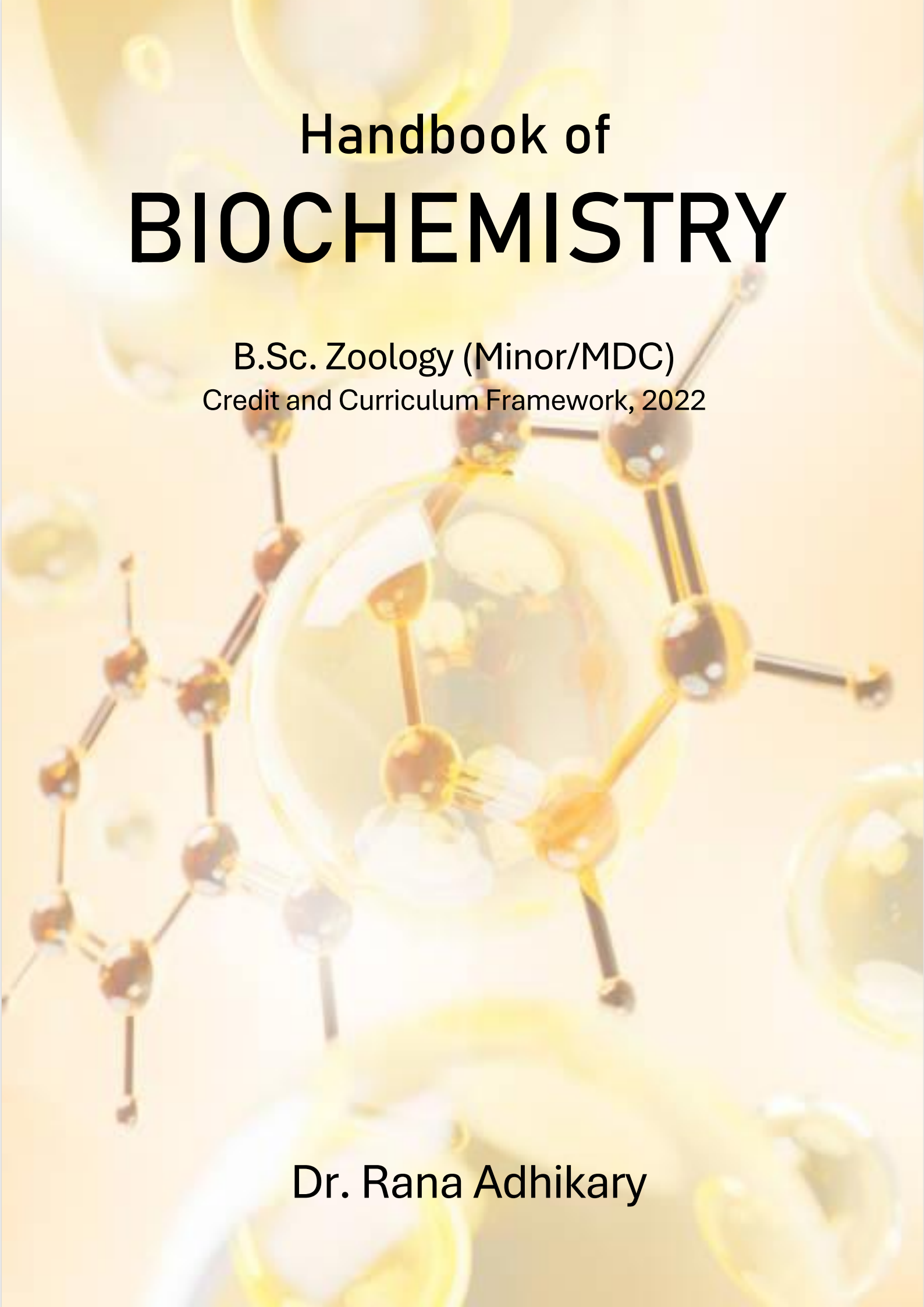
Handbook of
BIOCHEMISTRY

B.Sc. Zoology (Minor/MDC)
Credit and Curriculum Framework, 2022



Dr. Rana Adhikary

Handbook of
BIOCHEMISTRY

Paper: CC2/MN-2/MDC-MN-2

For students of Three Year/ Four Year B.Sc.
Zoology (MDC or Minor)

SEMESTER II (MDC/Minor)/ IV
(Minor) Under CCF curriculum
2022

Dr. Rana Adhikary

M.Sc. (Specialization: Immunology & Microbiology), Ph.D. (Science), IIT-GATE, CSIR-UGC NET (Twice, AIR: 25 & 45)

State Aided College Teacher at St. Paul's Cathedral Mission College
Former Whole Time Teacher at St. Paul's Cathedral Mission College
Former lecturer at PCMM College (Physiology & Biochemistry)
Former lecturer at Bhairab Ganguly College (Physiology)
Former research fellow at Immunology & Microbiology laboratory, University of Calcutta (DST-JRF, DST-SRF)

COPYRIGHT NOTICE

Dedicated to my beloved students.

CONTENTS

UNIT-1: CARBOHYDRATES

Structure, classification and properties of Monosaccharides (aldose ketose), Disaccharides and Polysaccharides. Isomerism of monosaccharide (D and L, optical isomers, furanose and pyranose, α and β anomers, epimers); Reducing and non-reducing sugars. Physiological importance of monosaccharides, disaccharides and polysaccharides.

Q1. Define carbohydrates. (2)

Ans: Carbohydrates or saccharides (Greek 'sackaron' means sweet) are those biomolecules that bear *hydrated carbons*. Carbohydrates are defined as ***simple organic compounds that carry alcoholic hydroxyl* (-OH) *group bearing carbon atoms, and one or more reactive aldehyde* (-CHO) *or ketone* (-C=O) *groups in addition***. Therefore, carbohydrates and their hydrolytic products are chemically either *polyhydroxyaldehydes* or *polyhydroxyketones*. All carbohydrates present with a molecular formula of $\mathbf{C_nH_{2n}O_n}$, where, hydrogen and oxygen atoms are present in 2:1 ratio. Examples include monosaccharides like glucose (*aldosugar*), fructose (*ketosugar*), disaccharides like sucrose, lactose and polysaccharides like starch, dextrin etc.

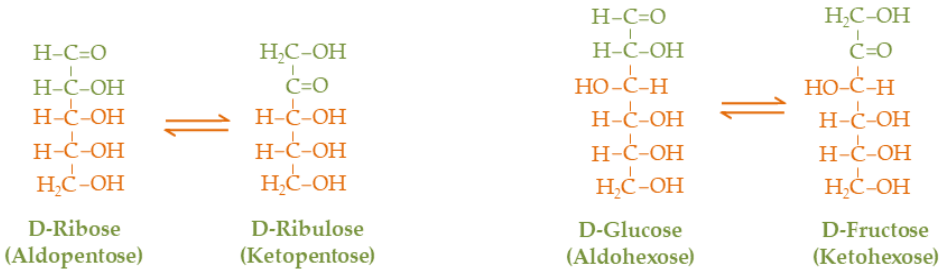
Q2. Describe the functions of carbohydrates. (3)

Ans: Carbohydrates primarily functions as energy sources, however they play many other important biological roles:

i) ***Production of energy:*** Carbohydrates such as glucose is readily oxidised to produce electron donors for mETC, which leads to production of high energy molecules like ATP through oxidative phosphorylation.
ii) ***Energy storage:*** Carbohydrates such as glycogen and starch are used for storage of energy in animals and plants respectively.
iii) ***Structural role:*** Carbohydrates like cellulose, chitin, hyaluronic acid, chondroitin sulphate are involved in formation of cell wall, glycocalyx and extracellular matrix in plants, bacteria and animals. Carbohydrates like glycosaminoglycans are important structural constituent of connective tissue.
iv) ***Cellular functions:*** Carbohydrates promote cell growth, intercellular communication and cell signaling.
v) ***Genetic role:*** Carbohydrates are important structural constituents of genetic materials, *e.g.*, ribose and deoxyribose in RNA and DNA respectively.
vi) ***Synthesis of other biomolecules:*** Metabolic products of some carbohydrates are used for synthesis of fatty acids and some amino acids. Carbohydrates also serve as nutrients, *e.g.*, lactose in milk.
vii) ***Protective role:*** Few carbohydrates give rise to antibiotics, *e.g.*, ***S. griseus*** produces streptomycin from D-glucose.

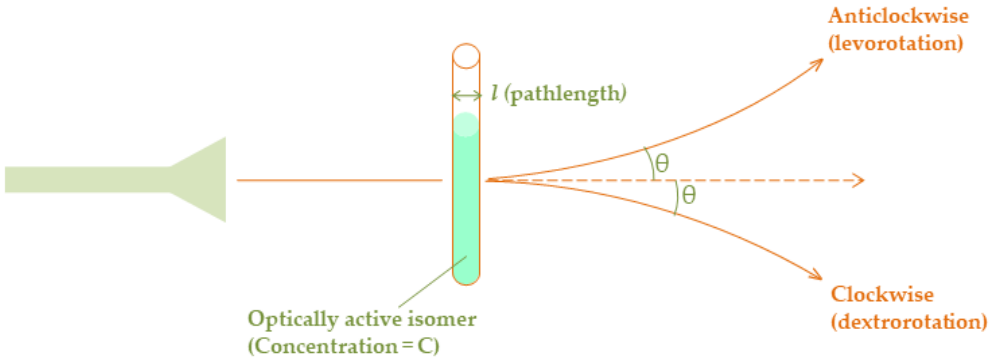
Q3. Define aldose-ketose isomerism. Give an example. (2)

Ans: Aldose-ketose isomerism is defined as existence of an aldose and a ketose with similar molecular formula. Example: Ribose **(*aldopentose*)** and ribulose **(*ketopentose*)** both are pentose sugars, containing five carbon atoms in their structure and their molecular formula are also similar, *i.e.*, $C_5H_{10}O_5$, yet these two differ from each other at the reactive group held at C^1-C^2 atoms in their linear chain structure. Glucose (***aldohexose***) and fructose (***ketohexose***) are another example**.** This is the simplest form of isomerism found in monosaccharides.



Q4. What is '*stereoisomerism*' or '*dl*' isomerism of monosaccharides? (3)

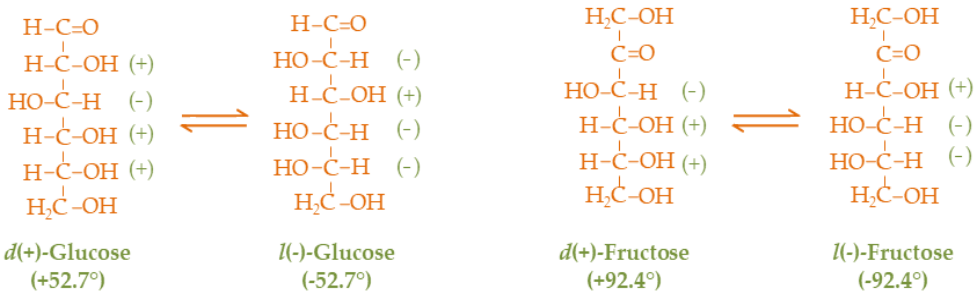
Ans: ***Optical isomerism***, also known as '***stereoisomerism***' (or '***dl***' isomerism) is defined as '*the existence of two optically active isoforms of same biomolecule*'. Optical isomers are also known as ***enantiomers***. An optically active form of a biomolecule refers to its ability to rotate the plane of vibration of polarized light, which is attributed to the presence of '***asymmetric***' or '***chiral***' atoms (most commonly carbon atoms) in their structure. An asymmetric carbon carries four different groups or atoms at its four valencies, which render the net intensity of the electromagnetic field around that carbon unequal in different directions, enabling them to deflect and rotate the plane of electromagnetic waves (*e.g.*, light). Therefore, the presence of one or more asymmetric carbons in a biomolecule may result in its existence into two optically active isoforms due to differences in the three-dimensional spatial arrangements of the groups or atoms held by the asymmetric carbons. A *plane polarized light* refers to the emergent light produced after passing an ordinary light through a *nicol prism* or a *polaroid film*. The difference between an ordinary light and a plane polarized light is that, in case of ordinary light the rays vibrate into different planes, whereas in case of polarized light different vibrational planes of different wavelengths are superimposed at a single plane. Polarized light is produced by polarimeter.



$$[\alpha]_D^{25} = \frac{100\,\theta}{l \times C}$$

α = specific rotation, at 25°C temperature for sodium D line
θ = observed rotation
l = pathlength (here diameter of sample tube)
C = Concentration of the optically active substance in $g.dl^{-1}$

An optically active isomer rotates the plane of vibration of polarized light, either ***clockwise*** (***dextrorotation***, which is designated with the prefix '*d*' or '+') or, ***anticlockwise*** (***levorotation***, which is designated with the prefix '*l*' or '-'). An optically active isomer capable of rotating the plane of polarized light in a clockwise direction is designated as ***'d' isomer*** and if it rotates the plane polarized light in anticlockwise direction, it is called ***'l' isomer***. The two optical isomers of a substance are non-superimposable mirror images of each other. However, the magnitude and direction of optical rotation of a substance are determined by the sum total of the dextrorotatory and levorotatory effects of all its asymmetric carbons. Example of stereoisomerism is presence of ***d* (+)-glucose** and ***l* (-)-glucose**. The specific optical rotation shown by *d*-glucose and *l*-glucose measured at 20°C with sodium D-line (589 nm) are +52.7° and -52.7° respectively. In case of fructose these values are +92.4° and -92.4° for *d* (+) and *l* (-) isomers respectively.



Q5. What are asymmetric carbons? (2)

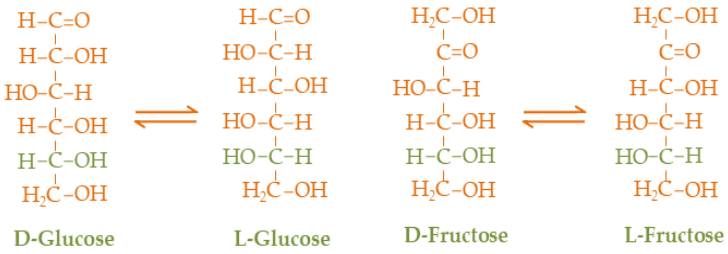
Ans: A carbon atom carrying four different groups or atoms on its four valencies is called an ***asymmetric carbon***. Since the intensity of net electromagnetic field around an asymmetric carbon becomes unequal in all directions, this renders them capable of rotating the plane of electromagnetic waves (*e.g.*, light). Therefore, presence of one or more asymmetric carbons may result in the existence of the biomolecule into two optically active isoforms.

Q6. Why glucose and fructose are called '*dextrose*' and '*levulose*' respectively? (2)

Ans: The magnitude and direction of optical rotation of an optically active isomer are determined by the sum total of the dextrorotatory and levorotatory effects of all its asymmetric carbons. Naturally occurring glucose molecule is dextrorotatory, hence glucose is also known as '*dextrose*'; on the other hand, naturally occurring fructose is always levorotatory and hence is known as '*levulose*'.

Q7. What is DL-isomerism of monosaccharides? (2)

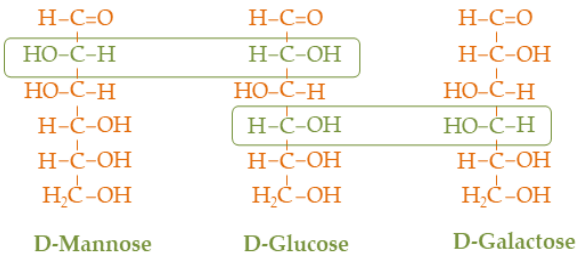
Ans: DL-isomerism of monosaccharides is defined as the *existence of two isomers of a same monosaccharide due to difference in spatial orientation of the groups or atoms around the '**penultimate carbon**'*. Penultimate carbon refers to the asymmetric carbon farthest from aldehydic or ketonyl group and nearest to the terminal carbon. In case of hexoses, it is fifth carbon atom in their linear chain form. Opposite rotations of hydrogen atom and hydroxyl (–OH) group held around a penultimate carbon, *i.e.*, left-handed or right-handed, results in DL isomerism.



In case of most of the monosaccharides found in eukaryotes, D-isoforms are biologically active. Two exceptions are L-fucose and L-iduronic acid. D-isomers of most of the monosaccharides exist into *dextrorotatory* (*d*) form; and may also exist into levorotatory (*l*) form; but the L-isoform essentially always exists into *levorotatory* (*l*) form in nature.

Q8. What do you mean by epimerism? (2)

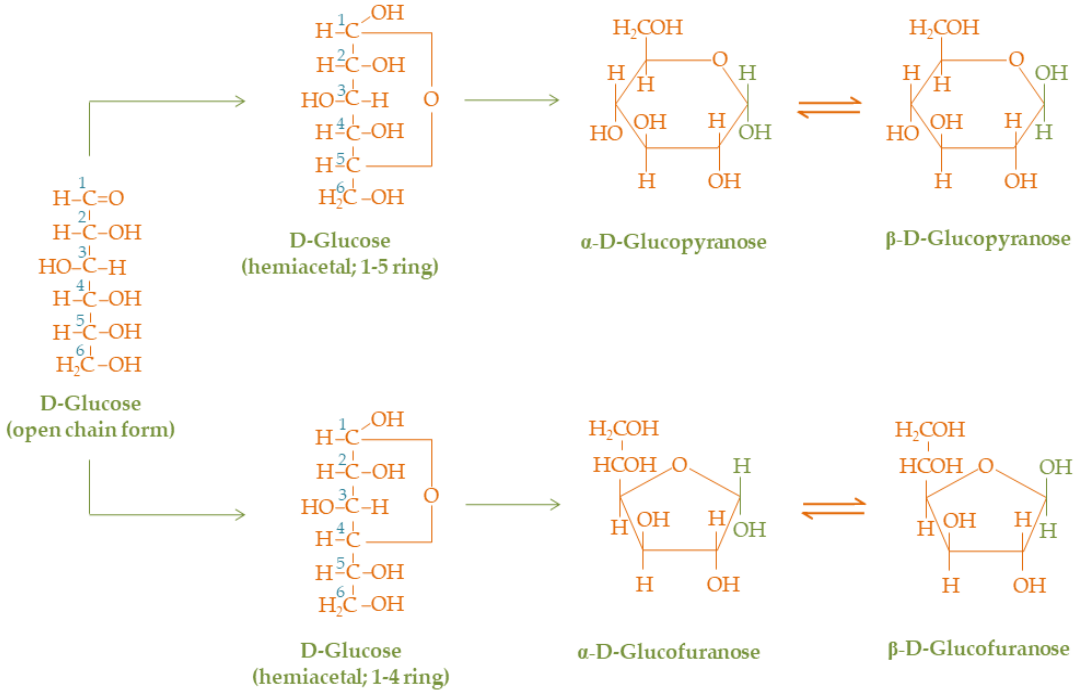
Ans: Epimerism is defined as the *existence of two monosaccharides with same molecular formula as two different isoforms due to difference in spatial orientation of the groups or atoms held by a specific asymmetric carbon*. Glucose and galactose have similar molecular formula and structure; however, the H and –OH around the C^4 asymmetric carbon shows an opposite rotation, *i.e.*, it is directed clockwise in case of glucose and anticlockwise in case of galactose. Since this type of epimerism involves a 4^{th} asymmetric carbon glucose and galactose are called 4-epimers of each other. Mannose and glucose appear to be '***2-epimers***' of each other.



Q9. Give a brief account of pyranose-furanose isomerism with an example. (5)

Ans: Pyranose-furanose isomerism of monosaccharides refers to the *existence of pentose and hexose sugars into a hexagonal or pentagonal rings structures known as pyran and furan respectively*. The aldehydic (-CHO) and ketonyl (-C=O)

groups in aldose and ketose sugars are highly reactive, and tend to react with hydroxyl (-OH) groups attached with the different carbons of same molecule in its linear chain from; which links the carbon atoms distant from each other giving rise to temporary '***tautomeric ring***' structures. This results into formation of '***hemiacetal***' or '***hemiketal***' in case of aldose or ketose sugars respectively. The *aldehydic* and *ketonyl* groups are converted to a new -OH group, and the carbonyl carbon and the alcoholic carbon gets bridged by an ether linkage (-C-O-C- linkage). Thus, the linear open chain structure of monosaccharide gets transformed into a closed ring form, *i.e.*, either a 6-member ***pyran*** (C^5 linked to O^1) (known as ***pyranose***) or a 5-member ***furan*** (C^4 linked to O^1) form (known as ***furanose***). The carbonyl carbon becomes a new asymmetric carbon with one hydrogen atom (H) and one hydroxyl group (-OH) attached to it and is called ***anomeric carbon***. The opposite orientation of hydrogen atom and hydroxyl group around this new asymmetric carbon gives rise to another type of isomerism known as ***anomerism***.



An example of pyranose-furanose isomerism is existence of D-glucose into six membered D-glucopyranose form and five membered D-glucofuranose form. The nucleophilic addition of C^5-OH of glucose and the carbonyl carbon (C^1) of reactive aldehyde group leads to formation of a hexagonal closed ring structure known as ***D-glucopyranose***. Since, the C^1 now becomes a new asymmetric carbon, the opposite orientation of H atom and -OH group around it gives rise to two isomers (***anomers***), one with -OH directed to the right (***α-D-glucopyranose***) and another with -OH directed left (***β-D-glucopyranose***). Similar nucleophilic addition of C^4-OH of glucose and the carbonyl carbon (C^1) of reactive aldehyde group leads to formation of a pentagonal closed ring structure which is called ***D-glucofuranose***. Opposite orientations of H atom and -OH group around the anomeric carbon will give rise two anomers, *i.e.*, ***α-D-glucopyranose*** and ***β-D-glucopyranose***. The α- and β-anomers of D-glucopyranose and D-glucofuranose shows difference in specific optical rotation.

- Into aqueous solutions including various biological fluids each hexose sugar exists into an equilibrium mixture of its open chain form, pyranose isomer and furanose isomer.
- Aldopentoses exists into an equilibrium mixture of its open chain form, and furanose isomer only. It almost never adopts pyran form.
- Ketopentoses, tetroses and trioses can never form pyranose or furanose isomers, therefore they exist in linear open-chain form only.

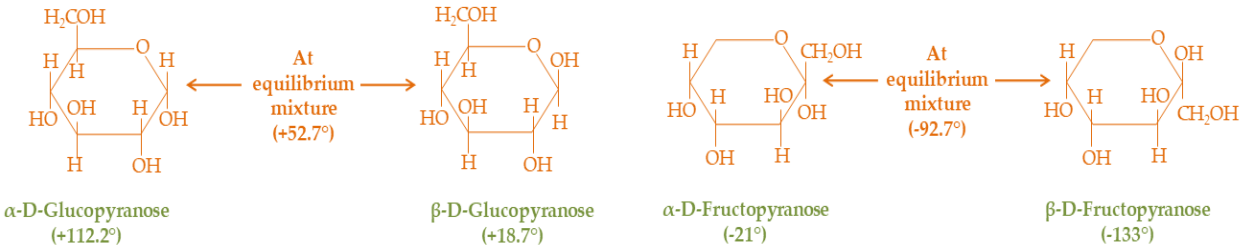
Q10. Define anomerism. (2)

Ans: During formation of pyran or furan rings due to nucleophilic addition of highly reactive aldehyde of ketonyl groups with C^5 or C^4 hydroxyl (-OH) group into a sugar molecule, the aldehydic or ketonyl C (*i.e.*, C^1 in aldoses and

C^2 in ketoses) becomes a new asymmetric carbon (also known as ***anomeric carbon***), the newly formed alcoholic -OH group is called an anomeric -OH group. *According to the spatial orientation of anomeric -OH group held by anomeric C, the pyranose or furanose ring structure of a sugar may exist into either an α-isoform or a β-isoform, which is known as* ***anomerism***. In case of ***α-anomers*** the anomeric -OH is oriented farthest from the $-CH_2OH$ positioned at the other end of the molecule; whereas, in case of ***β-anomers*** the anomeric -OH is oriented close to the $-CH_2OH$ at the other end of the molecule. The α- and β-anomers show difference in specific optical rotation, *i.e.*, ***α-D-glucopyranose*** and ***β-D-glucopyranose*** has optical rotation of +112.2° and +18.7° respectively.

Q11. Describe mutarotation with examples. (4)

Ans: Mutarotation is defined as ***dramatic changes in optical activity of freshly prepared solution of a reducing sugar until the net optical rotation becomes fixed at a particular value via attainment of a stable equilibria among its optical isomers, i.e., the α and β-anomers***. Into a solution of glucose, the α-anomers (α-D-glucopyranoses) show an initial specific rotation of +112.2°; this frequently changes to +18.7° upon standing due to conversion of few of the α-anomers into β-anomers (β-D-glucopyranoses). This type of interconversion continues until the solution attains equilibrium between the numbers of α- and β-anomers, which results in continuous changes in optical activity of the solution. When this type of equilibrium is reached the optical activity of the resulting solution of glucose is fixed into +52.7° known as ***mutarotation***. Another example of mutarotation is behavior of D-fructopyranose into its solution. The α-anomer of D-fructopyranose shows an optical rotation of -21° and the β-anomer has 133°; therefore, freshly prepared solution of fructose shows continuous changes in optical rotation of the solution until it stabilizes as -92.7°.



The interconversion between α- and β-anomers occurs spontaneously due to free rotation of the anomeric –OH and H around the new asymmetric carbon (anomeric C) in pyranose or furanose rings. Both the pyranose and furanose undergo mutarotation spontaneously. The Mutarotation is the property of reducing sugars (non-reducing sugars never show mutarotation), but reducing sugars like trioses, tetroses and ketopentoses remain unable to show mutarotation due to the fact that they never cyclize.

Q12. What are modified sugars? Give example. (2)

Ans: Modified sugars are chemically modified or conjugated forms of monosaccharides in our body that appear in vital biomolecules as major constituents. Modified sugars are classified into four groups, *e.g.*, deoxy sugar, amino sugar, uronic acids and amino sugar acids. Example: 2***-deoxyribose*** that acts as important constituent of DNA, and ***glucuronic acid***, a component of GAGs of extracellular matrix.

Q13. Define amino sugars with examples. (2)

Ans: Amino sugars are basic group (mostly $-NH_2$) containing sugars in their structure. There are two types of amino sugars, ***glycosoamines*** and ***glycosylamines.*** The basic difference between these two is in case of glycosoamines any -OH group is replaced by $-NH_2$ group, whereas in case of glycosylamine only the anomeric -OH group is replaced by $-NH_2$ group. Example: ***D-glucosamine*** is a glycosoamine and ***5-phosphoribosyl 1-amine*** is a glycosylamine.

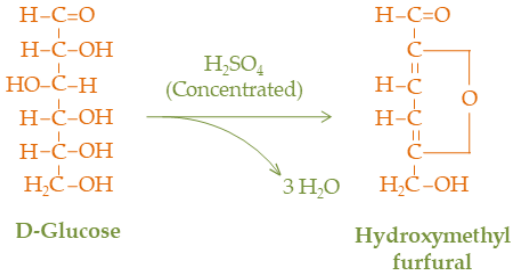
Q14. What are sugar acids? (2)

Ans: When the terminal aldehyde (-CHO) and/or alcoholic groups ($-CH_2OH$) in a sugar are oxidised into an acid (-COOH) group the derivative is called a ***sugar acid***. Sugar acids are of three types, *i.e.*, ***aldonic acid*** (oxidised into terminal –CHO group), ***uronic acid*** (oxidised into terminal $-CH_2OH$ group), and ***saccharic acid*** (oxidised into both

terminal –CHO group and CH_2OH group). Example: ***D-gluconic acid***, ***D-glucuronic acid*** and ***D-saccharic acid*** are examples of aldonic acid, uronic acid and saccharic acid respectively.

Q15. What are furfural compounds? State their importance. (2+2)

Ans: If the solution of a sugar is mixed with strong mineral acids (*i.e.*, H_2SO_4) it forms ***furfural derivatives***, *e.g.*, glucose being treated with concentrated H_2SO_4 forms hydroxymethyl furfurals. Furfurals form colored complexes with other compounds like α-napththol, thymol, recorcinol, orcinol, phloroglucinol etc.



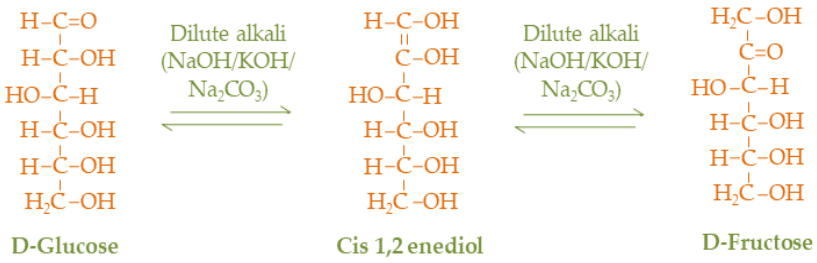
Formation of coloured furfurals often form the basis of different qualitative tests with monosaccharides we perform in laboratory, *e.g.*, ***Molisch's test***, ***Seliwanoff's test***.

i) ***Molisch's test:*** Addition of concentrated H_2SO_4 to a mixture of carbohydrate and Molisch's reagent (an alcoholic solution of α-napththol or thymol) leads to formation of a purplish ring at the junction between two adjacent layers. The lower layer of H_2SO_4 is heavier, and the alcoholic mixture forms the upper layer. However, this test is not specific particularly for the carbohydrates; many other molecules that contains aldehydic or ketonyl groups may give positive result with this test.

ii) ***Seliwanoff's test:*** This is specific test for the ketose sugars (*e.g.*, fructose). Seliwanoff's reagent contains resorcinol in diluted HCl. On boiling with Seliwanoff's reagent for 30 seconds only fructose can form furfural compounds because of their high sensitivity at even diluted HCl.

Q16. What are water enediols? (2)

Ans: In dilute alkali (NaOH or KOH or Na_2CO_3) the *aldoses* as well as *ketoses* undergo molecular rearrangement into their structures to form an intermediate that may frequently change into another sugar, *i.e.*, in case of glucose the ketonyl O of -CHO group receives proton from C^2, a double bond is formed between C^1-C^2 and in case of fructose the ketonyl O of C^2 receives proton from C^1 leading to formation of a C^1-C^2 double bond. Such intermediates are highly reactive and are known as '***water enediols***'.



Glucose and fructose both forms cis intermediates containing C^1-C^2 double bond, hence are known as ***cis-1,2 enediol***. Sugars with free aldehydic or ketonyl group can form enediols.

Q17. What do you understand by '*reducing sugar*'? (2)

Ans: Sugars that can form enediols on reaction with dilute alkali are called '***reducing sugars***'. The enediol forms are highly reactive and are easily oxidized by O_2 and other oxidizing agents to form sugar acids. The consequence of this oxidation is rapid reduction of other substances. Most of the monosaccharides are reducing agents, some disaccharides and as well as polysaccharides may also show reducing actions. The reducing action of sugars depend

on free aldehyde or ketonyl group in linear chain structure or presence of anomeric free alcoholic hydroxyl groups (-OH) in its closed ring structure.

Q18. What are glycoside linkages? (2)

Ans: The covalent bonds between a sugar and some other molecule, including another sugar via condensation of anomeric hydroxyl group (–OH) of the sugar and another hydroxyl group (-OH) of the other molecule after elimination of one molecule of water is known as ***glycosidic linkage***. A glycosidic linkage can be α or β, according to the configuration of hydrogen atom (H) and hydroxyl group (–OH) around the anomeric carbon of the preceding sugar in the oligosaccharyl or polysaccharyl unit.

Q19. State the occurrence and physiological importance of sucrose, trehalose, lactose and maltose. (4)

Ans: Disaccharides are produced by formation of glycosidic linkages between two monosaccharide units, *e.g.*, sucrose one molecule of α-D-glucopyranose and one molecule of β-D-fructofuranose condense via ***β-2,1 glycosidic linkage***; in case of trehalose, two molecules of α-D-glucopyranose are joined together via ***α-1,1 glycosidic linkage***; in lactose two molecules of α-D-glucopyranose are joined together via ***β-1,4 glycosidic linkage***; and in case of maltose an ***α-1,4 glycosidic linkage*** holds two molecules of α-D-glucopyranose.

***a) Sucrose*:** It is commonly known as '*table sugar*' and sometimes known as '*cane sugar*' as well.

i) ***Occurrence:*** Sucrose is widely distributed in nature and is the most abundant disaccharide in the human diet. It is obtained primarily from sugarcane and sugar beet, and is also present in fruits, vegetables, and plant sap.
ii) ***Physiological importance:*** Sucrose serves as a major dietary source of energy. It is hydrolysed into the small intestine by the enzyme sucrase (invertase) into glucose and fructose, which are readily absorbed. Excess consumption of sucrose is associated with dental caries, obesity, and metabolic disorders such as diabetes mellitus. In plants, sucrose acts as a transport form of carbohydrate.

***b) Trehalose*:**

i) ***Occurrence:*** Trehalose is found in fungi, yeast, insects, and some plants. It is also present in certain microorganisms, including Mycobacterium species.
ii) ***Physiological importance:*** Trehalose functions as a reserve carbohydrate and plays a protective role by stabilizing proteins and cellular membranes under conditions of desiccation, heat, and freezing. It is hydrolysed by the enzyme ***trehalase*** into glucose.

c) Lactose: Since milk is the major source of lactose it is also known as '*milk sugar*'.

i) ***Occurrence:*** Lactose is the principal carbohydrate of milk and is therefore also called milk sugar. It is present in significant amounts in mammalian milk.
ii) ***Physiological importance:*** Lactose is an important source of energy for infants. It is hydrolysed by the enzyme ***lactase*** in the intestinal mucosa into glucose and galactose. Galactose is essential for the synthesis of glycolipids and glycoproteins, particularly in nervous tissue. Deficiency of lactase results in lactose intolerance, leading to gastrointestinal disturbances such as diarrhoea and bloating.

d) Maltose: It is also known as '*malt sugar*'.

i) ***Occurrence:*** Maltose is not commonly found in free form in nature but is produced during the digestion of starch by the action of amylase. It is also present in germinating grains (malt).
ii) ***Physiological importance:*** Maltose is an intermediate in the digestion of polysaccharides. It is hydrolyzed by maltase in the intestine into two glucose molecules, which are absorbed and utilized for energy production. It is also important in fermentation processes.

