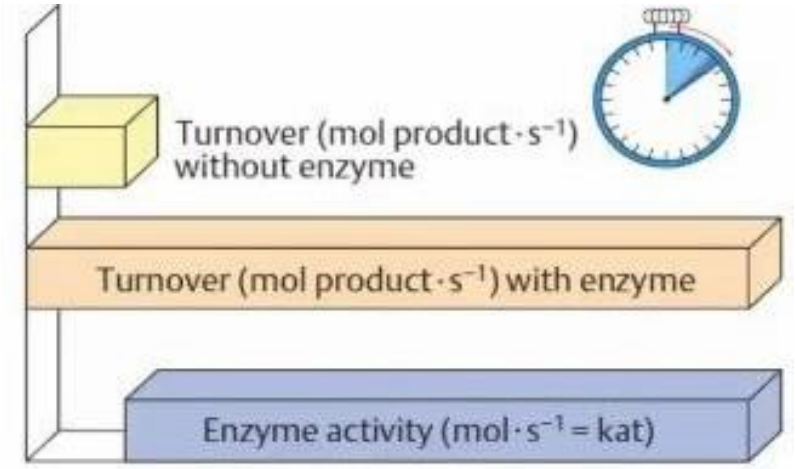
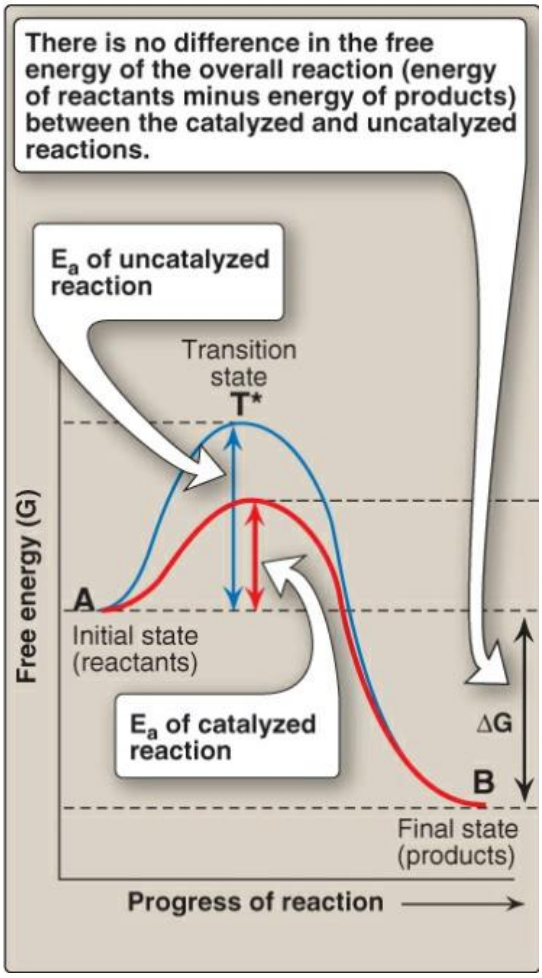
HOW ENZYMES WORK



- **Turnover (rate or velocity):** number of moles of substrate converted to product per second (mol/s.)
- **1 Katal:** amount of enzyme required to increase the turnover by 1 mol/s
- **IU:** amount of enzymes that catalyze conversion of 1µmol of substrate to product per minute



FACTORS AFFECTING REACTION VELOCITY

A. Substrate concentration

1. **Maximal velocity: The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time. Velocity is usually expressed as µmol of product formed per second. The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached (Fig. 5.6). The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.**

