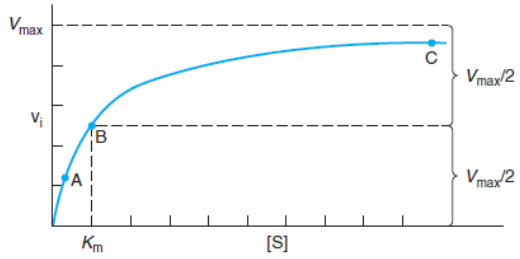
Biochemistry I – (E-April 2001)

1. **Explain the effects of different factors on rates of enzyme catalysed reactions (15M)**

The various factors which affect the enzyme activity and velocity as follows

Enzyme concentration: rate of enzyme velocity is directly proportional to enzyme concentration when all other kept optimal

Substrate concentration: the rate of enzyme velocity is very high during the initial reaction and after subsequent addition of substrate leads to loss of enzyme activity and velocity



The velocity is expressed in µM of substrate converted per minute

The maximum velocity obtained at substrate saturation level is called as Vmax

Km-Michaelis menten constant:

According to Michaelis theory, the formation of enzyme substrate complex is a reversible while the breakdown of complex to enzyme and product is irreversible

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

Km value is substrate concentration at half maximal velocity, means 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration

Km is independent of enzyme concentration. If enzyme concentration is double, the Vmax will be double, but ½ Vmax will remain same. In other words irrespective of enzyme concentration, 50% molecules are bound to substrate at that particular substrate concentration

Significance of Km:

Km is constant and characteristic feature of a particular enzyme for a specific substrate

Km denotes the affinity of enzyme for substrate. The lesser the km the more affinity for enzyme to substrate and vice versa

Kd dissociation constant

The affinity of an enzyme towards its substrate is inversely related to the dissociation constant

Kd = E+S ES complex E+P

Kd = k2/k1 and km = k2+k3/k1

Therefore, the smaller the tendency for the dissociation of the complex, the greater is the affinity of the enzyme for the substrate

Concentration of products

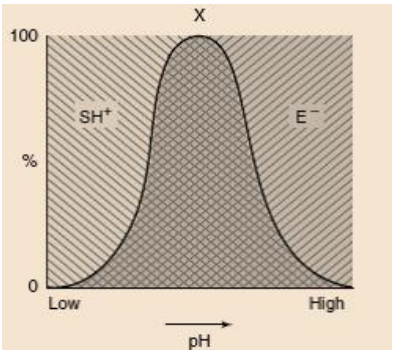
The rate of enzyme velocity is indirectly proportional to product concentration

Effect of temperature

The velocity of enzyme catalysed reaction is increases parallel upto a particular temperature due to supplying of activation energy and slowly falls in higher temperature due to degradation of enzyme molecule. The temp at which maximum amount of the substrate is converted to the product per unit is called the optimal temperature. Most of the enzymes works at optimal temperatures ranges betwee 37-50^{o}C , except some eg. Thermobacillus (Taq pol II). When we draw the plot a graph for velocity vs temp, we will get the bell shaped curve

Effect of pH

Each enzyme has a unique pH range for maximal activity, beyond these ranges enzyme velocity will slowdown. The optimal pH for most of the enzymes is pH 5-8.except pepsin with optimum pH of 1-2 and ALP has pH of 9-10 and acid phosphatase has pH 4-5 etc.



2. **Write short notes on (4X5=20)**
   a. **Sources of acetyl CoA (SN-April 2001)**

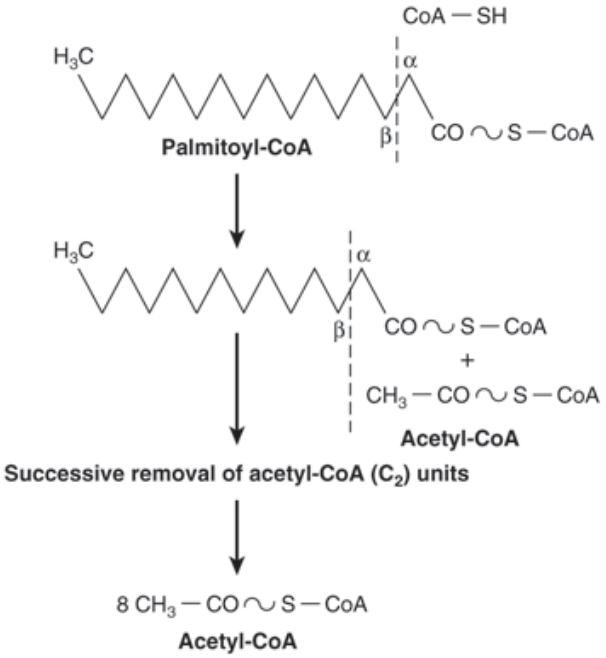
The various sources of acetyl CoA as follows –