Q20. Describe the structure, occurrence and physiological importance of starch. (5)

Ans: Starch is a homoglycan composed of glucose molecules and is found mainly into plants. Starch is the primary source of sugar for most of the people worldwide. Starch is a non-reducing carbohydrate and do not respond to Benedict's qualitative test for reducing sugars, but upon hydrolysis it shows reducing actions because hydrolysis yields maltose and glucose.

i) ***Structure:*** Starch appears as hydrophobic granules under microscope that are made up of concentric layer. Each such granule is composed of two polymeric units, some unbranched units (***α-amylose***) as well as branched units (***amylopectins***).

- ***α-Amylose:*** These comprise about 15-35% of starch molecules and are polymers of α-D-glucopyranose (as many as 600 in number) joined together via formation of ***α, 1-4 glycosidic linkages.*** These are water soluble, and are arranged into helical structures that form unbranched coils. At one end of each amylose chain, there is a free C^1-OH (***reducing end***) and a free C^4-OH at other end (***non-reducing end***).
- ***Amylopectins:*** These are water insoluble branched polymers, and are comprises about 65-85% of the starch molecules. Amylopectins are composed of liner chains of 25-30 molecules of α-D-glucopyranose joined together via formation of ***α, 1-4 glycosidic linkages.*** Around 10-200 such unbranched chains join with each other to form branched polymer of amylopectin. The joining of the linear chains with each other occurs via formation of ***α, 1-6 glycosidic linkages*** between glucose residues at branch points. Amylopectin molecules therefore carry greater number of glucose residues compared to amylose molecules. Amylopectins and amylose thus differ from each other structurally and biochemically in an extensive way.

ii) ***Occurrence:*** Starch is the principal storage carbohydrate in plants, especially in cereals (wheat, rice), tubers (potatoes), and legumes.

iii) ***Physiological importance:*** Starch is the most important dietary source of carbohydrates for humans. Hydrolysis of starch occurs into the GI tract by digestive enzyme ***amylases*** or may be done in vitro by heating in presence of mineral acids. Starch hydrolysis produce soluble forms of starch, which again yields smaller structures ***amylodextrin***, ***erythrodextrin***, ***achroodextrin*** and finally maltose. Amylases cannot yeild glucose from maltose, but under experimental conditions mineral acids hydrolyse maltose into glucose molecules. It provides sustained energy and contributes to maintaining blood glucose levels.

Q21. Describe the structure, occurrence and physiological importance of glycogen. (3)

Ans: Glycogen is a large, highly branched homopolysaccharide made up of glucose.

i) ***Structure:*** Glycogen is formed into liver (mainly) and to some extent in other tissues through an anabolic pathway, called ***glycogenesis***. There is a protein molecule present at the core of each glycogen, called ***glycogenin.*** Many unbranched linear chains composed of glucose molecules joined together by formation of ***α, 1-4 glycosidic linkages*** are attached to the serine residues of glycogenin. These straight chains are also attached to other small chains via formation of ***α, 1-6 glycosidic linkages*** at the branch points to form large branched molecules of glycogen

ii) ***Occurrence:*** Glycogen is the principal storage carbohydrate in animals, found mainly in the liver and skeletal muscles.

iii) ***Physiological importance:*** Glycogen serves as a readily mobilizable energy reserve. Liver glycogen maintains blood glucose levels during fasting. Muscle glycogen provides energy during muscular activity. Its branched structure allows rapid synthesis and degradation. During necessity glycogen molecules are hydrolysed to form glucose residues in a catabolic process referred to as ***glycogenolysis*** mainly into skeletal muscles and many other tissues.

Q22. Describe the structure, occurrence and physiological importance of cellulose. (3)

Ans: Cellulose is a linear homopolysaccharide molecule made up of glucose.

i) ***Structure:*** Cellulose is primarily composed of β-D-glucose units linked by ***β, 1-4 glycosidic linkages***. The chains are arranged in parallel and held together by hydrogen bonds, forming strong fibrils.

ii) ***Occurrence:*** Cellulose is the main structural component of plant cell walls and is abundant in cotton, wood, and plant fibers.

iii) ***Physiological importance:*** Cellulose is indigestible in humans due to the absence of cellulase enzyme. However, it acts as dietary fiber, promoting bowel movement, preventing constipation, and reducing the risk of colon diseases. In herbivores, it is digested by gut microbial enzymes.

Q23. What are glycoproteins? How do they differ from mucoprotein? State their physiological importance. (1+1+2)

Ans: Glycoproteins are conjugates of proteins with smaller oligosaccharyl units. On contrary mucoproteins are conjugates of proteins with long branched chain of carbohydrate. Therefore, in simple words, in a protein-carbohydrate conjugate when the carbohydrate content in below 4% it is usually a '***glycoprotein'***, and if the carbohydrate content is above 4% it is to be addressed as a ***'mucoprotein***'.

Physiological importance of glycoprotein: The oligosaccharyl conjugates present in glycoprotein are hydrophillic, which tends to alter the polarity of glycoproteins they are attached with and helps in modification of their biological activities. Several biologically important proteins come under the category of glycoproteins, *e.g.*, immunoglobulins, hormones like LH, FSH and TSH, intrinsic factor of castle, which is required for absorption of Vit B_{12} etc.

Q24. What are glycosaminoglycans (GAG) or mucopolysaccharides (MPS)? State their importance. (2)

Ans: ***Glycosaminoglycans***, also known as ***mucopolysaccharides*** are heteropolysaccharides formed of repeating units of ***amino sugars*** (glucosamine or galactosamine) and ***uronic acid*** (*glucuronic acid* or iduronic acid) in which the amino sugars can be further modified, *i.e.*, acetylated or sulphated. Glycosaminoglycans or mucopolysaccharides are very important components of proteoglycan present into extracellular matrix.

Q25. What are sialic acids? Give one example. (2)

Ans: Sialic acids are sugar acids found at the termini of oligosaccharyl chains attached to a glycoprotein, glycolipid or other glycosylated molecules. Example: ***N-acetylneuraminic acid.***

UNIT-2: PROTEINS

Amino acids: Structure, classification, general and electrochemical properties of α-amino acids; essential and non-essential amino acids; **Structures of protein:** Primary, secondary, tertiary and quaternary. Classification of proteins.

Q1. Define amino acids? (2)

Ans: Amino acids are α- amino organic acids in which a hydrogen atom attached to α- carbon of the carbon skeleton is substituted by an amino group, *e.g.*, glycine, alanine, glutamine. There also exist β- amino acids (*e.g., β-alanine*) and γ-amino acids (*e.g., γ- amino butyric acid* or *GABA*) in which the amino groups are held by β- and γ-carbons.

- *Standard amino acids:* All twenty amino acids, which appear into eukaryotic and prokaryotic proteins by conventional *translational machinery* in ribosomes, are called '***standard amino acids***'.
- *Modified amino acids:* Many amino acids appear in proteins through *post-translational modification* of existing standard amino acids within polypeptide chains after their synthesis are called '***modified amino acids***', *e.g.*, β-alanine, *β-amino isobutyric acid*, *γ-amino butyric acid* (GABA).

Q2. Name the 21st and 22nd amino acids. (2)

Ans: There are twenty amino acids, which occur in proteins of eukaryotes and prokaryotes by conventional translational machinery, and are called standard amino acids. In recent days two more amino acids, ***selenocysteine*** and ***pyrrolysine*** have been discovered, which are incorporated ***co-translationally*** into protein during translation and are designated as 21st and 22nd amino acids.

Q3. Classify amino acids with example. (5)

Ans: The twenty different naturally occurring standard amino acids are classified broadly into two groups according to the water solubility of their side chain groups.

a) Non-polar amino acids: Amino acids that contain non-ionizable, non-polar (water insoluble), hydrophobic (*water repelling*) hydrocarbon side chains are categorized under this class. These are further classified as follows:

i) Amino acids with non-polar aliphatic side chains: these amino acids usually have aliphatic hydrocarbon sidechains, *e.g.*, ***alanine, valine, leucine, isoleucine, methionine***.

ii) Amino acids with non-polar aromatic side chains: These amino acids carry phenyl or indole ring in sidechains, *e.g.*, ***phenyl alanine, tryptophan***.

iii) Imino acids: Imino acids contain imino groups (-NH) in their molecule, *e.g.*, ***proline*** is an imino acid that occurs in large proteins like collagens, immunoglobulins and offer them a structural flexibility.

b) Polar amino acids: The other amino acids contain water soluble, polar groups at their side chain. The side chain groups of polar amino acids are either non-hydrolyzable (uncharged) or are hydrolyzable (charged).

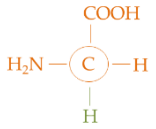
i) Amino acids with uncharged polar side chains: These amino acids bear aliphatic hydrocarbon sidechains, *e.g.*, ***serine, threonine, cysteine, asparagine, glutamine, tyrosine***.

- *Hydroxy amino acids:* Contain -OH groups *e.g.*, ***serine***, ***threonine***, ***tyrosine***.
- *Sulphur containing amino acids:* These amino acids possess thiol groups (-SH) in their sidechain, *e.g.*, ***cysteine***.
- *Amino acid amides:* Carry amide groups in their sidechain, *e.g.*, ***asparagine***, ***glutamine***.

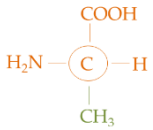
ii) Amino acids with charged polar side chains: These amino acids carry phenyl or indole ring in their sidechain.

- *Amino acids with positively charged sidechains:* These amino acids bear positively charged cationic groups in the hydrocarbon sidechains, *e.g.*, ***lysine, arginine, histidine***.
- *Amino acids with negatively charged sidechains:* These amino acids have negatively charged anionic groups in the hydrocarbon sidechains, *e.g.*, ***aspartate, glutamate***.

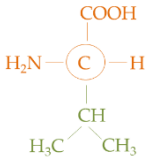
Non-polar amino acids



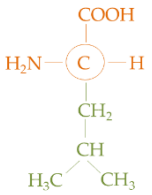
Glycine
(*α-amino, ethanoic acid*)



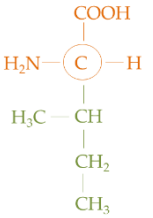
Alanine
(*α-amino, propionic acid*)



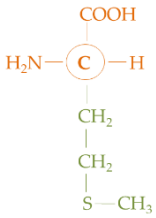
Valine
(*α-amino, iso-valeric acid*)



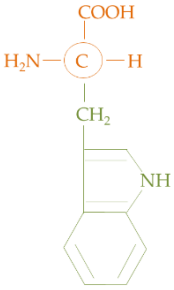
Leucine
(*α-amino, iso-caproic acid*)



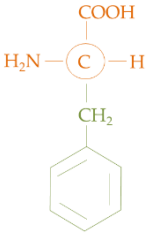
Isoleucine
(*α-amino, β-methyl valeric acid*)



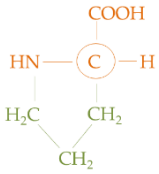
Methionine
(*α-amino, γ-methylthio, η-butyric acid*)



Tryptophan
(*α-amino, β-3-indole propionic acid*)

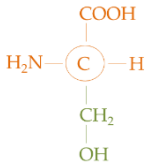


Phenyl alanine
(*α-amino, β-phenyl propionic acid*)

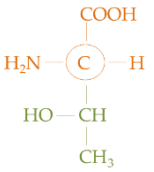


Proline
(*Pyrrolidone, 2-carboxylic acid*)

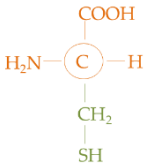
Polar uncharged amino acids



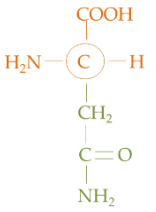
Serine
(*α-amino, β-hydroxy propionic acid*)



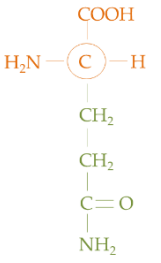
Threonine
(*α-amino, β-hydroxy butyric acid*)



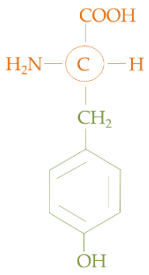
Cysteine
(*α-amino, β-mercapto propionic acid*)



Asparagine
(*α-amino succinic acid*)

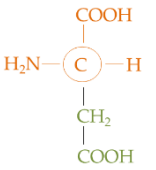


Glutamine
(*α-amino, δ-amido glutaric acid*)

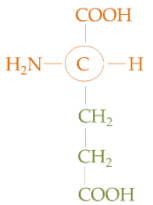


Tyrosine
(*α-amino, β-para hydroxy, phenyl propionic acid*)

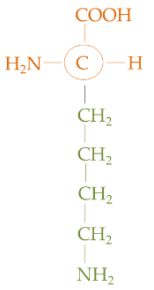
Polar charged amino acids



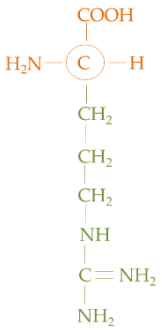
Aspartic acid
(*α-amino succinic acid*)



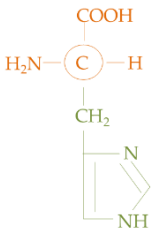
Glutamic acid
(*α-amino glutaric acid*)



Lysine
(*α, ε- diamine caproic acid*)



Arginine
(*α-amino, γ-guanidino valeric acid*)



Histidine
(*α-amino, β-imidazole propionic acid*)

Q4. What are essential amino acids? (2)

Ans: Few amino acids cannot be synthesized into the body, but are necessary for various physiological functions in body. There are ten such essential amino acids, *e.g.*, phenylalanine, threonine, methionine, tryptophan, valine, isoleucine, lysine, leucine, arginine and histidine (***phe-thre-met-try-va-iso-lysi-leuci-argi-histi***); among which arginine and histidine are ***semi-essential amino acids***.

Q5. Write a short note on modified amino acids. (5)

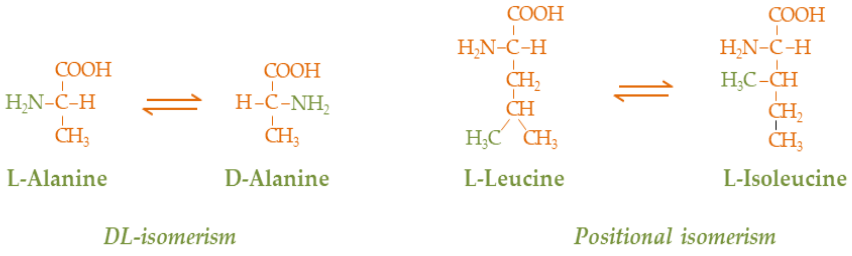
Ans: Modified amino acids appear within polypeptide chains after translation, *i.e.*, during post-translational modification of various amino acids. These post-translational modifications of standard amino acids include a wide array of chemical changes which are mostly *non-spontaneous reactions*. Modified amino acids are either post-translationally modified within the proteins and remain as a part of the protein; or are non-protein amino acid derivatives that act as free or metabolic products.

Examples of modified amino acids include ***β-alanine, βamino isobutyric acid, γ-amino butyric acid, δ-amino levulinic acid, taurine, ornithine, citrulline, 3,4-dihydroxy phenyl alanine, γ-carboxy glutamate, hydroxyproline, hydroxylysine, phosphoserine, phosphotyrosine, S-adenosyl methionine*** etc. Modified amino acids appear to serve various biochemical and physiologically important functions being a part of various biologically important proteins:

i) Modified amino acids confer structural integrity to the proteins, *e.g.*, ***hydroxylysine*** and ***hydroxyproline*** in collagens.
ii) Modified amino acids like ***phosphoserine, phosphotyrosine, phosphorthreonine*** helps in regulation of enzyme activity.
iii) Modified amino acids like ***phosphotyrosine*** of receptor tyrosine kinases are involved in signaling pathways.
iv) Gene expression is governed through modification of histones at lysine to form ***N-acetyl lysine***.
v) Modified amino acids like ***γ-carboxyglutamate*** is important for blood coagulation.
vi) ***Thyroid hormones*** are modified amino acids with pleiotropic hormonal functions. Pineal hormone ***melatonin*** is also a derivative of standard amino acid tryptophan.
vii) Many neurotransmitters, *e.g.*, ***GABA, adrenaline, nor-adrenaline*** are derived from modification of amino acids.
viii) Modified amino acid like creatine participate in cellular energy storage and transfer of the same.

Q5. What is DL-isomerism of amino acids? (2)

Ans: DL-isomerism of amino acids refers to the *existence of amino acids into D- and L-stereoisoforms based on the opposite orientation of amino (–NH_2) group and hydrogen (H) atom held around its chiral α-carbon*. Isomers in which the –NH_2 group is oriented at right-handed direction is called D-amino acid and isomers in which –NH_2 group is oriented at left-handed direction is called L-amino acid, *e.g.*, ***D-alanine*** and ***L-alanine***.



Amino acids may exist as positional isomers depending on the difference in position of specific groups. For example, leucine and isoleucine has same molecular formula, but they are positional isomer of each other because leucine bears –CH_3 substituent on its γ-carbon, whilst isoleucine bears –CH_3 substituent on its β-carbon.

Q6. *'Glycine is optically inactive'* – explain. (2)

Ans: Since the α- carbons in amino acids possess chirality, the strength of electromagnetic field remains unequal in all directions around it, hence it appears to be an asymmetric carbon. Due to presence of chiral α- carbons, almost all

amino acids endow optical activity in terms of deflection of the electromagnetic wave (*i.e.*, light) at specific direction. Being the smallest amino acid, glycine contains only one H-atom at its side chain, and which makes the α-C lose its chirality rendering glycine unable to rotate the direction of plane polarized light - *optically inactive*.

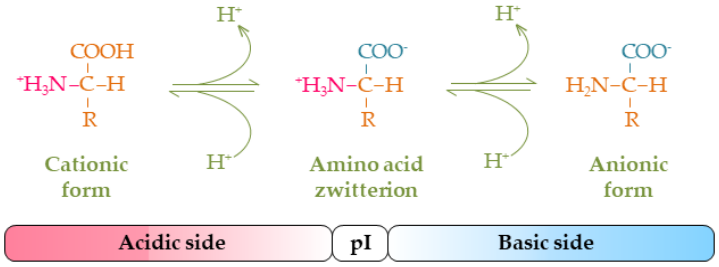
Q7. What do you understand by amphoteric nature of amino acids? (2)

Ans: The α-NH_2, and α-COOH groups as well as side chain -NH_2 and -COOH groups of amino acids are totally ionizable. Depending on the pH of the solution amino acid may *donate* or *accept* H^+ and get converted into an anion or cation respectively. Thus, amino acids exist as an *ampholyte*, and it is called ***amphoteric nature*** of amino acids.

Q8. What are zwitterions? What is isoelectric pH? (2)

Ans: Due to their amphoteric nature, an amino acid may *donate* or *accept* proton and get converted itself into an anion or cation respectively depending on the pH of solution. At a specific pH, the number of negative and positive charges on an amino acid becomes equal and the amino acid bears no net charge. Such an ionized amino acid carrying opposite charges with no net charge is called a ***zwitterion***, and the pH at which an amino acid exists into zwitterion form is its ***isoelectric pH*** (***pI***). Different amino acids exhibit different pI values.

- At acidic side of pI, amino acids exist as cations by accepting proton from surroundings.
- At the basic side of pI, amino acids exist as anions by donating proton to the surroundings.



A large number of amino acid zwitterions may get strongly held together by formation of electrostatic bonds between their oppositely charged ions, thereby stabilizing the formation of *random crystal lattices*. This imparts more and more resistance to structural disturbances in the lattice structure induced by application of heat, and *increases the melting point* of concerned amino acids.

Q10. Define proteins. (2)

Ans: Proteins are macromolecules made up of amino acids; more precisely proteins are polymer of L-α-amino acids joined together by peptide bonds. All naturally occurring animal proteins contain L-amino acids instead of D-amino acids. Proteins carry C, H, O, N and most of them also carries S, whereas few only carry P in addition. A ***polypeptide*** refers to a peptide chain containing more than 10 amino acids. On the other hand, a ***peptide*** refers to a simple chain containing 2-10 amino acids.

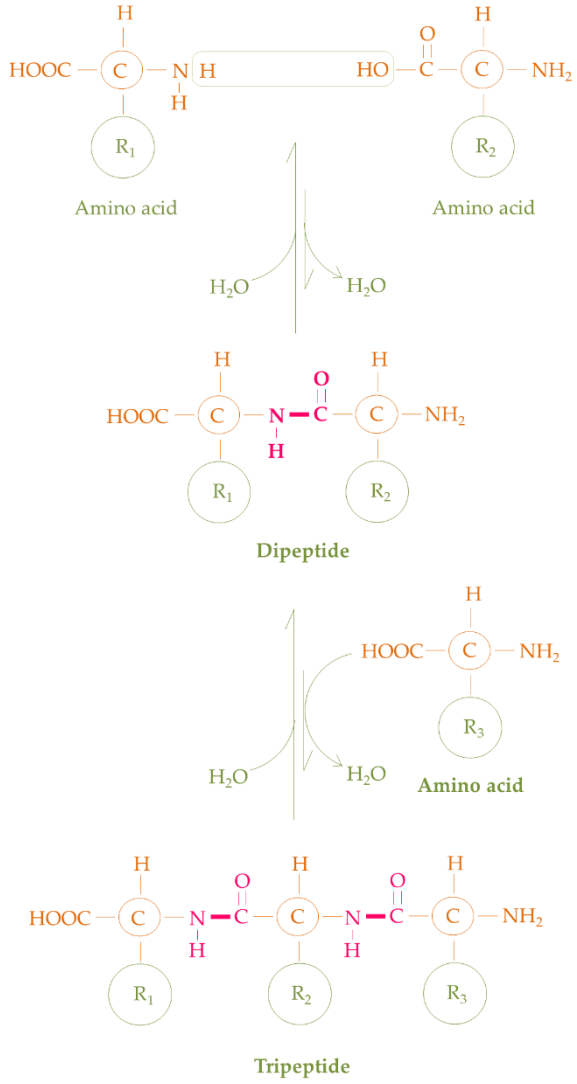
Q11. What do you understand by molecular configuration and conformation? (2+2)

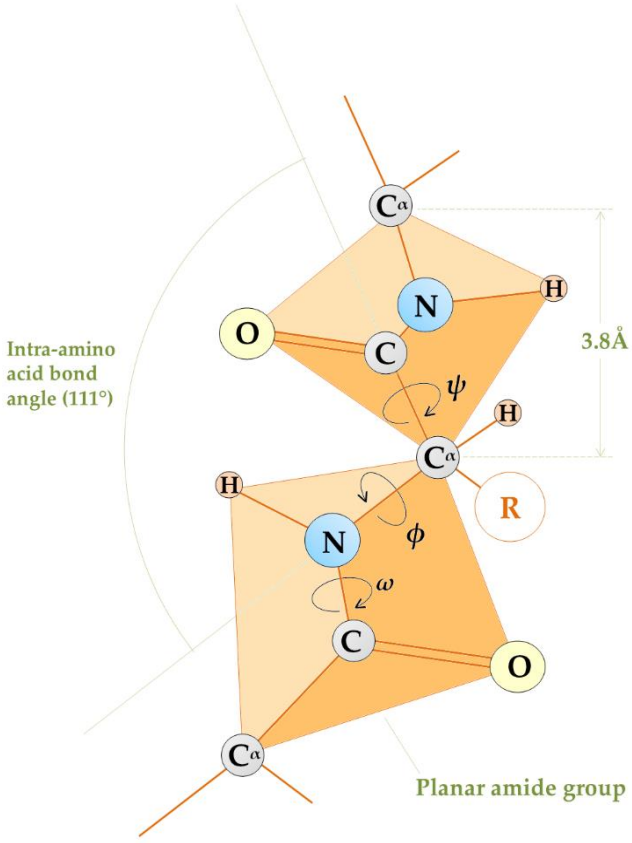
Ans: ***Configuration*** refers to the '*geometric relationship*' between given set of atoms, *e.g.*, D- and L-isoforms (isomers) of amino acids. Changes in configuration, and therefore interconversion of isomers require breakage of high energy and non-spontaneous covalent bonds. ***Conformation*** refers to the '*spatial relationship*' between every atom in a molecule; hence changes in conformation are possible without rupturing the covalent bonds. Changes in conformation are possible by simply rotating about single bonds, and retaining the configuration of the biomolecule.

Q12. Write a short note on peptide linkage. (5)

Ans: The linear sequence of amino acids in a single polypeptide chain is called the *primary structure* of proteins and peptide linkages are the major stabilising force**.** Peptide linkage formation involves ***nucleophilic acyl substitution reaction***, a dehydration type of condensation reaction. The unshared electron pair of the α-amino N of one amino

acid produce nucleophilic attack on α-carboxyl-C of another amino acid to release one mole of water and both the amino acids are joined together forming a linkage between them ***peptide linkage*** or ***amide linkage***. Peptide linkage between amino acids form linear polypeptide chains, hence is considered to be the major force for stabilisation of primary structure of proteins. Formation of peptide linkage is an endergonic process (**ΔG = +21 KJ/mol**).





So, the peptide linkages indicate joining points between amino acids in a peptide or polypeptide chain and from the preceding amino acid, a peptide linkage has six components, *i.e.*, the α-C of preceding amino acid, amide-N, amide-H, carbonyl-C, carbonyl-O and α-C of the amino acid. All these six components associated with one peptide linkage are together called the ***peptide unit*** or ***amide unit***. The lengths of different bonds in a peptide unit or amide unit are given below in the following table:

Bonds in a peptide or amide unit	Bond length
Carbonyl-C and amide-N (peptide linkage)	1.33 Å
α-C and carbonyl-C	1.53 Å
α-C and amide-N	1.45 Å
amide-N and amide-H	1.03 Å
Carbonyl-C and carbonyl-O	1.23 Å

Salient features of peptide linkage: Peptide linkage endows three unusual features as follows:

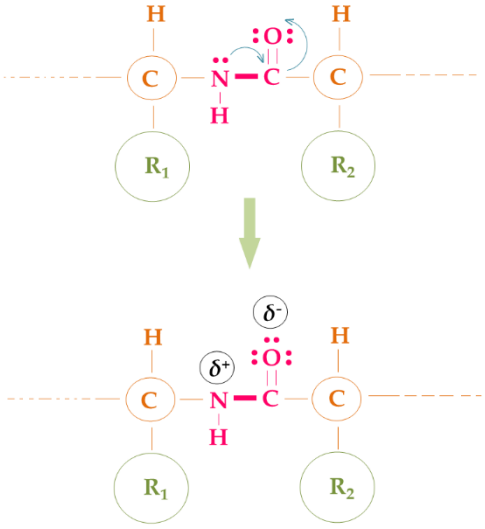
i) ***Partial double bond character:*** In a peptide unit or amide unit, the high electronegativity of carbonyl-O tends to snatch electron from the amide-N. As a consequence, the carbonyl-O gets a partial δ^- charge and the amide-N a partial δ^+ charge. Hence an additional mild electrostatic force starts to impact the strength of peptide linkage, *i.e.*, carbonyl-C starts to pull amide-N little closer, leading to shortening of the bond length, and this offers a 40% double bond character to the peptide linkage. The bond length between carbonyl-C and amide-N in a peptide unit is 1.33Å which is an intermediate value between standard C-N single bond (1.45 Å) and C-N double bond (1.27Å) but closer to the value of C-N double bond; hence is referred to as ***'partial double bond character'***.

ii) ***Rigidity:*** Due to the partial double bond nature of the peptide linkage, the entire peptide unit or amide unit the peptide linkage shows extreme ***resonance stabilization***. This results in ***restriction of rotation*** around the peptide linkage which confers a great ***rigidity*** to the peptide linkage and the entire amide unit. This rigid nature of peptide linkage restricts the rotation around it and keeps the value of angle of rotation fixed which is often denoted by ω. Based on the spatial orientation of amide-H and carbonyl-O around the peptide linkage, it can exist into either '*trans*' (ω = 180°) or '*cis*' form (ω = 0°). The *trans* form is predominant over *cis* form and is favoured by a ratio of 1000:1 due to ***'steric hindrance'*** or ***atomic collision***. The unusual *cis* form is preferred by *proline* and *hydroxyproline*.

iii) ***Coplanarity:*** Since, the rotation around peptide linkage is almost restricted, all six atoms in a *peptide unit* or *amide unit* tend to align at a similar plane, which is referred to as the ***planarity*** of peptide unit. It has been found that two successive planar amide groups also tend to be arranged in same plane which is called ***coplanarity*** of peptide units. The bond angle between successive planar amide groups accounts for 111°; this is the angle formed between C^α-C of one planar amide group and C^α-N of another planar amide group, and therefore is an ***intra-amino acid bond angle***. This intra-amino acid bond angle is nearly fixed at its value 111°.

Q13. Why peptide linkages are called *'partial double bonds'*? (2)

Ans: In a peptide unit or amide unit, the carbonyl-O being highly electronegative tends to snatch electron from the amide-N of the same. This provides carbonyl-O a small charge of δ^- and amide N a small δ^+ charge. Due to this electrostatic interaction between two ends of peptide bond it creates an unusual situation, in which carbonyl-C pulls amide-N little closer which provides 40% double bond character to the peptide linkage; and makes the peptide linkage a partial double bond in nature.



Q14. What are Ramachandran angles? State their significance. (2)

Ans: Ramachandran angles are torsion angles (*dihedral angles*), which are defined by 4 points in space. In case of two successive planar amide groups joined at C^α the angle of rotation around C^α-N is called ***phi*** ***(ϕ) angle*** and C^α-C is called ***psi*** ***(ψ) angle;*** these angles were described by ***G. N. Ramachandran*** and are also called ***Ramachandran angles***.

In proteins the two torsion angles ϕ and ψ describe the rotation of the polypeptide chain around the two bonds on both sides of the C^{α} atom, and therefore the flexibility of the polypeptide chain.

Q15. Why an amino acid within a polypeptide chain is called a residue? (2)

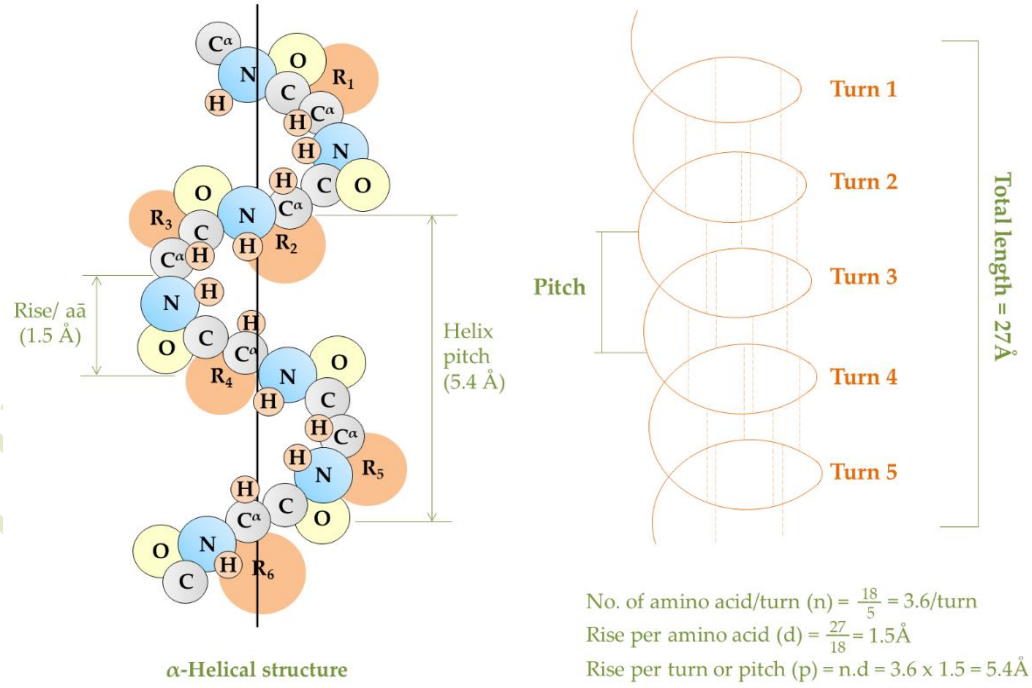
Ans: Since the formation of peptide linkage requires elimination of one H and one OH from participating amino acids, hence the molecular mass of amino acids within a peptide chain gets reduced, and they are better known as residue within the peptide.

Q16. What are 3_{10} helices? (2)

Ans: It is a short loop helical structure formed as a result of H-bonding between carbonyl-O and 10th H of the third amide plane, as the name implies. The 3_{10} helix is not that much unstable and can be found in proteins (2-5% of total helices).

Q17. Write a short note on α-helix. (5)

Ans: Helically arranged regions occurring in polypeptide chains have been emerged as fundamental structural units in many fibrous and most globular proteins. The geometry of helices is defined by its direction (***handedness***), ***pitch*** and *radius* of the helix. The helix can be either ***right-handed*** or ***left-handed*** depending on whether the groups are directed *clockwise* or *anticlockwise* around the axis of the helix. Pitch refers to the distance between two equivalent points in a complete turn of the helix. Helical structures are not intrinsically stable; and the stability to assume such a highly ordered helical conformation requires additional interactions like ***intra-chain H-bonding,*** which are non-covalent low energy spontaneous interactions. Different types of helices are found in secondary structures of proteins depending on differences in the pattern of H-bonding between amino acids, *e.g.*, ***2_7 ribbon***, ***3_{10} helix***, ***3.6_{13} helix*** (***α-helix***) and ***4.4_{16} helix*** (***π-helix***). The helical structure formed by H-bonding between carbonyl-O and 13th H atom of the fourth amide plane is called ***α helix***. Properties of α-helix were first described by Pauling and his team in 1930s and then by Corey in 1940s by X-ray crystallographic analysis of alpha keratin protein present in hair.



