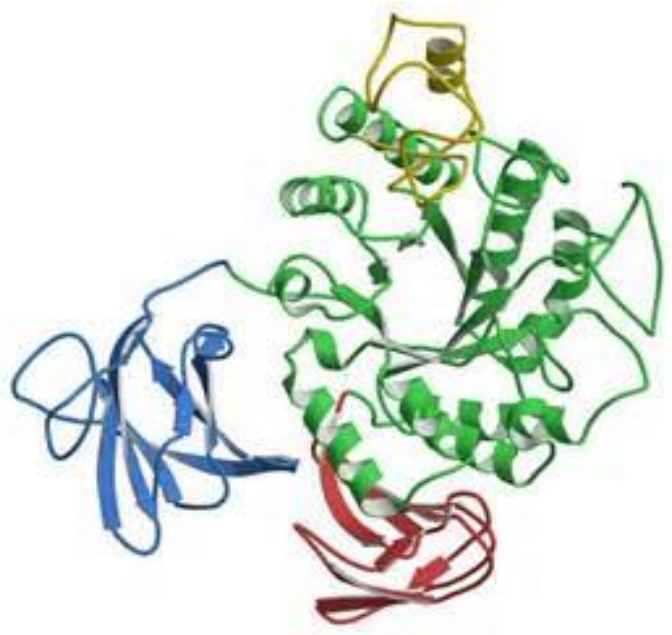
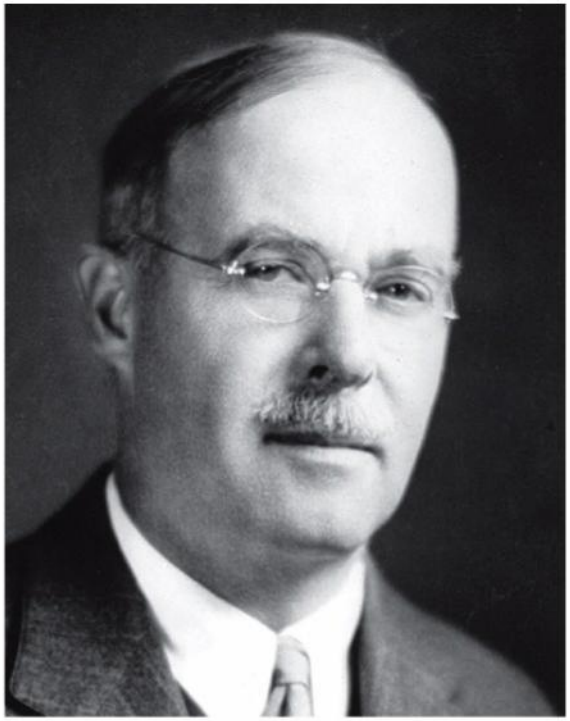
ENZYMES

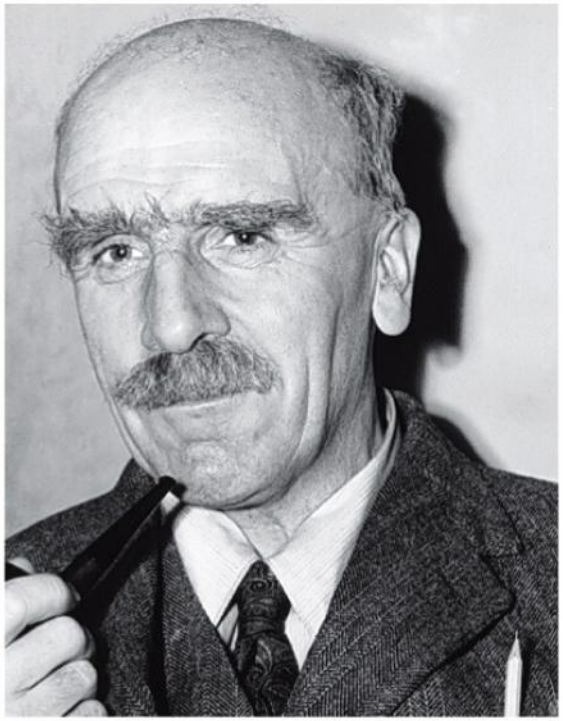


- Biomolecules synthesized by living cells
- Enzymes (Greek: en, in & Zyme, yeast)
- 1897 - Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol
- Frederick W. Kühne used word "**enzymes**"
- Isolation/crystallization of urease - James Sumner in 1926
- Sumner postulated that all E are proteins
- J. B. S. Haldane suggested - weak bonding interactions between E & S

