ENZYME
&
CLINICAL ENZYMOLOGY

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What is true about enzyme?

A. All are protein in nature
B. They are consumed in reaction
C. Increase Activation energy
D. Increase velocity of reaction

Which of The following is Enzyme?

A. Ribozyme

B. Abzyme

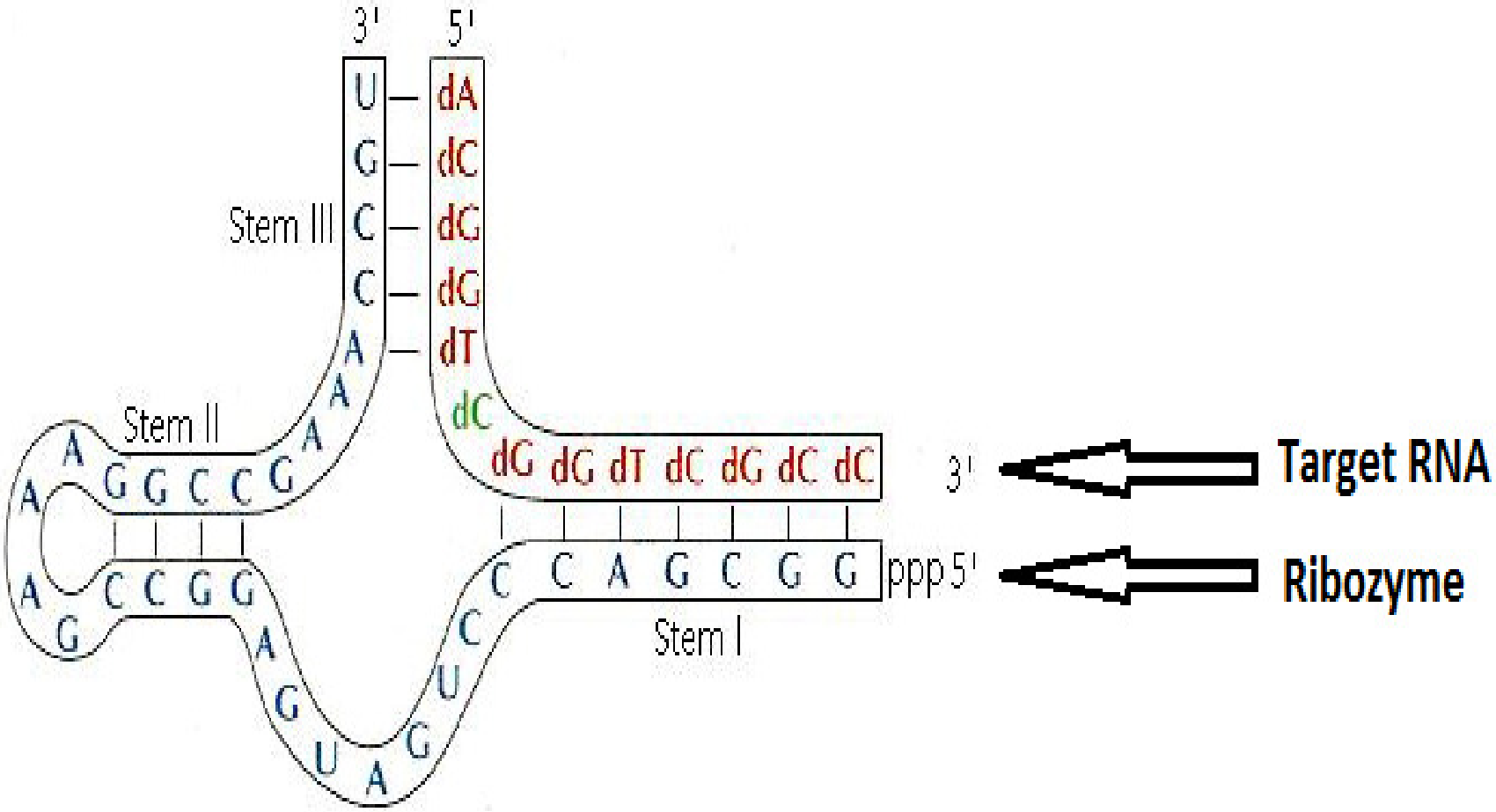
C. Thrombin

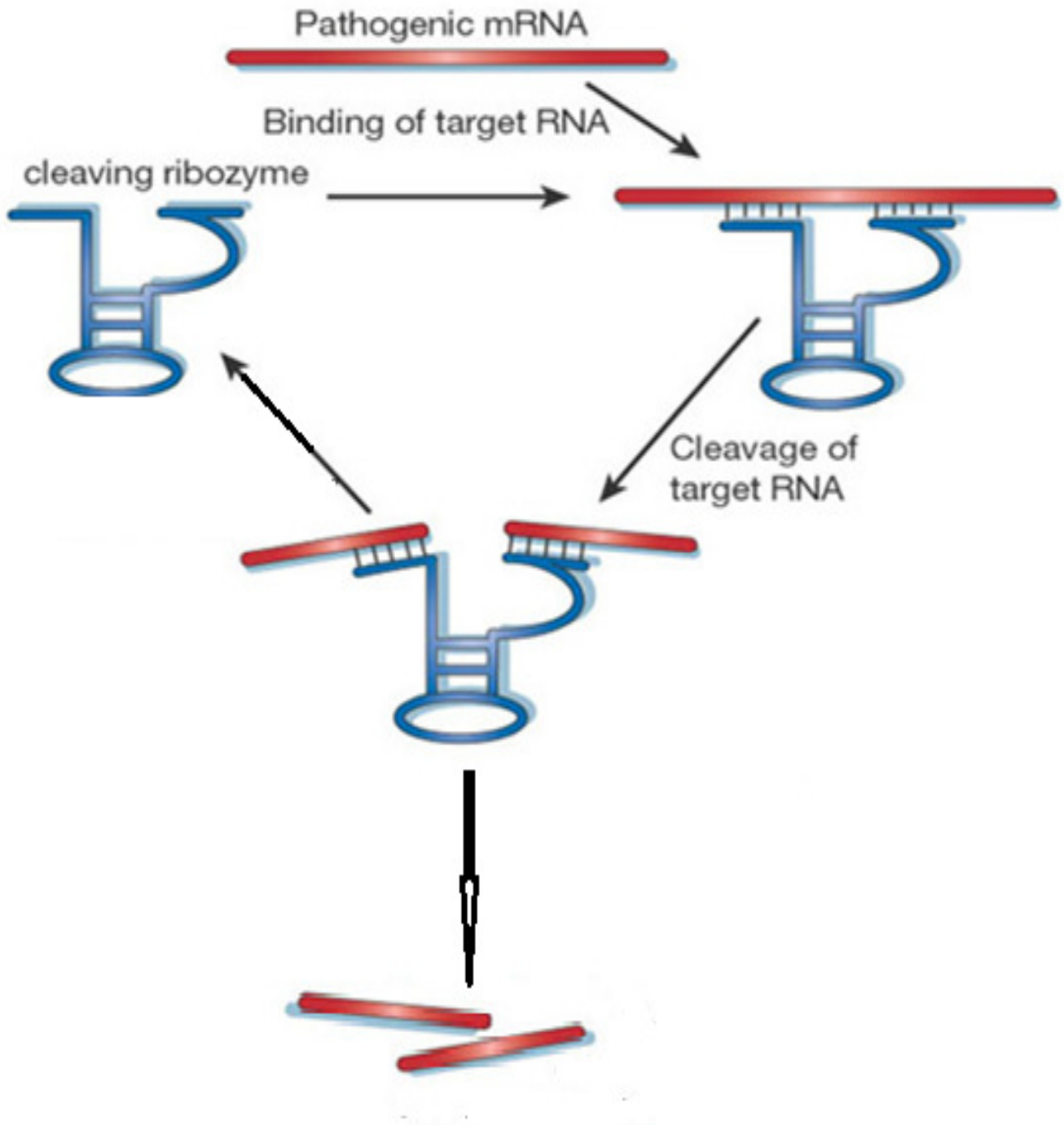
D. All of Above

Enzymes

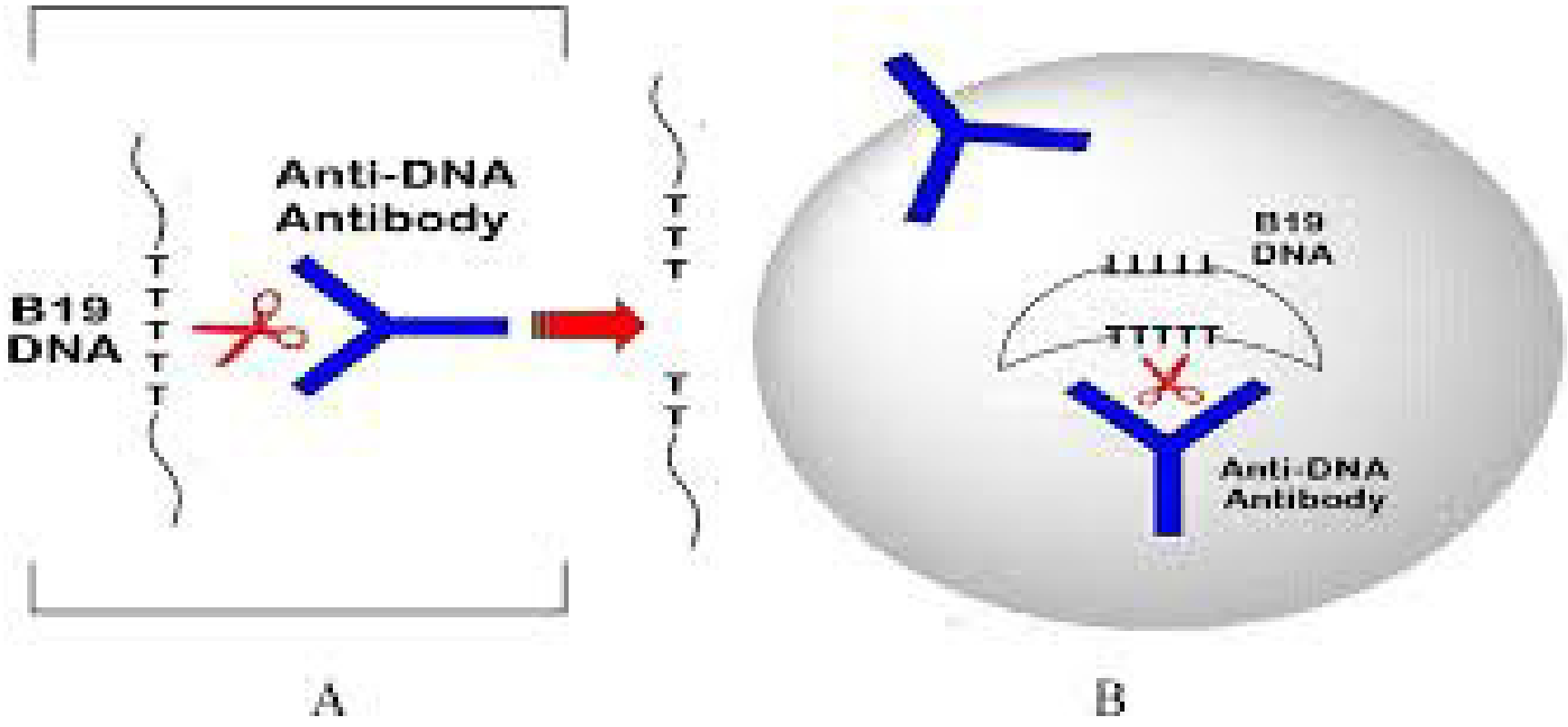
- **All Protein , Exception (Ribozyme)**
- **Increase Reaction Valocity**
- **Lowering Activation energy.**
- **Increase rates by 10^3-10^8**
- **Allow reactions to occur under much milder condition**
  - **Low Temperature**
  - **Low Atmospheric pressure**
  - **At physiological pH**

Ribozyme


3'
5'
U
G
C
C
A
dA
dC
dG
dG
dT
Stem III
Stem II
A
A
A
A
G G C C G
A
A
C C G G
G
A
G
U A
G
U
C
dC
dG dG dT dC dG dC dC
3'
Target RNA
C C A G C G G
ppp 5'
Ribozyme
Stem I


Pathogenic mRNA
Binding of target RNA
cleaving ribozyme
Cleavage of
target RNA

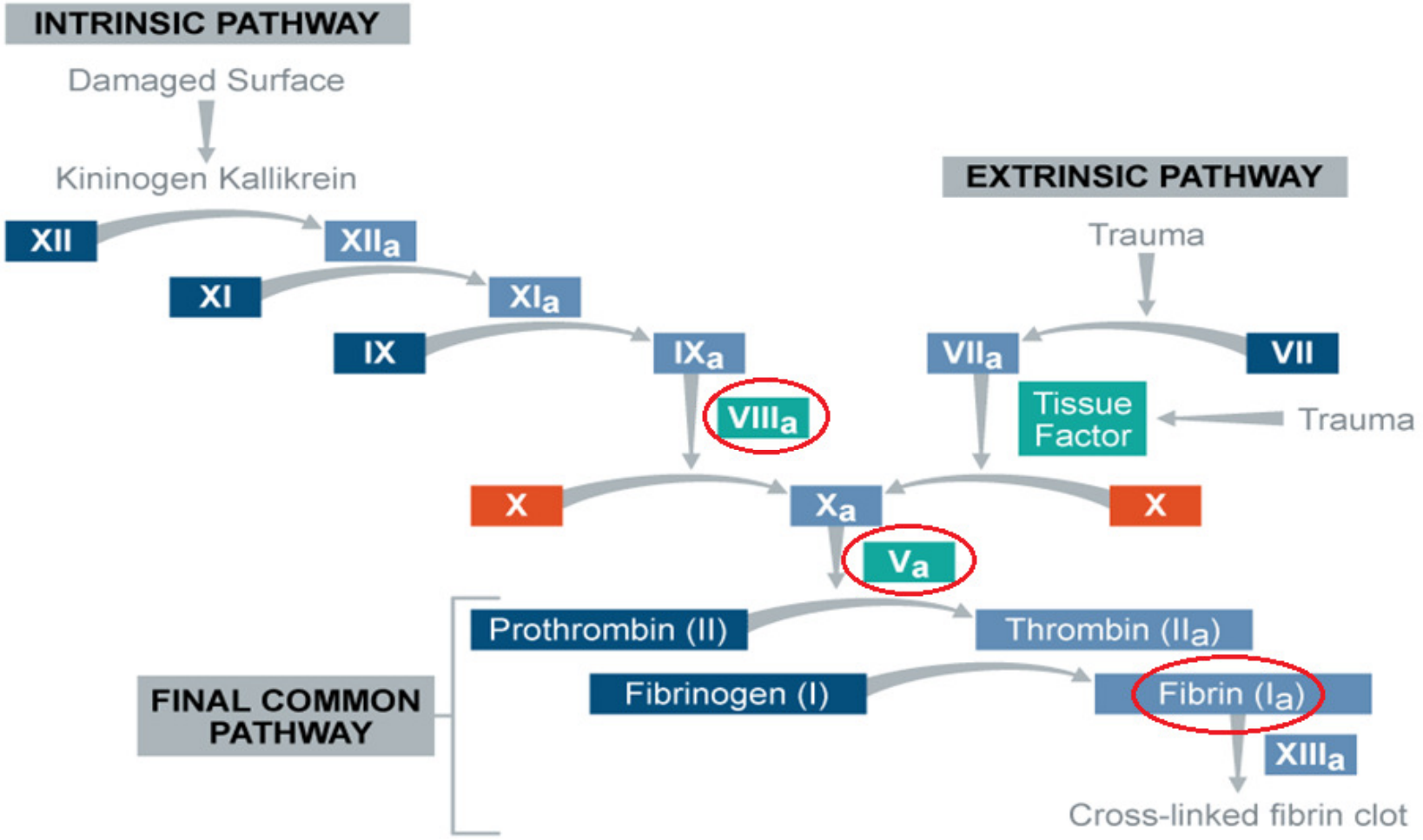
Abzyme


Anti-DNA
Antibody
B19
DNA
TTTTT
TTT
TT
B19
DNA
TTTTT
TTTTT
Anti-DNA
Antibody
A
B

- **Which of following clotting factor work as serine protease enzyme?**

A. Fibrinogen (Clotting Factor I)
B. Calcium (Clotting Factor IV)
C. Proaacelerin (Clotting Factor V)
D. Antihaemophilic factor (Clotting Factor VIII)
E. Fibrin stabilizing factor (Clotting Factor XIII)

Clotting factor - Serine Protease



All Clotting factor are " Serine Protease" enzyme

Clotting factor which is Not enzyme

1. Clotting Factor I = Fibrinogen
2. Clotting Factor IV = Calcium
3. Clotting Factor V = Proaccelerin (Labile Factor)
4. Clotting Factor VIII = Anti Haemophilic Factor

- They work like **"cofactor"**

C.F. XIII - Fibrin stabilizing factor - Transglutaminase

Which of the following is not a cofactor?

A. Thiamin Pyrophosphate

B. Pyridoxime phosphate

C. Biotin

D. Fibrinogen

Holo-enzyme means

A. Apoenzyme + Co-Enzyme
B. Apoenzyme + Co-factor
C. Apoenzyme + Metalloenzyme
D. All of Above

Holo-enzyme

Holo-enzyme = Apoenzyme + Non-protein component

- Apoenzyme = Enzyme (Protein moiety)
- Non – Protein Component
  - Co-enzyme = Organic molecule
    1. Co-Substrate (Loosely bound)
       - NADH, NADPH, FMN, FAD, Coenzyme A
    2. Prosthetic (tighly bound)
       - TPP, PLP, Biotin
  - Co-factor = Inorganic molecules
    - ✓ Metal ion e.g. Zn, Fe, Cu, Mn, Mg

Features of Co-enzymes

- Heat stable
- Low molecular weight
- After completion of r**e**action, come out from reaction.
- And participate in another reaction

Thiamine Pyrophasphate (TPP) is require as cofactor in,

A. Carboxylation
B. Decarboxylation
C. Transamination
D. Transketolase

Thiamine Pyrophasphate (TPP) is require as cofactor in,

A. Carboxylation = Biotin

B. Decarboxylation = TPP

C. Transamination = PLP

D. Transketolase = TPP

Oxidoreductase type of reaction require (as co-enzyme),EXCEPT

A. Riboflavin
B. Niacin
C. Pantothanic acid
D. Folic acid

Oxidoreductase type of reaction require (as cofactor),EXCEPT

A. Riboflavin = FAD, FMN = Oxidoreductase

B. Niacin = NAD, NADP = Oxidoreductase

C. Pantothanic acid = Coenzyme A = Acyl carrier

D. Folic acid = THF = Oxidoreductase & One Carbon carrier

Co – Enzyme , Cofactor & Prosthetic from Vitamins

✓ Thiamine	= TPP	= Decarboxylation & Transketolase
✓ Riboflavin	= FMN, FAD	= Oxida-reduction
✓ Niacin	= NAD, NADP	= Oxida-reduction
✓ Pyridoxin	= PLP	= Transamination
✓ Biotin	= Biocytin	= Carboxylation
✓ Folic acid	= THF	= Carrier of One Carbon
✓ Pantothenic acid	= Coenzyme A	= Acyl Carrier
✓ Vitamin B12	= Methylcobalamine	= Isomerization & H2 group transfer

Severe Iron deficiency can affect all of following , EXCEPT

A. Glutathione synthesis
B. Uric acid Synthesis
C. ATP synthesis
D. Collegen synthesis

Severe Iron deficiency can affect all of following , EXCEPT

A. Glutathione synthesis = Glutathione synthatase (Mg)

B. Uric acid Synthesis = Xanthine Oxidase (Fe)

C. ATP synthesis = Cytochrome Oxidase (Fe)

D. Collegen synthesis = Lysyl hydroxylase (Fe)

= Lysyl oxidasse (Cu)

Cofactor = Metalloenzyme

- Magnesium (Mg)
  - ✓ Hexokinase
  - ✓ Phosphofructokinase
  - ✓ Enolase
  - ✓ Glutathione Synthatase
- Maganese (Mn)
  - ✓ Hexokinase
  - ✓ Enolase
- Molybdenum (Mo)
  - ✓ Xanthine oxidase
  - ✓ Sulfite oxidase
- Iron (Fe)
  - ✓ Xanthine Oxidase
  - ✓ Cytochrome oxidase
  - ✓ Peroxidase
  - ✓ Catalase
  - ✓ Lysyl Hydroxylase
- Pottasium
  - ✓ Pyruvate Kinase
- Copper (Cu)
  - ✓ Cytochrome oxidase
  - ✓ Lysyl oxidase
  - ✓ Tyrosinase
  - ✓ Ceruloplasmin (Ferroxidase)
- Zinc (Zn)
  - ✓ Lactate dehydrogenase
  - ✓ Carbonic anhydrase
  - ✓ Alkaline phosphatase
  - ✓ Alcohol dehydrogenase
  - ✓ Glutamate dehydrogenase
- Selenium
  - ✓ Glutathione Peroxidase
- Nickle
  - ✓ Urease

Anaerobic Glycolysis can be inhibited due deficiency of

A. Magnesium
B. Manganese
C. Zinc
D. Potassium
E. All of Above

Anaerobic Glycolysis can be inhibited due deficiency of

A. Magnesium = HK, PFK, Enolase
B. Manganese = HK, Enolase
C. Zinc = LDH
D. Potassium = PK
E. All of Above

- **Non protein part of the enzyme is called**

A. Apo-enzyme
B. Co- enzyme
C. Holo-enzyme
D. Abzyme

Units of Enzyme

1 unit enzyme = Amount of enzyme that convert
1 micro mol of substrate per min into product

1 katal enzyme = Amount of enzyme that convert
1 mole of substrate per second into product

- **1 U = 1/60 micro katal = 16.67 nano katal.**

Velocity (Turn over) of Enzyme = V_0

Velocity of Enzyme Measure = micro mole / min

Catalase Velocity : 5 million micro mole / min

One catalase molecule convert approx. 5 million molecules of H_2O_2 into $H_2O + O_2$ per minute

Fastest to Slowest Velocity (Turnover) Enzyme

Catalase > Carbonic andydrase

> Acetylcholinesterase> Amylase

>LDH >Trypsin >Chymotrypsin

>DNA polymerase >Lysozyme

Velocity of the enzyme

A. indicate turn over of substrate to product
B. is indicated in micromole per min
C. indicate conc. of substrate required for half Vmax
D. indicate number of unit of enzyme require for substrate.

IUB (International Union of Biochemistry)

Classification of Enzyme

Enzyme Code = Four Digidts

1. **First (main class) = Type of Reaction**
2. **Second (subclasss) = Type of Group involved**
3. **Third (sub-subclasss) = denotes Substrate**
4. **Fourth = Individual enzyme name & serial number**

E.C. 1. Oxidoreductases

E.C. 2. Transferases

E.C. 3. Hydrolases

E.C. 4. Lyases

E.C. 5. Isomeraes

E.C. 6. Ligases

Which of the following enzymes is considered as NAD+ dependant Oxidoreductase ?

1. Isocitrate dehydrogenase
2. Alpha Keto Glutarate dehydrogenase
3. Succinate dehydrogenase
4. Malate dehydrogenase
5. Lactate dehydrogenase
6. Glucose 6 Phosphate dehydrogenase

A. 1 , 2, 3, 4

B. 1 , 2, 4, 5

C. 1 , 2, 5, 6

D. 1 , 2, 3, 6

Which of the following enzymes is considered as NAD+ dependant Oxidoreductase ?

1. Isocitrate dehydrogenase = NAD+
2. Alpha Keto Glutarate dehydrogenase = NAD+
3. Succinate dehydrogenase = FAD
4. Malate dehydrogenase = NAD+
5. Lactate dehydrogenase = NAD+
6. Glucose 6 Phosphate dehydrogenase = NADP+

A. 1 , 2, 3, 4

B. 1 , 2, 4, 5

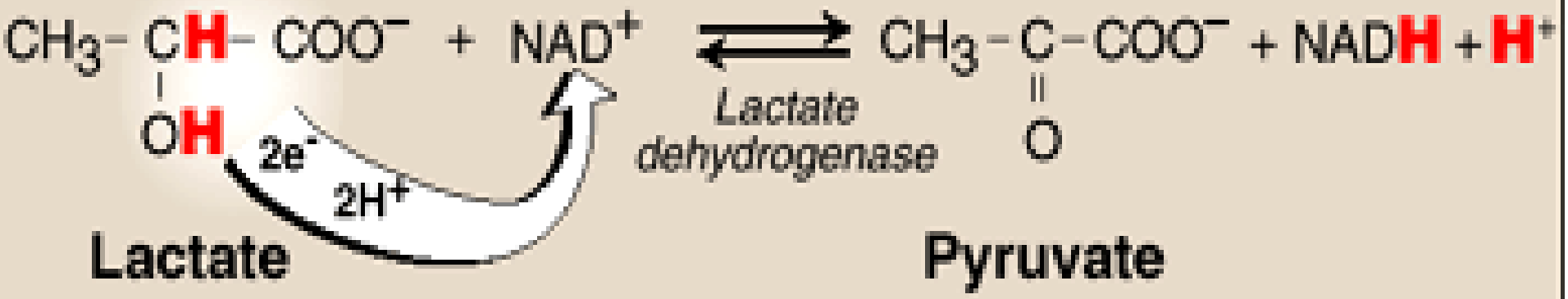
C. 1 , 2, 5, 6

D. 1 , 2, 3, 6

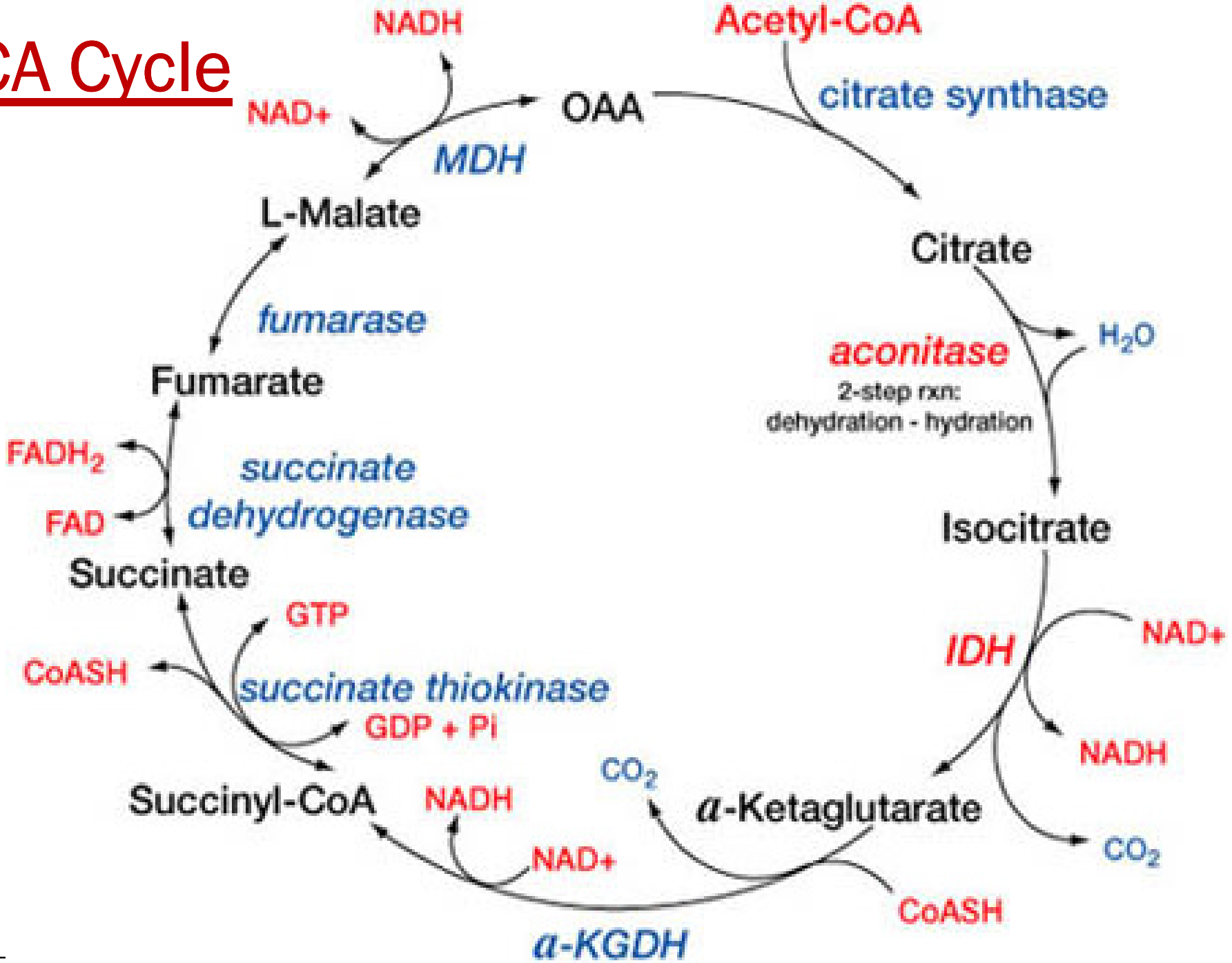
1. Oxidoreductases

- Catalyses a variety of oxidation-reduction reaction
- With help of NADH, NADPH , $FADH_2$, FMN
- Common names
  - Dehydrogenases - Oxidases
  - Peroxidases - Reductases