α-Helical structure

On an average, each of the α-helices has a length of 27Å with five turns and 18 amino acids. Hence, the number of amino acids present per turn of one α-helix is = 18/5 = 3.6, which is denoted as 'n'. The distance between two neighboring amino acids in α-helix is = 27Å/18 = 1.5Å, denoted as 'd'; and is also called the ***linear translation*** or ***rise***

per amino acid. If the rise per amino acid is 1.5Å and the number of amino acids per turn in α-helix is 3.6, then the '*pitch*' or '*rise per turn*' of the helix can easily be calculated by simply multiplying 'n' with 'd'. Hence the pitch of α-helix is = 3.6 x 1.5Å = 5.4Å, which is denoted as 'p'.

Small, uncharged, non-polar or hydrophobic amino acids such as alanine, leucine, and phenyl alanine are preferred to be present in α-helix. Presence of large, charged, polar or hydrophillic amino acids such as arginine, serine may destabilize the α-helical structure because of their bulky side chain groups and their tendency to bring water to the core. Since α-helices stabilized by hydrogen bonds water at its core becomes a disruptive force, hence non-polar or hydrophobic amino acids are usually found in α-helices. Proline and hydroxyproline should not be present and is never found in α-helix. The hydrogen bonding pattern of α-helix is strain free and nearly parallel to the helix axis in comparison to other helices and is therefore predominant form among all other helices in nature. Although α-helix can be directed left-handed or right-handed, the right-handed form is mostly preferred in naturally occurring proteins. Alpha helices are present in α-keratin proteins of hair, nail and skin; are found mostly in globular proteins. The α-helical structures are highly hydrophobic coiled coil barrel like structures and are often found in the transmembrane regions of many membrane-bound proteins.

Q18. Why proline and hydroxyproline are never found in α-helix? (2)

Ans: Since the formation of α-helices and β-pleated sheets rely on regular hydrogen bonding between the amide-H and carbonyl-O from peptide groups, presence of *proline* or *hydroxyproline* will produce structural irregularities and therefore high proportion of any of these two amino acids in α-helix or β-pleated sheets will disrupt the structure.

Q19. Why α-helices are the most abundant among other helices? (2)

Ans: Among all other helices like 2_7 ribbon, 3_{10} helix, 3.6_{13} or α-helix and π-helix, the α-form is the most abundant because of optimum H-bond length. In case of 3_{10} helix and π-helix the H-bond length is either too short or too long which does not support their structural integrity, making the α-helix most common form of helical structure in naturally occurring proteins.

Q20. Write a short note on β-pleated sheet. (5)

Ans: Beta (β) pleated sheets are extended secondary structures formed by more than one polypeptide chains arranged side by side either parallely or antiparallely. The β-pleated sheets have slightly zigzag appearance because of the regular bending of polypeptide backbones participating in its formation. A β-pleated sheet may consist of 2-15 peptide chains, each of which may consist of 5-16 amino acids which are hydrogen bonded to create an extended sheet like appearance. The hydrogen bonds are formed between several peptide chains hence are called ***inter-chain hydrogen bonding.*** There are two types of β-pleated sheets:

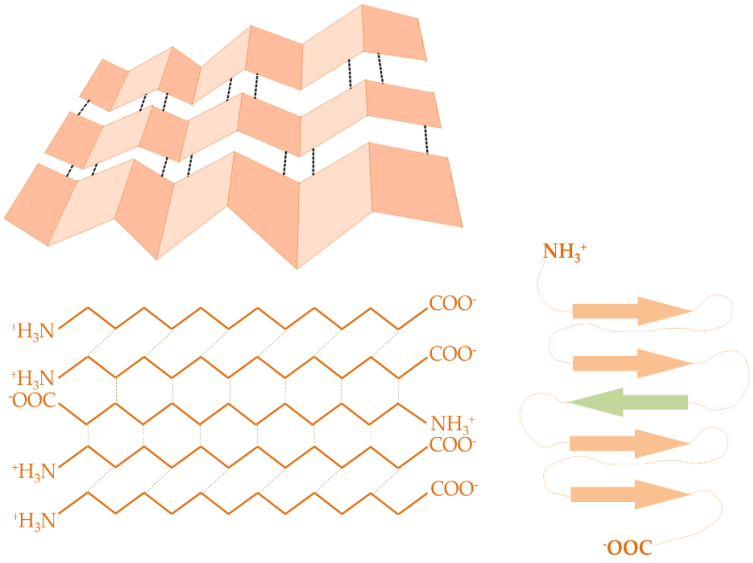
i) ***Parallel β-pleated sheets:*** In this type of β-pleated sheets the amide-N, α-C and carbonyl-C appears in same sequence into neighboring polypeptide stretches.
ii) ***Anti-parallel β-pleated sheets:*** The amide-N, α-C and carbonyl-C tend to appear in reverse order into subsequent polypeptide stretches.

Hydrogen bonds are formed here between the atom attached to the amide group of one amino acid and carbonyl-O of another amino acid of two separate adjacent polypeptide stretches. In case of parallel β-pleated sheets hydrogen bonds are slanted or slightly tilted forming an angle with the backbone axis of peptide chains, but in case of antiparallel β-pleated sheets hydrogen bonds are perpendicular to the backbone axis of the peptide chain. Probably this situation of irregularity in hydrogen bonds in parallel β-pleated sheets is the reason of distortion of their structure, rendering the parallel β-pleated sheets *more unstable* in comparison to anti-parallel β-pleated sheets.

- Proline and hydroxyproline if present may disrupt the sheet structure, as they do not contain any amide-H available for binding with carbonyl-O to form hydrogen bond.
- Formation of a stable β-pleated sheet structure requires small amino acids which are uncharged or bears slight charge.

- Temperature and physical distortion may convert α-helical structures of keratin in hair into β-pleated sheet structure.

The β-pleated sheets are found in fibrous proteins mostly, β-keratins of the reptilian claws, fish scales, spider web and silk fibroin; and are also found in central core of globular proteins.



Q21. *'Antiparallel β-pleated sheets are more stable than parallel β-pleated sheets'* – justify. (3)

Ans: In case of β-pleated sheets, hydrogen bonds are formed between the atom attached to the amide group of one amino acid and carbonyl-O of another amino acid of two separate adjacent polypeptide stretches. In case of parallel β-pleated sheet, the axes of hydrogen bonds are slanted or slightly tilted forming an angle with the backbone axis of polypeptide chains, but in case of antiparallel β-pleated sheets hydrogen bonds are perpendicular to the backbone axis of the polypeptide chain. Probably this irregularity in hydrogen bonds in parallel β-pleated sheets is the reason of slight distortion of regularity of their structure, rendering the parallel β-pleated sheets *unstable* in comparison to anti-parallel β-pleated sheets.

Q22. State the locations of α helices and β-pleated sheet? (2)

Ans: The α-helices are present in α-keratins of hair, nail and skin; are found mostly in globular proteins. On the other hand, β-pleated sheets are mostly found in fibrous proteins, like β-keratins of reptilian claw, fish scales, spider web and silk fibroin; and are also found in central core of globular proteins.

Q23. What are motifs? (2)

Ans: Secondary structures in proteins show hydrophobicity and for this reason adjacent helices and β-pleated sheets tend to cluster together. These types of structural associations are referred to as ***motifs***. Motifs are stabilized mainly by ***hydrophobic interactions*** and appear to be the structural units of super-secondary structure. Examples of motif include ***β-α-β motif***, ***4α motif***, ***β-barrel*** etc.

Q24. What are domains? (2)

Ans: The basic structural unit of tertiary structure is called ***domain*** which is formed by association of various motif structures being clustered with each other. Domains can fold independently into a stable three-dimensional tertiary structure and represent the functional units of proteins.

Q25. Describe the major physiochemical forces that stabilize a globular protein? (6)

Ans: Three major stabilising forces for a globular protein are– *peptide linkage*, *hydrogen bond* and *hydrophobic interactions*. Peptide linkages help in formation of primary structure of proteins, hydrogen bonding helps in formation of secondary structure of proteins, and hydrophobic interactions help in protein folding to form super-secondary and tertiary structures. However, additional physicochemical forces like *van-der Waal's forces*, *disulphide linkage*, *ionic interactions* (*i.e., salt bridges*) also help in stabilization of *three-dimensional conformation* of proteins.

Protein structure	Structural unit	Bonds involved in stabilisation
Primary (1°) structure	Amino acids	Peptide linkage
Secondary (2°) structure	Helical structure (mainly α-helix) and β-pleated sheet	• Inter-chain hydrogen bond • Intra-chain hydrogen bond
Super-secondary structure	Motif	• Hydrophobic interaction • Hydrogen bonds • van-der-Waal's forces
Tertiary (3°) structure	Domain	• Hydrophobic interaction • Hydrogen bonds • Salt bridges • van-der-Waal's forces • Disulfide linkages
Quaternary (4°) structure	Globular polypeptide chains	• Hydrophobic interaction • Disulfide linkages

i) ***Peptide linkage:*** Primary structure of a protein is defined as '*the linear sequence of amino acids in its polypeptide chain*'. Amino acids are joined together by formation of ***peptide bonds*** in between them. Peptide bonds share a partial double bond character which is so strong to keep amino acids joined with each other. Restriction of rotation around peptide linkage offers enormous stability to the backbone of polypeptide chain. Two amino acids are joined together by formation of one peptide linkage is called a *dipeptide unit*; similarly, three amino acids joined by formation of two peptide linkages called a *tripeptide unit* and so on.

ii) ***Hydrogen bonding:*** Hydrogen bonds are formed as a result of differential attraction of the atomic nuclei for their orbital electrons, a tendency of atomic nuclei which is also known as ***electrophilia***). Electrophilia is referred to as ***electronegativity***, and hydrogen bond is a bridge formed between two highly electronegative atoms that share hydrogen atom in between them. In case of polypeptide chains the free carbonyl-O of peptide unit may form hydrogen bonding with amide-N of another peptide unit sharing the amide-H in between them. The hydrogen bonding found in stabilizing secondary structure is of two types:

- ***Inter-chain H-bonding:*** occurs between free carbonyl-O of peptide unit may form hydrogen bonding with amide-N of another peptide unit among different polypeptide chains.
- ***Intra-chain H-bonding:*** occurs between free carbonyl-O of peptide unit may form hydrogen bonding with amide-N of another peptide unit within same polypeptide chain.

iii) ***Hydrophobic interactions:*** Hydrophobic interaction refers to the tendency of hydrophobic groups or molecules to cluster in an aqueous environment. The hydrophobic sidechains of non-polar amino acids from different secondary structures like α-helix, β-pleated sheet present in a polypeptide chain comes to close proximity with each other due to hydrophobic interactions between them, and forms higher order structures like ***motifs, domains***.

iv) ***Salt bridges:*** Salt bridges are combinations of ionic interactions and hydrogen bonds and are formed between oppositely charged side chains, *i.e.*, anionic (-COO^- end) and cationic (-NH^+ end). The basic amino acids usually have a -NH^+ group which may directly engage into an *electrostatic interaction* with -COO^- group of acidic amino acids. Besides this, H-atom attached with the -NH^+ group of basic amino acid can be shared with the O of -COO^- group of acidic amino acid forming a *hydrogen bond*. This combinatory force of ionic interaction and hydrogen bond is referred to as *salt bridge* which appears to be a very important stabilizing force for tertiary structure of proteins.

v) ***van-der-Waal's forces:*** Occurs between both polar and non-polar amino acids. Although these are weak forces, the cumulative outcome of huge number of van-der-Waal's forces operating within a protein molecule becomes impactful in determining its higher order structure, *i.e.*, super-secondary, tertiary and quaternary structures.
vi) ***Disulfide linkages:*** Most prominent covalent interaction found in tertiary structure, often found in extracellular proteins (the -SS- linkages are less stable at reducing pH of cytosol). Cysteine has a thiol group (-SH) in its side chain, which helps in formation of –SS- linkages between two cysteine residues forming a ***cystine.*** Disulfide linkages provide additional stability to higher order structure of proteins, especially in extracellular proteins.

Q26. Classify the proteins? (6)

Ans: Proteins are classified on different bases such as shape and size, functional properties, and solubility with physical properties.

Classification on the basis of shape and size: Proteins are divided into two major groups, fibrous and globular.

i) ***Fibrous proteins:*** Fibrous proteins are elongated, thread-like proteins in which the axial ratio (*length*:*width*) is usually greater than 10. They are generally insoluble in water and mainly perform structural and protective functions. These are long and narrow in shape, tough and insoluble that provide mechanical support and strength. These are usually present in connective tissues, *e.g.*, α-keratin of hair and nails, collagen of connective tissue and elastin of ligaments and tendons.
ii) ***Globular proteins:*** Globular proteins are spherical or compact proteins in which the axial ratio is less than 10. These proteins are usually soluble in water and are metabolically active. These are compact and rounded structure, that are water soluble in nature. Globular proteins participate in dynamic cellular functions. Examples include hemoglobin, myoglobin, enzymes, albumin.

Classification on the basis of functional properties: According to their physiological roles, proteins are classified as:

i) ***Defensive proteins:*** These proteins protect the body from harmful agents and diseases. Examples include immunoglobulins (antibodies).
ii) ***Contractile proteins:*** These proteins are involved in muscular contraction and movement. Examples include actin and myosin. These proteins form contractile filaments in muscle fibers.
iii) ***Respiratory proteins:*** These proteins help in transport and storage of respiratory gases. Examples include hemoglobin, myoglobin.
iv) ***Structural proteins:*** Structural proteins provide shape, support and strength to tissues. Examples include keratin in hair and nails, collagen in connective tissue, elastin in ligaments.
v) ***Hormonal proteins:*** Some proteins function as hormones and regulate physiological activities. Example: insulin, glucagon.
vi) ***Enzymatic proteins:*** These proteins act as biological catalysts and accelerate biochemical reactions. Examples include catalase, peroxidase, carbonic anhydrase etc.

Classification on the basis of solubility and physical properties: This is the most widely accepted classification of proteins. Proteins are divided into simple proteins, conjugated proteins and derived proteins.

a) Simple proteins: Simple proteins yield only amino acids upon complete hydrolysis. They are further classified into the following groups:

i) ***Protamines:*** These are small basic proteins rich in arginine. These are soluble in water, dilute acids and alkalies, non-coagulable by heat, strongly basic proteins. Examples include salmine, sardinine, clupeine.
ii) ***Histones:*** Histones are basic proteins associated with nucleic acids. These are soluble into water and dilute acids, insoluble in ammonia and are rich in arginine and histidine. Example: nucleohistones (H1, H2A, H2B, H3 and H4).
iii) ***Albumins:*** Albumins are acidic proteins soluble in water and dilute salt solutions. These are heat coagulable, precipitated by full saturation with ammonium sulfate, having an isoelectric pH around 4.7. Examples include ovalbumin of egg, lactalbumin of milk, legumelin of legumes.

iv) ***Globulins:*** Globulins are insoluble in pure water but soluble in neutral salt solutions. These are heat coagulable proteins, that are easily precipitated by half saturation with ammonium sulfate. Examples include ovoglobulin, lactoglobulin, legumin of legumes.

v) ***Gliadins:*** Gliadins are plant proteins soluble in 70–80% ethanol. These are insoluble in water, rich in proline and poor in lysine. Example include gliadin of wheat.

vi) ***Glutelins:*** Glutelins are plant proteins insoluble in water and neutral salt solutions but soluble in dilute acids and alkalies. These are heat coagulable proteins that are rich in glutamic acid. Examples include glutenin of wheat.

vii) ***Sclero-proteins (Albuminoids):*** These are fibrous proteins with high stability and low solubility that provide structural support.

- ***Keratins:*** Present in epidermal tissues, like hair, nails, wools, hoofs, and feathers.
- ***Collagens:*** Present in connective tissues. By boiling, collagen is converted into gelatin.
- ***Elastins:*** Present in elastic connective tissues, ligaments and tendons.

b) Conjugated proteins: Conjugated proteins contain a non-protein component called a prosthetic group in addition to amino acids. They are classified as follows:

i) ***Nucleoproteins:*** Contain nucleic acids as prosthetic groups. Examples may include deoxyribonucleoprotein, ribonucleoprotein.

ii) ***Mucoproteins:*** Contain mucopolysaccharides as prosthetic groups. Examples include mucin, gonadotropic hormones such as FSH, LH and HCG.

iii) ***Glycoproteins:*** Contain carbohydrates firmly attached to the protein molecule. Examples include immunoglobulins, certain membrane proteins.

iv) ***Lipoproteins:*** Contain lipids as prosthetic groups. Examples include plasma lipoproteins, membrane lipoproteins.

v) ***Metalloproteins:*** Contain metallic ions as prosthetic groups. Examples include ferritin (Fe^{3+}), carbonic anhydrase (Zn^{2+}), ceruloplasmin (Cu^{2+}).

vi) ***Chromoproteins:*** Contain colored prosthetic groups. Examples include hemoproteins (heme containing protein, *e.g.*, hemoglobin, cytochromes, catalases, peroxidases), flavoproteins (contain riboflavin derivatives, *e.g.*, respiratory flavoproteins), visual purple of retina (contains carotenoid pigment)

vii) ***Phosphoproteins:*** Contain phosphoric acid as prosthetic group. Examples include casein of milk, vitellin of egg yolk.

c) Derived proteins: Derived proteins are formed from native proteins by the action of heat, acids, alkalies, enzymes or other chemical and physical agents. They are divided into primary and secondary derived proteins.

i) ***Primary derived proteins:*** These are formed without cleavage of peptide bonds.

- ***Proteans:*** Insoluble products formed from proteins by action of water, dilute acids or enzymes. Examples include myosan from myosin, edestan from edestin.
- ***Metaproteins:*** Produced by the action of acids and alkalies on proteins. Examples include acid metaprotein, alkali metaprotein.
- ***Coagulated proteins:*** Produced by action of heat or alcohol on native proteins. Examples include cooked egg albumin, cooked meat proteins.

ii) ***Secondary derived proteins:*** These are formed by progressive hydrolysis of proteins involving cleavage of peptide linkages.

- ***Proteoses:*** These are water soluble heat coagulable proteins.
- ***Peptones:*** These are water soluble, not heat coagulable proteins.
- ***Peptides:*** Peptides contain a small number of amino acids joined by peptide bonds. These are water soluble, heat non-coagulable proteins.

UNIT-3: LIPIDS

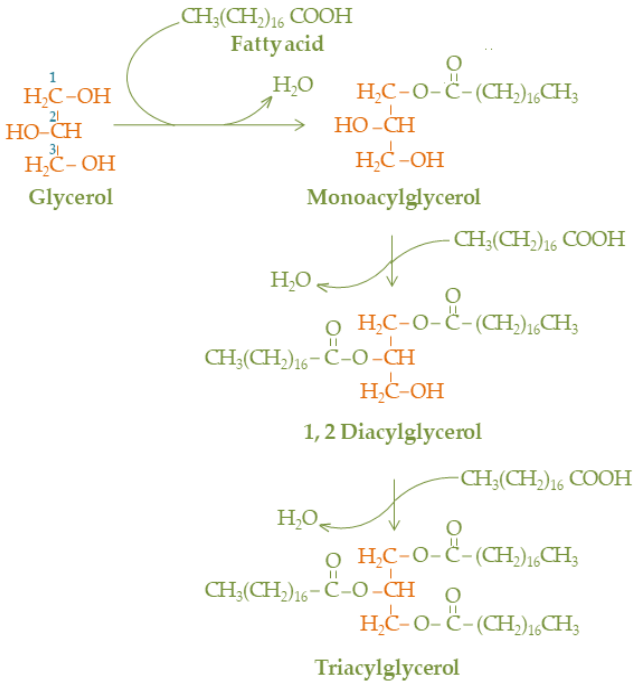
Classification of lipids. Saturated and unsaturated fatty acids, essential and non-essential fatty acids. Structure and formation of triglyceride. Iodine number and saponification number of fats.

Q1. What are lipids? (2)

Ans: ***Lipids are esters of fatty acids with alcohols***. So, the basic functional unit of a lipid molecule are glycerol or any other fatty alcohol and fatty acids. In many cases lipids may contain additional groups or molecules like phosphoric acid, nitrogenous bases and carbohydrates as their conjugates. Lipids usually represent a heterogeneous group of organic substances found both in eukaryotes and prokaryotes.

Q2. *'All fats are lipid but not all lipids are fat'*- justify. (2)

Ans: Lipids are esters of fatty acids with alcohols; on contrary fats are ***triacylglycerols***, lipids in which three fatty acid molecules are esterified specifically with one molecule of glycerol. Hence, any ester of fatty acid with any fatty alcohol must be considered as a lipid; on the other hand, in spite of the fact that fats are formed through esterification of fatty acids with alcohol, they must fulfil two specific criteria, first, there must be three fatty acids condensed with a fatty alcohol, and second, the fatty alcohol must be a ***glycerol***.



Q3. Classify lipids. (3)

Ans: According to ***Bloor's classification*** system lipids are broadly categorized into three different groups, *e.g.*, ***simple lipids***, ***compound lipids*** and ***derived lipids.***

i) Simple lipids: These are esters of fatty acids with alcohols.

a) Neutral fats or glycerides: These are esters of fatty acids with glycerol. According to the number of fatty acids attached to the glycerol, there are three types of glycerides –

- *Monoacylglycerol:* Contains only one molecule of fatty acid esterified to the glycerol.

- *Diacylglycerol:* Contains two fatty acid molecules esterified to the glycerol.
- *Triacylglycerol:* Contains three molecules of fatty acids esterified to glycerol. Triacylglycerols are also known as fats, and oils are those fats that remain in liquid state at room temperature.

b) Waxes: These are esters of long-chain fatty acids with higher monohydroxy long chain aliphatic alcohols. These esters are usually found in solid form. Examples include myricyl palmitate which is an ester of myricyl alcohol and palmitic acid found in bee wax. Cholesteryl esters and vitamin D esters are also examples of waxes.

ii) Compound lipids: When fats contain other groups in addition to the alcohol and fatty acids are referred as compound lipids. There are also different types of compound lipids –

a) Phospholipids: These are substituted forms of fats that contain phosphoric acid, and other groups like choline, ethanolamine, inositol, serine, or diphosphatidyl glycerol, *e.g.*, ***lecithin*** and ***cephalin***.
b) Glycolipids: These are compounds of lipids with carbohydrate moieties, along with an amino alcohol sphingosine. Glycolipid does not contain phosphates as in case of phospholipids, *e.g.*, ***cerebrosides*** and ***gangliosides***.
c) Sulfolipids: These are also substituted forms of lipids that contain sulphate groups.
d) Proteolipids: These are compounds of lipids with amino acids.
e) Lipoproteins: These are basically large compound of lipids with large proteins *e.g.*, **VLDL, LDL, IDL** and **HDL**.

iii) Derived lipids: These are basically hydrolytic end products of compound lipids as well as simple lipids. Although these are derived forms of lipids they retain all other general properties of lipids, *e.g.*, diacylglyerols.

iv) Miscellaneous lipids: Lipids like squalene, carotenoids and various hydrocarbons do not fall under standard categories of lipid mentioned above.

Q4. What are waxes? Give two examples. (2)

Ans: Waxes are defined as *solid esters of long chain fatty acids with long chain hydrocarbon fatty alcohols*. These are simple lipids that contain monohydric alcohol of higher molecular weight than that of glycerol. At physiological or room temperature waxes exist into solidified state. Waxes are soluble into fat only and are indigestible, i.e., do not have any nutritional value and are resistant to rancidity. Two examples of waxes are – ***myricyl palmitate***, which is commonly known as ***bee wax***, formed due to esterification myricyl alcohol and palmitic acid ($C_{15}H_{31}COOH$), and ***cetyl palmitate***, that contain cetyl alcohol and palmitic acid, commonly known as spermaceti wax which is used in manufacturing of candles.

Q5. How do waxes differ from oils? (2)

Ans: Waxes differ from oils in many ways, *i.e.*, oils are simple fat made up of three fatty acids esterified with one glycerol and waxes particularly contain one long chain polyhydrocarbon fatty alcohol esterified with one (*or more*) long chain fatty acids. Waxes exist into solid form and oils exist in liquid form at room temperature.

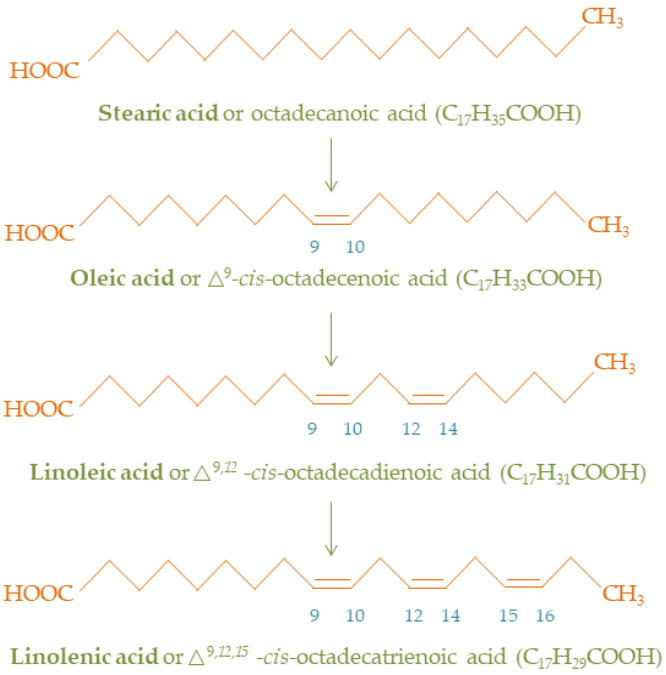
Q6. What are ω-3 and ω-6 fatty acids? State their importance. (2+1)

Ans: The last carbon into the hydrocarbon chain of any fatty acid is traditionally called '**ω carbon**'. In case of unsaturated fatty acids (MuFA or PuFA) the position of last double bond from the ω-carbon gave rise to another tradition for classification of unsaturated fatty acids, *i.e.*, if the last double bond in hydrocarbon chain is situated at C^3, C^6, C^7 or C^9, from ω-C the fatty acid is called ω-3, ω-6, ω-7 or ω-9 fatty acid respectively. The ω-3, ω-6 or ω-9 fatty acids have profound health benefits and fall into the category of essential fatty acids.

Q7. What are polyunsaturated fatty acids? Give examples. (2)

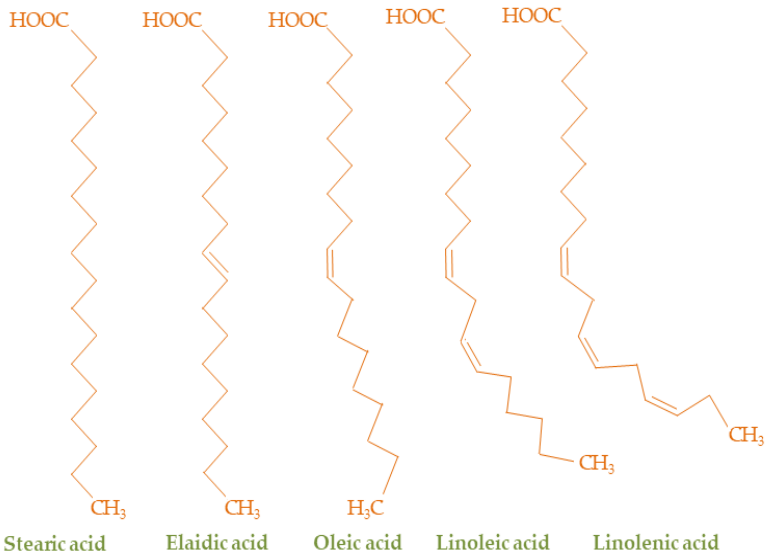
Ans: ***Polyunsaturated fatty acids*** (**PuFA**) are those fatty acids that contain two or more double bonds in their hydrocarbon chains. These are generated by serial oxidation of ***monounsaturated fatty acids*** (**MuFA**). PuFA are more desirable in diet because of their easy digestibility and health benefits. Examples include two isoforms of stearic acid,

i.e., ***linoleic acid*** ($\Delta^{9,12}$-*cis* octadecadienoic acid) and ***linolenic acid*** ($\Delta^{9,12,15}$-*cis* octadecatrienoic acid) that are formed by serial oxidation at C^{12} and C^{15} of the oleic acid respectively.



Q8. What are essential fatty acids? Give examples. (3)

Ans: Few omega (ω)-3 and ω-6 fatty acids *e.g.*, ***linolenic acid*** (ω-3), ***linoleic acid*** (ω-6) and ***arachidonic acid*** (ω-3) are essential for various physiological processes, *e.g.*, mitochondrial activity, reproductive functions, lowering cholesterol level into blood stream, synthesis of prostaglandins and leukotrienes, and maintenance of healthy skin. These fatty acids are PuFA that are formed from serial oxidation of oleic acid and monounsaturated form of arachidic acid. However, these fatty acids are not synthesized into human body due to *lack of mitochondrial desaturases that can add double bonds at the region between the terminal methyl group and existing double bond*. Hence, these fatty acids are necessary to be included in our regular diet and are called '***essential fatty acids***' (EFA). A long-term deficiency in EFA results in skin lesions that might develop eczema, serious damage of the arterial walls due to deposition of cholesterol leading to serious cardiovascular damage, fatty liver and liver cirrhosis, decreased immune function etc.



Q9. Classify fatty acids. (5)

Ans: Fatty acids are classified based on the composition of hydrocarbon chain, as follows:

i) Straight chain fatty acids: These are unbranched linear polyhydrocarbon organic acids. The number of the carbons in a polyhydrocarbon chain of fatty acids is given as 1,2,3… and so on starting from the carbon atom of terminal carboxy (-COOH) groups. Numbering is also given as α,β,γ… and so on starting from the carbon atom next to the terminal carboxy (-COOH) group. Straight chain fatty acids are of two types:

a) ***Saturated fatty acids:*** Refers to those which do not contain any double bond in their hydrocarbon chain. The general formula of the saturated fatty acids is $C_nH_{2n+1}COOH$; here n refers to number of carbons present in the fatty acid chain apart from the carbon atom of terminal –COOH group. The saturated fatty acids may contain 3 to 24 atoms of carbon in its hydrocarbon chain.

Saturated fatty acids with upto 10 carbon atoms are known as lower fatty acids, *e.g.*, acetic acid, butyric acid, caproic acid etc and are also volatile, *i.e.*, can be evaporated after passing steam over them. Saturated fatty acids that contain carbons greater than 10 are non-volatile. Saturated fatty acids can be classified into two groups -

- ***Even carbon fatty acids:*** They contain even number of carbon atoms at their hydrocarbon chains which includes terminal –COOH group also. Examples include butyric acid (C_4), caproic acid (C_6), caprylic acid (C_8), capric acid (C_{10}), lauric acid (C_{12}), palmitic acid (C_{16}), stearic acid (C_{18}), arachidic acid (C_{20}) etc.
- ***Odd carbon fatty acids:*** They contain odd numbers of carbon at their hydrocarbon chains including the terminal –COOH group. Examples include propionic acid (C_3), valeric acid (C_5), enanthic acid (C_7), pelargonic acid (C_9), undecyclic acid (C_{11}) margaric acid (C_{17}) etc. Straight chain volatile fatty acids with carbon number upto 10 are also known as lower fatty acids and non-volatile fatty acids with carbon number greater than 10 are known as higher fatty acids.

b) ***Unsaturated fatty acids:*** It refers to those which contain one or more double bonds in their hydrocarbon chains, which represents that unsaturated fatty acids are basically oxidized forms of saturated fatty acids. These are classified as -

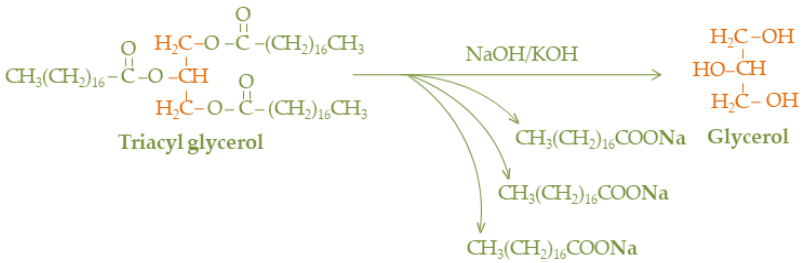
- ***Monounsaturated fatty acids (MUFA):*** These are unsaturated fatty acids which contain only one double bond in their hydrocarbon chains and are also known as monoenoic acids (here '*enoic*' indicates double bond), *e.g.*, oleic acid (Δ^9-*cis* octadecenoic acid) derived from stearic acid (octadecanoic acid) after oxidation.
- ***Polyunsaturated fatty acids (PUFA):*** These are also unsaturated fatty acids which contains two or more double bonds in their hydrocarbon chains. Examples include two isoforms of stearic acid, *i.e.*, ***linoleic acid*** ($\Delta^{9,12}$-*cis* octadecadienoic acid) and ***linolenic acid*** ($\Delta^{9,12,15}$-*cis* octadecatrienoic acid) that are formed by serial oxidation at C^{12} and C^{15} of the hydrocarbon chain respectively.

ii) Branched chain fatty acids: Apart from having unbranched straight chains, few odd and even carbon fatty acids occurring in natural lipids (*e.g.*, ***phytanic acid*** in butter) may also possess branchings as well.

iii) Substituted fatty acids: Replacement of one or more hydrogen atoms in a fatty acid chain by different groups (-OH, $-CH_3$) may generate substituted fatty acids, *e.g.*, ***hydroxy fatty acids*** and ***methyl fatty acids***. These substitutions alter the chemical and biological properties of fatty acids. A common example of substituted fatty acid is hydroxyl fatty acids, such as ***ricinoleic acid*** which contains a hydroxy group and is found into castor oil. Substituted fatty acids participate in formation of membrane structure, are involved into cellular signaling mechanisms and synthesis of biologically active compounds. Substituted fatty acids play crucial role in regulation of membrane fluidity.