Eduard Buchner, 1860–1917



James Sumner, 1887–1955



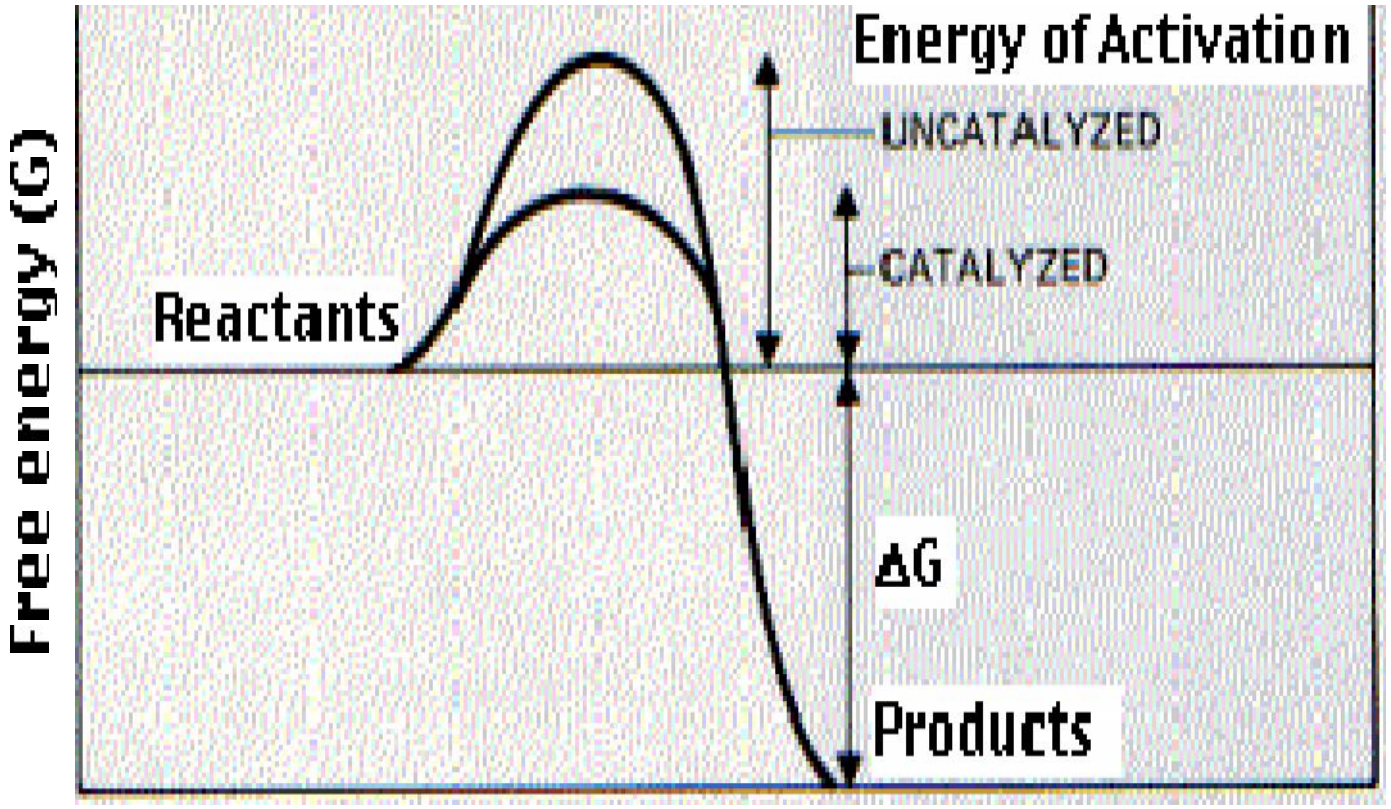
J. B. S. Haldane, 1892–1964

Unnumbered 6 p184

Lehninger Principles of Biochemistry, Fifth Edition

- E - catalyst for chemical reactions in biological system i.e. biological catalyst
- Most E protein in nature & RNA also acts as an E
- **Thomas Cech & Sidney Altman**
- RNA can act as highly specific E
- Nobel prize in **Chemistry** for **1989**
- They changed the deeply held principle of Biochemistry that **all E must be proteins.**
- Energy of activation, Substrate, catalytic site