Oxidation of pyruvate

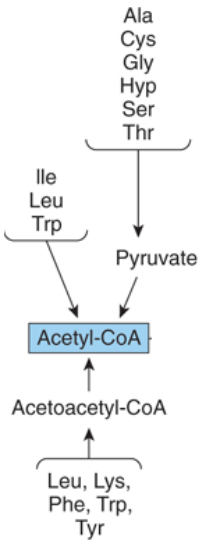
PDH

a. Pyruvate + NAD^+ + CoA ~~**Acetyl-CoA**~~ + NADH + H^+ + CO_2

b. Beta-Oxidation of FA



c. Catabolism of AA



b. Anaplerotic reactions (SN-April 2001)

TCA is a considered as major metabolic cycle in living organism. The several essential metabolites formed from intermediates of TCA cycle, which causes the deficiency of those intermediates and leads to cease of cycle. Therefore replenishment of deficient intermediates is termed as Anaplerosis

The various intermediates of TCA cycle used and refilled as follows

- Oxaloacetate and a-KG serve as Oxaloacetate and a-KG serve as precursor for the synthesis of aspartate and glutamate respectively. In order to replenish these intermediates the Anaplerotic reactions as follows –

Pyruvate + CO_2 + ATP → oxaloacetate + ADP + Pi catalysed by pyruvate carboxylase

Glutamate + NADP + H_2O → a-KG + NADPH + H + NH_4 by glutamate dehydrogenase

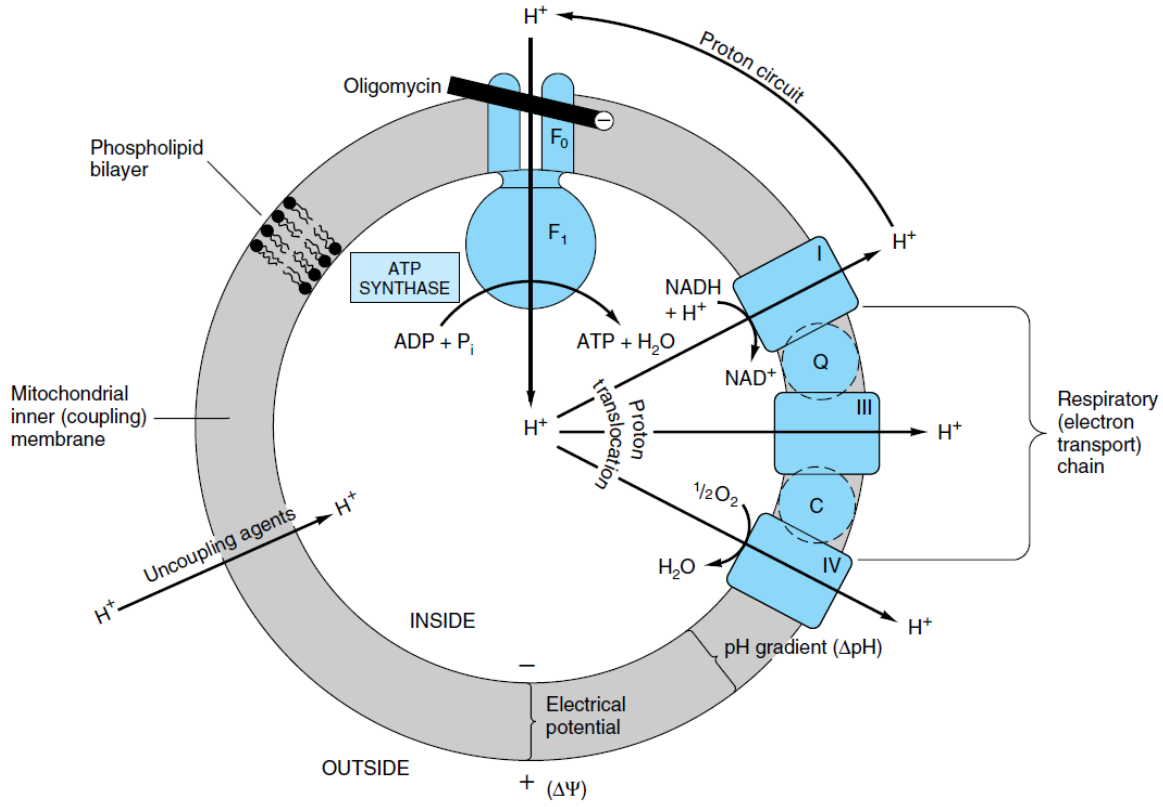
- Another Anaplerotic reaction for malate

Pyruvate +CO_2+NADPH +H → Malate + NADP + H_2O by malate dehydrogenase (malic enzyme)

c. Chemiosmotic hypothesis (SN-Feb 08,10)

The coupling of oxidation with phosphorylation is termed oxidative phosphorylation is called as oxidative phosphorylation. **Peter Mitchell** in 1961 proposed a theory to explain oxidative phosphorylation.

