10(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Blood Sugar, Tolerance |

| | | | | | |
|---|---|---|---|---|---|
|  Lavender | K2EDTA, K3EDTA | PET, Glass | 2,3,4,5,6,7,8,9(PET)<br>2,3,4,5,6,7,8,9,10(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Clinical Hematology |
|  Green | Lithium Heparin, Sodium Heparin | PET, Glass | 2,3,4,5,6,7,8,9(PET)<br>2,3,4,5,6,7,8,9,10(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Plasma Biochemistry Test, Rheology Measurement |
|  Blue | 3.2% Sodium Citrate | PET, Glass | 3.6,4.5,5.4,6.3,7.2,8.1,9(PET)<br>1.8,2.7,3.6,4.5(Glass) | 13 x 75<br>13 x 100<br>16 x 100 | Coagulation Test |
|  Black | 3.8% Sodium Citrate | PP + PET Glass | 1.28,1.6,2.4,3.2,4(Glass)<br>1.6,2.4(PP+PET) | 13 x 75<br>13 x 100<br>8 x 120 | Blood Sedimentation Rate Testing |

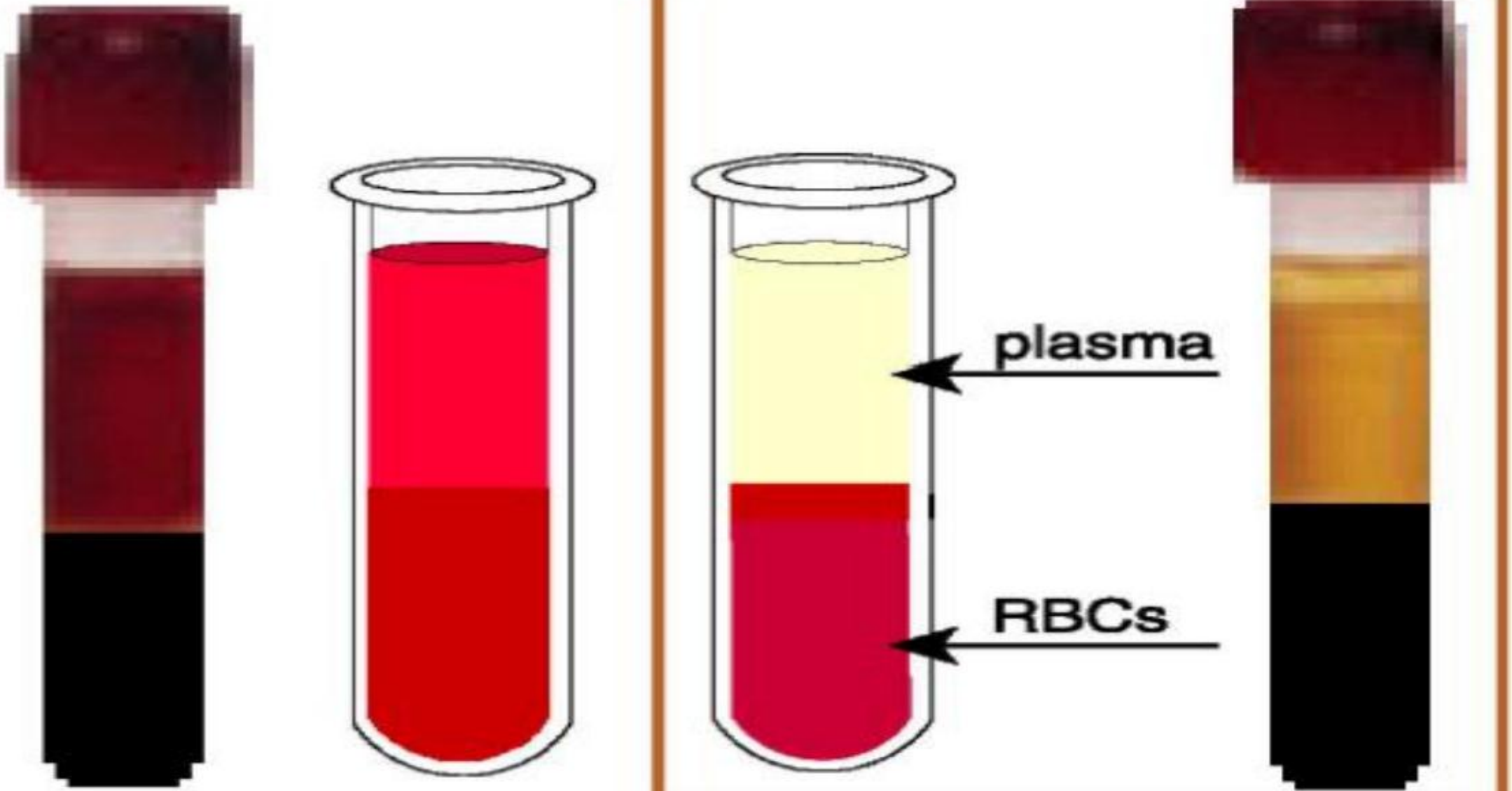
# Procedure of blood collection





# HOW TO AVOID HAEMOLYSIS OF THE SAMPLE ?

1. **Not using too fine needle.**
2. **Deliver يحرر the blood slowly into the tube (not through the needle).**
3. **Anti coagulated specimens should be mixed by inverting قلب the tube several times slowly (avoid excessive shaking).**




Haemolyzed sample

Proper sample

# Thank you for listening


**Practical Biochemistry**
**2nd Class , 1st Semester department of Medical Laboratory Technologies**

# Glycogen , Iodine Test

**Dr. Ahlam Said**

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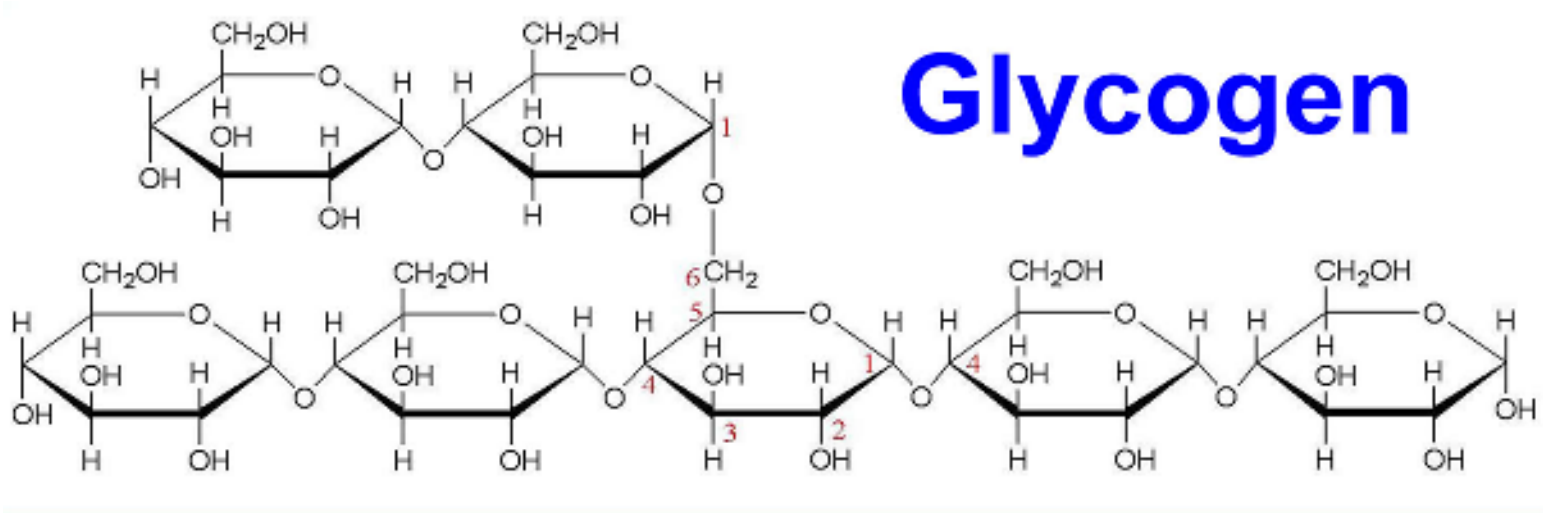
# Glycogen

Animals maintain a store of carbohydrates in the form of glycogen. The liver and muscles are the main sites of glycogen storage.

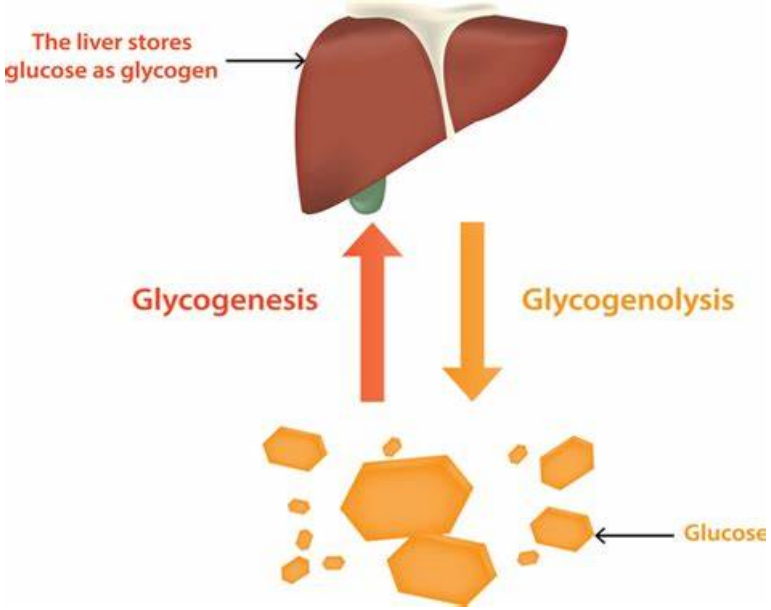
Liver glycogen used to maintain the blood glucose concentration, while muscle glycogen used as a fuel reserve during muscle contraction

Glycogen is a highly branched polymer of glucose, binding together with the glycosidic bonds of α(1-4) linkage and α(1-6) linkage.



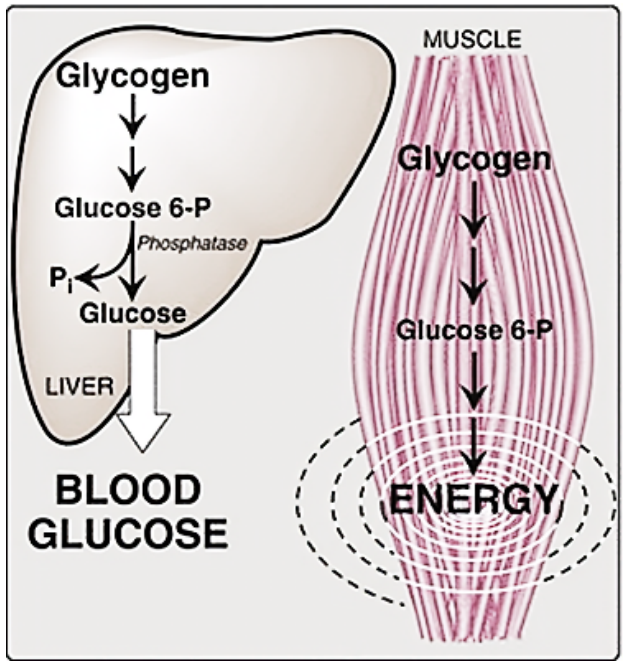
# Liver

As a meal containing carbohydrates or protein is eaten and digested, blood glucose levels rise, and the pancreas secretes insulin. Blood glucose from the portal vein enters liver cells (hepatocytes). Insulin acts on the hepatocytes to stimulate the action of several enzymes, including glycogen synthase. Glucose molecules are added to the chains of glycogen as long as both insulin and glucose remain plentiful. In this postprandial or "fed" state, the liver takes in more glucose from the blood than it releases.

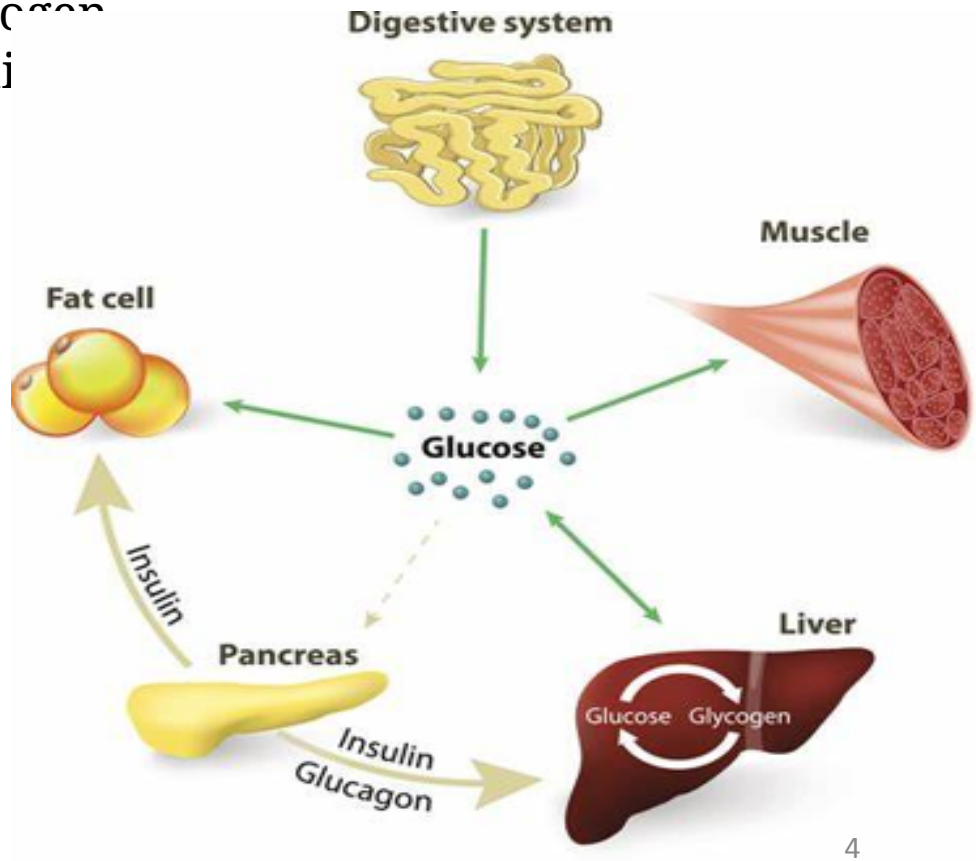



# Muscle

Muscle cell glycogen appears to function as an immediate reserve source of available glucose for muscle cells. Glycogen contained within skeletal muscle cells are primarily in the form of β particles. This is in contrast to liver cells, which, on demand, readily do break down their stored glycogen into glucose and send it through the blood stream as fuel for other organs.

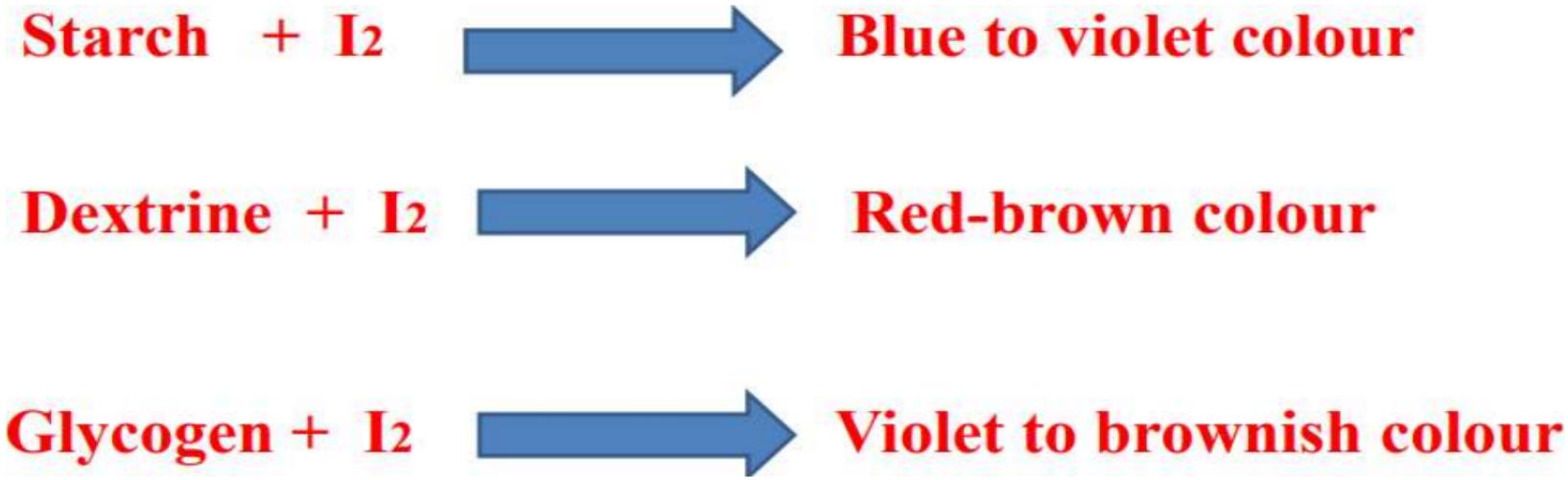


The body's cells need a constant supply of energy sources to function properly. **Glucose** is the only simple form of energy that cells use directly. Glucose comes from the breakdown of food found in various foods. The body uses as much glucose as it needs for various functions, and stores the rest for later use. Before storing it, the body must combine simple glucose units into a new, complex sugar called **glycogen**. Glycogen is then stored in the **liver** and **muscles**. When the body needs extra fuel, it breaks down the glycogen stored in the liver back into glucose units that cells can use. The work of glycogen is controlled and broken down according to the body's need. This process is called **glycogen metabolism**.



