# S.5 Bio notes

## ENZYMES

Enzymes are biological catalysts-that is they speed up the rate of chemical reactions in living organisms without themselves being changed at the end of the reaction. Enzymes are biological catalysts because they are protein molecules made by living organisms.

They increase the rate of reactions by a factor of between $10^6$ to $10^{12}$ times, allowing the chemical reactions that make life possible to take place at normal temperatures.

Enzymes are vitally important because in their absence reactions in the cells (metabolic reactions) would be too slow to sustain life.

Enzymes catalyze both types of metabolic reaction i.e. **catabolic reactions** (involving the breakdown of molecules and usually release energy and often involve oxidation e.g. respiration or hydrolysis e.g. digestion) and **anabolic reactions** (involving synthesis of molecules and usually require energy and often involve condensation e.g. glutamine synthetase, which catalyses the synthesis of the amino acid glutamine from glutamic acid and ammonia)

There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction.

**Substrates**= the molecules that bind to the enzyme

**Products**=new substances formed

### Properties of enzymes

Enzymes are protein in nature and their characteristics reflect the properties of the protein:

- *All enzymes are globular proteins*
- *Being proteins, they are coded for by DNA*
- *They are biological catalysts i.e. speed up chemical reactions within living cells*
- *Their presence does not alter the nature or properties of the end product(s) of the reaction*
- *They are very efficient i.e. they generally work very rapidly. A very small amount of catalysts bring about the change of a large amount of substrate*
- *Enzymes are not destroyed by the reactions they catalyse and so can be used again.*
- *Enzymes are highly specific i.e. an enzymes catalyses only a single reaction e.g. catalase only catalyses the decomposition of hydrogen peroxide.*
- *The catalysed reaction is reversible i.e. an enzyme can can work in either direction. Metabolic reactions are reversible and the direction in which they proceed depends on the relative amount of substrate and the product present*

- *The activity of enzyme is affected by PH; temperature; substrate concentration and enzymes concentration (to be considered in detail-practical work)*
- *The rate of enzyme-controlled reactions may be decreased by the presence of inhibitors.*
- *Enzymes possess active sites. These are precise places on the surfaces of the enzymes, to which substrates become attached.*

## Active sites

In one part of the enzyme molecule, there is an area called **active site** where the substrate molecule can bind. The binding of the substrate to the active produces an **enzyme-substrate complex.**

The three dimensional shape of the active site fits the substrate exactly, so only one type of the substrate can bind with the enzyme. The enzyme is therefore **specific** for that substrate.

The R groups of amino acids at the active site form temporary bonds with the substrate molecule. This pulls the substrate slightly out of shape, causing it to react and form **products**

**Enzymes work by lowering the activation energy**

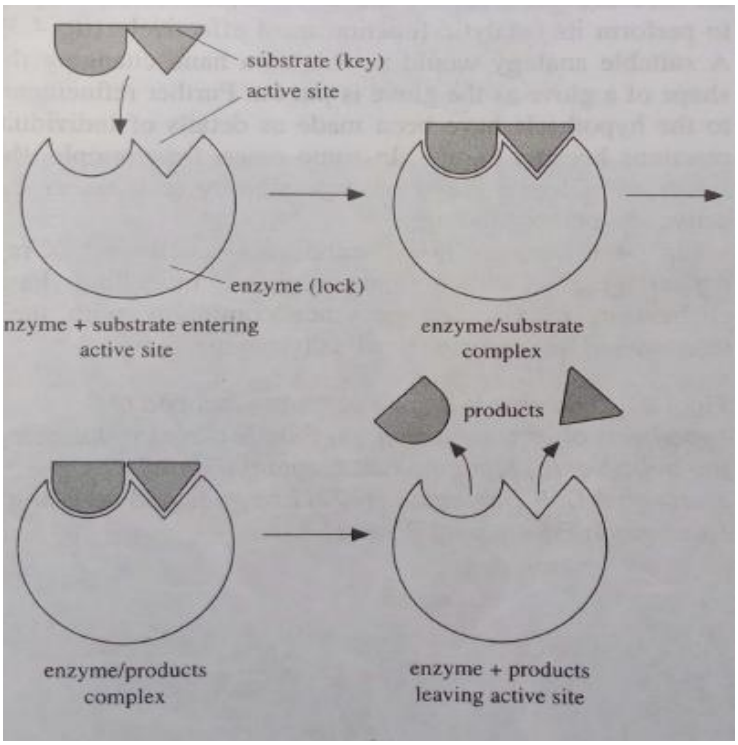
Activation energy is the energy required for the substrates to change themselves into products. Enzymes reduce activation energy needed, making the metabolic reactions in organisms to take place at lower energy level. They do this by distorting the shape of the substrate when it binds at the enzyme's active site.

## Mechanisms of enzyme action

Two hypotheses have been proposed to explain the mode of action of enzymes which include:

1. **Lock and Key hypothesis** (Emil Fisher, 1894)
   This hypothesis proposes that: Each enzyme molecule has an **active site** which has a precise/specific shape/configuration into which only certain specific substrate molecules with complementary structures fit exactly. Like a key into a lock, only the correct size and shape of the substrate (the key) would fit into the active site of the enzyme (the lock).