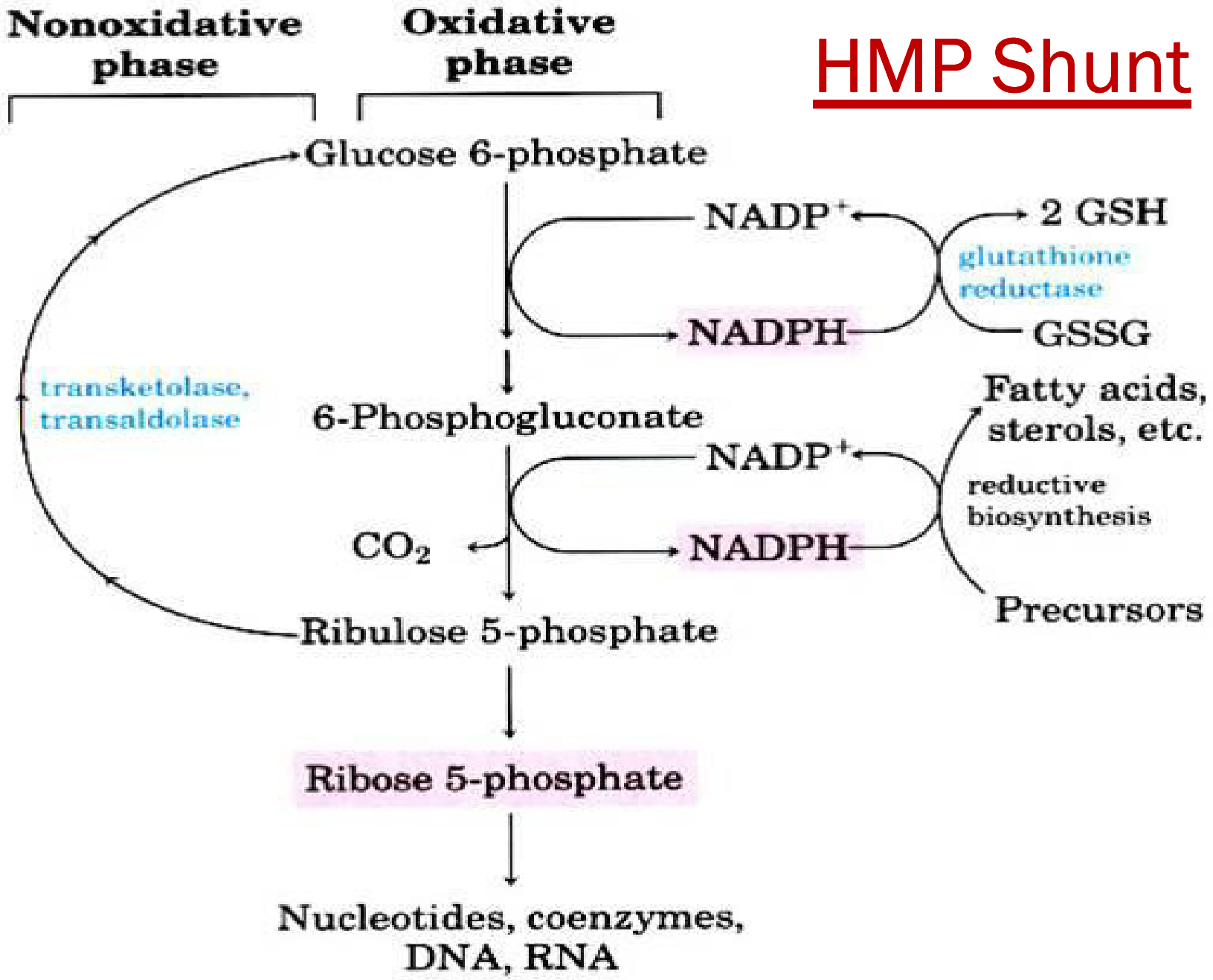
1. Oxidoreductases Catalyze oxidation-reduction reactions, such as:



TCA Cycle


NADH
Acetyl-CoA
NAD+
OAA
citrate synthase
MDH
L-Malate
Citrate
fumarase
aconitase
H_2O
2-step rxn:
dehydration - hydration
Fumarate
$FADH_2$
succinate
FAD
dehydrogenase
Isocitrate
Succinate
GTP
NAD+
IDH
CoASH
succinate thiokinase
GDP + Pi
NADH
CO_2
Succinyl-CoA
NADH
α-Ketaglutarate
CO_2
NAD+
CoASH
α-KGDH

HMP Shunt


Nonoxidative phase
Oxidative phase
Glucose 6-phosphate
NADP+
2 GSH
glutathione reductase
NADPH
GSSG
transketolase, transaldolase
6-Phosphogluconate
Fatty acids, sterols, etc.
NADP+
reductive biosynthesis
CO2
NADPH
Precursors
Ribulose 5-phosphate
Ribose 5-phosphate
Nucleotides, coenzymes, DNA, RNA

Oxidoreductase

1. **NAD+ dependent**
    1. Pyrvate dehydrogenase
    2. Isocitrate dehydrogenase
    3. Alpha Ketoglutarate dehydrogenase
    4. Malate dehydrogenase
2. **NADP+ dependent**
    1. Glucose 6 Phsophate dehydrogenase
    2. 6 Phosphogluconate dehydrogenase
3. **FAD+ dependent**
    1. Succinate dehydrogenase

Oxidoreductase

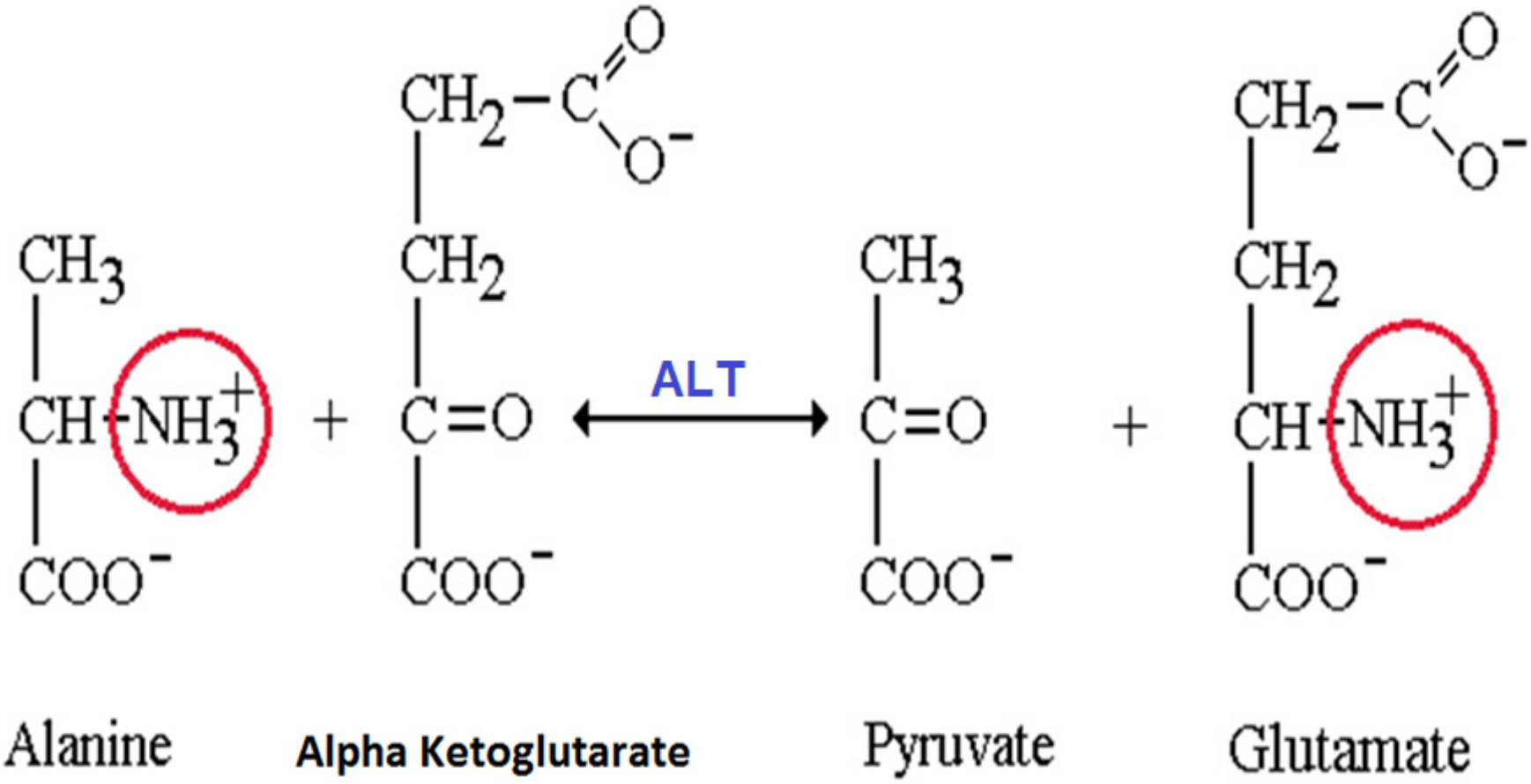
4. **Other**
    1. Xanthine oxidase
    2. Tyrosinase
    3. Phenylanaline hydroxylase
    4. Homogentisate oxidase
    5. Peroxidase
    6. Catalase

2. Transferases

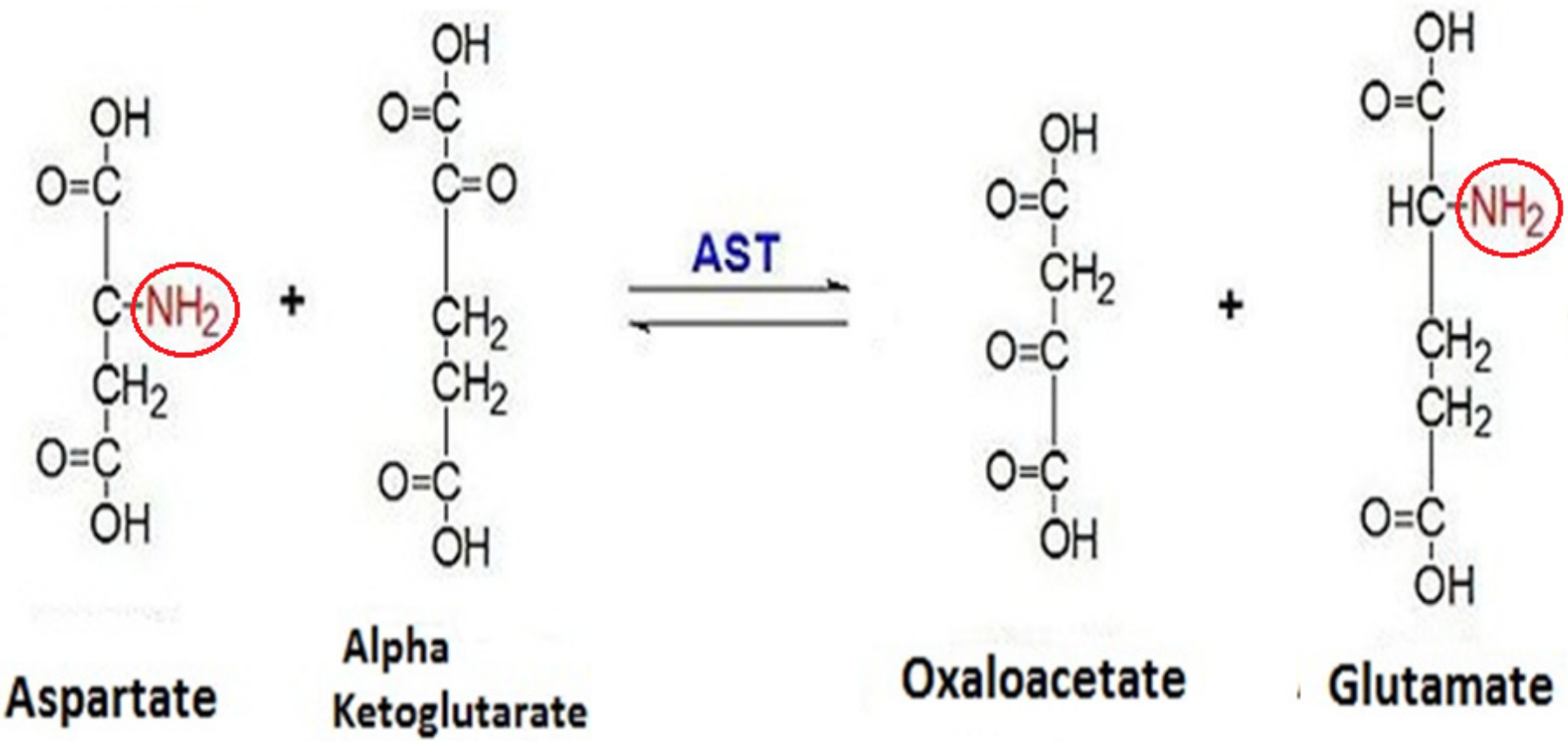
- **Transfer of**
  - **Amino**
  - **Carboxyl**
  - **Phosphoryl**
  - **Methyl**
  - **Acyl**
  - **Glycosyl**
- **Kinase transfer Phosphate group**

- **Example**
  - **GPT (ALT)**
  - **GOT (AST)**
  - **Hexokinase**
  - **Glucokinase**
  - **Pyruvate Kinase**
  - **Transketolase**
  - **Transaldolase**
  - **Transcarboxylase.**

Alanine Amino-transferase
Alanine Transaminase (ALT)
Glutamate Pyruvate Transaminase (GPT)



Aspartate Amino-transferase
Aspartate Transaminase (AST)
Glutamate Oxaloacetate Transaminase (GOT)

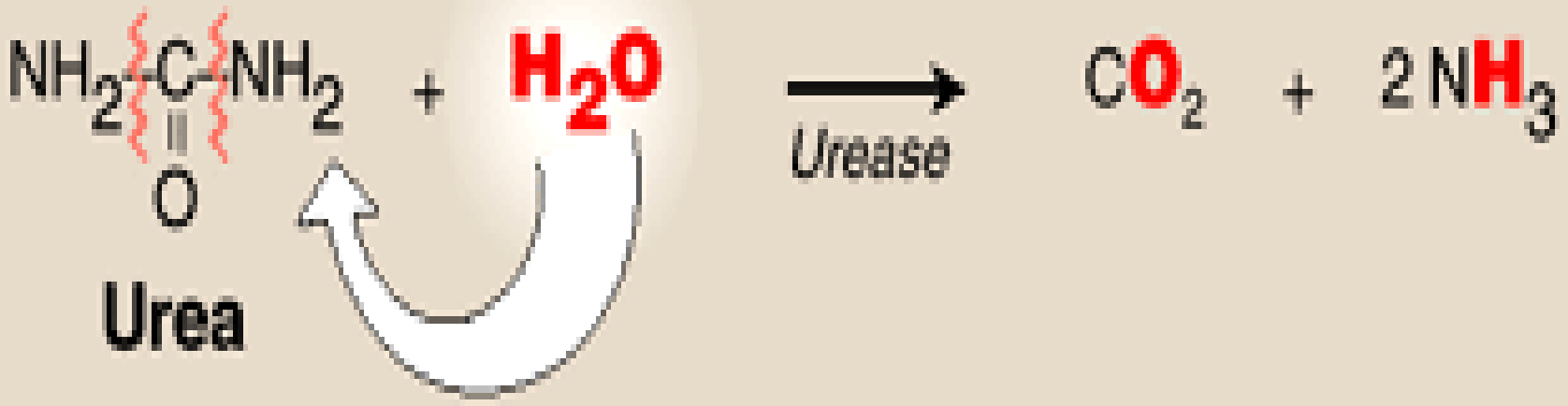


3. Hydrolases

- **Cleavage of C-C, C-O, C-N & other Covalant bonds**
- **By *addition of water*.**
- **Example = All Digestive Enzyme**
  - Protease (Trypsin, Chymotrypsin,Pepsin ,Collagenase)
  - Esterase
  - Amylase
  - Lipase
  - Phosphatase
  - Urease
  - Arginase
- ***Amylase , Lipase, Protease, Cellulase = Present in Detergent***

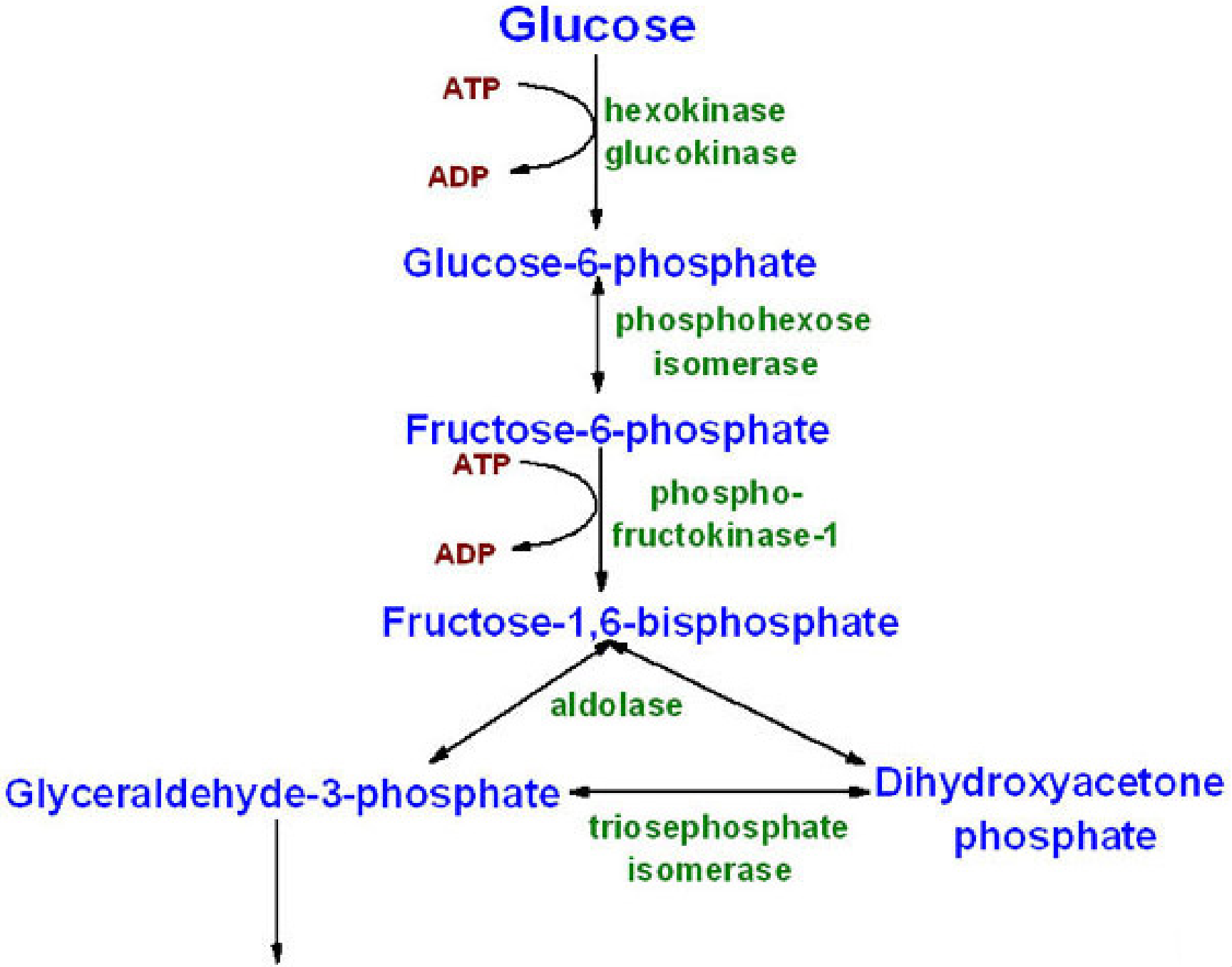
3. Hydrolases

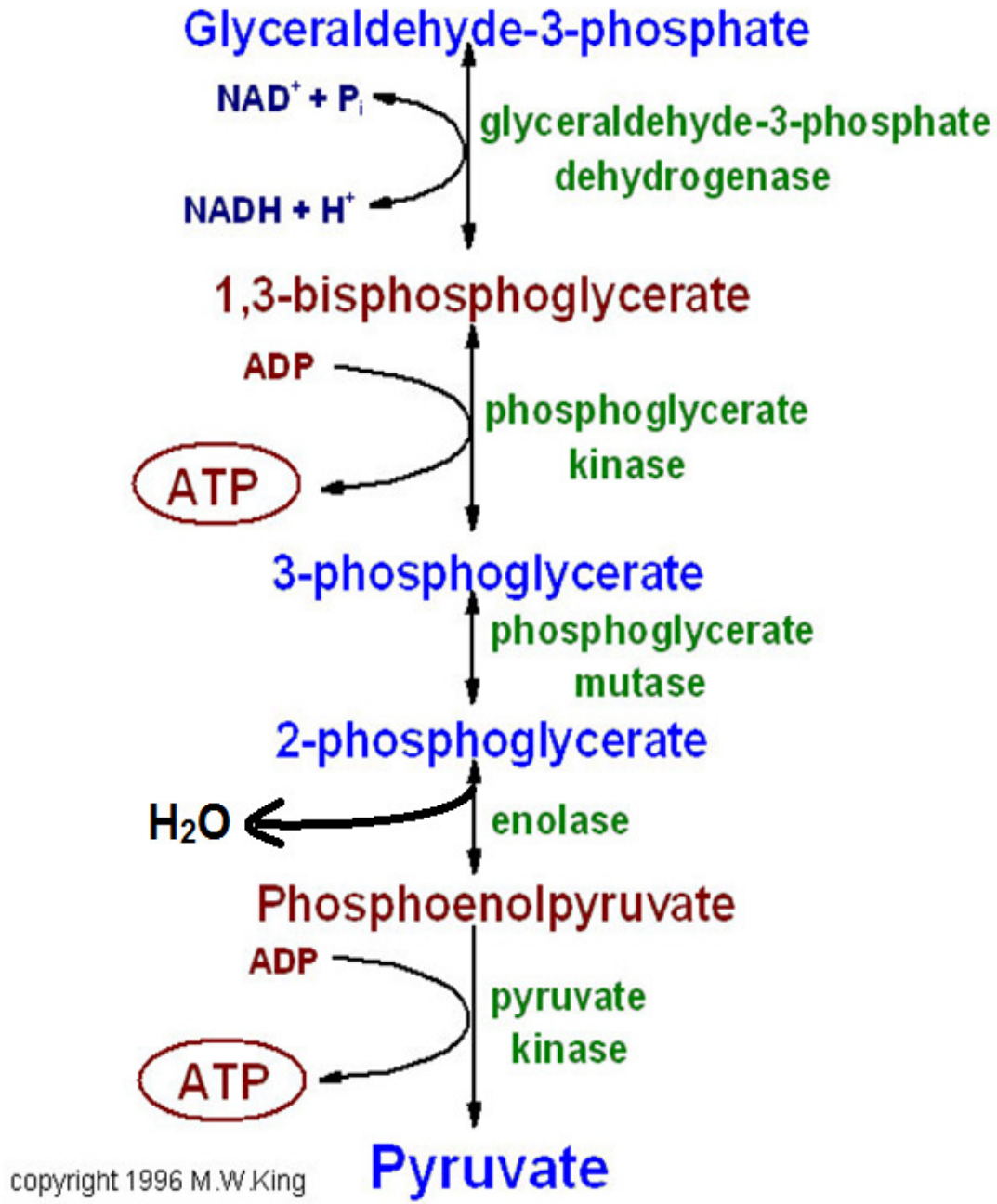
Catalyze cleavage of bonds by addition of water, such as:

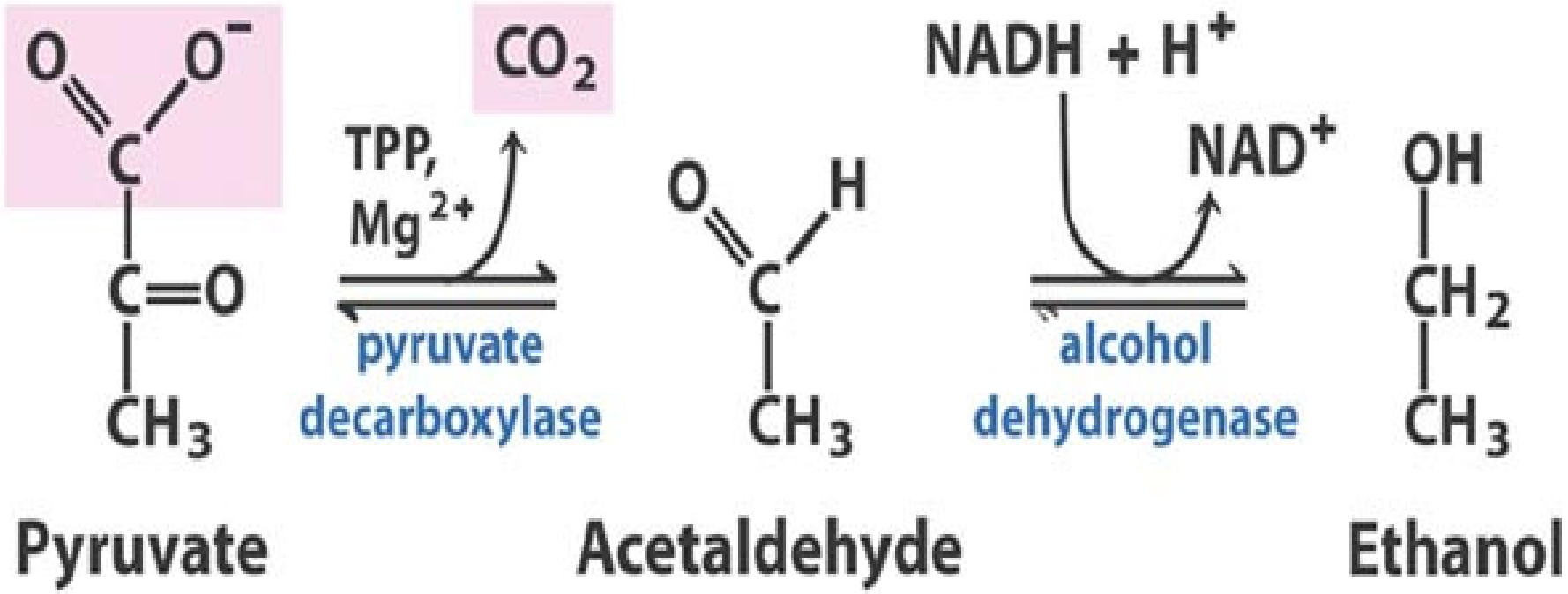


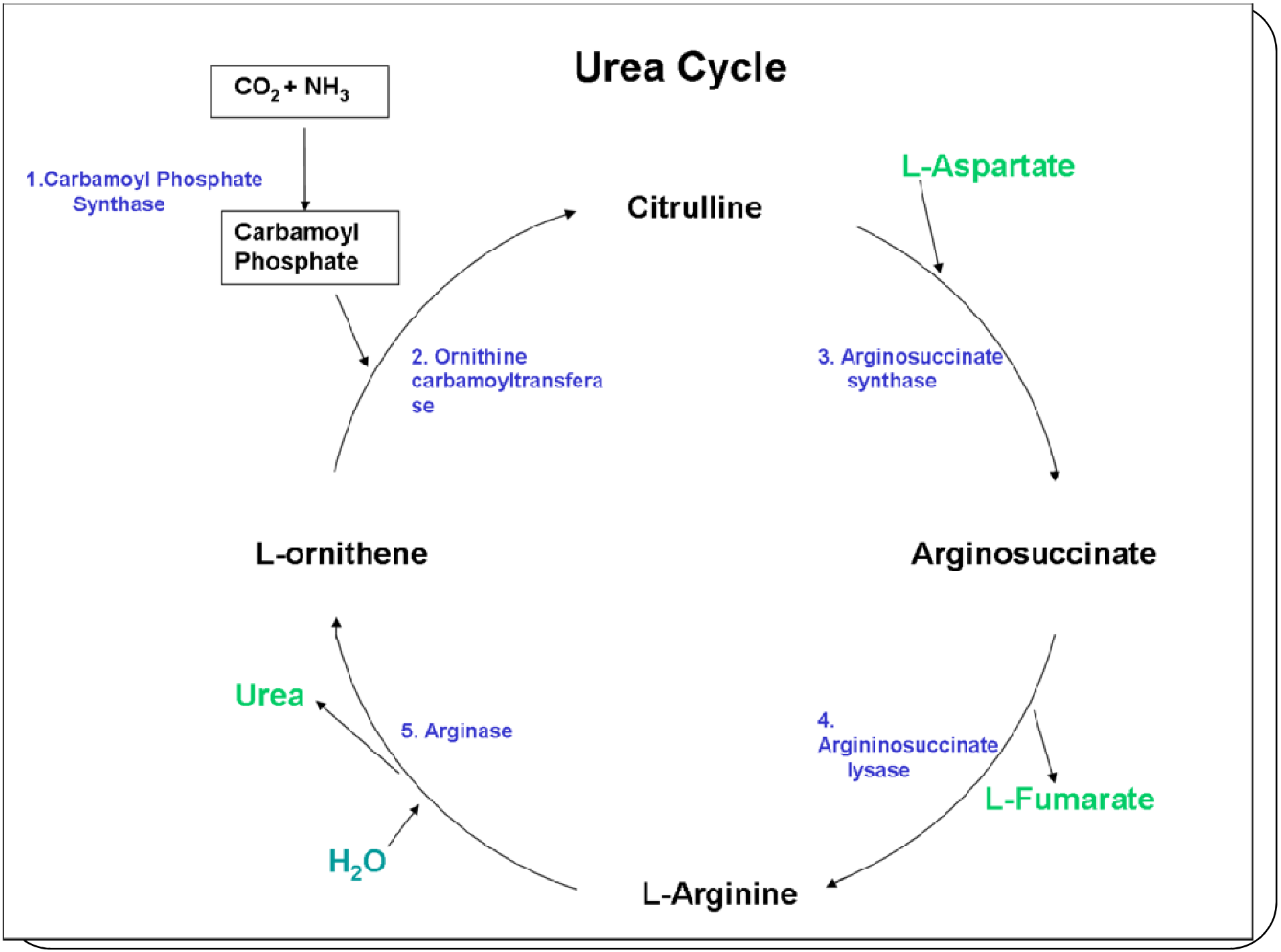
4. Lyases

- **Cleavage of C-C, C-O, C-N & other Covalant bonds**
- **By atomic elimination and Generating double bond.**
- **Without adding water**
- **Example**
  - **Aldolase**
  - **Enolase**
  - **Fumarase**
  - **Arginosuccinase**
  - **Pyruvate decarboxylaes**
  - **HMG CoA lyase**