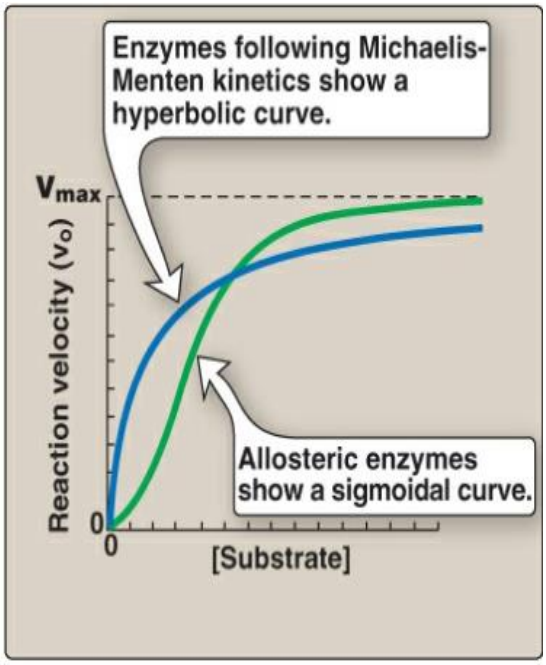
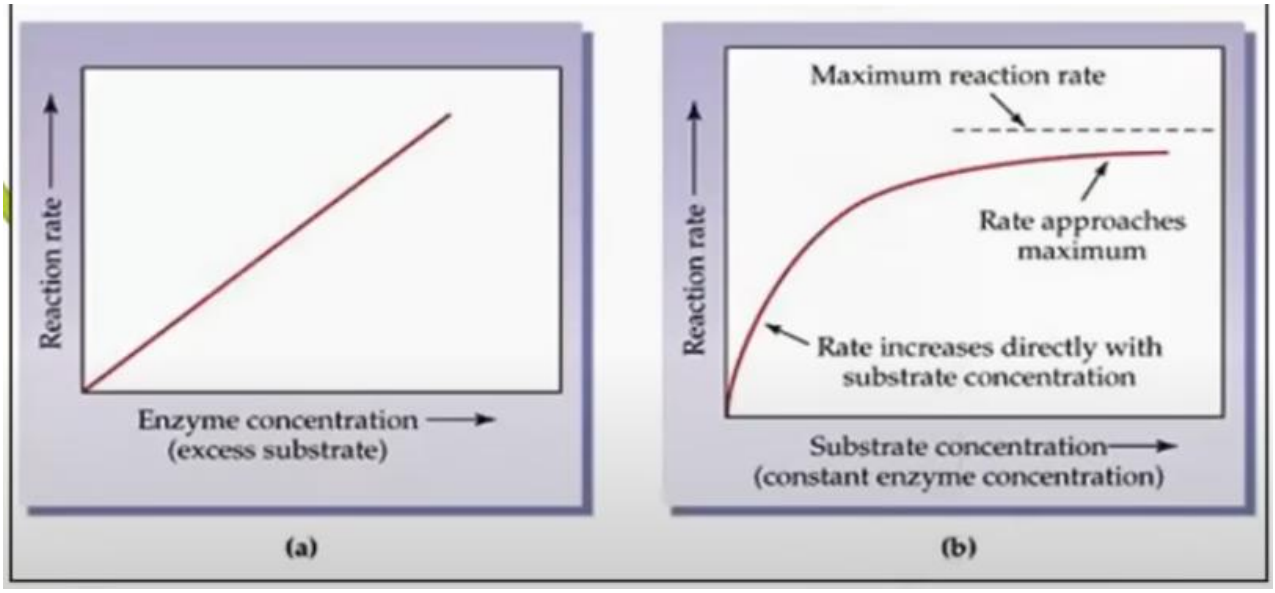


Figure 5.6
Effect of substrate concentration on reaction velocity.

2. **Shape of the enzyme kinetics curve: Most enzymes follow Michaelis–Menten kinetics , in which a plot of initial reaction velocity(vo) against substrate concentration is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin. In contrast, allosteric enzymes do not follow Michaelis–Menten kinetics and instead show a sigmoidal curve (see Fig. 5.6) that is similar in shape to the oxygen-dissociation curve of hemoglobin.**



B. Temperature

1. **Velocity increase with temperature: The reaction velocity increases with temperature until a peak velocity is reached (Fig. 5.7). This increase is the result of the increased number of substrate molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.**
2. **Velocity decrease with higher temperature: Further elevation of the temperature causes a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme (see Fig. 5.7).**

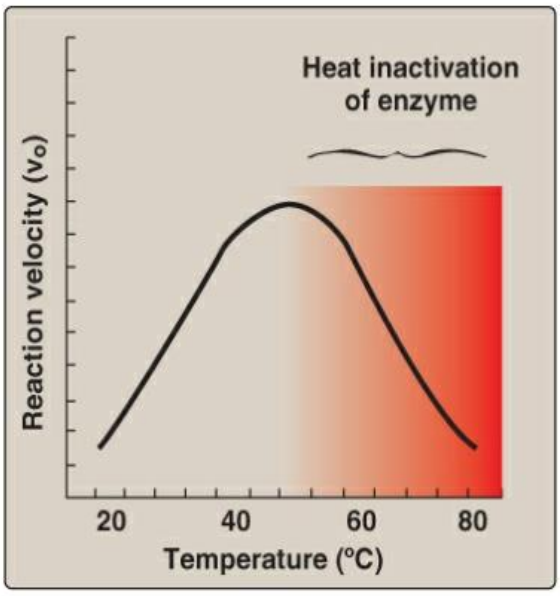


Figure 5.7
Effect of temperature on an enzyme-catalyzed reaction.