- When the complementary substrate enters the active site, a temporary enzyme-substrate complex is formed. The enzyme-substrate complex briefly forms the enzyme-product complex which then forms the products. The products have a different shape from the substrate and once formed, they escape from the active site leaving it free to become attached to another substrate molecule since the enzyme is left unchanged.
- This shows the **high specificity** of enzymes, however it is too rigid. So it does not explain how enzyme carries out its catalytic function.




*BS 3r ed. Pg 117*

Lock and key hypothesis can explain:

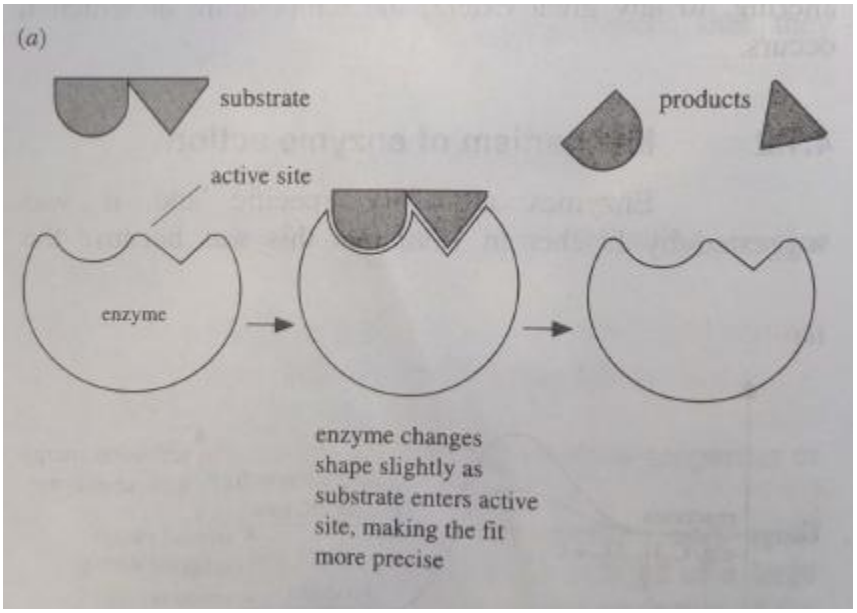
- the specificity of the enzymes i.e. the active site has specific shape into which only substrates with complementary shapes fit.
- why enzymes can be used over and over again, since the enzymes are left unchanged
- to an extent why the rate of enzyme-controlled reaction is limited by increasing substrate concentration.
- why and how the enzymes can be inhibited.
- why enzymes can be denatured by high temperatures and pH change

**2. Induced fit hypothesis (Koshland, 1959)**

This is an alternative suggested recent modification to the Lock and key hypothesis. It adds an idea that: the active site of the enzyme changes shape/modified slightly as the substrate molecule interacts with the enzyme in order to suit the substrate's shape. The amino acids which make up the active site are moulded into a precise shape/configuration that is catalytically active and affects the shape of the substrate enabling the enzyme to perform its catalytic function most efficiently by lowering the activation energy.

- A suitable analogy is that of a hand changing the shape of a glove as is put on or the way clothing is flexible and can mould itself to fit the shape of the wearer.
- This hypothesis is more acceptable since it gives an understanding on how enzymes work to catalyze reactions.

*Illustration (BS 3rd ed. pg 118 fig 4.3 (a).*




## Naming and classifying of enzymes

Most enzymes are named by attaching the suffix –ase to the name of the substrate on which it acts e.g. maltase acts on maltose, lipase acts on lipids, urea acts on urease.

Pepsin and trypsin attach proteins and are generally known as proteases and those which attach carbohydrates are generally known as carbohydrases

Enzymes can be classified/grouped based on:

**(a) Location/ where they carry out their effect:**

- Intracellular enzymes found inside the cells where they control metabolism;
- Extracellular enzymes produced by cells but achieve their effects outside the cells. These include digestive enzymes that break food in the gut. Some enzymes occur freely in the solutions, while others are fixed to the cell membrane inside the cell.

**(b) Type of reaction they catalyse:**

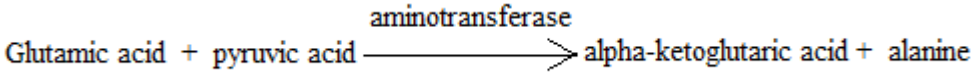
The modern classification of enzymes is based on the type of reaction being catalysed. There are six major classes:

1. **Oxidoreductases** catalyse redox reaction (biological oxidation and reduction) by transfer of hydrogen, oxygen, or electrons from one molecule to another for example:

$$\text{Ethanal} + \text{NADH} + \text{H}^+ \xrightarrow{\text{alcohol dehydrogenase}} \text{ethanol} + \text{NAD}^+$$

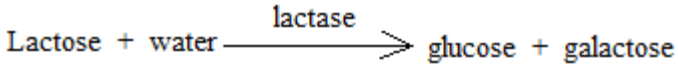
Hydrogen is simultaneously lost from NADH and gained by ethanol. NADH is oxidized to $NAD^+$, and ethanol is reduced to ethanol. This particular process takes place in anaerobic respiration in yeasts and plants.