Q10. What is saponification number? State its biochemical importance. (3)

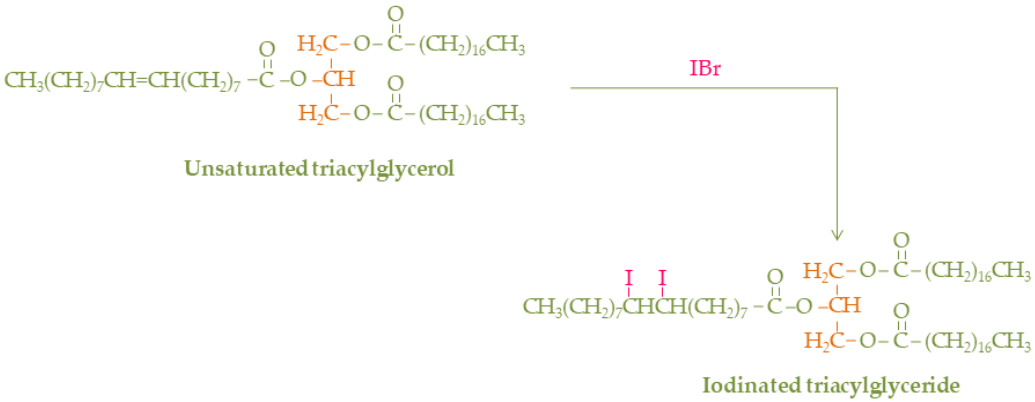
Ans: Saponification of fat refers to the hydrolytic removal of fatty acids from glycerol on reaction with alkali (NaOH or KOH solution). The fatty acids are released as metallic salts of Na^+ or K^+, which are known as '***soap***'. Soaps are cleansing agents because of their emulsifying action. Some soaps of high molecular weight and a considerable degree of unsaturation are selective germicides, others such as ***sodium ricinoleate***, have detoxifying activity against diphtheria and tetanus toxins.



By definition, '***the number of milligrams of KOH required to saponify the free and combined FA in one gram of a given fat is called its saponification number***'. A fat containing short chain fatty acids will have more –COOH group present into per gram dry weight in comparison to the long chain fatty acids and it will take up more alkali and hence will show higher ***saponification number***. Therefore, the saponification number represents the number of fatty acids present in a given amount of fat, *i.e.*, high saponification number indicates presence of more free fatty acids and low saponification number indicates lesser number of free fatty acids. ***Thus, the fat containing short chain fatty acids will have more –COOH groups per gram than long chain fatty acids and this will take up more alkali and hence will have higher saponification number.*** Butter containing a larger proportion of short chain fatty acids, such as butyric and caproic acids, has relatively high saponification number from 220 to 230. Oleomargarine, with more long chain fatty acids, has a saponification number of 195 or less.

Q11. Define iodine number. Write down its significance. (3)

Ans: Iodine number is defined as '***the number of grams of iodine absorbed by 100 grams of fat***'. Iodine number is a measure of the degree of unsaturation, *i.e.*, number of double bonds in a fat. The more is the iodine number, greater will be the degree of unsaturation.



Halogenation is an additive reaction of unsaturated fatty acids. On reaction with IBr, linoleic acid uptakes iodine at its double bond and becomes saturated and forms *stearic acid tetraiodide*. The determination of iodine number ***is useful to the chemist in determining the quality of oil or its freedom from adulteration.*** Iodine number of ***cotton seed*** oil varies from 103 to 111, that of ***olive oil*** from 79 to 88, and that of ***linseed oil*** from 175 to 202. ***A commercial lot of olive oil which has iodine number higher than 88 might have been adulterated with cotton seed oil***. Again, a batch of linseed oil with iodine number lower than 175 might also have been adulterated with the cotton seed oil.

UNIT-4: ENZYMES

Nomenclature, classification and properties: Cofactors and co-enzymes. Effect of temperature, pH, substrate concentration, enzyme concentration on enzyme action, Isozyme and pro-enzyme, Mechanism of enzyme action (lock and key model, Induced fit model). Enzyme kinetics: Derivation of Michaelis-Menten equation with its significance, Lineweaver-Burk plot and its significance. Enzyme inhibition – competitive, non-competitive. allosteric/feedback and its effect on Vmax and Km.

Q1. Define enzymes. (2)

Ans: Enzymes are important group of biomolecules, synthesized by living cells, better known as organic catalysts of biological system (***biocatalysts***), which can catalyze specific thermodynamically possible biochemical reactions, both inside and outside of the cell. Few salient features of enzymes are listed below:

- Always synthesized by living cells.
- Capable of catalyzing reactions both outside and inside of the cells.
- Organic catalysts that catalyze biochemical reactions – ***biocatalysts***.
- Most of the enzymes are biochemically protein in nature with high molecular weight.
- Enzymes can catalyze reactions of either a specific substance or a group of related substances – called ***substrates***.
- Enzymes are capable of catalyzing changes in ***covalent*** bonds, not the ***non-covalent*** bonds within their substrates, in spite of the fact that energy content of covalent bonds is greater than non-covalent bonds.
- Enzymes accelerate *thermodynamically possible* reactions by *lowering the activation energy*.

Q2. Classify enzymes based on their functions. (3)

Ans: Enzyme commission in the year 1999 classified enzymes on the basis of mode of their action into six groups, *i.e.*, ***oxidoreductases, transferases, hydrolases, lyases, isomerases*** and ***ligases*** (abbreviated as '**OTHLIL**'). In the year 2018 a seventh group of enzymes, *i.e.*, ***translocases***, was introduced.

i) ***EC-1 or oxidoreductases:*** Group of enzymes which are involved in oxidation and reduction of their substrates, *e.g.*, ***alcohol dehydrogenases. lactate dehydrogenases.***

ii) ***EC-2 or transferases:*** Group of enzymes which catalyze transfer of a specific group from one substrate to another substrate, *e.g.*, ***alanine transaminases (AST).***

iii) ***EC-3 or hydrolases:*** Group of enzymes which bring about hydrolysis of their substrates, *e.g.*, ***pepsin, trypsin.***

iv) ***EC-4 or lyases:*** Group of enzymes which facilitates removal of small fractions from large molecules of their substrates, *e.g.*, ***fumarases, pyruvate decarboxylases.***

v) ***EC-5 or isomerases:*** Group of enzymes which are involved in isomerization of their substrates, *e.g.*, ***phosphohexose isomerases, phosphotriose isomerases.***

vi) ***EC-6 or ligases:*** Group of enzymes which are involved in joining of two of their substrates, *e.g.*, ***DNA ligases, glutamine synthases.***

vii) ***EC-7 or translocases:*** Group of enzymes that catalyze translocation of ions or molecules across plasma membrane, separation of substances within membrane in an energy-coupled process *e.g.*, ***ATP synthases, Na^+-K^+ ATPases.***

Q3. What are zymogens? (2)

Ans: Most of the enzymes synthesized by living cells are not active, they remain into inactive large precursor molecules, known as ***zymogens*** or ***pro-enzymes***. Zymogens are subsequently cleaved proteolytically, to release the few additional parts from both C-terminal and N-terminal regions, known as *making peptides* to obtain the final active form of enzyme molecules. Two very common examples may include ***trypsinogen***, the precursor of *trypsin* and ***pepsinogen***, the zymogen of *pepsin*.

Q4. What are co-enzymes and prosthetic groups? (3)

Ans: Few enzymes do not require any additional non-protein substance for their activities, but few other enzymes require additional non-protein substances for their action. The protein part of such enzymes is called ***apo-protein*** or ***apo-enzyme*** and the additional non-protein substances are either ***co-enzymes*** or ***prosthetic groups***.

- If the non-protein part is bound *loosely* with the apo-enzyme through weak *non-covalent* interactions like hydrogen bond, hydrophobic interactions, van der Waal's forces, then it is called a ***co-enzyme***, *e.g.*, biotin, NAD^+, $NADP^+$, co-enzyme A.
- In contrast, if the non-protein part is bound *tightly* with the apo-enzyme via formation of *covalent* interactions like ionic interaction, then it is considered to be a ***prosthetic group***, *e.g.*, FAD^+, biotin, heme.

Holo-enzyme (*entire molecule*) = Apo-enzyme (*protein part*) + Co-enzyme/Prosthetic group (*non-protein part*)

The co-enzymes and prosthetic groups bind to enzymes in order to facilitate their catalytic activities, *viz.*, *transfer of electrons*, *transfer of hydrogens* and *transfer of groups*. During these events co-enzymes and prosthetic groups may accept or donate specific groups, electrons and hydrogens; however, after completion of enzyme action the co-enzyme and prosthetic groups recover immediately.

	Coenzyme	Prosthetic group
Nature of binding	Loosely bound with the apo-enzyme hence are associated temporarily, *i.e.*, can be dislodged easily from apo-enzyme	Tightly and permanently bound with the apo-enzyme, *i.e.*, cannot be separated easily from apo-enzyme
Chemical bonds	Bound via weak non-covalent forces	Bound via strong covalent forces
Mechanism of action	Transfers groups, electrons or atoms	Participates directly into catalysis
Specificity	Low, *i.e.*, a single coenzyme may work with many different enzymes	High, *i.e.*, a single coenzyme may work with one enzyme only
Regeneration	Often regenerated by another enzyme system after reaction	Regenerated within the same enzyme complex

Q5. What are co-factors? State their role in enzymatic reactions. (2)

Ans: Co-factors are usually non-protein chemical substances or small metal ions temporarily associated with enzymes via covalent or non-covalent interactions which are essential of enzymatic actions, *e.g.*, Fe^{2+} for ***catalase***, Mn^{2+} for ***arginase***. Cofactors associates occasionally with enzymes. Cofactors are essential because many amino acid side chains present alone into active site of enzyme cannot perform certain complex biochemical reactions efficiently. The cofactors extend the chemical capabilities of enzymes. Cofactors participate in enzyme action by helping in:

i) Substrate binding.
ii) Electron transfer.
iii) Group transfer reactions.
iv) Stabilization of enzyme structure.
v) Formation of enzyme–substrate complex and catalytic transformation of substrate into product.

Q6. What are active sites on enzymes? (2)

Ans: Substrates bind enzymes at a specific site on it, known as ***active site***, also termed as ***catalytic site***. Active sites possess a specific three-dimensional shape that provides a non-polar hydrophobic cleft to bind substrate. The active sites contain specific amino acid residues (usually hydrophobic) that creates a non-polar environment and enables the enzyme to bind with the substrate in a specific manner, *i.e.*, determines the substrate specificity. Such active site amino acids also determine the specificity of reaction catalyzed by an enzyme. These active site amino acid residues are often help in binding with metal ions, coenzyme and prosthetic groups.

Q7. Describe the two models for enzyme-substrate interactions. (3+3)

a) Fischer's template for lock and key hypothesis: It was proposed by Emil Fischer in 1890. According to this model, the enzyme contributes highly specific rigid active sites, into which the substrate molecules bind. The active sites itself thus provides a rigid and pre-shaped template that fits correctly with the size and shape of the substrate molecule, just like a key fit into its lock, and hence it is known as ***"lock-and-key"*** hypothesis for ES complex formation. The enzyme represents the lock and the substrate represents the key. Only the correctly shaped substrate can enter the active site and form the enzyme–substrate complex. The model assumes that the active site is preformed and does not undergo any structural alteration during substrate binding. When the substrate enters the active site, temporary interactions such as hydrogen bonds, ionic interactions, hydrophobic interactions and van der Waals forces stabilize the enzyme–substrate complex. The catalytic reaction then proceeds, leading to conversion of substrate into products. After the reaction, the products leave the active site while the enzyme remains unchanged and can participate in another catalytic cycle. The model successfully explained several important properties of enzymes, including:

i) ***Substrate specificity:*** Enzymes act only on specific substrates because only substrates with complementary geometry can fit into the active site.
ii) ***Formation of enzyme–substrate complex:*** The hypothesis clearly emphasized the importance of temporary enzyme–substrate association during catalysis.
iii) ***High catalytic efficiency:*** Proper fitting of substrate into the active site allows optimal orientation for chemical transformation.
iv) ***Stereospecificity:*** Many enzymes can distinguish between optical isomers because only one stereochemical form can properly fit into the active site.

However, despite its historical importance, the Lock and Key model has several limitations:

- It considers the enzyme structure to be completely rigid, whereas proteins are actually flexible molecules.
- It cannot adequately explain the transition-state stabilization of substrates.
- It fails to explain how enzymes can bind substrates with slightly different structures.
- The model cannot account for allosteric regulation and conformational changes observed experimentally.

b) Koshland's induced fit hypothesis: It was proposed by Daniel Koshland in 1959. According to this model, the enzymes possess flexible active sites, which are not rigid and pre-shaped. The substrate molecule may not have structural similarities with the active site, and they bring some conformational changes at their active sites upon their binding to the enzymes. The active sites have no rigid and preformed structure to fit the substrate and the substrates change their binding sites to attain a final catalytic shape and form. The induced fit model claims that the substrate first approaches the enzyme and establishes weak initial interactions with active site residues of enzyme. These interactions induce conformational changes in the enzyme structure. The active site adjusts itself around the substrate to form a tightly bound enzyme–substrate complex. Catalysis occurs more efficiently because catalytic groups become properly aligned and products are released and the enzyme returns to its original conformation. This model emphasizes that enzymes are dynamic molecules rather than rigid structures. The induced fit hypothesis explains several important aspects of enzyme catalysis:

i) ***Flexibility of enzyme structure:*** Proteins possess dynamic three-dimensional structures. Substrate binding can alter tertiary or quaternary structure of the enzyme.
ii) ***Enhanced catalytic efficiency:*** Conformational changes bring catalytic amino acid residues into correct orientation, thereby lowering activation energy more effectively.
iii) ***Transition-state stabilization:*** The altered active site stabilizes the transition state of the reaction, which is a major factor responsible for enzymatic catalysis.
iv) ***Broad substrate specificity:*** Some enzymes can act on structurally related substrates because the active site can adapt slightly to different molecular structures.
v) ***Allosteric regulation:*** The model helps explain allosteric modulation, cooperative binding and feedback regulation in many enzymes.

A classic example is hexokinase, where binding of glucose induces structural changes that exclude water molecules from the active site and facilitate phosphorylation of glucose. The induced fit model is now considered more accurate than the Lock and Key model because it better reflects the dynamic nature of proteins and explains a wider range of enzymatic phenomena. Nevertheless, the earlier lock and key concept still remains useful for understanding the fundamental principle of enzyme specificity.

Q8. What are rate limiting enzymes (RLE)? (2)

Ans: Rate limiting or committed step of metabolic pathways is that reaction which determines the rate and direction of the entire metabolic pathway. The enzymes which catalyze the rate limiting step of one metabolic pathway are known as ***rate limiting enzymes.*** Hence, the reaction catalyzed by the *rate limiting enzymes* (*rate limiting step* or *committed step*) determines the rate and direction of the entire metabolic pathway. Example: ***Phosphofructokinase 1*** of glycolytic pathway**.**

- Among all the enzymes of relevant metabolic pathway RLEs possess lowest substrate affinity (highest K_m).
- The activity or the synthesis of RLEs is regulated by *in vivo* processes; hence RLEs are regulated enzymes themselves.
- The rate limiting enzymes catalyze the rate limiting reactions unidirectionally and hence regulate the direction of the entire metabolic pathway.
- The reaction catalyzed by rate limiting enzymes (***rate limiting step***) is often the initial step of a metabolic pathway. Therefore, the intermediates of earlier steps will never be accumulated as in case of a ***feed-back inhibition*** or repression of the rate limiting enzyme.

Q9. What are isoenzymes? Give example (2)

Ans: Isoenzymes are the physically distinct forms of the same enzyme, which differ from each other structurally (*i.e.*, amino acid sequences), electrophoretically and immunologically, but they are similar in function (*i.e.*, catalyze same chemical reaction of one specific substrate). Example include five isoenzymes of ***lactate dehydrogenases.*** Each of the LDH isozymes is made up of four polypeptide chains (also called polypeptide subunits) – hence are tetramers. The subunits are of two types – H and M; and different isoforms of LDH are composed of different proportions of H and M chains.

Q10. What are ribozymes? Give example (2)

Ans: These are basically large RNA molecules found in prokaryotes and invertebrates which functions as enzymes; *e.g.*, self-splicing introns of pre-RNA transcripts. Ribozymes can be associated with proteins and in such cases the role of the protein component is to promote the interaction between the ribozyme and its substrate. Examples may include ***28S rRNA*** and ***23S rRNA*** present into large subunit (**60S**) *eukaryotic ribosome* and large subunit (**50S**) *prokaryotic ribosome* respectively that endow *peptidyl transferase* activity, *i.e.*, the ability to form peptide bond between amino acids**.**

Q11. What are abzymes? (2)

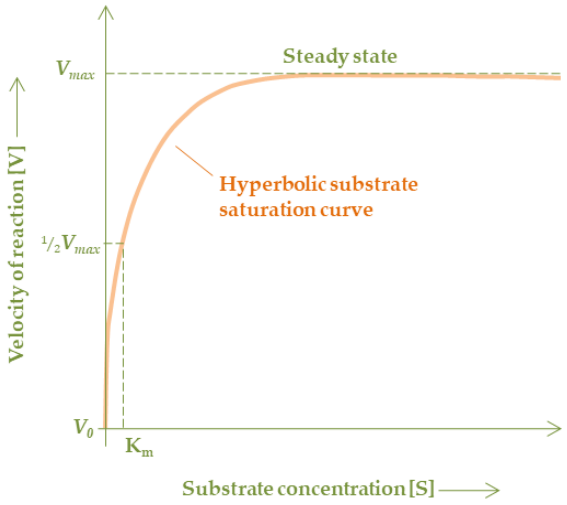
Ans: Synthetic *monoclonal antibodies* (mAbs), produced into laboratory from a single clone of B-lymphocytes that are equipped with catalytic efficiency, similar to an enzyme, hence are also known as ***abzymes*** or ***CatmAbs***. Although most of the abzymes are synthetic, few abzymes are produced naturally within human body.

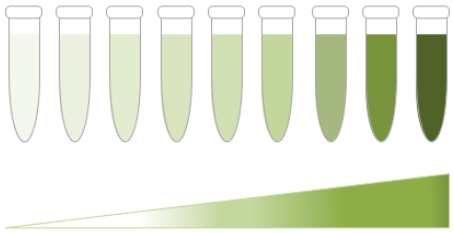
- Most of the abzymes are produced synthetically through ***hybridoma technology.***
- Abzymes are also found in normal humans (*e.g.*, ***anti-vasoactive intestinal peptide autoantibodies***), and in case of pathogenesis of autoimmune diseases like systemic lupus erythematosus (SLE).

Since these are equipped with only weak and modest catalytic activity they are not being used for practical purposes, but experimental uses of CatmAbs have shown effective killing of HIV viruses.

Q12. Derive the Michaelis-Menten equation. (5)

Ans: As per the observation and interpretation of Michaelis and Menten, when the rate of an enzyme (***V***) catalyzed thermodynamically possible non-spontaneous reaction is plotted against substrate concentration, the rate of reaction increases gradually until it reaches a maximum value and becomes stationary giving rise to a ***hyperbolic substrate-saturation curve***. According to the Michaelis-Menten postulates for a ***single-substrate enzyme reaction***, it is *reversible* and a *two-step reaction*, *i.e.*, at first step a substrate (S) binds with the enzyme (E) at its active site to form a transient enzyme-substrate (ES) complex, the substrate gets converted itself into the product (P) via physicochemical changes and at the second step ES complex is quickly dissociated release the enzyme (E) and the product (P). The k_1 is the rate constant for the reaction in which enzyme combines with substrate to for ES complex, and k_2 is the rate constant for the reaction in which ES complex is broken down to release unchanged substrate from the enzyme. There is another rate constant, k_3, which represents the rate of reaction in which ES complex releases the enzyme and product, hence it is the rate constant for product formation.





$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

k_1 = Rate constant for ES formation

k_2 = Rate constant for ES breakdown back into enzyme and substrate

k_3 = Rate constant for ES breakdown into enzyme and product

In accordance to the second order reaction kinetics, the '*velocity of ES formation*' is $v = k_1[E][S]$ and '*velocity of ES breakdown*' is $v = k_2[ES] + k_3[ES]$. According to the steady state assumptions of enzyme catalyzed reaction kinetics the amount of ES formation is counterbalanced by the amount of ES breakdown.

Therefore,

$$k_1[E][S] = k_2[ES] + k_3[ES] \text{ or } k_1[E][S] = [ES](k_2 + k_3)$$

$$\text{or, } \frac{[E][S]}{[ES]} = \frac{[k_2][k_3]}{[k_1]}$$

The fraction $\frac{[k_2][k_3]}{[k_1]}$ is also defined as ***Michaelis-Menten constant*** (K_m). Therefore,

$$K_m = \frac{[E][S]}{[ES]} \quad \ldots\ldots\ldots\ldots \text{(i)}$$

Let, the total enzyme concentration is [E_t] and at any time point during enzyme catalyzed reaction the amount of free enzyme (E) will be the amount of ES complex subtracted from the amount of total enzyme (E_t). Therefore,

$$[E] = [E_t] - [ES]$$

Putting this value of [E] into equation no (i) we get,

$$K_m = \frac{[E_t][S]-[ES][S]}{[ES]}$$

$$\text{or, } K_m = \frac{[E_t][S]}{[ES]} - \frac{[ES][S]}{[ES]}$$

$$\text{or, } K_m = \frac{[E_t][S]}{[ES]} - [S]$$

$$\text{or, } K_m + [S] = \frac{[E_t][S]}{[ES]}$$

$$\text{or, } [ES] = \frac{[E_t][S]}{K_m + [S]} \quad \ldots\ldots\ldots\ldots \text{(ii)}$$

Since, the product formation in an enzyme catalyzed reaction depends on k_3, we may write $v = k_3$ [ES]. Therefore,

$$[ES] = \frac{v}{k_3} \quad \ldots\ldots\ldots\ldots \text{(iii)}$$

Combining equation number (ii) and (iii) we get,

$$\frac{v}{k_3} = \frac{[E_t][S]}{K_m + [S]}$$

$$\text{or, } v = \frac{k_3[E_t][S]}{K_m + [S]} \quad \ldots\ldots\ldots\ldots \text{(iv)}$$

When the enzyme catalyzed reaction reaches ***maximum velocity*** (**V_{max}**) rate of product formation becomes $V_{max} = k_3$ [ES]. When Vmax is reached, all the enzyme molecules present into reaction mixture are supposed to be saturated with substrate and exists as ES complex. Therefore, at maximum velocity of enzyme catalyzed reaction [E_t] = [ES] and we may write $V_{max} = k_3$ [E_t]. Putting this value of k_3 [E_t] into equation number (iv) we get,

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad \ldots\ldots\ldots\ldots \text{(v)}$$

This equation [equation number (V)] represents the velocity of reaction and product formation at any point of an enzyme catalyzed reaction and is widely known as ***Michaelis-Menten equation*** (**MME**).

Q13. What is K_m? Write down its significance. (4)

Ans: By definition, ***Michaelis-Menten constant*** (**K_m**) is the substrate concentration which is required to attain half of the maximum velocity of an enzyme-catalyzed reaction.

At half of the maximum velocity ($\frac{V_{max}}{2}$), the **MME** may be re-written as $\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$ or $K_m + [S] = 2[S]$ or $K_m = [S]$

Michaelis-Menten constant (K_m) has several biochemical importances:

i) K_m indicates the substrate affinity of enzymes, *i.e.*, higher is the K_m lower will be the substrate affinity of enzyme, *i.e.*, high substrate concentration is required to attain half of the V_{max}.

ii) Lower is the Km higher will be the substrate affinity of enzyme, *i.e.*, small amount of substrate is required to attain half of the V_{max}.

iii) K_m often indicates the substrate preferences of enzymes.

iv) K_m denotes binding ability of the substrates.

v) Low K_m indicates that enzyme can act on very small quantities of substrate (*i.e.*, ***high efficiency***); whereas, high K_m indicates ***low efficiency*** of enzymes.

vi) Since isoenzymes of an enzyme molecule have differences in K_m, it is often used as an indicator to distinguish between isoenzymes.

Q14. What do you mean by initial rate and maximum velocity of enzyme catalyzed reaction? (2)

Ans: The rate of reaction at the beginning point for any enzyme catalyzed reaction is too low due to lowest concentration of substrate ([S]=0) and is known as ***initial rate*** (V_0). The rate of enzyme catalyzed reaction increases with rise in substrate concentration and become stationary when all the enzyme molecules is saturated with substrate; ***maximum velocity*** (V_{max}) is rate of reaction when all the enzyme molecules is saturated with substrate molecules.

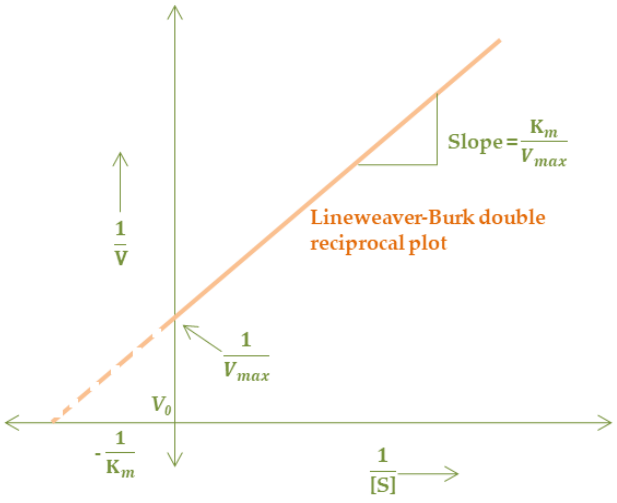
Q15. Describe any one linear transformation of Michaelis-Menten equation. (3)

Ans: Limitations of Michaelis-Menten equation, *i.e.*, requirement of experimentally impossible too high and too low concentrations of substrates, scattered values and difficulty with extrapolation of the hyperbolic substrate-saturation curve, have greatly provoked researchers to transform the outcomes of enzyme catalyzed reactions to overcome them. One famous transformation among them is '***Lineweaver-Burk double reciprocal plot***' (***LB plot***). It was done simply by plotting inverse values of reaction velocity against the inverse values of respective substrate concentrations, hence is called *double reciprocal plot*. Plotting of inverse values of reaction velocity (v) along the X-abscissa and inverse values of substrate concentration ([S]) along the Y-abscissa a straight line is obtained, hence it is called *linear transformation*.

According to the Michaelis-Menten equation, $v = \frac{V_{max}[S]}{K_m + [S]}$

Taking the inverse values of both sides we get, $\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$

$$\text{or, } \frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]} \quad \text{or, } \frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



This equation is known as Lineweaver-Burk (LB) double reciprocal equation. Comparing this equation with the standard equation of straight line (***y* = m*x* + c**), the Y-intercept gives value of $\frac{1}{V_{max}}$ whereas the slope gives the value of

$\frac{K_m}{V_{max}}$ and the X-intercept gives the value of $\frac{1}{[S]}$ that corresponds to the maximum velocity, *i.e.*, $\frac{1}{V_{max}}$ of the enzyme catalyzed reaction. The major advantage of LB-plot is the straight line obtained can be plotted using any range of [S] versus *v*. The points obtained are no more scattered randomly and extrapolation becomes possible.

Q16. What is turn over number (K_{cat})? (2)

Ans: The ***turn over number*** or ***catalytic constant*** (k_{cat}) of an enzyme is defined as the maximum number of moles of substrate converted into products per mole of enzyme in unit time. To determine turn over number of an enzyme, the reaction must proceed at maximum velocity and in accordance with principles of zero order reaction. This is true when [ES] is maximum, *i.e.*, when all the enzyme molecules are saturated with substrates.

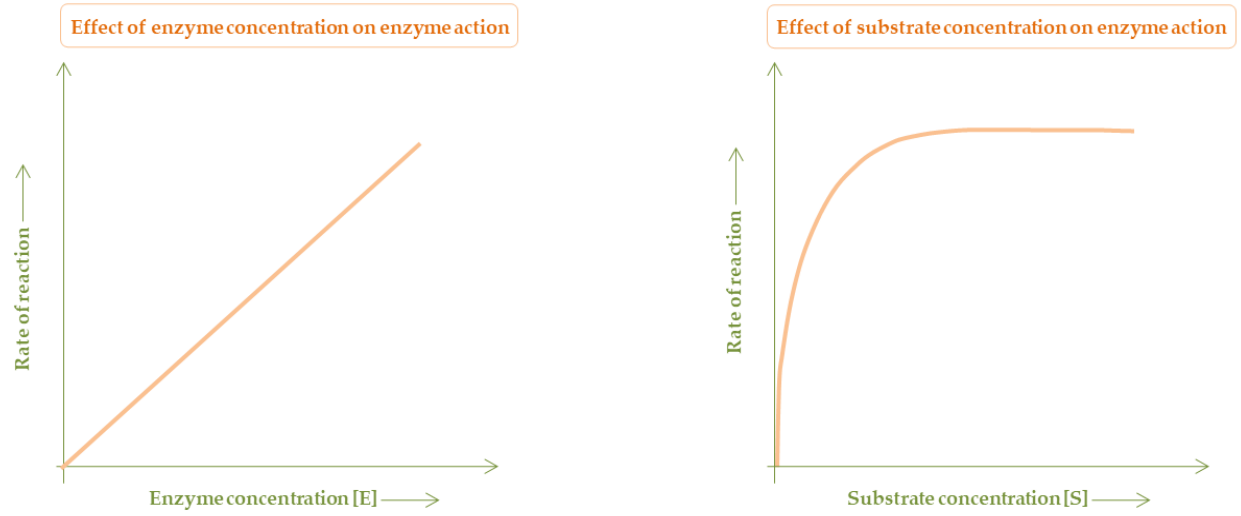
It is expressed as $k_{cat} = \frac{V_{max}}{[E_t]}$. It indicates catalytic efficacy of an enzyme and depends on temperature, pH and substrate concentration. ***Catalases*** have highest k_{cat} values. and ***carbonic anhydrases*** also possess exceptionally high k_{cat} value.

Q17. Describe the effects of enzyme concentration and substrate concentration on enzyme action. (3+3)

Ans: Among the various factors affecting enzyme action, two primary factors are enzyme concentration and substrate concentration, that mainly regulate ES complex formation.

a) Effect of enzyme concentration: The rate of an enzyme-catalyzed reaction is greatly influenced by the concentration of enzyme molecules present in the reaction system. Under conditions where substrate is available in excess, an increase in enzyme concentration produces a proportional increase in reaction velocity. This relationship exists because increasing enzyme concentration increases the total number of active sites available for substrate binding. Consequently, more enzyme–substrate complexes are formed simultaneously, resulting in enhanced product formation per unit time. Thus, when substrate concentration remains sufficiently high, the reaction velocity becomes directly proportional to enzyme concentration, *i.e.*, $v \propto [E]$. Graphically, plotting enzyme concentration against reaction velocity yields a straight line passing through the origin, indicating a linear relationship. However, this proportionality persists only so long as adequate substrate molecules are available. If substrate concentration becomes limiting, further increase in enzyme concentration no longer increases the rate of reaction because many enzyme molecules remain free and unoccupied. Therefore, enzyme concentration determines the maximum catalytic capacity of a biochemical system when substrate supply is abundant.

b) Effect of substrate concentration: Substrate concentration exerts profound influence on enzyme-catalyzed reactions and forms the basis of Michaelis–Menten enzyme kinetics. When enzyme concentration is kept constant and substrate concentration is gradually increased, the reaction velocity initially rises rapidly. At low substrate concentration, numerous free enzyme active sites remain available.



Therefore, addition of substrate greatly enhances the probability of enzyme–substrate collisions and ES complex formation. Under such conditions, the reaction rate increases almost proportionally with substrate concentration. As substrate concentration continues to rise, more enzyme active sites become occupied progressively. Consequently, the number of free enzyme molecules decreases gradually, and the rate of increase in reaction velocity becomes slower. Finally, a stage is reached where almost all enzyme molecules are associated with substrate molecules, forming enzyme–substrate complexes. At this point the enzyme becomes saturated with substrate and the reaction attains its maximum velocity (V_{max}). Beyond this saturation point, further increase in substrate concentration fails to increase reaction velocity because no additional free active sites remain available for catalysis. The relationship between substrate concentration and reaction velocity is therefore represented by a characteristic hyperbolic substrate saturation curve. This behavior is explained by the steady-state assumption of Michaelis–Menten kinetics, according to which the concentration of ES complex remains nearly constant during most of the reaction. Thus, substrate concentration regulates enzyme activity by controlling the extent of enzyme saturation and ES complex formation.

Q18. State the effects of temperature and pH on rate of enzyme catalyzed reaction. (6)

Ans: Enzymes are highly specialized biological catalysts composed predominantly of proteins. Their catalytic efficiency depends upon their precise three-dimensional structural organization, especially the active site where substrate binding and catalysis occur. Since proteins are extremely sensitive to environmental conditions, any alteration in the surrounding physicochemical environment may significantly influence the structure as well as the catalytic activity of enzymes. Enzyme activity is greatly influenced by temperature and pH.

a) Effect of temperature: Over a limited range of temperature, the rate of enzyme catalyzed reaction increases with rise in temperature, and above it the rate of reaction decreases. Temperature is one of the most important environmental factors affecting enzyme action. Since enzymes are proteins, temperature influences both the kinetic behavior of molecules and the structural stability of enzymes. The relationship between temperature and enzyme activity generally produces a bell-shaped curve.

