1. What is enzyme inhibition? Describe different kinds of enzyme inhibitions with example (SN-Feb 11)

Inhibition of catalytic activity of enzyme is called enzyme inhibition, and the inhibiting molecules are called enzyme inhibitors (I). Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for study of the mechanism of enzyme action.

Enzyme inhibitions classified based on Inhibitor site of action on the enzyme, on whether or not they chemically modify the enzyme, or on the kinetic parameters they influence. Kinetically, we distinguish two classes of inhibitors based upon whether raising the substrate concentration does or does not overcome the inhibition.

Enzyme broadly classified into 3 categories –

I. Reversible inhibition – which include Competitive, non-competative and uncompetitive inhibition
II. Irreversible inhibition
III. Allosteric inhibition/regulation

I. Competitive inhibition:

The inhibitor molecules resembles like substrates thus called substrate analogs. Inhibitor binds the catalytic site of enzyme and blocks its activity. The effects of competitive inhibitors can be overcome by raising the concentration of the substrate.

Eg: Succinate dehydrogenase catalyses the removal of one hydrogen atom from each of the two methylene carbons of succinate. Both succinate and its structural analog malonate can bind to the active site of succinate dehydrogenase, forming an ES or an EI complex, respectively. However, since malonate contains only one methylene carbon, it cannot undergo dehydrogenation.

In competitive inhibition Vmax will be lowered and Km value is increases

![Lineweaver-Burk plot](image)
Non-competitive inhibition: the inhibitor binds at a site of the active site on enzyme. This binding impairs enzyme activity. Here the inhibitor is not structural analog, but has strong affinity towards enzyme. Inhibitors do not interfere with enzyme-substrate formation. But it disrupts the formation of enzyme-product complex. In this case Vmax is lowered but Km does not change.

Eg: heavy metal ions – Hg, Pb

Uncompetitive inhibition: it is rare, here inhibitor does not bind with enzyme but it binds with enzyme-substrate complex. In this type of inhibition both Vmax and Km are decreased.

II. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible.

Eg: toxic compounds such as

Iodoacetate is irreversibly bind to –SH groups of papain and glyceraldehyde 3-P dehydrogenase and inactivates them.

Diisopropyl fluorophosphates (DFP) is known as nerve gas, developed by germanscientists during II World war. It irreversibly binds to serine proteases, acetylcholine esterase

III. Allosteric inhibition

In Gk “allo” mean other. Some of the enzymes have additional sites for binding allosteric molecule is known as allosteric sits, which decides the enzyme activity. Such enzymes are called as allosteric enzymes. The molecules which bind these sites and alter the activity of enzyme are called as allosteric modulators. Hence this type of inhibition called as Allosteric regulation

Positive allosteric modulator: This increases the enzyme activity

Negative allosteric modulator: This decreases the enzyme activity

Types of Allosteric enzymes:

K-Class: the allosteric modulator alters the Km not the Vmax

V-Class: the modulator alters the Vmax but not the Km
<table>
<thead>
<tr>
<th>S.No</th>
<th>Allosteric enzyme</th>
<th>Allosteric activator</th>
<th>Allosteric inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ALA synthase</td>
<td></td>
<td>Heme</td>
</tr>
<tr>
<td>2</td>
<td>Aspartate-transcarbamoylase</td>
<td>ATP</td>
<td>CTP</td>
</tr>
<tr>
<td>3</td>
<td>HMG CoA reductase</td>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td>4</td>
<td>PFK</td>
<td>AMP,F-2,6-P</td>
<td>ATP, citrate</td>
</tr>
<tr>
<td>5</td>
<td>Acetyl CoA carboxylase</td>
<td>Citrate</td>
<td>acylCoA</td>
</tr>
<tr>
<td>6</td>
<td>Citrate synthase</td>
<td></td>
<td>ATP</td>
</tr>
</tbody>
</table>
2. Describe in detail the synthesis and utilization of ketone bodies. Name any three conditions of increased production of ketone bodies and mention laboratory evaluation of ketoacidosis (SN-Aug 08)

The ketone bodies are mainly three, namely Acetacetate, B-Hydroxybutyrate and acetone. The later one is volatile in nature. Ketone bodies are water soluble and on oxidation they produce high range of energy.