2. **Transferases** catalyse the transfer of a group from one compound to another, for example:



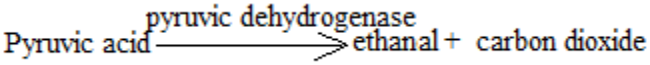

The R group on the amino acid, glutamic acid, is exchanged with the R group on a keto acid, pyruvic acid. A new amino acid alanine, is formed along with a new keto acid, alpha-ketoglutaric acid. This specific type of process is called transamination and it enables us to make non-essential amino acids from the essential amino acid.

3. **Hydrolases** catalyse the spilting of large substrate molecules into smaller products. Water is involved in the reaction : it is a hydrolysis, for example:



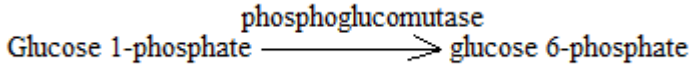

The disaccharide lactose is broken down into two monosaccharides by the addition of water: Hydrolases enable many condensation reactions (such as the polymerization of glucose to glycogen) and hydrolytic reactions (such as the digestion of proteins to amino acids) to take place.

4. **Lyases** catalyse the addition of a group across a double bond. For example:



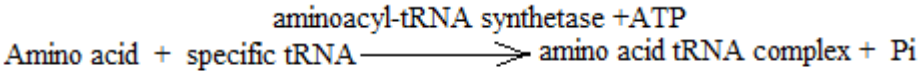

Pyruvic acid is converted into ethanol and carbon dioxide by breakage of its double bond and the addition of a new group to the free bonds. This particular reaction takes place during the fermentation of sugar by yeast. The ethanol is then converted to ethanol (alcohol)

5. **Isomerases** catalyse rearrangements within a molecule, converting one isomer to another, for example:




The position of a phosphate group in the glucose 1-phosphate molecule is changed to form the isomer glucose 6-phosphate. This reaction takes place during respiration.

6. **Ligases** catalyse bond formation between two compounds. The reaction uses energy that comes from the hydrolysis of ATP to ADP and phosphate, for example:




An amino acid is joined to a tRNA molecule. This particular process is called tRNA activation and it is an essential step in protein synthesis.

**A table summerising classification of enzymes based on the type of reaction**:

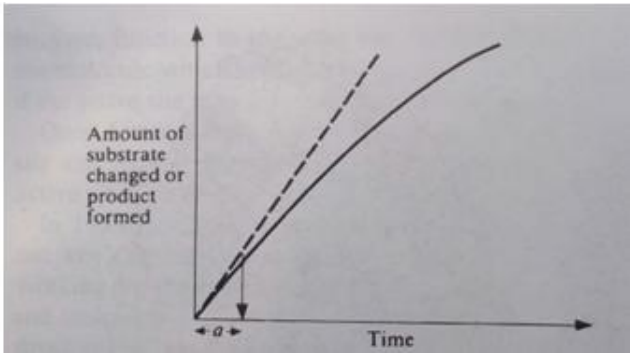
| **Enzyme group** | **Type of reaction catalysed** | **Enzyme examples** |
|---|---|---|
| 1. Oxidoreductases | Transfer of O and H atoms between substances | Dehydrogenases<br>Oxidases |

| | | |
|---|---|---|
| 2. Transferases | Transfer of a chemical group from one substance to another | Transaminases<br>Phosphorylases |
| 3. Hydrolases | Hydrolysis reactions | Peptidases<br>Lipases<br>Phosphotases |
| 4. Lyases | Addition or removal of a chemical group other than hydrolysis | Decarboxylases |
| 5. Isomerases | Rearrangement of groups within a molecule | Somerases<br>Mutases |
| 6. Ligasse | Formation of bonds between molecules using energy derived from the breakdown of ATP. | Synthetases |

## The rate of enzyme reaction

The rate of an enzyme reaction is measured by the amount of substrate changed, or amount of product formed, during a period of time.

The rate is determined by measuring the slope of the tangent to the curve in the initial stages of the reaction. The steeper the slope, the greater is the rate. If the activity is measured over period of time, the rate of reaction usually falls, most commonly as a result of a fall in substrate concentration.




**Question:** *Why are initial rates used when investigating factors which affect the enzyme reaction?*

## Factors affecting the rate of enzyme reaction

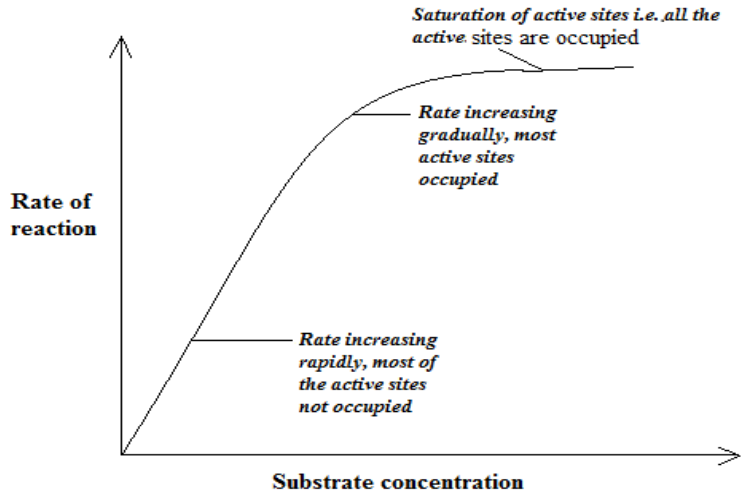
These factors are:

-*Temperature*
-*pH*
-*Enzyme concentration*
-*Substrate concentration*
-*Enzyme Inhibitors*

## Substrate concentration

For a given enzyme concentration, the rate of an enzyme reaction increases rapidly, then gradually with increasing substrate concentration until a point when the rate remains constant. At low substrate concentration, the active site of the enzyme molecules are not all used-there are simply not enough substrate molecules to occupy them all.

