Enzymology- An overview-1

Enzymes- An introduction

- Biologic (organic catalysts) polymers that catalyze the chemical reactions.

- Enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.

- With the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins.

- In addition to being highly efficient, enzymes are also extremely selective catalysts.

-Thermolabile, site specific, with a high turn over number compared to the inorganic catalysts.

Nomenclature of enzymes

-In most cases, enzyme names end in –ase

-The common name for a hydrolase is derived from the substrate

Urea: remove -a, replace with -**ase** = urease

Lactose: remove - **ose**, replace with - **ase** = lactase

- Other enzymes are named for the substrate and the reaction catalyzed

Lactate dehydrogenase

Pyruvate decarboxylase

- Some names are historical - no direct relationship to substrate or reaction type

Catalase

Pepsin

Chymotrypsin

Trypsin

Classification of Enzymes

- Enzyme Commission (EC) – according to **International Union of Biochemistry and Molecular Biology (IUBMB)**

- Each enzyme was given 4 digit numbes [1.2.3.4]

1st one of the 6 major classes of enzyme activity

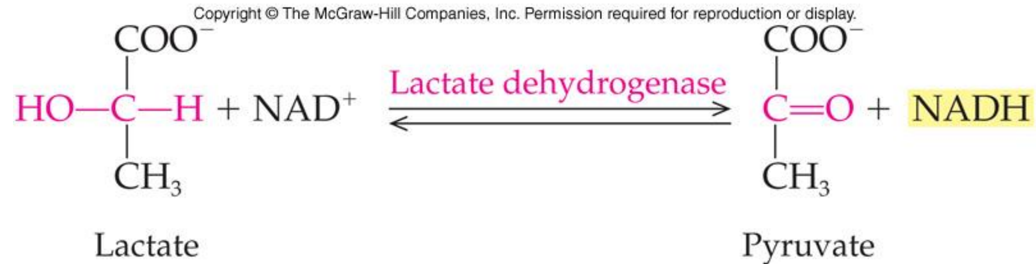
2nd the subclass (type of substrate or bond cleaved)

3rd the sub-subclass (group acted upon, cofactor required, etc...)

4th a serial number… (order in which enzyme was added to list)

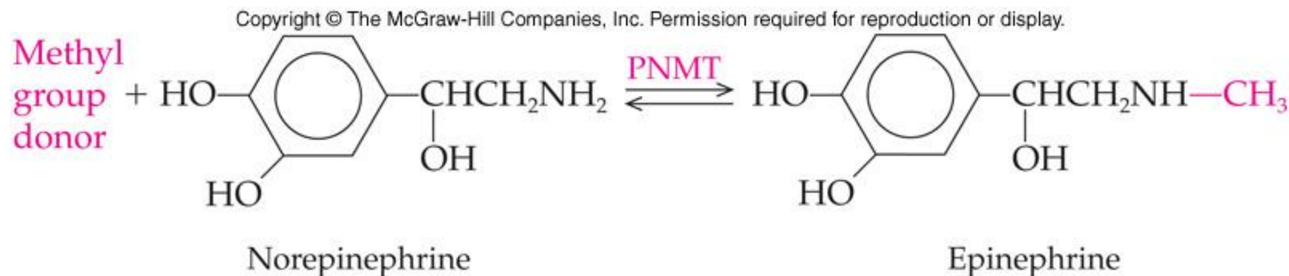
1- Oxidoreductases (**EC.1**) catalyze redox reactions, such as (Alcohol dehydrogenase [EC 1.1.1.1])

- Reductases
- Oxidases



2- Transferases (**EC.2**) transfer a group from one molecule to another, such as (Hexokinase [EC 2.7.1.2])

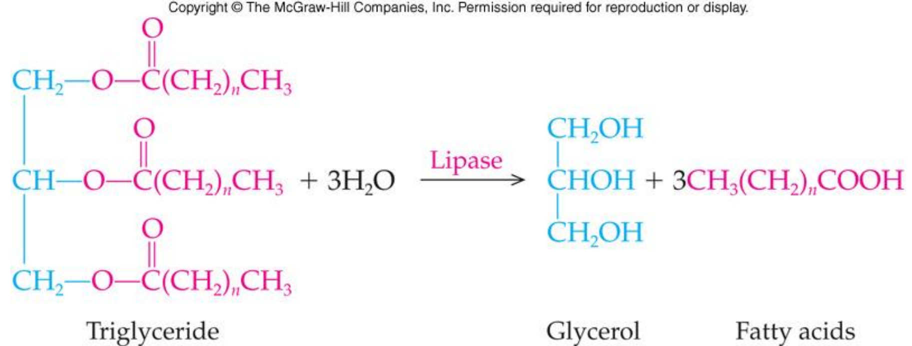
-Transaminases catalyze transfer of an amino group
- Kinases transfer a phosphate group



3- Hydrolases (**EC.3**) cleave bonds by adding water, such as

(Alkaline phosphatase [EC 3.1.3.1])