# Iodine Test :

The general test for polysaccharide. When iodine is added to polysaccharide the colour will be form depending on the kind of polysaccharides, if:

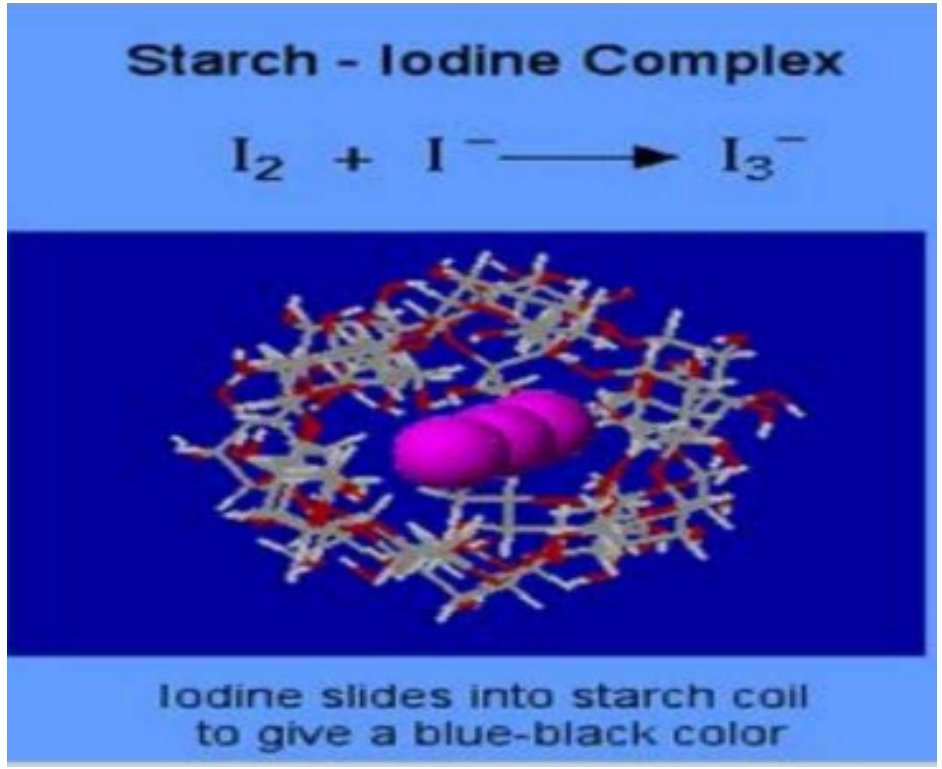



**Principles :** Iodine can form complex with the helical structure (coil-like) of the polysaccharide.

## Conditions for conducting the test

1. In acid medium or in neutral medium because in alkaline medium no colour will produce and react with iodine molecule and dissociate.

2.Test should be done in a cold not in hot, When we heating the solution a coil expand and the iodine molecule escape that results the colour disappear in heating and reappear on cooling

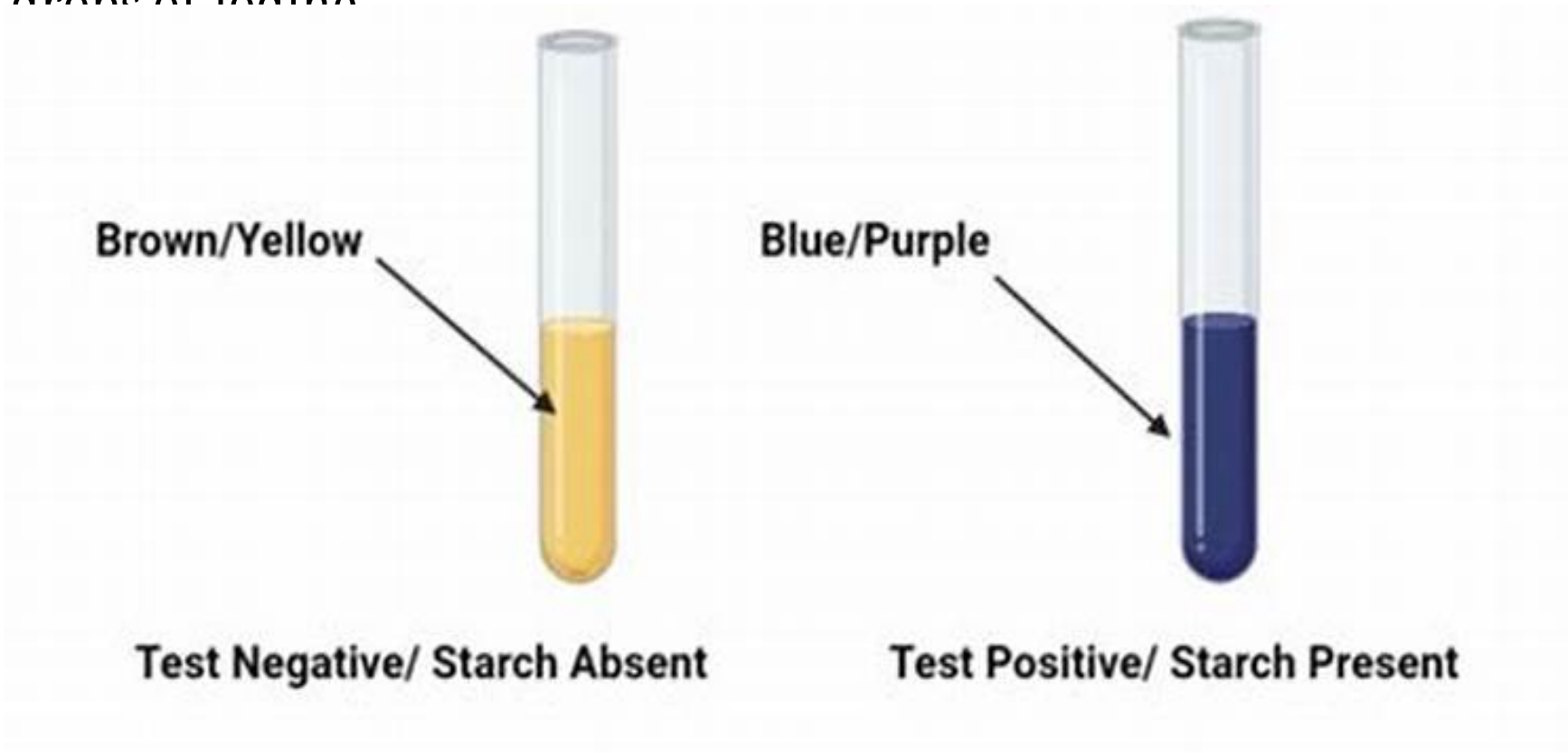


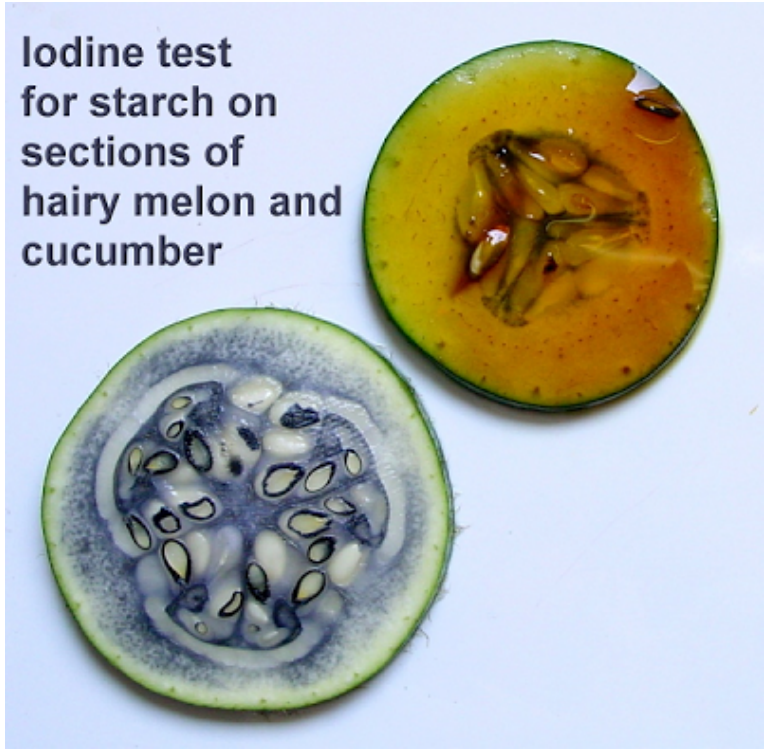
# Procedure

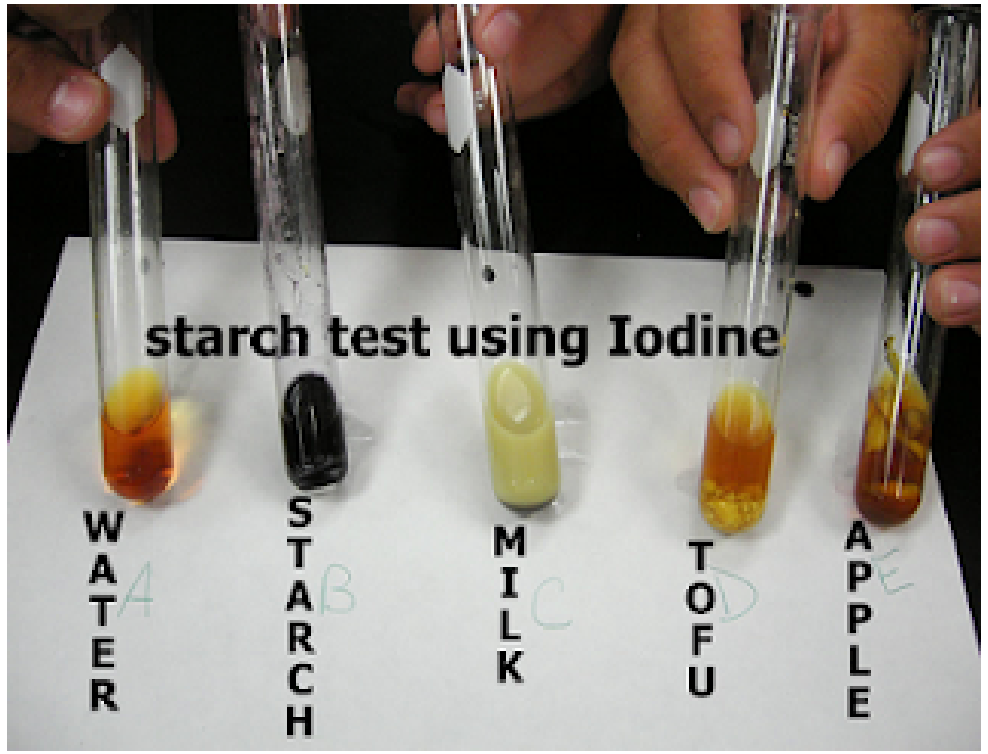
1. Remove apart of fresh liver.
2. Rinse it under the tap and dry it on a piece of filter paper.
3. Cut it into small pieces and then grind it for about one minute.
4. Add 5ml of 10% Trichloroacetic acid (TCA) and grind again for about two minute.
5. Filter the solution using filter paper.
6. Test for filtrate (glycogen).

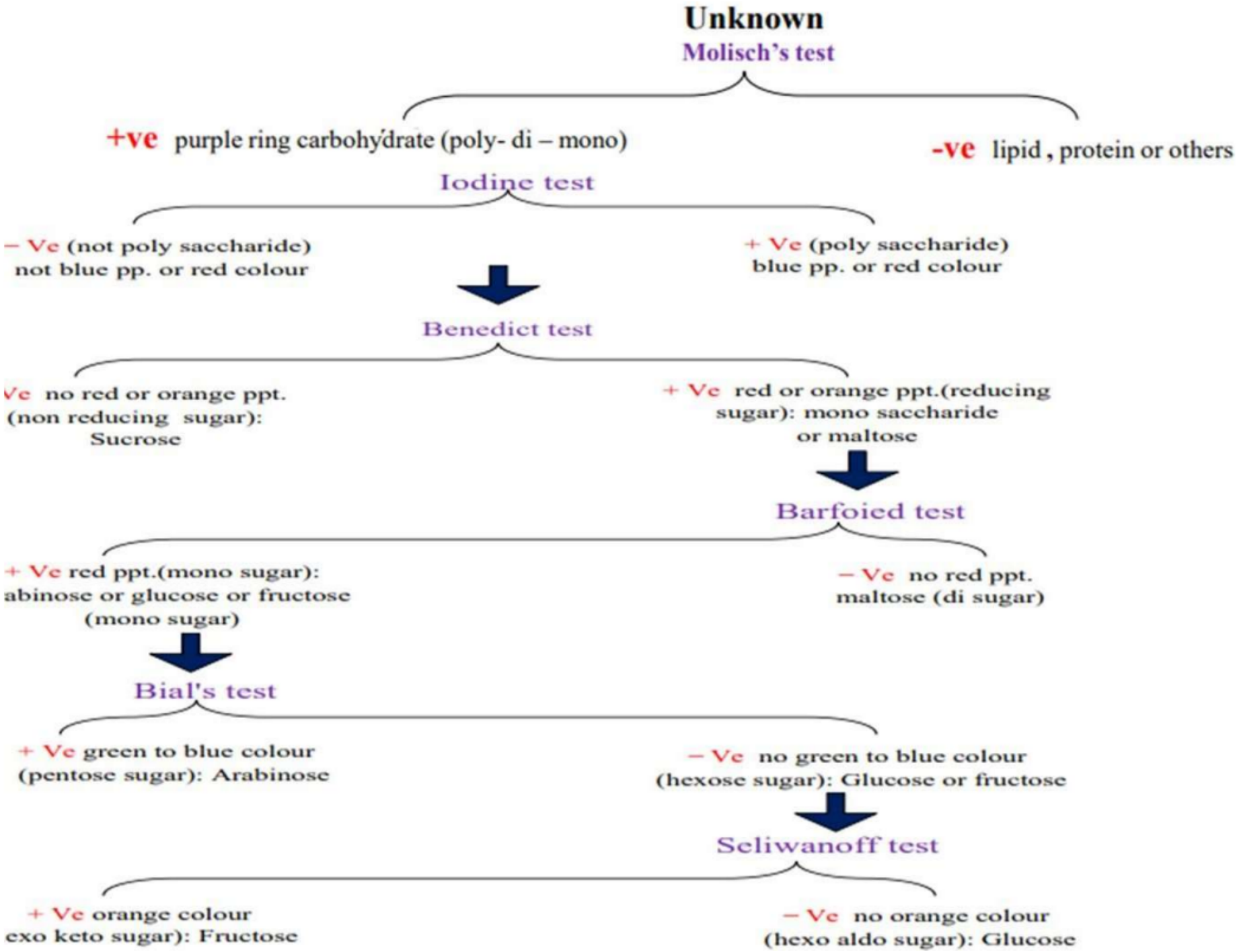
# Procedure of Iodine Test:

1- Place 1 ml of test solution (sample: such as liver ) in dry test tube.
2- add (3-4) drops of iodine




Iodine test
for starch on
sections of
hairy melon and
cucumber


starch test using Iodine
WATER
STARCH
MILK
TOFU
APPLE


Unknown
Molisch's test
+ve purple ring carbohydrate (poly- di – mono)
-ve lipid , protein or others
Iodine test
– Ve (not poly saccharide)
not blue pp. or red colour
+ Ve (poly saccharide)
blue pp. or red colour
Benedict test
Ve no red or orange ppt.
(non reducing sugar):
Sucrose
+ Ve red or orange ppt.(reducing
sugar): mono saccharide
or maltose
Barfoied test
+ Ve red ppt.(mono sugar):
abinose or glucose or fructose
(mono sugar)
– Ve no red ppt.
maltose (di sugar)
Bial's test
+ Ve green to blue colour
(pentose sugar): Arabinose
– Ve no green to blue colour
(hexose sugar): Glucose or fructose
Seliwanoff test
+ Ve orange colour
exo keto sugar): Fructose
– Ve no orange colour
(hexo aldo sugar): Glucose

# H.W

**1**. The …….. and ……. are the main sites of glycogen storage.

**2**. Liver glycogen used to maintain the ……concentration, while muscle ……..used as a fuel reserve during muscle contraction