As the substrate concentration increases, the rate increases rapidly since more and more active sites become occupied, forming more enzyme-substrate complex and hence products per unit time . After certain concentration, the rate increases gradually since most of the active sites are bound with the substrate and few left to be occupied by the substrate. At high substrate concentration a point is reached when increasing the substrate concentration does not increase the rate of reaction since all the active sites of the enzyme molecules are virtually saturated (all being used) with the substrate. Thus any extra substrate has to wait until the enzyme-substrate complex has released the product before it may itself enter the active site of the enzyme. Therefore, increasing the substrate concentration further cannot increase the rate of reaction as the amount of enzyme is limiting factor.

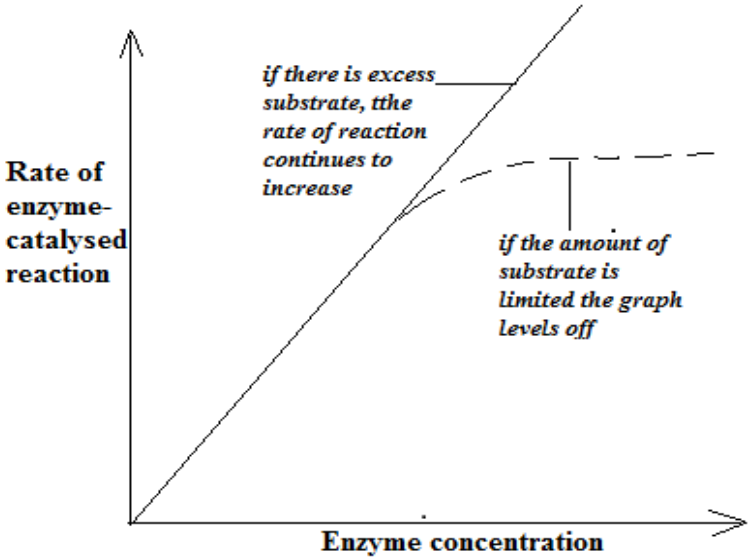



## Enzyme concentration

Provided that the substrate concentration is maintained at a high level, and other conditions such as pH and temperature are kept constant, the rate of reaction increases with increase in enzyme concentration. This is because more active sites are provided to bind with substrates.

If the amount of substrate is restricted, it may limit the rate of reaction. The addition of further enzyme does not increase the rate because all the substrates have been converted into products.

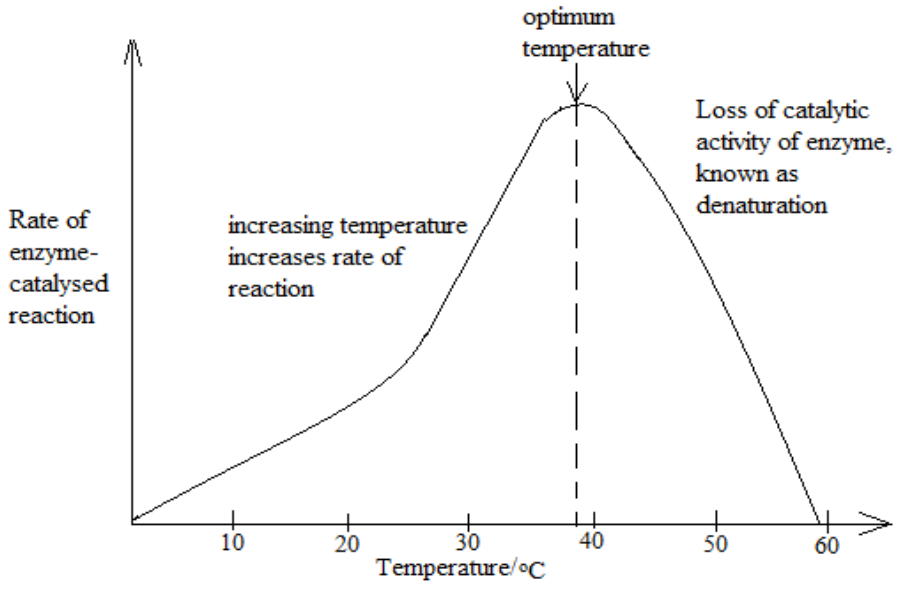
Note: Normally, the reactions are catalyzed by enzyme concentrations which are much lower than substrate concentration. This is because the active site of an enzyme can be used again and again.




## Temperature

The rate of enzyme-controlled reaction increases with increase in temperature until a maximum, and then the rate decrease until it stops completely

**A graph showing the effect of temperature on the rate of an enzyme-catalysed reaction**




At low temperature, the rate of reaction is low; enzyme and substrate molecules have little kinetic energy. They move slowly and so collide infrequently.