Generation of proton gradient and synthesis of ATP: the complexes I, III and IV expel proton form inside to outside of the mitochondria membrane. This causes the electrochemical potential difference across the membrane, once established it inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the ATP synthase. This in turn depends on availability of ADP and P_i.



d. Acute intermittent porphyria (SN-April 2001)

It is one of the major disorders in heme synthesis. It is an inherited autosomal dominant trait

The enzyme PBG-deaminase involved in the conversion of porphobiliogen to uroporphyrinogen.

The levels of ALA and PBG are elevated in blood and urine. Fresh urine is colourless, but on standing it become colour due to photooxidation of PBG to porphobilin. Because this reason urine sample should collected freshly and transported in dark colour bottle

Patients have acute abdominal pain. In some cases neurological symptoms like sensory and motor disturbances, agitation ad confusion.

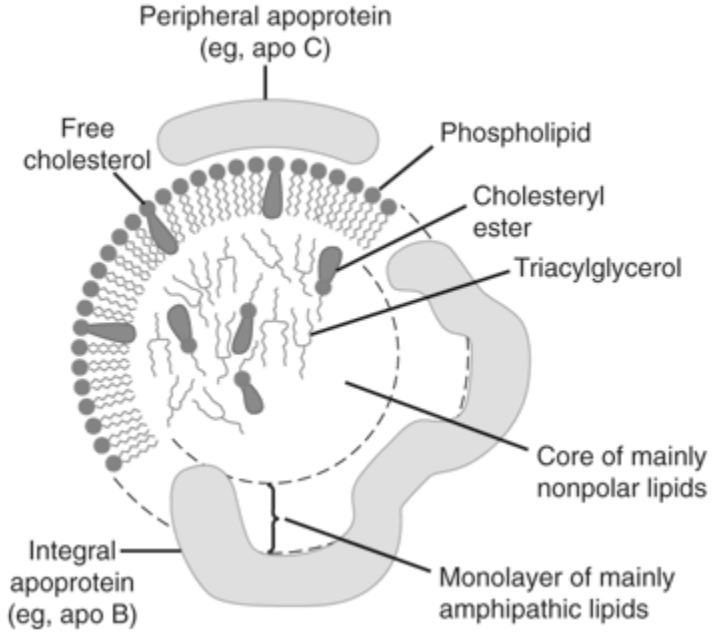
Female have less severe manifestations than male, since female sex hormones have a stimulatory effect on ALA synthase

Treatment: starvation, carbohydrate rich diet, drugs like barbiturates

3. **How are lipoproteins classified? What are their functions? Describe the metabolism of low density lipoproteins (SN-April 2001)**

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. Chylomicrosns – 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 are 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 catabolyzed by LCAT, that means cholesterol present in the peripheral transported to liver for further catabolism.

LDL metabolism:

LDL molecules are derived from mostly VLDL, but small part is directly synthesized directly from liver.

LDL molecules enter into the liver through LDL receptors and metabolized. These receptors are clathrin coated pits and specific for Apo B100. When apo B100 binds to the receptor the receptor-LDL complex is internalised b endocytosis. These vesicles bind and fuse with lysosomes and hydrolysed by hydrolytic enzymes.

4. **Write short notes**
    a. **Antioxidants (SN-April 2001)**

Antioxidnats are the molecules which supress the adverse actions of oxidants such as ROS. Antioxiantsmay be considered as scavengers of free radicals

Types of antioxidants

Preventive antioxidants: they block the initial reaction of red radicals – catalase, glutathione peroxidase

Chain breaking antioxidants: they break the chain reactions of lipid peroxidation eg – super oxide dismutase, vitamin e, uric acid

Plasma antioxidants: beta carotene, vitamin c, bilirubin, uric acid, ceruloplasmin, transferrin

Cell membrane antioxidants: vitamin E

Intracellular antioxidants: sod, catalase, glutathione peroxidase

Antioxidant vitamins:

Vitamin E (Tocopherol) act as lipid peroxidation chain breaking antioxidant

Vitamin C (Ascorbic acid) act as natural aqueous phase antioxidant

Antioxidant enzymes:

Superoxide dismutase: This enzyme scavenges superoxide radicals by catalyzing the conversion of two of superoxide radicals into less toxic hydrogen peroxide and molecular oxygen in the presence of Mn

Catalase: it degrades H2O2 into water

Glutathione peroxidase:it detoxifies hydrogen peroxide into water in the presence of reduced glutathione

Reduced glutathione: it assists the various oxido reduction reactions

b. Vitamin K (SN-April 2001)

Common name: anti haemophilic vitamin,

Chemical name: phylloquinone

Structure: The vitamin k is made up of quinone ring attached to isoprenoidunits.