- At low temperatures, enzyme and substrate molecules possess relatively low kinetic energy. Consequently, intermolecular collisions occur less frequently and with insufficient force to achieve efficient enzyme–substrate interaction. As a result, formation of ES complexes remains low and the reaction proceeds slowly. With gradual rise in temperature, the kinetic energy of enzyme and substrate molecules increases. Molecular movement becomes more rapid and collisions occur more frequently and effectively. This increases the probability of successful enzyme–substrate interactions, thereby enhancing ES complex formation and reaction velocity.
- According to collision theory, increased molecular motion results in increased catalytic activity. Therefore, the rate of enzyme-catalyzed reactions rises progressively with increase in temperature. At a certain temperature, the reaction velocity becomes maximum. This temperature is known as the ***optimum temperature*** of the enzyme. For most of the enzymes, optimum temperature lies close to normal body temperature, *i.e.*, approximately 37°C.
- At the optimum temperature the apo-enzyme structure remains stable and substrate binding becomes maximal, therefore the catalytic efficiency reaches its highest level.
- However, if temperature rises beyond the optimum level, enzyme activity begins to decline rapidly. This decline in enzymatic activity occurs because high temperature disrupts the weak non-covalent interactions responsible for maintaining the secondary, tertiary, and quaternary structure of proteins. These interactions include: hydrogen bonds, ionic interactions, hydrophobic interactions, and van der Waal's forces. Disruption of these bonds alters the three-dimensional conformation of the enzyme, particularly the active site. Consequently, substrate binding decreases and catalytic activity is progressively lost. At very high temperatures, irreversible denaturation of enzyme proteins may occur, resulting in permanent loss of enzymatic activity.

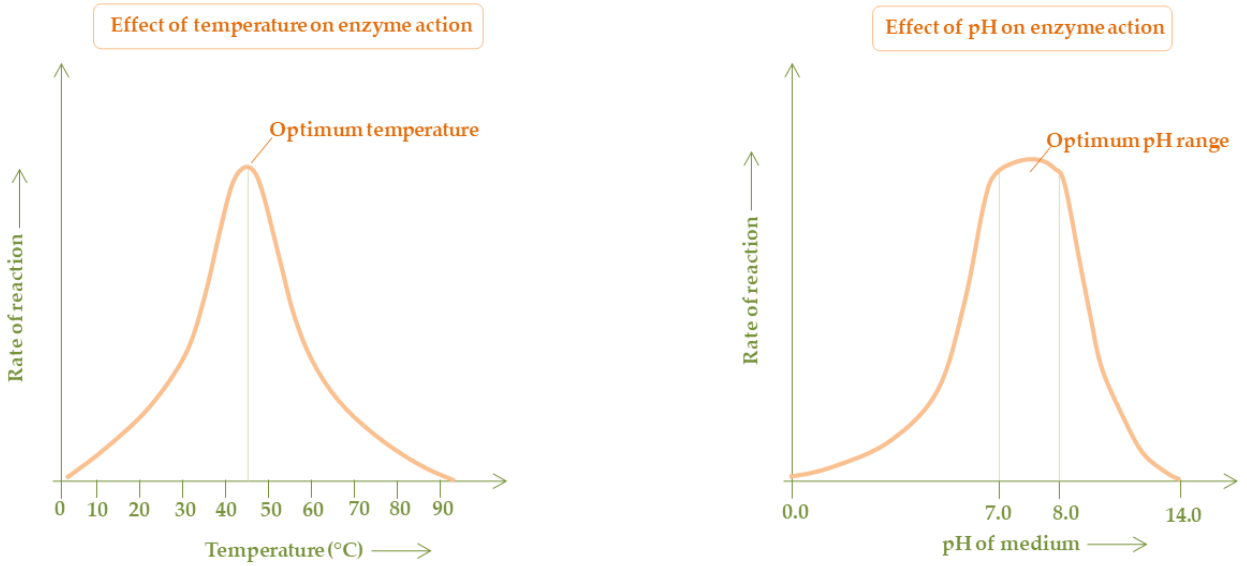
Therefore, ambient temperature affects enzyme action by influencing molecular collisions and structural integrity.

b) Effect of pH: Hydrogen ion concentration or pH exerts profound influence on enzyme activity because enzymes contain ionizable amino acid side chains essential for substrate binding and catalysis. Alteration in pH may change:

- Ionization status of amino acid residues

- Electrical charges on enzyme molecules
- Ionic interactions and salt bridges
- Three-dimensional conformation of enzymes into protein part of enzyme (*i.e.*, ***apo-enzyme***)

Therefore, both the structural stability and catalytic efficiency of enzymes are strongly dependent upon pH. Every enzyme exhibits maximum catalytic activity within a particular pH range known as the ***optimum pH***, which is often 7.0 to 8.0 in humans. At this optimal pH range, the ionization status of active site amino acid residues becomes most favorable for substrate binding and catalytic activity. Examples may include ***pepsin***, a hydrolytic enzyme with an optimum pH around 2.0 and ***trypsin***, another hydrolytic enzyme that have an optimum pH around 8.0.



At highly acidic or highly alkaline pH, enzyme activity declines markedly. Such decline occurs due to several mechanisms. Firstly, alteration in pH changes the ionization state of amino acid side chains present at the active site. This may interfere with substrate binding and catalytic function, thereby altering substrate affinity and Michaelis constant (K_m). Secondly, extreme pH may disrupt ionic bonds and salt bridges responsible for maintaining higher-order protein structure. As a result, conformational changes occur within the enzyme molecule, leading to distortion of the active site. Thirdly, drastic changes in pH may dissociate prosthetic groups or cofactors from apoenzymes, producing inactive enzyme forms. Finally, severe acidic or alkaline conditions may cause denaturation of enzyme proteins, resulting in irreversible loss of enzymatic activity. Thus, pH regulates enzyme activity primarily through its effects on ionization state, structural conformation, and stability of enzyme molecules.

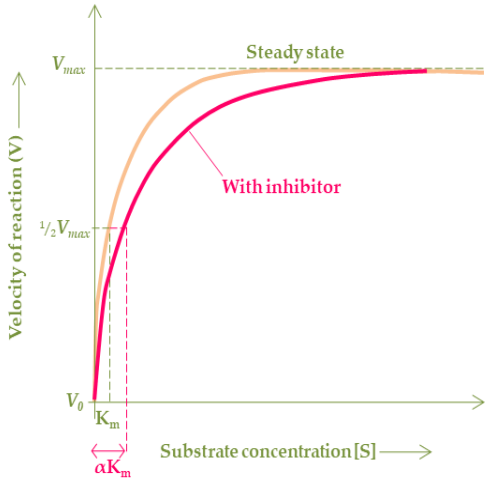
Q19. What is temperature coefficient (Q_{10})? (2)

Ans: ***Temperature coefficient*** (***Q_{10}***) is a factor by which the rate of an enzyme catalyzed reaction increases with every 10°C rise in temperature within a limited physiological range. In biological system, the rate of an enzyme catalyzed reaction doubles with every 10°C rise in temperature, and becomes half 10°C decline in temperature.

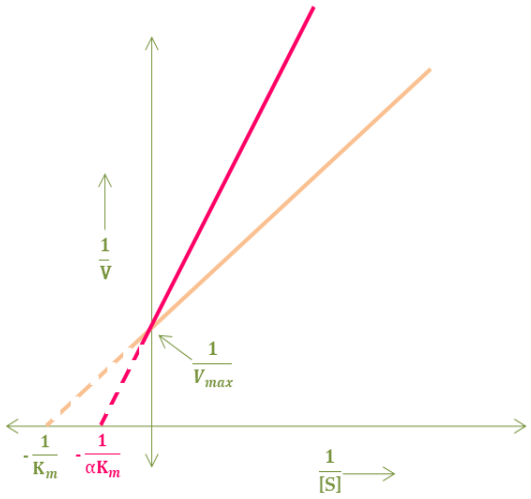
Q20. What are reversible competitive enzyme inhibition and reversible non-competitive enzyme inhibition? (6)

Ans: In case of reversible competitive inhibition, the inhibitor is a structural analogue of the substrate; and hence there is a competition between the inhibitor and substrate to occupy the active sites. Since, increasing inhibitor concentration competitively displace the substrate and *vice versa* with increase in substrate concentration, this type of enzyme inhibition is categorized as a *reversible inhibition.* Due to the competition between the substrate and inhibitor it is also known as ***reversible competitive inhibition.*** The inhibitor binds to free enzyme after dissociating substrate from it, and forms EI complex in place of ES complex, thus there is no formation of product. However, this effect of inhibitor can be reversed by addition of substrates, and V_{max} is reached by increasing substrate concentration. ***However, higher concentration of substrate is required than usual and therefore there is increase in K_m, which depicts decrease in substrate affinity, without any change in V_{max}.***

Competitive enzyme inhibition



Effect of competitive inhibitors on hyperbolic substrate saturation curve



Effect of competitive inhibitors on Lineweaver-Burk double reciprocal plot

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$
$$+$$
$$I$$
$$\Big\Vert k_i$$
$$EI$$

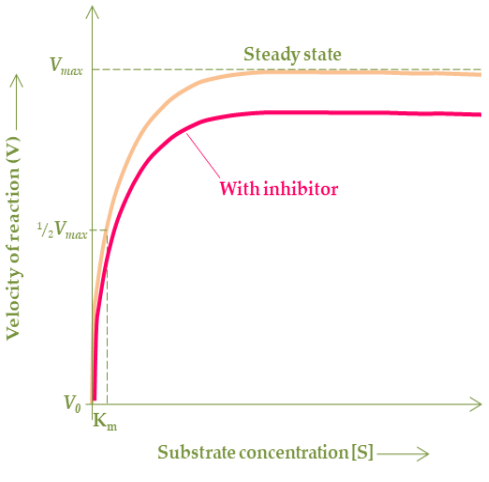
k_1 = Rate constant for ES formation

k_2 = Rate constant for ES breakdown back into enzyme and substrate

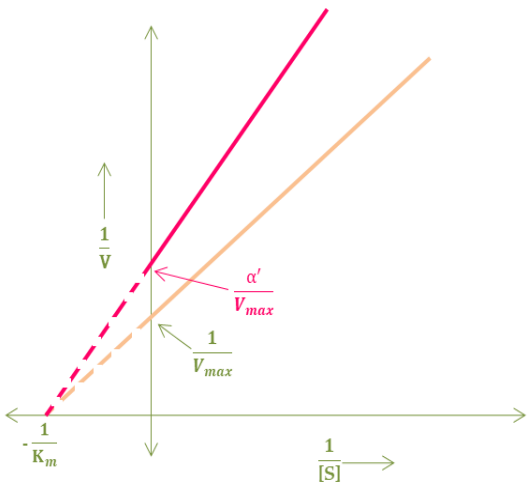
k_3 = Rate constant for ES breakdown into enzyme and product

k_i = Rate constant for formation of EI complex

Non-competitive enzyme inhibition



Effect of non-competitive inhibitors on hyperbolic substrate saturation curve



Effect of non-competitive inhibitors on Lineweaver-Burk double reciprocal plot

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$
$$+ \qquad\qquad +$$
$$I \qquad\qquad I$$
$$\Big\Vert k_i \qquad \Big\Vert k_i'$$
$$EI \qquad\quad ESI$$

k_1 = Rate constant for ES formation

k_2 = Rate constant for ES breakdown back into enzyme and substrate

k_3 = Rate constant for ES breakdown into enzyme and product

k_i = Rate constant for formation of EI complex

k_i' = Rate constant for formation of ESI complex

In case of reversible non-competitive inhibition, the inhibitor binds to the free enzyme or the enzyme-substrate complex in a site other than the active site in such a manner that induces some conformational changes in the enzyme molecule so that the substrate cannot bind to the enzyme or already bound substrates are not released from the enzyme-substrate complex. ***This reduces the V_{max}, as V_{max} cannot be attained even after increasing the substrate concentration; but the affinity for substrate is not changed so K_m remains unaltered.***

Q21. State the differences between reversible competitive, non-competitive and uncompetitive inhibitions? (6)

Ans: All these three different types of reversible enzyme inhibition mechanisms endow several differences as follows:

	Competitive inhibition	Non-competitive inhibition	Uncompetitive inhibition
Structure of inhibitors	Inhibitor shares structural homology with substrate.	Inhibitor shares no structural homology with substrate.	Inhibitor shares no structural homology with substrate.
Competition between substrates and inhibitors	Inhibitor competes with substrate to occupy the active site.	Inhibitor does not compete with substrate to occupy the active site.	Inhibitor does not compete with substrate to occupy the active site.
Binding site for the inhibitor	Inhibitor binds to the active site on enzyme.	Inhibitor binds to a site other than the active site on enzyme.	Inhibitor binds to a site other than the active site on enzyme.
Interaction between inhibitor and enzyme	Inhibitor binds with the free enzyme only.	Inhibitor binds with the free enzyme as well as ES complex.	Inhibitor binds with the ES complex only.
Effects on substrate affinity (K_m)	K_m increases, *i.e.*, substrate affinity decreases.	K_m remains unaltered, *i.e.*, substrate affinity also remains unaltered.	K_m decreases, *i.e.*, indicates an abnormal rise in substrate affinity.
Effects on rate of reaction (V_{max})	The V_{max} remains unaltered, which means V_{max} can be attained on increasing the substrate concentration.	V_{max} decreases significantly, which indicates a powerful modification of active site.	V_{max} decreases significantly, which indicates a powerful modification of active site.
Structural alterations into enzyme	Three-dimensional conformation of enzymes remains unaltered.	Three-dimensional conformation of enzymes is altered temporarily.	Three-dimensional conformation of enzymes is modified temporarily.
Degree of inhibition	Degree of inhibition depends on molar concentration of both substrate and inhibitor.	Degree of inhibition does not depend on substrate concentration, rather it depends on inhibitor concentration alone.	Degree of inhibition completely depends on timing of inhibitor binding, *i.e.*, substrate must bind prior to inhibitor binding.

Q22. What is reversible non-covalent modification? Give example. (2)

Ans: Reversible non-covalent modification are those modifications, in which enzymes once activated or deactivated can be deactivated or reactivated through changes in non-covalent interactions. Example: A classic example of reversible non-covalent modification is '***allosterism***'.

Q23. Write a short note on allosterism. (5)

Ans: ***Allosterism*** *is a type of reversible non-covalent interaction.* Few enzymes are activated or inhibited after binding of some low molecular weight ligands to specific sites on enzymes which are physically distinct from the active sites or catalytic sites of the same enzyme, and called ***allosteric sites*** ("***allo***" means other; and "***steric***" means position). Such type of enzyme is called ***allosteric enzymes. Allosteric modulators*** are some low molecular weight ligands that bind

to allosteric sites of allosteric enzymes to modulate their functions either positively or negatively. The allosteric modulators are of two types –

- ***Allosteric activators:*** binding of these ligands enhance the catalytic activities of the allosteric enzymes.
- ***Allosteric inhibitors:*** binding of these ligands decrease the catalytic activities of the allosteric enzymes.

Salient features of allosteric enzymes:

i) Allosteric enzymes contain separate sites for binding with allosteric modulators, known as allosteric sites. Such allosteric sites are different from active sites from many aspects. Allosteric modulators have no specific active sites from denaturation.

ii) In case of few allosteric enzymes, separate allosteric sites for different modulators are available. Allosteric activators bind to a separate site and allosteric inhibitors will also bind to a separate site on the allosteric enzyme.

iii) An allosteric enzyme possesses specific allosteric sites for specific allosteric modulators, which means just like active sites the allosteric sites are also highly specific.

iv) An allosteric enzyme may have more than one substrate binding site.

v) ***Heterotropic effect:*** An allosteric modulator may have positive or negative co-operativity with the substrate of that allosteric enzyme; *e.g.*, if we consider an allosteric activator, it actually enhances the binding of substrate with the enzyme (***positive co-operativity*** *of allosteric activator and substrate*); if we consider an allosteric inhibitor, it actually decreases the binding of substrate with the enzymes (***negative co-operativity*** **of allosteric inhibitor and substrate**). Since such type of co-operativities do not exist between two different forms or partners of enzymes, *i.e.*, substrate and allosteric modulator which are very distinct from each other structurally and functionally, thus these types of co-operation are known as heterotropic effect.

vi) ***Homotropic effect:*** An allosteric enzyme may have positive or negative co-operativities for its substrates. In such situation the binding of one substrate molecule to the active site of the allosteric enzyme brings some three-dimensional conformational change in its structure, which may increase or decrease the affinity of the allosteric enzyme for another substrate. Since such type of positive or negative co-operativities exist between similar type of biomolecular substances (substrates only), these type of co-operativities are known as homotropic effect.

vii) Feedback allosteric inhibition: In such type of allosteric modulation an intermediate or a product of metabolic pathway allosterically inhibits the catalytic activity of an enzyme.

viii) Feed forward allosteric activation: In such type of allosteric modulation an intermediate or a product of metabolic pathway enhances the catalytic activity of an enzyme allosterically.

ix) Mutation, radiation, exposure to microwave, alteration in pH, temperature may destroy allosteric properties retaining the catalytic activity of the enzyme, which clearly indicate that allosteric sites are highly different from active sites. In many cases, allosteric enzymes containing more than one polypeptide chain, usually the allosteric sites and active sites may appear separate subunits.

Q24. What do you understand by homotropic and heterotropic effects? (2)

Ans: An allosteric enzyme may have co-operativities between its substrates. The binding of substrate molecule to one of the substrate binding sites in the enzyme brings conformational changes which promote binding of another substrate to the substrate binding site of the same enzyme, this is known as ***homotropic effect***. An allosteric modulator may have positive or negative co-operativities with the substrate, *i.e.*, a *positive allosteric modulator* (*activator*) enhances binding of substrates with the enzymes or increase the catalytic efficiency of the allosteric enzymes, on contrary *negative allosteric modulator* (*inhibitor*) lowers the binding of substrates and decrease catalytic efficiency of the allosteric enzymes. This type of co-operativity between the allosteric modulators and substrates is known as ***heterotropic effect***.

Q25. Write a note on feed-back allosteric inhibition. (5)

Ans: ***Feedback allosteric inhibition***, also known as ***feedback inhibition*** or ***end-product inhibition***, is an important regulatory mechanism of metabolism in which the final product of a metabolic pathway inhibits the activity of an enzyme involved in an earlier step of the same pathway. The inhibition usually occurs through allosteric interaction, where the end product binds to a regulatory site other than the active site of the enzyme. This mechanism is

extremely important for maintenance of metabolic balance and prevention of unnecessary synthesis of cellular products. In feedback inhibition, the end product acts as an *allosteric inhibitor*. When the concentration of the final product becomes sufficiently high, it binds reversibly to the allosteric site of a regulatory enzyme to reduce the catalytic activity of the enzyme and consequently decrease the overall rate of the metabolic pathway. The enzyme inhibited is usually the first committed enzyme or the *rate-limiting enzyme* of the pathway. Few important features of feedback inhibition are:

i) ***Allosteric regulation:*** The inhibitor binds at an allosteric site rather than the active site.
ii) ***Reversible process:*** The inhibition disappears when end-product concentration decreases.
iii) ***Controls metabolic flux:*** Prevents excessive accumulation of metabolic products.
iv) ***Energy conservation:*** Avoids unnecessary expenditure of ATP and precursor molecules.
v) ***Rapid regulation:*** Provides immediate control over enzymatic activity.
vi) ***Usually affects regulatory enzymes:*** Especially the first irreversible or committed step of a pathway.

Examples of feedback allosteric inhibition may include inhibition of ***threonine deaminase*** by *isoleucine*, which occurs during biosynthesis of the amino acid isoleucine. When isoleucine accumulates, it binds allosterically to the enzyme threonine deaminase, the first enzyme of the pathway, thereby inhibiting its activity. As a result, further synthesis of isoleucine is reduced. An example of feedback inhibition is regulation of cholesterol biosynthesis; during cholesterol synthesis, excess *cholesterol* inhibits the enzyme ***HMG-CoA reductase***, the rate-limiting enzyme of the pathway. This regulation helps maintain cholesterol homeostasis in cells.

Physiological and biochemical importance: Feedback inhibition helps to maintains metabolic homeostasis, helps in conservation of cellular energy, prevents overproduction of metabolites, coordinates interconnected metabolic pathways, protects cells from accumulation of toxic intermediates and enables rapid metabolic adaptation. Feedback inhibition is therefore one of the most important self-regulatory mechanisms in physiology.

Q26. What are K-series and V-series allosteric enzymes? Give examples (2)

Ans: Allosteric enzymes are classified as K-series and V series (or M series) enzymes. K-series allosteric enzymes are those in which the substrate affinity is decreased by the allosteric modulators, and K_m is raised without any change in V_{max}, *e.g.*, ***ATCase, PFK-1.*** On contrary in case of V-series allosteric enzymes, the substrate affinity is unaltered but the V_{max} is decreased by allosteric modulators, *e.g.*, ***acetyl CoA-carboxylase.***

UNIT-5: CARBOHYDRATE METABOLISM

Glycolysis, Citric acid cycle, Pentose phosphate pathway, Gluconeogenesis from lactate and glycerate, Glycogenesis and glycogenolysis. (Pathways with name of enzymes and significance).

Q1. Give a brief description of sequence of biochemical reactions of glycolysis. (6)

Ans: Glycolysis is the central metabolic pathway responsible for the breakdown of glucose into pyruvate which occurs into almost every cell in our body. The pathway of glycolysis was first elucidated by **Gustav Embden**, **Otto Meyerhof** and **Jakub K Parnas** during 1930 and is therefore also known as ***Embden-Meyerhof-Parnas pathway*** or ***EMP pathway***. The stoichiometry of the glycolytic pathway is:

$$\textbf{Glucose + 2ADP + 2NAD}^{+}\textbf{ + 2Pi = 2Pyruvate + 2ATP + 2NADH + 2H}^{+}\textbf{ + 2H}_2\textbf{O}$$

Site of glycolysis: All reactions of glycolytic pathway exclusively take place into the cytoplasm. Reactions of glycolytic pathway can be categorized into two broad phases, *i.e.*, ***non-oxidative phase*** or *preparatory phase* (***"pay-in"*** phase) and ***oxidative phase*** or *ATP generation phase* (***"pay-off"*** phase).

a) Non-oxidative (Pay-in) phase of glycolysis:

i) One molecule of glucose is phosphorylated at its C^6 position to form *glucose-6-phosphate*, with the help of enzyme ***hexokinase*** (or ***glucokinase***). The enzyme transfers one phosphate group from ATP to the glucose in presence of Mg^{2+} as cofactor. This reaction is irreversible and is often considered to be a rate limiting step in glycolysis. Phosphorylation of glucose by hexokinases lead to retention of glucose into cytoplasm, as the negatively charged phosphate group of glucose-6-phosphate does not allow diffusion of it across negatively charged surface of plasma membrane (***phosphate trapping***).

ii) The glucose-6-phosphate is next isomerized into *fructose-6-phosphate* (***keto isomer***), in a reversible rection catalyzed by the enzyme ***phosphohexose isomerase***.

iii) At the next stage fructose-6-P is phosphorylated into fructose-1, 6-bis phosphate with in an irreversible phosphotransfer reaction, catalyzed by enzyme ***phosphofructokinase-1*** **(PFK-1)**, an allosteric enzyme in presence of Mg^{2+} as cofactor. This reaction costs one high energy bond from one ATP molecule. This is one of the most important rate limiting step of glycolysis.

Here ends the "*pay-in*" phase of glucose oxidation that generates *fructose- 1, 6 bisphosphate* from glucose at a cost of two high energy bonds.

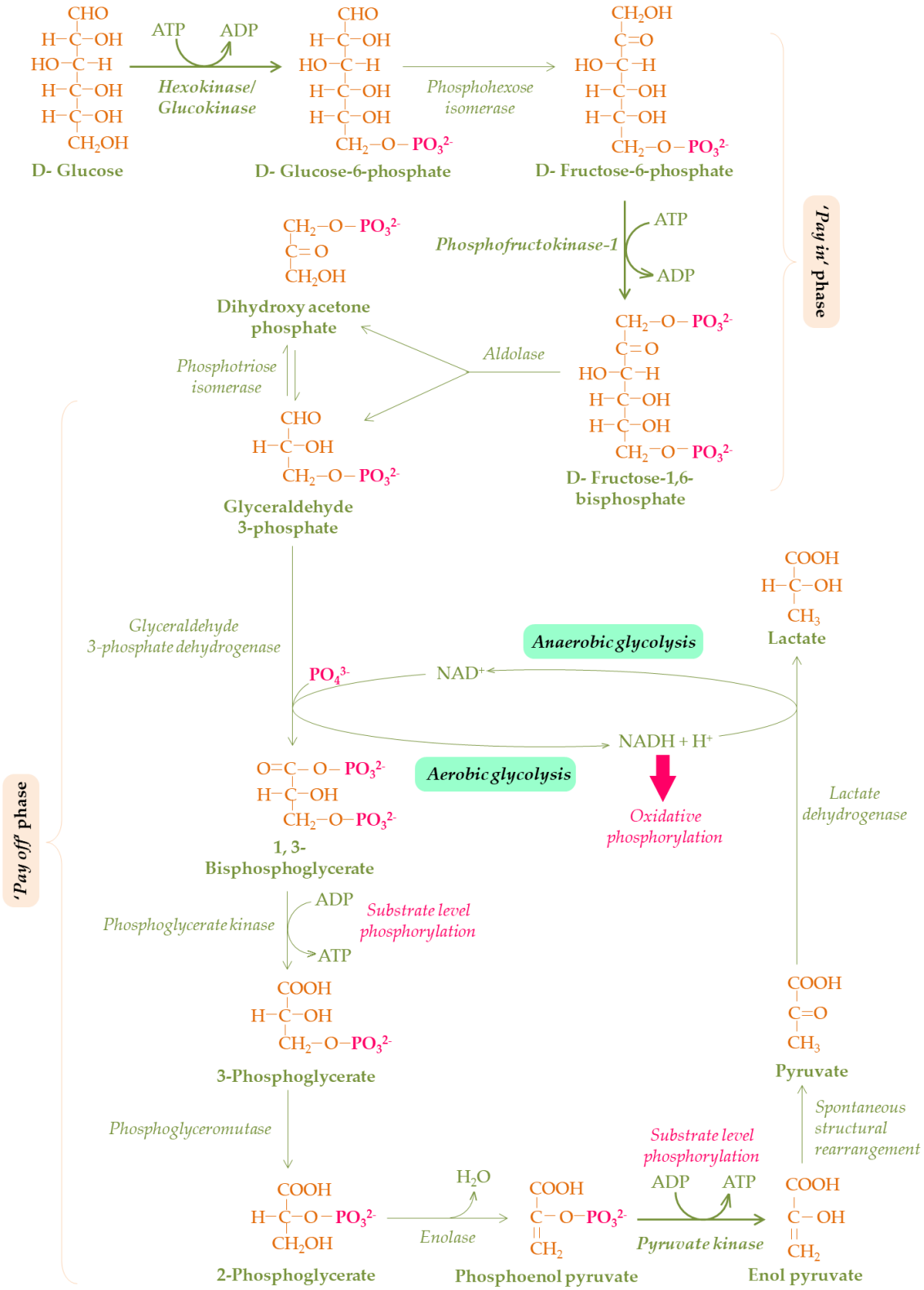
b) Non-oxidative (Pay-off) phase of glycolysis:

i) At the very first step of "*Pay-off*" phase of glycolysis, the intermediate fructose- 1,6 bisphosphate is cleaved two molecules of 3C compounds, *i.e.*, one molecule of *glyceraldehyde-3-phosphate* and one molecule of *dihydroxy acetone phosphate* (DHAP), with the help of a lyase called ***aldolase*** in a reversible reaction. The enzyme may catalyse aldol condensation reaction of glyceraldehyde-3-phosphate (aldehyde) and dihydroxy acetone phosphate (ketone), and also the reverse reaction, as depicted here. Interconversion between glyceraldehyde-3-phosphate and dihydroxy acetone phosphate occurs via isomerization catalyzed by the enzyme ***phosphotriose isomerase***. In summary, one molecule of 6C sugar hereby generates two molecules of 3C glyceraldehyde-3-phosphate.

ii) Glyceraldehyde-3-phosphate is next oxidized into *1,3-bisphosphoglycerate (1,3-BPG)*, with the help of enzyme ***glyceraldehyde-3-phosphate dehydrogenase***, in a reversible redox reaction, with help of NAD^+, and free inorganic phosphate (PO_4^{3-}). In this reaction NAD^+ is converted into NADH + H^+ after accepting electrons from the substrate. Two molecules of glyceraldehyde-3-P generate two molecules of NADH + H^+.

iii) Thus produced 1,3-bisphosphoglycerate is next converted into *3-phosphoglycerate* in a phosphotransfer type of reversible reaction catalyzed by ***phosphoglycerate kinase***, which transfers the phosphate group from C^1 of the substrate to an ADP molecule, producing a high energy ATP molecule (***substrate level phosphorylation***).

iv) Next occurs a mutation of arrangement of atoms and groups to form *2-phosphogycerate* from 3-phosphoglycerate, in a reversible reaction catalyzed by ***phosphoglyceromutase*** in presence of Mg^{2+} as cofactor.



v) In the next step, 2-phosphoglycerate is dehydrated to produce *phosphoenol pyruvate*. One H from C^2 and OH from C^3 of 2-phosphoglycerate are removed in form of one molecule of water, in a reversible reaction catalyzed by ***enolase*** to produce phosphoenol pyruvate**,** in presence of Mg^{2+}/Mn^{2+} as cofactor.

vi) Phosphoenol pyruvate is transformed into *enol pyruvate* with the help of enzyme ***pyruvate kinase*** in a phosphotransfer type of irreversible reaction, and generates one molecule of ATP (*substrate level phosphorylation*),

via transfer of phosphate group from phosphoenol pyruvate to one molecule of ADP. This is another rate limiting step of glycolysis.

vii) *The* enol pyruvate is spontaneously changed into *pyruvic acid*, after a spontaneous intramolecular atomic rearrangement.

Production of two molecules of pyruvic acid marks the ends of "*pay-off*" phase of glycolytic pathway. The fate of newly produced pyruvic acid depends on whether oxygen is present in adequate amount or not, *i.e.*, whether the cell is operating aerobically on anaerobically.

- *In presence of oxygen* **(*aerobic glycolysis*)**, NADH + H^+ donates its electrons in mitochondrial electron transport (ETC) chain (since ETC is active in presence of molecular oxygen), and pyruvic acid is fed into TCA cycle.
- *In absence of sufficient amount of oxygen* **(*anaerobic glycolysis*)**, NADH + H^+ is never fed into mitochondrial ETC, and is utilized for conversion of pyruvic acid into lactic acid, with the help of enzyme ***lactate dehydrogenase***, in a redox type of reaction.

Q2. Describe the energetics of anaerobic and aerobic glycolysis. (4)

Ans: Glycolytic pathway can be segregated into two major phases, *i.e.*, non-oxidative phase where two molecules of high energy ATP is consumed for phosphate trapping of glucose, formation of fructose-1,6-bisphosphate, hence is known as '***pay in***' phase and oxidative phase where two substrate level phosphorylation occurs and generates ATP during formation of 3-phosphoglycerate and enol pyruvate, hence is known as '***pay off***' phase. Beside this, an electron donor of ETC, *i.e.*, $NADH+H^+$ is also produced during oxidative phase of glycolysis which may contribute to additional amounts of ATP depending on the availability of oxygen. One molecule of glucose gives rise to two molecules of *glyceraldehyde-3-phosphate* at the beginning of '***pay off***' phase, each of which independently undergo oxidative reactions, *i.e.*, total four substrate level phosphorylation reactions take place and two molecules of $NADH+H^+$ are produced when oxidative phase completes.

Reactions of glycolysis		**ATP production**
Non-oxidative phase	***Hexokinase/glucokinase*** (for phosphorylation)	**- 1 ATP**
	Phosphofructokinase-1 (for phosphorylation)	**- 1 ATP**
Oxidative phase	***Glyceraldehyde-3P-dehydrogenase*** (oxidation of $2NADH+H^+$ into mETC)	**+ 6 ATP**
	Phosphoglycerate kinase (substrate level phosphorylation)	**+ 2 ATP**
	Pyruvate kinase (substrate level phosphorylation)	**+ 2 ATP**
	Net gain	**= 10 – 2 = 8 ATP**

The energetics of glucose oxidation might vary according to the concentration of oxygen into surrounding tissue, *i.e.*,

- ***During aerobic glycolysis***, $NADH+H^+$ is fed into mitochondrial ETC to produce ATP, therefore the net gain of high energy ATP is (10-2) = 8
- ***During anaerobic glycolysis***, $NADH+H^+$ produced by *glyceraldehyde 3-phosphate dehydrogenase* is utilized for conversion of pyruvate into lactate and fail to donate its electrons into mitochondrial ETC and therefore the net gain becomes (4-2) = 2 high energy ATP molecules.