Glucose
ATP
ADP
hexokinase
glucokinase
Glucose-6-phosphate
phosphohexose
isomerase
Fructose-6-phosphate
ATP
ADP
phospho-
fructokinase-1
Fructose-1,6-bisphosphate
aldolase
Glyceraldehyde-3-phosphate
triosephosphate
isomerase
Dihydroxyacetone
phosphate

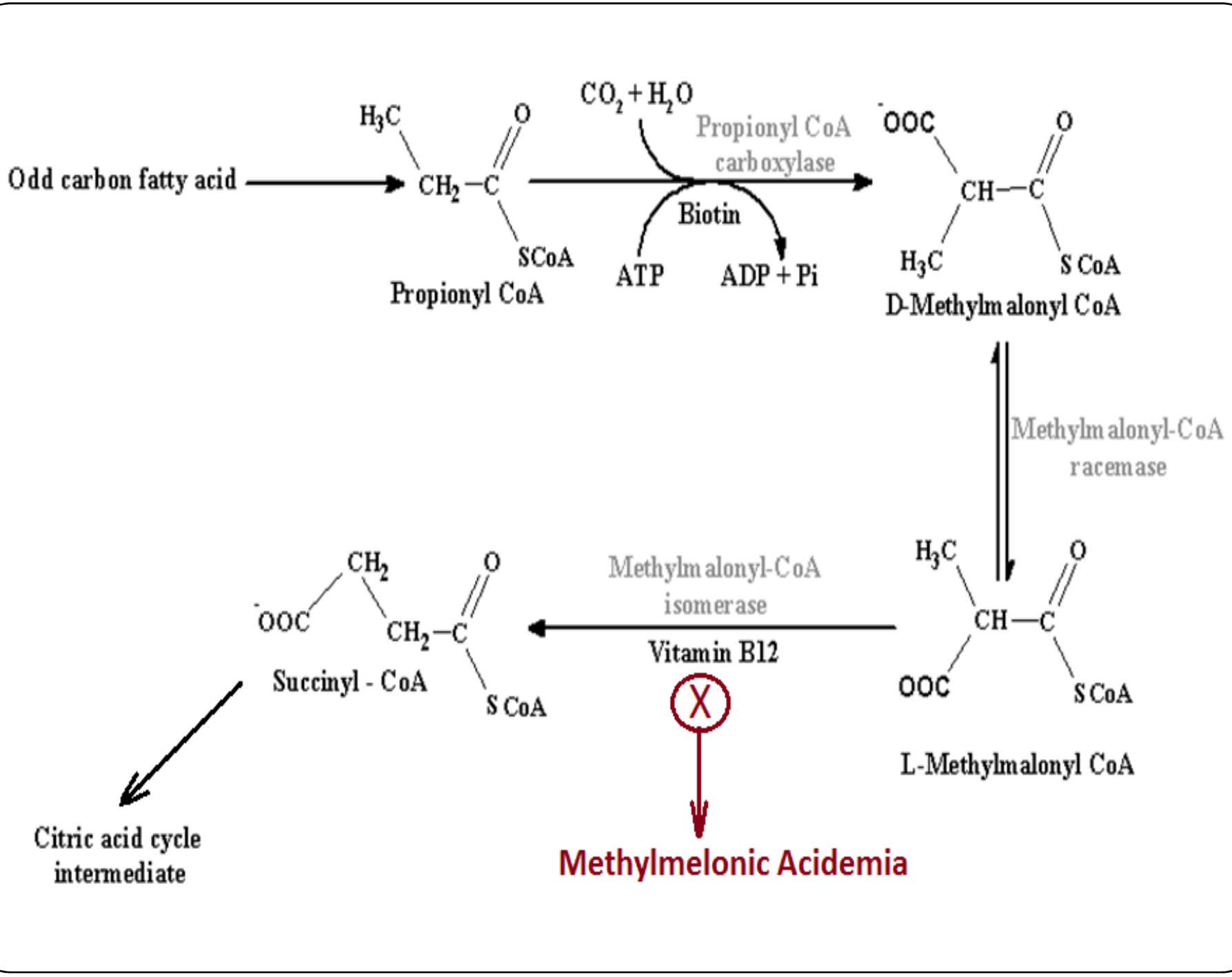



O
O⁻
C
C=O
CH3
CO2
TPP,
Mg2+
pyruvate
decarboxylase
O
H
C
CH3
NADH + H+
NAD+
alcohol
dehydrogenase
OH
CH2
CH3
Pyruvate
Acetaldehyde
Ethanol


Urea Cycle
$CO_2 + NH_3$
1.Carbamoyl Phosphate Synthase
Carbamoyl Phosphate
2. Ornithine carbamoyltransfera se
Citrulline
L-Aspartate
3. Arginosuccinate synthase
Arginosuccinate
4. Argininosuccinate lysase
L-Fumarate
L-Arginine
5. Arginase
Urea
H_2O
L-ornithene

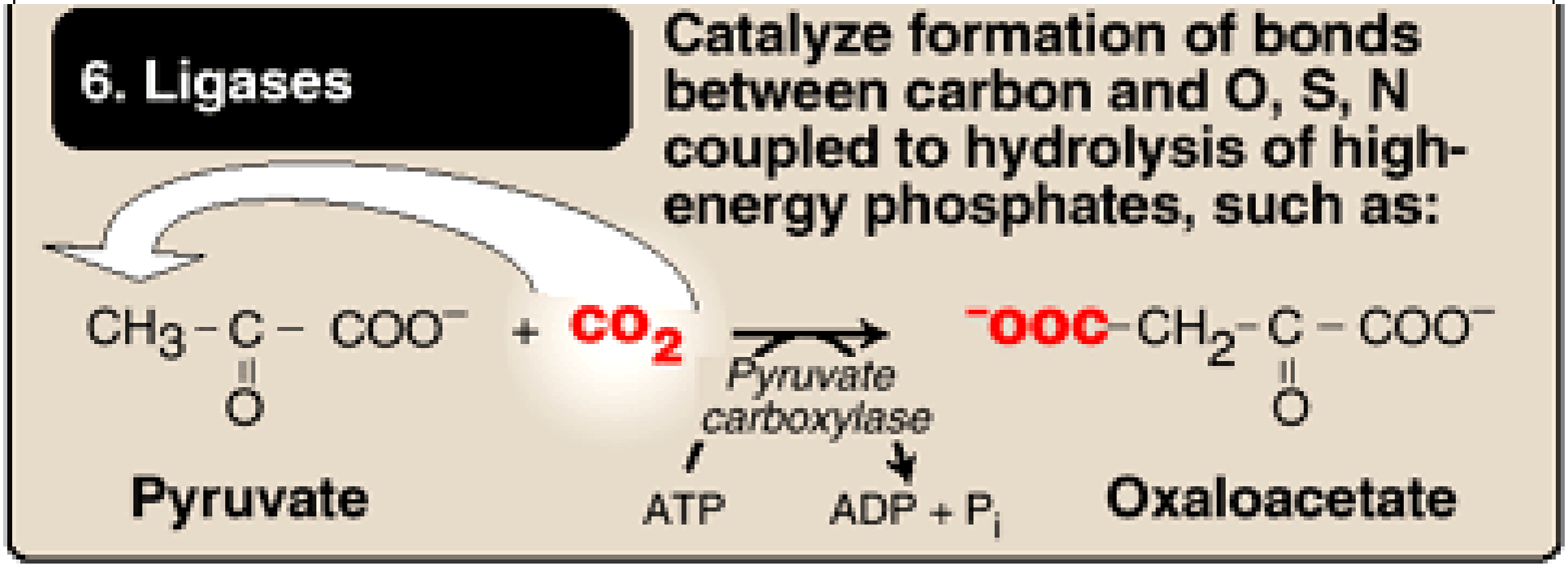
5. Isomerases

- **Optical, Geometric or Positional changesof substrate**
- **Example**
  - **Racemases**
  - **Epimerases**
  - **Triose phosphate isomerase**


Odd carbon fatty acid
H_3C
CH_2
C
O
SCoA
Propionyl CoA
$CO_2 + H_2O$
Propionyl CoA carboxylase
Biotin
ATP
ADP + Pi
^-OOC
CH
C
O
H_3C
S CoA
D-Methylmalonyl CoA
Methylmalonyl-CoA racemase
H_3C
CH
C
O
OOC
S CoA
L-Methylmalonyl CoA
Methylmalonyl-CoA isomerase
Vitamin B12
X
CH_2
^-OOC
CH_2
C
O
S CoA
Succinyl - CoA
Citric acid cycle intermediate
Methylmelonic Acidemia

6. Ligases

- **Link two substrate Usually with help of ATP**
- **Example**
  - **Synthatase**
  - **Acetyl CoA carboxylase**
  - **DNA Ligase**

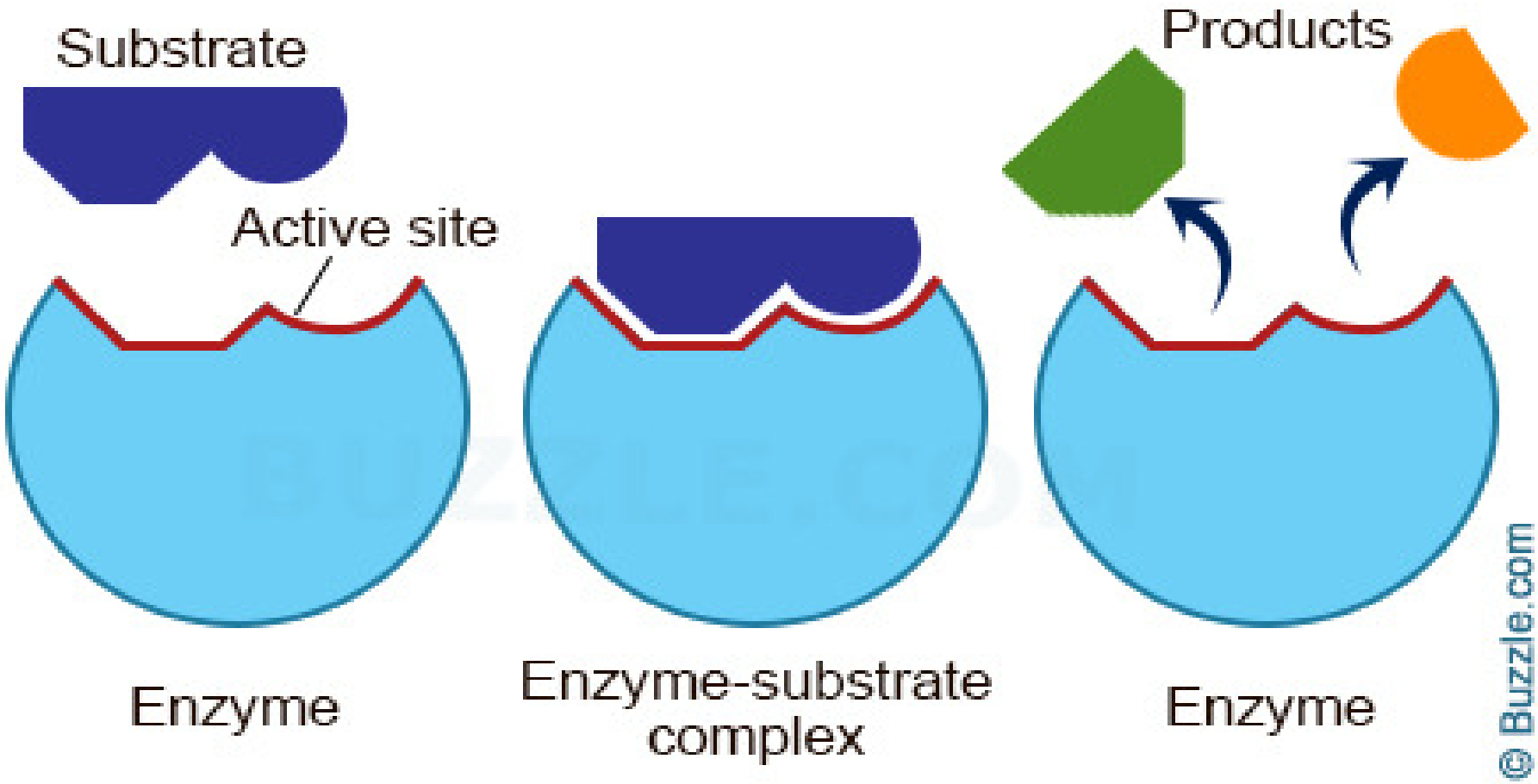


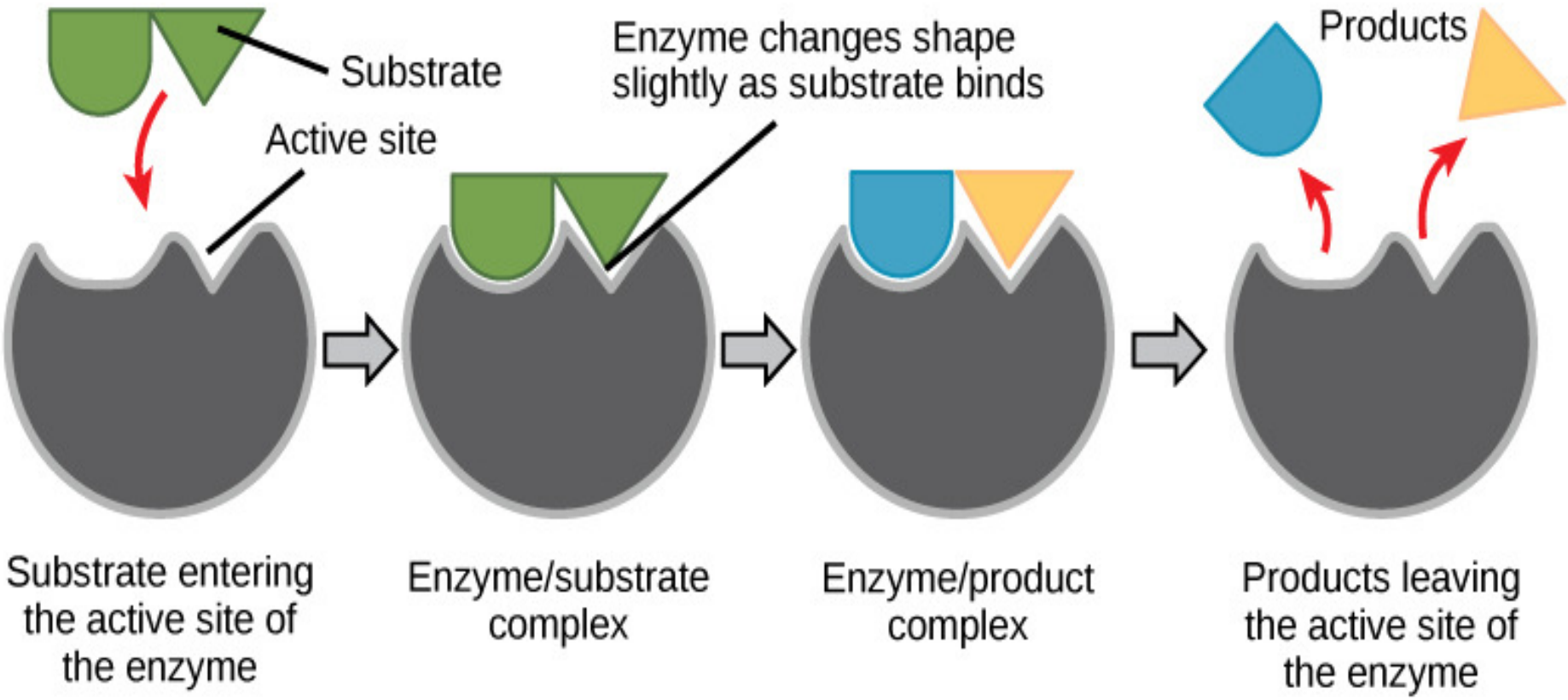
Enzymes which move a molecular group from one molecule to another are known as

A. Oxido-reductase
B. Transferase
C. Hydratase
D. Lyase

Active Site for Enzyme

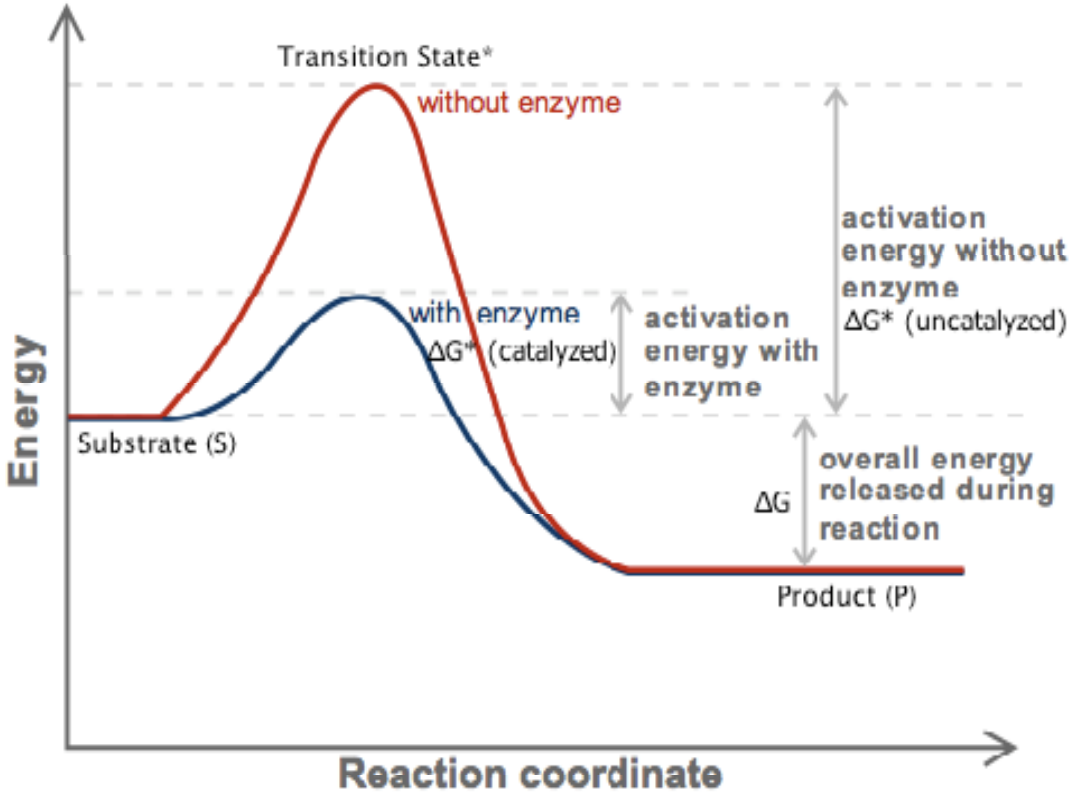
- Special pocket of Enzyme molecules
- Three-dimensional surface
- Complementory Amino acid side chain in Enzyme & Substrate




Substrate
Active site
Enzyme changes shape slightly as substrate binds
Products
Substrate entering the active site of the enzyme
Enzyme/substrate complex
Enzyme/product complex
Products leaving the active site of the enzyme

Mode of action of Enzymes

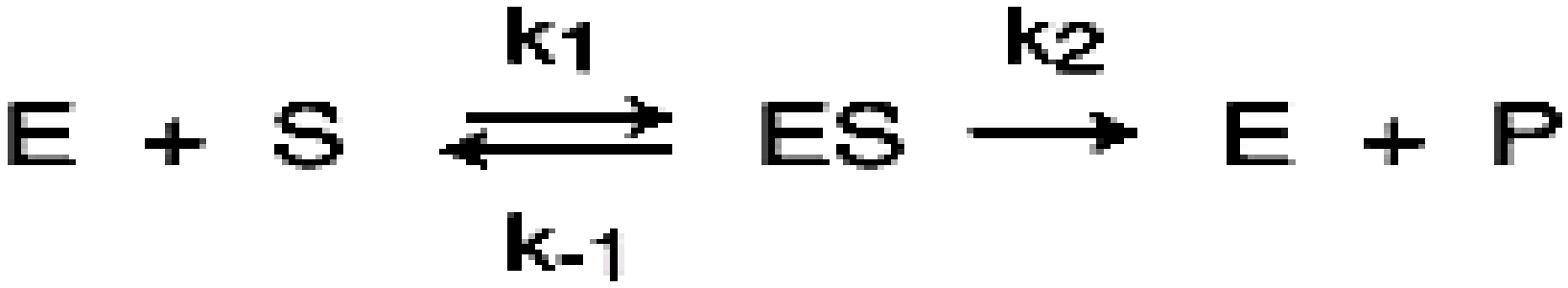
- **Reactions have an energy barrier**
- **That energy barrier separate substrates and products.**
- **Energy barrier = *free energy of activation***



If any enzymatic reaction require high " free energy of activation", it means

A. It is slow reaction
B. It is fast reaction
C. It generates more energy
D. None

Michaelis-Menten Equation



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

Velocity (Turn over) of Enzyme (reaction)

Velocity of Enzyme Measure = micro mole / min

V_{max} = Maximum velocity of the reaction.

Catalase Velocity : 5 million micro mole / min

One catalase molecule convert approx. 5 million molecules of H_2O_2 into $H_2O + O_2$ per minute

Fastest to Slowest Velocity (Turnover) Enzyme

Catalase > Carbonic andydrase

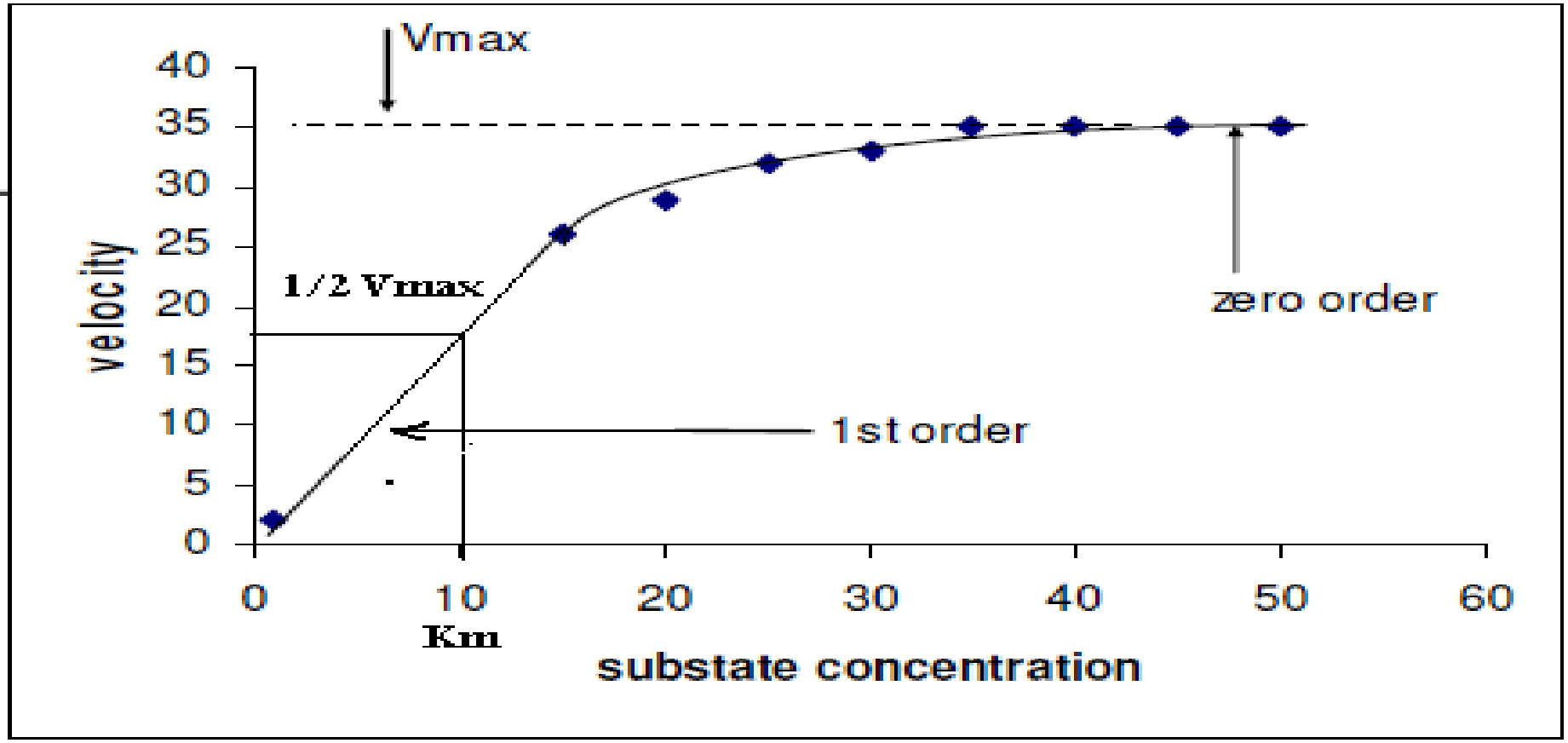
> Acetylcholinesterase> Amylase

>LDH >Trypsin >Chymotrypsin

>DNA polymerase >Lysozyme

Km (Michaelis constant)

- Substrate concentration require to achieve $\frac{1}{2}V_{max}$
- **Reflects Affinity of Enzyme for substrate.**
- Low Km = Less Substrate require for $\frac{1}{2}V_{max}$
  = Indicate High Affinity
- High Km = High Substrate require for $\frac{1}{2}V_{max}$
  = Indicate Low Affinity



- **Kcat** = Turnover number of 'S' to 'P'
- **Km** = Affinity of Enzyme towards substrate
- **Kcat / Km** = Catalytic Efficiency of Enzyme

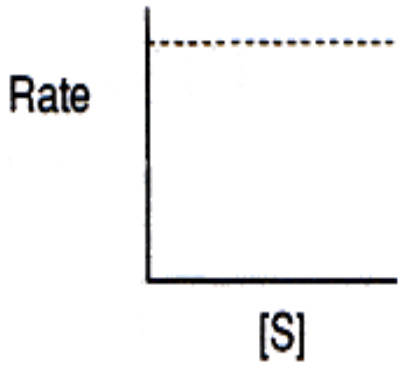
What is true about "Faster catalytic reaction"?

A. Less "Free energy of activation" require
B. Less Vmax
C. Less Kcat/km for enzyme
D. Less Km value for enzyme

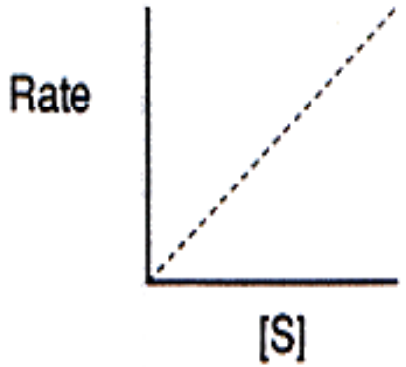
- **Faster Reaction**
  - **Less "Free energy of activation" require**
  - **Easily reach Transition state**
  - **High Kcat/km**
- **Slow Reaction**
  - **More "Free energy of activation"**
  - **Difficult to reach Transition state**
  - **Low Kcat/km**

Type of Reaction

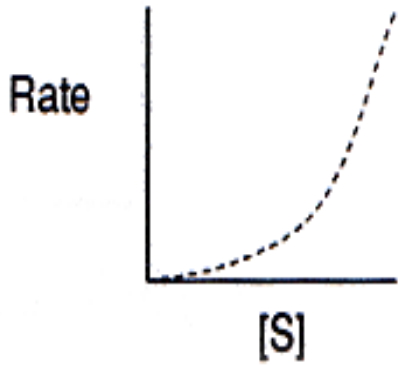
Zero order: Rate is independent of [S], i.e., proportional to $[S]^0$ (= 1)



First order: Rate is proportional to [S], i.e., $[S]^1$ (= [S]).