Energy of activation



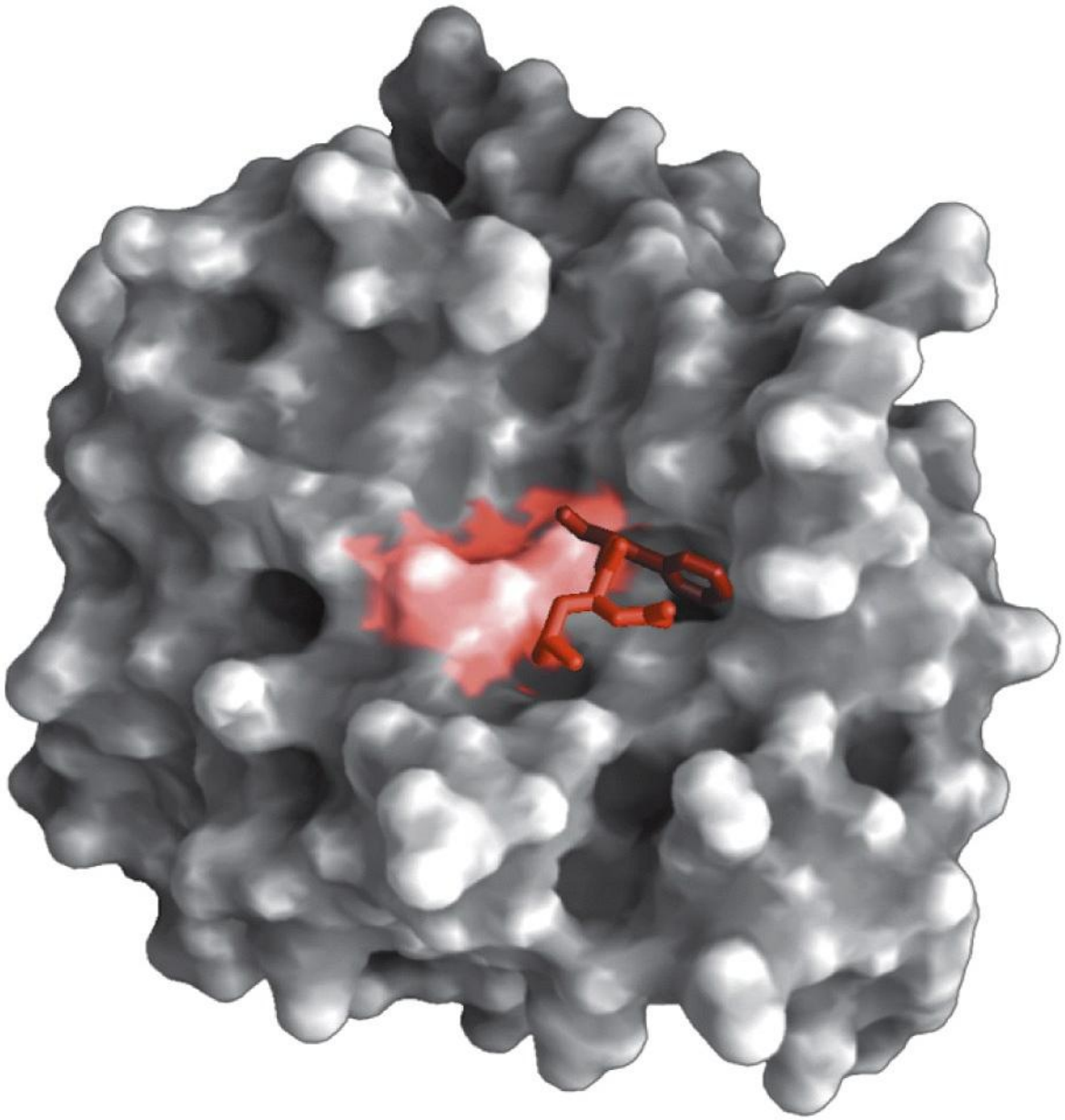


Figure 6-1
Lehninger Principles of Biochemistry, Fifth Edition

Steps in enzyme catalyzed reaction

1) Binding of substrate to E – ES complex

2) Formation of Product

- Enzyme reversibly combines with its substrate to form an ES complex that subsequently breaks down to product, regenerating the free enzyme.

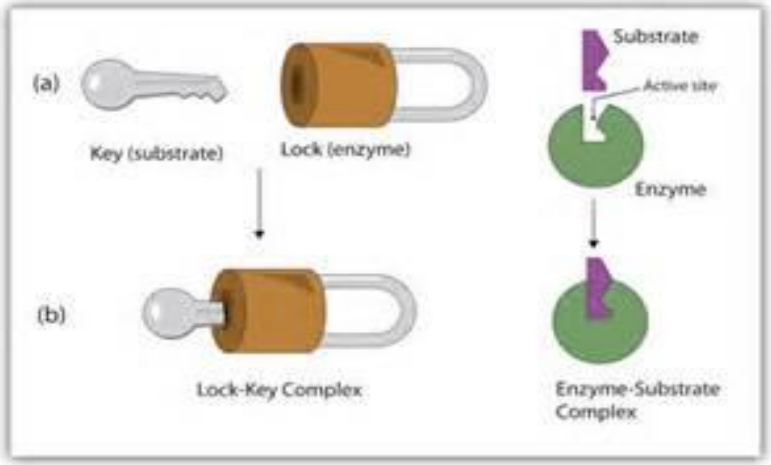
Where:

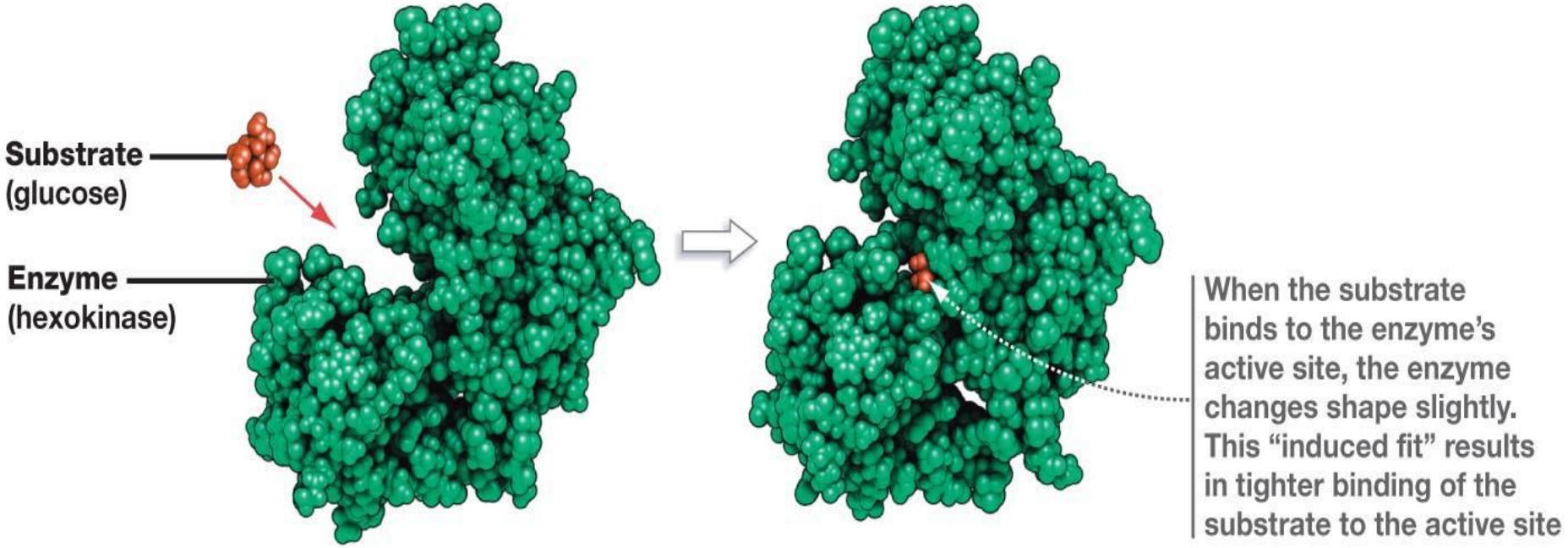
S = substrate

E = enzyme

ES = enzyme-substrate complex, P = product

1.LOCK & KEY THEORY – EMIL FISCHER, 1896
2.INDUCED FIT THEORY – DANIAL KOSHLAND, 1958





- E capable of increasing the reaction rates from 10^3 to as high as 10^{14} times faster than the uncatalyzed reactions
- In general, E capable of acting on 100 to 1,000 substrate molecules per second
- No. of substrate molecules acted upon by an E in one second is called its **turnover number**.
- **Rate or velocity of an enzyme-catalyzed reaction** is the number of substrate molecules, which are converted to product molecules per unit time; it is usually expressed as µmole product formed per minute.

Units of E activity

1. IU
2. Katal

Chemical nature of E

- Pure Protein only
- E = Protein + non-protein part

Holoenzyme = Apoenzyme + Cofactor

- Cofactor: Organic , inorganic
- Prosthetic group???

Holoenzymes

1. Apoenzyme - Protein portion
2. Cofactor – non-protein portion

Cofactor can be

- inorganic ion (ex: metal ions)
- organic molecule; called Coenzyme (ex: NAD^+ /$NADH+H^+$ nicotinamide adenine dinucleotide, FAD^+/$FADH_2$Flavin adenine dinucleotide)

Michaelis Menten equation

- Relationship between substrate & E concentration
- Proposed in 1913, Leonor Michaelis & Maud Menten
- Describes how reaction velocity varies with substrate concentration

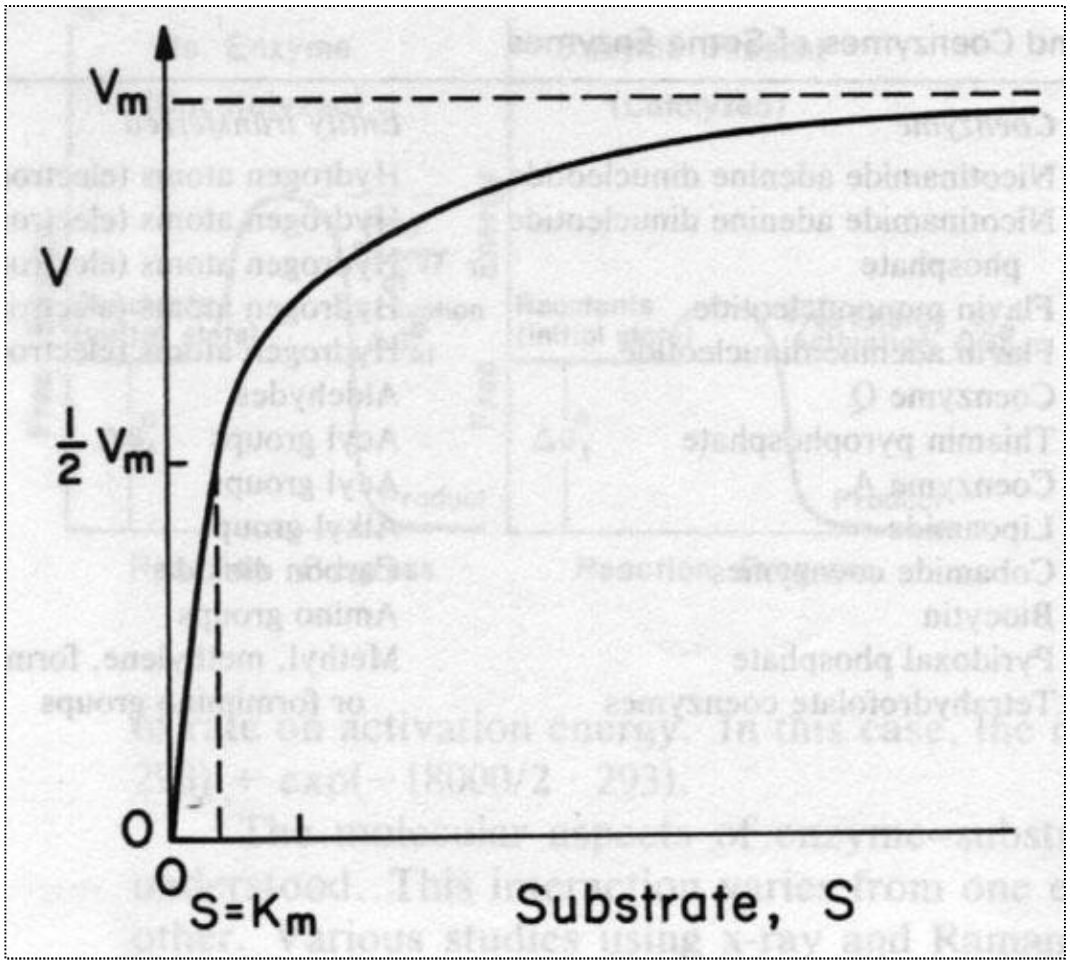
$$V_o = (V_{max}\,[S]\,) / (K_m + [S])$$

V_o = initial reaction velocity (dependent on [S])

V_{max} = maximal velocity (velocity under substrate conc.)