**Synthesis of Ketone bodies (Ketogenesis):**

ketogenesis is takes place during high production of FA from liver

Site: mitochondrial matrix of liver cells

Precursor: acetyl CoA

Reactions: two molecules of acetyl CoA condense to form acetoacetyl CoA, this reaction is catabolized by thiolase

Acetoacetyl Co A condenses with another Acetyl CoA to produce b-hydroxy b-methyl glutaryl CoA (HMG CoA) synthase. This reaction is catalysed by HMG CoA synthase; it is key regulatory enzyme of ketogenesis

HMG CoA is cleaved to acetoacetate and acetyl CoA by the action of HMG CoA lyase

Acetoacetate can undergo spontaneous decarboxylation to form acetone

Acetoacetate can be reduced by dehydrogenase to b-hydroxybutyrate

**Utilization of Ketone bodies:**

Ketone body utilization is takes place in extra hepatic tissues for energy production. Tissues like heart, renal cortex, prefer KA than Glucose for energy production.

**Ketolysis reactions:**

Acetoacetate is activated to acetoacetyl CoA by thiophorase enzyme

Acetoacetate + Succinyl CoA → Acetoacetyl CoA + Succinate

Then the acetoacetyl CoA enter into TCA cycle to produce energy

Conditions which lead to elevated KA: DeabetesKeto Acidosis, Prolonged Fasting, Muscle wasting disease

**Laboratory evaluation of KA:**

Ketone bodies appear in urine under pathological conditions such as diabetes mellitus, persistent vomiting, Von Gierke’s disease and alkalosis. The major ketone bodies are three, acetoacetic acid, beta hydroxybutyric acid and acetone.
Ketone bodies in urine are measured by Rothera’s test, rapid tests such as ketostix strips and acetest tablets.

**Rotheras test:**

Principle: freshly prepared sodium nitroprusside reacts with ketone bodies and forms a purple coloured ferropentacyanide complex. This test is specific for acetoacetate and beta hydroxybutyrate.
3. Write short notes:
   a. Lipoproteins and their functions (SN-Mar 2002)

Lipoproteins are conjugated proteins. These are spherical bodies and made up of lipid and proteins. The outer layer poses polar heads PL, apoproteins and inner core contains non polar lipids such as TAG, tails of PL, cholesterol esters.

**General structure of lipoproteins**

They are classified according to their density into 4 major types

1. Chylomicrons – these are very larger molecules and lower density than VLDL, these contains high concentrations of lipid and lower concentrations of proteins
2. Very low density lipoproteins (VLDL)/pre beta lipoproteins – these are synthesized in liver. VLDL involved in transport of TAG from liver to peripheral tissues for energy
3. Low density lipoproteins (LDL)/beta lipoproteins – these molecules are rich in cholesterol and apo B100. LDL is directly linked to CVD risk. Since it involved in transport of cholesterol from liver to peripheral tissues.
4. High density lipoproteins (HDL) – these are very smaller but very high density, which contains lower concentrations of lipid and higher concentrations of proteins. HDL involved in reverse cholesterol transport catabolized by LCAT, that means cholesterol present in the peripheral transported to liver for further catabolism.
b. Significance of HMP shunt pathway (SN-feb 07,10, Aug-10)

1. HMP shunt is essential for production of pentoses, namely ribose -5 – phosphate, which is starting molecules in purine and pyrimidine biosynthesis.

2. It produces NADPH which is utilized for reductive biosynthesis of fatty acids, reduction reactions in detoxification, phagocytosis, and stabilization of iron in ferrous form.