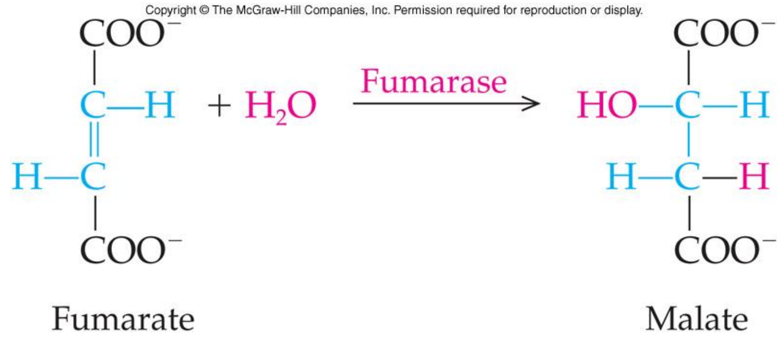
- Phosphatases
- Peptidases
- Lipases



4- Lyases (**EC.4**) catalyze removal of groups to form double bonds or the reverse break double bonds, such as

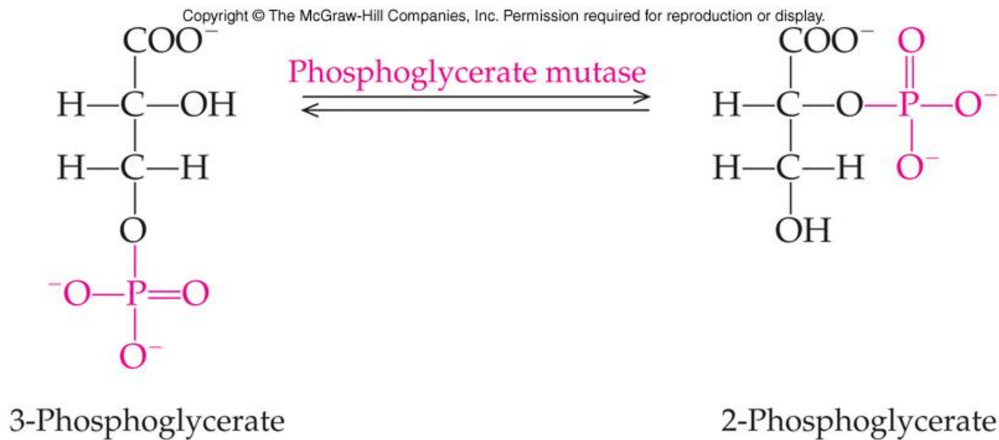
(Pyruvate decarboxylase [EC 4.1.1.1])

- Decarboxylases
- Synthases

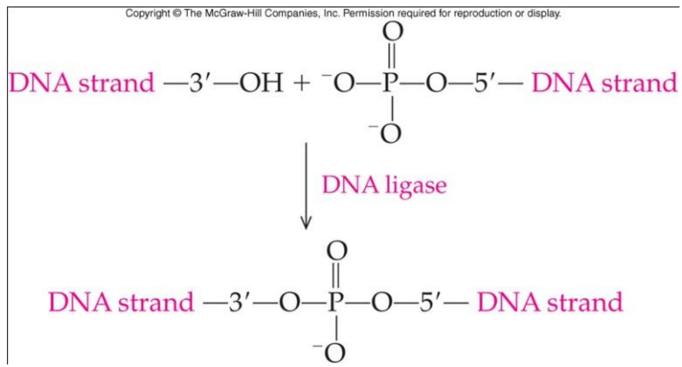


5- Isomerases (**EC.5**) catalyze intramolecular rearrangements, such as (Alanine racemase [EC 5.1.1.1])