K_m = Michaelis constant

[S] = concentration of substrate


V_m
V
$\frac{1}{2} V_m$
O
O
$S = K_m$
Substrate, S

ENZYMES CLASSIFICATION

TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Table 6-3
Lehninger Principles of Biochemistry, Fifth Edition

FACTORS AFFECTING ENZYME ACTIVITY

1. Enzyme Concentration
2. Substrate Concentration
3. Effect of temperature
4. Effect of pH
5. Effects of products of reaction
6. Presence of cofactors
7. Presence of inhibitors

Enzyme Concentration

- Rate of reaction proportional to the amount of E (reaction of first order)
- E concentration -fall or rise depending on E synthesis &/or degradation (hormones & metabolites regulation)
- E conc. do not change in a short time but take hours to days to take place.

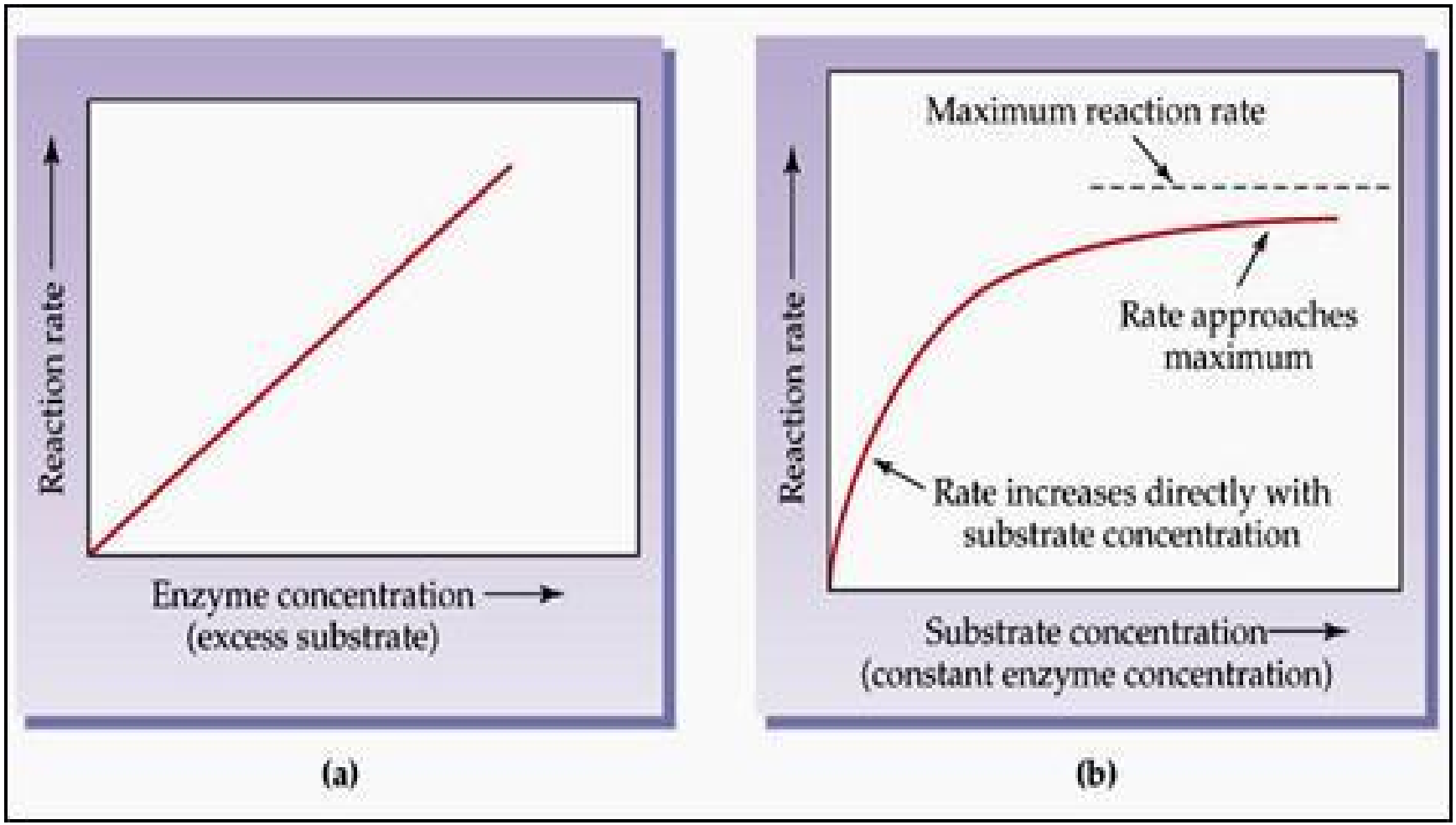
Substrate Concentration

- As S conc. increase, rate of reaction rises **(reaction of first order)**
- At very high S conc., FOR any further increase in S conc. no further increase in rate of reaction observed **(reaction of zero order/saturation effect)**

Reason for this saturation effect

At high S conc., all active sites of E present become saturated with the S

Effect of Enzyme and Substrate concentrations on Enzyme Activity



Effect of Temperature

- E reactions in man occur - **37 °C**
- Optimum temp.
- Plant enzymes - 60°C - Optimum temperature
- E reactions-chemical reactions, rate increased initially by a rise in temp.