As the temperature increases, the kinetic energy of the substrate and enzyme molecules increases and so they move faster. This increases the frequency of successful collisions between the

enzyme and substrate molecules, more products are formed per unit time and hence increasing the rate of reaction. The temperature that promotes maximum activity is referred to as the optimum temperature.
Above the optimum temperature, the atoms which make up the enzyme molecules vibrate. This breaks the hydrogen bonds and other forces which hold the molecules in their precise shape. The three-dimensional shape of the enzyme molecules including the active site is altered to such an extent that their active sites no longer fir the substrate. The enzyme is said to be denatured and loses its catalytic properties.

Most mammalian enzymes have an optimum temperature of about 37-40$^{o}$C, but enzymes with higher optima exist. For example the enzymes of bacteria living in hot spring may have an optimum temperature of 70$^{o}$C or higher. Also many artic alpine plants have enzymes which function efficiently at temperature around 10$^{o}$C.
**Note**: if the temperature is reduced to near or below freezing point, enzymes are inactivated, not denatured. They will gain their catalytic activity when higher temperatures are restored.
***Question: The graph below shows the quantity of products formed by enzyme-controlled reactions at different temperatures***

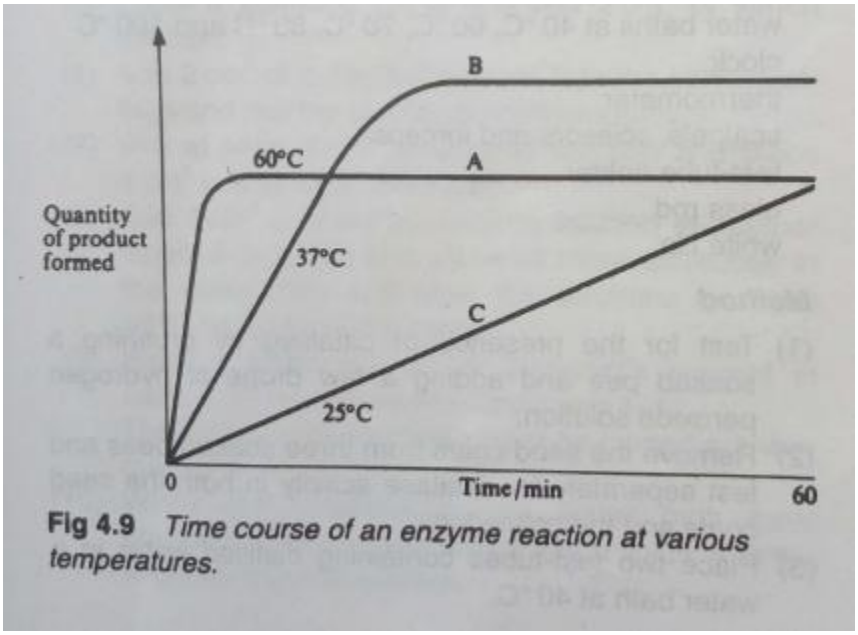
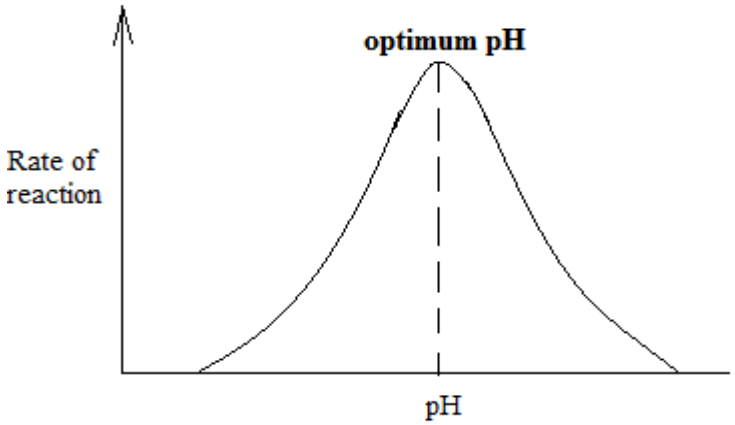


**Fig 4.9** *Time course of an enzyme reaction at various temperatures.*

*Explain the shapes of the curves given for the enzyme reaction at different temperatures?*

**P$^{H}$**
Under conditions of constant temperature, every enzyme function efficiently over a particular range, often this is a narrow range. The optimum PH is that PH at which the maximum rate of reaction occurs. When the PH is altered above or below this, the rate of enzyme activity reduces

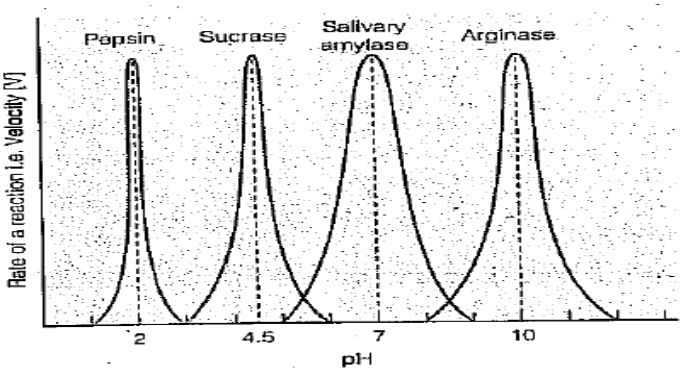
Graph showing effect of pH on the rate of enzyme-controlled reaction