3. Importance of NADPH:
   - NADPH is utilized by glutathione reductase, which keeps the glutathione in reduced state. The reduced glutathione is essential for peroxidases, catalase activity. These enzymes convert 2 mol of H2O2 to two mol of water.
   - NADPH is necessary for to maintain RBC membrane integrity.
   - NADPH utilized by WBC’s for phagocytosis.
   - NADPH is necessary for fatty acid and reductive biosynthesis of steroids.

After 120 days RBC degraded and heme is released. The iron present in heme is reutilized and remaining porphyrin ring is catabolized to bilirubin in the liver, which is an ultimate end product of heme catabolism.

Breakdown of HEME/Generation of BILIRUBIN

The prophyrin ring is degraded in microsomes of reticulo endothelial cell of liver, spleen, and bone.

Each day about 6g of Hb is catabolized, from which about 250 mg of bilirubin is formed.

**Microsomal heme oxygenase system:**

- The components present in this system are – heme oxygenase, molecular O2, NADPH
- This enzyme is induced by heme
- The alpha methylenyl bridge present between pyrrole rings I and II is cleaved and liberated as CO2
- The ferric iron liberated is become ferrous and it taken up by transferrin
- The so formed linear tetrapyrrole is called as BILIVERDIN, which is in green colour
- In mammals which is further reduced to BILIVERDIN, which is in red yellow colour by NADPH dependent biliverdin reductase

Bilirubin is water insoluble and is taken up by albumin and transported to sinusoidal surface of the liver. One molecule of albumin can bind two mol of bilirubin.

The bilirubin uptake by the liver is a carrier mediated active process.

The water insoluble bilirubin conjugates with two molecules of glucoronic acid and become water soluble nontoxic diglucoronide-bilirubin complex and is then secreted into bile.
**d. Fate of acetyl CoA**

Acetyl CoA is generated from various sources such as oxidation of pyruvate, catabolism of carbon skeleton of AA and oxidation of FA will go to different types of metabolisms

Fate of Acetyl CoA

- Enter into TCA cycle for energy production
- Ultimate precursor for cholesterol synthesis
- Ultimate precursor for ketone body synthesis
- Precursor for synthesis acetylcholine
- Used as acetyl donor in xenobiotic reactions catalysed by acetyltrasferase
e. Functions and deficiency symptoms of vit A

Biochemical functions of Vitamin A:

The major functions of vitamin A are carried by aldehyde form:

1. Vision: George wald first elucidated the visual pathway in retinal hence the name wald's visual cycle.

**Wald's visual cycle:**

The retina contains rods and cones. Rods meant for dim light vision and cones meant for bright light and clear vision. Rods contain visual purple called rodopsin, and cones contain 3 visual pigments called porphyropsin for red colour, iodopsin for green, and cyanopsin for blue colour. All these pigments undergo the same pathway to generate nerve impulse.

Rodopsin is made up of protein part opsin and nonprotein part 11-cis-retinal. When light strikes the pigment divided into two parts opsin and all-trans-retinal. In the conversion of 11-cis-retinal to all-trans-retinal there is generation so many intermediates namely prelumirodopsin, lumirhodopsin, metarhodopsini, 2, 3, and finally to all-trans retinal and opsin. Among which metarodopsin 2 is important because it activates the inactive transdusin. The activated transdusin activates the inactive phosphodiesterase. The activated phosphodiesterase degrades the cyclic GMP to linear form, which is important for opening of sodium channel. So, a dieresis in cGMP leads to closing of channel and finally generation of AP.

The all-trans-retinal is generated in the retina is partially converted to 11-cis-retinol by retinal isomerase. The most of all-trans-retinal transported to liver and converted to 11-cis-retinal by two liver enzymes, namely alcohol dehydrogenase, and isomerase.
Other functions:

1. Alcohol and acid forms of vitamin A acts as steroid hormone and controls the cell growth and differentiation.

2. Retinyl esters are necessary for synthesis of glycoproteins, mucopolysaccharde and hence it necessary to maintain proper healthy epithelium.