**3**. Iodine test use to detect of ……..
1. Water.
2. Oligosaccharides.
3. Monosaccharides.
4. Polysaccharide.

**4.** In iodine test, red brown color be formed that indicate to presence the starch. (True or False)

# H.W

**5**. Iodine Test should be done in a cold not in hot, Why?

**6**. What is the Principle of Iodine test ?

**7**. Iodine test should be done in neutral medium, Why?

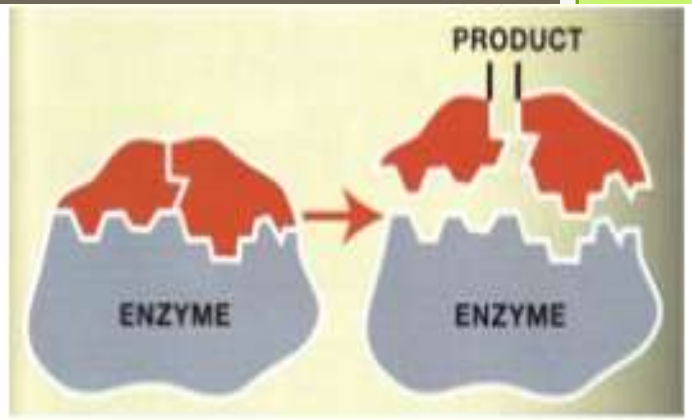
# Enzyme and CoEnzyme

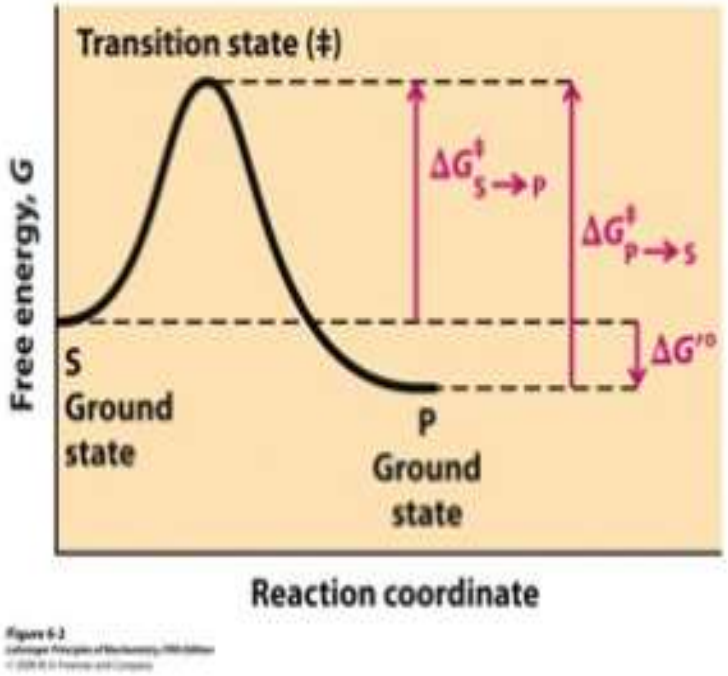
Enzymes are proteins that catalyze (i.e., **increase the rates of** ) chemical reactions. Nearly all known enzymes are proteins. However, certain RNA molecules can be effective biocatalysts too. These RNA molecules have come to be known as ribozymes.

In enzymatic reactions, the molecules at the beginning of the process are called **substrates** , and the enzyme converts them into different molecules, called the **products** .

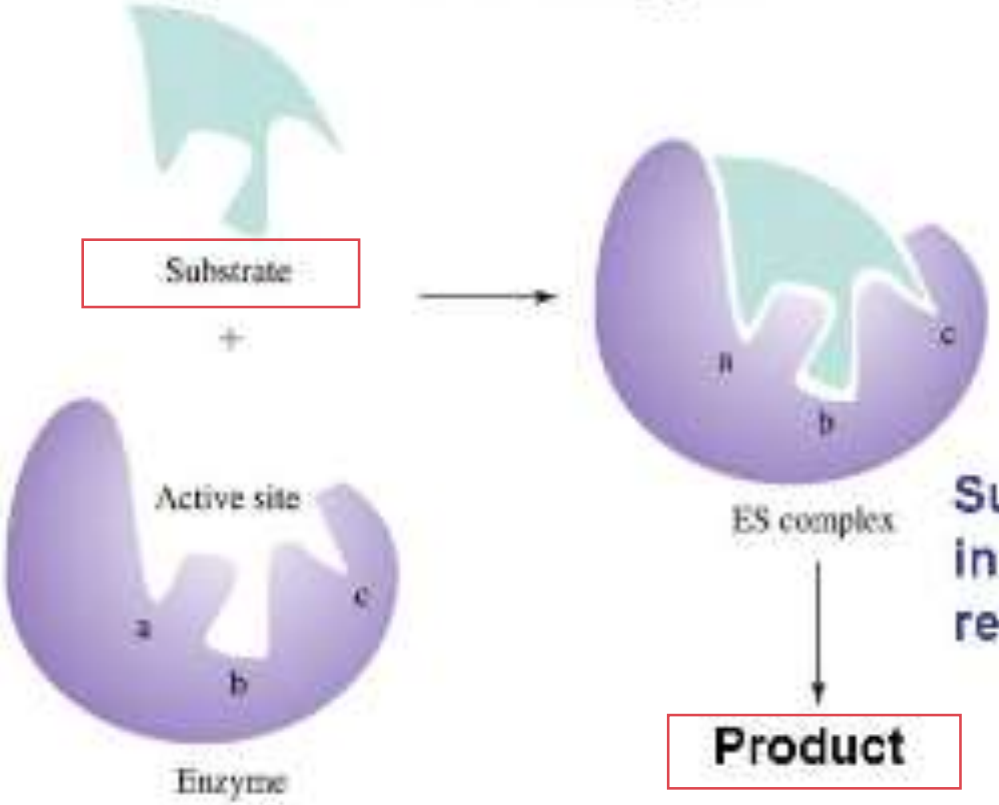
Like all catalysts, enzymes work by **lowering the activation energy** for a reaction, thus dramatically increasing the rate of the reaction. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions.

As with all catalysts, **enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions** . However, enzymes do differ from most other catalysts by being much more specific.



# Enzyme Catalysis




Active site - part of enzyme to which the substrate binds and the reaction takes place

bind

Substrate – a reactant in an enzyme-catalyzed reaction

Enzyme-substrate (ES) complex – the intermediate formed when the substrate is bind at the active site of an enzyme

# General Characteristics of Enzymes

- **ENZYME**
  - Usually a **protein**, acting as **catalyst in specific biochemical reaction**

- Each cell in the human body contains 1,000s of different enzymes
  - Every reaction in the cell requires its own specific enzyme

- Most enzymes are globular proteins
  - A few enzymes are made of RNA
    - Catalyze biochemical reactions involving nucleic acids

- Enzymes undergo all the reactions of proteins
  - Enzymes denaturation due to pH or temperature change
    - A person suffering high fever runs the risk of denaturing certain enzymes

خضع

تمسخ

Denaturation

# Enzyme Structure

- **SIMPLE ENZYMES**

  Composed only of protein

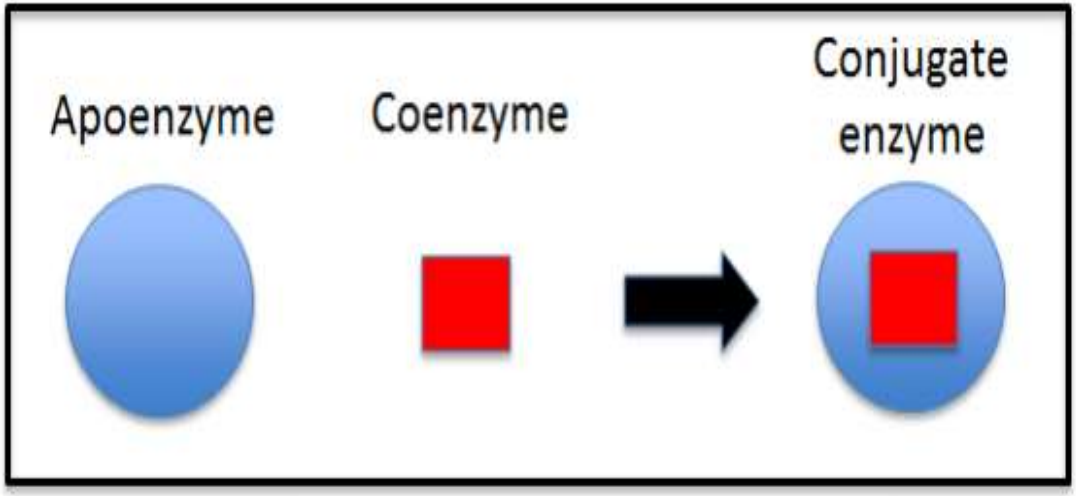
مترافق

- **CONJUGATED ENZYMES**

  Composed of:

  – **Apoenzyme**
    - Conjugate enzyme without its cofactor
    - Protein part of a conjugated enzyme

  – **Coenzyme (Cofactor)**
    - Non-protein part of a conjugated enzyme




- The apoenzyme can't catalyze its reaction without its cofactor.
  – The combination of the apoenzyme with the cofactor makes the conjugated enzyme functional.
- **Holoenzyme** = apoenzyme + cofactor العامل المساعد
  – The **biochemically active** conjugated enzyme.

# Enzyme definitions

| Term | Definition |
| --- | --- |
| **Enzyme (simple)** | Protein only enzyme that facilitates a chemical reaction |
| **Coenzyme** | Compound derived from a vitamin (e.g. $NAD^+$) that assists an enzyme in facilitating a chemical reaction |
| **Cofactor** | Metal ion (e.g. $Mg^{2+}$) that that assists an enzyme in facilitating a chemical reaction |
| **Apoenzyme** | Protein only part of an enzyme (e.g. isocitrate dehydrogenase) that requires an additional coenzyme to facilitate a chemical reaction (not functional alone) |
| **Holoenzyme** | Combination of the apoenzyme and coenzyme which together facilitating a chemical reaction (functional) |

يسهل

# Enzyme Nomenclature

- Enzymes are named according to the
  type of reaction they catalyze and/or their substrate
- **Substrate** = the reactant upon which the specific enzyme acts
  - Enzyme physically binds to the substrate

لاحقة

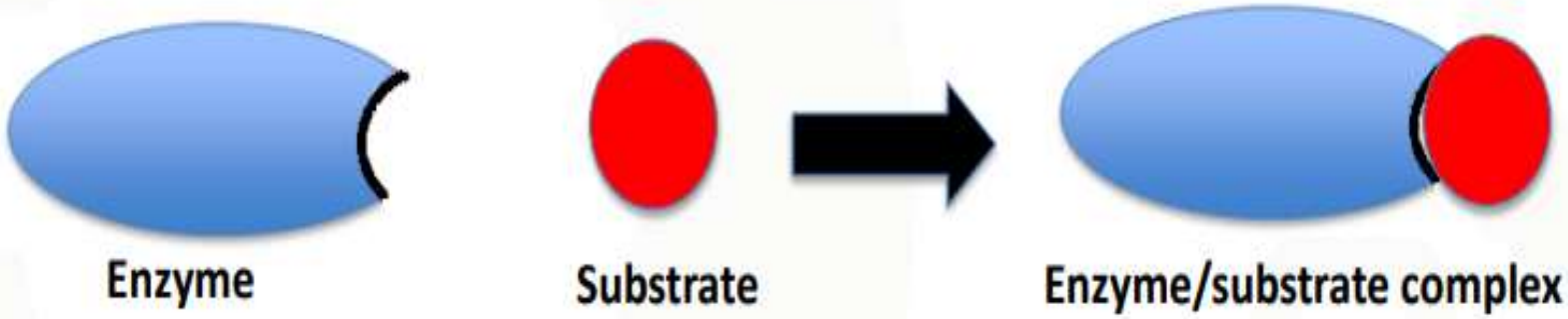
- **Suffix of an enzyme –ase**
  - *Lactase, amylase, lipase* or *protease*
    - Denotes an enzyme
- Some digestive enzymes have the suffix **–in**
  - *Pepsin, trypsin & chymotrypsin*
    - These enzymes were the first ones to be studied

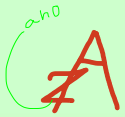
بادئة

- **Prefix** denotes the type of reaction the enzyme catalyzes
  - *Oxidase: redox reaction*
  - *Hydrolase: Addition of water to break one component into two parts*

هوية

- **Substrate identity** is often used together with the reaction type
  - *Pyruvate carboxylase, lactate dehydrogenase*



| Enzyme Class | Reaction Catalyzed | Examples in Metabolism |
|---|---|---|
| **Oxidoreductase** | Redox reaction (reduction & oxidation) | Examples are **dehydrogenases** catalyse reactions in which a substrate is oxidised or reduced |
| **Transferase** | Transfer of a functional group from 1 molecule to another | **Transaminases** which catalyze the transfer of amino group or kinases which catalyze the transfer of phosphate groups. |
| **Hydrolase** | Hydrolysis reaction | **Lipases** catalyze the hydrolysis of lipids, and proteases catalyze the hydrolysis of proteins |
| **Lyase** | Addition / removal of atoms to / from double bond | **Decarboxylases** catalyze the removal of carboxyl groups |
| **Isomerase** | Isomerization reaction  | **Isomerases** may catalyze the conversion of an aldose to a ketose, and mutases transfer functional group from one atom to another within a substrate. |
| **Ligase**  | Synthesis reaction (Joining of 2 molecules into one, forming a new chemical bond, coupled with ATP hydrolysis) | **Synthetases** link two smaller molecules are form a larger one. |

ENDEAVOUR

# 6 Major Classes of Enzymes

## Based on the type of reaction they catalyze

**The table explains the functions of enzymes and how they are classified and named.**

# Enzyme Active Site

- **Active site**
  - The specific portion of an enzyme (location) where the substrate binds while it undergoes a chemical reaction

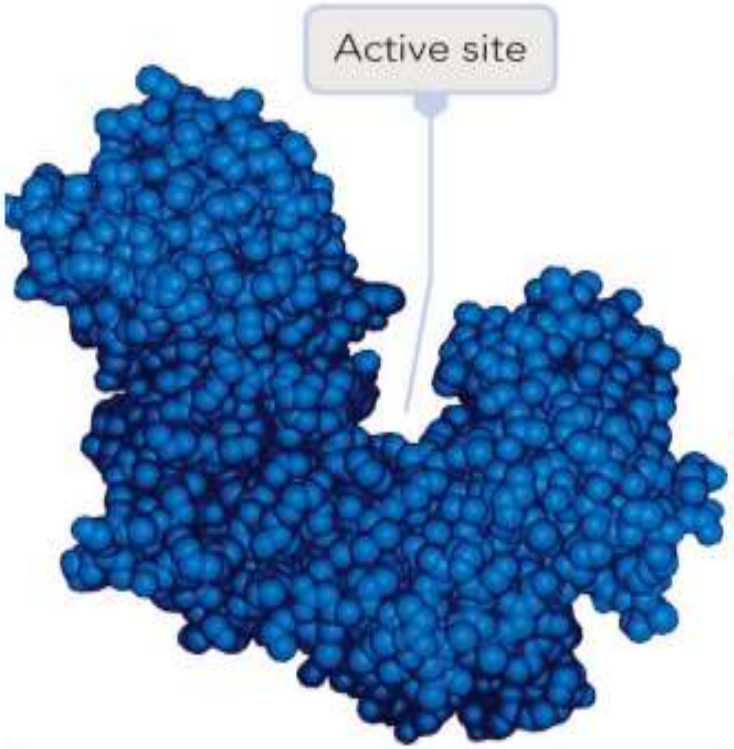
ربط خضع

- The active site is a 3-D 'crevice-like' cavity formed by secondary & tertiary structures of the protein part of the enzyme
  - Crevice formed from the folding of the protein
    - Aka binding cleft

قابلة للطي

  - An enzyme can have more than only one active site

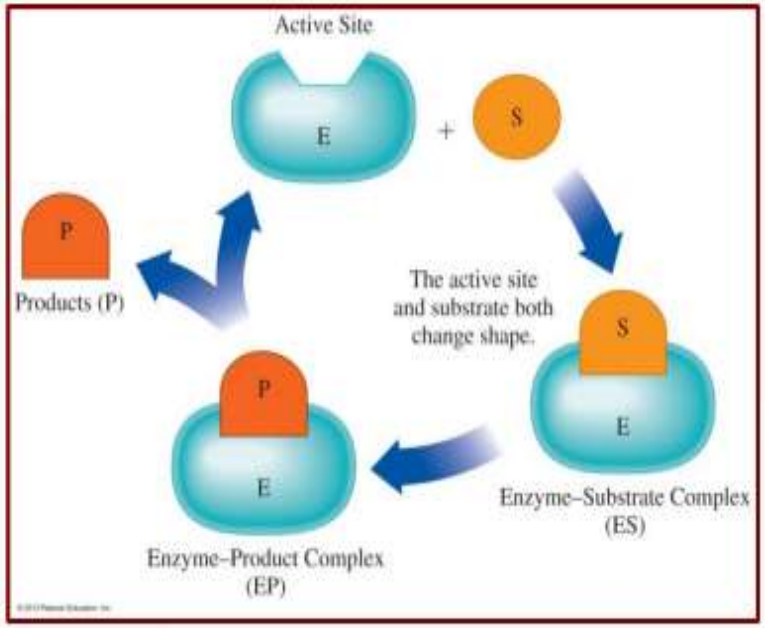
  - The amino acids R-groups (side chain) in the active site are important for determining the specificity of the substrate