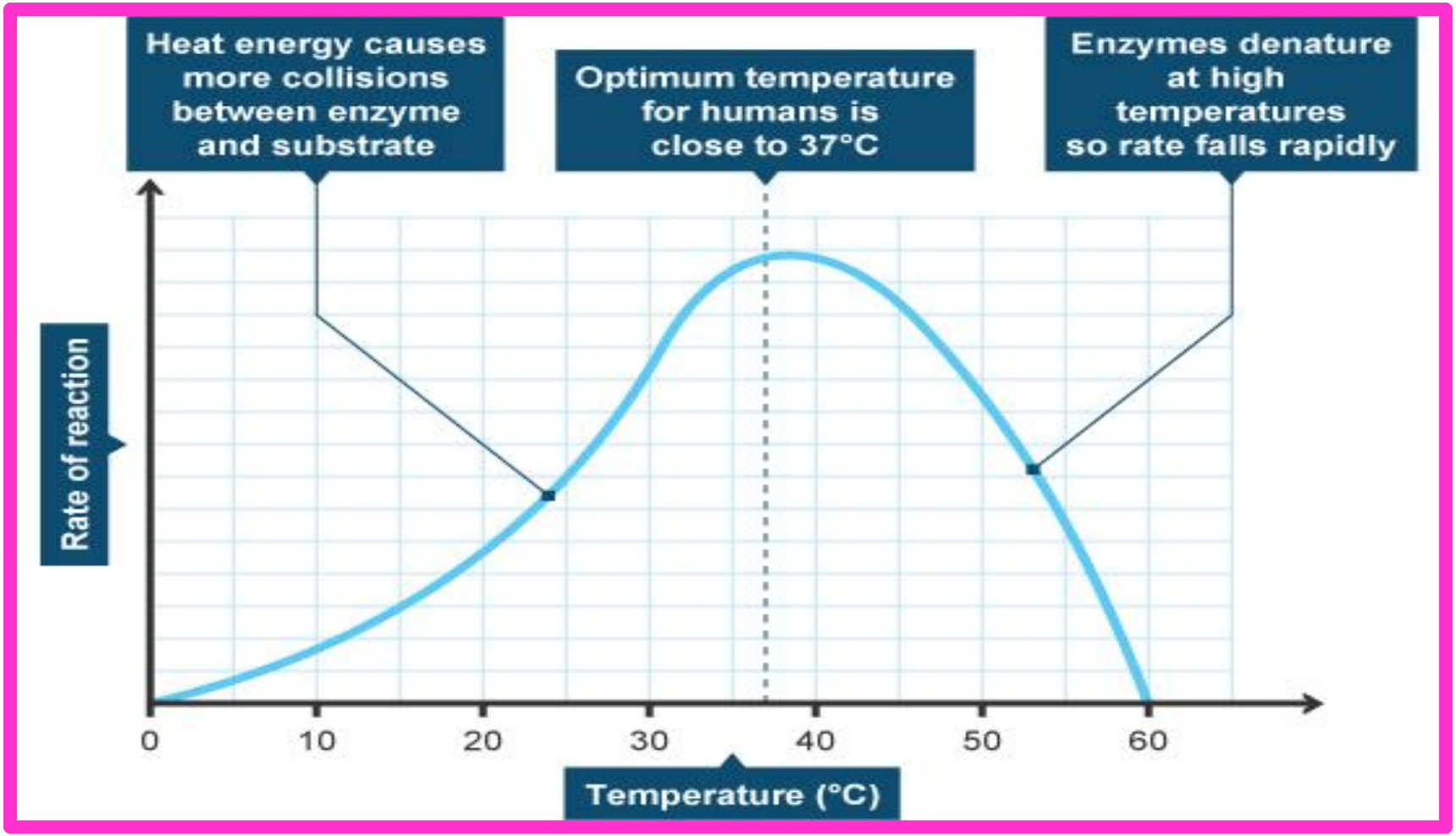
Reason:

Increase in temp. increase the thermal motion of molecules, increase fraction of molecules having sufficient internal energy to enter the transition state

- **BUT** Rise in E activity with rise in temp. limited
- High temp., the enzymes **DENATURE**

- Q_{10} - temperature coefficient
- Expression of the increase in reaction rate for a 10^{o} C rise in temperature
- Majority of E - a Q_{10} value of **3** or lower
- Q_{10} value of 3 means that a rise of 10^{o}C increases the reaction rate **3** times

Effect of Temperature



Effect of pH

Most E - optimum pH near 7

- Optimum pH of
  - pepsin = 1.5
  - salivary amylase = 6.4 – 6.9
  - trypsin = 8 – 9
- Extreme changes in pH may actually denature the enzyme

At optimum pH, important proton donating or proton accepting groups in enzyme catalytic site are in their required state of ionization