$H^+$ and $OH^-$ affect the ionic bonds that hold the enzyme molecules in shape. This causes a change in the shape of the enzyme, and its active site. The rate of reaction is maximum at optimum pH: At optimum pH, the bonds within the enzymes are influenced by $H^+$ and $OH^-$ in such a way that the shape of their active sites is most complementary to the shape of their substrates/hydrogen concentration favours appropriate three dimensional structures of the enzyme/active sites to bind with the substrate.
When pH decreseas below optimum, acidity and the concentration of $H^+$ ions increases. This increases positive charges which affect the negatively charged carboxyl groups ($COO^-$ groups) disrupting the ionic bond. If the PH increase above the optimum pH, the concentration of the $H^+$ decreases which alter the ionic charge of the basic group ($NH_3^+$ group) and also disrupting the ionic bond. The disruption of the ionic bonds alters the three dimensional/precise shape of the enzyme including the active sites such that the enzyme loses its catalytic function/can longer accommodate the substrate and is denatured.

Each enzyme works best at a particular pH, and deviations from this optimum may result into denaturation

Graph showing the effect of pH on the rate of reaction of four different enzymes.



Different enzymes have different optimum PH values.
Most enzymes maintain their tertiary structure within in a narrow PH range, generally around 7. However, some enzymes require different range of temperature, e.g. pepsin found in the stomach has an optimum PH of 2.

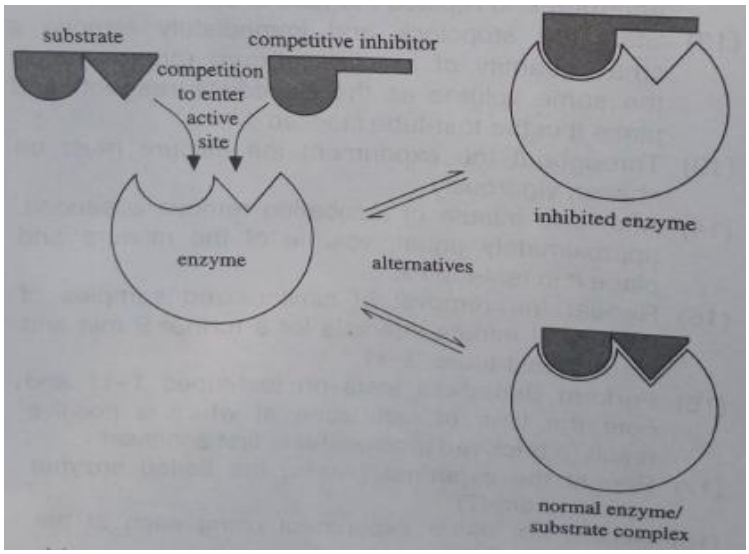
## Enzyme Inhibition

There are a variety of small molecules which reduce the rate of an enzyme-controlled reaction. They are called enzyme inhibitors. It is important to realize that inhibition is a normal part of the regulation of enzyme activity within cells. Many drugs and poisons also act as enzyme inhibitors.

Enzymes inhibitors are substances that slow down or stop the rate of enzyme-controlled reactions. They are of two types .i.e. *competitive inhibitors* and *non-competitive inhibitors. Non-competitive inhibitors may be reversible or non-reversible.*

### (i) Competitive inhibitors

A competitive inhibitor has a structure which is sufficiently similar to that of the normal substrate, so it is able to fit into the active site. When the competitive inhibitor enters the active site, it prevents the true substrate from entering the site to form the enzyme-substrate complex. The genuine substrate and the inhibitor therefore compete for a position in the active site thereby slowing down the rate of enzyme reaction.
(Illustration BS 3rd ed. Pg 124)




A characteristic feature of competitive inhibition is that if the substrate concentration is increased, the rate of reaction increases i.e. less inhibition occurs. This is because, as the substrate and inhibitor are in direct competition, the greater the proportion of substrate molecules the greater their chance of finding the active sites, leaving fewer to be occupied by the inhibitor. An example of competitive inhibitor is malonic acid. It competes with succinate for the active site of siccinic dehydrogenase, an important enzyme in the Kreb's cycle. Suphonamide

antibiotics which are used to destroy or prevent the growth of pathogenic bacteria without damaging host tissue competitively bind to the enzymes which are essential for the growth of pathogenic (disease-causing) bacteria.

**(ii) Non-competitive inhibitors**

Non-competitive inhibitor has no structural similarity with the substrate, and does not bind with the enzyme at the active site instead binds at some other points, changing the shape of the enzyme including the active site such that the formation of enzyme-substrate complex is prevented. It does not therefore compete with the substrate for active site but makes it impossible for catalysis to take place.

The rate of reaction decreases with increasing inhibitor concentration since more enzymes become inhibited. When the inhibitor saturation is reached, the rate of reaction will almost be nil. A characteristic feature of this type of inhibition is that, an increase in substrate concentration does not affect the rate of reaction unlike competitive inhibition. This is because the inhibitor and the substrate are not in direct competition for the active sites.