3. Retinol and retinoic acid are involved in synthesis of transferritin.

4. It is involved in immunity.

5. Involved in cholesterol synthesis.

6. β-Carotene is acts as antioxidant at stops the oxidative reactions.

Deficiency of vitamin-A:

The deficiency of vitamin –A is unusual, and is related to vision, growth.

The primary affected organ is eye; the features are as follows –1. The first symptom of deficiency is nictolopia (night blinded ness).

2. In severe deficiency leads to epithelial degeneration and condition called xerophthalmia (dryness of conjunctiva and corneas), and finally leads to keratization of epithelial tissues of lacrimal glands.

3. If xerophthalmia persists long time it leads to keratomalacia (ulceration and degeneration of cornea).

4. Other manifestations of vitamin are retardation of growth, degeneration of germinal epithelium of gonads, keratization of epithelial tissues, and formation of urinary stones.
f. Isoenzymes and their diagnostic importance

The enzymes with similar function and physically distinct from each other are called isoenzymes are the products of different genes are known as trueisoenzymes.eg: salivary and pancreatic amylase

The characteristic features of Iso-enzymes are tissue specific and differ in affinity to the substrate. Hence study of isoenzymes is useful to understand diseases of different organs.

Identification techniques for isoenzymes: electrophoresis, heat stability, inhibitors, substrate specificity, cofactor, compartmentalization, use of specific antibodies

Diagnostic important isoenzymes:

Lactaedehydrogenase (LDH):

LDL catalyses the conversion of pyruvate to lactate and vice versa. LDL is concentrated in RBC cell; therefore minor haemolysis causes the false value. Normal values ranges from 100-200 U/L.

Differential diagnosis of LDH: the elevation of LDH is seen in haemolytic anaemia, hepatocellular damage, muscular dystrophy, cancer etc.

It has 5 tissue specific isoenzymes.

LDH is tetramer made up of two H(heart) bands and two M(muscle) bands. Both of these are same molecular wt. and with minor amino acid variations.

With two different polypeptide chains therefore 5 combinations of H and M are possible, namely H4, H3M, H2M2, M3H, M4. The tissue specificity and diagnostic importance of these 5 isoenzymes is as follows

H4 form found in heart, which is useful for diagnosing heart disease

M4 form found in muscle, hence it is useful in diagnosing muscle diseases

Creatine kinase (CK)

It catalysis the synthesis of creatine phosphate from creatine and ATP. Normal blood ranges from 15-100 IU/L, it is made up of 2 polypeptides namely M & B. therefore 3 combinations of isoenzymes are possible. They are MM found in skeletal muscle, MB found in heart and BB found in brain

CK sub form is highly elevated in muscular dystrophies, acute cerebrovascular injuries. It is most reliable factor in diagnosing AMI
Alkaline phosphatase (ALP)

It is nonspecific enzyme which hydrolyses aliphatic, aromatic and heterocyclic compounds at pH 9-10 in the presence of Mn and Mg. ALP produced by osteoblasts for the calcification process. Normal serum levels are 40-125 u/L. moderate increase seen in hepatic diseases, and very high levels are seen in extrahepatic obstruction or intrahepatic obstructions, and very high levels are seen in bone diseases.

ALP has nearly 6 types of Iso-enzymes

- Alpha 1 ALP: it is about 10% total ALP, and is increased in obstructive jaundice
- Alpha 2 ALP: it is about 20% of total ALP
- Alpha 2 heat stable ALP: it is about 1% of total ALP. It is heat stable above 65°C
- Pre beta ALP: it is about 50% of total ALP. It is elevated in bone diseases
- Gamma ALP: it is about 10%, it is increased in ulcerative colitis
- Leucocyte ALP: it is increased in lymphomas and decreased in chronic myeloid leukaemia
g. Galactosemia (Mar-2002)