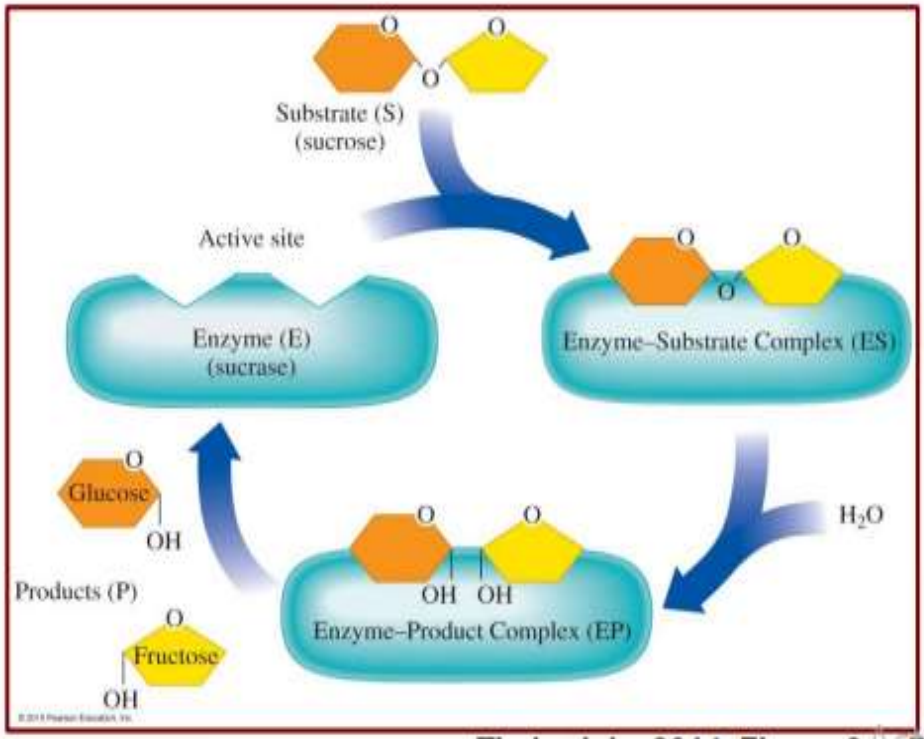

Stoker 2014, Figure 21-2 p750

# Enzyme – Substrate Complex

- When the substrate binds to the enzyme active site an **Enzyme-Substrate Complex** is formed temporarily
  - Allows the substrate to undergo its chemical reaction much faster




Timberlake 2014, Figure 4, p.738



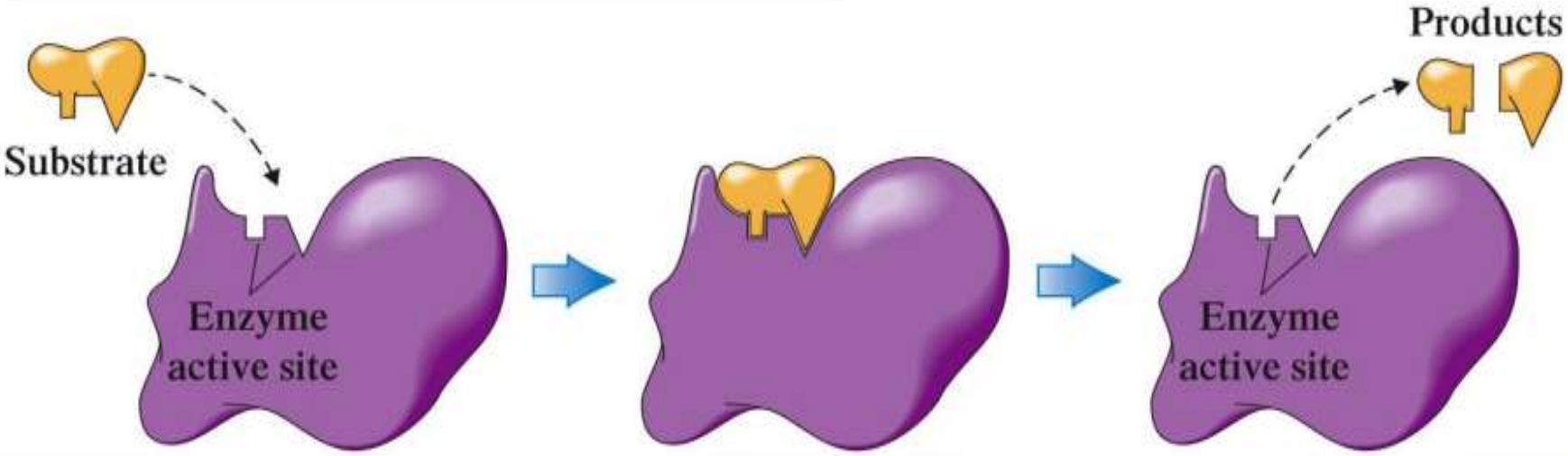

Timberlake 2014, Figure 3, p.737

قفل

# Lock & Key Model of Enzyme Action

- The active site is fixed, with a rigid shape (LOCK)
- The substrate (KEY) must fit exactly into the rigid enzyme (LOCK)
- Complementary shape & geometry between enzyme and substrate
  - Key (substrate) fits into the lock (enzyme)
- على Upon completion of the chemical reaction, the products are released from the active site, so the next substrate molecule can bind

الناتج يجب أن يحتوي على منطقة نشيطة

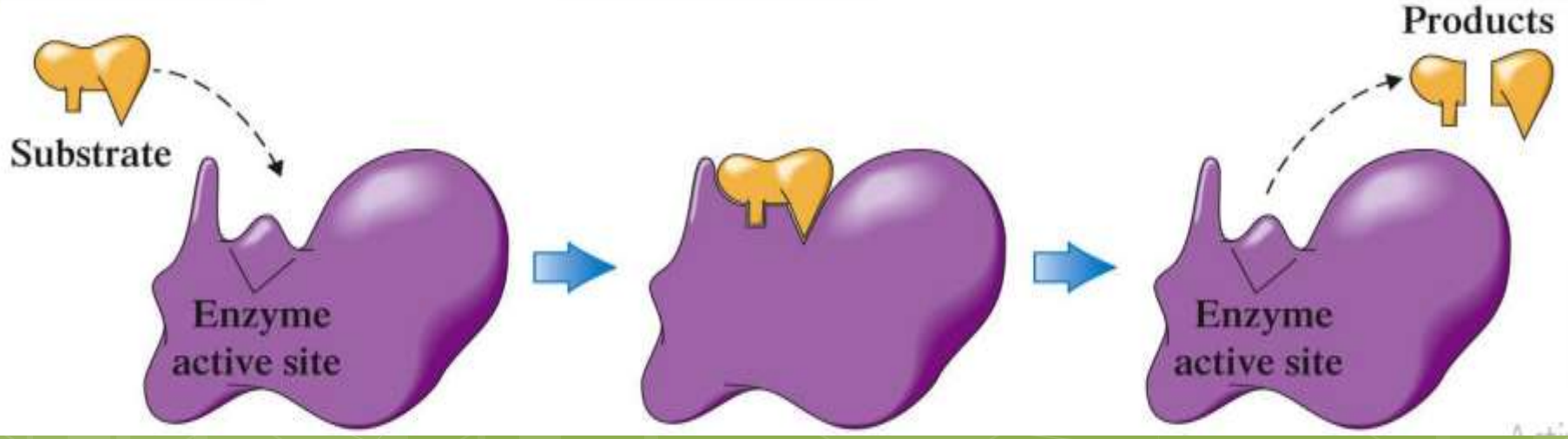



Stoker 2014, Figure 21-3 p750

Stoker 2014, Figure 21-4 p751

# Induced Fit Model of Enzyme Action

- Many enzymes are flexible & constantly change their shape
  - The shape of the active site changes to accept & accommodate the substrate
    - Conformation change in the enzyme's active site to allow the substrate to bind
    - Analogy: a glove (enzyme) changes shape when a hand (substrate) is inserted into it



# Enzyme Specificity

- **Absolute Specificity**
  - An enzyme will catalyze a particular reaction for only one substrate
  - Most restrictive of all specificities
    - Not common
      - ***Catalase*** has absolute specificity for hydrogen peroxide ($H_2O_2$)
      - ***Urease*** catalyzes only the hydrolysis of urea

- **Group Specificity**
  - The enzyme will act only on similar substrates that have a specific functional group
    - ***Carboxypeptidase*** cleaves amino acids one at a time from the carboxyl end of the peptide chain
    - ***Hexokinase*** adds a phosphate group to hexoses

# How does enzyme work

- **The Effect of Enzymes on the Activation Energy of a Reaction**
- An enzyme speeds a reaction by lowering the activation energy, changing the reaction pathway, This provides a lower energy route for conversion of substrate to product

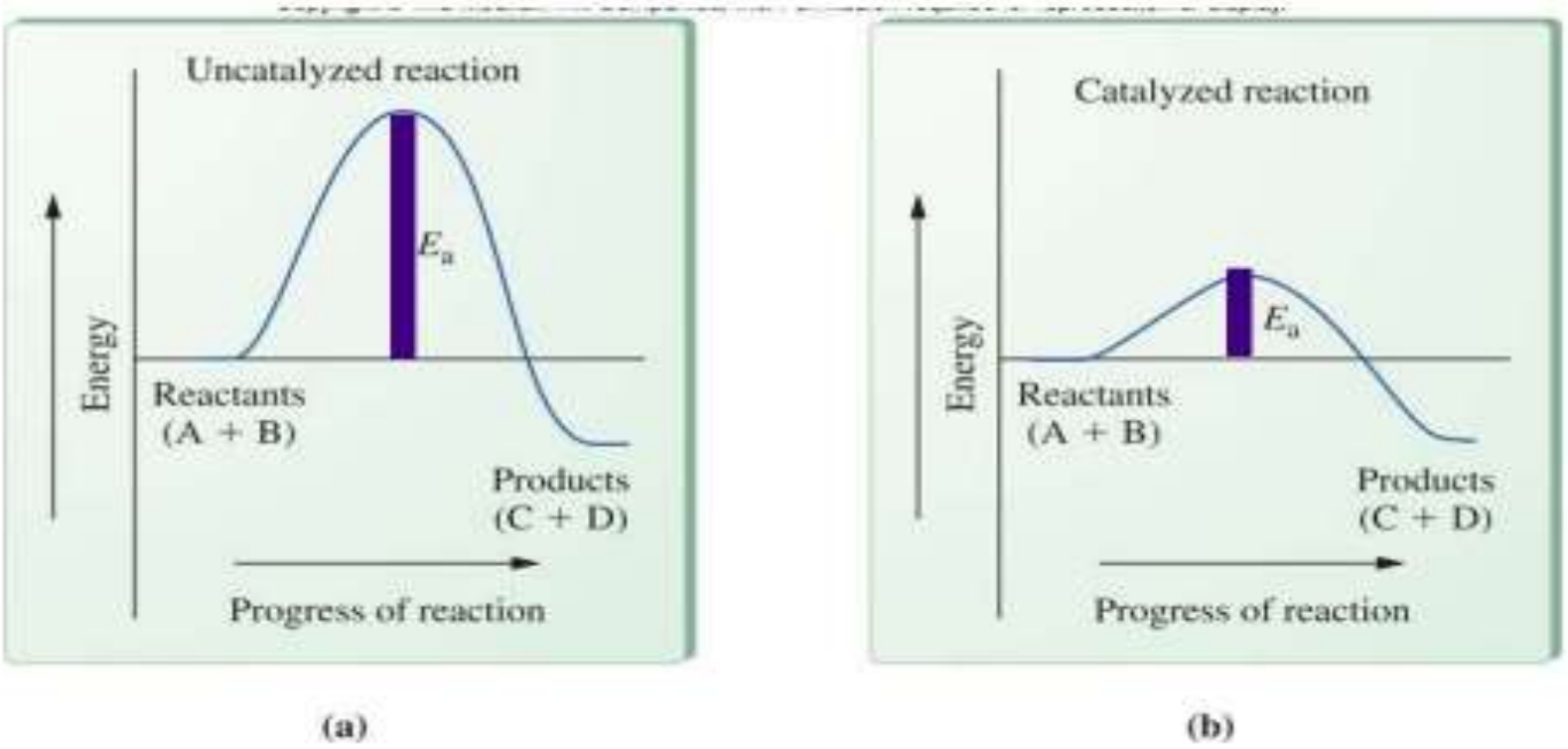
يوفر
طريق
تحويلات

- Every chemical reaction is characterized by an equilibrium constant, Keq, which is a reflection of the difference in energy

$$K_{eq} = \frac{[B]^b}{[A]^a} = \frac{[\text{product}]^b}{[\text{reactant}]^a}$$

$$aA \rightleftharpoons bB$$

- **Diagram of Energy Difference Between Reactants and Products**




- The uncatalyzed reaction has a large activation energy, *Ea* , seen at left
- In the catalyzed reaction, the activation energy has been lowered significantly increasing the rate of the reaction.

Theory Biochemistry
2nd class , 1st semester
Department of dentistry

# BIOCHEMISTRY
## Enzymes and Coenzymes

*Prof. Farha Khalaf Omar*

# Factors Affecting Enzyme Activity

**Enzyme activity**

- Measure of the rate at which an enzyme converts substrate to products in a biochemical reaction

***4factors affect enzyme activity:***

- Temperature
- pH
- Substrate concentration: [substrate[
- Enzyme concentration: [enzyme[

# Temperature (t(

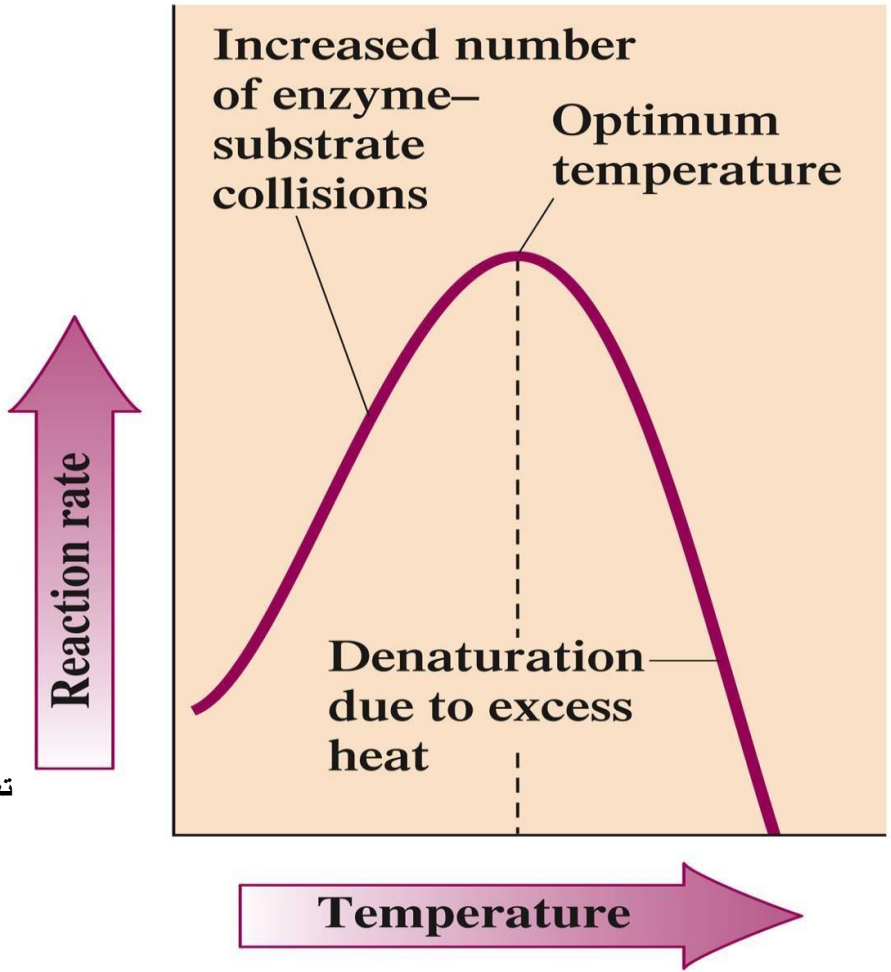
- With increased **t** the $E_{KIN}$ increases
  - More collisions
  - Increased reaction rate

- ***Optimum temperature*** ***($t_{OPT}$)*** is the **t,** at which the enzyme exhibits maximum activity
  - The $t_{OPT}$ for human enzymes = $37^0$C

- When the **t** increases beyond $t_{OPT}$
  - Changes in the enzyme's tertiary structure occur, inactivating & denaturing