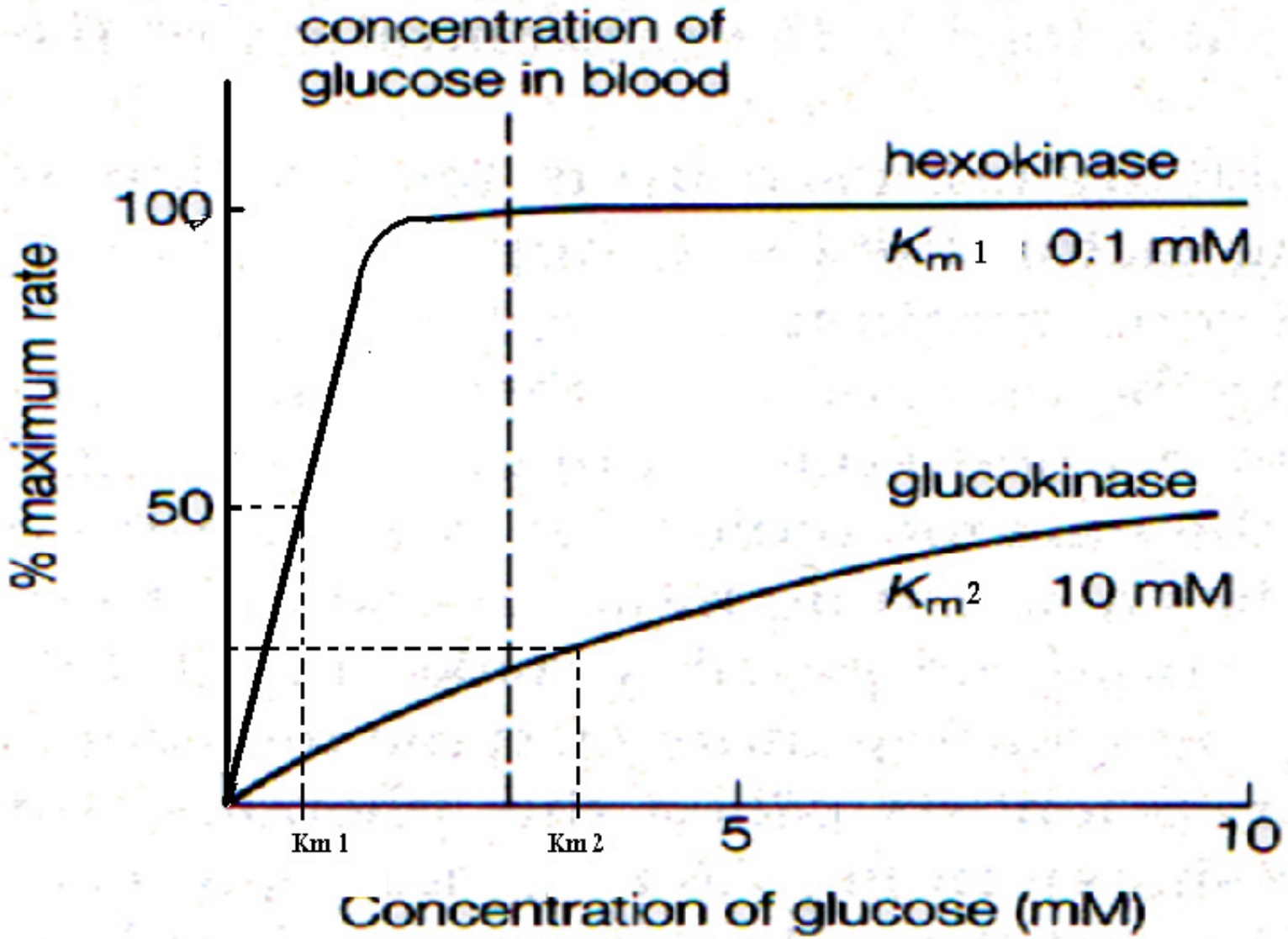


Second order: Rate is proportional to [S]×[S] (i.e., $[S]^2$).



In first order kinetic, velocity of reaction is

A. directly proportional to [S].
B. Inversely proportional to [S].
C. not depend on [S].
D. proportion to $[S]^2$


concentration of
glucose in blood
hexokinase
$K_{m\,1}$: 0.1 mM
glucokinase
K_{m2} 10 mM
100
50
% maximum rate
Km 1
Km 2
5
10
Concentration of glucose (mM)

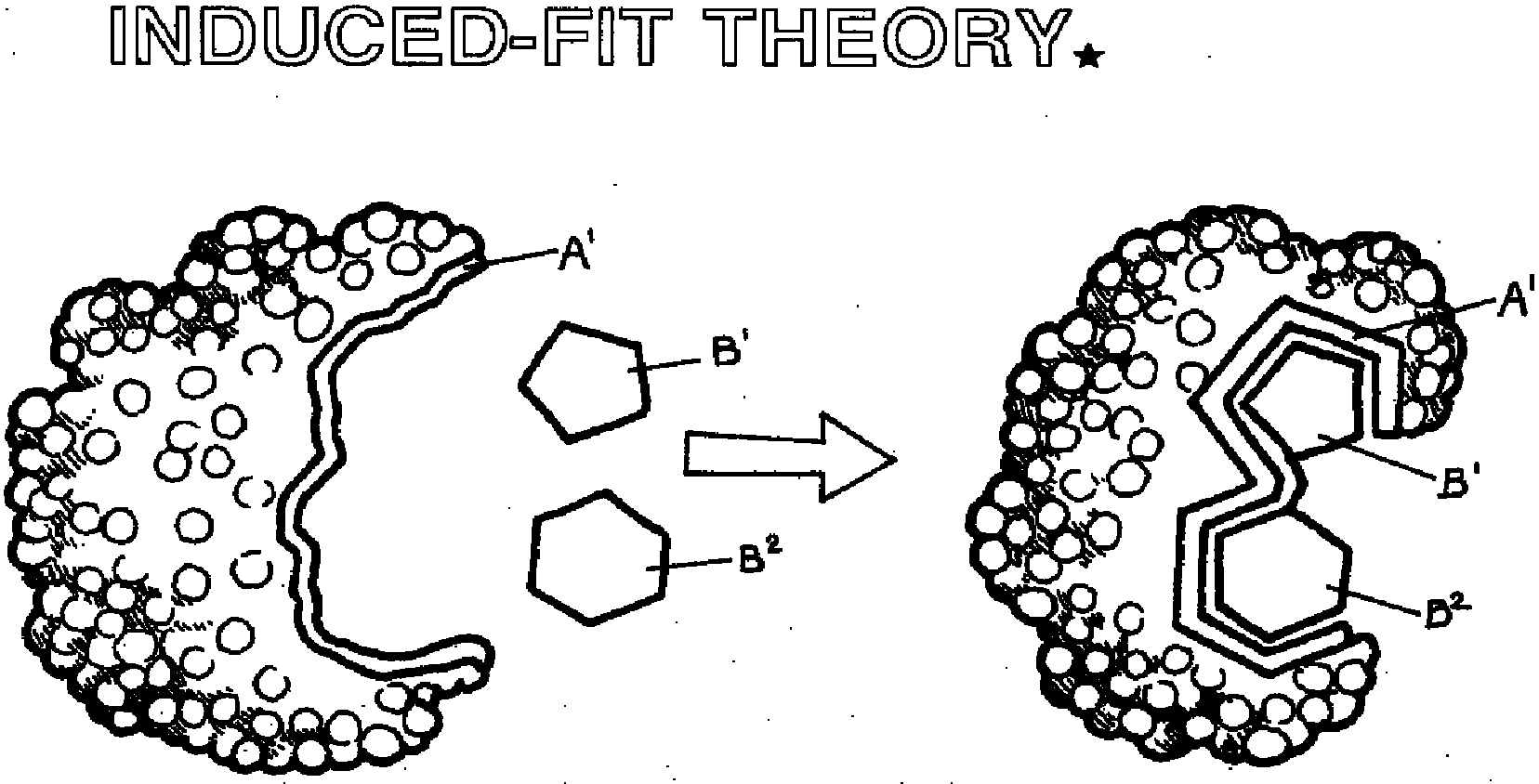
Hexokinase Vs Glucokinase

- Non Specific
- Act on any hexose sugar
- For Glycolysis
- Present in every cell
- Low km
- High affinity
- Activated even with low concentration of glucose

- Specific
- Act on only Glucose
- For Glycogen synthesis
- Present in hepatic cell
- High km
- Low affinity
- Activated with only high concentration of glucose

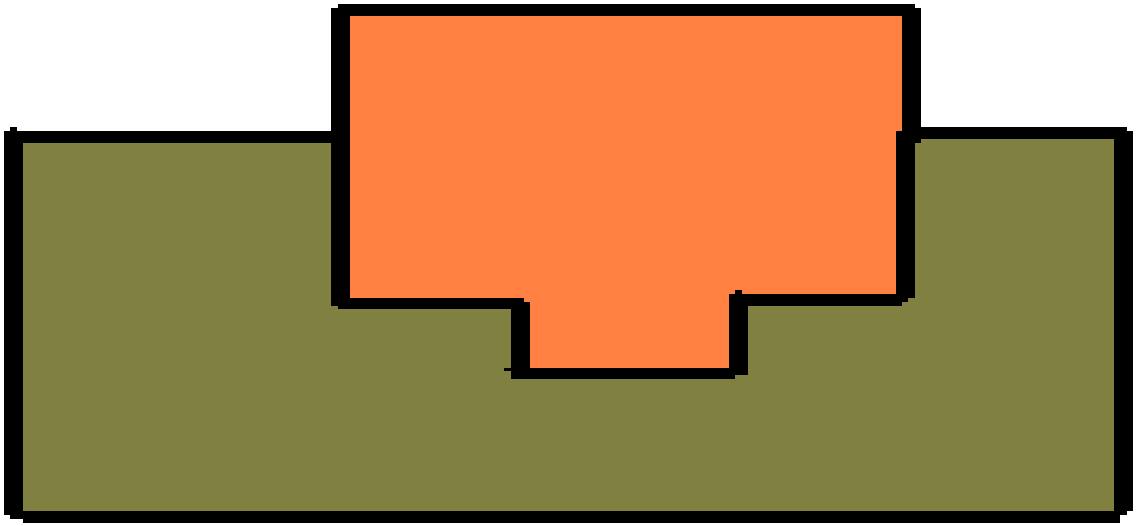
Koshland's Induce Fit Theory

- After binding of substrate to enzyme at specific site, there will be more confermational change in the enzyme.
- e.g. Hand Gloves.



Fischer's Template Theory

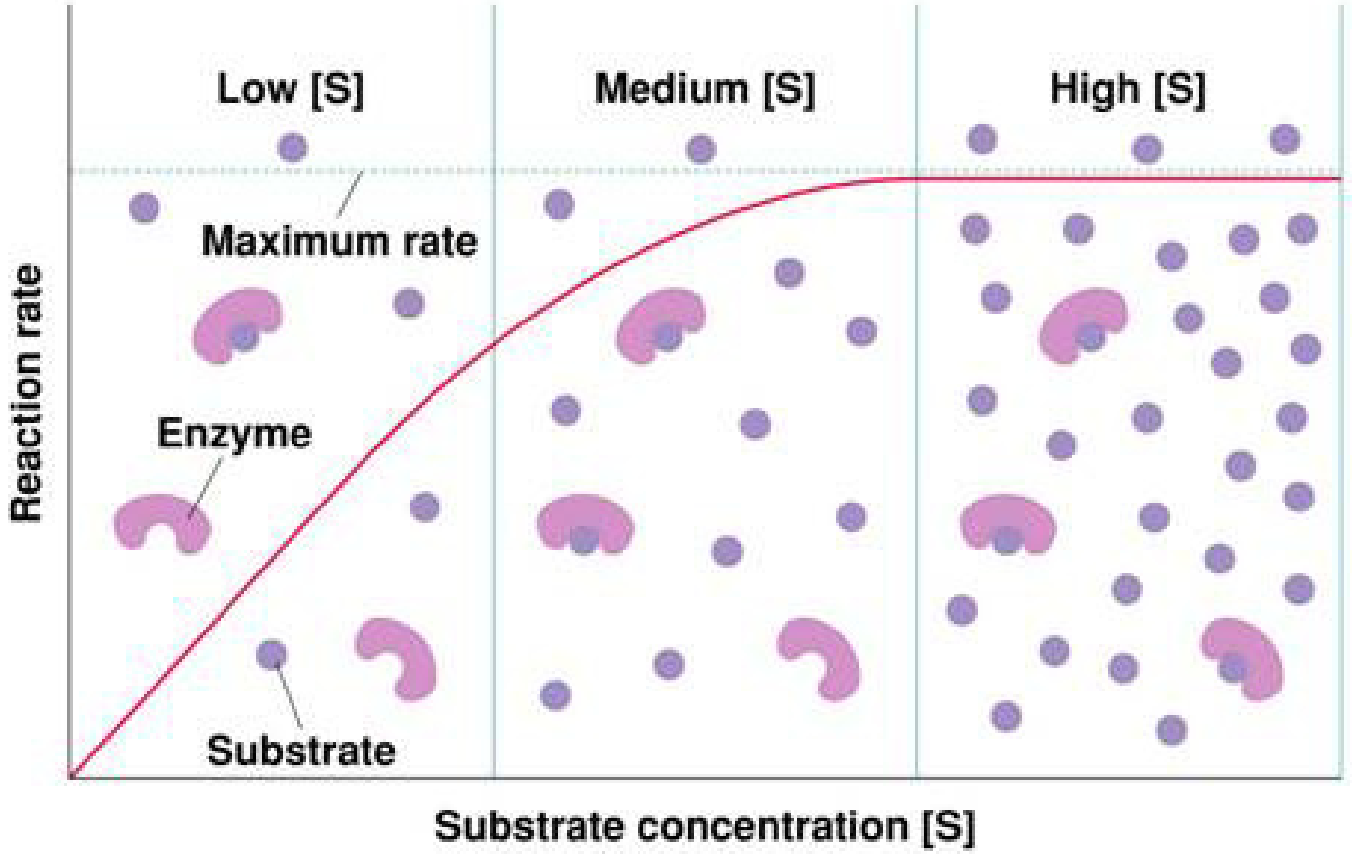
- Active site of the enzyme is complementary to the substrate.
- E.g. Lock & Key



Factors Affecting Enzyme Reaction Activity and It's Velocity

1. Substrate concentration

- Velocity increases with [S]
- Until V_{max} is reached.
- High [S] = enzyme Saturated with substrate.



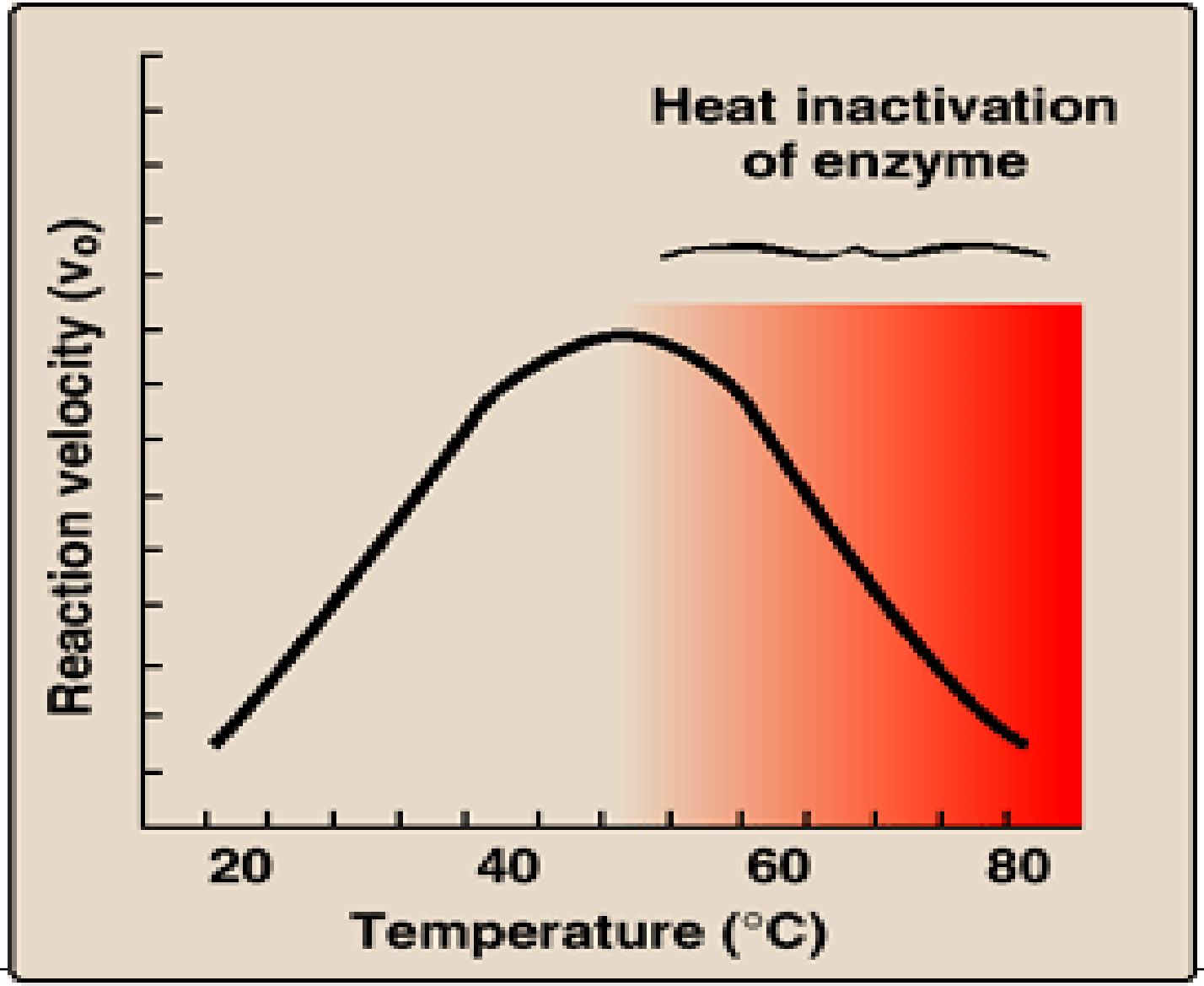
2. Temperature

- **Optimum temperature (Human)= 35° - 40°C.**
- Enzymes denature = above 40°C temperature.
- **Maximum reaction velocity at Optimum temperature.**

In PCR

- Enzyme to act at 70-90°C
- **Enzyme from Hot spring Bacteria**
- **Thermus Equatics** = Optimum temperatures of 70°C.
- **Taq Polymerase** = PCR

Effect of Temperature on Enzyme activity



Q_{10} Temperature Coefficient

- The rate of change in Reaction velocity as increase the temperature by 10 °C
- **Biological Value of Q_{10} = 2 -3**

3. Enzyme concentration

- Velocity (Rate of the reaction) is directly proportional to [E] at all [S].

- Half [E] $= V_o$ & V_{max} are reduced to half.

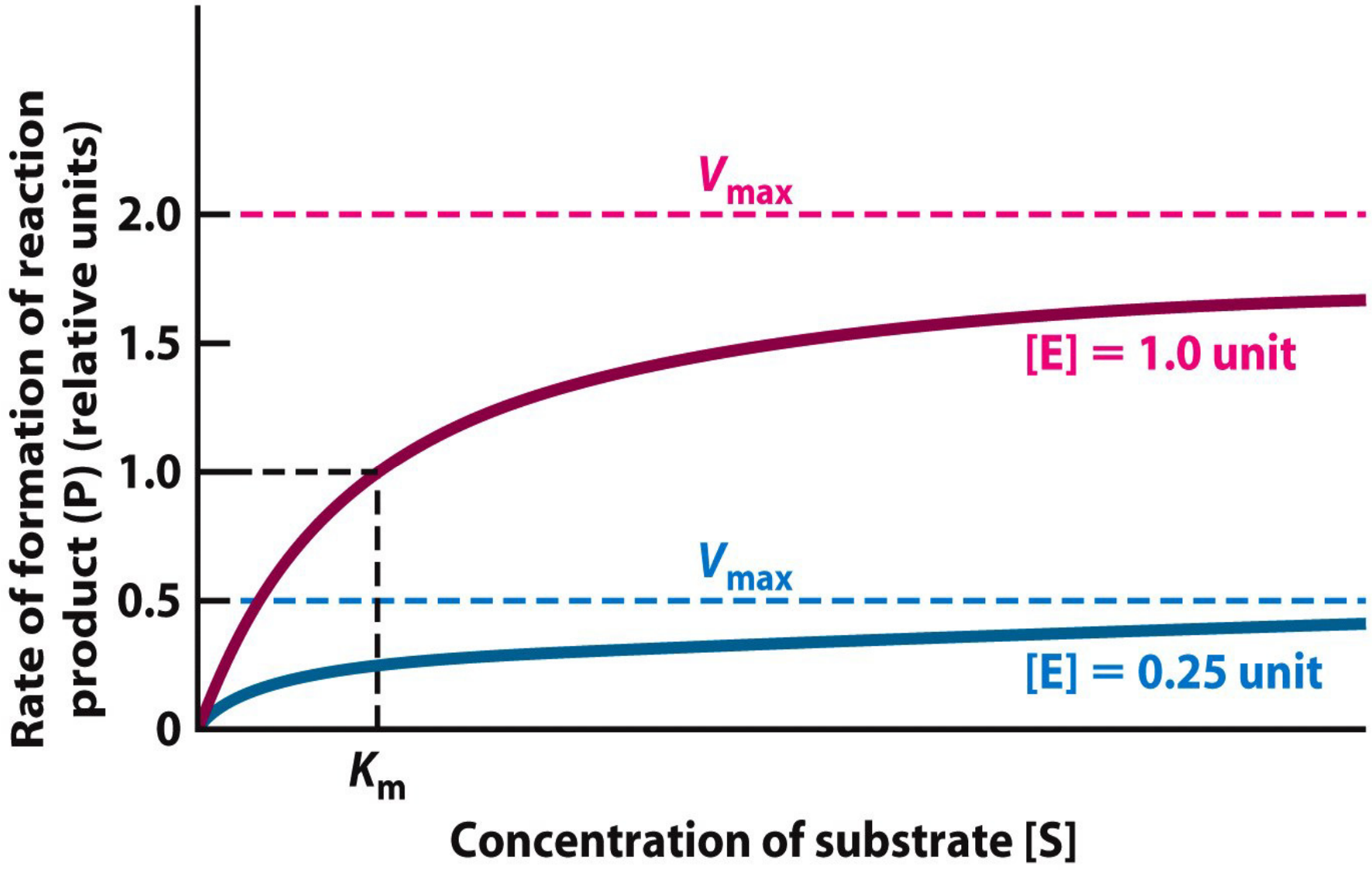


Figure 3-22a
Molecular Cell Biology, Sixth Edition

Substrate concentration is always more than Km in

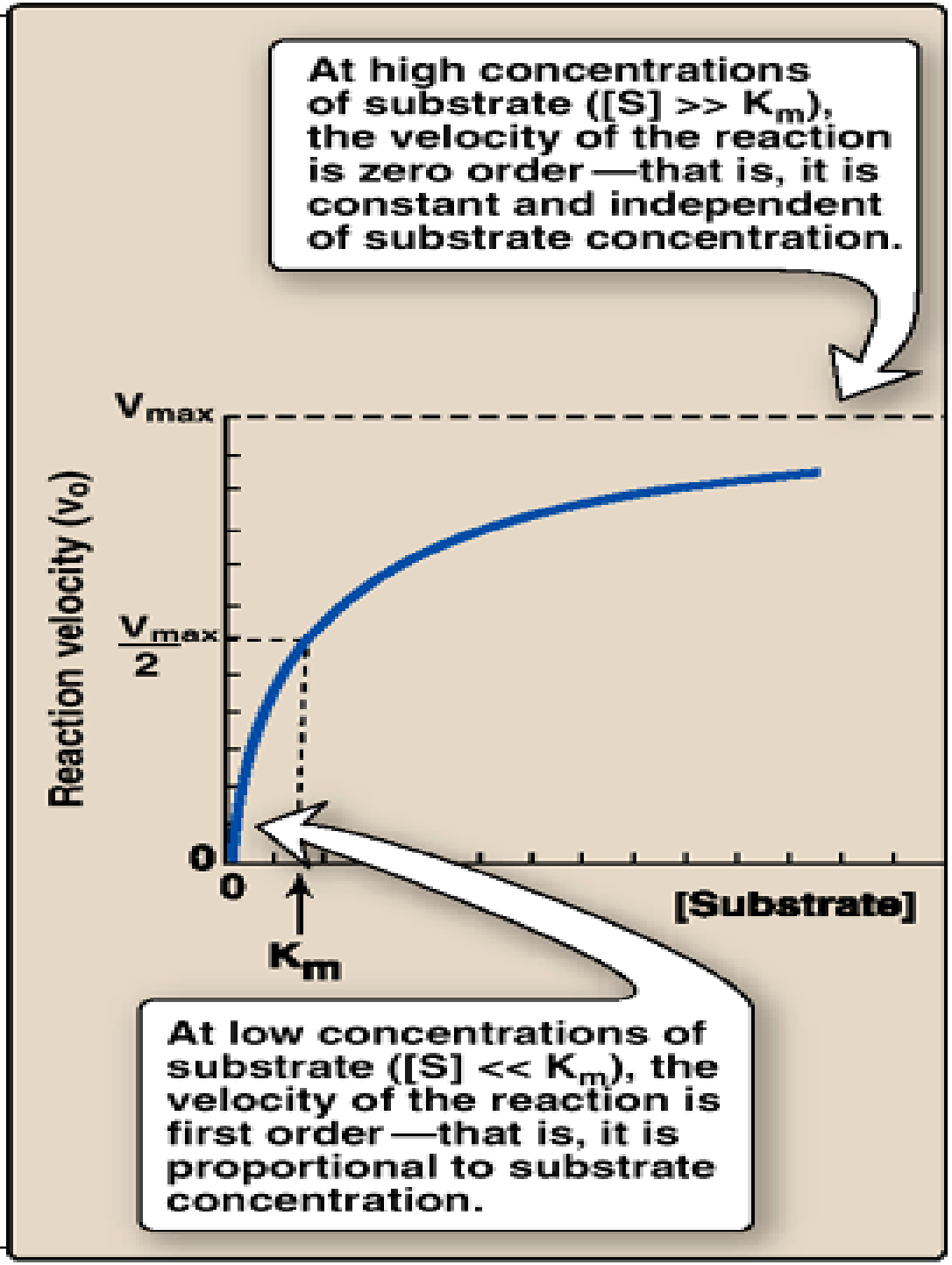
A. Zero order kinetic
B. First order kinetic
C. Second order kinetic
D. Third order kinetic

First Order Reaction

- [S] <<< K_m
- V α [S]

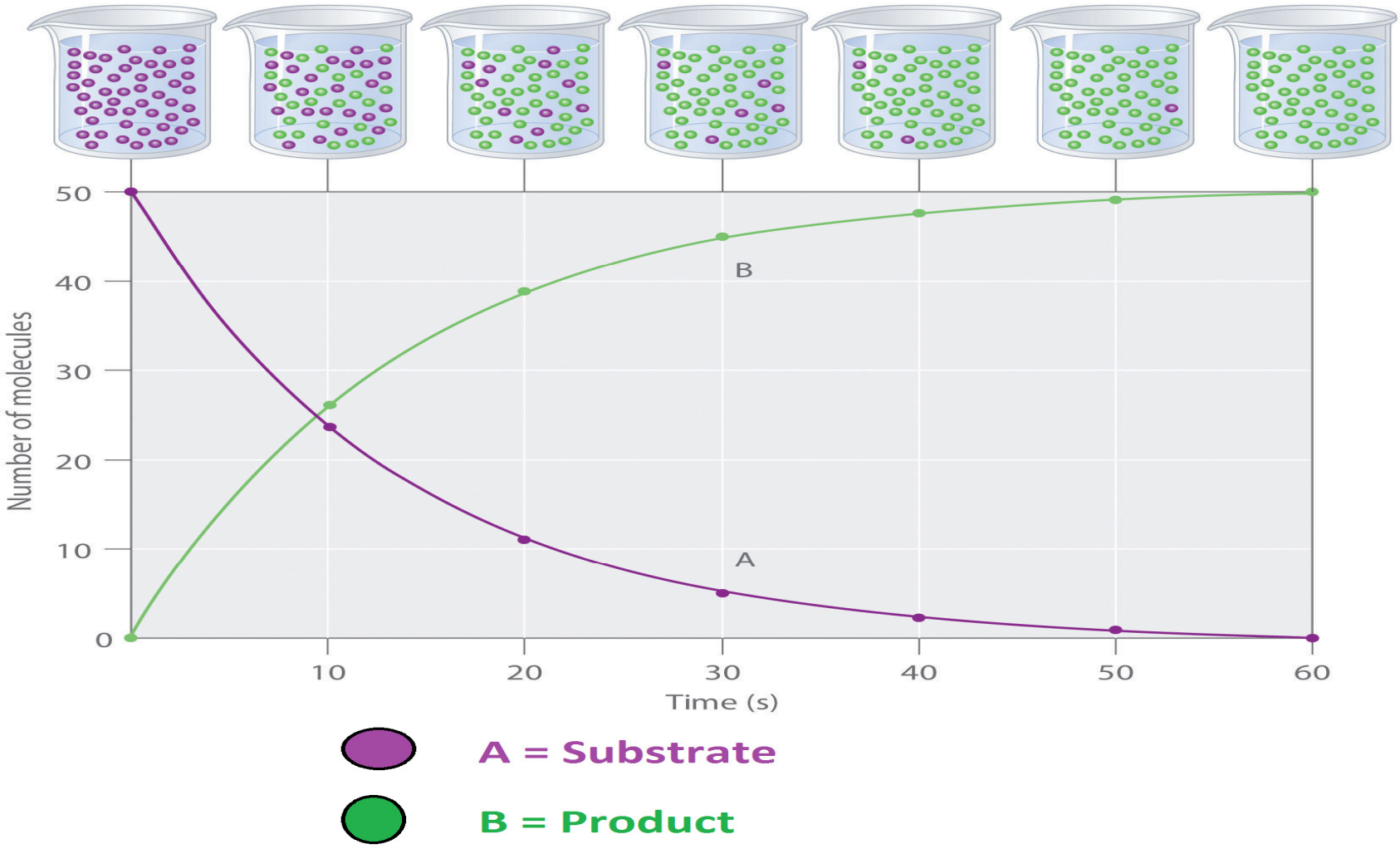
Zero Order Reaction

- [S] >>> K_m
- V remains constate [S]
- V = Vmax



4. Product concentration

- Increase Product Conc. = Velocity Slow .
- Higher Product conc. = Inhibits reaction.