Q3. Describe the rate limiting steps of glycolysis. (4)

Ans: Among several reactions of glycolytic pathway, only a few are physiologically irreversible and highly regulated. These irreversible reactions serve as the major rate-limiting and regulatory steps of glycolysis, and are as follows:

a) Hexokinase reaction: The first regulatory step of glycolysis is catalyzed by the enzyme ***hexokinase*** (or ***glucokinase*** in liver cells). In this reaction, glucose is phosphorylated to form *glucose-6-phosphate* using one molecule of ATP. This

phosphorylation traps glucose within the cell and commits it to intracellular metabolism. Since the reaction is essentially irreversible under physiological conditions, it acts as an important control point for glycolytic flux. Hexokinase is inhibited by its product, *glucose-6-phosphate*, thereby preventing excessive accumulation of phosphorylated glucose inside the cell.

b) Phosphofructokinase-1 reaction: The most important regulatory and rate-limiting step of glycolysis is catalyzed by ***phosphofructokinase-1*** (**PFK-1**); hence it serves as the principal rate limiting step of glycolysis. This enzyme converts *fructose-6-phosphate* into *fructose-1,6-bisphosphate* by transferring a phosphate group from ATP. Because this reaction is highly exergonic and irreversible, PFK-1 serves as the major controlling enzyme of glycolysis. PFK-1 is regulated allosterically according to the energy status of the cell. Intracellular ATP concentration in muscle cells plays a major role in this regulation. When ATP concentration rises, ATP binds to an allosteric regulatory site on PFK-1 distinct from the active site. This decreases the affinity of the enzyme for fructose-6-phosphate and thereby reduces the rate of glycolysis. Consequently, a fall in ATP concentration increases the activity of PFK-1 and stimulates glycolysis to meet cellular energy demands. Another important regulator of PFK-1 is *fructose-2,6-bisphosphate*. When *fructose-6-phosphate* is abundant, the enzyme ***phosphofructokinase-2*** (**PFK-2**) converts part of it into *fructose-2,6-bisphosphate*. This compound acts as a powerful allosteric activator of PFK-1. It not only increases the activity of PFK-1 but also counteracts the inhibitory effect of ATP on the enzyme. Thus, *fructose-2,6-bisphosphate* enhances glycolysis through multiple mechanisms. During conditions in which both glycolysis and β-oxidation occur at high rates, large amounts of intermediates enter the tricarboxylic acid (TCA) cycle, leading to increased ATP and citrate production. Elevated ATP and citrate concentrations inhibit PFK-1 allosterically, thereby slowing the overall rate of glycolysis. This mechanism ensures proper coordination between carbohydrate and lipid metabolism. AMP plays a crucial role in regulation of PFK-1 activity. Physiological concentrations of AMP oppose the inhibitory effect of ATP on the enzyme. Therefore, a high ATP/AMP ratio suppresses glycolysis, whereas a low ATP/AMP ratio stimulates glycolysis. In this way, PFK-1 functions as a sensitive metabolic sensor that adjusts glycolytic activity according to cellular energy requirements.

c) Pyruvate kinase reaction: The final irreversible step of glycolysis is catalyzed by ***pyruvate kinase***. In this reaction, *phosphoenolpyruvate* (PEP) transfers its high-energy phosphate group to ADP to produce ATP and pyruvate. This reaction is another important regulatory point of glycolysis. Pyruvate kinase is activated by *fructose-1,6-bisphosphate* through a feed-forward mechanism, ensuring coordinated progression of the pathway. Conversely, ATP and alanine inhibit the enzyme when the cellular energy supply is adequate. In the liver, ***pyruvate kinase*** activity is also hormonally regulated through reversible phosphorylation.

Q4. State the differences between hexokinase and glucokinase. (3)

Ans: Both of these two enzymes can phosphorylate glucose molecules into *glucose-6-phosphate* in an irreversible energy consuming reaction. However, there exists many differences between these two enzymes

	Hexokinase	**Glucokinase**
Distribution	Present into most of the tissues	Present mainly into liver and pancreas (β-cells of islets of Langerhans)
Cellular localization	Mostly cytoplasmic	Also cytoplasmic, but sometimes may be associated with nuclear proteins (in liver)
Physiological role	Provides glucose utilization to meet general cellular energy demand.	Helps in regulation of blood glucose after a meal. Hence after a carbohydrate rich meal activity of glucokinase rises markedly.
Affinity for glucose (K_m)	High (*i.e.*, very low K_m); hence are active at low blood sugar level.	Low (*i.e.*, high K_m); hence shows activity at relatively high blood sugar level.
Maximum velocity (V_{max})	Low V_{max}.	High V_{max}.
Substrate specificity	Wide, *i.e.*, many hexoses.	Narrow, *i.e.*, highly specific for glucose.

Regulation	Inhibited by glucose-6-phosphate (feed-back inhibition).	Not inhibited by glucose-6-phosphate.
Physiological significance	Ensures continuous ATP supply into tissues like brain and muscle.	Prevents hyperglycemia.

Q5. What is '*phosphate trapping*'? State its significance. (2+1)

Ans: Phosphate trapping refers to a biochemical phenomenon in which a molecule, after entering the cell, becomes phosphorylated and is thereby prevented from diffusing back across the plasma membrane. The addition of a phosphate group increases the polarity and negative charge of the molecule, making it unable to pass easily through the negatively charged surface and hydrophobic core of lipid bilayer of the cell membrane due to electrostatic repulsion. As a result, the phosphorylated compound becomes effectively '*trapped*' within the cell. A classic example of phosphate trapping occurs during the first step of glycolysis. After glucose enters the cell through glucose transporters, it is rapidly phosphorylated to glucose-6-phosphate by the enzyme hexokinase or glucokinase using ATP as the phosphate donor. Phosphate trapping is physiologically very important because it ensures cellular uptake, retention and efficient intracellular utilization of glucose. Phosphate trapping also serves as the metabolic commitment step for glucose utilization.

Q6. Trace the metabolic pathway of TCA cycle. (6)

Ans: The ***tricarboxylic acid*** (**TCA**) ***cycle***, also known as the ***citric acid cycle*** or ***Krebs cycle***, is the central metabolic pathway of aerobic respiration.

Site of TCA cycle: It occurs mainly into the mitochondrial matrix of eukaryotic cells, although one enzyme of the cycle, ***succinate dehydrogenase***, is located in the inner mitochondrial membrane.

Preparatory reactions: Before entering the actual TCA cycle, pyruvate produced during glycolysis undergoes preparatory reactions inside the mitochondrial matrix.

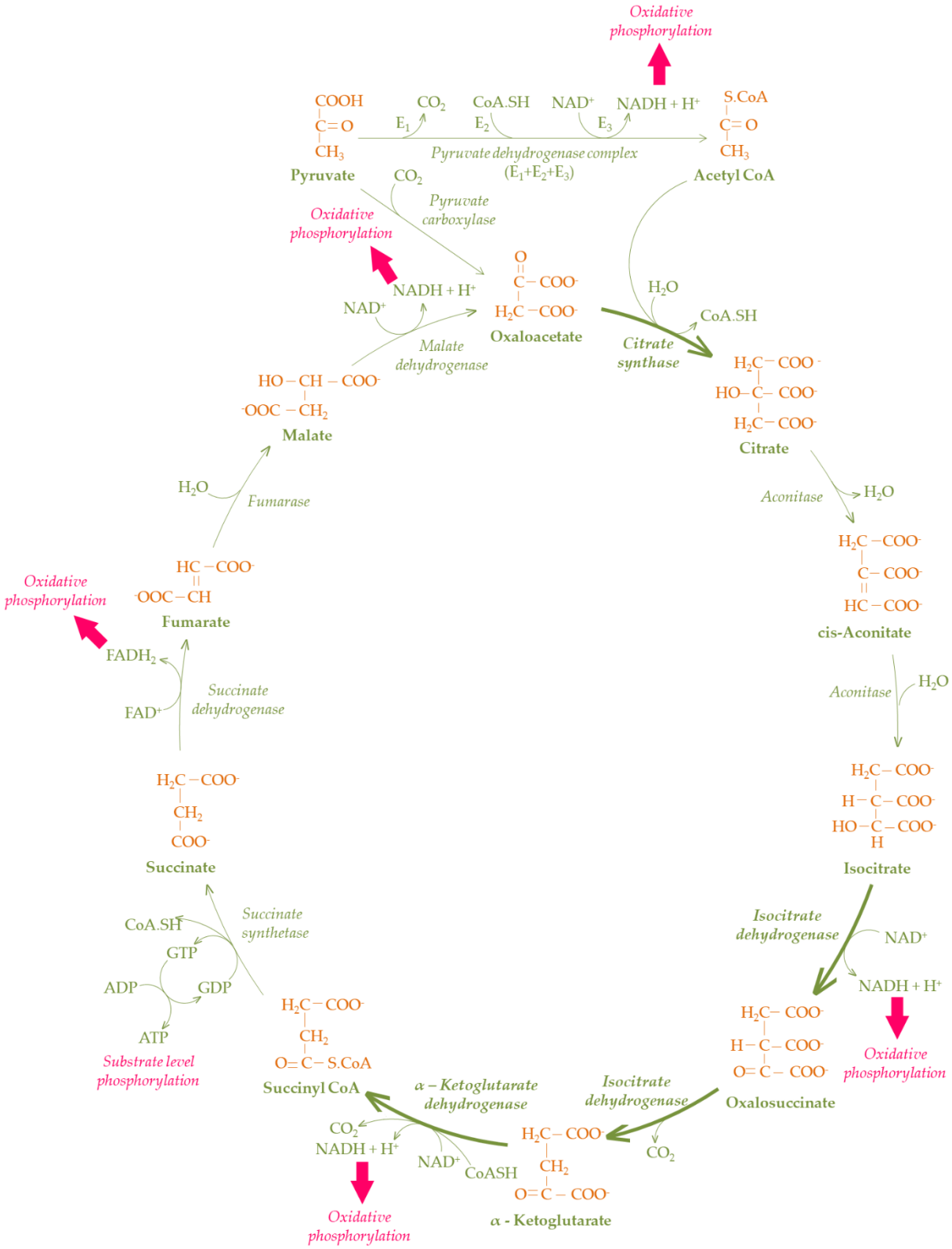
- The first preparatory reaction is the oxidative decarboxylation of pyruvate to acetyl-CoA catalyzed by the ***pyruvate dehydrogenase (PDH) enzyme complex***. During this reaction, NAD^+ is reduced to NADH + H^+. The pyruvate dehydrogenase complex is a mitochondrial enzyme complex, that belongs to the oxidoreductase class of enzymes, and the reaction catalyzed by it is irreversible. PDH enzyme complex is constituted by the enzymes, *i.e.*, E_1, E_2, and E_3. The E_1 catalyzes decarboxylation of pyruvate, E_2 catalyzes addition of coenzyme A (CoA-SH) and E_3 catalyzes oxidation of the substrate and formation of NADH+H^+.
- Pyruvate may also undergo carboxylation to form *oxaloacetate* in a reaction catalyzed by another mitochondrial enzyme ***pyruvate carboxylase***. This enzyme belongs to the *ligase* category and catalyzes an irreversible anaplerotic reaction that replenishes oxaloacetate for continuation of the cycle.

Reactions of TCA cycle takes place mostly into mitochondrial matrix, except one, and the reactions are as follows:

i) The first reaction of the TCA cycle involves condensation of one molecule of acetyl-CoA with one molecule of oxaloacetate in the presence of water to form *citrate*. The reaction is catalyzed by ***citrate synthase*** with simultaneous release of coenzyme A. Citrate synthase is a mitochondrial enzyme that belongs to the *transferase* class of enzymes. This reaction is highly exergonic and irreversible and acts as one of the major regulatory steps of the cycle.

ii) The citrate formed is subsequently converted into *isocitrate* through a reversible isomerization reaction catalyzed by ***aconitase***. The enzyme first dehydrates citrate to form cis-aconitate and then hydrates it again to produce isocitrate. Aconitase is a mitochondrial enzyme that belongs to the *lyase* category of enzymes.

iii) Isocitrate then undergoes oxidative decarboxylation to produce *α-ketoglutarate* in a reaction catalyzed by ***isocitrate dehydrogenase***. This mitochondrial enzyme belongs to the *oxidoreductase* class. The enzyme ***isocitrate dehydrogenase*** removes two hydrogen atoms from C^3 of the isocitrate to produce *oxalosuccinate*, and donates the hydrogen atoms to NAD^+ producing NADH + H^+. It also catalyzes decarboxylation of oxalosuccinate to produce *α-ketoglutarate*, in presence of Mg^{2+}/Mn^{2+} as cofactor.



iv) The α-ketoglutarate formed subsequently undergoes another oxidative decarboxylation reaction to form *succinyl-CoA*. This reaction is catalyzed by the ***α-ketoglutarate dehydrogenase complex***, which belongs to the *oxidoreductase* category. During the reaction, NAD^+ is reduced to NADH + H^+ with liberation of carbon dioxide. This reaction is irreversible and serves as another important rate limiting step of TCA cycle.

v) Succinyl-CoA is then converted into *succinate* in a reversible reaction catalyzed by ***succinyl-CoA synthetase***, also known as ***succinate thiokinase***. This enzyme belongs to the *ligase* class. During the reaction, the high-energy thioester bond of succinyl-CoA is cleaved, and the released energy is utilized for phosphorylation of GDP into GTP. This is the only *substrate-level phosphorylation* step of the TCA cycle.

vi) Succinate is then oxidized to *fumarate* in a reversible reaction catalyzed by ***succinate dehydrogenase***. During this reaction, two hydrogen atoms are removed from succinate and transferred to FAD to produce $FADH_2$. Succinate dehydrogenase belongs to the *oxidoreductase* class of enzymes. Unlike the other enzymes of the cycle, this enzyme is embedded in the inner mitochondrial membrane and functions as Complex II of the electron transport chain.

vii) The fumarate produced is subsequently hydrated to *malate* into mitochondrial matrix in a reversible reaction catalyzed by ***fumarase***, also known as ***fumarate hydratase***. Fumarase belongs to the *lyase* category of enzymes.

viii) Finally, malate is oxidized to regenerate *oxaloacetate* in a reaction catalyzed by ***malate dehydrogenase***. This enzyme belongs to the oxidoreductase class. During the reaction, NAD^+ is reduced to NADH + H^+. Although the reaction is reversible, it proceeds forward in the cell because oxaloacetate is continuously utilized in the citrate synthase reaction.

Thus, regenerated oxaloacetate again combines with acetyl-CoA to initiate another turn of the cycle. Among all the reactions of the TCA cycle, the reactions catalyzed by ***citrate synthase***, ***isocitrate dehydrogenase***, and ***α-ketoglutarate dehydrogenase*** are irreversible. Of these, the ***isocitrate dehydrogenase*** reaction is considered the principal rate-limiting step of the cycle. One complete turn of the cycle generates three molecules of NADH, one molecule of $FADH_2$, one molecule of GTP, and two molecules of carbon dioxide, thereby making the TCA cycle the major energy-producing pathway of aerobic metabolism.

Q7. Describe the energy yeild of TCA cycle. (3)

Ans: Two molecules of pyruvic acid produced into glycolysis enters into TCA cycle in presence of mitochondrial oxygen. One complete turn of the cycle generates three molecules of NADH, one molecule of $FADH_2$, one molecule of GTP, and two molecules of carbon dioxide, thereby making the TCA cycle the major energy-producing pathway of aerobic metabolism.

Reactions of TCA cycle		ATP production	
		Old concept	Modern concept
1.	***Isocitrate dehydrogenase*** (Isocitrate to oxalosuccinate) [1st oxidative stage]	+ 3 ATP	+ 2.5 ATP
2.	***α-Ketoglutarate dehydrogenase*** (α-Ketoglutarate to succinyl CoA) [2nd oxidative stage]	+ 3 ATP	+ 2.5 ATP
3.	***Succinate synthetase*** (Succinyl CoA to succinate) [Substrate level phosphorylation]	+ 1 ATP	+ 1 ATP
4.	***Succinate dehydrogenase*** (Succinate to fumarate) [3rd oxidative stage]	+ 2 ATP	+ 1.5 ATP
5.	***Malate dehydrogenase*** (Malate to oxaloacetate) [4th oxidative stage]	+ 3 ATP	+ 2.5 ATP
	Net gain	= 12 ATP	= 10 ATP

Q8. *'TCA cycle is called the final common pathway of metabolism'* - explain. (4)

Ans: The tricarboxylic acid (TCA) cycle often called the final common pathway of metabolism because it represents the oxidative pathway through which the major nutrients *i.e.*, carbohydrates, lipids, and proteins are metabolized.

- Carbohydrates first undergo glycolysis in the cytoplasm to produce *pyruvate*. Pyruvate then enters the mitochondria where it undergoes oxidative decarboxylation by the ***pyruvate dehydrogenase complex*** to form *acetyl-CoA*. This acetyl-CoA subsequently enters the TCA cycle for complete oxidation into carbon dioxide and water.

- Lipids also contribute to the TCA cycle. Fatty acids are degraded through β-oxidation within mitochondria, producing multiple molecules of *acetyl-CoA*. These *acetyl-CoA* molecules then enter the TCA cycle and undergo oxidation. *Glycerol*, another component of lipids, may enter glycolysis and eventually contribute to TCA cycle intermediates through pyruvate formation. Inside the TCA cycle, acetyl-CoA combines with *oxaloacetate* to form citrate, and through a sequence of enzymatic reactions, the acetyl group becomes completely oxidized. During this process, carbon dioxide is liberated and large quantities of reducing equivalents in the form of NADH and $FADH_2$ are generated. These reduced coenzymes subsequently donate electrons to the electron transport chain, leading to ATP synthesis through oxidative phosphorylation.
- Proteins contribute to the cycle after digestion into amino acids. Following deamination or transamination, different amino acids are converted into *pyruvate*, *acetyl-CoA*, or directly into TCA cycle intermediates such as *oxaloacetate*, *α-ketoglutarate*, *succinyl-CoA*, and *fumarate*. These intermediates then participate in the cycle for further oxidation and energy production.

The TCA cycle therefore serves as the common meeting point for the catabolism of carbohydrates, fats, and proteins. Since the degradation products of all these nutrients eventually enter this cycle and undergo complete oxidation, it is regarded as the '*final common oxidative pathway of intermediary metabolism*'.

Q9. Discuss about the amphibolic nature of TCA cycle. (4)

Ans: The ***tricarboxylic acid*** (**TCA**) cycle is often described as an '***amphibolic pathway***' because of its dual role in cellular metabolism. It participates both in *catabolism*, where complex molecules are broken down to release energy, and in *anabolism*, where intermediates of the cycle are utilized for the synthesis of various biomolecules. Thus, the TCA cycle occupies a central position in intermediary metabolism.

i) ***Catabolic role of TCA cycle:*** In its catabolic role, the TCA cycle acts as the final common oxidative pathway for carbohydrates, lipids, and proteins. Glucose undergoes glycolysis to form *pyruvate*, which is converted into *acetyl-CoA* before entering the cycle. Fatty acids are degraded through β-oxidation to generate *acetyl-CoA*, while many *amino acids* are converted into *pyruvate*, *acetyl-CoA*, or TCA cycle intermediates. Inside the cycle, *acetyl-CoA* is completely oxidized into carbon dioxide through successive *dehydrogenation* and *decarboxylation* reactions. During these reactions, high-energy electrons are transferred to NAD^+ and FAD to form NADH and $FADH_2$, which subsequently donate electrons to the electron transport chain for ATP production through oxidative phosphorylation. Therefore, the cycle serves as a major energy-generating pathway of aerobic cells.

ii) ***Anabolic role of TCA cycle:*** Besides its catabolic function, the TCA cycle also performs important anabolic functions because several intermediates of the cycle serve as precursors for biosynthesis. *Citrate* formed in the mitochondria may move into the cytoplasm where it provides *acetyl-CoA* for *fatty acid* and *cholesterol* synthesis. The *α-ketoglutarate* and *oxaloacetate* act as precursors for the synthesis of several amino acids through transamination reactions. The *α-ketoglutarate* gives rise to *glutamate, glutamine, proline*, and *arginine*, whereas *oxaloacetate* forms *aspartate, asparagine, methionine, lysine*, and *threonine*. *Succinyl-CoA* is utilized for the synthesis of *porphyrins* and *heme* compounds, which are essential components of *hemoglobin* and *cytochromes*. *Malate* may be transported to the cytoplasm and converted into *oxaloacetate*, which subsequently participates in gluconeogenesis for glucose synthesis. Thus, intermediates of the TCA cycle are continuously withdrawn for various biosynthetic purposes.

Therefore, the TCA cycle is termed '*amphibolic*' because it simultaneously participates in both degradation of nutrients for energy production and synthesis of important biomolecules required for growth, repair, and cellular maintenance.

Q10. What are anaplerotic reactions? (2)

Ans: The intermediates of TCA cycle are continuously removed from the cycle for anabolic reactions; hence, the cell must replenish them to maintain normal cycle activity. Such replenishing reactions are known as ***anaplerotic reactions***. One of the most important anaplerotic reactions is the *carboxylation* of *pyruvate* to *oxaloacetate* catalyzed by ***pyruvate carboxylase***. This reaction restores *oxaloacetate* levels and ensures continuous functioning of the cycle even when intermediates are diverted toward biosynthesis.

Q11. Describe the rate limiting steps of TCA cycle. (4)

Ans: The ***tricarboxylic acid*** (**TCA**) cycle is regulated mainly at the irreversible reactions that possess large negative free-energy changes. These reactions function as the principal control points of the cycle and determine the overall rate of aerobic oxidation of *acetyl-CoA*. The major rate-limiting and regulatory enzymes of the TCA cycle are ***citrate synthase***, ***isocitrate dehydrogenase***, and ***α-ketoglutarate dehydrogenase complex***. Among them, the reaction catalyzed by isocitrate dehydrogenase is considered the principal rate-limiting step of the cycle. Through regulation by ATP, ADP, NADH, calcium ions, and metabolic intermediates, these enzymes coordinate the rate of the cycle according to the energy requirements of the cell.

a) Citrate synthase reaction: The first important regulatory step is catalyzed by ***citrate synthase*** in the mitochondrial matrix. In this reaction, oxaloacetate condenses with acetyl-CoA in the presence of water to form *citrate* with simultaneous release of *coenzyme A*. *Citrate synthase* belongs to the transferase category of enzymes, and the reaction is irreversible because of its highly exergonic nature. The activity of citrate synthase is regulated according to the energy status of the cell. High concentrations of ATP, NADH, citrate, and succinyl-CoA inhibit the enzyme *allosterically*. Since citrate is the product of the reaction, accumulation of citrate indicates reduced utilization of TCA intermediates and suppresses further entry of acetyl-CoA into the cycle. Conversely, availability of substrates such as oxaloacetate and acetyl-CoA stimulates the reaction.

b) Isocitrate dehydrogenase reaction: The second and most important rate-limiting step is catalyzed by isocitrate dehydrogenase, an oxidoreductase enzyme located in the mitochondrial matrix. In this reaction, isocitrate undergoes oxidative decarboxylation to produce α-ketoglutarate with simultaneous formation of $NADH + H^+$ and release of carbon dioxide. The enzyme requires Mg^{2+} or Mn^{2+} as cofactors, and the reaction is irreversible. This reaction is regarded as the principal rate-limiting and committed step of the TCA cycle because it strongly determines the overall rate of cycle activity. Isocitrate dehydrogenase is activated by ADP and Ca^{2+} ions, which signal increased energy demand in the cell. In contrast, ATP and NADH inhibit the enzyme. A high ATP/ADP ratio therefore slows the cycle, whereas a fall in cellular energy charge stimulates oxidation of isocitrate and increases TCA cycle activity.

c) α-Ketoglutarate dehydrogenase reaction: Another rate limiting step is catalyzed by ***α-ketoglutarate dehydrogenase complex***, which belongs to the oxidoreductase class. This enzyme complex catalyzes the *oxidative decarboxylation* of α-ketoglutarate to *succinyl-CoA* with simultaneous reduction of NAD^+ to $NADH+H^+$ and release of CO_2. The reaction occurs into the mitochondrial matrix and is irreversible. The *α-ketoglutarate dehydrogenase complex* resembles the pyruvate dehydrogenase complex both structurally and mechanistically and requires several coenzymes including *thiamine pyrophosphate*, *lipoic acid*, FAD, NAD^+, and *coenzyme A*. The enzyme is inhibited by its products, *succinyl-CoA* and NADH, and is activated by Ca^{2+} ions. Increased concentrations of ATP indirectly suppress its activity by increasing NADH production.

Q12. State the energetics of complete oxidation of a glucose molecule. (3)

Ans: A glucose molecule into cytoplasm primarily enters glycolytic pathway to produce two molecules of pyruvic acid; however, its fate depends largely on the level of oxygen into cells.

i) ***Aerobic oxidation:*** Glucose is oxidised in glycolysis to form two molecules of pyruvate, and in presence of oxygen the mitochondrial ETC remains functional and lactate is never formed from pyruvate, and $NADH + H^+$ is free to donate their electrons in ETC. Net ATP production is (10-2) = 8. Each of two pyruvate molecules are converted into acetyl CoA forming two $NADH + H^+$ which produce 6 ATP after donating their electrons to mitochondrial ETC coupled with ***ATP synthase***. Finally in TCA, these two acetyl CoA are fed to produce 12 ATP per cycle, and therefore, 24 ATP molecules in total. Hence the gross production of ATP becomes (8 + 6 + 24) = 38 ATP [***according to old concept of bioenergetics***]. According to the modern concept of bioenergetics it becomes (7 + 5 + 20) = 32 ATP molecules**.**

ii) ***Anaerobic oxidation:*** Glucose is oxidised in glycolysis to form two molecules of pyruvate, and in absence of sufficient oxygen $NADH + H^+$ cannot donate its electrons into mitochondrial ETC, and reduces pyruvate into lactate. Therefore, the net ATP production is (4-2) = 2. Since no pyruvate molecules remains free to be fed into TCA cycle, the gross production remains 2 ATP only per glucose molecule oxidised.

Q13. Describe the pentose phosphate pathway. (3)

Ans: ***Pentose phosphate pathway*** (***PPP***) or ***hexose monophosphate*** (***HMP***) shunt is an alternative pathway for the oxidation glucose in eukaryotic cells; which is concerned with production of mainly ribose sugars, some amount of reducing equivalents (NADPH) and a lesser amount of ATP also. There are some other synonyms of this pathway; *e.g., pentose cycle, phosphogluconate pathway* and ***Warburg-Dickens-Lipman pathway*** etc.

Site of pentose phosphate pathway: It occurs in some specialized tissues, *e.g.,* red blood cells, adipose tissues, liver, testes, ovary, adrenal cortex and in lactating mammary glands. It is a multicyclic process where three molecules of glucose-6-phosphate enters and three molecules of each of the CO_2 and 5C pentose sugars are produced; which are rearranged to form two molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3-phosphate. Here oxidation is carried out via dehydrogenation with production of six molecules of NADPH. The stoichiometry is:

3 Glucose-6- phosphate + NAD^+ = 3 CO_2 + Glucose-6-phosphate + Glyceraldehyde-3- phosphate + 6 NADPH + 6 H^+

Different stages of pentose phosphate pathway: All the reaction of pentose phosphate pathway are divided into two distinct phases –

- Oxidative phase: which involve production of 5C pentose sugars.
- Non-oxidative phase: This involves interconversion of pentose sugars to form D-ribose-5-phosphate and D-xylulose-5-phsopahte.

a) Oxidative phase: Glucose is the starting material for pentose phosphate pathway; which is first phosphorylated at C^6 position to form *glucose-6-phosphate* in a reaction catalyzed by ***glucokinase*** or ***hexokinases*** with consumption of one high energy bond**.** Glucose-6-phosphate undergoes subsequent dehydrogenation reactions. There are two oxidative stages in pentose phosphate pathway.

i) First oxidative stage: At the first oxidative stage glucose-6-phosphate is oxidized into *6-phosphogluconic acid* through formation of an intermediate compound *6-phsophogluconolactone*. The enzyme ***glucose-6-phosphate dehydrogenase*** transfers two hydrogen atoms from C^1 and C^5 positions of glucose-6-phosphate to NAD^+ which results in cyclization of the substrate leading to formation of *6-phosphogluconolatone* and NADPH. The product 6-phosphogluconolatone is immediately hydrated to form *6-phosphogluconic acid* in a reaction catalyzed by ***gluconolactone hydratase***.

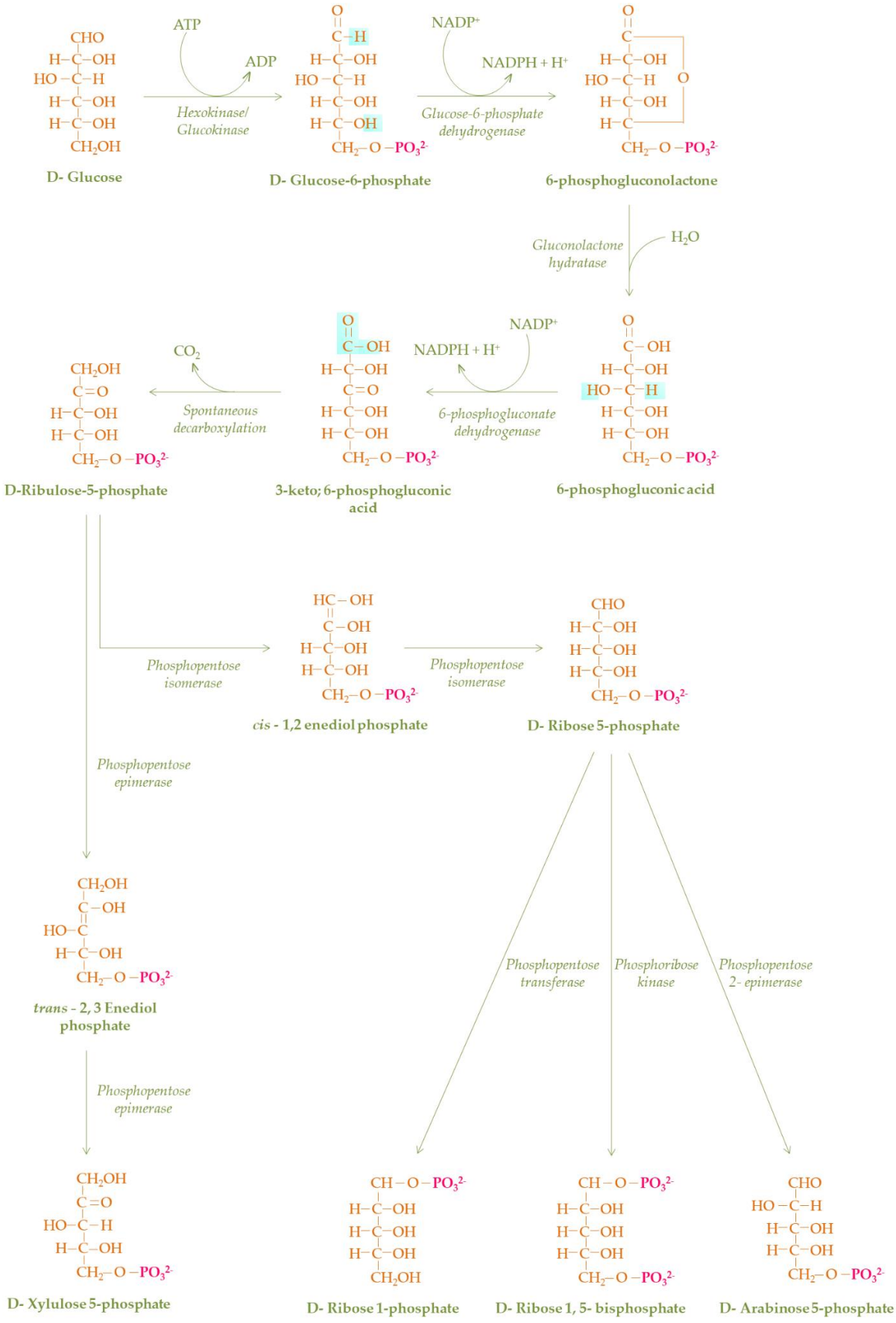
ii) Second oxidative stage: In the second oxidative stage 6-phosphogluconic acid is oxidized to form D-ribulose-5-phosphate. The enzyme ***6-phosphogluconate dehydrogenase*** transfers two hydrogen atoms from C^3 position of 6-phosphogluconic acid into NAD^+ and results in formation of *3-keto; 6-phosphogluconic acid* with subsequent production of NADPH. The product is very unstable and undergoes immediate decarboxylation with a net loss of one carbon to form 5C pentose sugar – *D-ribulose-5-phosphate*.

b) Non-oxidative phase: The non-oxidative phase of pentose phosphate pathway is also composed of two stages –

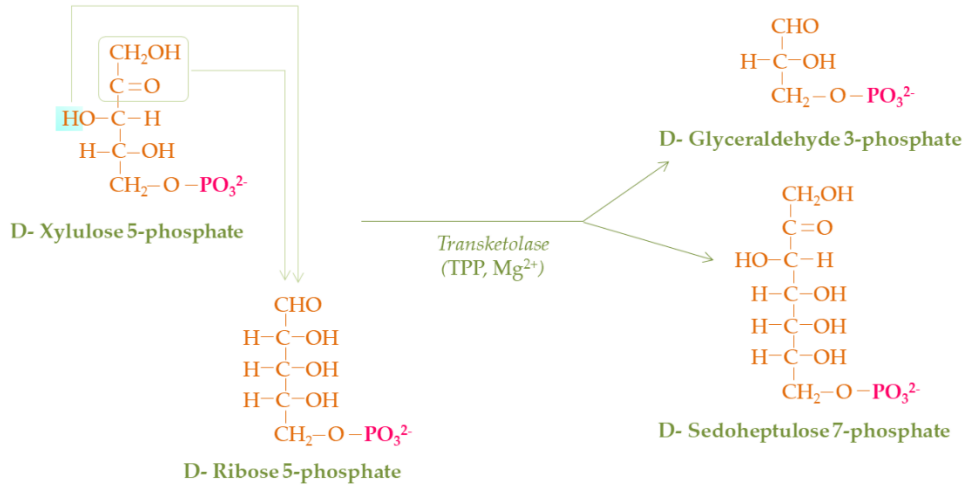
i) Interconversion of pentose sugars: D-ribulose-5-phosphate formed during oxidative phase of PPP is an aldopentose, which is subsequently isomerized into its keto isomer *D-ribose-5-phosphate* in a reaction catalyzed by the enzyme ***phosphopentose isomerase***. The reaction involves formation of an intermediate *cis - 1, 2 enediol phosphate*.