تغييرات في البنية الثلاثية للانزيم تؤدي الى تعطيله (هدمه)وتغييرطبيعته مثل الحمى

- Little activity is observed at low **t**



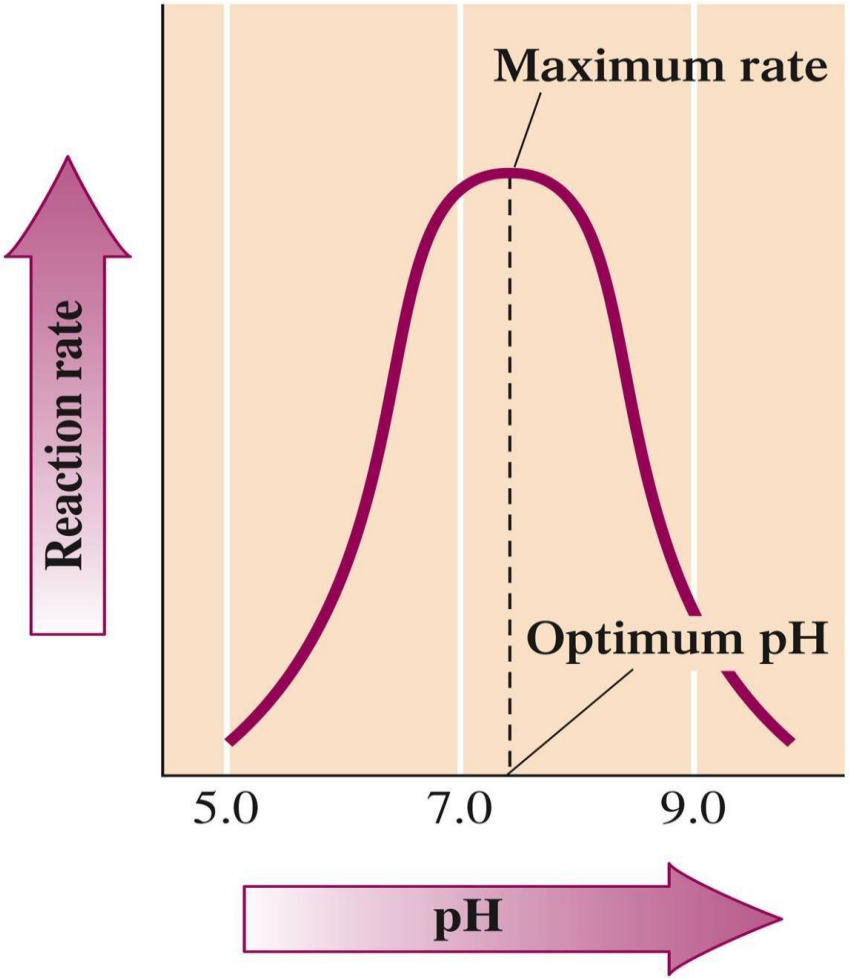
# pH

- ***Optimum pH ($pH_{OPT}$)*** is the **pH**, at which the enzyme exhibits maximum activity

- Most enzymes are active over a **very narrow pH range**
  - Protein & amino acids are properly maintained
    البروتينات والأحماض الأمينية تحافظ عليها بشكلها الصحيح
  - Small changes in pH (low or high) can result in enzyme denaturation & loss of function

- Each enzyme has its characteristic ***$pH_{OPT}$***, which usually falls within physiological pH range 7.0 - 7.5
  معظم الانزيمات تعمل في PH7.4متعادلة

- ***Digestive enzymes are exceptions:***
  - ***Pepsin*** (in stomach) – ***$pH_{OPT}$*** = 2.0
  - ***Trypsin*** (in SI) – ***$pH_{OPT}$*** = 8.0



# Substrate Concentration

- If [enzyme] is kept constant & the [substrate] is increased
  - The reaction rate increases until a ***saturation point*** is met
    - At saturation the reaction rate stays the same even if the [substrate] is increased
  - **At saturation point** substrate molecules are bound to all available active sites of the enzyme molecules

عند نقطة التشبع ترتبط جزيئات S بجميع المواقع النشطة لجزيئات الانزيم

- Reaction takes place at the active site
  - If they are all active sites are occupied the reaction is going at its maximum rate

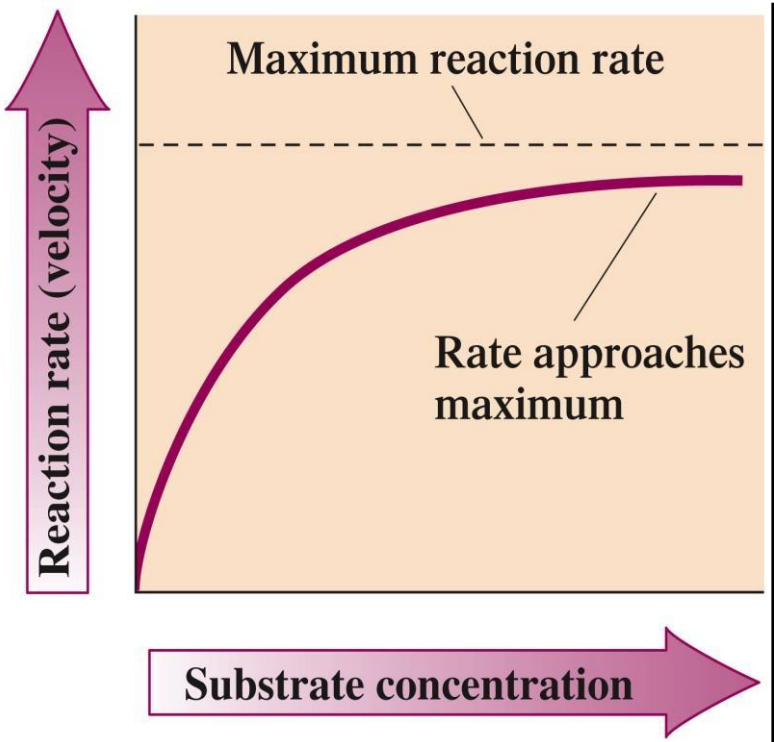
اذاكانت جميع المواقع مشغولة فان التفاعل يجري بالحد الاقصى له

    - Each enzyme molecule is working at its maximum capacity

يعمل كل جزئ انزيم باقصى طاقة له

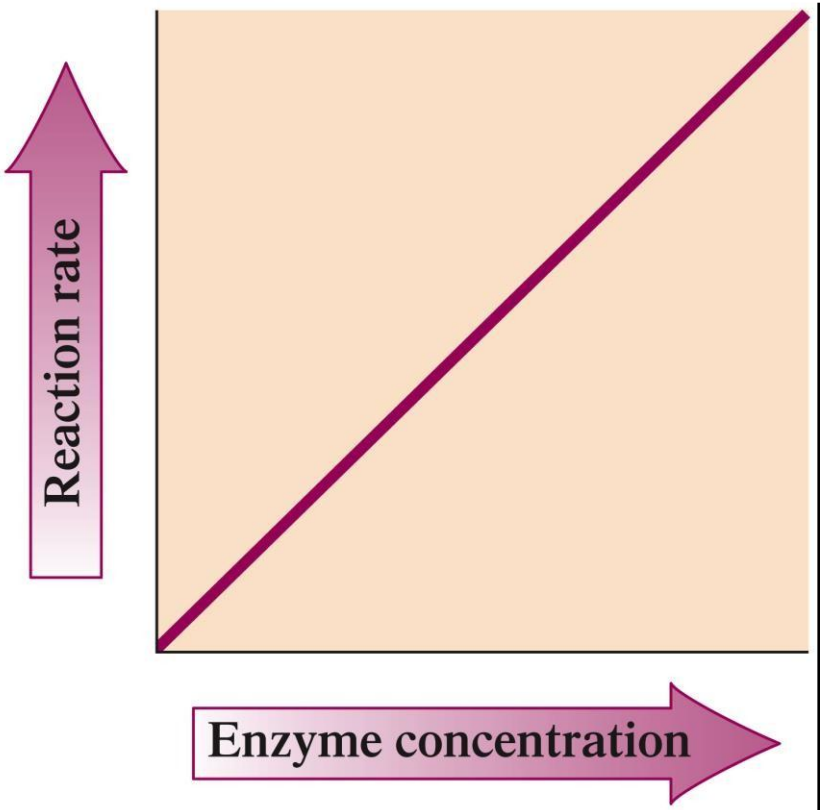
  - The incoming substrate molecules must "wait their turn"

يجب على جزيئات الركيزة الواردة ان تنتظر دورها



# Enzyme Concentration

- If the [substrate] is kept constant & the [enzyme] is increased
  - The reaction rate increases
  - The greater the [enzyme], the greater the reaction rate
- **RULE:**
  - The rate of an enzyme-catalyzed reaction is always directly proportional to the amount of the enzyme present
- ***In a living cell:***
  - The [substrate] is much higher than the [enzyme[
    - Enzymes are not consumed in the reaction
    - Enzymes can be reused many times

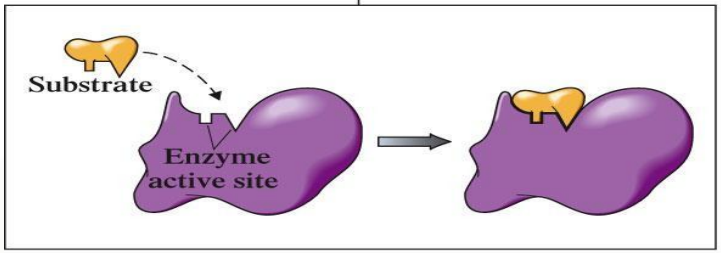


## THE MECHANISM OF ENZYME ACTION

Formation of an enzyme–substrate complex as an intermediate species provides an alternative pathway, with lower activation energy, through which a reaction can occur.

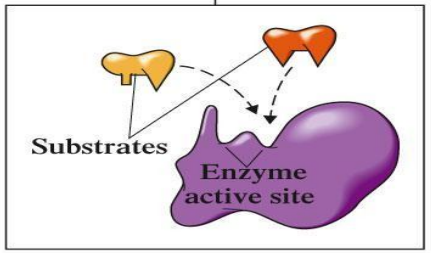
### Lock-and-Key Model

The active site has a fixed geometric shape. Only a substrate with a matching shape can fit into it.




### Induced-Fit Model

The active site has a flexible shape that can change to accept a variety of related substrates. Enzymes vary in their degree of specificity for substrates.




## FACTORS THAT AFFECT THE RATE OF ENZYME ACTIVITY

### Temperature

Reaction rate increases with temperature until the point at which the protein is denatured and activity drops sharply.

### pH

Maximum enzymatic activity is possible only within a narrow pH range; outside this pH range, the protein is denatured and activity drops sharply.

### Concentration of Substrate

Reaction rate increases with substrate concentration until full saturation occurs; then the rate levels off.

### Concentration of Enzyme

Reaction rate increases with increasing enzyme concentration, assuming enzyme concentration is much lower than that of substrate.

# Enzyme Inhibition

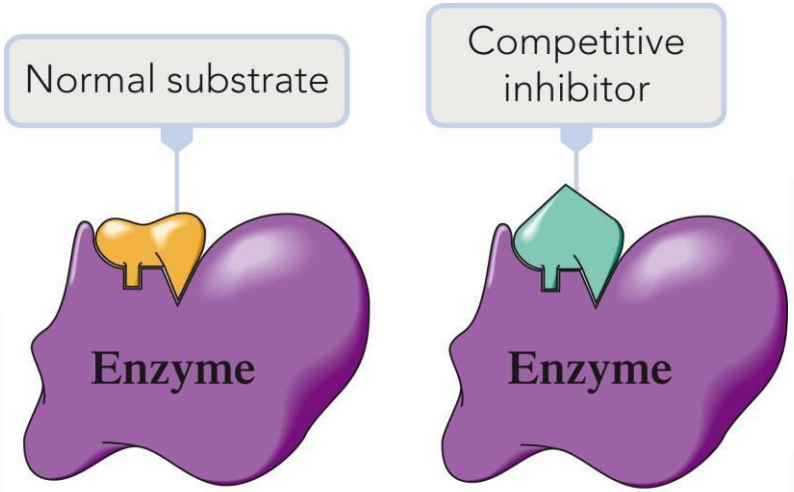
- **ENZYME INHIBITOR**
  - A substance that slows down or stops the normal catalytic function of an enzyme by binding to the enzyme

مادة تبطئ أو توقف الوظيفة التحفيزية الطبيعية للإنزيم عن طريق الارتباط بالإنزيم

- ***Three types of inhibition:***
  - Reversible competitive inhibition مثبط تنافسي عكسي
  - Reversible non-competitive inhibition مثبط غير تنافسي عكسي
  - Irreversible inhibition تنافسي غير عكسي

# Reversible Competitive Inhibition

- A ***competitive inhibitor*** resembles the substrate
  - Inhibitor competes with the substrate for **binding to the active site of the enzyme**
  - If an inhibitor is bound to the active site:
    - Prevents the substrate molecules to access the active site
      - Decreasing / stopping enzyme activity
- The binding of the competitive inhibitor to the active site is a reversible process
  - Add much more substrate to outcompete the competitive inhibitor
- ***Many drugs are competitive inhibitors:***
  - **Anti-histamines** inhibit *histidine decarboxylase*, which converts histidine to histamine



# Reversible Noncompetitive Inhibition

- A **non-competitive inhibitor** decreases enzyme activity by **binding to a site** on the enzyme **other than the active site**

•يعمل المثبط غير التنافسي على تقليل نشاط الإنزيم عن طريق الارتباط بموقع على الإنزيم غير الموقع النشط

  - The non-competitive inhibitor alters the tertiary
  - structure of the enzyme & the active site

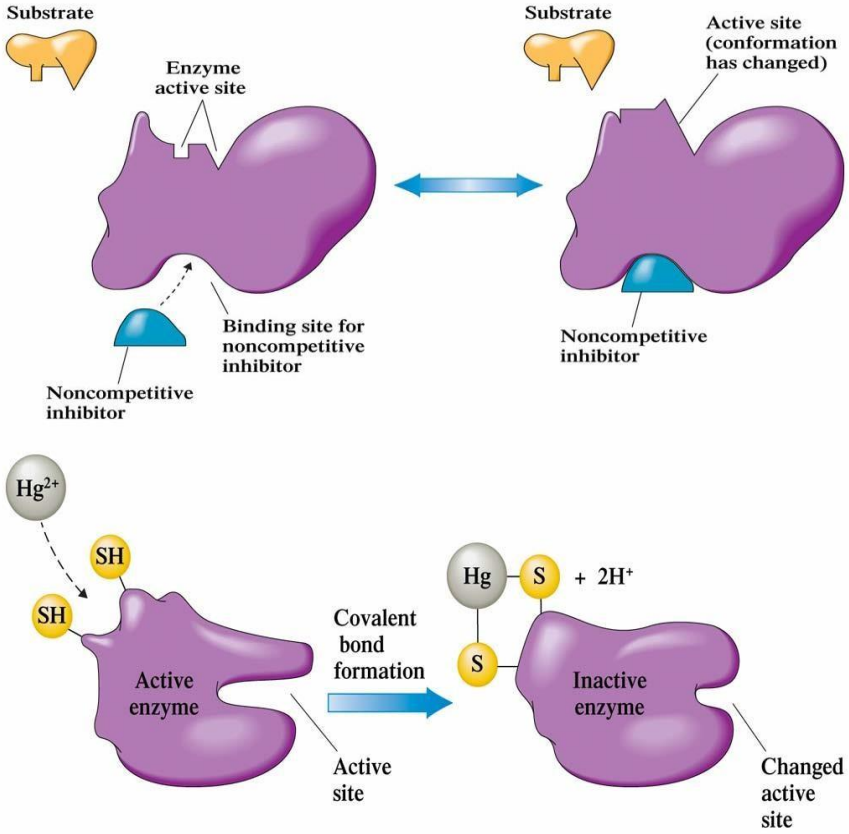
–يقوم المثبط غير التنافسي بتغيير البنية الثلاثية للإنزيم والموقع النشط

    - Decreasing enzyme activity
    - Substrate cannot fit into active site

  - Process can be reversed only by lowering the [non-competitive inhibitor[

–لا يمكن عكس العملية إلا عن طريق خفض [المثبط غير التنافسي]

- **Example:**
  - **Heavy metals** $Pb^{2+}$ & $Hg^{2+}$ bind to –SH of Cysteine, away from active site
    - Disrupt the secondary & tertiary structure




Stoker 2004, Figure 21.12, p.634

# Irreversible Inhibition

- An ***irreversible inhibitor*** inactivates an enzyme
  by **binding to** its **active site by a strong covalent bond**

**مثبط لا رجعة فيه يثبط نشاط الإنزيم عن طريق الارتباط بموقعه النشط بواسطة رابطة تساهمية قوية**

  - **Permanently deactivates the enzyme** **يقوم بتعطيل الإنزيم بشكل دائم**

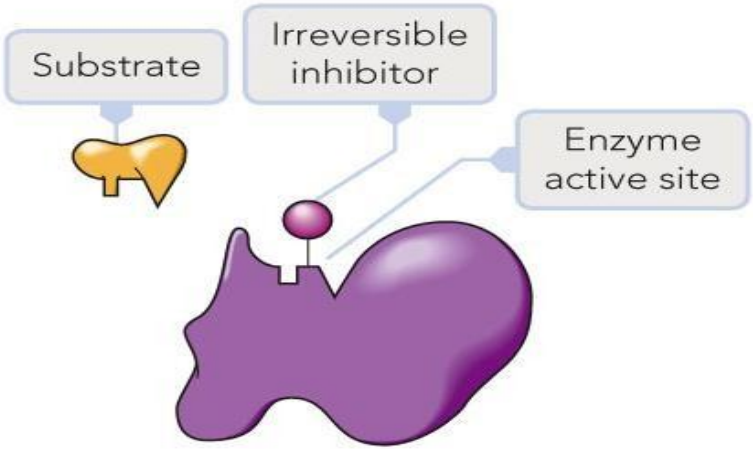
  - Irreversible inhibitors do not resemble substrates