Enzyme activity increase with , Except

A. Increase substrate concentration
B. Increase enzyme concentration
C. Increase temperature (not more than optimmum)
D. Increase product concentration

5. pH

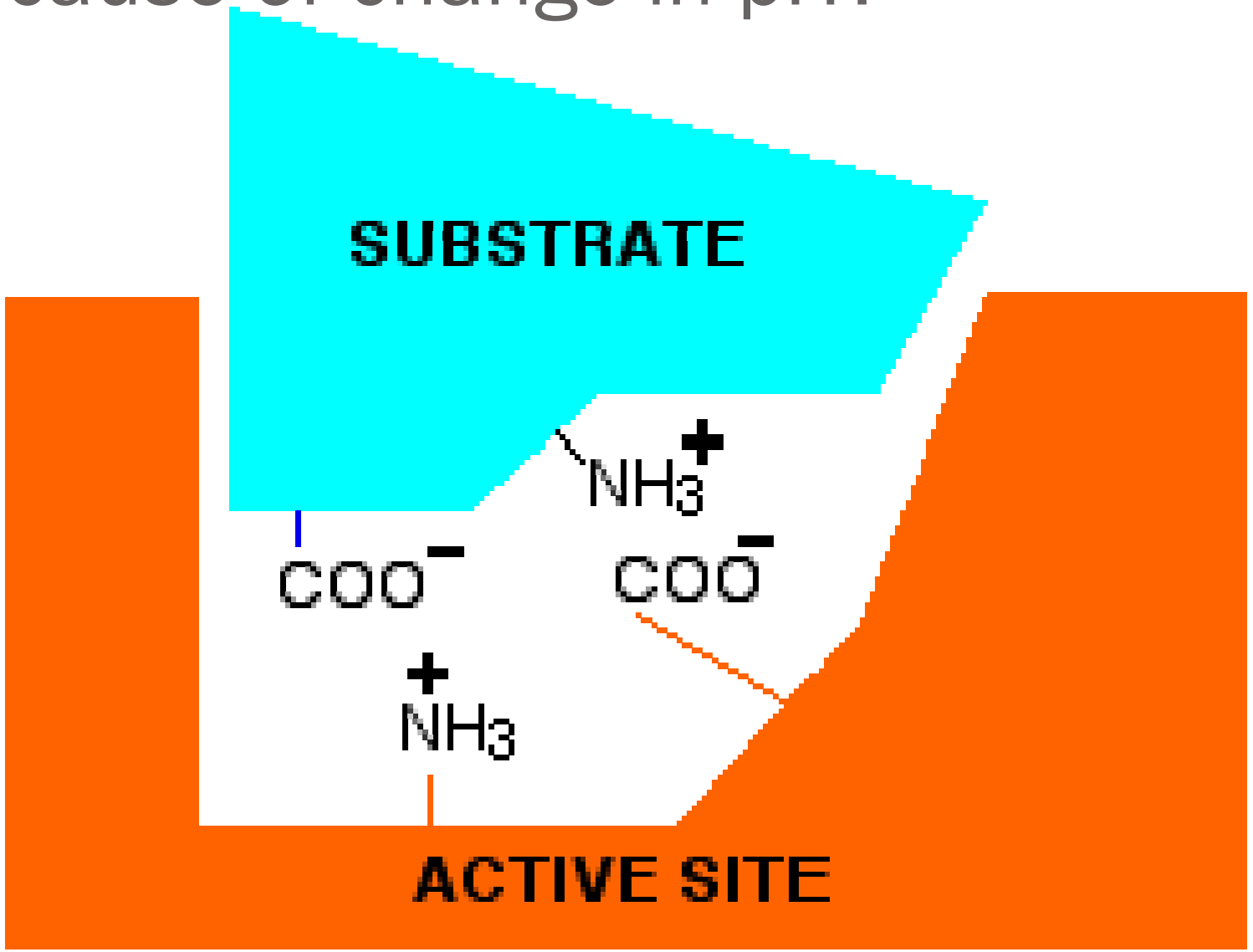
- [H^+]
  - Change Active site & Bonds
  - Configuration change
  - Change Velocity
  - Can denature enzyme

Different Optimum pH

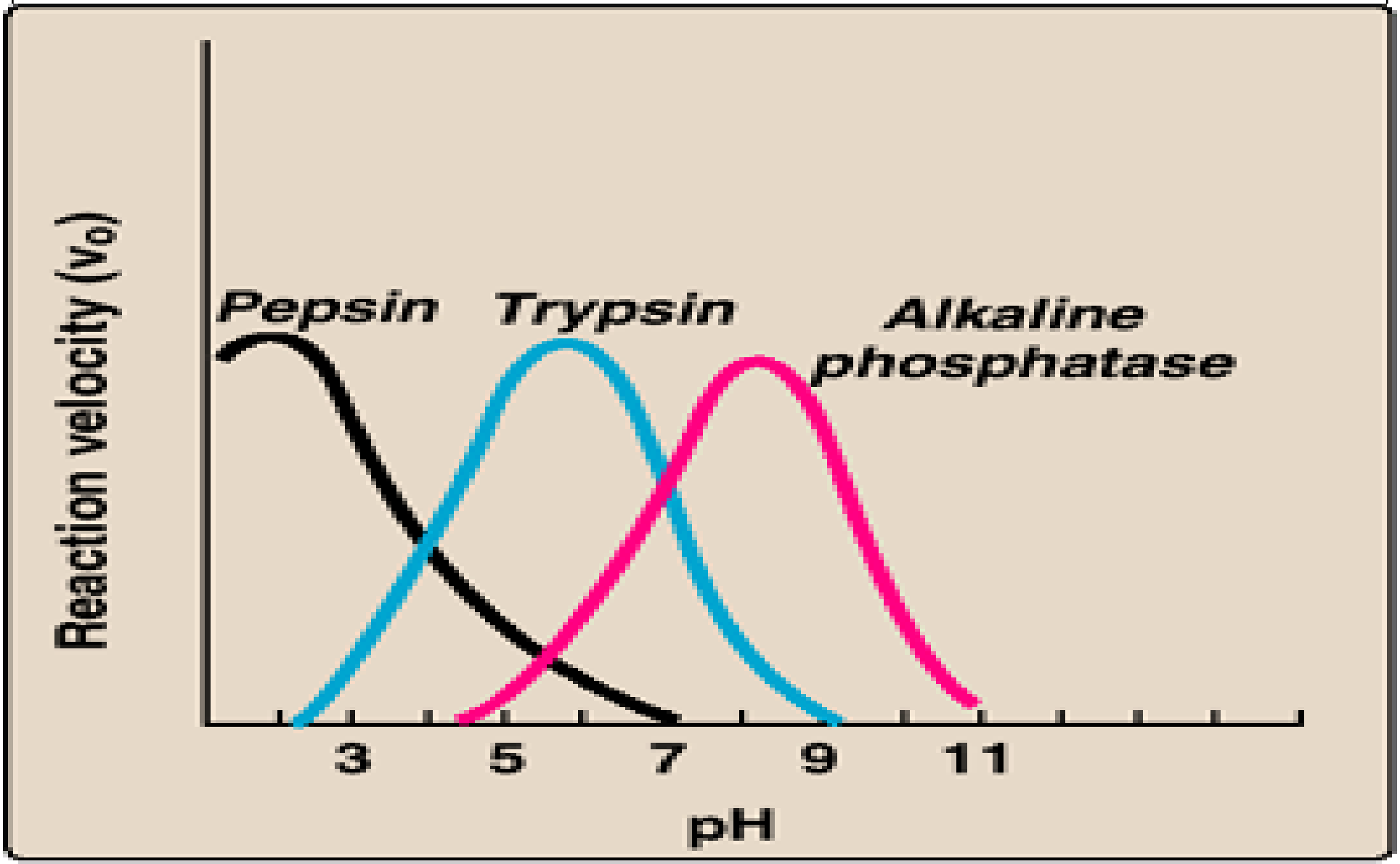
for

Different enzyme.

What change can occur at active site, because of change in pH?



Different enzyme with it’s optimum pH



6. Enzyme activation

- In presence of certain metallic ions, some enzyme shows higher activity.
  - Salivary amylase = chloride
  - Lipase = calcium
- Pro-enzyme (Zymogen) to active form
  - Trypsinogen = Trypsin
  - Chymotrypsinogen = Chymotrypsin
  - Lysosomal enzymes

Protein Activation

  - Coagulation factors
  - Complementary components

7. Enzyme Inhibition

Inhibitors

- Reduce the rate of enzymic reactions
- Work at low concentrations
- Block the enzyme but they do not usually destroy it
- Many drugs and poisons are inhibitors of enzymes

The effect of enzyme inhibition

Reversible inhibitors

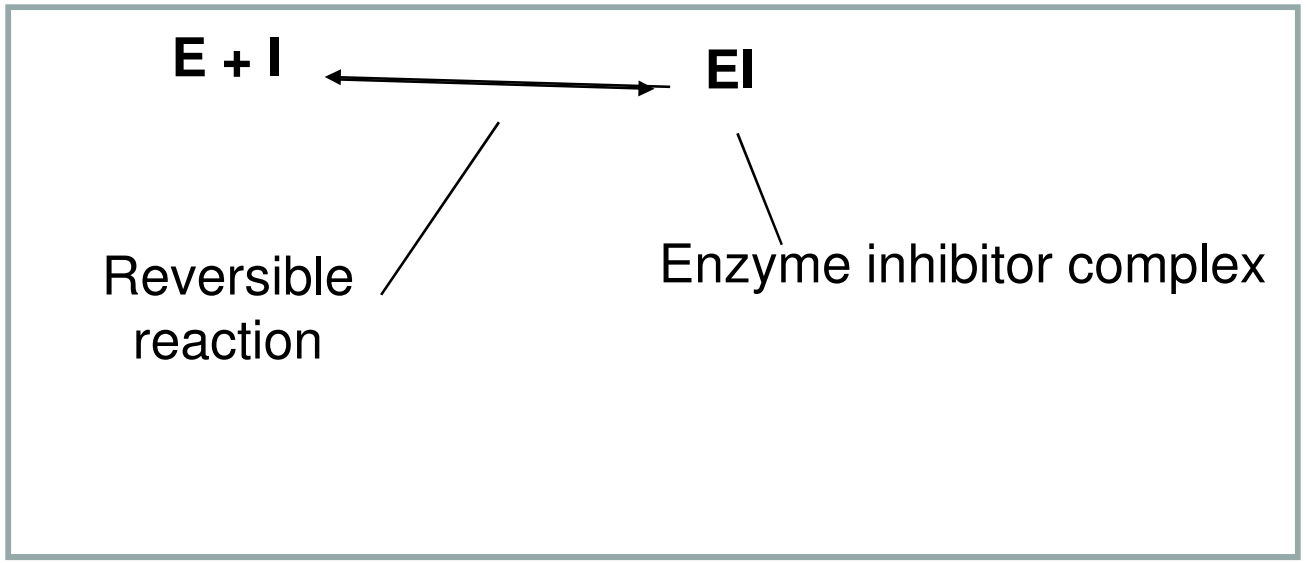
1. **Competitive inhibitors**
2. **Non – competitive inhibitors**

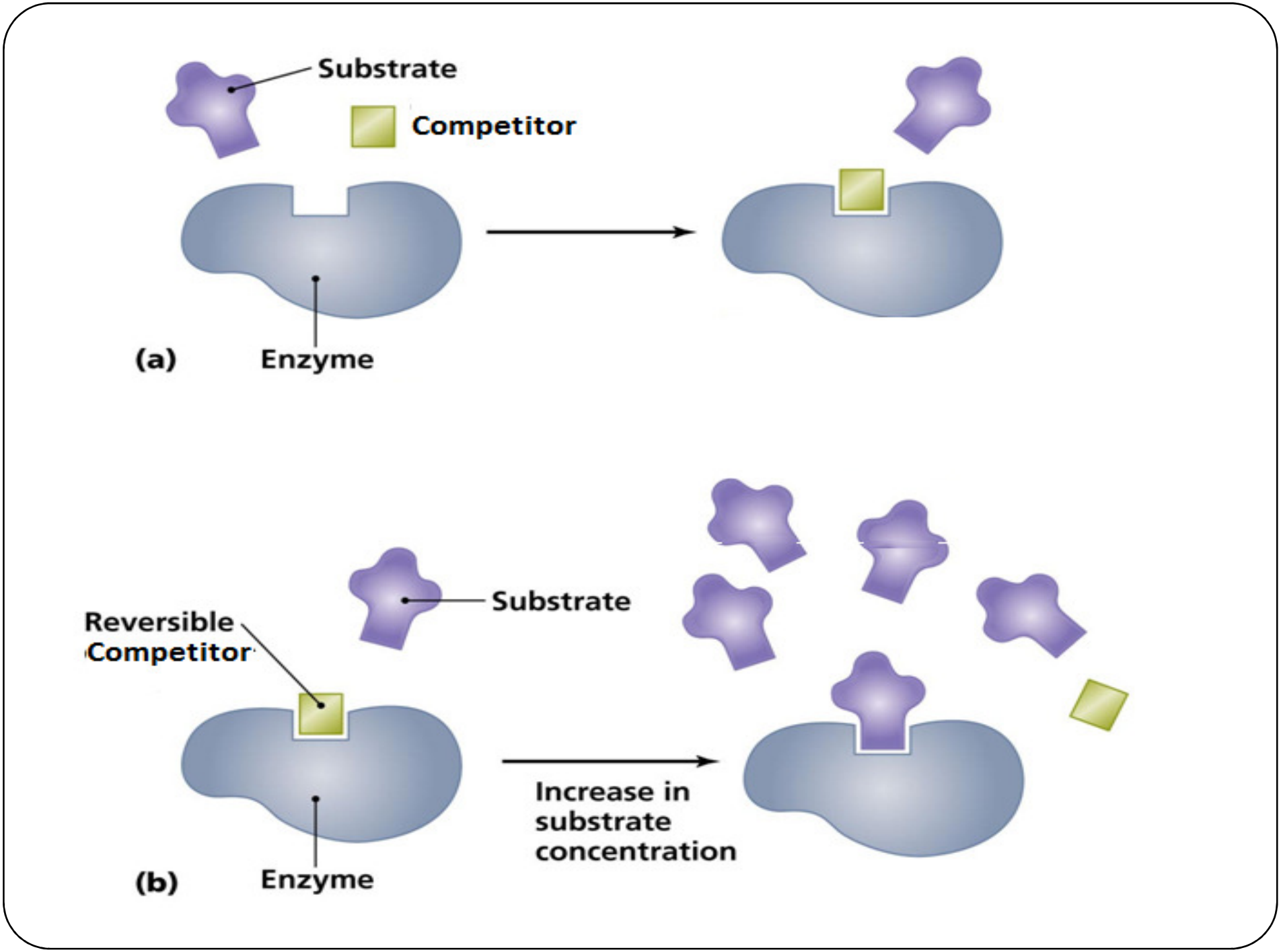
Irreversible inhibitors

- **Combine with functional groups at active site**
- **Irreversibly**

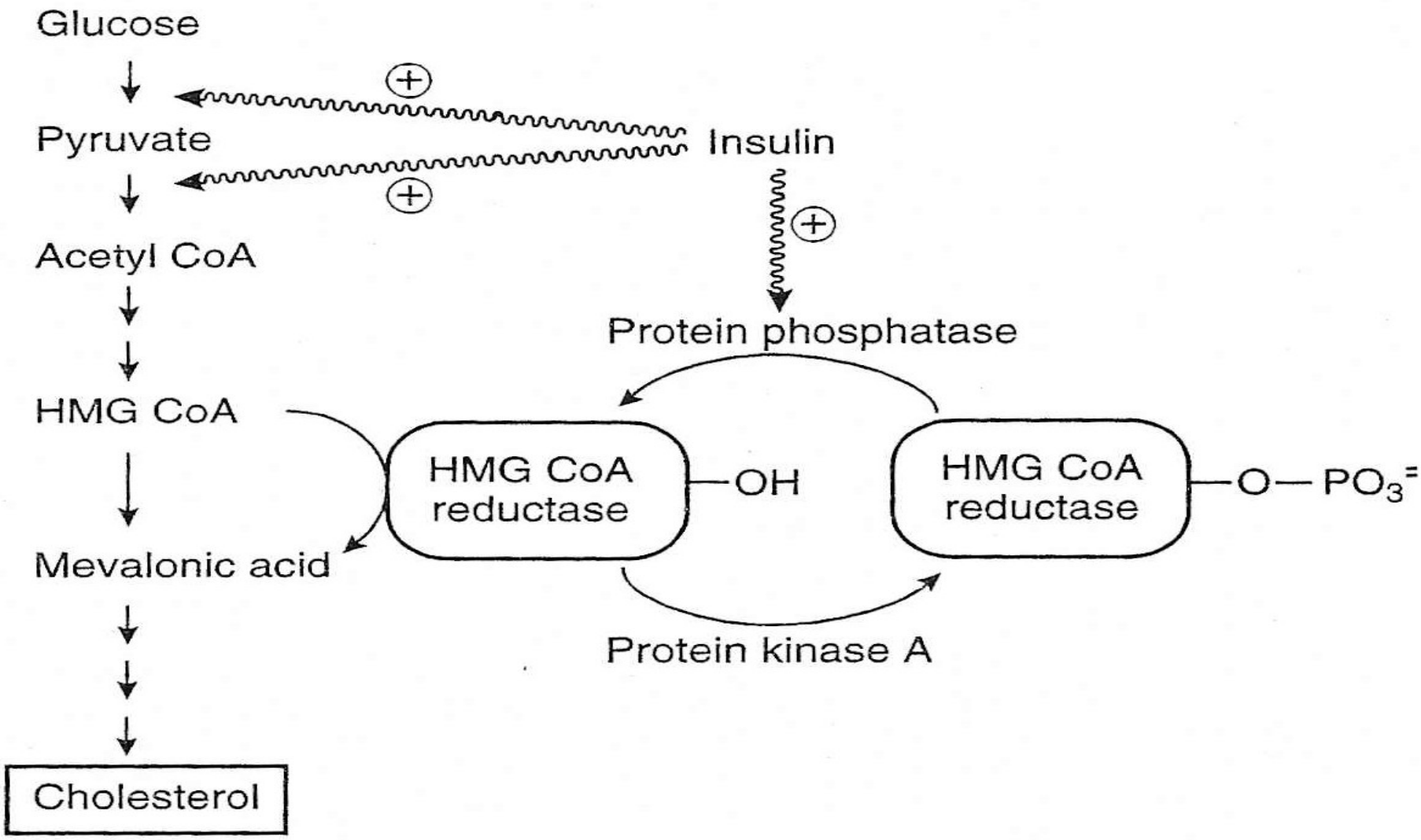
Compatitive Inhibition

- Inhibitor = Structurally resemble to substrate.
- Compete with the substrate molecules for the active site.
- Inhibitor action is proportional to its concentration

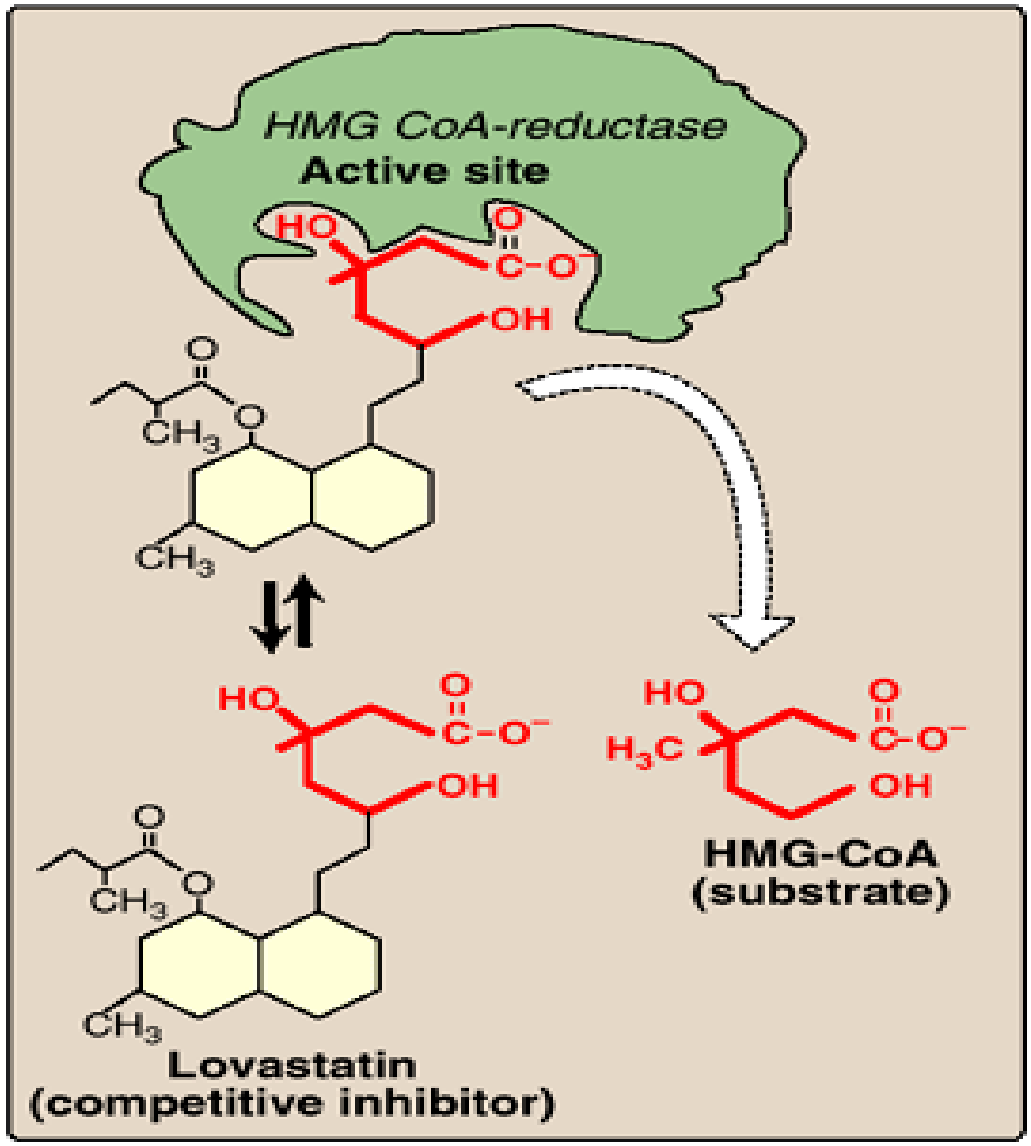



Substrate
Competitor
(a)
Enzyme
Reversible Competitor
Substrate
Increase in substrate concentration
(b)
Enzyme

Cholesterol Regulation



Competitive Inhibition



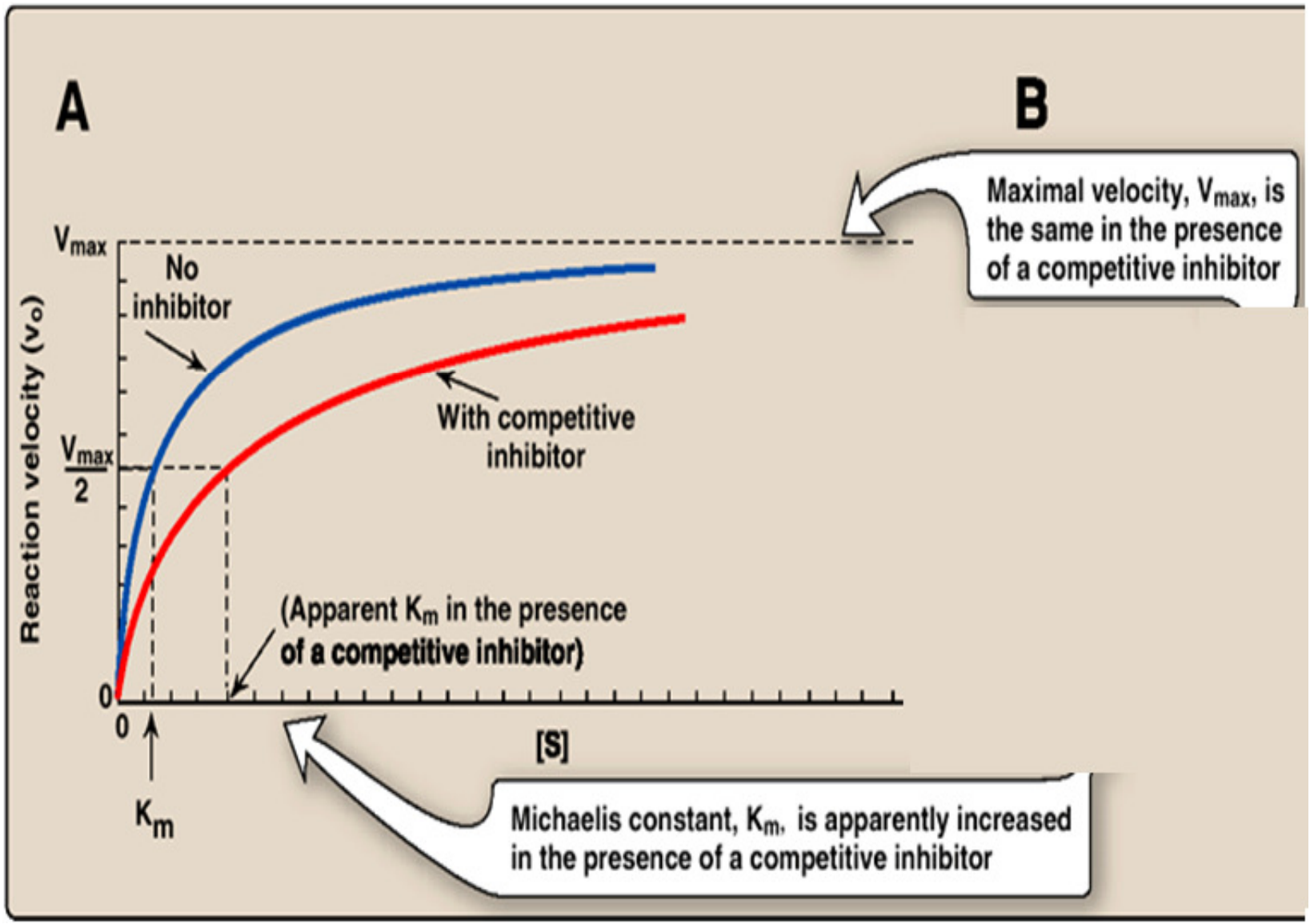
Statin use in hypercholesterolemia, because it makes

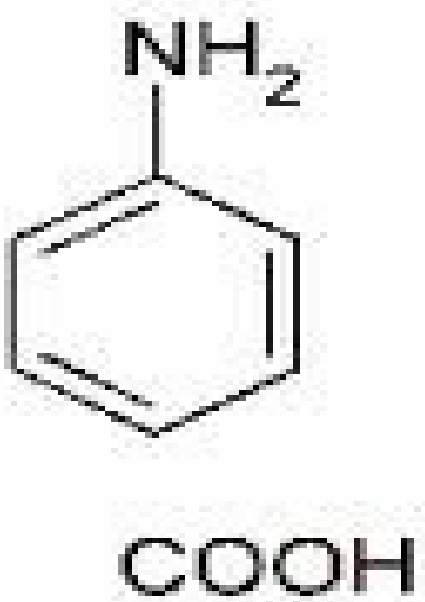
A. Competitive inhibiton as it is analogues to HMG CoA
B. Competitive inhibiton as it is analogues to HMG CoA reductase
C. Non-competitive inhibiton as it is analogues to HMG CoA
D. Non-competitive inhibiton as it is analogues to HMG CoA reductase

Vmax decrease in ,

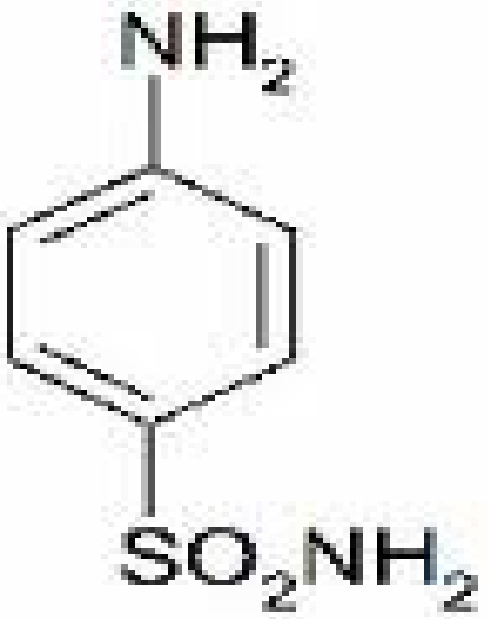
A. Competitive inhibition
B. Non - Competitive inhibition
C. Un - Competitive inhibition
D. Suicide inhibition
E. B & C
F. All of Above