Most poison are non-competitive inhibitors for example cyanide is a non-competitive inhibitor it attaches itself to the copper prosthetic group of cytochrome oxidase, thereby inhibiting respiration

*Assignment: Draw an illustration to show non-competitive* inhibition

***Non-competitive inhibitors may be reversible or irreversible.***

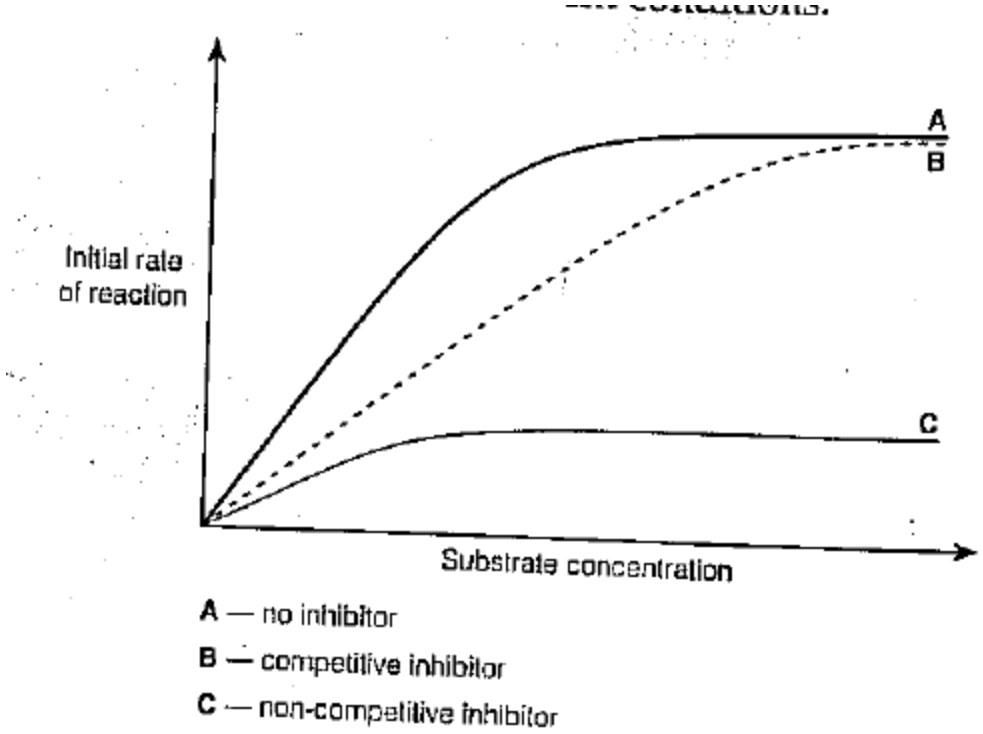
Reversible inhibitor does leave the enzyme damaged and so the enzyme regain its catalytic function when the inhibitor leaves it.

Irreversible inhibitors leave the enzysme permanently damaged and so unable to carryout it catalytic function. Irreversible inhibitors include the heavy metals such mercury ($Hg^{2+}$) and silver ($Ag^{+}$) which cause disulphide bonds to break. These bonds help to maintain the shape of the enzyme molecule. Once broken, the enzyme molecule's structure becomes irreversibly altered with the permanent loss of its catalytic properties. Certain iodine-containing compounds completely inhibit some enzymes.

**Assignment:**

1. *(a) What is meant by*
   - *(i) Competitive inhibition*
   - *(ii) Non-competitive inhibition*

   *(b) Compare competitive and non-competitive inhibitors*
2. *The graph shows the relationship between substrate concentration and the initial rate of an enzyme-catalysed reaction under different conditions.*




*(a) Suggest why the initial rate of the reaction was measured in each case.*

*(b) Explain*

*(i) The shape of curve A;*

*(ii) The difference between the shapes of curve B and curve C.*

*(c) (i) Explain what is meant by induced fit model of enzyme action.*

*(iii) Suggest how this may provide a better explanation for the effects of a non-competitive inhibitor than the lock and key model*

## Control of metabolic reaction

One of the most common ways of regulating metabolic pathways in cells is by means of allosteric enzymes.

### Allosteric enzymes

These are enzymes which are designed to change shape. They are regulated by compounds which act as non-competitive inhibitors called **allosteric inhibitors**. The allosteric inhibitors bind to the enzyme at specific sites well away from the active site called **allosteric sites** where they modify enzyme activity by causing a reversible change in the structure of the enzyme's active site. This in turn affects the ability of the substrate to bind the enzyme.