- Epimerases
- Mutases

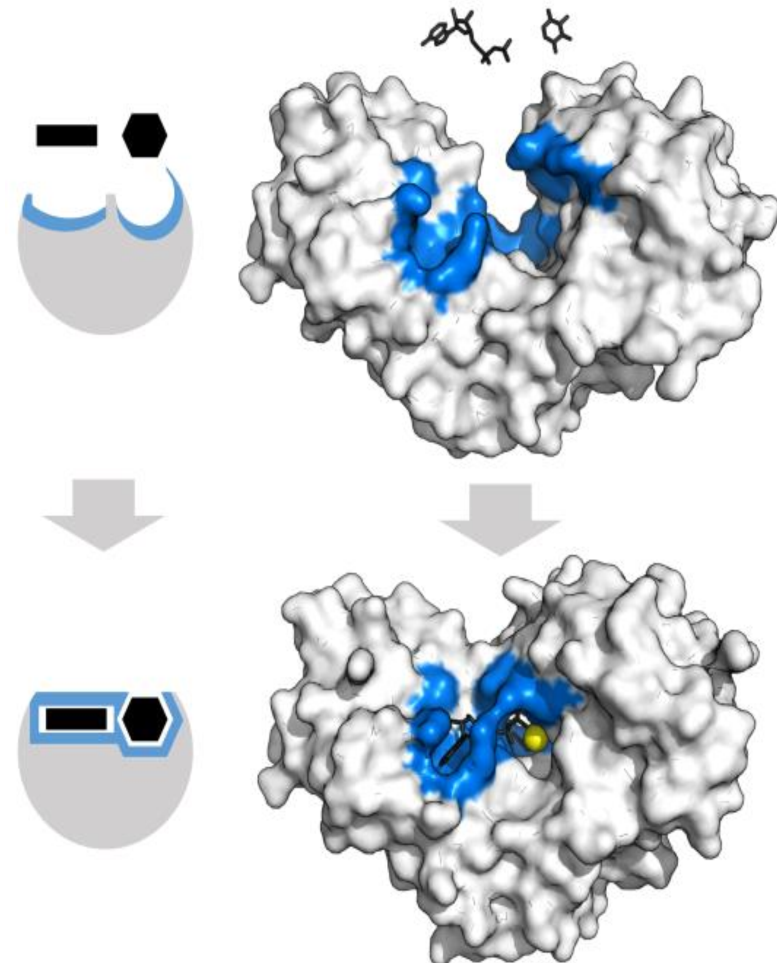


6- Ligases (**EC.6**) catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken, such as (Isoleucine-tRNA ligase [EC 6.1.1.5])



Active site

- Takes the form of a cleft or pocket
- Takes up a relatively small part of the total volume of an enzyme
- Substrates are bound to enzymes by multiple weak attractions
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site
- The active sites of multimeric enzymes are located at the interface between subunits and recruit residues from more than one monomer

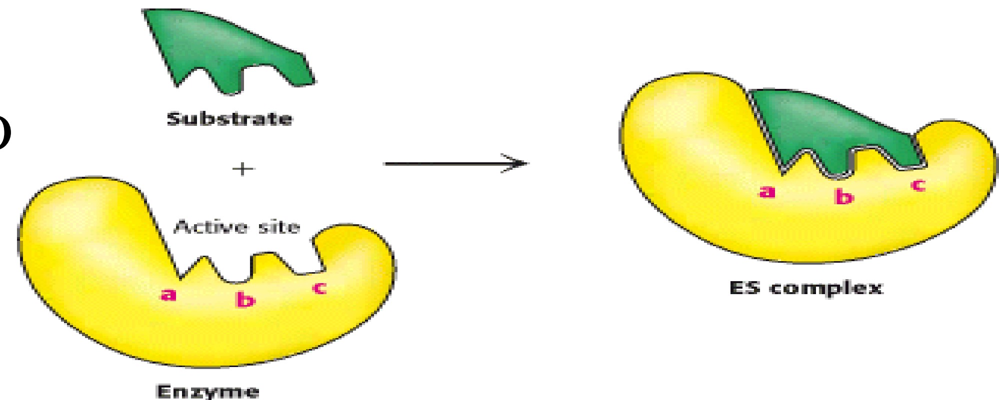


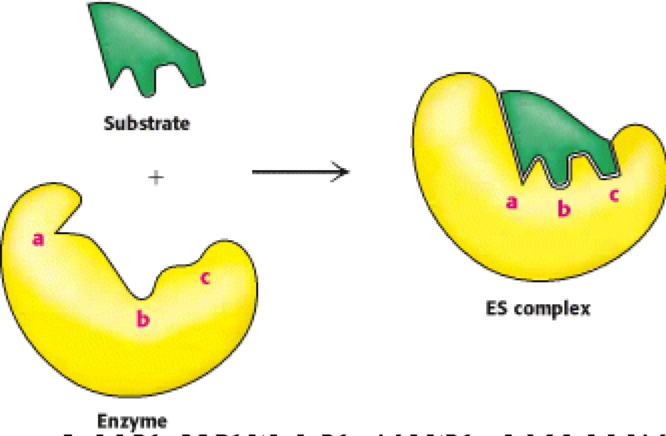
Enzyme substrate binding

-Two models have been proposed to explain how an enzyme binds its substrate: the lock-and –key model and the induced-fit model.

- Lock-and-Key Model of Enzyme-Substrate Binding, in this model, the active site of the unbound enzyme is complementary in shape to the substrate.

-"lock and key model" **accounted for the exquisite specificity of enzyme-substrate interactions**, the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany catalysis.





f Enzyme-Substrate Binding

zyme changes shape on substrate

a shape complementary to the substrate only after the substrate has been bound.

- When a substrate approaches and binds to an enzyme they induce a conformational change, a change analogous to placing a hand (substrate) into a glove (enzyme).