In Compatitive Inhibiton
Km Increases & Vmax remains unchanged



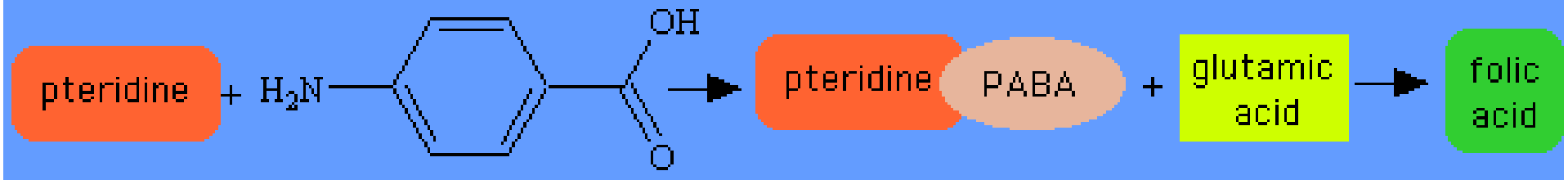


PABA
(metabolit)



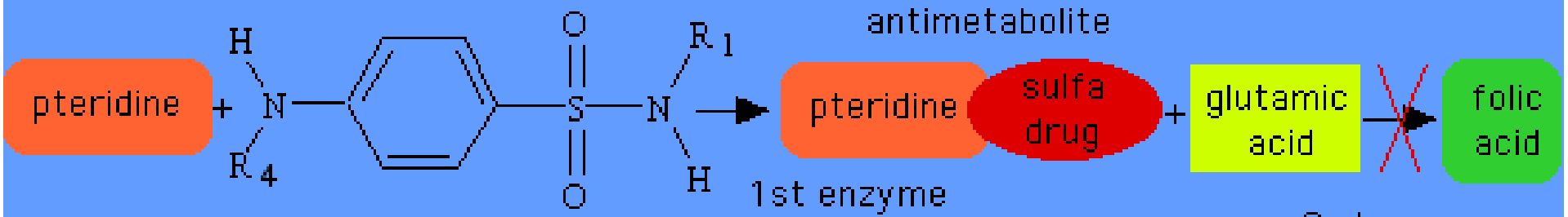
Sulfonamid
(antimetabolit)

Sulfa Drug - Antimetabolite



normal reaction

para-aminobenzoic acid
PABA

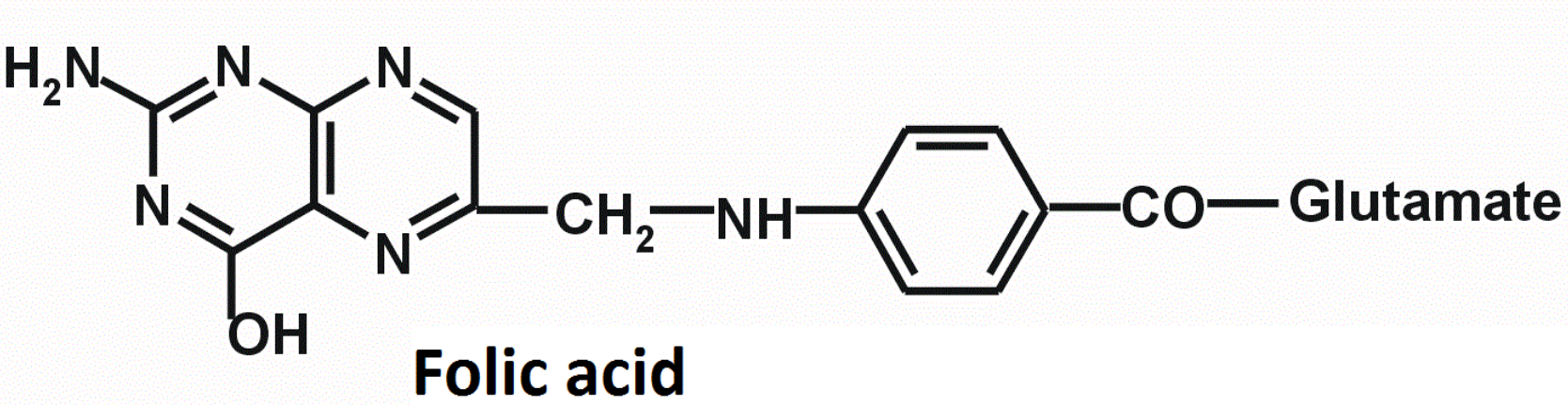


1st enzyme
"fooled"

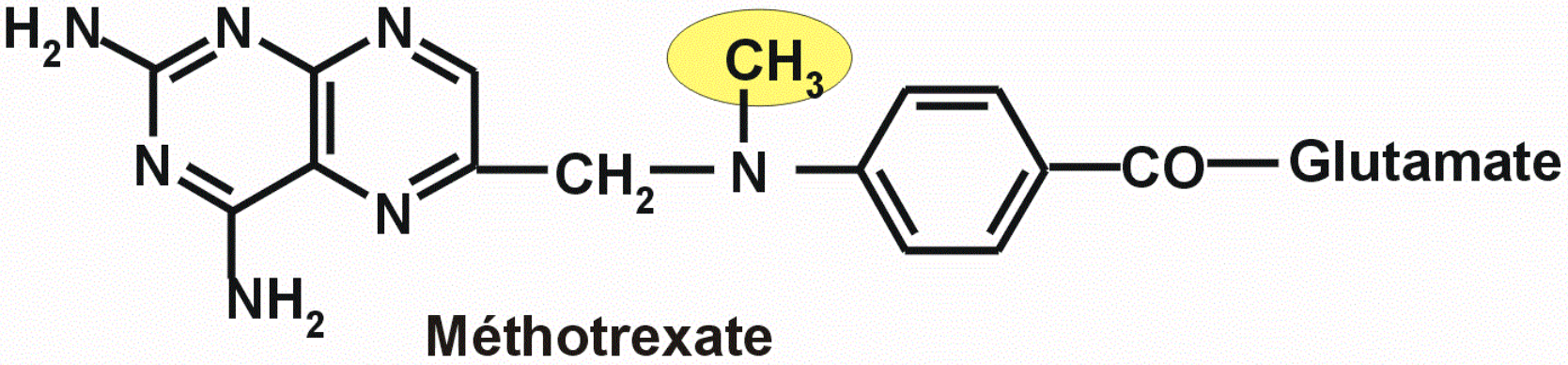
2nd enzyme
inhibited

Sulfonamide base structure

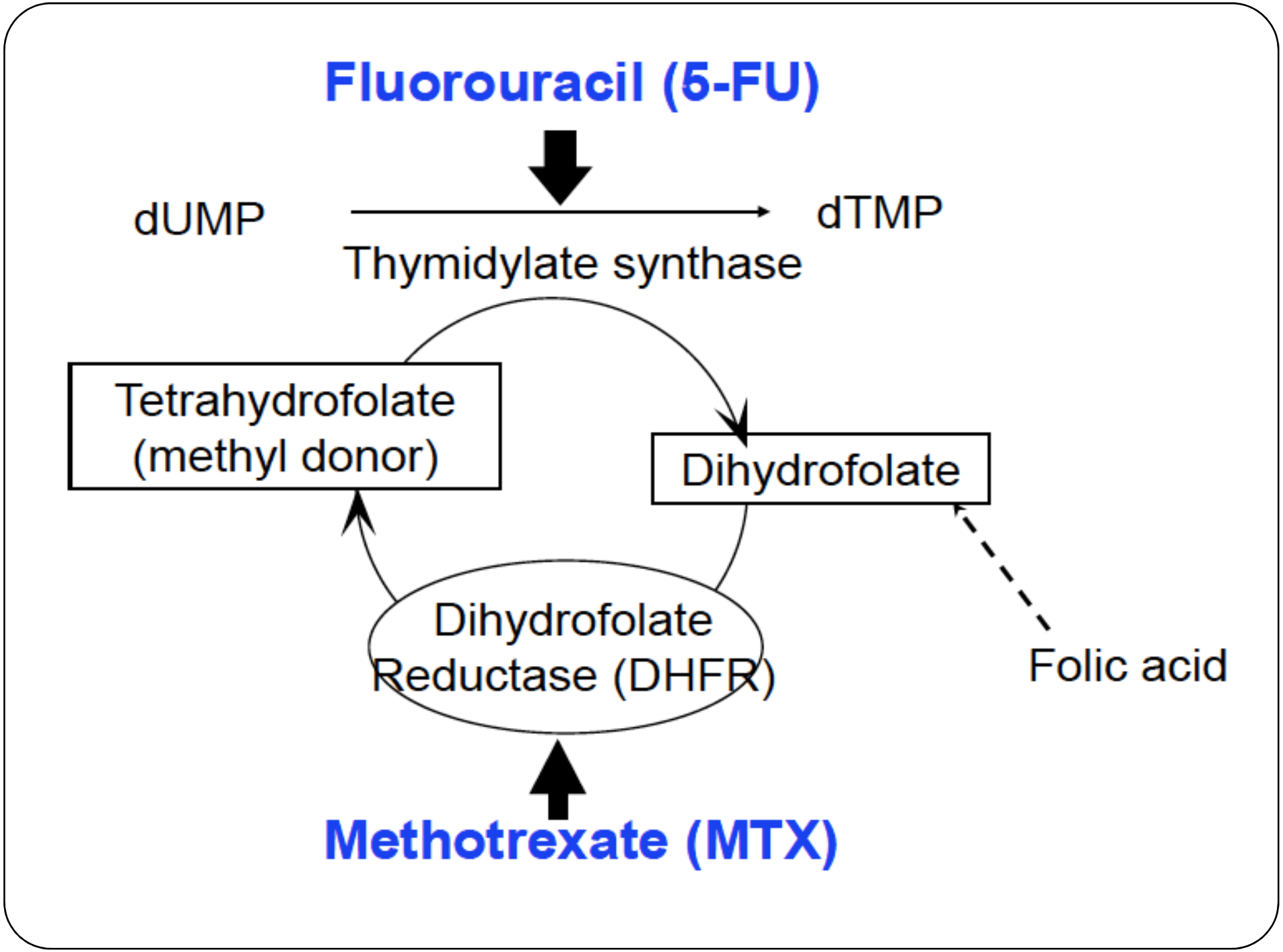
Methotrexate =Folic Acid Analogues

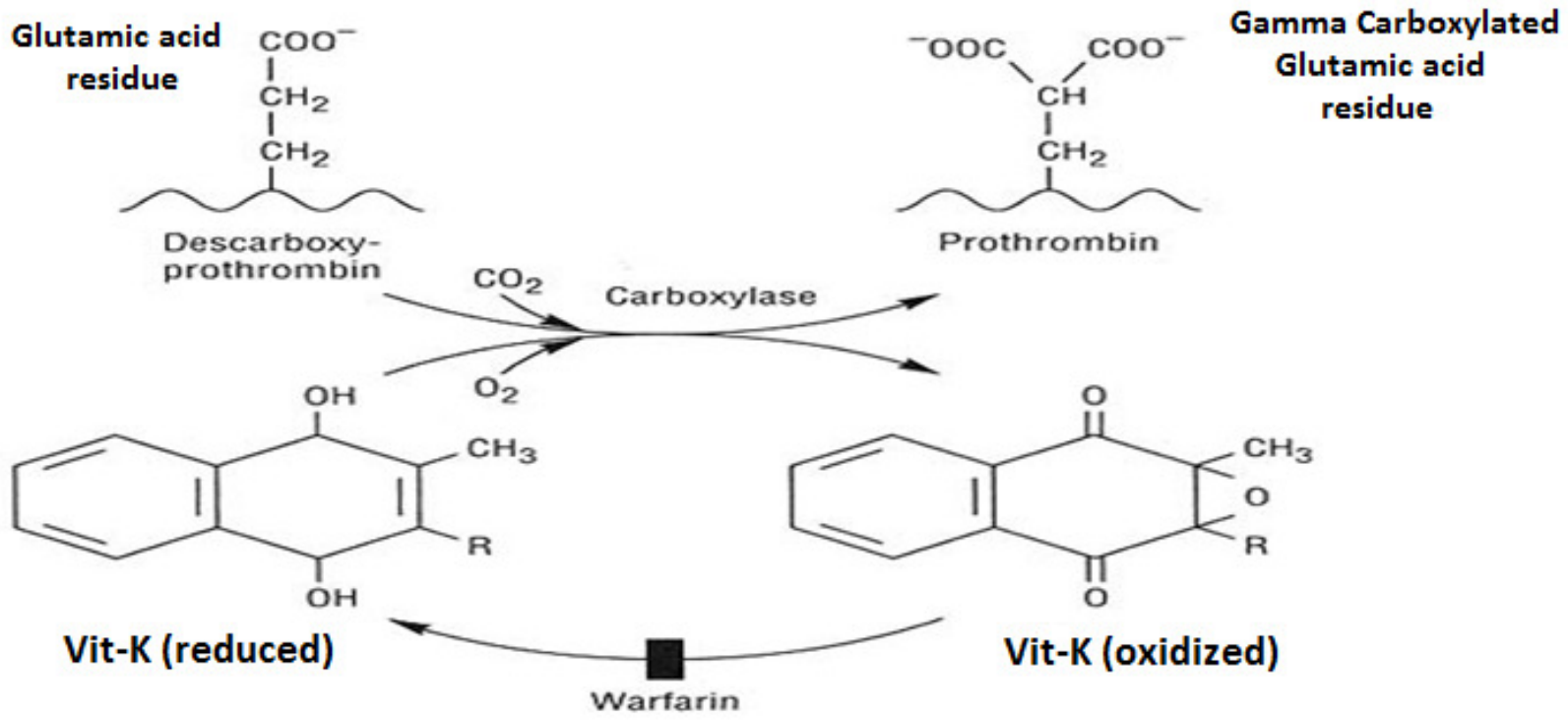


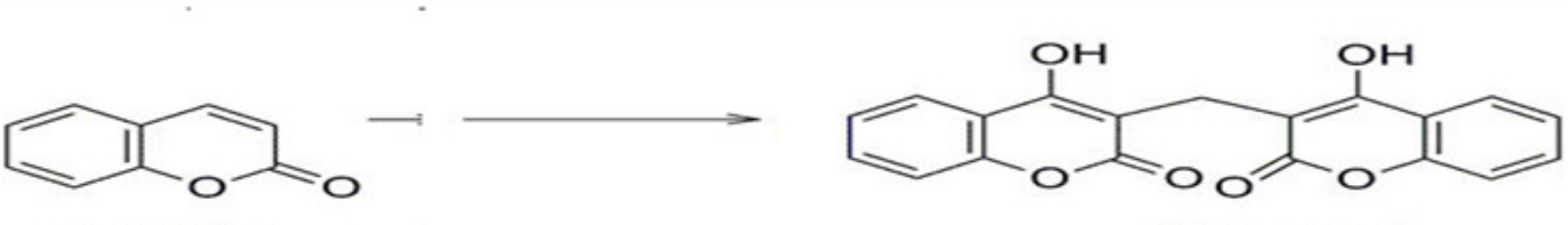
Folic acid

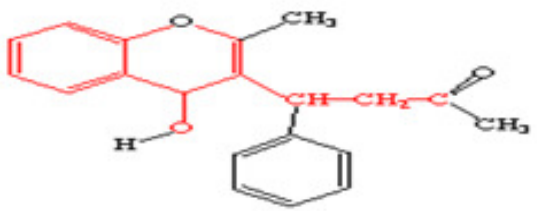


Méthotrexate


Fluorouracil (5-FU)
dUMP
dTMP
Thymidylate synthase
Tetrahydrofolate
(methyl donor)
Dihydrofolate
Dihydrofolate
Reductase (DHFR)
Folic acid
Methotrexate (MTX)







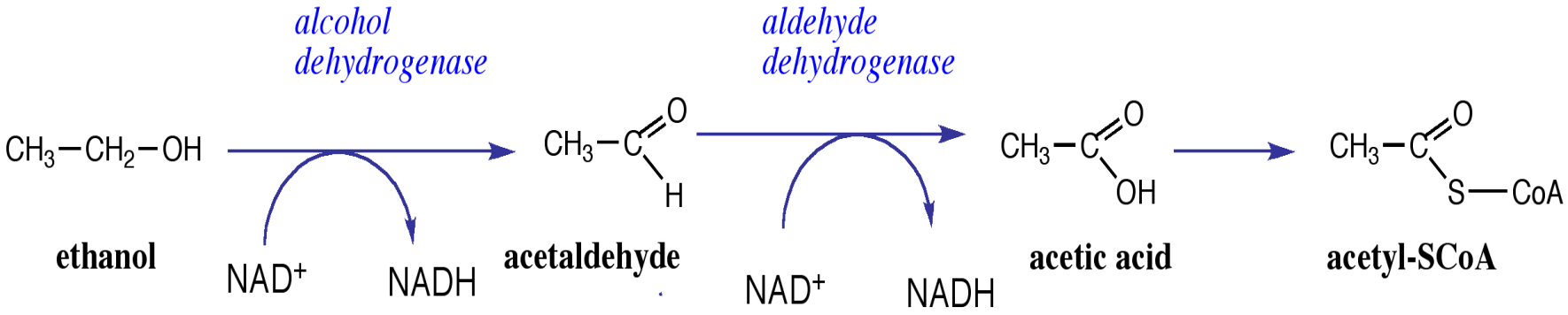
Warfarin

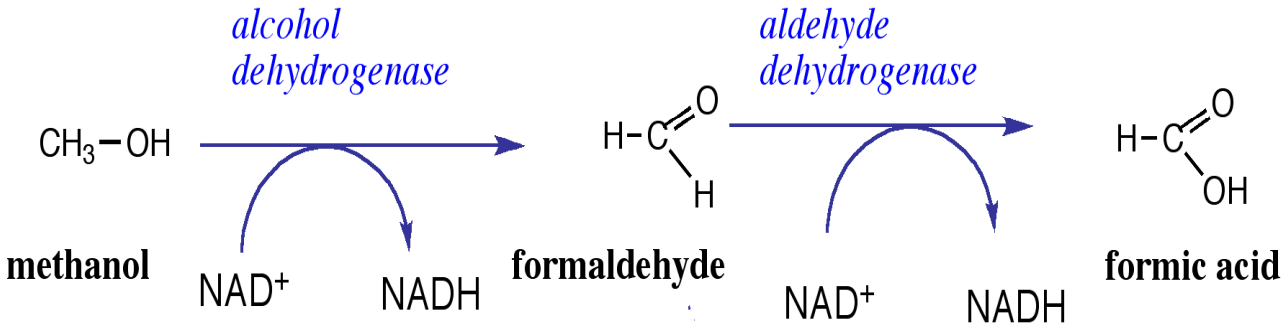
Vitamin K Analogue

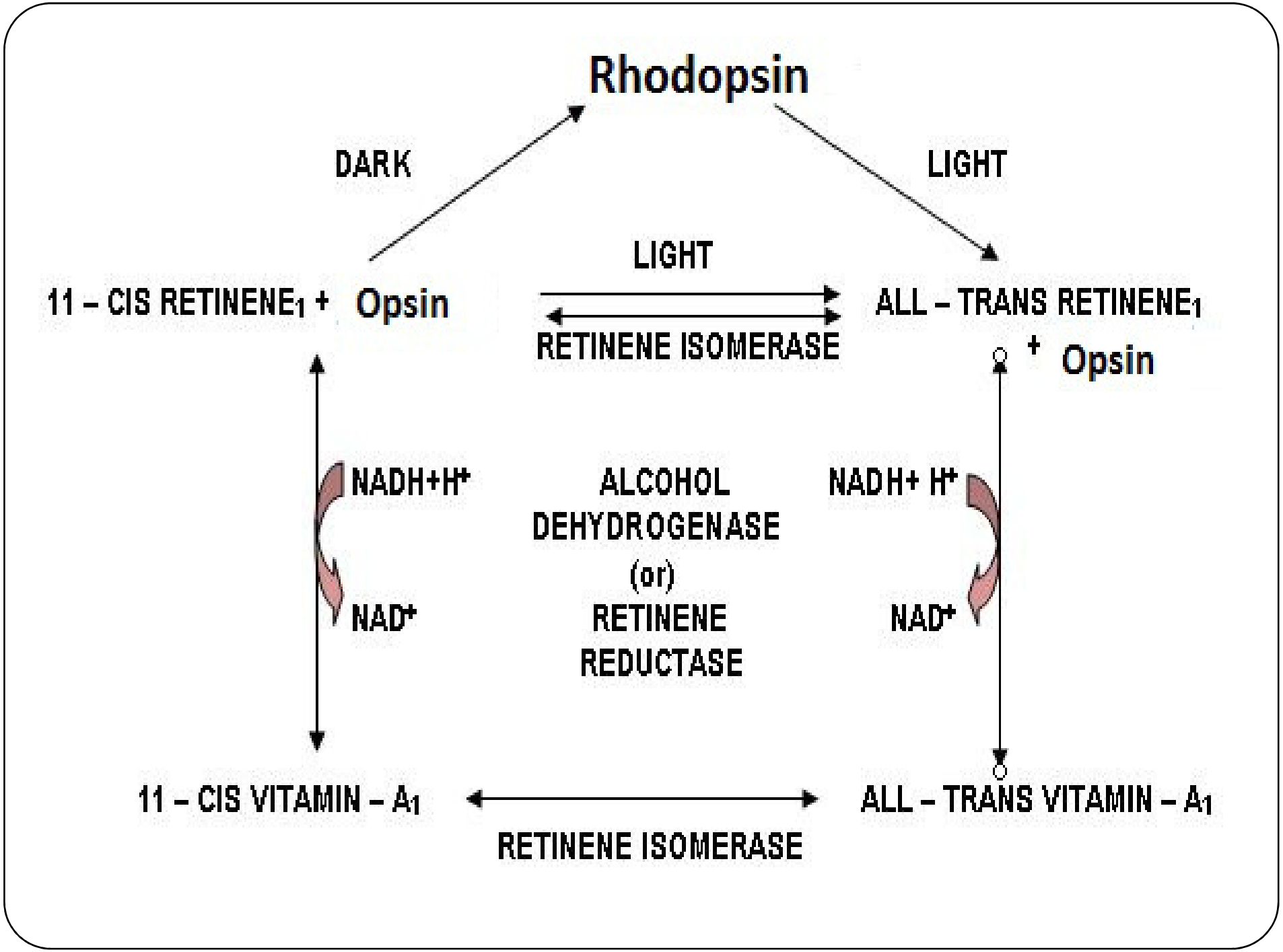
Vitamin K analogues

- **Dicoumarol and Warfarine**
  - Structurally analogues to Vitamin K.
  - Inhibit formation of reduce vitamin-k
  - So, decrease formation of carboxylation of glutamic acid of the prothrombin
  - Subsequently, activation of clotting factor is inhibited
  - So both drug work as Anti-coagulant.

Alcohol Metabolism






Rhodopsin
DARK
LIGHT
LIGHT
11 – CIS RETINENE1 + Opsin
RETINENE ISOMERASE
ALL – TRANS RETINENE1
+ Opsin
NADH+H+
NAD+
ALCOHOL
DEHYDROGENASE
(or)
RETINENE
REDUCTASE
NADH+ H+
NAD+
11 – CIS VITAMIN – A1
RETINENE ISOMERASE
ALL – TRANS VITAMIN – A1

Example : Competitive Inhibition

Sulphonamides

- Sulphonamide is analogues to PABA.
- Antibacterial agent
- In bacteria, PABA + Pteroyl glutamic acid = Folic acid (require for bacterial growth)
- Drug is non-toxic to human cells.

Statin Drugs (Atorvastatin, Simvastatin)

- HMG CoA analogues
- Inhibit HMG CoA reductase
- Decrease serum cholesterol level

Methotrexate

- Folic acid analogues
- Inhibit *dehydrofolate reductase* enzyme.
- Inhibit purine – pyrimidine synthesis
- Inhibit cell division.
- Use as Chemotherapy (Anti-cancer drug)

Example : Competitive Inhibition

Isonicotinic acid Hydrazide (INH)

- Analogue to Pyridoxal.
- Drug for tuberculosis (AKT)
- Inhibit Pyridoxal kinase enzyme
- which convert Pyridoxal into PLP.

Dicoumarol and Warfarine

- Structurally analogues to Vitamin K.
- Anti-coagulant.

Methanol

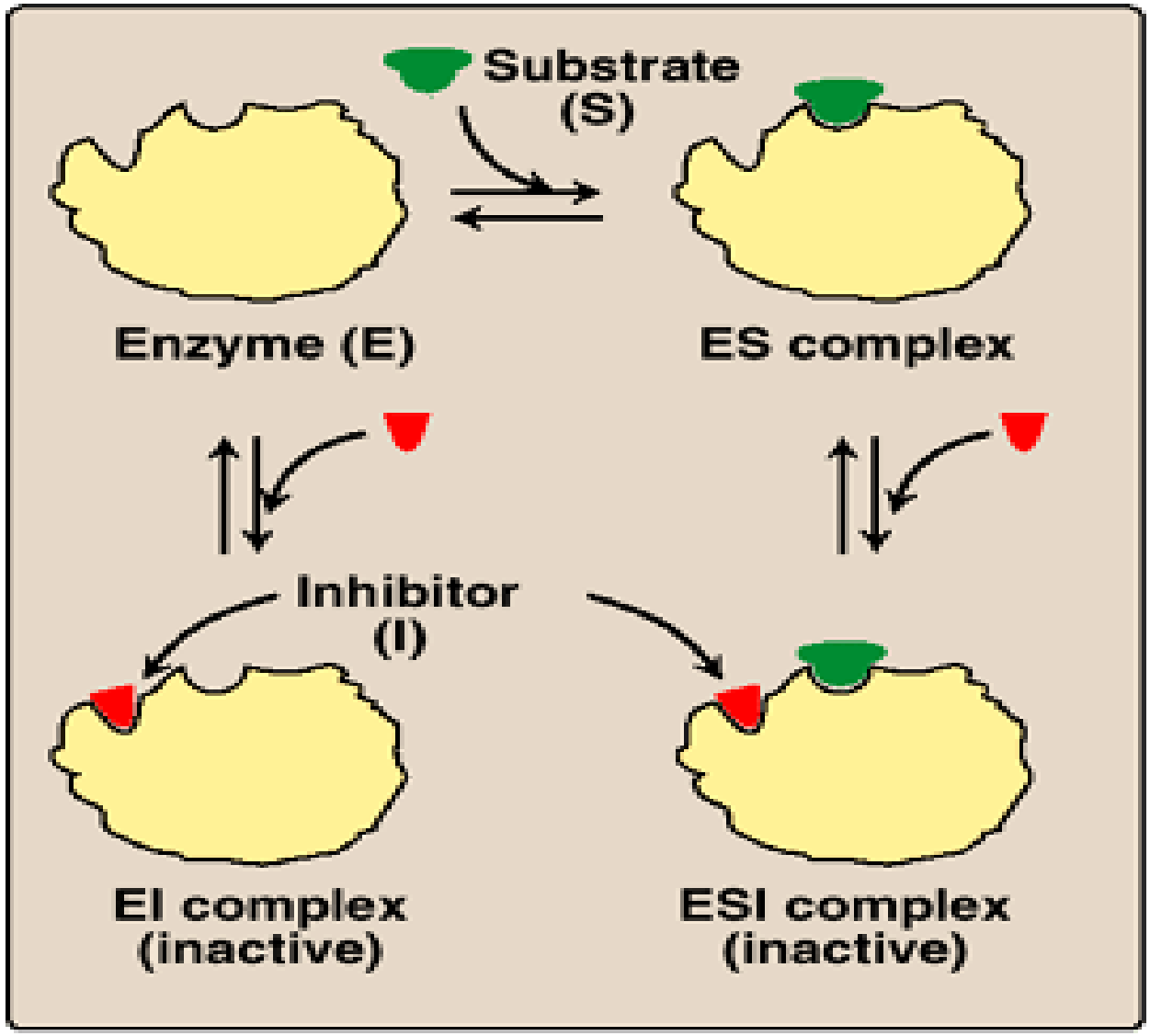
- Ethanol is analogues to methanol
- Methanol converted into formaldehyde & formic acid.
- Enzyme Alcohol dehydrogenase
- Formaldehyde causes sudden death & blindness.
- Ethanol has high affinity for ADH than Methanol
- Ethanol is use as antidote in methanol poisoning.