C. pH

1. **pH effect on active site ionization: The concentration of protons ([H+]) affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or unionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form (-NH_3^+). Because this group is deprotonated at alkaline pH, the rate of the reaction declines.**

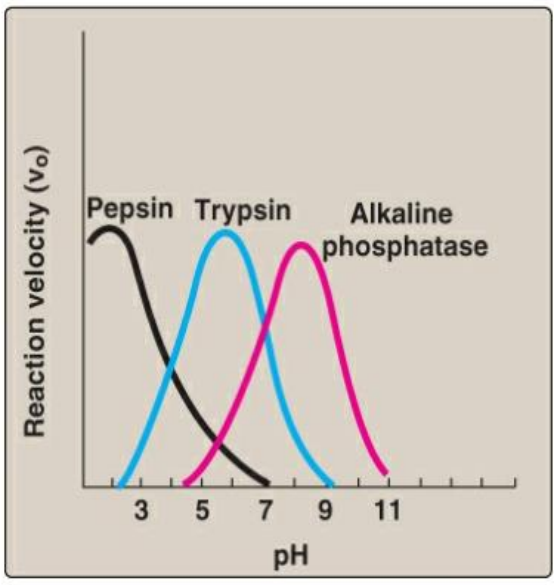


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

2. **pH effect on enzyme denaturation: Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.**
3. **Variable pH optimum: The pH at which maximal enzyme activity is achieved is different for different enzymes and often reflects the [H+] at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Fig. 5.8).**

ENZYME KINETICS:

Michaelis-Menten equation:- **The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:**

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} ES \xrightarrow{K_2} E + P$$