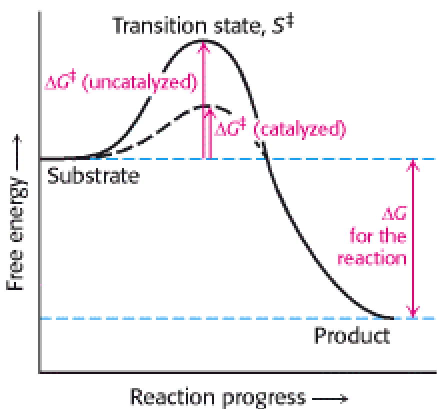
Mechanism of Action of Enzymes

- Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.
- The basic enzymatic reaction can be represented as follows:

- Where E represents the enzyme catalyzing the reaction, S the substrate, the substance being changed, and P the product of the reaction.
- The mechanism of action of enzymes can be explained by two perspectives:

1- Thermodynamic changes

2- Processes at the active site



mic changes

accelerate reaction rates

transition states

ed ΔG F for

formation of the transition states.

-The lower activation energy means that more molecules have the required energy to reach the transition state.

Processes at the active site

1- **Catalysis by proximity**: for the molecules to react they must come within bond-forming distance of one another. When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration.

Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.

2- **Acid base catalysis**: the ionizable functional groups of aminoacyl side chains of prosthetic groups contribute to catalysis by acting as acids or bases.

- General acid catalysis involves partial proton transfer from a donor that lowers the free energy of the transition state.
- General base catalysis involves partial proton abstraction from an acceptor to lower the free energy of the transition state.

3- **Catalysis by strain**: enzymes that catalyze the lytic reactions involve breaking a covalent bond typically bind their substrates in a configuration slightly unfavorable for the bond that will undergo cleavage.

4- **Covalent catalysis**: accelerates reaction rates through transient formation of enzyme-substrate covalent bond. Three stages in covalent catalysis:

1- Nucleophilic reaction between enzyme and substrate
2- Electrophilic withdrawl of electrons from substrate
3- Elimination reaction (reverse of stage 1)

5- Metal Ion catalysis

- Two classes of metal ion dependent enzymes:

1- Metalloenzymes contain tightly bound transition metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+})

2- Metal-activated enzymes loosely bind metal ions (alkali or alkaline metal including Na^+, K^+, Mg^{2+} and Ca^{2+})

- Metal ions enhance catalysis in three major ways:

1- Binding to and orienting substrates for reaction as Mg^{2+} binding to ATP

2- Mediating redox reaction through changes in oxidation state such as reduction of O_2 to H_2O through electron transfer

3- Electrostatic stabilization or shielding of negative charges as Mg^{2+} binding to ATP

6- Electrostatic catalysis

- Enzymes seem to arrange active site charge distributions to stabilize the transition states of catalyzed reactions
- Substrate binding generally excludes water from an enzyme active site generating a low dielectric constant within the active site
- Electrostatic interactions are stronger
- pka's can vary by several pH units due to proximity of charged groups

- Alternative form of electrostatic catalysis: several enzymes as superoxide dismutase apparently use charge distributions to guide polar substrates to their active sites

Enzyme Specificity

- In general, there are four distinct types of specificity:

1- Absolute specificity: the enzyme will catalyze only one reaction.

2- Group specificity: the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups

3- Linkage specificity: the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure

4- Stereo chemical specificity: the enzyme will act on a particular steric or optical isomer.

Cofactors

- Cofactors can be subdivided into two groups: metals and small organic molecules

- Cofactors that are small organic molecules are called coenzymes.

- Most common cofactor are also metal ions.

- If tightly bound, the cofactors are called prosthetic groups.

- Loosely bound Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate

Prosthetic groups

- Tightly integrated into the enzyme structure by covalent or non-covalent forces. e.g.;
  Pyridoxal phosphate
  Flavin mononucleotide(FMN)
  Flavin adenine dinucleotide(FAD)
  Thiamin pyrophosphate (TPP)
  Biotin
  Metal ions – Co, Cu, Mg, Mn, Zn

- Metals are the most common prosthetic groups

Role of metal ions

- Enzymes that contain tightly bound metal ions are termed – Metalloenzymes

- Enzymes that require metal ions as loosely bound cofactors are termed as metal-activated enzymes

- Metal ions facilitate:
  - Binding and orientation of the substrate
  - Formation of covalent bonds with reaction intermediates
  - Interact with substrate to render them more electrophilic or nucleophilic

Coenzymes

- They serve as recyclable shuttles—or group transfer agents—that transport many substrates from their point of generation to their point of utilization.

- The water-soluble B vitamins supply important components of numerous coenzymes.

- Chemical moieties transported by coenzymes include hydrogen atoms or hydride ions, methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).

Diagnostic significance of enzymes

1- Enzymes can act as diagnostic markers of underlying diseases .

2- Enzymes can also act as reagents for various biochemical estimations and detections

Enzymes as diagnostic markers

1- **Functional plasma enzymes** (Plasma derived enzymes):

- Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood.