Inability to metabolize galactose occurs in the **galactosemias**, which may be caused by inherited defects of galactokinase, uridyl transferase, or 4-epimerase (Figure 21–6A), though deficiency of **uridyl transferase** is the best known. Galactose is a substrate for aldose reductase, forming galactitol, which accumulates in the lens of the eye, causing cataract. The general condition is more severe if it is the result of a defect in the uridyl transferase, since galactose 1-phosphate accumulates and depletes the liver of inorganic phosphate. Ultimately, liver failure and mental deterioration result. In uridyl transferase deficiency, the epimerase is present in adequate amounts, so that the galactosemic individual can still form UDPGal from glucose. This explains how it is possible for normal growth and development of affected children to occur despite the galactose-free diets used to control the symptoms of the disease.
h. Cyclic AMP

Cyclic AMP was the first intracellular signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP. Different peptide hormones can either stimulate or inhibit the production of cAMP from adenylyl cyclase. ADH, β-Adrenergic, Calcitonin, CRH, FSH, Glucagon, hCG, LH, LPH, MSH, PTH, TSH stimulates adenylyl cyclase and stimulates production of cAMP. Adenylyl cyclase converts ATP to cAMP.

In eukaryotic cells, cAMP binds to a protein kinase called protein kinase A (PKA) that is a heterotetrameric molecule consisting of two regulatory subunits (R) and two catalytic subunits (C). cAMP binding results in the following reaction:

![Diagram of second messenger action of cAMP]

**Fig. Mechanism of second messenger action of cAMP**

The R2C2 complex has no enzymatic activity, but the binding of cAMP by R dissociates R from C, thereby activating the latter. The active C subunit catalyses the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The end result is the protein is phosphorylated. This causes a change in its function. Eg. Epinephrine stimulates cAMP production leading to phosphorylation of phosphorylase kinase which is the active form, leading to glycogenolysis.

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases
i. RDA

The daily need for essential nutrients has been published by the food and nutrition board of the National Research Council as Recommended Dietary Allowances (RDA). The allowances are to provide for individual variations among most normal persona as they live under their usual environment conditions. They do not allow for extra requirements in illness or pathologic disorder. Diets should be based on a variety of foods, both to cover known requirements and to provide other nutrients for which human requirements have been less well defined. All nutritional requirements must be met to prevent deficiency diseases and ill health. Ignorance or poor economic conditions are almost always the underlying cause of failure to satisfy nutritional requirements. On the other hand, certain common diseases are associated with excess intake of nutrients. Obesity, Diabetes, CVD, cancer due to high fat intake, cerebrovascular disease due to high salt intake
j. **Essential FA (SN-Mar 2002)**

Not all the fatty acids are essential to the body, since some of them are synthesized by the body, but some we may need to get through diet which is called as essential fatty acids. All the essential fatty acids are unsaturated fatty acids, which mean their aliphatic chain, contain one or more double bonds. These FA named by adding the suffix *enoic* after systematic name. UFA exhibit geometrical isomerism at the double bonds. All natural FA have *cis* configuration.

There are two types of Essential fatty acids

**Mono unsaturated FA (MUFA):**

- Oleic acid (*cis*-9-octadecenoic acid) present in all fats
- Palmitoleic (*cis*-9-hexadecenoic acid) present in most fats and very high in olive oil

**Poly unsaturated FA (PUFA):**

- Linoleic acid (all-*cis*-9,12-octadecadienoic acid) – present in corn, peanut, cotton seed, soy bean and other plant oils
- Linolenic acid (all-*cis*-6,9,12-octadecatrienoic acid) – present in oils of eveningprimrose, borage arachidonic acid (all-*cis*-5,8,11,14-eicostetraenic acid) – found in animal fats (PLs).
- Arachidonic acid is semi essential because it can produce form linoleic acid.

Deficiency of unsaturated fatty acid leads improper synthesis of skin, this is called as phrynoderma or toad skin, it is characterized by horny eruptions on the posterior and lateral parts of limbs, back and on the buttocks, hair loss, poor wound healing.