Thus produced D-ribose-5-phosphate undergoes further chemical remodelling to produce different other types of pentose sugars as follows –

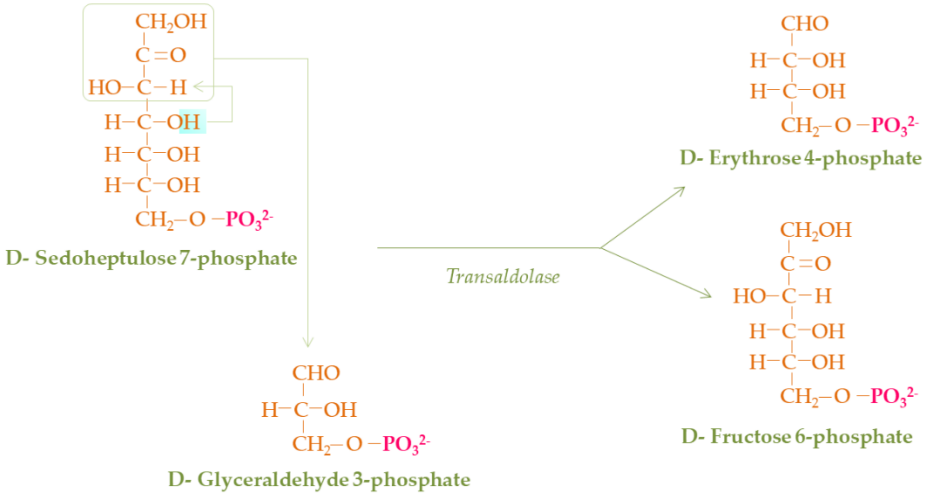
- D-ribose-5-phosphate is epimerized into *D-arabinose-5-phosphate* (2-epimer of ribose) in a reaction catalyzed by ***phosphoribose 2-epimerase***.
- In another reaction the D-ribose-5-phosphate may also be phosphorylated at its C^1 position to form *D-ribose 1, 5-bisphosphate* under influence of the enzyme ***phosphoribose kinase***.
- *D-ribose-1-phosphate* may also be formed from D-ribose-5-phosphate in a phosphotransfer reaction catalyzed by ***phosphopentose transferase***.



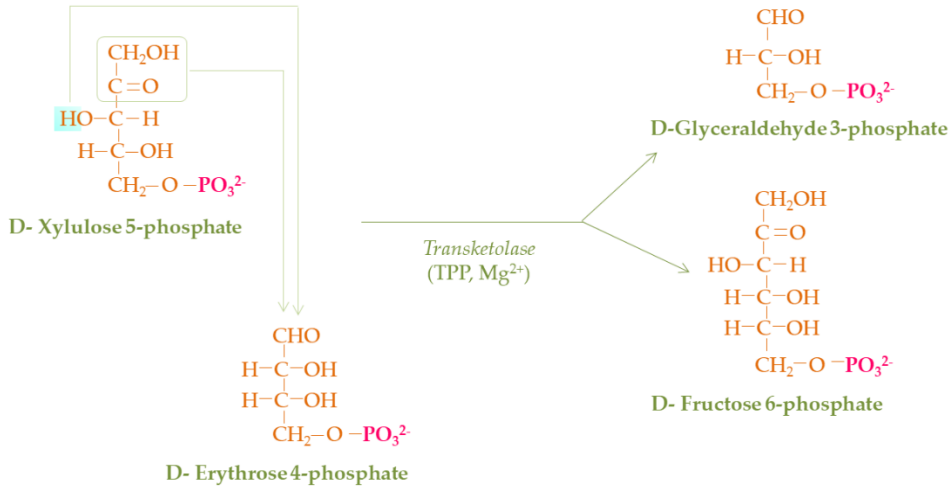
First transketolase reaction



Transaldolase reaction



Second transketolase reaction



D-ribulose-5-phosphate may be epimerized into *D-xylulose-5-phosphate* (3-epimer of ribulose) another ketopentose. The reaction is catalyzed by the enzyme ***phosphopentose epimerase***, by alteration of the configuration of H and –OH around C^3 through formation of a transient intermediate *trans - 2, 3 enediol phosphate*.

ii) Formation of hexose phosphates: This phase involves reformation of hexose sugars from the pentose sugars of PPP, *e.g.*, D-ribose-5-phosphate and D-xylulose-5-phosphate forms *D-fructose-6-phosphate* to carry out the PPP again. This is catalyzed by two peculiar enzymes, *i.e.*, ***transketolase*** and ***transaldolase*** in three separate stages.

- Firstly, the enzyme ***transketolase*** transfers a 2C moiety from D-xylulose-5-phosphate to D-ribose-5-phosphate resulting in formation of one molecule of each of *D-glyceraldehyde-3-phosphate* and *D-sedoheptulose-7-phosphate*. The enzyme transketolase is a homodimer, which utilizes *thiamine pyrophosphate* (*TPP*) and Mg^{2+} as cofactors. The TPP acts to accept ketol group from the donor molecule and then by proper rearrangement of electrons from the product aldehydes. The ketol-TPP addition product then reacts with an acceptor aldehyde to form the product ketol, *i.e.*, to regenerate itself.
- Secondly, another type of enzyme, ***transaldolase*** catalyzes transfer of a 3C moiety (*dihydroxy acetone*) from D-sedoheptulose-7-phosphate to D-glyceraldehyde-3-phosphate resulting in formation of one molecule each of *D-fructose-6-phosphate* and *D-erythrose-4-phosphate*. The acceptor molecule may also be D-ribose-5-phosphate in place of D-glyceraldehyde-3-phosphate.
- The transaldolase reaction is immediately followed by another transketolase reaction catalyzed by the same enzyme ***transketolase.*** In the second transketolase reaction the D-erythrose-4-phosphate and D-xylulose-5-phosphate are utilized to produce *D-fructose-6-phosphate* and *D-glyceraldehyde-3-phosphate*, thereby regenerating the hexose sugar again. The enzyme transfers a 2C moiety from D-xylulose-5-phosphate to the C^1 of D-erythrose-4-phosphate generation a hexose sugar, D-fructose-6-phosphate; D-glyceraldehyde-3-phosphate is also generated as a byproduct of this reaction. The D-fructose-6-phosphate is also isomerized into *D-glucose-6-phosphate* to run the cycle over again.

Q14. State the physiological significance of pentose phosphate pathway. (6)

Ans: ***Pentose phosphate pathway*** (***PPP***) or ***hexose monophosphate*** (***HMP***) shunt is an alternative pathway for the oxidation glucose in eukaryotic cells and possess several physiological importances:

a) Formation of reducing equivalent NADPH: Although pentose phosphate pathway is an alternative pathway for oxidation of glucose in cells, it is never meant for energy production; as the reducing equivalent generated in this pathway, *i.e.*, NADPH is incapable of donating electrons to the components of mitochondrial ETC. NADPH is generated during the first two oxidative phases of PPP via the activity of enzymes ***glucose-6-phosphate dehydrogenase*** and ***6-phosphogluconate dehydrogenase***. Although NDPH is incapable of donating its electrons to the mitochondrial ETC; they are good reducing agents. Hence NADPH is utilized in various reductive biosynthesis mechanisms, *e.g.*,

i) NADPH acts as a co-enzyme for ***glutathione reductase*** during conversion of oxidized glutathione into reduced glutathione
ii) NADPH also acts as a co-enzyme for the enzyme ***methemoglobin reductase*** for conversion of methemoglobin into hemoglobin.
iii) Extra-mitochondrial fatty acid synthesis
iv) Steroid and cholesterol biosynthesis
v) Sphingolipid biosynthesis
vi) Uronic acid pathway

b) Role in erythrocyte fragility: Pentose phosphate pathway is red blood cells or erythrocytes gives rise to intracellular NADPH, which is a potent reducing agent. NADPH is capable of reducing a tripeptide *glutathione* (GS-SG) into GSH. This reaction is catalyzed by ***glutathione reductase***. Thus produced GSH is immediately oxidized into its previous form (GS-SG) in a reaction catalyzed by another enzyme ***glutathione peroxidase***. Glutathione peroxidase transfers the hydrogen atoms from GSH into H_2O_2; in order to neutralize the latter into H_2O and GSH is converted into its oxidised form again. Whenever the cells lack NADPH; the intracellular GSH level falls and reoxidation of

glutathione does not take place, so the H_2O_2 being produced by metabolic activities within physiologically active cells starts to accumulate. The H_2O_2 is toxic for the cell from the context of its capabilities to destroy membrane lipids; which makes the plasma membrane leaky. H_2O_2 also reduces the conversion of methemoglobin (oxidized form of hemoglobin; Fe^{3+}) into hemoglobin (Fe^{2+}). These all decreases average life span of erythrocytes. People suffering from genetic deficiency of the enzyme ***glucose-6-phosphate dehydrogenase*** shows serious perturbance in intracellular NADPH balance, and lacks glutathione mediated protection against H_2O_2 toxicity. The plasma membrane of erythrocytes becomes more prone to damage making them more fragile and susceptible to ***hemolysis***.

c) Production of pentoses: Pentose phosphate pathway (or ***hexose monophosphate pathway***) provides huge amount of pentose sugars, *e.g.*, D-ribose-5-phosphates. Such phosphopentose are easily dephosphorylated and the pentose sugars are used for biosynthesis of nucleic acids. Thus, PPP provides a good source to provide starting materials for nucleic acid biosynthesis.

d) Role in phagocytosis: Reactions of pentose phosphate pathway are increased manifold in leucocytes, and phagocytic cells *e.g.*, neutrophils, eosinophils, macrophages during phagocytosis. NDPH generated in during PPP is utilized by ***NADPH oxidase*** in producing *superoxide anions* (O_2^-) for destroying phagocytosed materials within phagolysosome.

Q15. Describe the gluconeogenesis from lactate and glycerate. (6)

Ans: ***Gluconeogenesis*** or ***neo-glucogenesis*** refers to the metabolic process where glucose is formed from non-carbohydrate sources, *e.g.*, lactate, glycerate, glycerol, amino acids etc. Gluconeogenesis from lactate takes place partially into the cytoplasm and partially into the mitochondria.

i) Lactate is produced during *anaerobic glycolysis* from pyruvate, by ***lactate dehydrogenase***. This lactate is may also be re-oxidized into pyruvate by LDH in the cytoplasm. Pyruvate produced in this way, enters into the mitochondrial matrix along with a proton (H^+) via a ***pyruvate-proton symporter***.

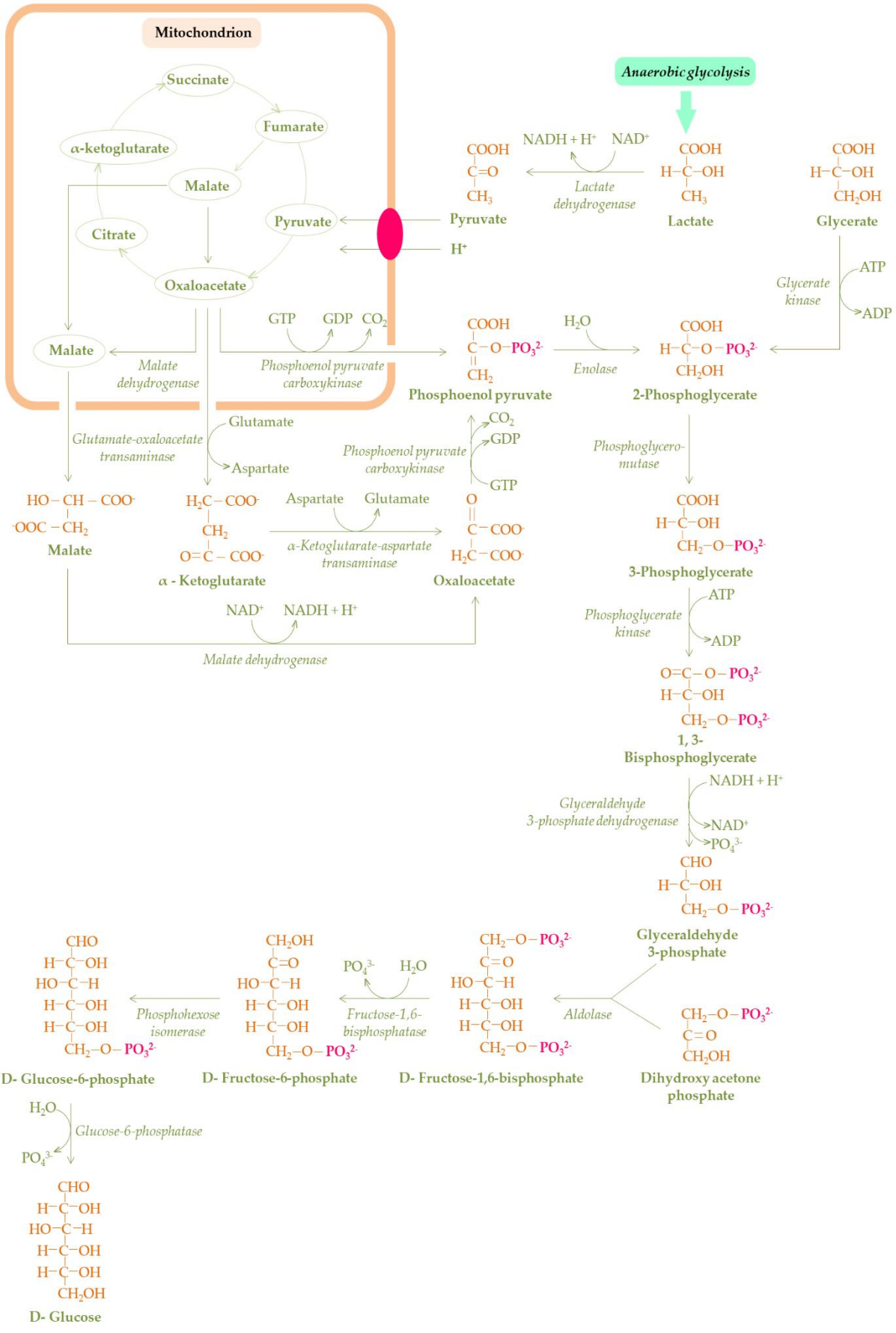
ii) Pyruvate present in the mitochondrion is converted into *oxaloacetic acid* (OAA) via the action of ***pyruvate carboxylase***. This enzyme carboxylates pyruvate and for this purpose uses the bond energy of one ATP and by breaking one high energy bond in it and converting it into ADP and inorganic phosphate (PO_4^{3-}). Pyruvate carboxylase is a key enzyme of gluconeogenic pathway. This enzyme complex contains biotin, and forms biotin-biotin-carboxy complex and then reacts with the substrate and carboxylates it.

iii) In humans the enzyme ***phosphoenol-pyruvate carboxykinase*** (**PEPCK**) decarboxylates and phosphorylates OAA into *phosphoenol pyruvate* (PEP) using a phosphate group derived from GTP. The phosphoenol pyruvate is transported into the cytoplasm through a carrier protein of inner mitochondrial membrane. Phosphoenol pyruvate may also be produced from oxaloacetate into two other separate mechanisms, as follows:

iv) OAA may get oxidized into *malate* by ***malate dehydrogenase*** and is transported through the mitochondrial membrane into cytosol. Into cytosol, this malate is again converted into OAA by *malate dehydrogenase* and OAA may be converted into phosphoenol pyruvate by cytoplasmic PEPCK.

v) OAA may also get transaminated into *aspartate* and is transported into cytoplasm. This is catalysed by a transaminase, known as ***glutamate-oxaloacetate transaminase***. Into this transamination reaction it produced one molecule of α-ketoglutarate, which is transported into cytoplasm. Another transamination reaction catalyzed by cytoplasmic ***α-ketoglutarate-aspartate transaminase*** produces oxaloacetate into cytoplasm, which is in turn converted into phosphoenol pyruvate by cytoplasmic PEPCK.

vi) Into cytoplasm, the phosphoenol pyruvate is readily hydrated by the glycolytic enzyme ***enolase*** producing *3-phosphoglycerate*. This 3-phosphoglycerate is next isomerized into *2-phosphoglycerate* by another glycolytic enzyme ***phosphoglycerate mutase***. The enzyme ***phosphoglycerate kinase*** converts 2-phosphoglycerate into *1,3-bisphosphoglycerate* in a reaction that consumes one ATP molecule. The 1,3-bisphosphoglycerate is dephosphorylated and reduced into *glyceraldehyde-3-phosphate* by the enzyme ***glyceraldehyde-3-phosphate dehydrogenase*** which costs consumption of one molecule of $NADH+H^+$. Glyceraldehyde-3-phosphate is partly converted into *dihydroxyacetone phosphate* (DHAP) by ***phosphotriose isomerase***. The enzyme ***aldolase B*** condenses glyceraldehyde-3-phosphate and into dihydroxyacetone phosphate to produce *fructose 1,6-bisphosphate*, a six-carbon symmetrical sugar.



vii) ***Fructose 1,6 bisphosphatase*** another key enzyme of gluconeogenesis, which hydrolytically removes PO_4^{3-} from C^1 to convert it into *fructose-6-phosphate*. Fructose-6-phosphate is next isomerized into *glucose-6-phosphate* by the enzyme ***phosphohexose isomerase***. Finally, the enzyme ***glucose-6-phosphatase*** converts glucose-6-phosphate into *glucose* after removal of PO_4^{3-}hydrolytically from the substrate.

Gluconeogenesis from glycerate is comparatively simple. Glycerate produced from lipid breakdown and as a consequence of other metabolic pathways is converted directly into *2-phosphoglcyerate* after addition of an inorganic phosphate (PO_4^{3-}) to the C^2 position in a reaction catalyzed by the enzyme ***glycerate kinase*** located into cytoplasm. Formation of glucose from 2-phosphoglcyerate follows the same reaction sequence as occurs during gluconeogenesis from lactate.

Q16. Describe the metabolic reactions of glycogenesis. (6)

Ans: Synthesis of glycogen form glucose is called ***glycogenesis***. Glycogen is a branched chain polysaccharide found in animal cells and its synthesis has profound physiological importance in the context of conservation of energy. Glycogen is made up of glucose molecules joined together via formation of α-1,4 and α-1,6-glycosidic linkages.

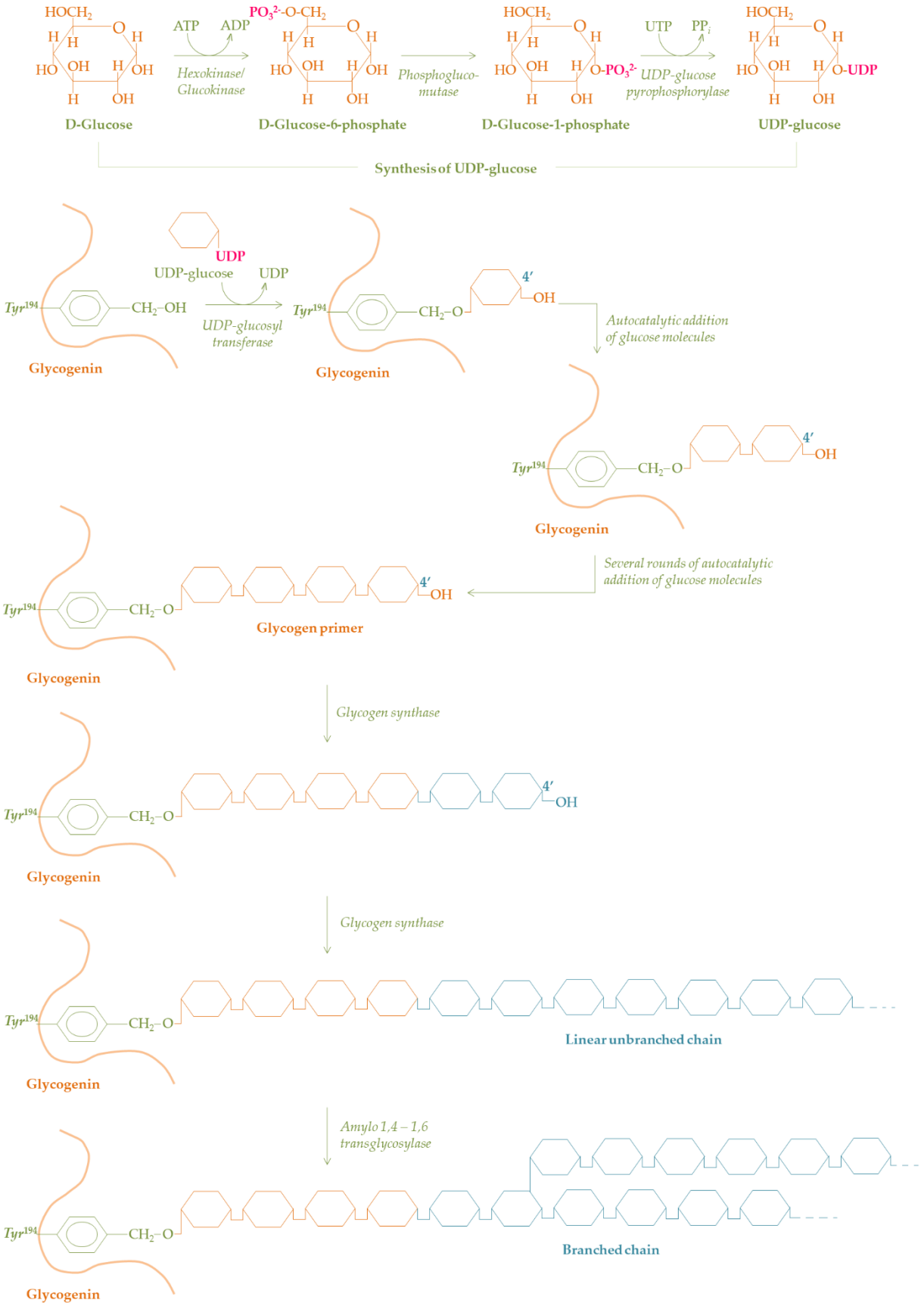
Site of glycogenesis: Liver and skeletal muscles.

Different stages of glycogenesis: Glycogenesis can be divided into three major phases, *i.e.*, ***activation of glucose, synthesis of glycogen primer*** and ***polymerization phase***.

Activation of glucose: Before the polymerization begins D-glucose molecules are converted into *glucose-6-phosphates*, through a reaction catalyzed by enzyme ***glucokinase*** (or ***hexokinase***). This enzyme transfers a phosphate moiety from ATP to C^6 of glucose to form glucose-6-phosphate (Mg^{2+} acts as a cofactor in this reaction). Glucose-6-phosphate is then converted into *glucose-1-phosphate*, in a reaction catalyzed by the enzyme ***phosphoglucomutase***. This enzyme contains a ***phosphoserine residue*** at its active site. The phosphate moiety is first transferred to the C^1 of glucose-6-P converting it into *glucose 1, 6-bisphosphate*. At the next step, phosphate moiety is transferred from C^6 position of glucose 1, 6-bisphosphate to active site serine residue of the enzyme, converting it into glucose-1-phosphate. Another enzyme, ***UDP-glucose pyrophosphorylase*** catalyzes release of two phosphoric acid moieties (in form of *pyrophosphate* or *PPi*), one from an UTP molecule and another from the glucose-1-phosphate, thereby converting it into ***UDP-glucose***, a highly reactive compound.

Synthesis of glycogen primer: Before the enzyme ***glycogen synthase*** forms an unbranched chain of glucose molecules joined with each other by α-1,4-glycosidic linkages. For this purpose, glycogen synthase requires a ***glycogen primer*** (already existing glucose polymer). Synthesis of glycogen primer requires presence of a core protein called ***glycogenin***. In humans, the most common form is ***glycogenin-1***, particularly abundant in skeletal muscle, although ***glycogenin-2*** is also present mainly in liver tissue. Glycogenin possesses intrinsic '***glycosyltransferase activity***', which enables it to catalyze the attachment of glucose residues to itself, this is known as *autocatalysis*. During the initiation process, glycogenin utilizes UDP-glucose as the activated glucose donor. The C^1 of first glucose molecule becomes covalently attached to the hydroxyl group of a specific tyrosine residue (Tyr^{194}) of glycogenin through an *O-glycosidic linkage*. After this initial attachment, glycogenin sequentially adds several more glucose residues, usually forming a short oligosaccharide chain containing approximately 8–10 glucose units, which is known as ***glycogen primer***. The C^1-OH (reducing end) of oligosaccharyl chain remains attached to the glycogenin and the C^4-OH (non-reducing end) of it remains free.

Polymerization of glucose: After synthesis of short oligosaccharyl chain on glycogenin (*i.e.*, after synthesis of the glycogen primer), the enzyme ***glycogen synthase*** starts to add glucose residues to the C^4 non-reducing end of glycogen primer. The enzyme ***glycogen synthase*** splits UDP-glucose into UDP and glucose. The energy released in this process is then utilized into formation of bond among glucose molecules. Several rounds of addition of glucose occurs under the activity of glycogen synthase. After addition of 11 or more than 11 molecules of glucose to the C^4 ends of glycogen primer, the enzyme ***amylo 1,4-1,6 transglycosylase*** (also known as ***branching enzyme***) splits a segment from newly formed straight chain, and transfers it elsewhere in the straight chain through formation of α-1,6-glycosidic linkages. Polymerization and branching continue until it gives rise to a bulky polymer of glycogen.



Q17. What is glycogenin? State its physiological importance. (3)

Ans: Glycogenin is a specialized self-glycosylating protein that plays a fundamental role in the initiation of glycogen synthesis. It serves as the core primer molecule upon which glycogen particles are formed. Since glycogen synthase, the major enzyme responsible for glycogen elongation, cannot initiate synthesis de novo, glycogenin provides the initial short chain of glucose residues required for the commencement of glycogenesis. Thus, glycogenin acts as the molecular foundation for glycogen granule formation in animal cells. Structurally, glycogenin is a dimeric protein located at the center of every glycogen particle. In humans, the most common form is glycogenin-1, particularly abundant in skeletal muscle, although glycogenin-2 is also present mainly in liver tissue. The protein possesses intrinsic glycosyltransferase activity, enabling it to catalyze the attachment of glucose residues to itself.

Physiological importance of glycogenin: The importance of glycogenin in carbohydrate metabolism is immense. Its primary significance lies in its indispensable role in the initiation of glycogen biosynthesis. Without glycogenin, glycogen synthase cannot begin glycogen formation efficiently because it requires a pre-existing glucose chain as substrate. Glycogenin therefore ensures the proper storage of glucose in the form of glycogen within liver and muscle cells. Glycogenin is also important for maintaining cellular energy homeostasis. In skeletal muscles, glycogen reserves provide a rapid source of glucose during muscular contraction and strenuous exercise, while in the liver glycogen acts as a buffer to maintain blood glucose concentration during fasting.

Q18. Describe the metabolic reactions of glycogenolysis. (6)

Ans: ***Glycogenolysis*** refers to the breakdown of glycogen and release of glucose residues.

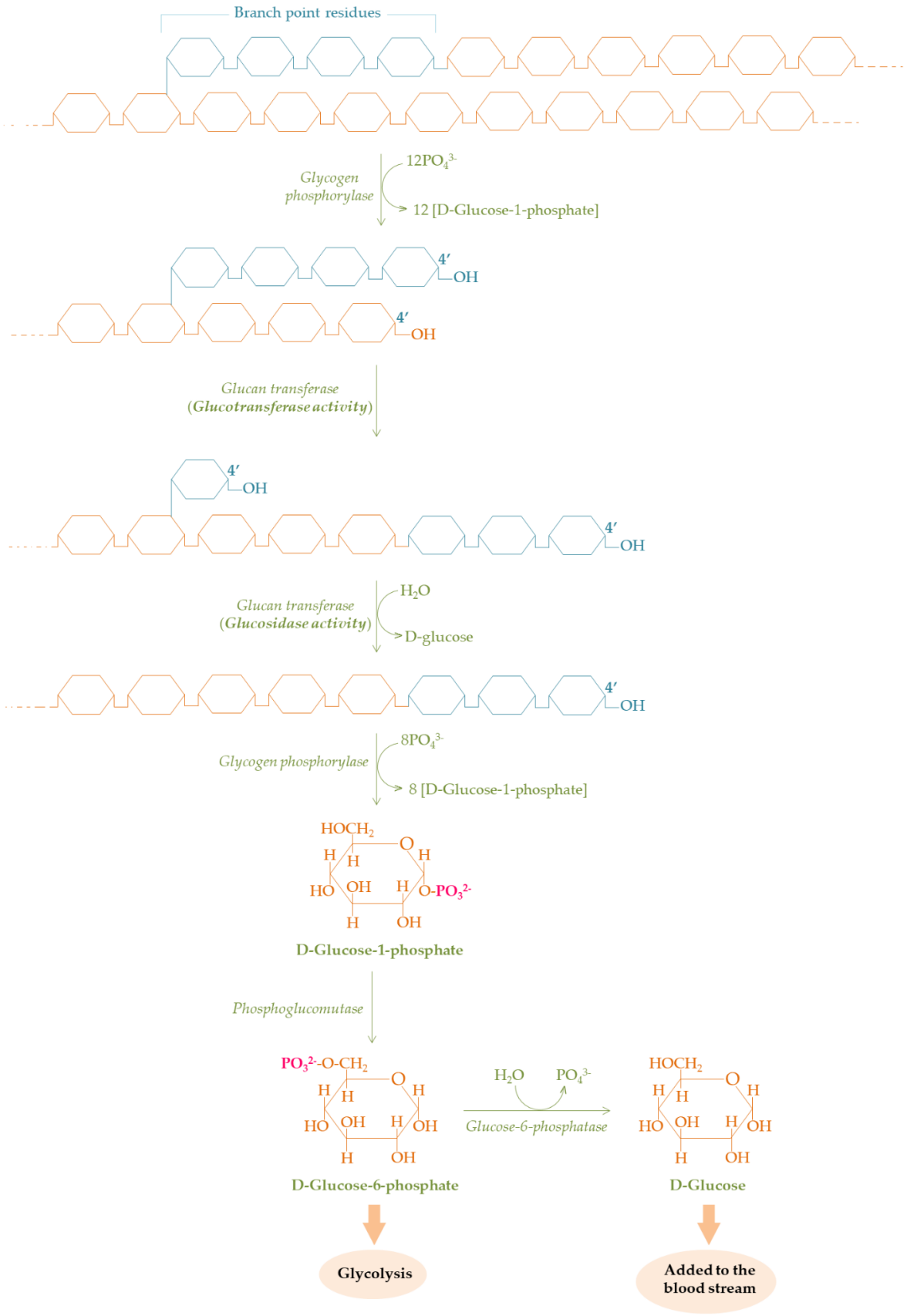
Site of glycogenolysis: Skeletal muscle and liver.

Different stages of glycogenolysis: Liver has the highest content of glycogen in contrast to the muscle tissue per unit mass. Since muscle mass is greater than liver, muscle contains a higher amount of glycogen than liver.

Glycogenolysis is carried out by the action of enzyme ***glycogen phosphorylase***. This enzyme phosphorolytically remove glucose moieties one by one from non-reducing end of glycogen, by breaking down the α-1,4-glycosidic linkages. However, this phosphorolytic reaction catalyzed by ***glycogen phosphorylase*** does not require consumption of ATP, rather high concentration of free intracellular inorganic phosphoric acid (PO_4^{3-}) is enough to drive this reaction. Thus, the released glucose residues are phosphorylated, highly active and charged molecules. Glucose is released as *glucose-1-phosphate*. ***Glycogen phosphorylase***, is unable to break α-1,6-glycosidic linkages which it frequently encounters at branching point. The activity of ***glycogen phosphorylase*** ceases at 4^{th} glucose residue from each branching points. The removal of glucose molecules from branching point requires presence of another enzyme, ***glucan transferase*** (also known as ***debranching enzyme***). ***Glucan transferase*** possess two different activities:

- ***Glucotransferase activity*:** transfers three glucose residues from the end of the branch to the second branch.
- ***Glucosidase activity*:** hydrolytically removes the last glucose molecule at branching point, cleaving α-1,6-glycosidic linkages. The glucose molecule released by glucosidase activity of ***glucan transferase*** is not in phosphorylated form [*hence theoretically it seems that muscle can add free glucose to blood stream; however, in actual the hexokinase activities in muscle converts glucose into glucose-6-phosphate, which is committed and fed into glycolysis*].

Glucose is released in form of glucose-1-phosphate, which is converted into glucose-6-phosphate in a reaction catalyzed by the enzyme ***phosphoglucomutase*.** The enzyme contains an active site *phosphoserine* residue into its active site, during its action the phosphate group from phosphoserine residue is transferred to C^6 of the glucose-1-phosphate to form *glucose- 1,6 bisphosphate* as an intermediate, at the next step the phosphate group from C^1 of the glucose- 1,6 bisphosphate is transferred to the serine residue at the active site of phosphoglucomutase to restore phosphoserine residue in the enzyme, thereby releasing *glucose-6-phosphate*. Liver, kidney contains ***glucose-6-phosphatase***, which converts it into glucose to add it freely into the blood stream. Skeletal muscles lack ***glucose-6-phosphatase***; therefore, it cannot add glucose into blood stream. Glucose-6-phosphate formed into skeletal muscles is fed into glycolytic pathway. Liver and kidneys also contain ***glucokinase/hexokinase*** which convert glucose into glucose-6-phosphate and add it into various metabolic pathways.