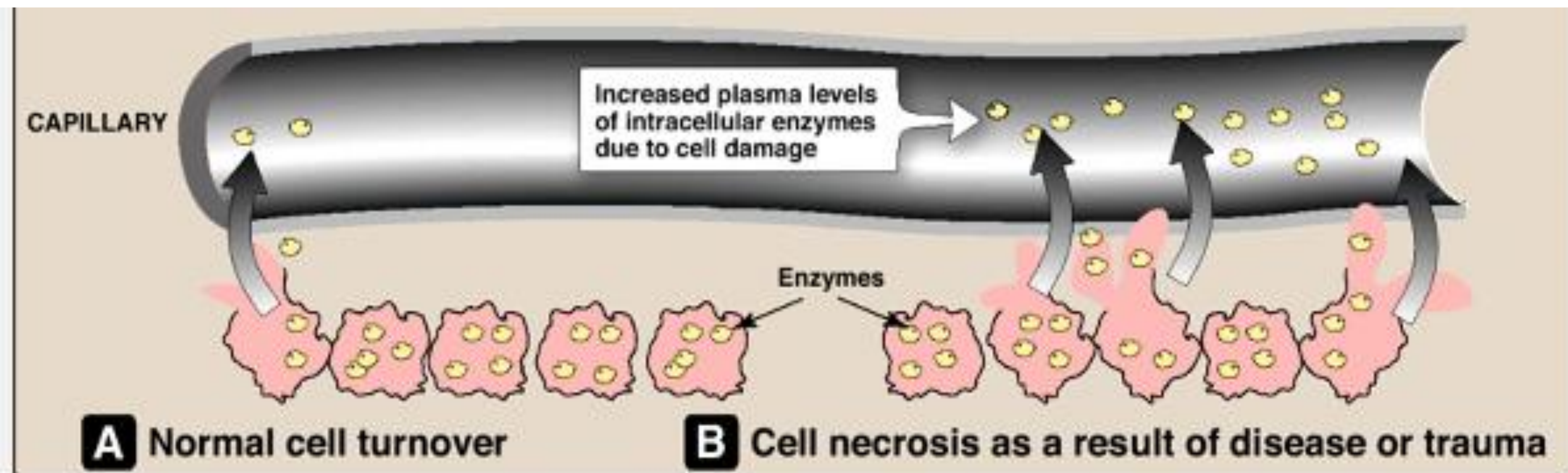
Examples of these functional plasma enzymes include lipoprotein lipase, pseudo cholinesterase, and the proenzymes of blood coagulation and blood clot dissolution .The majority of these enzymes are synthesized in and secreted by the liver.

2- **Nonfunctional plasma enzymes** (Cell derived enzymes):

- Plasma also contains numerous other enzymes that perform no known physiologic function in blood.

-These apparently nonfunctional plasma enzymes arise from the routine normal destruction of erythrocytes, leukocytes, and other cells.

-Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes.



Isoenzymes (Isoenzymes)

- Are homologous enzymes that catalyze the same reaction but have differences in enzymatic properties.
- Often different isoenzymes are found in different locations in a cell or in different organs/tissues of an organism.
- They are from different polypeptide chains that coded by different genes and so, they are affected by different activators and different inhibitors in different tissues.

e.g.:

Lactate dehydrogenase isoenzymes,

- The enzyme interconverts lactate and pyruvate (LDH)
- Humans have two isoenzymic chains for lactate dehydrogenase: LDH (M) found in muscle and LDH (H) found in heart.
- M is optimized to work under anaerobic conditions and H optimized to work under aerobic conditions.

-There are 5 different isoenzymes.
-The relative ratio of the isoenzymes depends on the location in the organism as well as the developmental stage.

Isoenzyme	Tissue origin
LDH1 (H4)	Cardiac and kidney
LDH2 (H3M)	Cardiac, kidney, brain and RBCs
LDH3 (H2M2)	Brain, lung and WBCs
LDH4 (HM3)	Lung, skeletal muscle
LDH5 (M4)	Skeletal muscle and liver

CK/CPK Isoenzymes

- There are three Isoenzymes.
- Measuring them is of value in the presence of elevated levels of CK or CPK to determine the source of the elevation.
- Each isoenzyme is a dimer composed of two protomers ‘M’ (for muscles) and ‘B’(for Brain).

-These isoenzymes can be separated by, electrophoresis or by ion exchange chromatography.

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean % in blood
MM(CK3)	Least	Skeletal muscle Heart muscle	97-100%
MB(CK2)	Intermediate	Heart muscle	0-3%
BB(CK1)	Maximum	Brain	0%

Enzyme Kinetics

- It is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme-catalyzed reactions and the study of the factors affecting these rates.
- The rate of a chemical reaction is described by the number of molecules of reactant(s) to be converted into product(s) in a specified time period which is dependent on the concentration of the chemicals involved in the process and on rate constants that are characteristic of the reaction.