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

Where

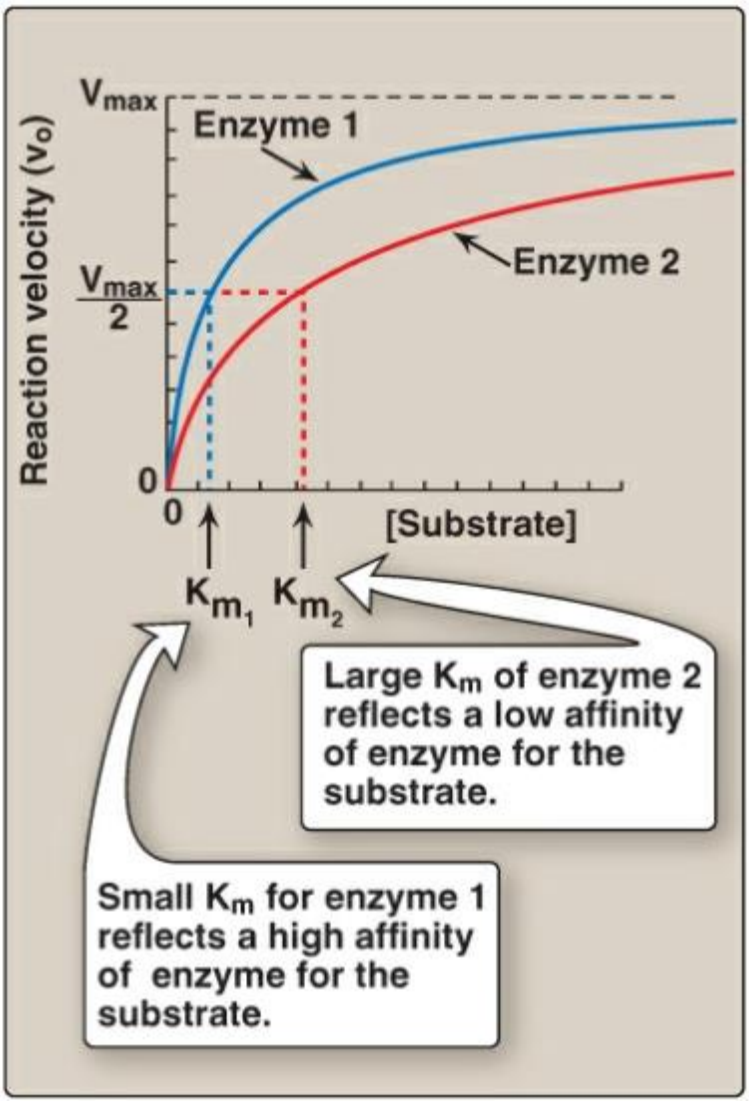
V_o = initial reaction velocity

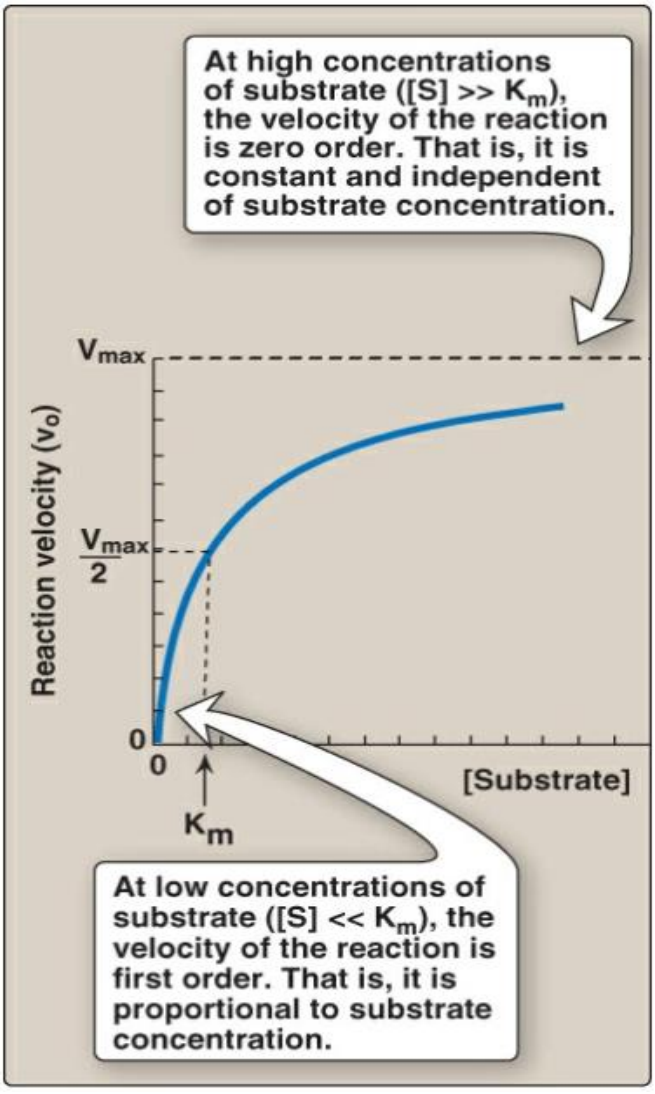
Vmax = maximal velocity

Km = Michaelis constant = $(k_{-1} + K_2)/ k_1$

[S] = substrate concentration

1. **The substrate concentration ([S]) is much greater than the concentration of enzyme so that the percentage of total substrate bound by the enzyme at any one time is small.**
2. **The concentration of the ES complex does not change with time (the steady-state assumption), that is, the rate of formation of ES is equal to that of the breakdown of ES (to E + S and to E + P). In general, an intermediate in a series of reactions is said to be in steady state when its rate of synthesis is equal to its rate of degradation.**
3. **Initial reaction velocities (vo) the rate of the reaction is measured as soon as enzyme and substrate are mixed. At that time, the concentration of product is very small, and therefore, the rate of the reverse reaction from product to substrate can be ignored.**





- ***K_m – the Michaelis constant*** **is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate.**

  - **Km is numerically equal to the substrate concentration at which the reaction velocity is equal to 1/2 Vmax.**

- **Km does not vary with the concentration of enzyme.**
- **Low Km reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme- that is, reach a velocity that is 1/2 Vmax .**
- **Large or high Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.**

Reaction order:

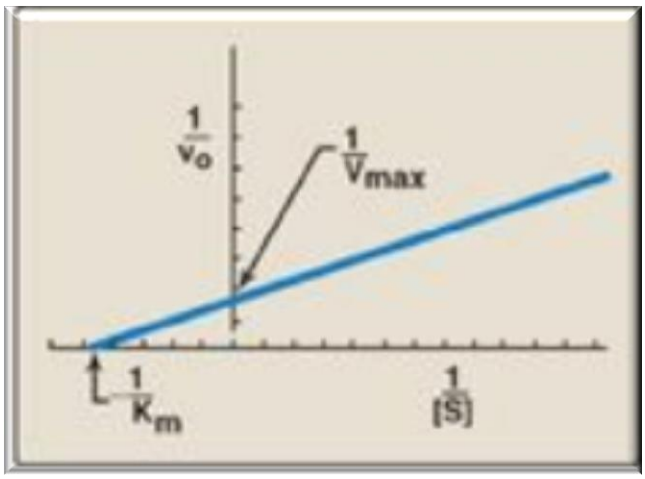
1. **When [S] is much less (<<) than Km, the velocity of the reaction is approximately proportional to the substrate concentration.(first order)**
2. **When [S] is much greater (>>) than Km, the velocity is constant and equal to Vmax. The rate of reaction is then independent of substrate concentration because the enzyme is saturated with substrate and is said to be zero order.**

- ***Lineweaver-Burke plot :-*** **When vo is plotted against [S], it is not always possible to determine when Vmax has been achieved because of the gradual upward slope of the hyperbolic curve at high substrate concentrations..**
- **if $1/v_o$ is plotted versus 1/[S], then a straight line is obtained**

The equation describing the Lineweaver-Burk plot is:-

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Where the intercept on the x axis is equal to - 1/Km, and the intercept on the y axis is equal to 1/Vmax.



Inhibition of enzyme activity

Any substance that can decrease the velocity of an enzyme-catalyzed reaction is called an inhibitor. 2 type:

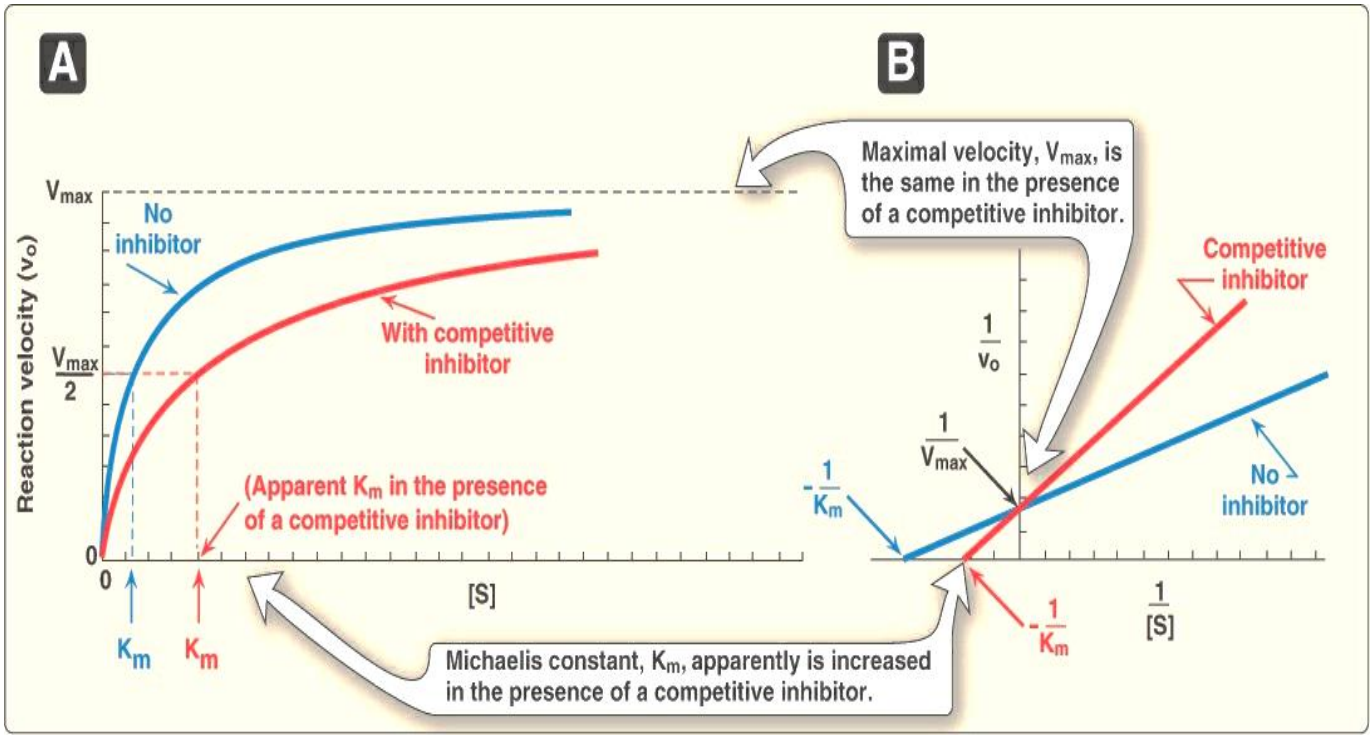
a) ***Irreversible inhibitors*** **bind to enzymes through covalent bonds.**