**–لمثبطات التي لا رجعة فيها لا تشبه الركائز**

- Addition of excess substrate doesn't reverse this process
  - Cannot be reversed

- **Chemical warfare** (nerve gases(

- **Organophosphate insecticides**



## ENZYME INHIBITORS

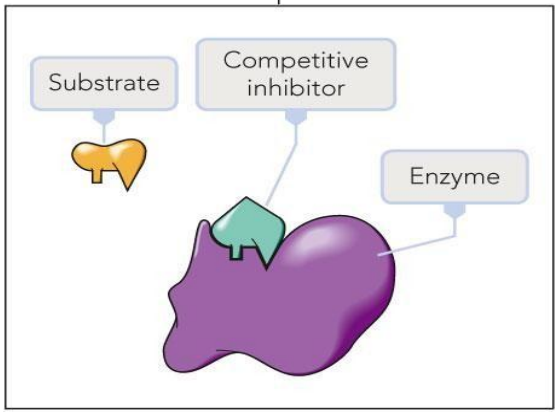
Substances that bind to an enzyme and stop or slow its normal catalytic activity

جزئ يشبه الى حد كبير الركيزة يرتبط بالموقع النشط ويمنع الركائز من احتلاله مؤقتا بالتالي يمنع التفاعل

جزئ يرتبط بموقع على انزيم ليس هو الموقع النشط ولاتزال الركيزة الطبيعية تحتل الموقع النشط ولكن الانزيم لايستطيع تحفيزالتفاعل بسبب وجودا لانهيبتر

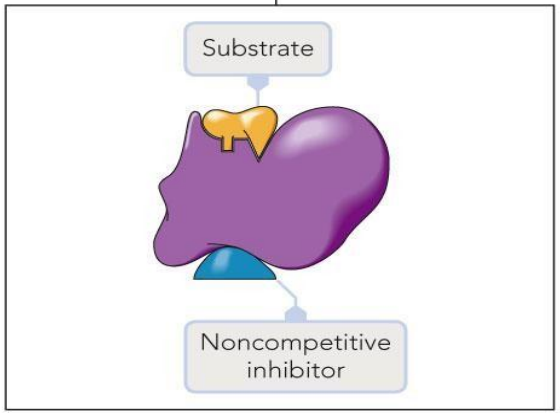
### Competitive Enzyme Inhibitor

A molecule closely resembling the substrate. Binds to the active site and temporarily prevents substrates from occupying it, thus blocking the reaction.



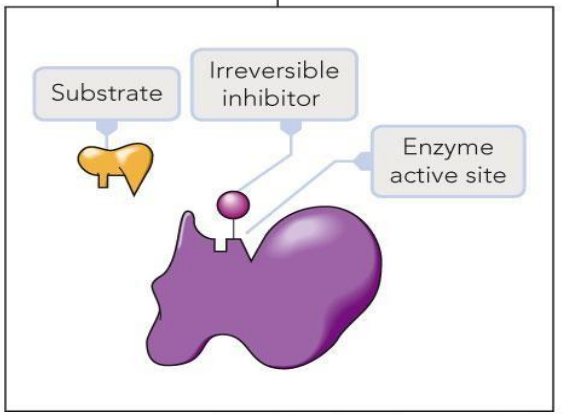

### Noncompetitive Enzyme Inhibitor

A molecule that binds to a site on an enzyme that is not the active site. The normal substrate still occupies the active site but the enzyme cannot catalyze the reaction due to the presence of the inhibitor.




### Irreversible Enzyme Inhibitor

A molecule that forms a covalent bond to a part of the active site, permanently preventing substrates from occupying it.




Stoker 2014, p760

# AI - Noor University College

## Department of Dentistry

## Biochemistry -2nd class

**Prof. Farha Khalaf Omar**

**Lec. 2**

# Enzyme Nomenclature

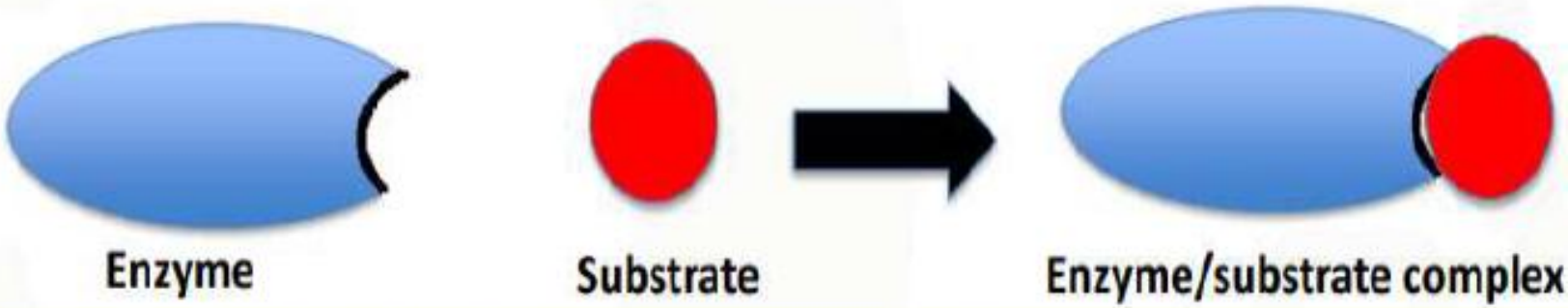
- Enzymes are named according to the
  type of reaction they catalyze and/or their substrate
- **Substrate** = the reactant upon which the specific enzyme acts
  - Enzyme physically binds to the substrate

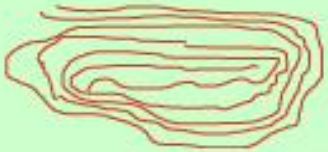
لاحقة
- **Suffix of an enzyme** **–ase**
  - *Lactase, amylase, lipase* or *protease*
    - Denotes an enzyme

انزيمات هضمية
- Some digestive enzymes have the suffix –
  - *Pepsin, trypsin & chymotrypsin*
    - These enzymes were the first ones to be stu

بادئة
- **Prefix** denotes the type of reaction the enzyme catalyzes
  - *Oxidase: redox reaction*
  - *Hydrolase: Addition of water to break one component into two parts*

هوية
- **Substrate identity** is often used together with the reaction type
  - *Pyruvate carboxylase, lactate dehydrogena*



| Enzyme Class | Reaction Catalyzed | Examples in Metabolism |
|---|---|---|
| **Oxidoreductase**<br>انزيمات الاكسدة والاختزال | Redox reaction (reduction & oxidation) | Examples are **dehydrogenases** catalyse reactions in which a substrate is oxidised or reduced |
| **Transferase**<br>انزيمات النقل | Transfer of a functional group from 1 molecule to another | **Transaminases** which catalyze the transfer of amino group or kinases which catalyze the transfer of phosphate groups. |
| **Hydrolase**<br>انزيمات التحلل المائي | Hydrolysis reaction | **Lipases** catalyze the hydrolysis of lipids, and proteases catalyze the hydrolysis of proteins |
| **Lyase**<br>انزيمات الفصل او الحذف | Addition / removal of atoms to / from double bond | **Decarboxylases** catalyze the removal of carboxyl groups |
| **Isomerase**<br>انزيمات التشكل/ تحول الهدف Sالى متشكل اخر | Isomerization reaction  | **Isomerases** may catalyze the conversion of an aldose to a ketose, and mutases transfer functional group from one atom to another within a substrate. |
| **Ligase**<br>انزيمات الارتباط/انشاء رابطة جديدة بين مركبين مختلفين  | Synthesis reaction (Joining of 2 molecules into one, forming a new chemical bond, coupled with ATP hydrolysis) | **Synthetases** link two smaller molecules are form a larger one. |

ENDEAVOUR

# 6 Major Classe of Enzymes

## Based on the type o reaction they cataly

**The table explains the functions of enzymes and how they are classified and named.**

# Enzyme Active Site

- **Active site**
  - The specific portion of an enzyme (location) where the substrate binds while it undergoes a chemical reaction
    ربط خضع

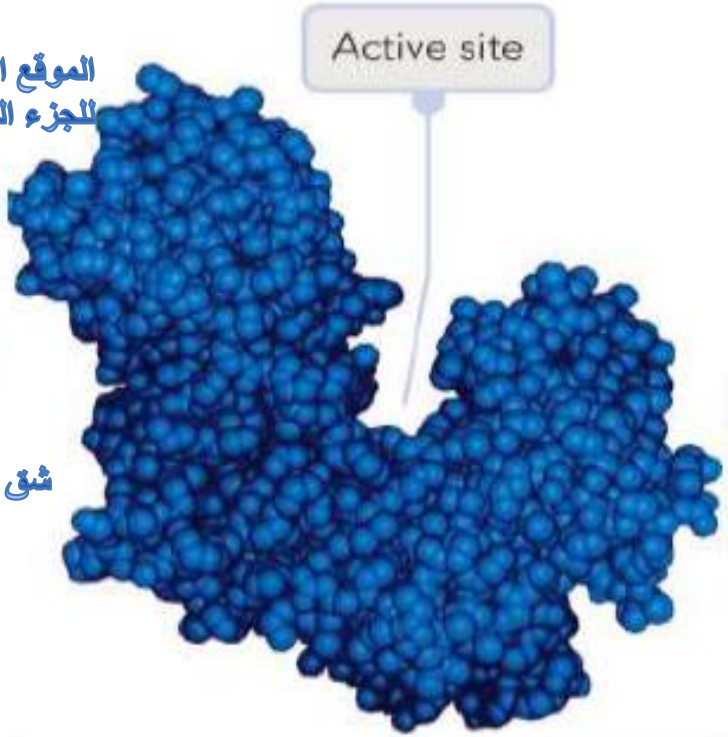
الموقع النشط عبارة عن تجويف ثلاثي البعاد يشبه الشق يتكون من هياكل ثانوية او ثلاثية للجزء البروتيني من الانزيم

- The active site is a 3-D 'crevice-like' cavity formed by secondary & tertiary structures of the protein part of the enzyme
  - Crevice formed from the folding of the protein
    - Aka binding cleft
      قابلة للطي

شق يتكون من البرتين القابل للطي يعرف اسم الشق

  - An enzyme can have more than only one active site

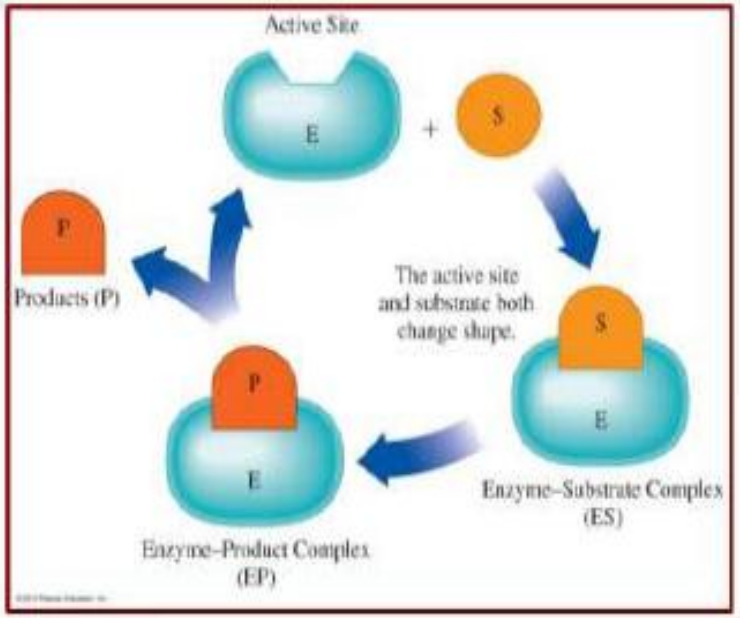
  - The amino acids R-groups (side chain) in the active site are important for determining the specificity of the substrate



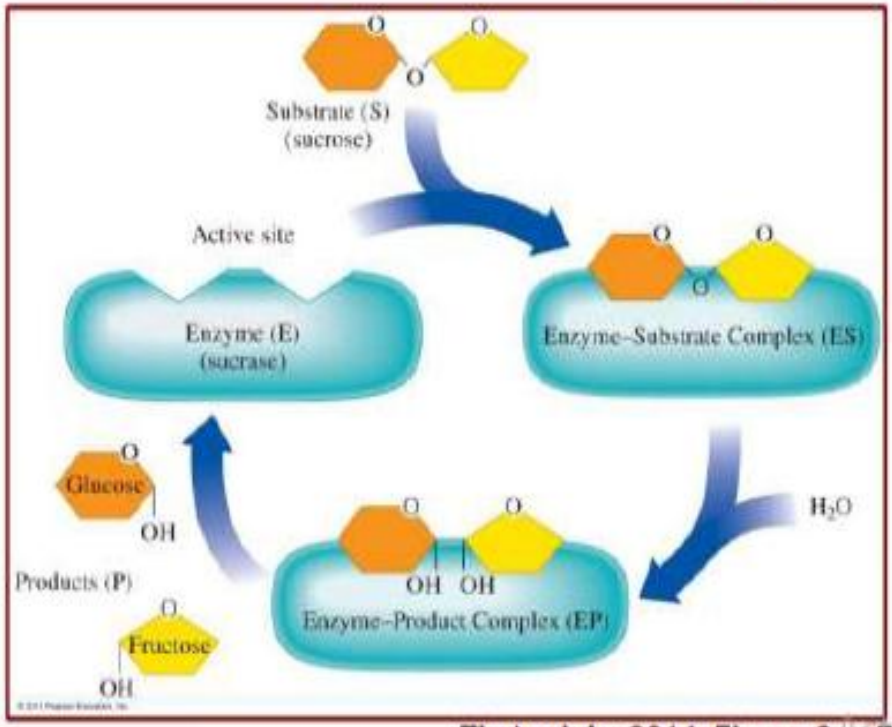

Stoker 2014, Figure 21-2 p750

# Enzyme – Substrate Complex

- When the substrate binds to the enzyme active site an **Enzyme-Substrate Complex** is formed temporarily
  - Allows the substrate to undergo its chemical reaction much faster



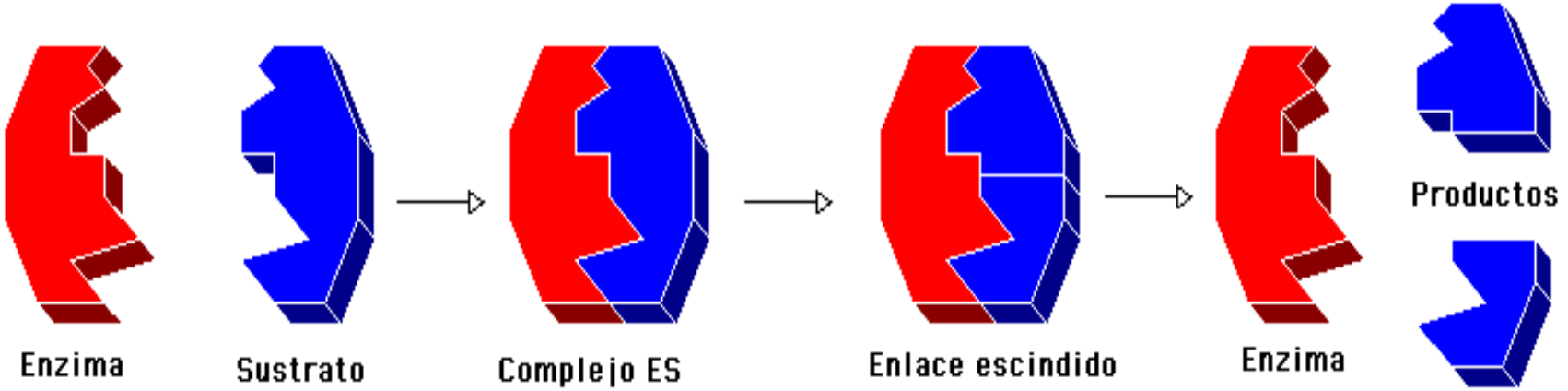

Timberlake 2014, Figure 4, p.738




Timberlake 2014, Figure 3, p.737

# Enzymatic specialization التخصص الانزيمي

The specific specifications for the structure of the active centers in enzymes are what determine the phenomenon of specialization in their activation activity. The scientist Emil Fischer proposed the term “lock and key,” which is equivalent to the term “induced fit” proposed by the scientist Daniel Koshland.