***(Illustration BS 3rd ed. Pg 125)***

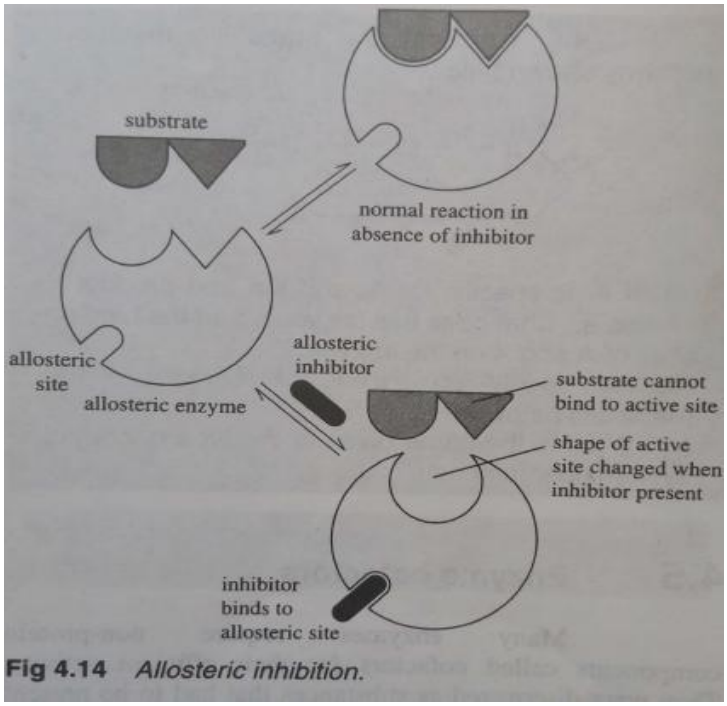


**Fig 4.14** *Allosteric inhibition.*

Allosteric enzymes can therefore exist in two different forms, i.e. an inactive form where the inhibitor binds to the allosteric site changing the shape of the enzyme in such a way that the substrate will not fit into the active site and the active form when the inhibitor is absent.

An example is provided by one of reactions of glycolysis (the series of reaction that form the first part of cell respiration). The purpose of cell respiration is to produce ATP. When ATP is at a high concentration, it inhibits one of the enzymes of glycolysis allosterically switching off the pathway. However, when metabolism increases and more ATP is used up, the overall concentration of ATP decreases and pathway once again comes into operation because the inhibitor ATP, has been removed. This as example of end-product inhibition.

**End-product inhibition (negative feedback)**

When the end-product of a metabolic pathway begins to accumulate, it may act as an allosteric inhibitor of the enzyme controlling the first step of the pathway. Thus the product starts to switch off its own production as it builds up. The process is self-regulatory. As the product is used up, its production is switched back on again. This is called end-product inhibition and is an example of a negative feedback mechanism.

**Importance of inhibitors**

- They control metabolic pathways by regulating the steps in them e.g. allosteric inhibition and end-product inhibition
- They are used in agriculture as pesticide
- Used in medicine as drugs
- They can be used to break particular reaction thereby enabling biochemists to reconstruct metabolic reactions. This is essential in scientific research.

## Enzyme cofactors

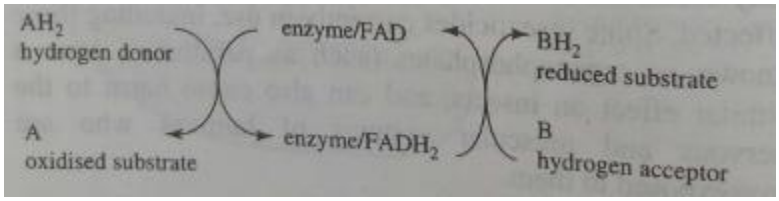
A cofactor is a non-protein substance/component essential for some enzymes to function efficiently. Cofactors vary from simple inorganic ions to complex organic molecules, and they remain unchanged at the end of a reaction or are regenerated by a later process.

There are three types of cofactors:

1. *Inorganic ions (enzyme activators)*
2. *Prosthetic groups*
3. *Coenzymes.*

**Enzyme activators (inorganic ions)**
Activators are inorganic ions which are necessary for the functioning of certain enzymes. They mould the enzymes or the substrate into a shape that allows an enzyme-substrate complex to be formed, hence increasing the chances of a reaction occurring between them and therefore increasing the rate of reaction catalysed by that particular enzyme. For example salivary amylase activity is increased in the presence of chloride ions, enzyme thrombokinase which converts prothrombin into thrombin during blood clotting is activated by calcium ($Ca^{2+)}$ ions.

**Prosthetic groups (for example FAD, haem)**
Prosthetic groups are organic molecules that are tightly bound to the enzymes on a permanent basis and they assist in the catalytic functioning of their enzymes, as in flavin adenine dinucleotide (FAD). This contains riboflavin (vitamin B2), the function of which is to accept hydrogen. FAD is concerned with cell oxidation pathway and is part of the respiratory chain in respiration.



**Haem** is iron-containing prosthetic group. It has the shape of the flat ring ( a' porphyrin ring' as found in chlorophyll) with an iron atom at its centre.
It has a number of biologically important functions which include the following:

- Electron carrier. Haem is the prosthetic group in cytochromes where it act as an electron carrier
- Oxygen carrier. Haemoglobin and myoglobin are oxygen-carrying proteins that contain haem groups
- Found in other enzymes such as catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide into water and oxygen.

**Coenzymes (for example NAD, NADP, Coenzyme A, ATP)**
Like prosthetic groups, coenzymes are organic molecules which act as cofactors but unlike the prosthetic groups they do not remain attached to the enzyme between reactions. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. All coenzymes are derived from vitamins.
NAD (nicotinamide adenine dinucleotide). This is derived from vitamin nicotinin acid (niacin) and it can exist in both a reduced and oxidized form. In an oxidized state it functions as a hydrogen acceptor.