Enzymology- An overview-2

Factors affecting Enzyme activity

- Numerous factors affect the reaction rate:

Temperature

-The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature

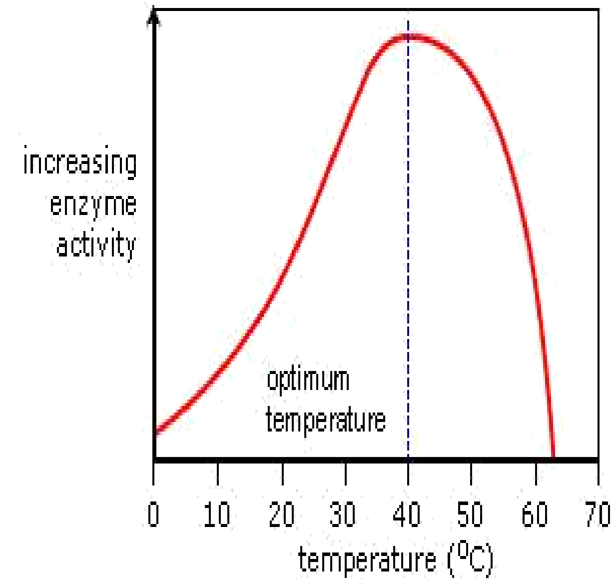
- Most animal enzymes rapidly become denatured at temperatures above 40°C

- The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C

-The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature.

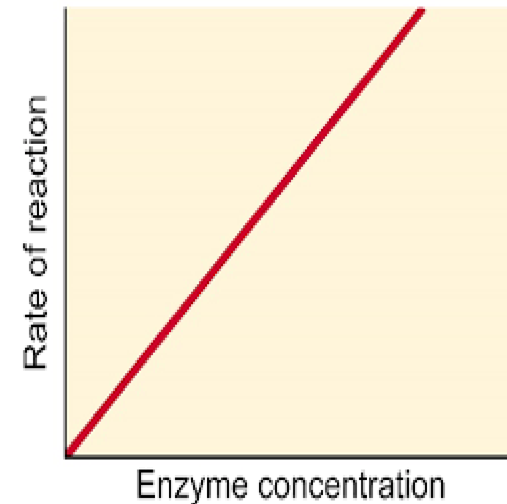
Effect of Temperature

- For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.



Effect of enzyme concentration

- As the amount of enzyme is increased, the rate of reaction increases.
- If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.
- Reaction rate therefore increases then it levels off.



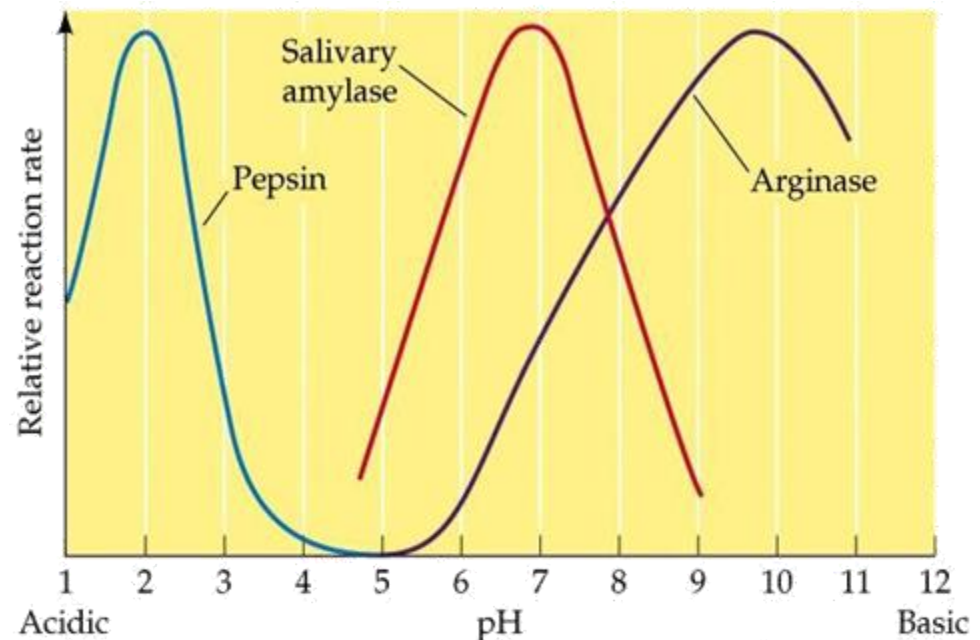
Effect of pH on enzyme activity

-The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration

- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.

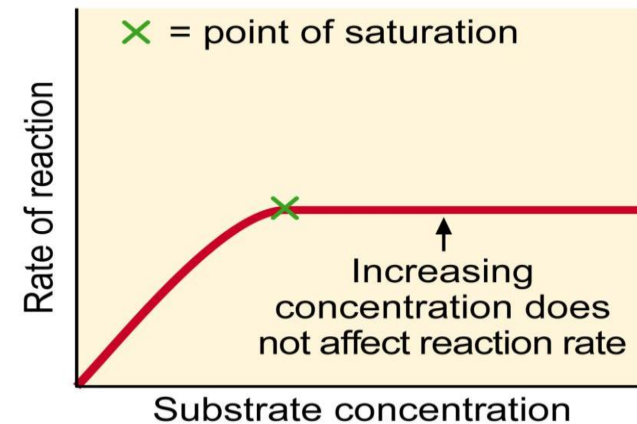
-The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both.