- Activity of E decrease sharply on either side of the optimum pH
- Changes in pH of the medium act by increasing or decreasing the ionized or unionized states of the E and / or the substrate

$$Enz^- + SH^+ \longrightarrow EnzSH$$

At low pH, $Enz^- + H^+ \longrightarrow EnzH$

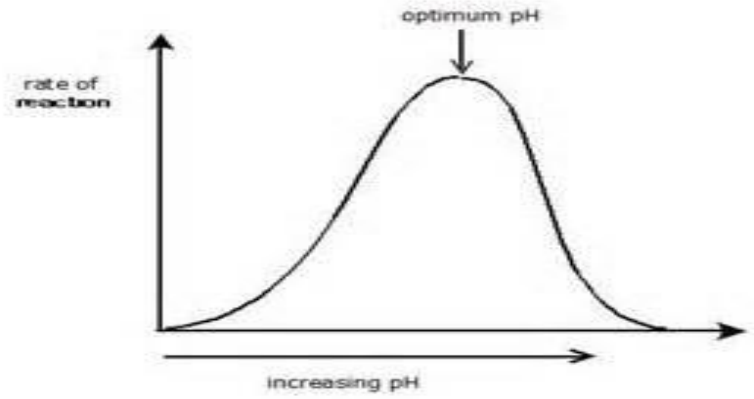
uncharged enzyme cannot form complex with SH^+

$\longrightarrow$

At high pH, $SH^+ \longrightarrow S + H^+ (H_2O)$

uncharged substrate cannot form complex with E

- pH varied - a change in the conformation of E may occur
- A charged group far from catalytic site may be necessary to maintain an active tertiary & quaternary structure
- As the charge on this group is changed, the protein may **unravel (open)** or become more compact or dissociate into subunits – all with a resulting loss of activity (**Denaturation**)



Effects of products of reaction

Suppose an enzyme catalyzes the reaction;

$$A + B \longleftrightarrow C + D$$

- If products C & D removed as fast as formed, then reaction is 100% complete
- If not removed, reaction will remain incomplete. **WHY??????????**

Two reasons:

- **Firstly,** the E reactions are usually reversible & some A & B are being re-formed
- **Secondly,** the products of reaction having some structural resemblance to the substrate will bind some enzyme, thus slowing the rate of activity

Cofactors

- Some enzymes associate with a nonprotein cofactor

- **Functions**

1. Needed in enzymatic activity
2. They tend to have specific geometries
3. Aid in positioning the groups involved in a reaction for optimum catalysis.
4. regenerated for further reaction

- Examples : metal ions or organic molecules

Role of Metal ions

1. either maintaining or producing active structural conformation (3-D shape)
2. formation of ES complex
3. making structural change in S molecule
4. accept or donate electrons

Examples

Fe^{2+} (cytochrome oxidase, catalase, peroxidase)

Cu^{2+} (cytochrome oxidase)

Zn^{2+} (Carbonic anhydrase, Alcohol dehydrogenase)

Mg^{2+} (Hexokinase, Pyruvate kinase)

Inhibitors

- Presence of certain substances called inhibitors may block or reduce the reaction rate
- They interfere with