1. **Non-competitive**

- **Inhibitor is not analogue to substrate.**
- **Inhibitor & substrate bind at different sites**
- **Inhibitor can bind either free enzyme or ES complex.**
- **Not influenced by the conc. of the substrate.**

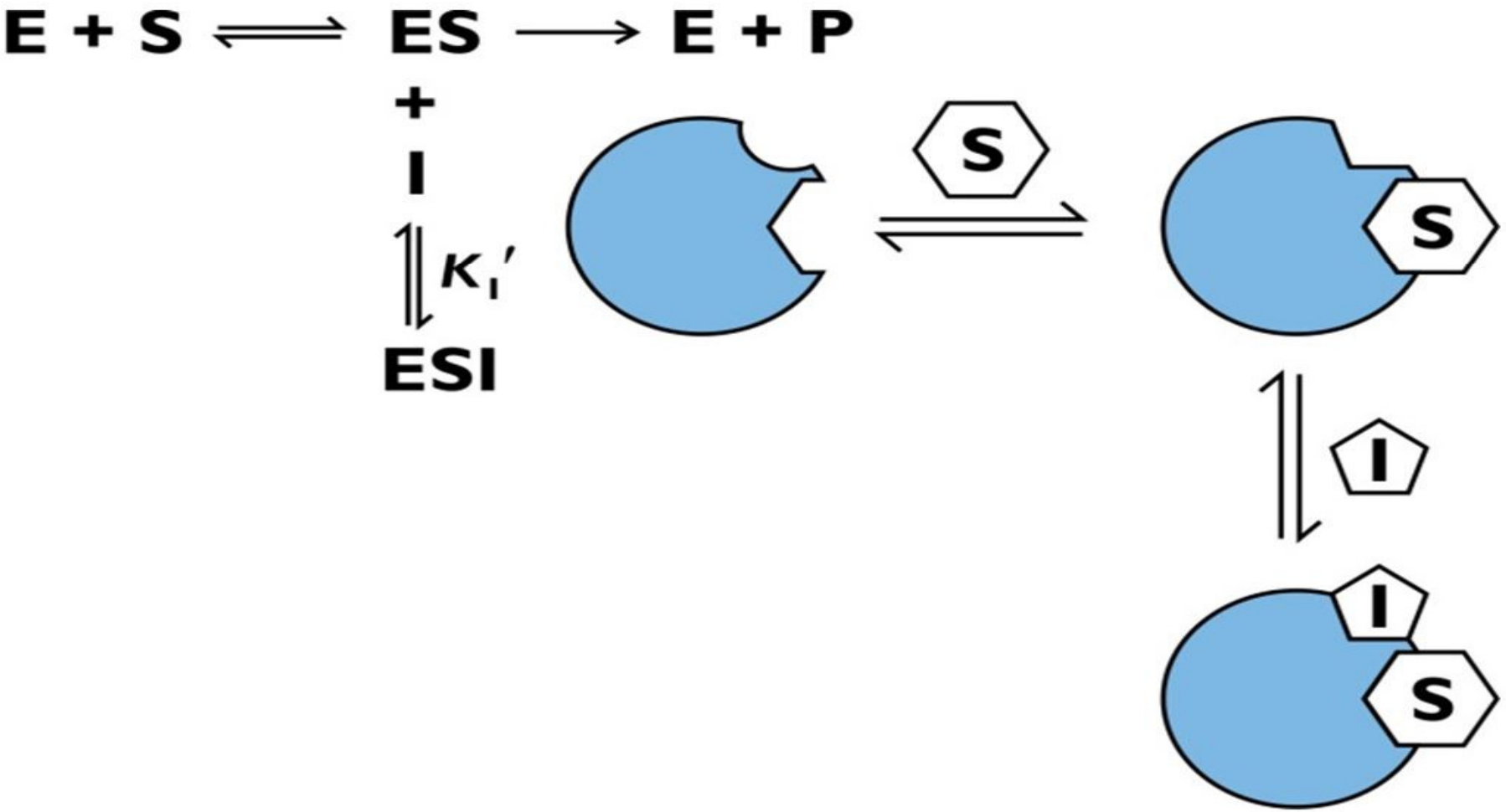
- **Vmax = Decrease**
  - **Inhibition can not overcome by substrate**
- **Km = Not changed**
  - **Not infer with substrate for active site**

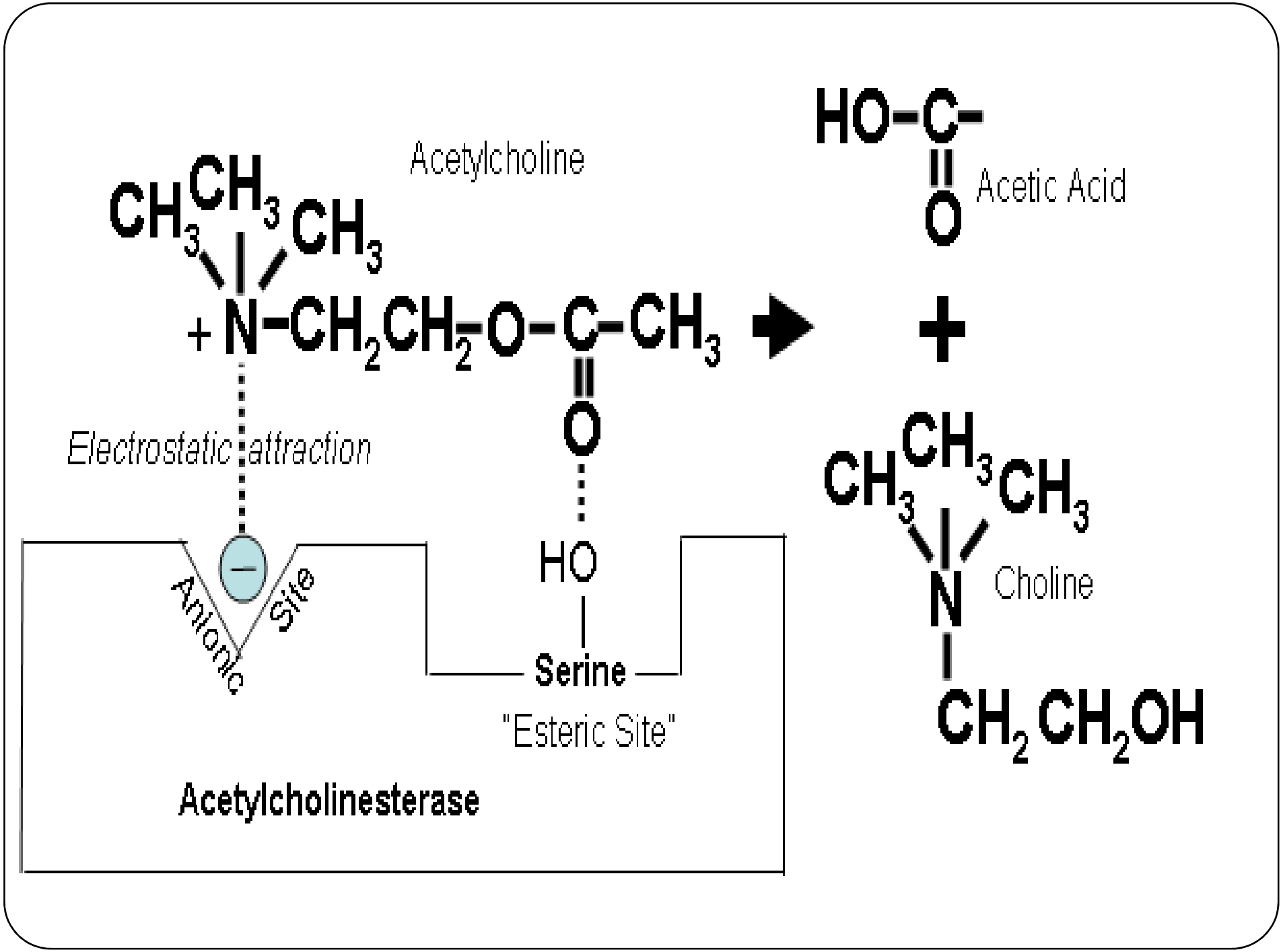
Non-competitive



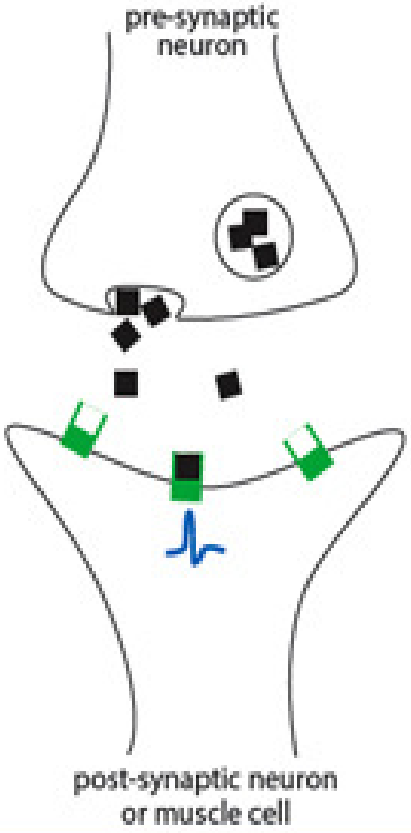
Un-competitive

- Inhibitor bind to ES complex
- Both Km and Vmax decrease



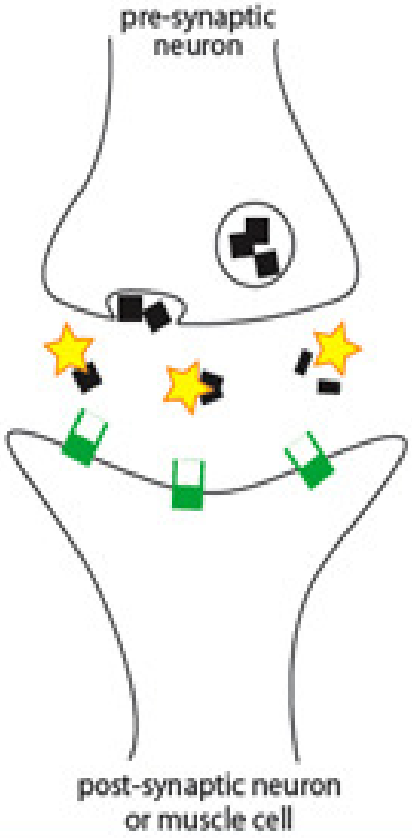

Acetylcholine
Acetic Acid
Choline
Electrostatic attraction
Anionic Site
Serine
"Esteric Site"
Acetylcholinesterase

Acetylcholine signaling at synapse



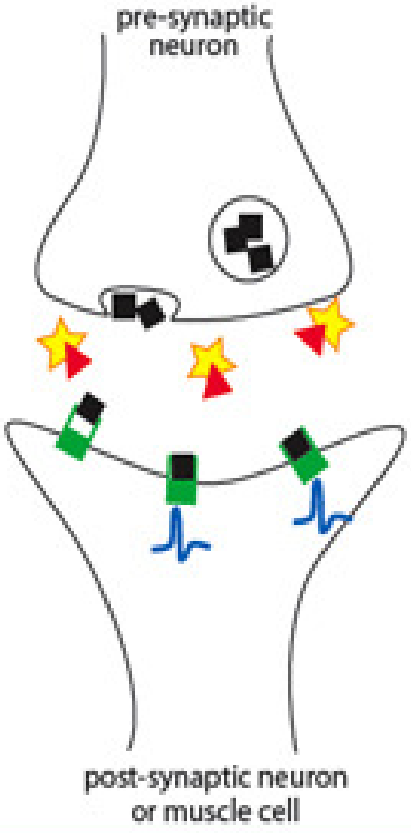
- Acetylcholine (ACh)
- ACh Receptor
- Signal transmission

ACh Esterase STOPS signaling process



- ACh
- ACh Receptor
- Signal transmission
- ACh Esterase

OP's inhibit ACh Esterase



- ACh
- ACh Receptor
- Signal transmission
- ACh Esterase
- Organophosphate pesticide (OP)

1. **Non-competitive:**

Examples

- **Cyanide** combines with the Iron in Cytochrome oxidase
- **Lead** inhibit Ferrochelate of heme synthesis.
- **Heavy metals**, **Ag** or **Hg**, combine with **–SH** groups.
- **Fluoride** inhibit Enolase, Glycolysis.
- ***Di-isopropyl fluoro phosphatase* inhibit *acetylcholine-esterase.***

These can be removed by using a chelating agent such as EDTA

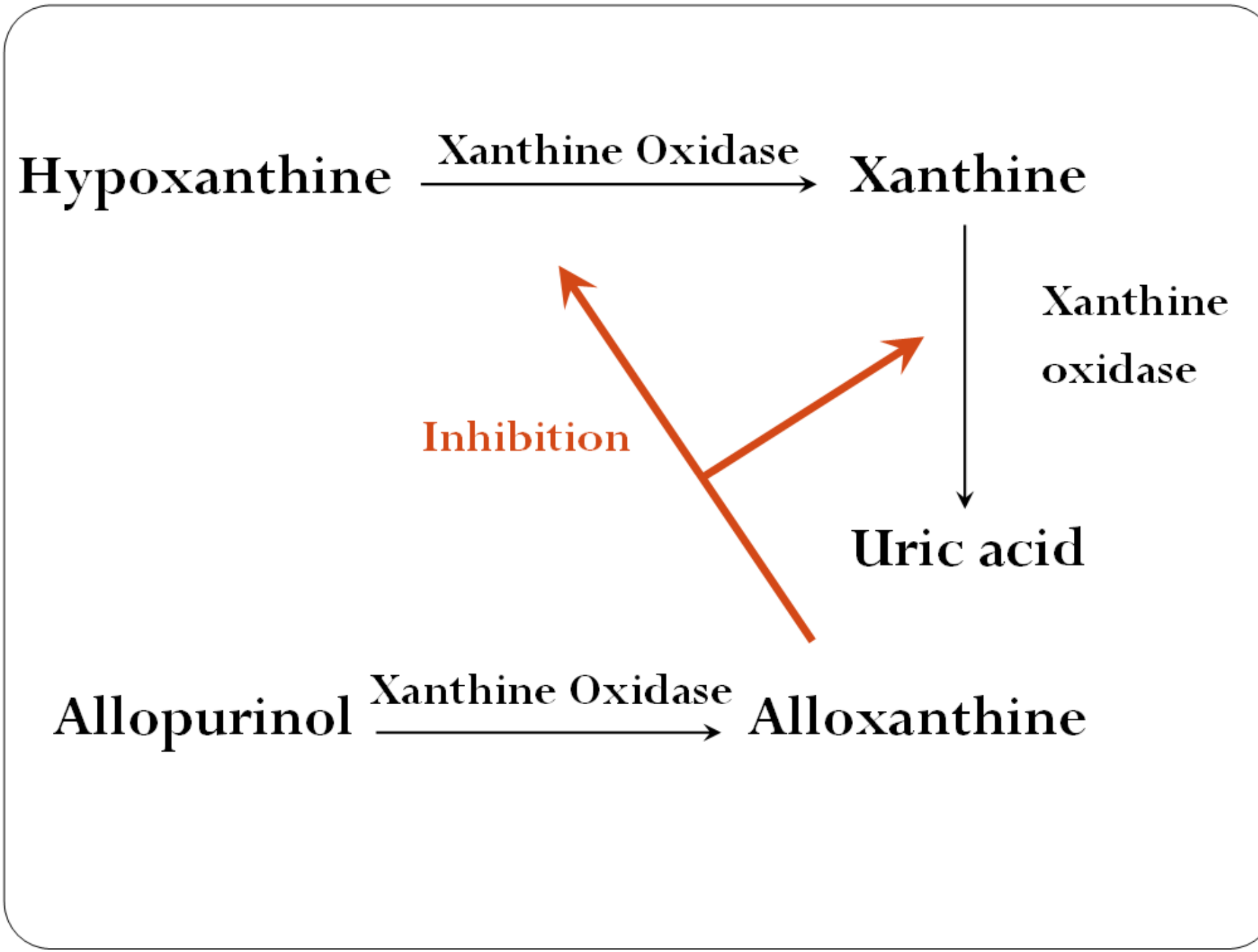
BAL (British Anti Lewisite, Dimercaprol)

- **Use as antidote for heavy metal poisoning**
- **It have several – SH group to neutralized heavy metal**

Suicide Inhibition

- Inhibitor = Structural analogues
- Make Compatitive inhibiton
- Than converted to more effective inhibitor
- Which inhibit it's own enzymatic reaction.
- **Allopurinol** = Drug for gouty arthritis.
- **Aspirin**
- **5-Fluro-Uracil**

- **Difluro Methyl Ornithine (DFMO)** = Inhibit Ornithine decarboxylase

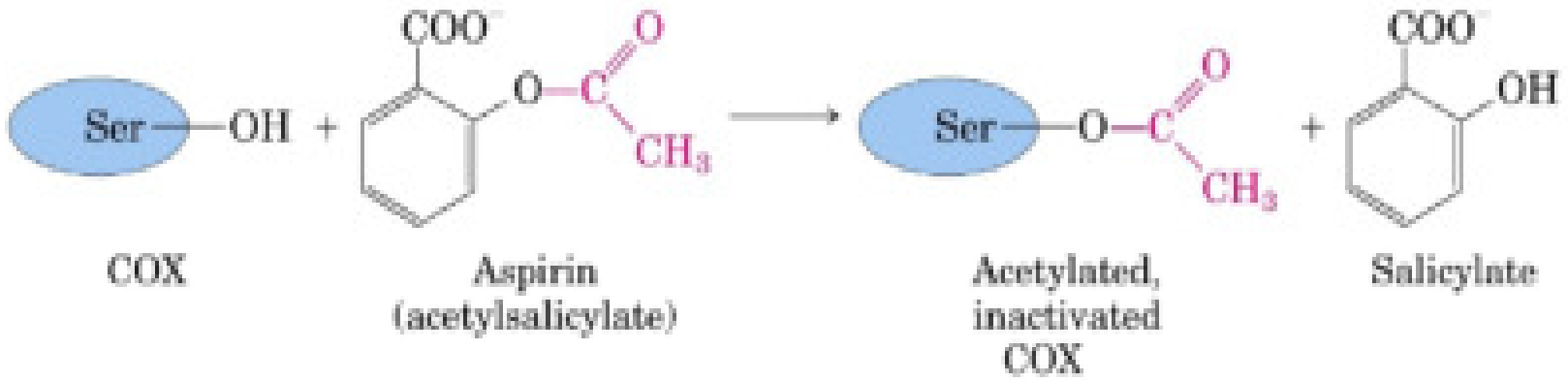

Hypoxanthine
Xanthine Oxidase
Xanthine
Xanthine oxidase
Inhibition
Uric acid
Allopurinol
Xanthine Oxidase
Alloxanthine

Vmax decrease in ,

A. Competitive inhibition
B. Non - Competitive inhibition
C. Un - Competitive inhibition
D. Suicide inhibition
E. B & C
F. All of Above

Vmax decrease in ,

A. Competitive inhibition = unchange
B. Non - Competitive inhibition = decrease
C. Un - Competitive inhibition = decrease
D. Suicide inhibition = unchange
E. B & C
F. All of Above


Ser
OH
+
COO⁻
O
O
C
CH3
Ser
O
C
O
CH3
+
COO⁻
OH
COX
Aspirin
(acetylsalicylate)
Acetylated,
inactivated
COX
Salicylate

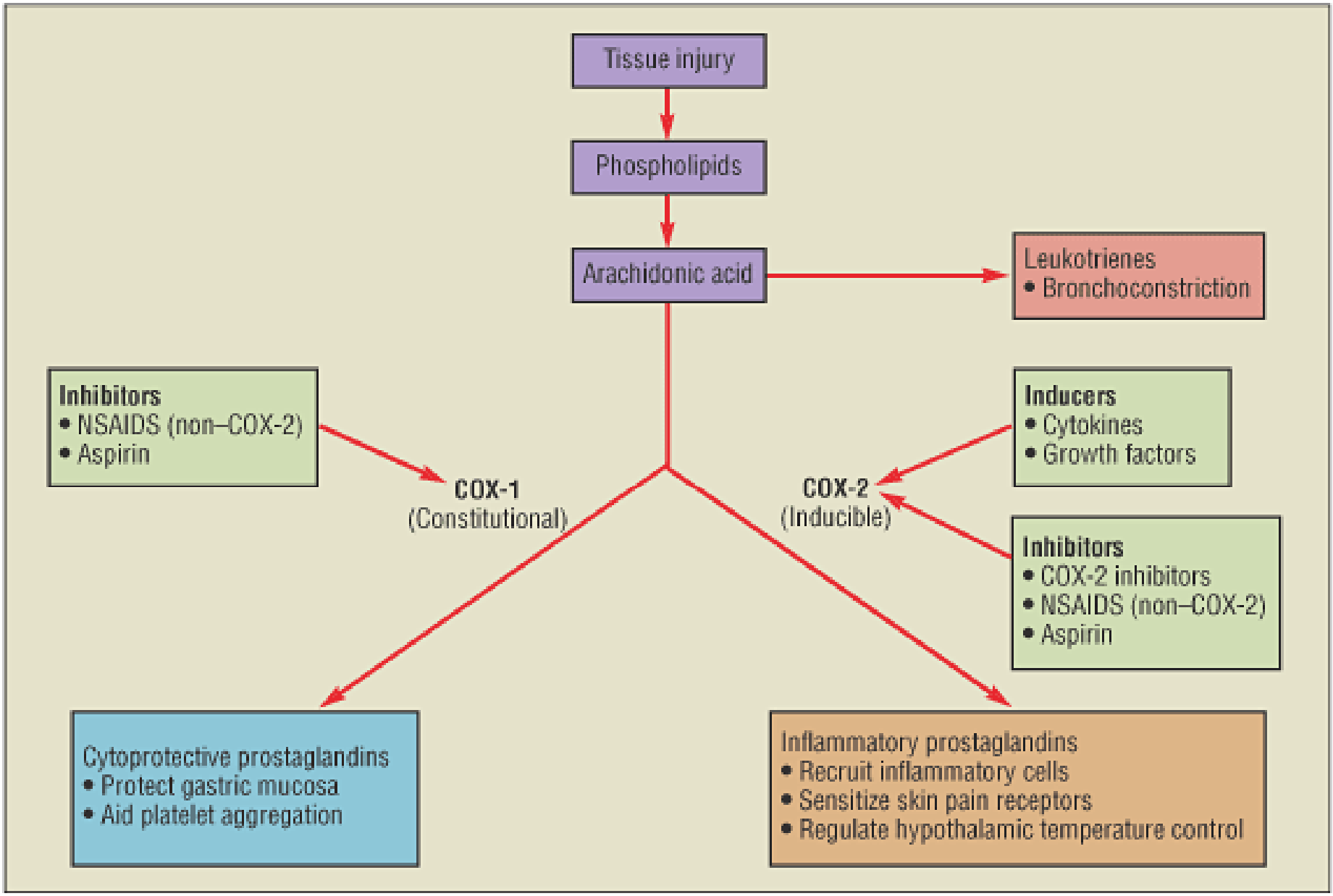
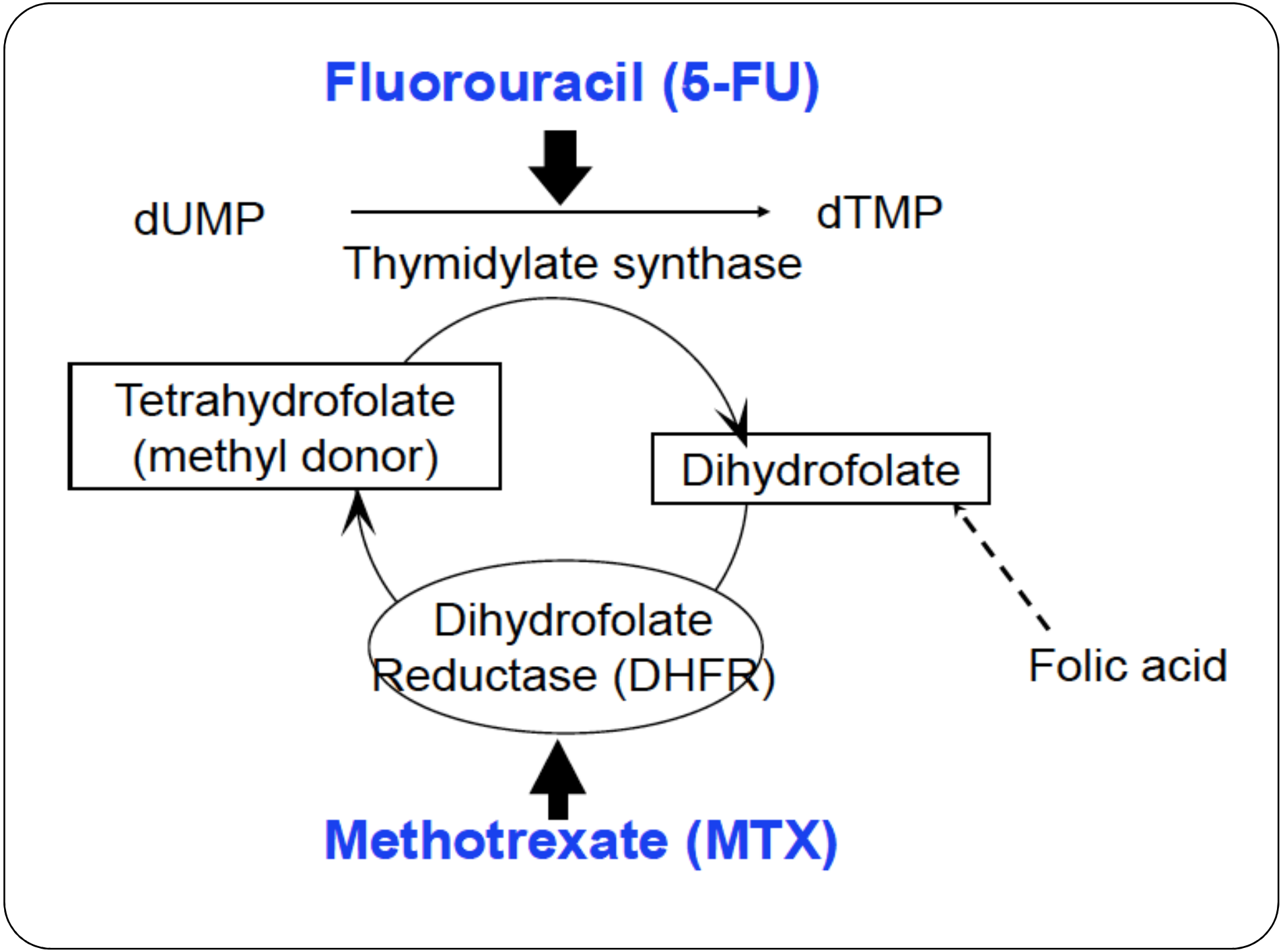
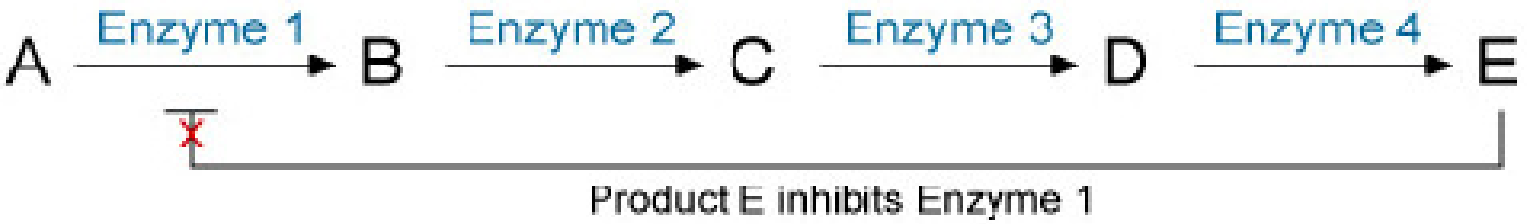


FIGURE 1. Algorithm of the biochemical pathway shows that the formation of prostaglandins occurs via both cyclooxygenase enzymes (COX-1 and COX-2).