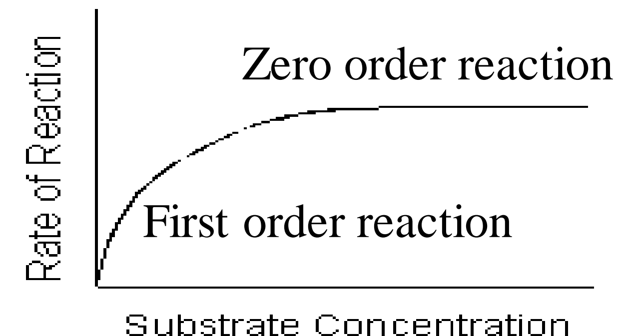
- Except for Pepsin, acid phosphatase and alkaline phosphatase, most enzyme have optimum pH between 5 to 9.



Effect of substrate concentration

- At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.
- Higher concentrations cause more collisions between the molecules.
- The rate of reaction increases (First order reaction).
- The maximum velocity of a reaction is reached when the active sites are almost continuously filled.
- Increased substrate concentration after this point will not increase the rate.
- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).





The shape of the curve that relates activity to substrate concentration is hyperbolic.

Michaelis-Menten Kinetics

-The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction [V_i], the concentration of substrate [S] and two constants, V max and km (which are set by the particular equation).

-The symbols used in the Michaelis-Menten equation refer to the reaction rate [V_i], maximum reaction rate (V max), substrate concentration [S] and the Michaelis-Menten constant (km).

Michaelis-Menten equation

-The dependence of initial reaction velocity on [S] and Km may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

$$v_1 = \frac{V_{max}[S]}{\{K_m + [S]\}}$$

1- When [S] is much less than km, the term km + [S] is essentially equal to km.

Since V max and km are both constants, their ratio is a constant (k).

In other words, when [S] is considerably below km, V max is proportionate to k[S].

The initial reaction velocity therefore is directly proportionate to [S].

2- When [S] is much greater than km, the term km + [S] is essentially equal to [S].

Replacing km + [S] with [S] reduces equation to

$$Vi = Vmax$$

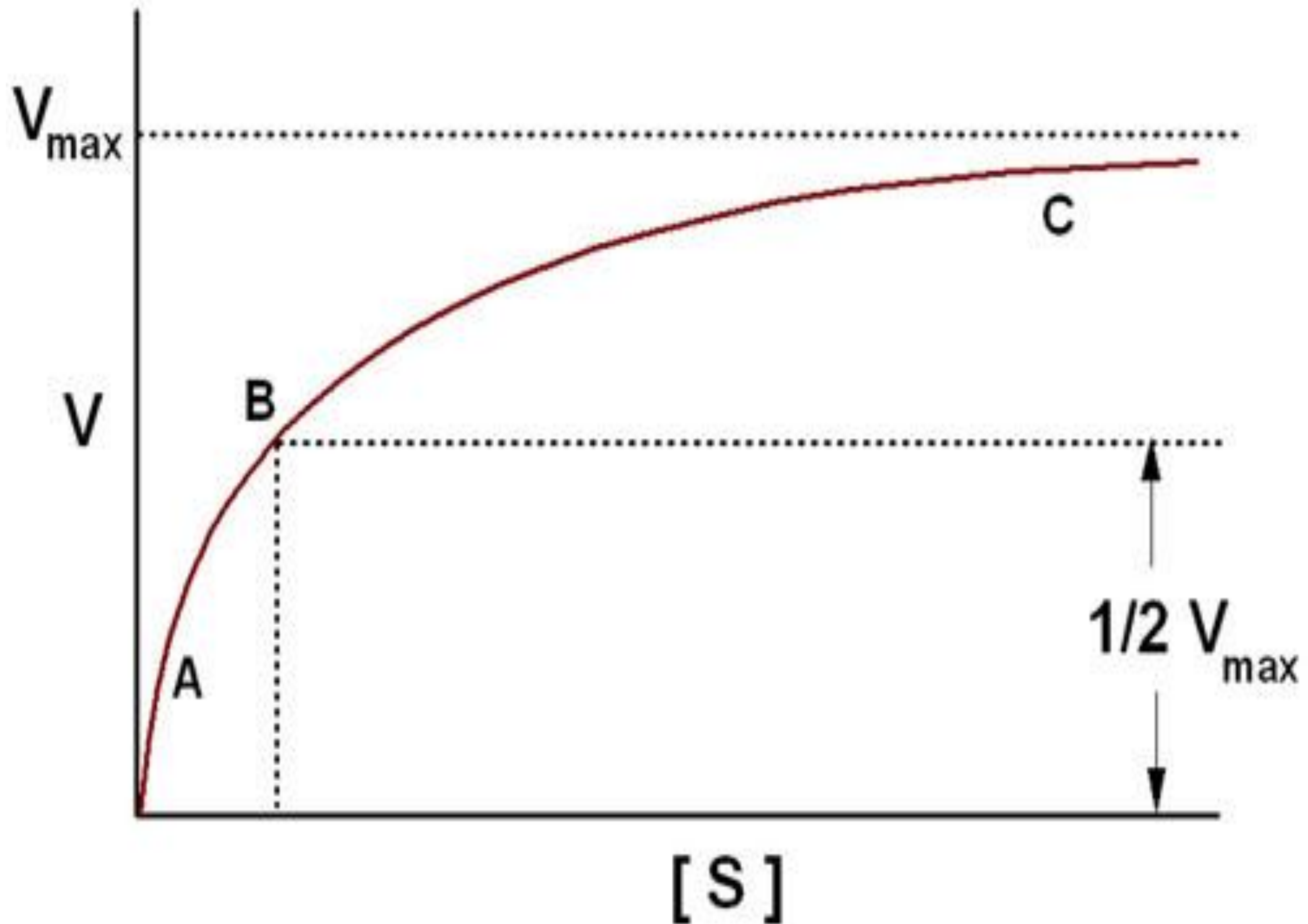
- Thus, when [S] greatly exceeds km, the reaction velocity is maximal (V max) and unaffected by further increases in substrate concentration.