1. the formation of ES OR
2. Breakdown of ES complex

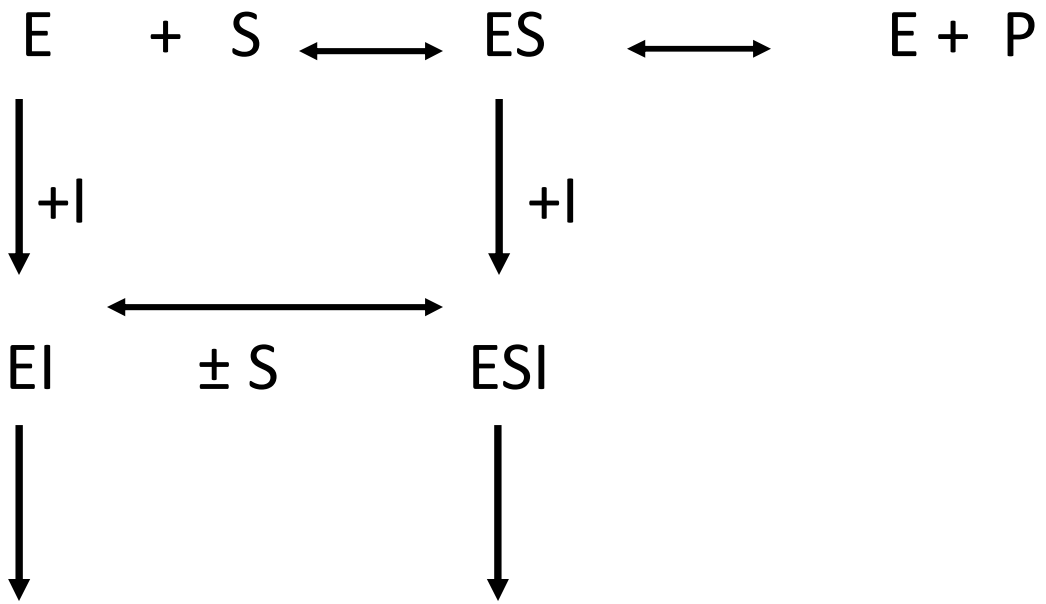
- Such phenomenon called inhibition

Enzyme inhibition - two types

1. Irreversible Inhibition
2. Reversible Inhibition

1. Irreversible Enzyme inhibition

Kinetic scheme



DEAD-END COMPLEX

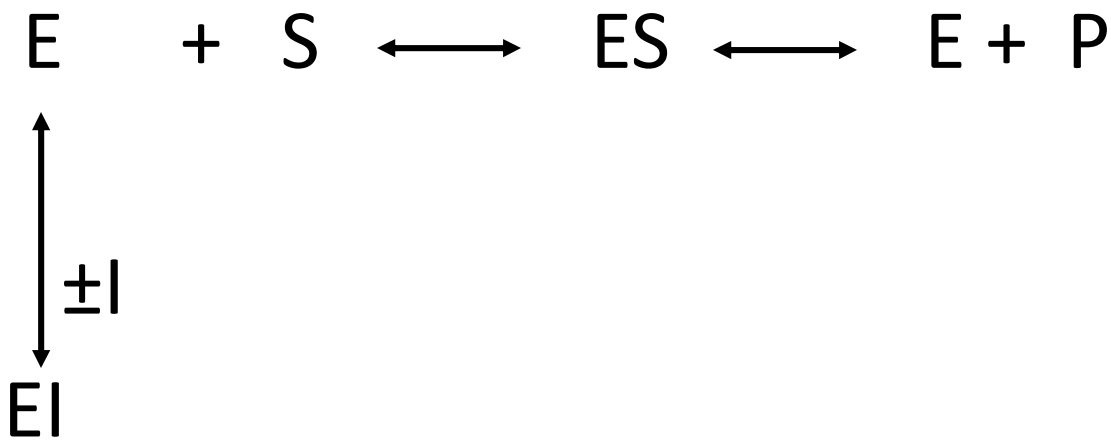
- Strong covalent bonds are produced between an E & I

2. Reversible Inhibition

Three subtypes

1. Competitive
2. Noncompetitive
3. Uncompetitive

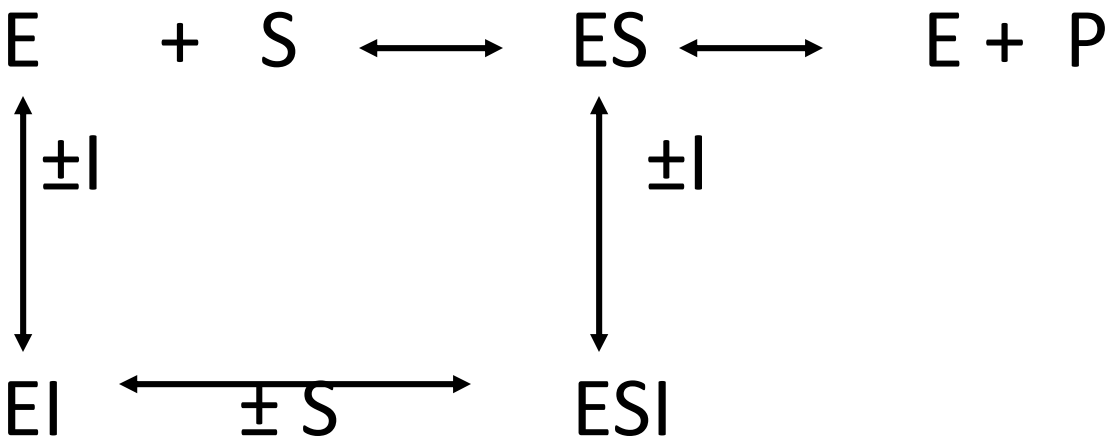
1.Competitive inhibition



Kinetic scheme

- Inhibitor (I) resemble the substrate (S)
- I compete with S to bind to the active site
- I interferes with ES formation

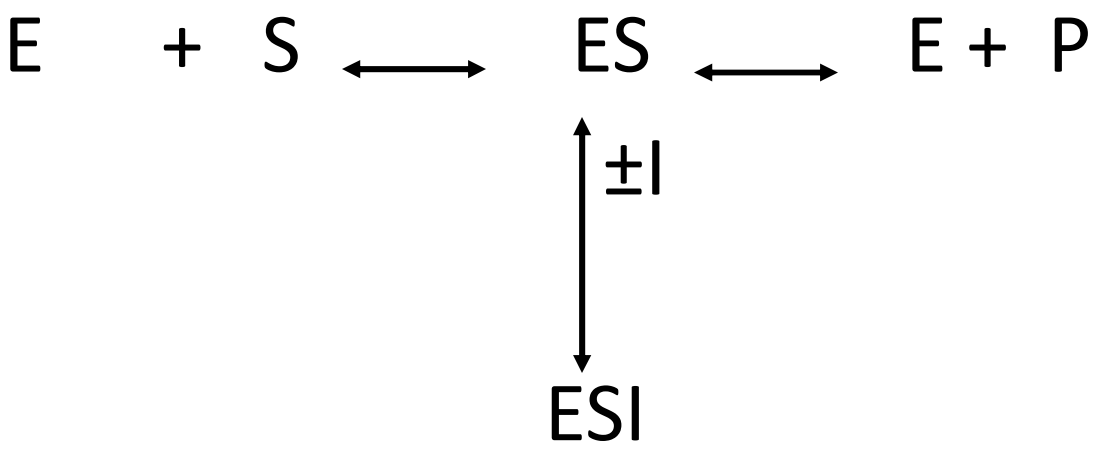
2.Non-Competitive inhibition



Kinetic scheme

- I & S bind simultaneously to E
- I bind with E at a site other than active site, involve an **allosteric site**
- I affects E 3-D structure
- I interferes with the breakdown of ES complex

3. Uncompetitive inhibition



Kinetic scheme

- I react only with the ES complex
- I affects E 3-D structure
- I interferes with breakdown of ES