Vitamin k is 3 types.

K1 – phylloquinone (naphthoquinone ring+4 isoprenoid units) it isfound in plants

K2 – menaquinone (napthaquinone ring+ 6 isoprenoid units) it isfound in animals.

K3 – menadione (no isoprenoid units)

Metabolism of vitamin:

The vitamin is synthesized by intestinal bacteria so need not to takethrough diet. Its absorption is dependent on chylomicrons and bile salts,and it is finally stored in liver.

Functions:

Vitamin – K is involved in post-translational modifications of clottingproteins (clotting factors) such as factor II, VII, IX, X etc. which undergoes posttranslational modifications prior to their action such as carboxylation.

Carboxylation of clotting factors and significance;

All the pre-translational clotting factors contain glutamic acids at theirends of polypeptide chains, the glutamic acids are converted to γ-carboxyglutamic acid in the presence of carboxylase, O2, CO2, vitamin –K(quinone form), NADP+H+.These carboxilatedclotting factors now possess two negativecharges on their γ-carboxyglutamate, which intern chelates with twopositive charges of Ca2+; now this complex bind to phospholipids ofplatelets, the final effect is conversion of prothrombin to thrombin.

Other functions of Vitamin – K:It is involved in electron transport chain and oxidativephosphorylation.

Deficiency of Vitamin – K:

The deficiency is uncommon, because its adequate supply is madeby intestinal bacteria, and also though diet. However, the deficiency mayoccur due to malabsorption syndrome, diarrhoea, and antibiotic drugs.The major feature of deficiency is increased both bleeding time and clottingtimes.

c. Von Gierke's disease (SN-April 2001)

- It is also called as Glycogen storage disease I and it is most common attack 1 in 1 Lack births.
- Glucose 6-phosphatase enzyme is deficient in patients. Hence the glucose cannot be released from liver during overnight fasting and leads to hypoglycaemia
- G-6-P may divert to glycogen synthesis. Therefore, the accumulation of large amount of glycogen in liver leads to enlargement of liver and cirrhosis. Due to this children my die in early age
- The excess G-6-P is then diverted to HMP shunt with increasing production of pentoses and nucleotides
- The more Nucleotides and the large amount of formation of Uric Acids, which is charactarized as hyperuricemia
- Other symptoms include, gyperlipidemia, lactic acidosis and ketosis

Treatment: small quantity of food at frequent intervals

d. Classification of Enzymes

In early days enzymes are classified and named according to their type of reaction catalysed, followed by the suffix *-ase.* For example, dehydrogen*ases* remove hydrogen atoms, prote*ases*hydrolyse proteins, and isomer***ase***catalyse rearrangements in configuration. International union of biochemistry and molecular biology suggested the system of nomenclature of enzymes. It is complex but unambiguous and internationally accepted.

As per thissystem enzyme are represented as EC (enzyme class) number with 4 digits

First digit represent the class, second for the subclass, third for sub-sub class, and fourth represents specific enzyme in list

The enzymes are classified major classes

1. **Oxidoreductases –** these class of enzymes catalyse oxidations and reductions

   AH2 + B → A + BH2

   Eg: alcohol dehydrogenase, oxidases, reductases

2. **Transferases**– these class of enzymes catalyse transfer of moieties between substrate such as glycosyl, methyl, or phosphoryl groups

   A-R + B → A + B-R

   Eg: hexokinase, transaminase

3. **Hydrolases**– these class of enzymes catalyze*hydrolytic* cleavage of C—C, C—O, C—N, and other bonds by adding of water

   Eg: Acetyl choline + H2O → Choline + Acetate catalysed by Acetyl choline esterase

4. **Lyases –** These class of enzymes catalyze cleavage of C—C, C—O, C—N, and other bonds by *atom elimination,* leaving double bonds.

   Eg: Frucsotse-1,6bisphosphate → Glyceraldehyde -3-phosphate + DHAP by aldolase

5. **Isomerases –** these class of enzymes catalyze geometric or structural changes within a molecule

   Eg: Recemases, epimerase, cis-trans isomerases

6. **Ligases** -these class of enzymes catalyze the joining together of two molecules coupled to the hydrolysis of ATP)

   Eg: Acetyl CoA carboxylase

   Acetyl CoA + CO2 + ATP →Malonyl CoA + ADP +Pi