b) ***Reversible inhibitors*** **bind to enzymes through non covalent bonds and, thus, dilution of the enzyme–inhibitor complex results in dissociation of the reversibly bound inhibitor and recovery of enzyme activity, (Competitive and Noncompetitive).**

1. Competitive inhibition:-

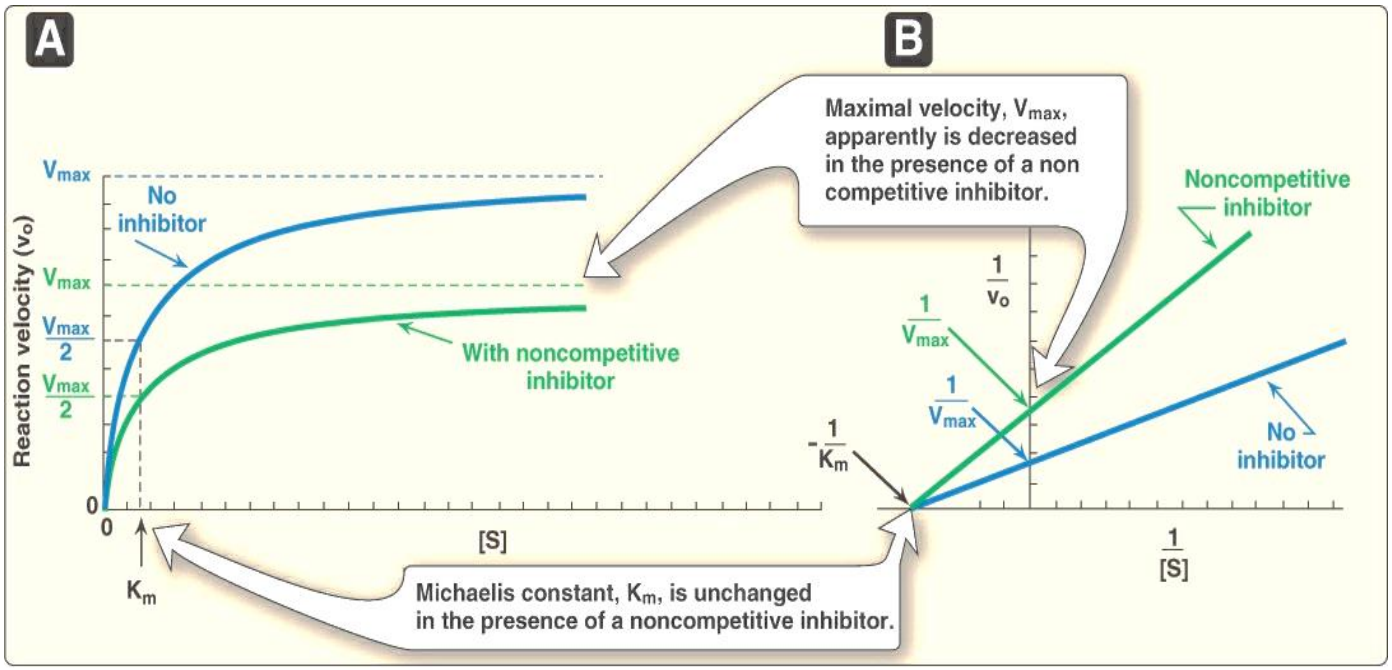
This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and therefore, inhibitor competes with the substrate for the site.e.g: Statins drugs, sulfa drugs.