This theory says that the base material does not bind easily to the fixed active site. The side chains of the amino acids that make up the active site of the enzyme will shape themselves to give the correct site with the precise shape that helps the enzyme to perform its catalytic function and bind to the base material

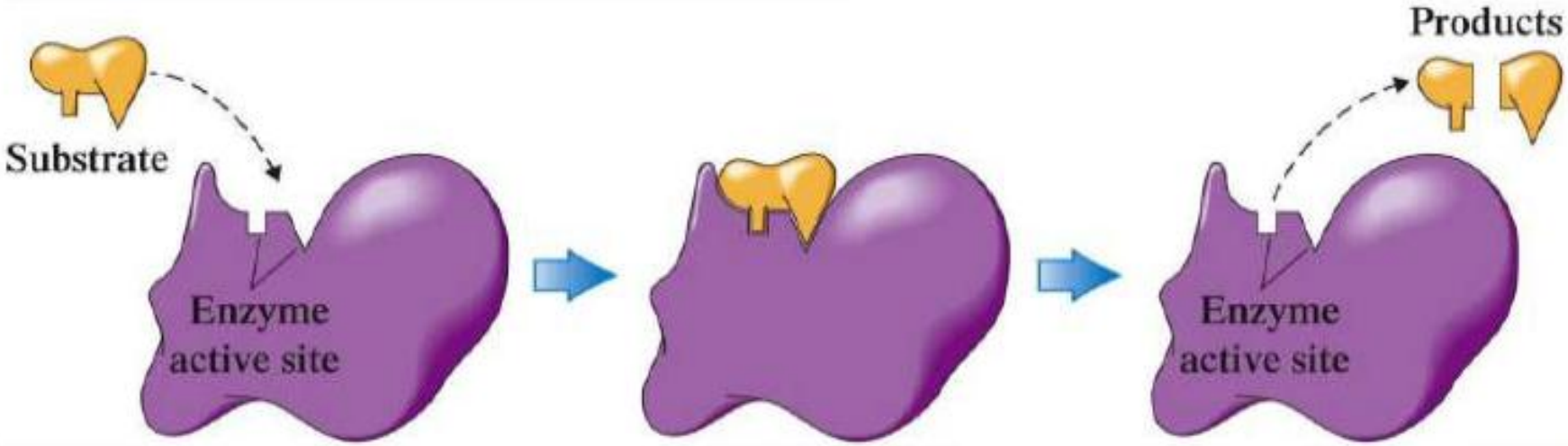
**هذه النظرية تقول بأن المادة الأساس لا ترتبط بسهولة مع الموقع النشط الثابت الشكل، فالسلاسل الجانبية للأحماض الأمينية المكونة للموقع النشط للأنزيم سوف تشكل نفسها لتعطي الموقع الصحيح والشكل الذي يساعد الأنزيم ليؤدي وظيفته المحفزة و يرتبط بالمادة الأساس.**

قفل

# Lock & Key Model of Enzyme Action

- The active site is fixed, with a rigid shape (LOCK)
- The substrate (KEY) must fit exactly into the rigid enzyme (LOCK)
- Complementary shape & geometry between enzyme and substrate
  - Key (substrate) fits into the lock (enzyme)
- على Upon completion of the chemical reaction, the products are released from the active site, so the next substrate molecule can bind

الناتج يجب أن يحتوي على منطقة نشيطة




Stoker 2014, Figure 21-3 p750

Stoker 2014, Figure 21-4 p751

# Induced Fit Model of Enzyme Action

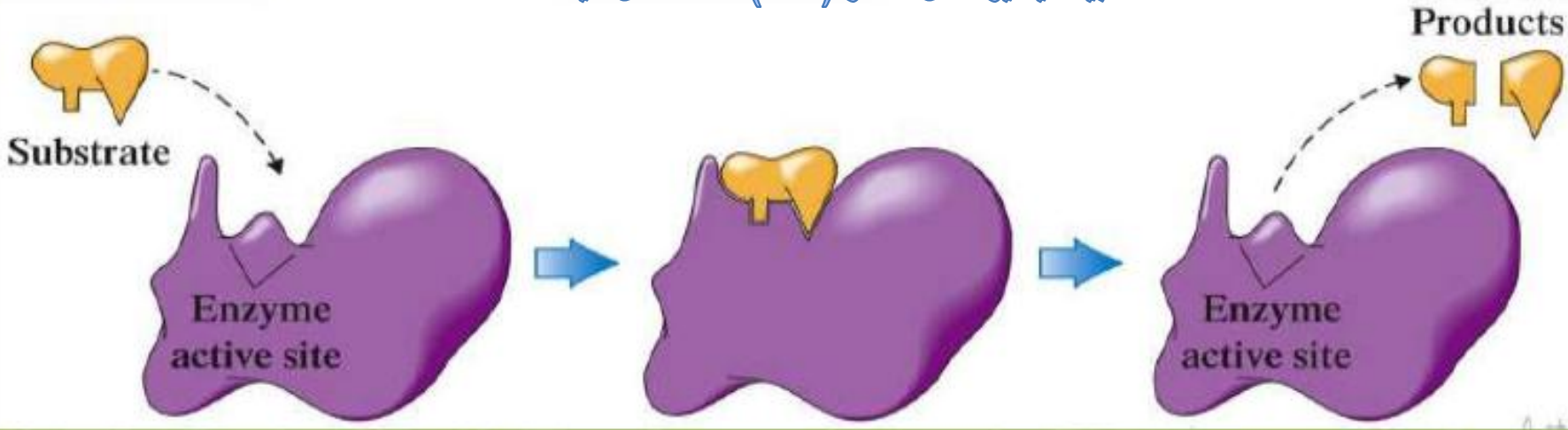
النموذج الملائم المستحث للانزيم

- Many enzymes are flexible & constantly change their shape
  - The shape of the active site changes to accept & accommodate the substrate
    - Conformation change in the enzyme's active site to allow the substrate to bind

شكل الركيزة في الموقع النشط للانزيم للسماح للركيزة بالارتباط

- Analogy: a glove (enzyme) changes shape when a hand (substrate) is inserted into it

تشبيه: يتغير شكل القفاز (الكف) عند ادخال اليد



# Enzyme Specificity


- **Absolute Specificity**
  - An enzyme will catalyze a particular reaction for only one substrate
  - Most restrictive of all specificities
    - Not common
      - ***Catalase*** has absolute specificity for hydrogen peroxide ($H_2O_2$)
      - ***Urease*** catalyzes only the hydrolysis of urea

- **Group Specificity**
  - The enzyme will act only on similar substrates that have a specific functional group
    - ***Carboxypeptidase*** cleaves amino acids one at a time from the carboxyl end of the peptide chain
    - ***Hexokinase*** adds a phosphate group to hexoses

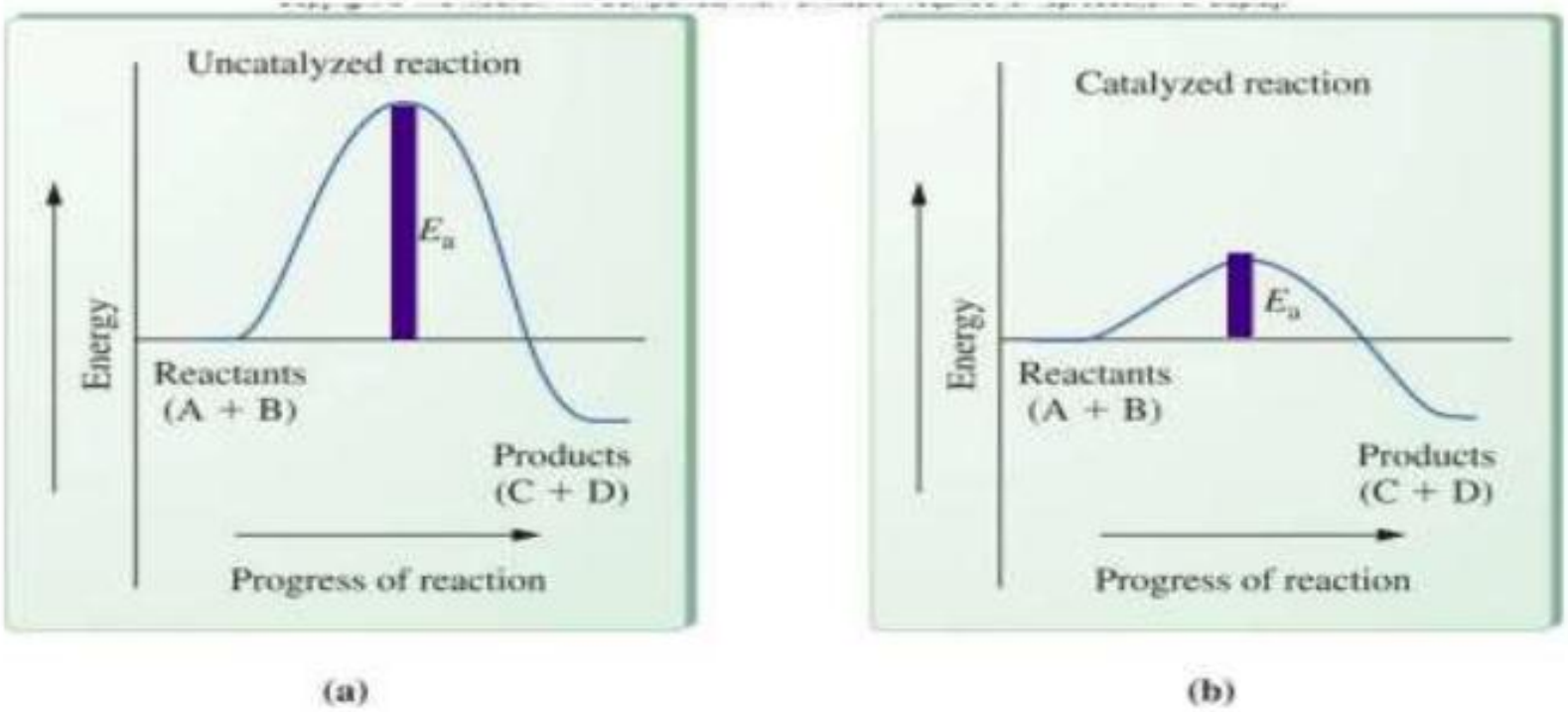
# How does enzyme work

- **The Effect of Enzymes on the Activation Energy of a Reaction**
- An enzyme speeds a reaction by lowering the activation energy, changing the reaction pathway, This provides a lower energy route for conversion of substrate to product
- Every chemical reaction is characterized by an equilibrium constant, Keq, which is a reflection of the difference in energy

$$K_{eq} = \frac{[B]^b}{[A]^a} = \frac{[\text{product}]^b}{[\text{reactant}]^a}$$

$$aA \rightleftharpoons bB$$

- **Diagram of Energy Difference Between Reactants and Products**




- The uncatalyzed reaction has a large activation energy, *Ea* , seen at left
- In the catalyzed reaction, the activation energy has been lowered significantly increasing the rate of the reaction.

## Answer true or false for the following sentences.

1. Some enzymes can even make the conversion of substrate into product.

2.In the enzymatic reaction , the molecules at the beginning of the process are called products

## Chose the correct answer

1.combination of the apoenzyme **and** coenzyme which together facilitating chemical reaction **(**apoenzyme **,** Holoenzyme, cofactor )

2.catalase has absolute specificity for (urea, hydrogen peroxide ,carboxyl)

Theory Biochemistry
2nd class , 1st semester
Department of dentistry

# BIOCHEMISTRY
# Enzymes and Coenzymes

*Prof. Farha Khalaf Omar*

# Allosteric Enzymes

- Allosteric enzymes have a quaternary structure
  - Are composed of 2 or more protein chains
  - Possess 2 or more binding sites

- ***2 types of binding sites:***

  - One binding site for the substrate
    - Active site

  - Second binding site for a regulator molecule
    - Regulatory site

- Active & regulatory binding sites are distinct from each other in shape & location

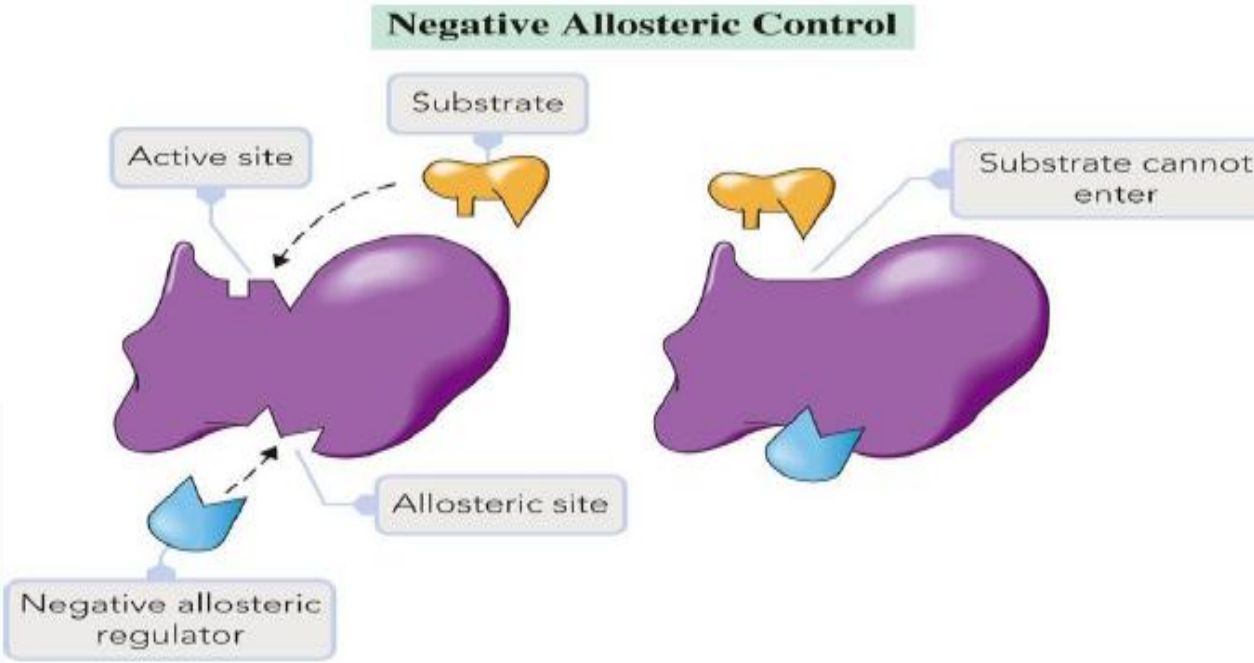
- Binding of a regulator molecule to its regulatory site causes changes in 3-D structure of the enzyme & the active site

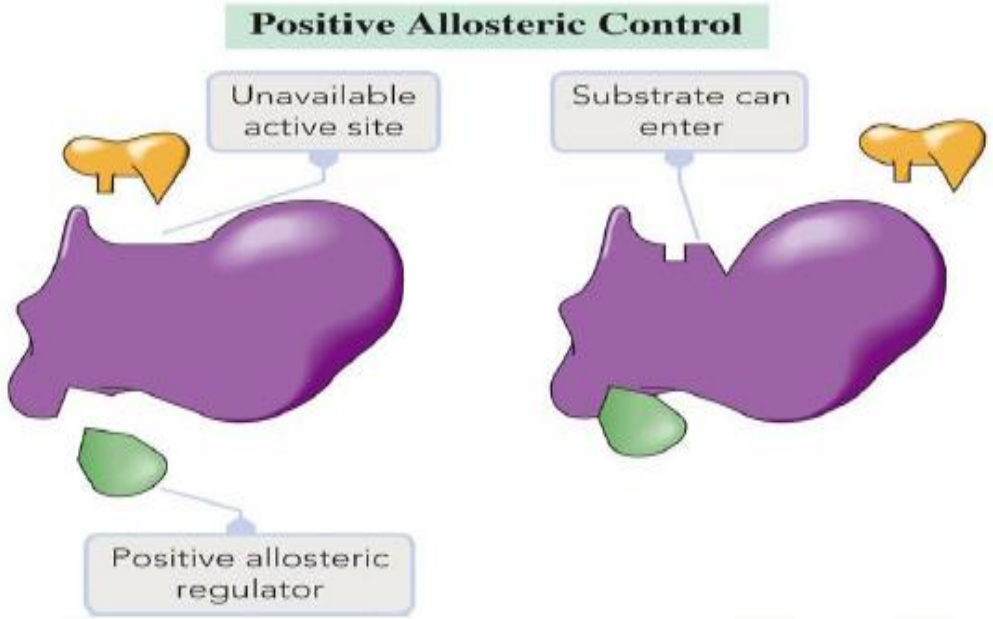
- Binding of a **Positive regulator**
  up-regulates enzyme activity
  - Enhances active site, more able to accept substrate

ينظم نشاط الانزيم ويعززالموقع النشط يجعله اكثر قدرة على قبول ال s

- Binding of a **Negative regulator** (non-competitive inhibitor)
  down-regulates enzyme activity
  - Compromises active site, less able to accept substrate

# The different effects of Positive & Negative regulators on an Allosteric enzyme

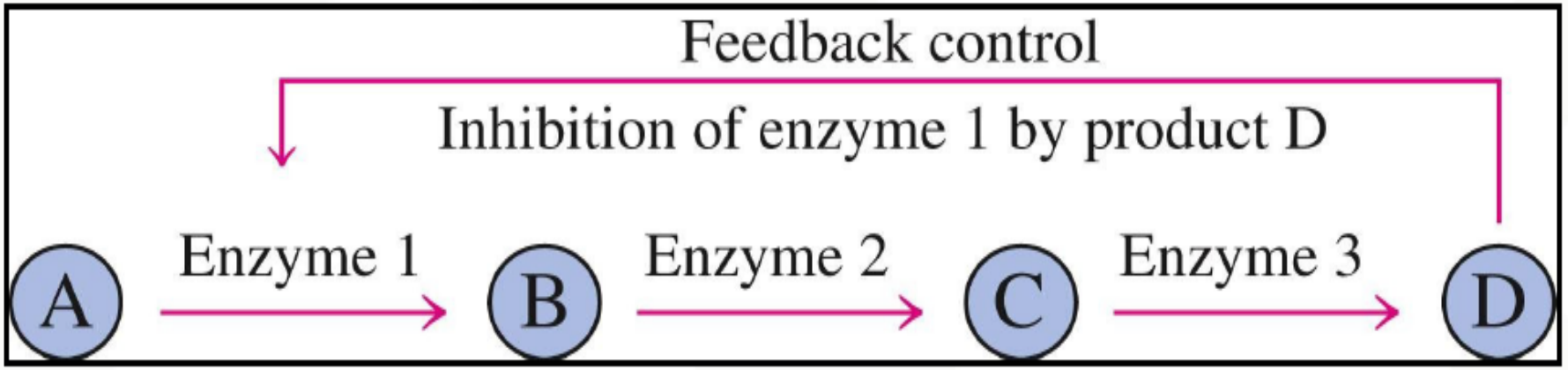


# Feedback Control

- A process in which activation or inhibition of one of the earlier reaction steps in a reaction sequence is controlled by a product of this reaction sequence.
  - One of the mechanisms in which allosteric enzymes are regulated
  - Most biochemical processes proceed in several steps & each step is catalyzed by a different enzyme
    - The product of each step is the substrate for the next step / enzyme.