3- When [S] = km

Equation states that when [S] equals km, the initial velocity is half-maximal.

Equation also reveals that km is a constant and may be determined experimentally from—the substrate concentration at which the initial velocity is half-maximal.

Plot of substrate concentration versus reaction velocity



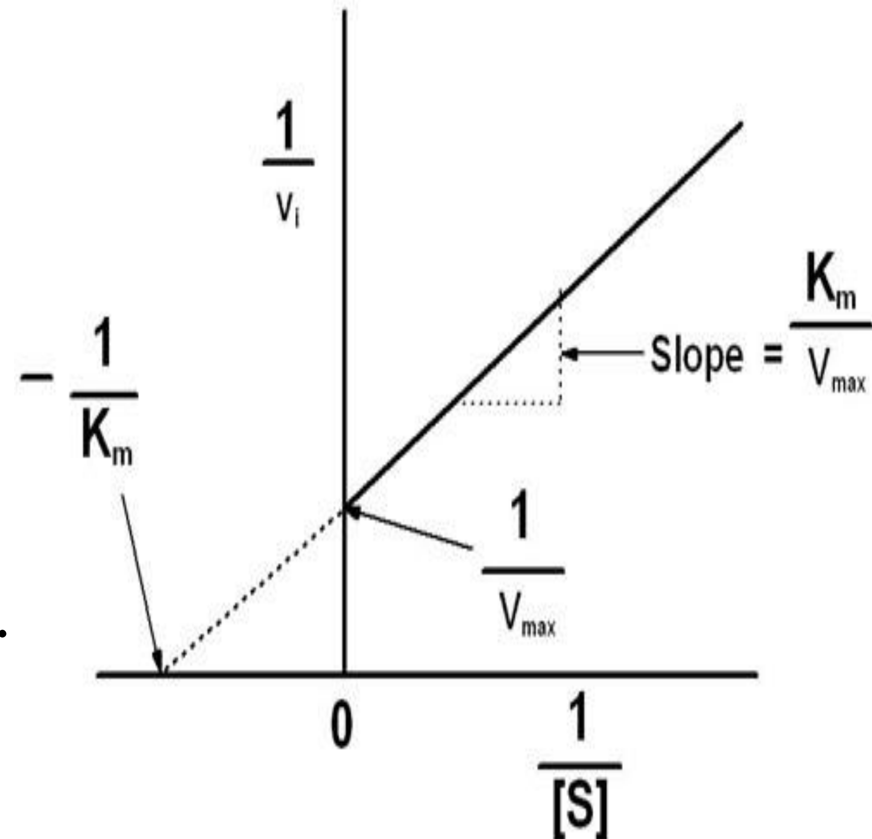
Lineweaver-Burk Plot

- A Linear Form of the Michaelis-Menten Equation is used to determine km & V max.

$v_i = \frac{V_{max}[S]}{K_m + [S]}$ Invert $\frac{1}{v_i} = \frac{K_m + [S]}{V_{max}[S]}$ factor $\frac{1}{v_i} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$ and simplify $\frac{1}{v_i} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$

Lineweaver-Burk Plot

- A plot of $1/V_i$ as *y* as a function of $1/[S]$ as *x* therefore gives a straight line whose *y* intercept is 1/ V max and whose slope is km / V max.
- Such a plot is called a double reciprocal or Lineweaver-Burk plot.



Km and its significance

-The Michaelis constant K_m is the substrate concentration at which V_i is half the maximal velocity (Vmax/2) attainable at a particular concentration of enzyme

- It is specific and constant for a given enzyme under defined conditions of time , temperature and pH

- K_m determines the affinity of an enzyme for its substrate, lesser the Km for is the affinity and vice versa, it is inversely proportionate to the affinity

- K_m value helps in determining the true substrate for the enzyme.