- **Effect on Vmax: no change**
- **Effect on Km: increases Km (in the presence of a competitive inhibitor, more substrate is needed to achieve half Vmax).**



2. Non- competitive inhibition:- inhibitor and substrate bind at different sites on the enzyme. e.g.: heavy metals and oxidizing agents.

- **Effect on Vmax: decrease the apparent Vmax**
- **Effect on Km: no change (do not interfere with the binding of substrate to enzyme**



Regulation of enzyme activity

1. ***Allosteric Regulation (non-covalent modification):-*** **Effectors that bind non-covalently at a site other than the active site.**

a) **Homotropic effectors: substrate positively feedback the enzyme**

b) **Heterotropic effectors: product negatively feedback the enzyme**

2. ***Covalent modification:*** **phosphorylation and dephosphorylation (by the addition or removal of phosphate groups from specific serine ,threonine, or tyrosine residues of the enzyme)**

3. ***Induction and repression of enzyme synthesis:*** **cells can regulate the amount of enzyme present by altering the rate of enzyme degradation (repression) or, the rate of enzyme synthesis (induction).**

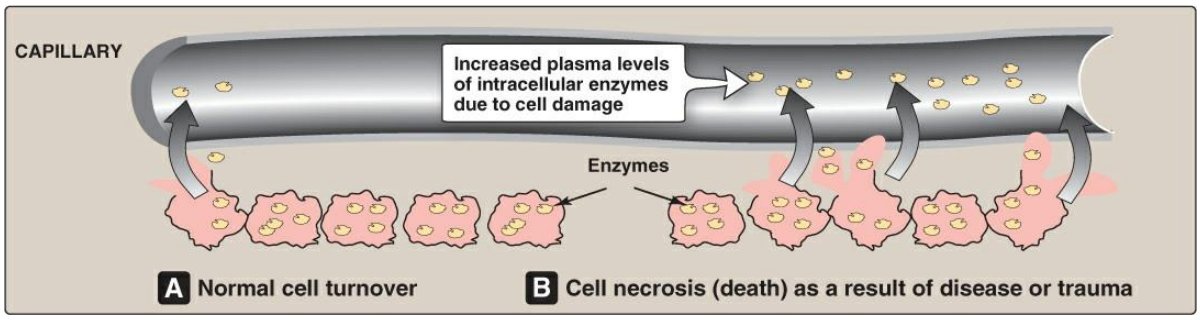
NB. 1 and 2 affect the enzyme activity (rapid) while 3 affects the amount of enzyme itself (slow)

Enzymes in clinical diagnosis

Plasma enzymes can be classified into two major groups.

1) **Functional Enzymes: they are enzymes that act on substrate normally present in plasma (coagulation enzymes, LCAT, LPL) 2)**

2) **Non-functional Enzymes: they are enzymes that act on substrate normal cell turnover e.g: ALT, AST, ALP amylase.**



- **Isoenzymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction (differ in some physical or chemical properties)**
- **Formed of two or more polypeptide chains (Differ in AA sequence).**
- **Different polypeptide chains are products of different genes.**
- **May be separable on the basis of charge (electrophoresis) or the molecular weight (ultracentrifugation).**
- **They are tissue specific**

1. **e.g: Creatine Kinase (CK) , Lactate Dehydrogenase (LDH)**

Creatine Kinase (CK)

- **Creatine Kinase is a dimer made of 2 monomers occurs in the tissues**
- **Skeletal muscle contains M subunit.**
- **Brain contains B subunit.**
- **So there are 3 different isoenzymes are formed.**

Isoenzyme	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases
CK-2	MB	Myocardium / Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle	Muscular dystrophy After surgery

Lactate Dehydrogenase (LDH)

- **LDH is a tetrameric protein and made of two types of subunits.**
- **Heart contains 2 H subunits**
- **Skeletal muscle contains 2 M subunits.**
- **So there are 5 different isoenzymes are formed**

Isoenzyme	Composition	Present in	Elevated in
LDH1 (H_4)	HHHH	Myocardium, RBCs	Myocardial Infarction
LDH2 (H_3M_1)	HHHM	Myocardium, RBCs, kidney	
LDH3 (H_2M_2)	HHMM	Brain, Lung, WBCs	
LDH4 (H_1M_3)	HMMM	Lung, Skeletal muscle	
LDH5 (M_4)	MMMM	Skeletal muscle, Liver	Skeletal muscle & liver diseases