Observe animation of feedback control




**Reaction 1:** converts reagent A into product B

**Reaction 2:** converts reagent B into product C

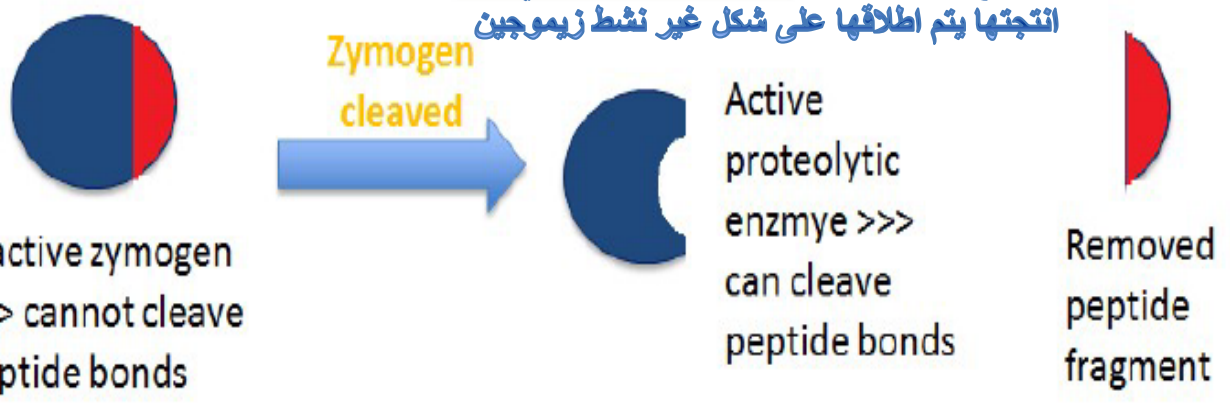
**Reaction 3:** converts reagent C into product D

# Proteolytic Enzymes & Zymogens

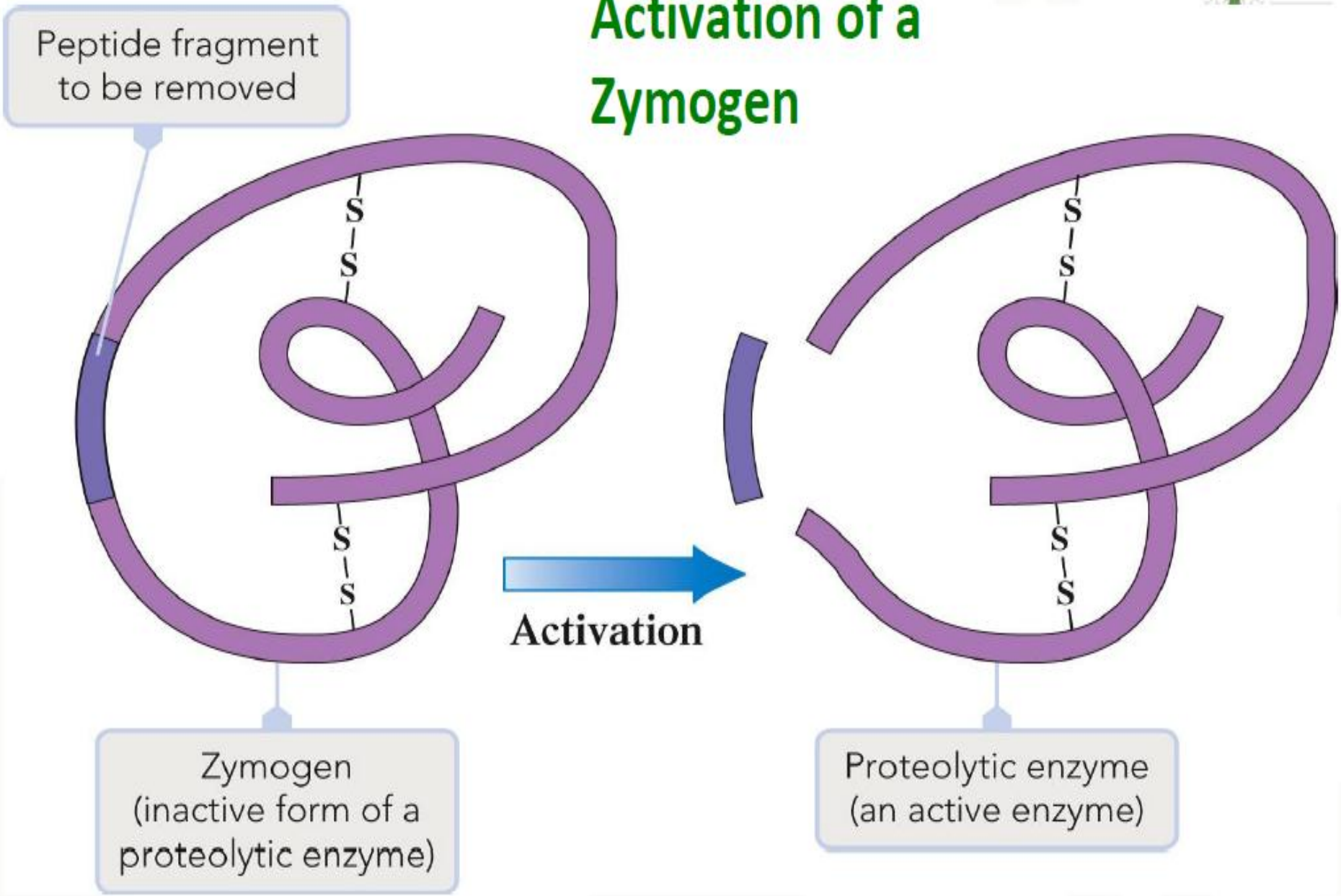
- 2nd mechanism of allosteric enzyme regulation
  - Production of an enzyme in an inactive form
  - Activated when required (right time & place)
    - Activated aka "turned on"
- **Proteolytic enzymes** catalyze breaking of peptide bond in proteins
  - To prevent these enzymes from destroying the tissues, that produced them, they are released in **an inactive form = ZYMOGENS**

- Most digestive & blood-clotting enzymes are proteolytic
  معظم الانزيمات اهضمية وتخثر الدم هي بروتينية
  - Blood clotting enzymes break down proteins within the blood so that they can form the clot
    تقوم انزيمات تخثر الدم بتكسير بروتينات الموجودة لتتمكن من تشكيل الجلطة
    - Platelets interspersed with tangled protein (collagen and thrombin)
- Activation of a zymogen requires the removal of a peptide fragment from the zymogen structure
  - Changing the 3-D shape & affecting the active site
    - E.g. Pepsiongen (zymogen)
      >>> Pepsin (active proteolytic enzyme)

لمنع هذه الانزيمات من تدمير الانسجة التي انتجتها يتم اطلاقها على شكل غير نشط زيموجين



# Activation of a Zymogen




Stoker 2014, Figure 21-14 p76

# Covalent Modification of Enzymes

- Covalent modifications are the 3rd mechanism of enzyme activity regulation
  - A process of altering enzyme activity by covalently modifying the structure of the enzyme
    - Adding / removing a group to / from the enzyme

- Most common covalent modification = addition & removal of phosphate group:
  - Phosphate group is often derived from an ATP molecule

    - Addition of phosphate = **phosphorylation** is catalyzed by a ***Kinase*** enzyme

    - Removal of phosphate = **dephosphorylation** is catalyzed by a ***Phosphatase*** enzyme

  - ***Glycogen synthase:*** involved in synthesis of glycogen
    - Deactivated by phosphorylation

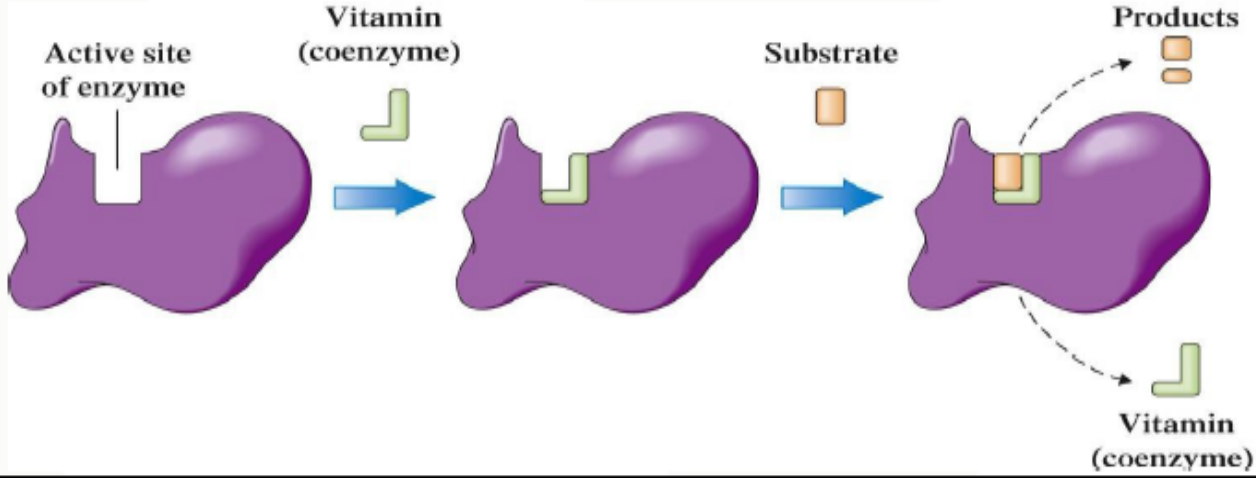
  - ***Glycogen phosphorylase:*** involved in breakdown of glycogen
    - Activated by phosphorylation.

# Vitamins as Coenzymes

- Many enzymes require B vitamins as coenzymes
  - Allow the enzyme to function

- Coenzymes serve as temporary carriers of atoms or functional groups
  - Coenzymes provide chemical reactivity that the apoenzyme lacks
  - Important in metabolism reactions to release energy from foods
    - E.g. redox reactions where they facilitate oxidation or reduction

- B vitamins don't remain permanently bonded to the apoenzyme
  - After the catalytic action the vitamin is released & can be repeatedly used by various enzymes
  - This recycling reduces the need for large amounts of B vitamins



# Drugs Inhibiting Enzyme Activity

- Many prescription drugs inhibit enzymes

- **ACE Inhibitors**
  - Inhibit Angiotensin-Converting Enzyme
    - Lowers blood pressure

- **Sulfa drugs**
  - Antibiotics acting as competitive inhibitors of bacterial enzymes
    - Involved in conversion of PABA to Folic acid
      - Deficiency of folic acid retards bacterial growth, eventually killing them

- **Penicillin's**
  - β-lactam antibiotics inhibit *transpeptidase*
    - Transpeptidase enzyme strengthens the cell wall
      - Forms peptide cross links between polysaccharides strands in bacterial cell walls
      - Without transpeptidase enzyme (inhibited by Penicillin) >>> weakened cell wall, bacteria

# Medical Uses of Enzymes

- Enzymes can be used in diagnosis & treatment of certain diseases

- ***Lactate dehydrogenase*** **(LDH)** is normally not found in high levels in blood, as it is produced in cells
  - Increased levels of LDH in the blood indicate myocardial infarction (MI) (Heart attack)

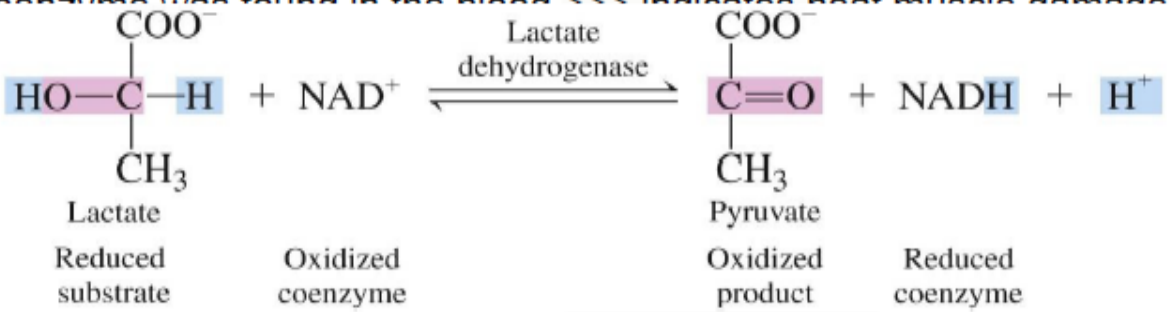
  - **Tissue plasminogen activator (TPA)** activates the enzyme *plasminogen* that dissolves blood clots
    - Used in the treatment of MI

- There is no direct test to measure urea in the blood
  - ***Urease*** converts urea into ammonia, which is easily measured & is used as urea indicator
    - Blood Urea Nitrogen (BUN) is used to measure kidney function

  - High urea levels in the blood indicate kidney malfunction

# Isoenzymes

- Isoenzyme catalyze the same reaction in different tissues in the body
  - e.g. *lactate dehydrogenase* (LDH) consists of 5 isoenzymes
    - Each isoenzyme of LDH has the same function
      - Converts lactate to pyruvate
    - $LDH_1$ isoenzyme is more prevalent in heart muscle
    - $LDH_5$ form is found in skeletal muscle & liver
- Isoenzymes can be used to identify the damaged or diseased organ or tissue
  - It is a marker for a particular location
- If $LDH_1$ isoenzyme was found in the blood >>> indicates heart muscle damage



## Table 21.3 Selected Blood Enzyme Assays Used in Diagnostic Medicine

| Enzyme | Condition Indicated by Abnormal Level |
|---|---|
| lactate dehydrogenase (LDH) | heart disease, liver disease |
| creatine phosphokinase (CPK) | heart disease |
| aspartate transaminase (AST) | heart disease, liver disease, muscle damage |
| alanine transaminase (ALT) | heart disease, liver disease, muscle damage |
| gamma-glutamyl transpeptidase (GGTP) | heart disease, liver disease |
| alkaline phosphatase (ALP) | bone disease, liver disease |

Stoker 2014, Table 21-3 p7

## Table 21.7 Selected Important Coenzymes in Which B Vitamins Are Present

| B Vitamin | Coenzymes | Groups Transferred |
|---|---|---|
| **thiamin** | thiamin pyrophosphate (TPP) | aldehydes |
| **riboflavin** | flavin mononucleotide (FMN)<br>flavin adenine dinucleotide (FAD) | hydrogen atoms |
| **niacin** | nicotinamide adenine dinucleotide ($NAD^+$)<br>nicotinamide adenine dinucleotide phosphate ($NADP^+$) | hydrogen atoms |
| **pantothenic acid** | coenzyme A (CoA) | acyl groups |
| **vitamin $B_6$** | pyridoxal-5-phosphate (PLP)<br>pyridoxine-5′-phophate (PNP)<br>pyridoxamine-5′-phosphate (PMP) | amino groups |
| **biotin** | biotin | carbon dioxide (carboxyl group) |
| **folate** | tetrahydrofolate (THF) | one-carbon groups other than $CO_2$ |
| **vitamin $B_{12}$** | methylcobalamin | methyl groups, hydrogen atoms |

Stoker 2014, Table 21-7 p780