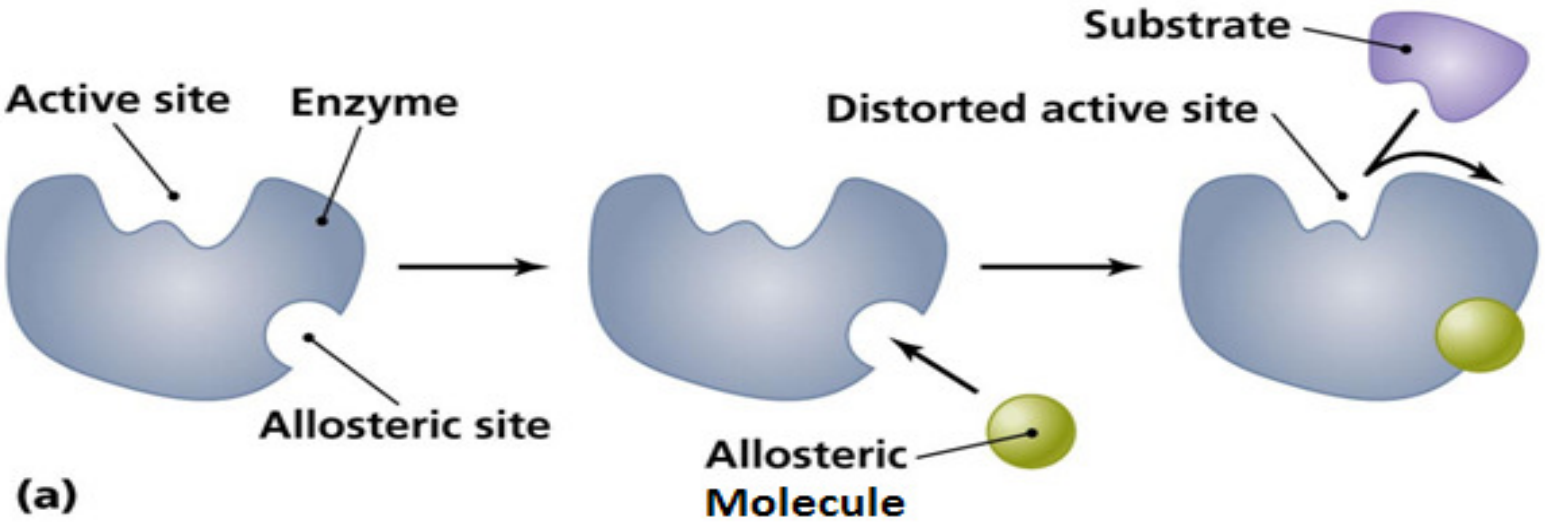

Fluorouracil (5-FU)
dUMP
dTMP
Thymidylate synthase
Tetrahydrofolate
(methyl donor)
Dihydrofolate
Dihydrofolate
Reductase (DHFR)
Folic acid
Methotrexate (MTX)

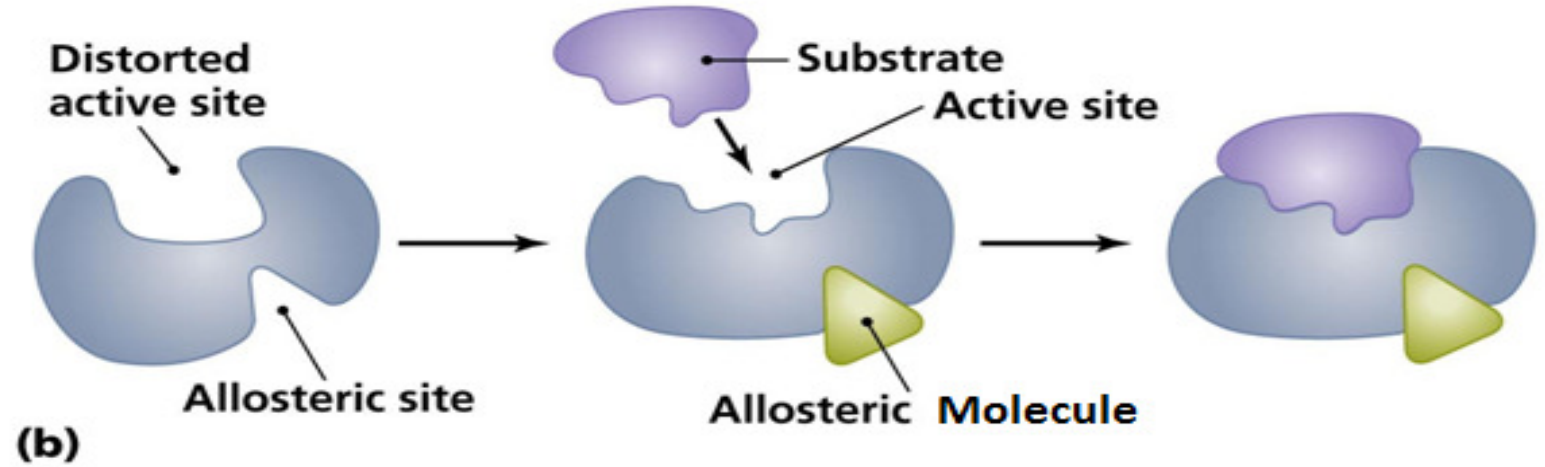
Feedback inhibition



8. Allosteric Regulation

- **EFFECTOR (MODIFIER)**
- Bind to site other than active site.
- Affect Both
    - Affinity (Km) OR
    - Catalytic activity (Vmax).
- **Negative effectors**
- **Positive effectors.**
- ***Allosteric enzyme play role as regulatory enzyme (Key enzyme , Rate limiting enzyme) in cycle of reaction.***

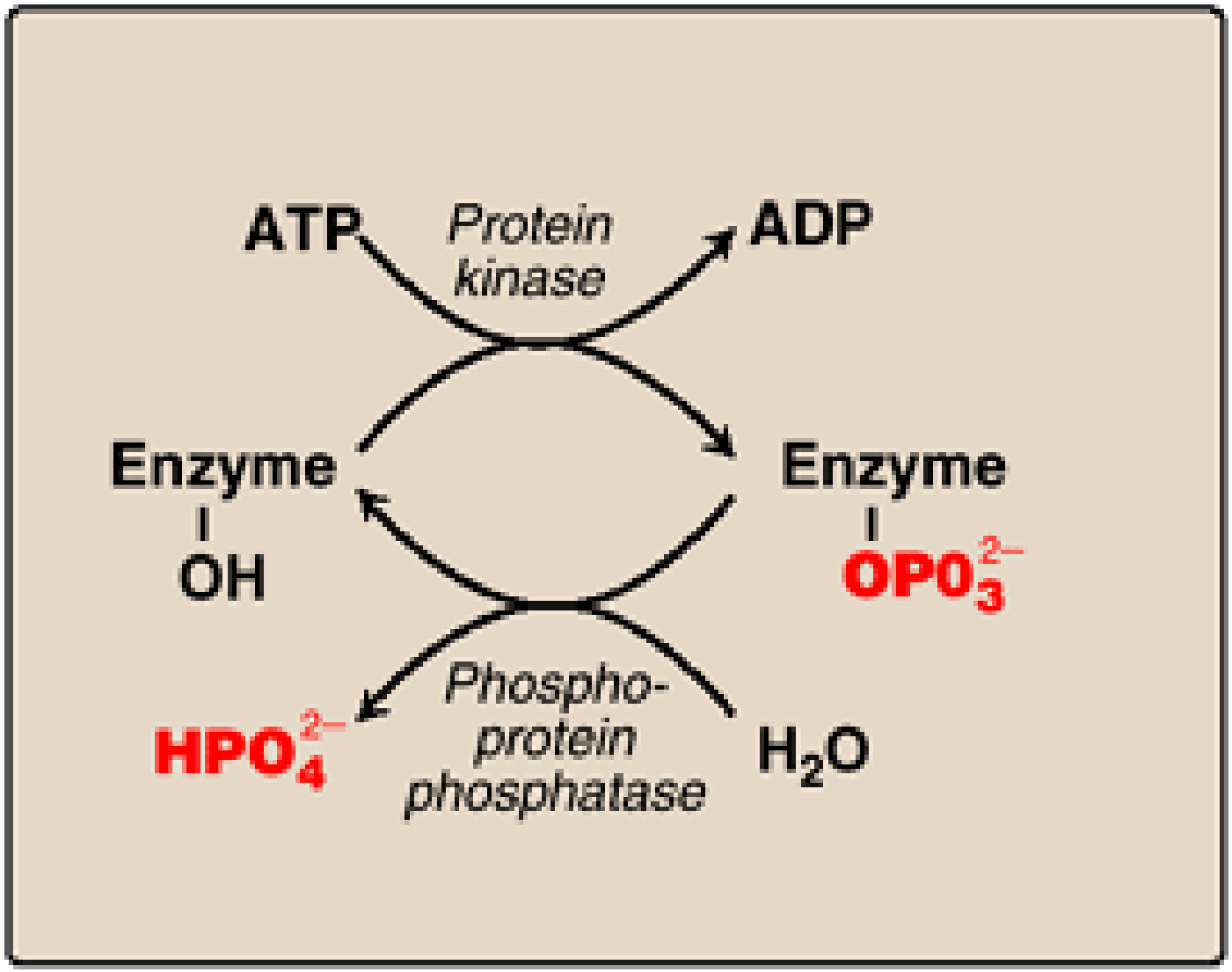

Substrate
Active site
Enzyme
Distorted active site
Allosteric site
Allosteric
Molecule
(a)


Distorted
active site
Substrate
Active site
Allosteric site
Allosteric
Molecule
(b)

9 . covalent modification

- **Most frequently by the addition or removal of phosphate groups.**
- **Phosphorylation reactions = use ATP**
    1. **Phosphorylation – Dephosphorylation**
    2. **Adenylation – Deadenylation**
    3. **Ribosylation**
    4. **Uridylation**
    5. **Methylation**

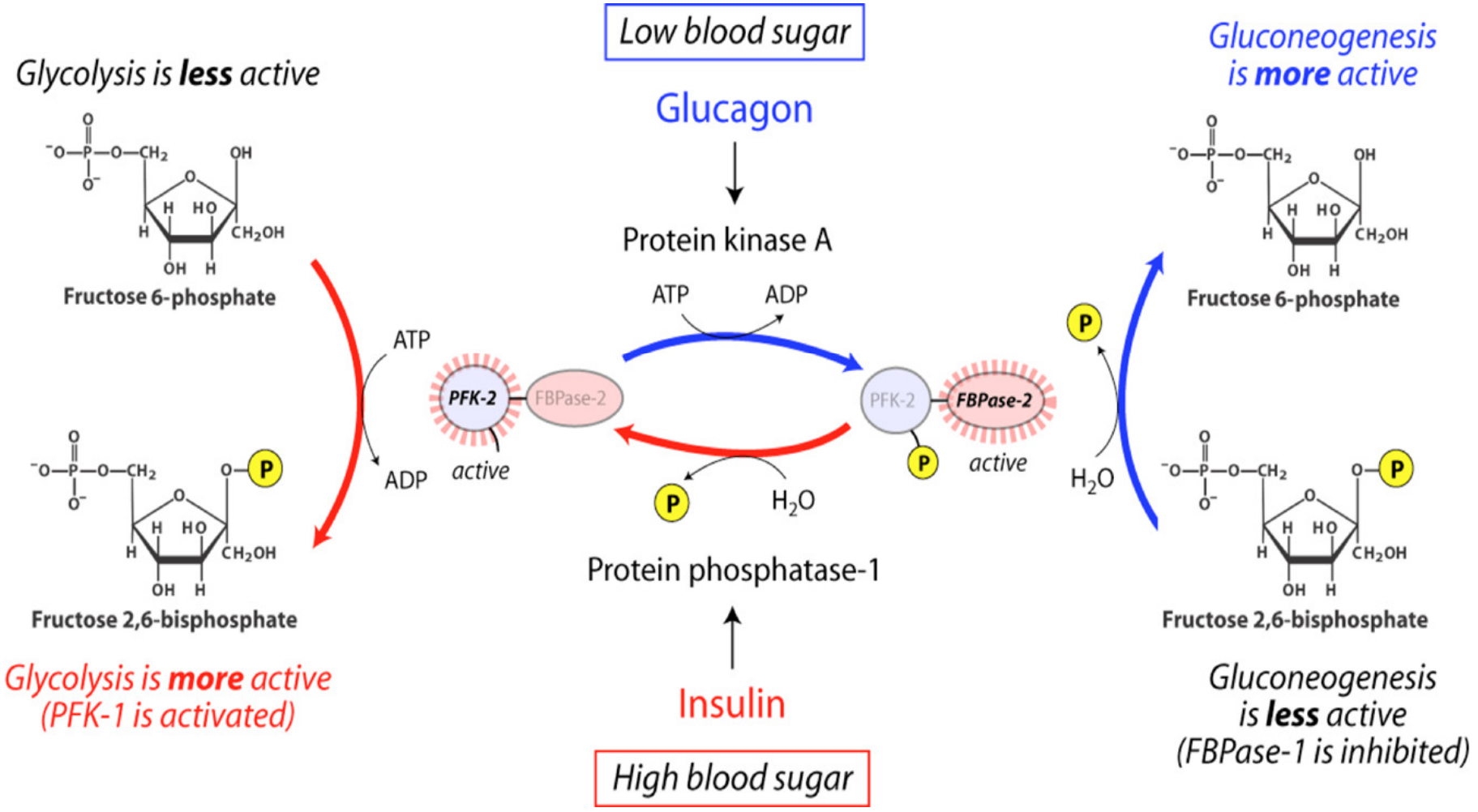
covalent modification



- **Active in Dephospharylate form**
  - PFK-1
  - Pyruvate Kinase
  - Glycogen synthase
  - HMG CoA reductase
- **Active in Phosphorylate form**
  - Fructose 1-6 bisphaphatase
  - Glycogen phosphorylase
  - Glucose 6 phosphatase

- **Active in Dephospharylate form**
- **(Decrease Glucose) (Insulin)**
  - PFK-1
  - Pyruvate Kinase
  - Glycogen synthase
  - HMG CoA reductase
- **Active in Phosphorylate form**
- **(Increase Glucose) (Glucagon)**
  - Fructose 1-6 bisphaphatase
  - Fructose 2-6 bisphaphatase
  - Glycogen phosphorylase
  - Glucose 6 phosphatase

Regulation of Glycolysis by Covalent modification



Which of following enzyme is active in phosphorylated form?

A. Hexokinase
B. Phosphofructokinase – 1
C. Fructose 1 – 6 bisphosphatase
D. Glycogen synthase

Which of following enzyme is active in phosphorylated form? (Increase Glucose)

A. Hexokinase (decrease Glucose)

B. Phosphofructokinase – 1 (decrease Glucose)

C. Fructose 1 – 6 bisphosphatase (increase Glucose)

D. Glycogen synthase (decrease Glucose)

10. Induction & 11. Repression

- Regulate the amount of enzyme activity.
- Efficiency of enzyme = Not affected.
- Act at Gene level.
- **Altering rate of enzyme synthesis**.
- Increase enzyme synthesis = ***Induction***
- Decrease enzyme synthesis = ***Repression***
- Induction / Repression = Slow **(hours to days)**
- Allosteric regulation = Fast **(seconds to minutes)**

Example

- ALA synthase (key enzyme of Heme synthesis)
- Autoregulated by the heme
- Heme act as repressor molecule on ALA synthase gene

Induction of ALA synthase , due low haemoglobin level can increases,

A. ALA synthase Vo
B. ALA synthase Kcat/Km
C. ALA synthase concentration
D. All of Above

Induction of ALA synthase , due low haemoglobin level can increases,

A. ALA synthase Vo
B. ALA synthase Kcat/Km
C. ALA synthase concentration
D. ALA synthase Km

12. Compartmentalisation

- Certain enzymes are present
  - In mitochondria &
  - In cytoplasm.
- Some of Cycle occurs in Both Mitochndria & Cytoplasm,
  - Urea cycle
  - Heme synthesis
  - Gluconeogenesis.

Iso-enzyme
and
Clinical Enzymology

What is matching with Isoenzyme?

A. Same Chemically form
B. Same Physical characteristic
C. Does same catalytic reaction
D. Has single polypeptide unit

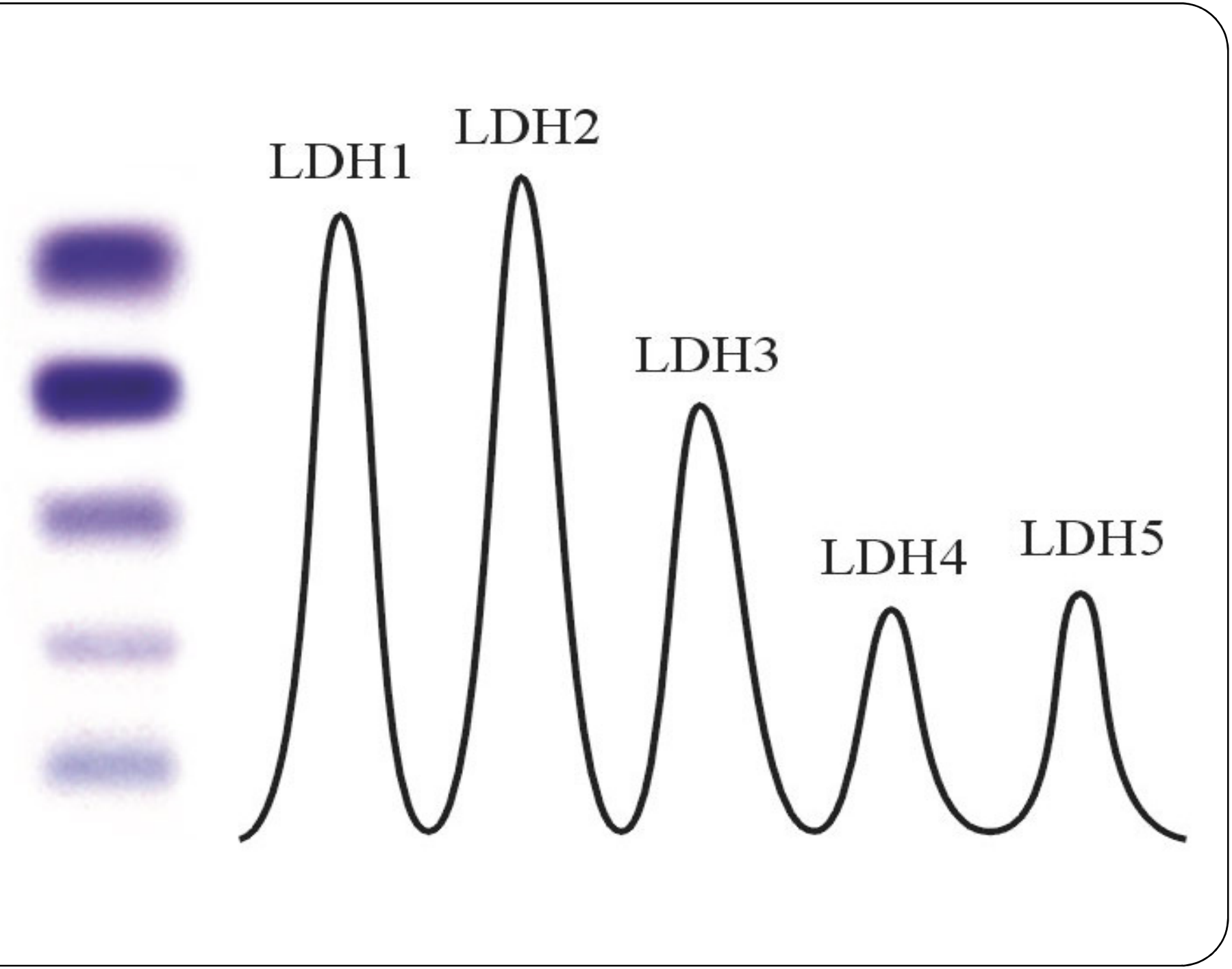
ISOENZYMES

- Catalyze the same reaction
- Two or more polypeptide chains
- Different polypeptide chains are products of different genes
- Differ in AA sequence and physical properties
- May be separable on the basis of charge
- Are tissue specific
- “They are physical distinct forms of the same enzyme activity”

Identification of Iso-enzymes

1. Electrophoresis
2. Heat stability
3. Inhibitors
4. Substrate specificity , Km value
   - e.g. Hexokinase & Glucokinase
5. Cofactor requirement
   - e.g. Mitochondrial ICD – NAD^+ dependent Cytoplasmic ICD – $NADP^+$ dependent
6. Tissue location
7. Specific antibody

Type of LDH	Composition	Fraction of LDH in %	Location
LDH 1	HHHH	20-30 %	Myocardium
LDH 2	HHHM	30-40%	RBC
LDH 3	HHMM	20-25%	Lung
LDH 4	HMMM	10-15%	Kidney & Pancrease
LDH 5	MMMM	5-15%	Skeletal muscle & Liver


LDH1
LDH2
LDH3
LDH4
LDH5

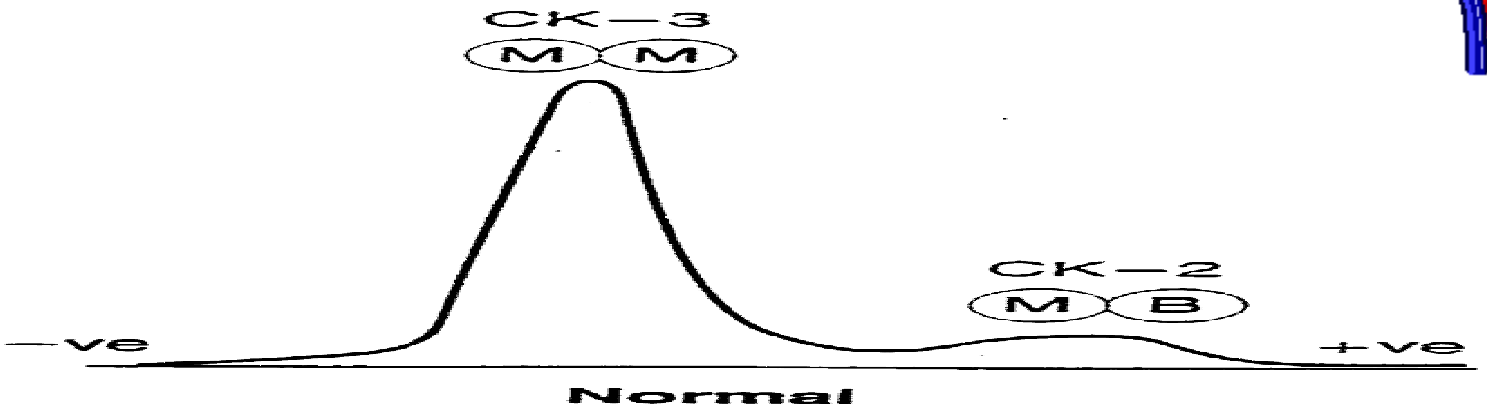
Creatine Kinase - Dimer

Type of CK	Composition	Location
CK- 1 (CK-BB)	BB	Brain
CK- 2 (CK-MB)	MB	Myocardium
CK- 3 (CK-MM)	MM	Skeletal Muscle

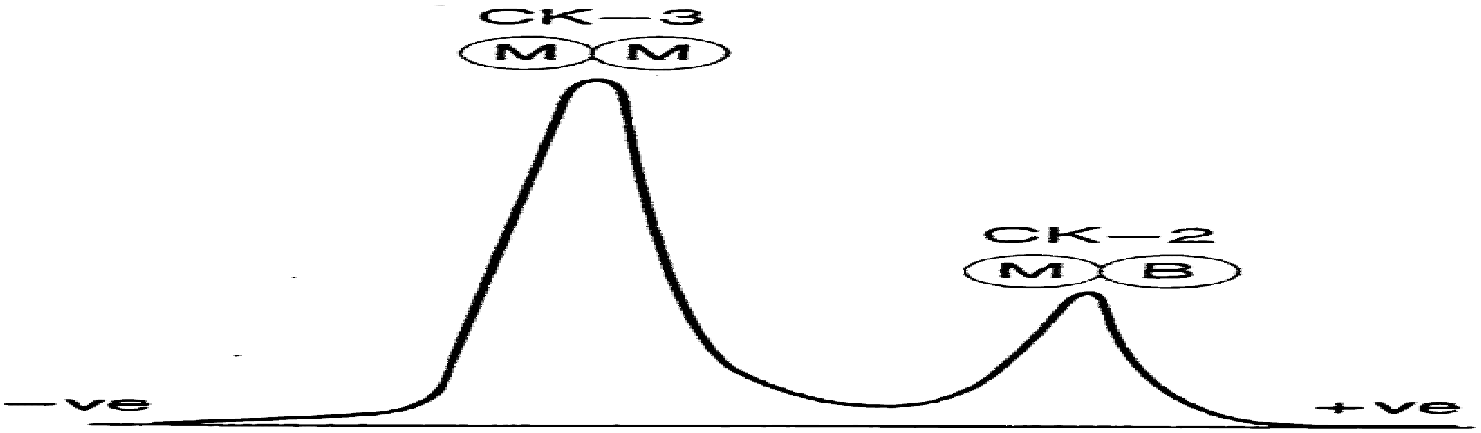
CK-2 & CK-3 in normal subject &

After 24 hours of Myocardial Infarction

Creatine Kinase isoenzymes in blood

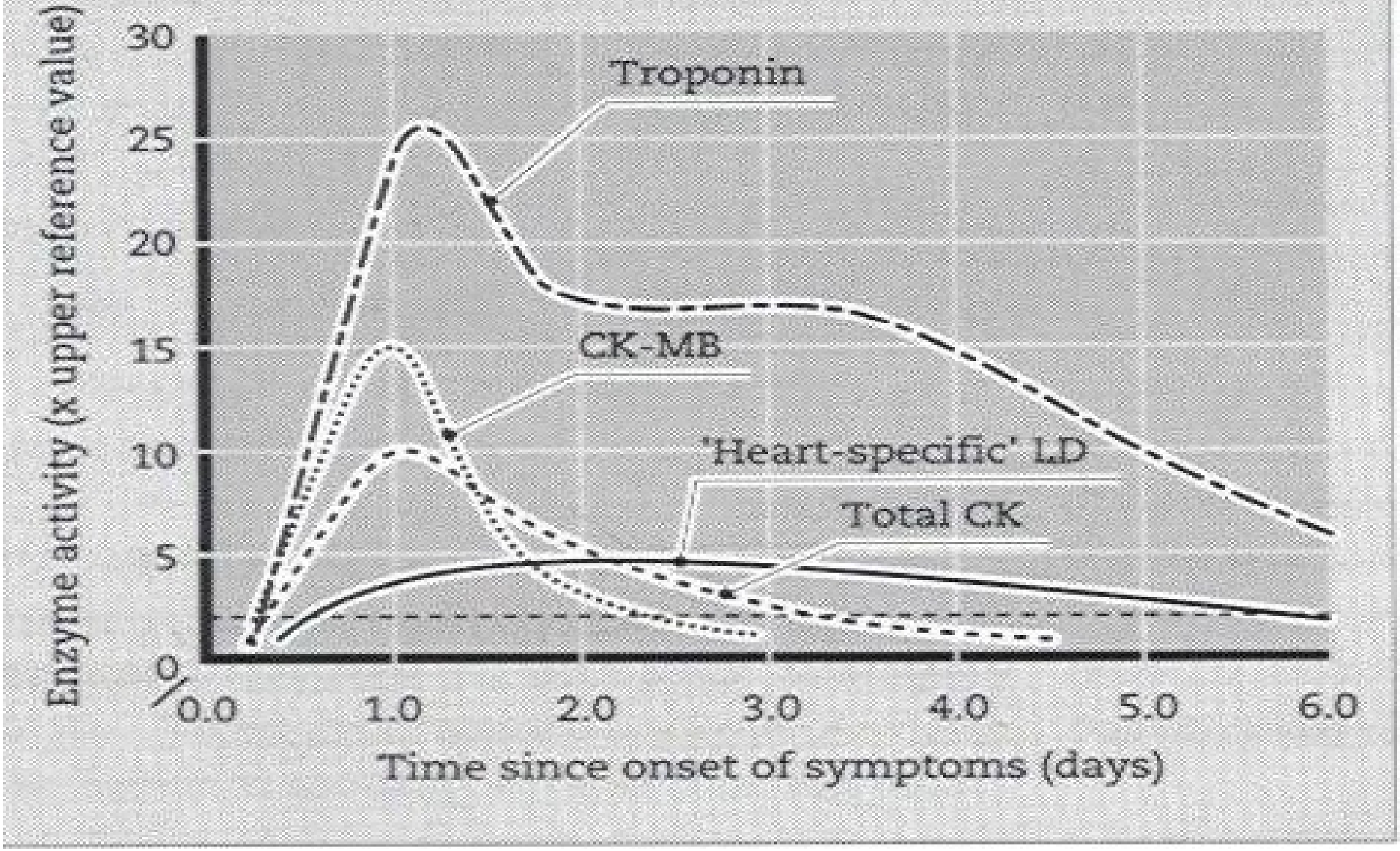


Normal



Patient 24 hrs after myocardial infarction

ENZYME ACTIVITY AFTER MYOCARDIAL INFARCTION



In Acute myocardial infaction, which of following enzyme rises?

A. LDH- 1 & CK – MM
B. LDH- 2 & CK – MM
C. LDH -1 & CK – MB
D. LDH- 2 & CK – MB

Plasma Enzymes Changes After Myocardial Infarction

Enzyme	Abnormal activity (hour)	Peak value (hour)	Duration (days)
C-Troponin I (not enzyme)	**4 - 6**	12 – 24	**10 - 14**
CK-MB	**4 - 6**	12 - 24	1.5 - 3
Total CK	6 - 12	18 - 30	2 - 5
AST(GOT)	6 - 12	20 - 30	2 - 6
LDH-1	8 - 18	30 - 48	5 - 14

In Acute myocardial infaction, which enzyme is considered as specific marker for diagnosis?

A. **LDH**
B. **AST**
C. **CK-MB**
D. **Cardiac Troponin – I**

Isoenzymes of Alkaline Phosphatase

Depending on number of sialic acid residue

1. Alpha – 1 ALP (10%)	**Biliary Canaliculi**
2. Alpha – 2 heat labile ALP (25%)	**Hepatic cells**
3. Alpha – 2 heat stable ALP (1%)	**Regan Isoenzyme Placental cell**
4. Pre – beta ALP (50%)	**Bone disease**
5. Gamma – ALP (10%)	**Intestinal cells**
6. Leucocyte ALP	**Leucocyte**

- **Decrease in chronic myeloid leukemia**
- **Increase in lymphoma**

Organ Specific Enzyme	
Heart	CK-MB , AST (GOT) , LDH
Liver	ALT , AST , LDH , Alkaline Phosphatase Gamma Glutamyl Transferase
Pancrease	Lipase ,Amylase
Muscle	Aldolase , CK-MM , CK-Total , AST
Bone	Alkaline Phosphatase
Prostate	Acid Phosphatase (Prostate isoform – inhibited by Tartrate)
RBC	LDH Acid Phosphatase (Erythrocyte isoform – inhibited by formaldehyde & cupric ion)

Diagnostically Important Enzyme	Principal Sources
Alanine aminotransferase(ALT)	Liver
Aspartate aminotransferase(AST) I (cytosol) & II (mitochondria)	Liver, Gall Bladder, Erythrocytes Skeletal muscle, Heart, Kidney,
Gamma Glutamyl Transferase	Hepatobilliary tract, Kidney
5' Nucleosidase	Hepatobiliary tract
Alkaline Phosphatase (ALP)	Bone, Gall Bladder ,Liver, Intestinal mucosa, Placenta, Kidney
Acid Phosphatase	Prostate, Erythrocytes
Amylase	Pancrease ,Salivary glands,Ovaries
Lipase	Pancrease

For diagnosis of Acute Viral Hepatitis,

Which of the following enzyme is specific ?

A. ALT

B. AST

C. Alkaline Phosphatase

D. Gamma Glutamyl transfarase

For diagnosis of Acute Viral Hepatitis,

Which of the following enzyme is specific ?

A.	ALT	=Liver
B.	AST	=Liver, Gall Bladder, Heart
C.	Alkaline Phosphatase	=Bone ,Liver,Gall Bladder
D.	Gamma Glutamyl transfarase	=Liver (induce by Drug & Alcohol), Kidney

Enzyme as Therapeutic Agents

1. **Streptokinase & Urokinase**
   - **Lysis intravascular clot**
   - **Use in myocardial infarction**
2. **Pepsin & Trypsin**
   - **Use in patient having indigestion**
3. **Asparaginase**
   - **Used as anticancer drugs.**

Enzyme as Diagnostic Agents

1. **Glucose oxidase & Peroxidase (GOD-POD)**
2. **Urease**
3. **ELISA test**
4. **Restricted Endonuclease**


Thank you