UNIT-6: PROTEIN METABOLISM

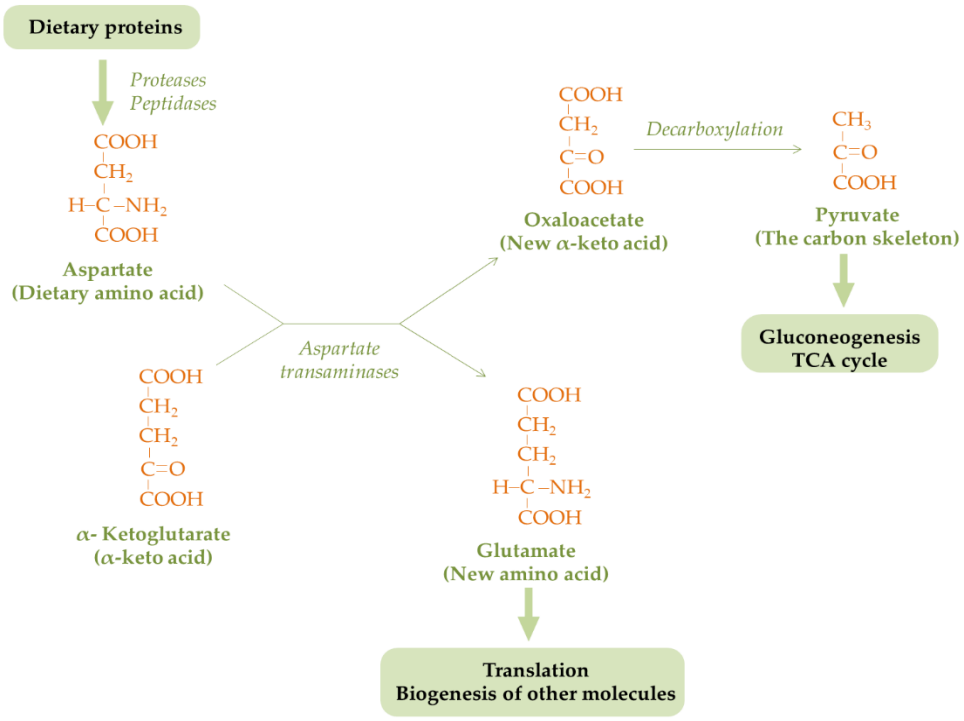
Transamination, Deamination and its types (Pathways with name of enzymes and significance). Fate of C-skeleton of Glucogenic and Ketogenic amino acids.

Q1. Describe the process of transamination with an example. (5)

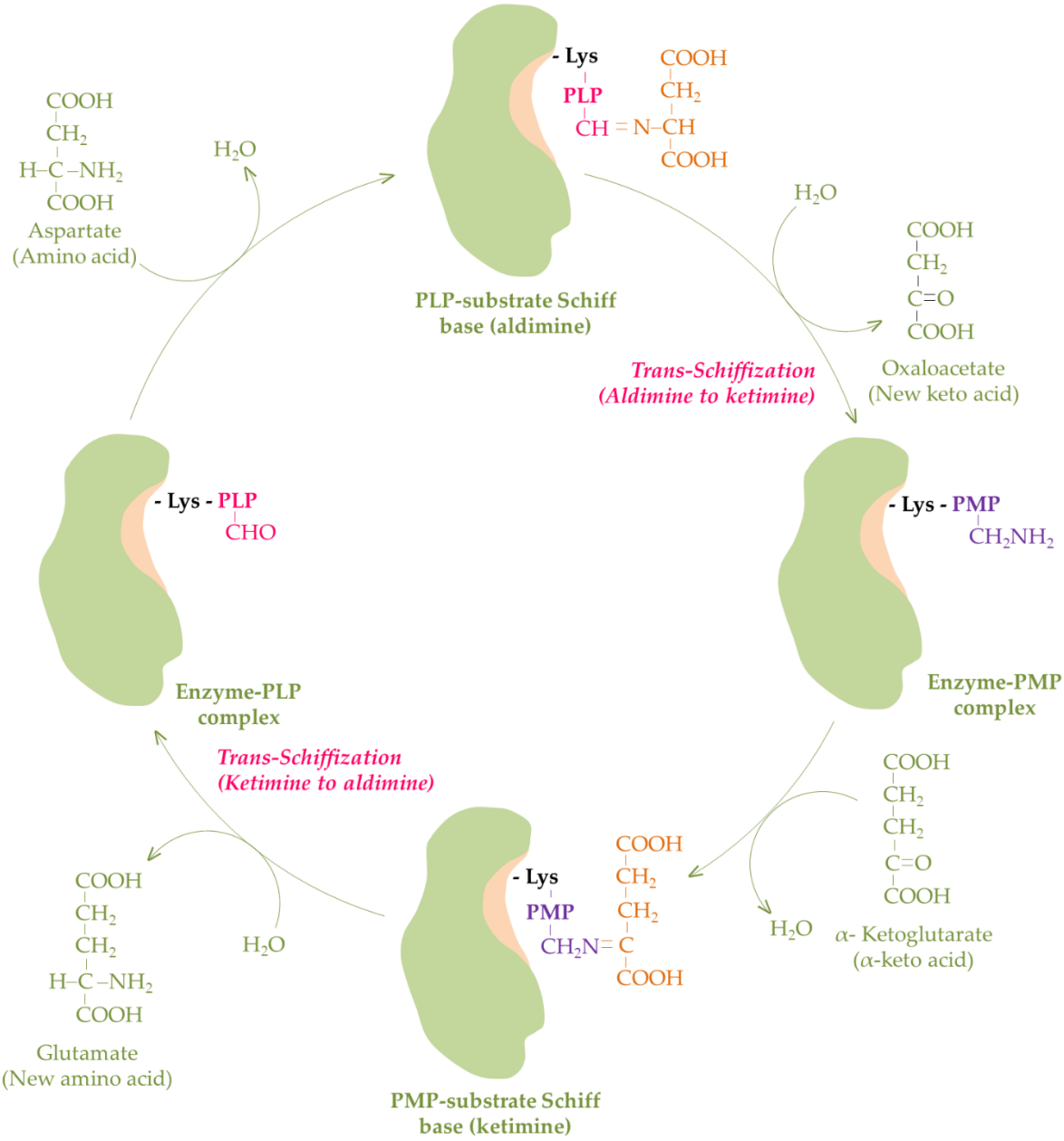
Ans: *Transamination* refers to a reversible biochemical reaction in which the α-NH_2 of one amino acid is transferred to one α-keto acid to form a new amino acid and a new keto acid. The mechanism of transamination was first described by ***Braunstein*** and ***Kritzmann.*** Although transamination is one of the mechanisms for N-catabolism of amino acids, no ammonia is released during this process. Transamination reactions are carried out by specific enzymes, called ***transaminases***. These enzymes carry ***pyridoxal phosphate*** (PLP) as the prosthetic group. PLP is attached to the active site lysine of the enzyme with its –CHO group by forming a Schiff base.

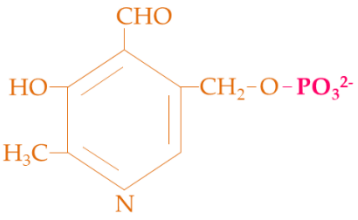
Site of the transamination: The major sites of transamination are mitochondria and cytosol of cells from liver, kidney, heart and brain. Transamination takes place in almost all tissues to little extent.

Biochemical reaction during transamination: In a typical transamination reaction the enzyme ***transaminase*** requires two substrates, *i.e.*, one α-amino acid derived from dietary proteins, which bear an amino group at its α-carbon and one α-keto acid, which bears a keto group at its α-carbon. The enzyme transaminase catalyzes transfer of the α-NH_2 group from the α-amino acid to itself first; this leads to conversion of the α-amino acid into a new α-keto acid. The α-keto acids thus produced immediately undergo further modifications, *e.g.*, ***spontaneous decarboxylation*** in order to complete the catabolism of α-amino acid derived from dietary proteins leaving the carbon skeleton, which in turn may undergo *gluconeogenesis*, giving rise to glucose or may enter into the *TCA cycle*. The enzyme containing α-NH_2 group now reacts with one α-keto acid inside the cell and transfers the α-NH_2 group to it leading to formation of a new amino acid. Such produced α-amino acid is often utilized by translational machineries for synthesis of proteins or for biosynthesis of some bioactive molecules.

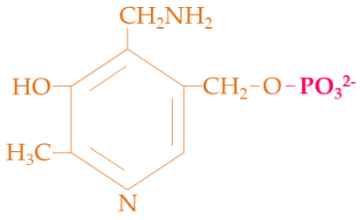


There are different transaminases identified in humans which are specific for catalyzing transamination reactions. ***Aspartate transaminase*** is specific for transfer of α-NH_2 group from aspartate (α-amino acid) to α-ketoglutarate (α-keto acid) to generate *pyruvate* as a new α-keto acid and *glutamate* as a new α-amino acid.





Pyridoxal phosphate
(PLP)



Pyridoxamine phosphate
(PMP)

Mechanism of reaction: These are ***double-displacement (ping-pong) bi-substrate reactions***. The enzyme binds with its two substrates - α-amino acid and α-keto acid at separate sites to the PLP prosthetic group.

i) Substrate α-amino acid replaces the active site lysine from the linkage and gets involved in formation of Schiff base with –CHO group of PLP (*trans-Schiffization reaction*) to form an ***aldimine*** complex. PLP is still not dislodged from the enzyme rather it remains bound to the enzyme via non-covalent linkages. Tautomerization of the ***aldimine*** into ***ketimine*** leads formation of α-keto acid, which is released hydrolytically; and PLP gets changed into PMP (***pyridoxamine phosphate***).

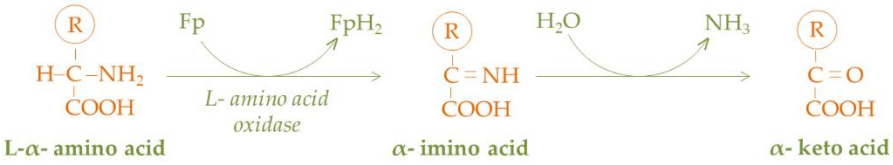
ii) Substrate α-keto acid binds to the PMP forming another *Schiff base* – ***ketimine;*** another *trans-Schiffization* reaction takes place. Tautomerization of this ***ketimine*** changes it into ***aldimine***. Now, the freed lysine residue attacks on this *Schiff base*, and then through another trans-Schiffization reaction gets involved into *Schiff base*, releasing the α-amino acid.

Limitations: Mammalian transaminases act specifically on L-α-amino acids. Amino acids like threonine, lysine, proline and hydroxyproline never take part in transamination reaction.

Q2. What is deamination of amino acids? Classify the deamination reactions. (2+3)

Ans: Deamination refers to a reversible reaction in which the α-NH_2 from amino acids is removed in form of *ammonia* (NH_3). Deamination of amino acids is found into cytosol of cells of liver and kidney. Deamination reactions are broadly categorized into two groups, *i.e., oxidative deamination* and *on-oxidative deamination*.

a) Oxidative deamination: Oxidative deamination is carried out by ***D-*** and ***L-amino acid oxidases*** (categorized based on whether they act on D- or L- amino acids), which oxidatively liberate NH_3 from amino acid substrates. The ***L-amino acid oxidases*** catalyze transfer of H^+ and e^- from the amino acids to their flavin prosthetic groups, and change the amino acids into imino acids. Transfer of H^+ and e^- from the amino acids oxidise them. The imino acids thus formed are highly reactive and unstable. Therefore, undergo hydrolytic liberation of NH_3 upon reaction with H_2O from the surrounding medium, and gets converted itself subsequently into *α-keto acid*.



b) Non-oxidative deamination: Few amino acids are deaminated *non-oxidatively* by specific enzymes, and can form NH_3. These reactions may contribute to NH_3 formation, but do not play major role in NH_3 formation. Three types of non-oxidative deamination have been described:

i) *Amino acid dehydrase mediated deamination:* The –OH containing amino acids *e.g., serine, threonine* and *homoserine* are deaminated by specific enzymes, called ***amino acid dehydrases*** which require *pyridoxal phosphates* (PLP) as co-enzymes. The enzyme catalyzes a primary dehydration followed by spontaneous deamination.

ii) *Deamination of histidine:* Histidine is non-oxidatively deaminated by the specific enzyme ***histidase*** to form NH_3 and *urocanic acid*.

iii) *Amino acid desulfhydrase mediated deamination:* Sulphur containing amino acids, *e.g., cysteine* and *homocysteine* are deaminated by a primary desulfhydration, removing H_2S, leading to formation of *imino acid* which is spontaneously hydrolysed.

Q3. What are glycogenic and ketogenic amino acids? (4)

Ans: Based on their metabolic fates, the amino acids present into body are categorized into three classes – *glycogenic, ketogenic* and *glycogenic-ketogenic amino acids*.

- *Glycogenic amino acids:* carbon skeleton of those amino acids form gluconeogenic intermediates (*e.g.,* propionate, pyruvate, oxaloacetate etc).

- ***Ketogenic amino acids:*** carbon skeleton of those amino acids form ketone bodies (*e.g.,* acetoacetate).
- ***Glycogenic-ketogenic amino acids:*** carbon skeleton of those amino acids partly form gluconeogenic intermediates and partly ketone bodies.

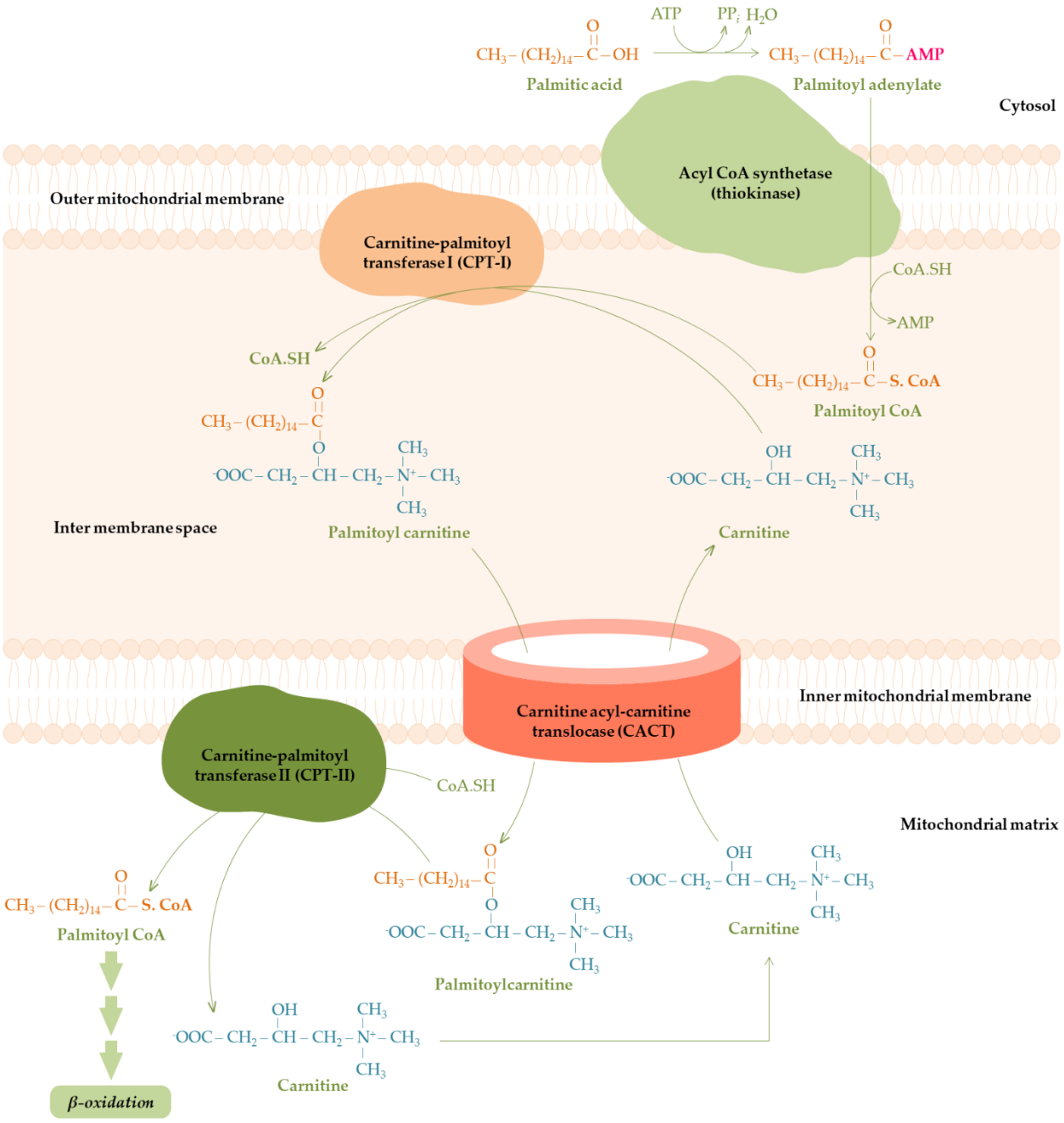
Glycogenic amino acids	Ketogenic amino acids	Glycogenic-ketogenic amino acids
• **Glycine** • **Alanine** • **Serine** • **Cysteine** • **Valine** • **Methionine** • **Glutamate** • **Aspartate** • **Histidine** • **Arginine** • **Proline** • **Hydroxyproline**	• Lysine • Leucine	• Phenyl alanine • Tyrosine • Tryptophan • Isoleucine • Threonine

UNIT-7: LIPID METABOLISM

β-oxidation of fatty acids – a. Palmitic acid {saturated(C16:0)}, b. Linoleic acid {unsaturated(C18:2)}; Fatty acid biosynthesis.

Q1. Describe the reactions of β-oxidation of a C16 saturated fatty acid (palmitic acid). (5)

Ans: Fatty acids derived from lipolysis by lipases are broken down into two carbon units (*i.e.,* acetyl CoA) into the mitochondria. Such type of oxidation might be initiated at different positions of both the saturated and unsaturated fatty acids, *i.e.,* if it begins from the C^3 or β-carbon of the linear fatty acid chain then the mechanism is referred to as ***β-oxidation***. ***Palmitic acid*** or ***hexadecenoic acid*** (**$C_{15}H_{31}COOH$**) is a 16-carbon saturated fatty acid (C16:0) which undergoes seven rounds of β-oxidation to generate eight molecules of acetyl CoA.



The whole process of β-oxidation of palmitic acid is divided into three different phases, *i.e.*, ***activation of palmitic acid, transport of palmitic acids into mitochondrial matrix*** and ***β-oxidation reactions.***

a) Activation of palmitic acid: At the first stage of β-oxidation, palmitic acid is *thioesterified* for activation into fatty palmitoyl-CoA molecules in a two-step reaction catalyzed by an enzyme fatty ***acyl CoA synthetase*** (formerly known as ***thiokinase***) located into the outer mitochondrial membrane.

- One high energy phosphoanhydride linkage of one ATP molecule is cleaved to release one molecule of pyrophosphate (PP_i) and the AMP is transferred to the –COOH end of the palmitic acid, leading to formation of a *palmitoyl-adenylate complex*. The pyrophosphate (PPi) released in this process is further cleaved into two inorganic phosphates (PO_4^{3-}) by the action of enzyme ***pyrophosphatase***.
- The enzyme *acyl CoA synthetase* next catalyzes replacement of AMP attached to the palmitoyl-adenylate with one molecule of coenzyme A (CoA-SH) and converts it into *palmitoyl-CoA* after replacement of AMP with CoA-SH.

b) Transport of fatty acids into mitochondrial matrix: Transport of palmitoyl-CoA from cytosol into mitochondrial matrix is a carrier-mediated multi-step process, which occurs into following stages:

- The palmitoyl-CoA easily gets transported through the outer mitochondrial membrane via simple passive diffusion due to its hydrophobic nature. However, the transport of palmitoyl-CoA through the inner mitochondrial membrane is assisted by a carrier molecule, ***carnitine*** (*β-hydroxy-γ-trimethyl ammonium butyrate*).
- There is a transmembrane enzyme, ***carnitine-acyl transferase I*** or ***carnitine-palmitoyl transferase I*** (**CAT-I** or **CPT-I**) situated at the outer mitochondrial membrane, which conjugates one molecule of *carnitine* with palmitoyl-CoA to form *palmitoyl-carnitine complex*, after removal of CoA.SH.
- There is a large transmembrane protein, known as ***carnitine-acylcarnitine translocase*** (**CACT**) located into the inner mitochondrial membrane, that transports *palmitoyl-carnitine* from intermembrane space into the mitochondrial matrix in exchange of one free *carnitine*. This reverse translocation is crucial for replenishment of free carnitine level into the intermembrane space.
- The ***carnitine-acyl transferase II*** or ***carnitine-palmitoyl transferase II*** (**CAT-II** or **CPT-II**) is an integral membrane protein embedded at the inner surface of inner mitochondrial membrane, which catalyzes replacement of the *carnitine* attached to the palmitoyl-carnitine with one molecule of CoA.SH, to regenerate the *palmitoyl-CoA* into the mitochondrial matrix.

c) β-oxidation reactions: After transport of palmitoyl-CoA from cytosol into mitochondrial matrix in a carrier-mediated process, it undergoes four major reactions among which two are oxidative and one is lytic reaction. The reaction sequence is as follows:

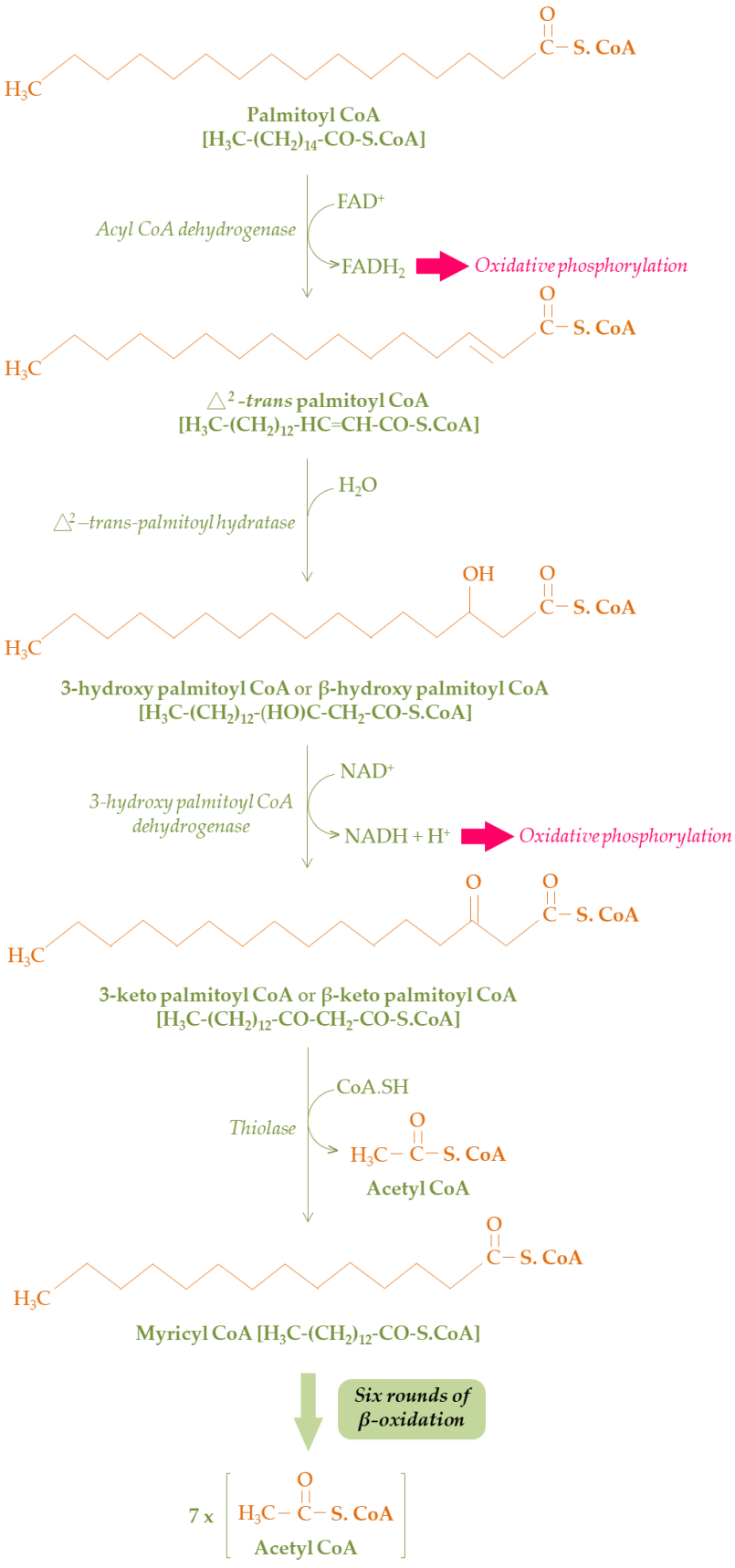
i) Into the mitochondrial matrix, palmitoyl-CoA is first oxidized in a redox reaction catalyzed by the enzyme ***acyl-CoA dehydrogenase*** to form Δ^2-*trans palmitoyl-CoA*, and generates one molecule of $FADH_2$. In this step, hydrogen atoms from C^2 and C^3 of palmitoyl CoA are transferred to FAD^+, leading to formation of a *trans* double bond between C^2 (α) and C^3 (β) positions. This is the first oxidative step of β-oxidation.

ii) Hydration of Δ^2-*trans* palmitoyl-CoA in a reaction catalyzed by the enzyme ***Δ^2-trans enoyl-CoA hydratase***, yields L-(+)3-*hydroxy palmitoyl-CoA*, with addition of one –OH group at C^3 (β) position and one H atom at the C^2 (α) position.

iii) In another oxidation reaction, catalyzed by ***3-hydroxy acyl-CoA dehydrogenase*** the L-(+)3-hydroxy palmitoyl-CoA is converted into 3-*keto palmitoyl-CoA*, after removal of two H atoms from C^3 (β) position and simultaneous generation of $NADH + H^+$. This is the second oxidative step of β-oxidation.

iv) Finally, the enzyme ***thiolase*** brings about '*thiolytic cleavage*' of 3-keto palmitoyl-CoA through addition of one CoA.SH molecule to release a two-carbon unit of *acetyl CoA* and a shorter fourteen carbon fatty acyl-CoA (*myristyl-CoA* or $C_{13}H_{27}COOH$).

Repeated oxidation of fatty acid releases one acetyl-CoA in each cycle. Since palmitic acid ($C_{15}H_{31}COOH$) is a 16-carbon saturated fatty acid (C16:0), it may which undergo maximum seven rounds of β-oxidation to generate eight molecules of acetyl CoA, seven molecules of $FADH_2$ and $NADH+H^+$ as electron donors for mitochondrial electron transport chain (mETC).



Q2. Describe the energy yield of β-oxidation of a C16 saturated fatty acid (palmitic acid). (3)

Ans: The energy production during β-oxidation relies on aerobic processes. In presence of O_2 the $FADH_2$ and $NADH+H^+$ generated at the stages of oxidation of fatty acyl-CoA into the mitochondrial matrix donate their electrons into mitochondrial ETC and generates ATP. Each of the acetyl CoA molecule enters TCA cycle when sufficient amount of O_2 is present and to generate a bulk of ATP. For example, palmitic acid is a 16-carbon saturated fatty acid; therefore, complete oxidation of it yields eight molecules of acetyl CoA (2-carbon) and requires 7 rounds of β-oxidation, and therefore 7 molecules of $FADH_2$ and 7 molecules of $NADH + H^+$ are generated.

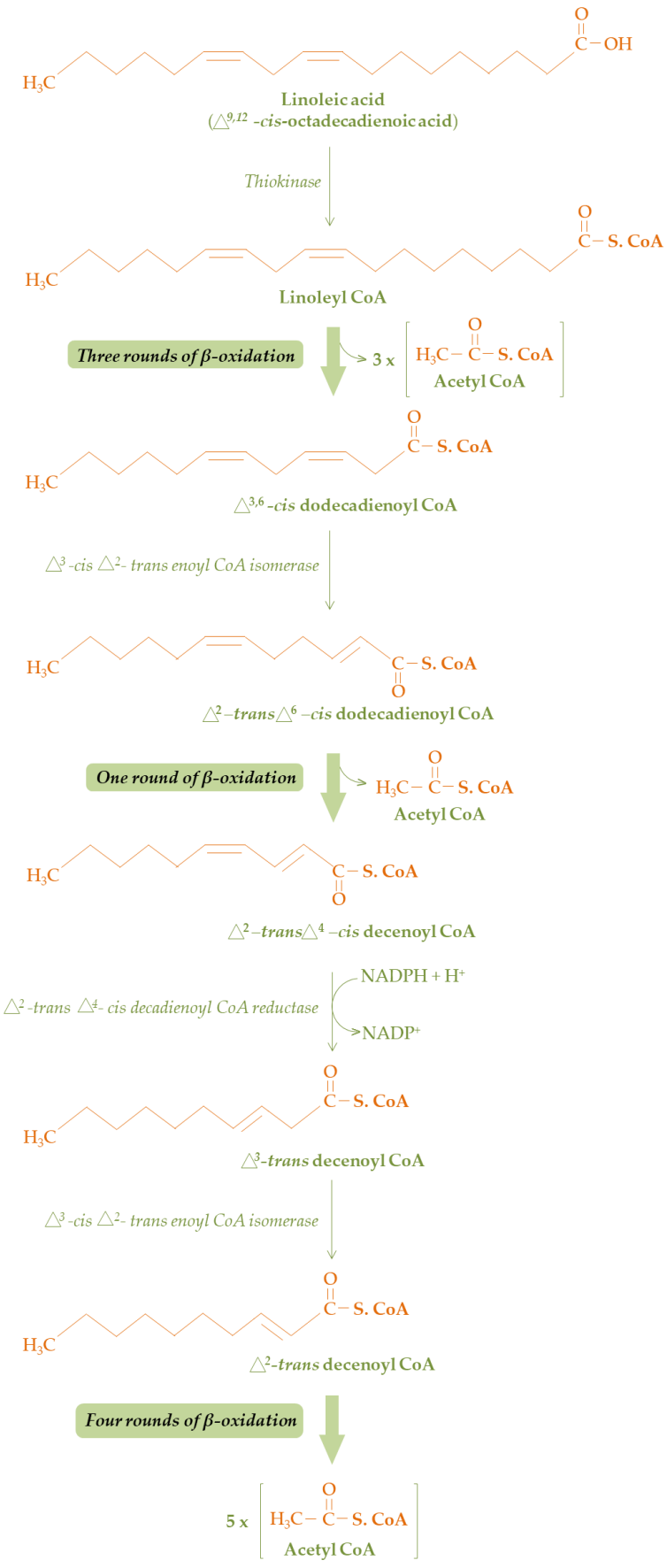
Reactions of β-oxidation of palmitic acid			**ATP production**	
			Old concept	**Modern concept**
1.	***Acyl CoA synthetase (P***almitic acid to palmitoyl-CoA) [Activation phase]	**- 2 bonds**	**- 2 ATP**	**- 2 ATP**
2.	***Acyl CoA dehydrogenase*** (palmitoyl CoA to Δ^{2-} *trans* palmitoyl-CoA) [1st oxidative stage]	**7 $FADH_2$**	**+ 14 ATP**	**+ 10.5 ATP**
3.	***3-hydroxy acyl CoA dehydrogenase*** (3-hydroxy palmitoyl-CoA to 3-keto palmitoyl-CoA) [2nd oxidation stage]	**7 NADH + 7 H^+**	**+ 21 ATP**	**+ 17.5 ATP**
4.	***Acetyl CoA enters TCA cycle***	**8 rounds**	**+ 96 ATP**	**+ 80 ATP**
		Net gain	**= (131 - 2) = 129 ATP**	**= (108 - 2) = 106 ATP**

Q3. Discuss about the β-oxidation of linoleic acid. (6)

Ans: Linoleic acid ($\Delta^{9,12}$-*cis*-octadecadienoic acid) is a 18-carbon polyunsaturated fatty acid. It contains two *cis*-double bonds between C^9-C^{10} and C^{12}-C^{13}. Linoleic acid undergoes β-oxidation mainly into the mitochondrial matrix, after activation in the cytosol or outer mitochondrial membrane and transport through the ***carnitine shuttle***. Before β-oxidation begins, linoleic acid is first activated into *linoleyl-CoA*. This reaction occurs at the cytosolic side of outer mitochondrial membrane and is catalysed by ***acyl-CoA synthetase*** or ***thiokinase***. In this reaction, linoleic acid reacts with one molecule of CoA-SH and ATP to form *linoleyl-CoA*, AMP and PPi. The activated linoleyl-CoA is then transported into the mitochondrial matrix via carnitine shuttle, which involves ***CPT-I***, ***carnitine-acylcarnitine translocase*** and **CPT-II**.

Into the mitochondrial matrix, linoleyl-CoA undergoes several rounds of ordinary β-oxidation. In the first round of β-oxidation, linoleyl-CoA is acted upon by ***acyl-CoA dehydrogenase***, which produces a *trans*-double bond between the α (C^2) and β (C^3) carbons, thereby leading to formation of Δ^2-*trans*-$\Delta^{9,12}$-*cis-octadecatrienoyl-CoA*. In this step, one molecule of FAD^+ is reduced into $FADH_2$. At the next step, ***enoyl-CoA hydratase*** adds water across the Δ^2-*trans* double bond of the former to convert it into L-(+)-β-*hydroxy linoleyl-CoA*. Thus produced L-(+)-β-hydroxy linoleyl-CoA is oxidized by ***β-hydroxyacyl-CoA dehydrogenase***, using NAD^+, to form β-*keto linoleyl CoA* and $NADH+H^+$. Finally, the enzyme ***thiolase*** cleaves the β-keto linoleyl-CoA with addition of one molecule of CoA-SH, releasing one molecule of *acetyl-CoA* and producing a shorter (16-carbon) unsaturated acyl-CoA, $\Delta^{7,10}$-*cis-hexadecadienoyl-CoA*. This molecule undergoes another ordinary round of β-oxidation into the mitochondrial matrix. After this round, another *acetyl-CoA* is released producing a 14-carbon molecule, $\Delta^{5,8}$-*cis-tetradecadienoyl-CoA*. A further ordinary β-oxidation step removes another two-carbon unit as acetyl-CoA, leaving behind a 12-carbon chain of $\Delta^{3,6}$-*cis-dodecadienoyl-CoA*.

At this point, normal β-oxidation cannot proceed directly because the double bond is in the Δ^3-*cis* position, whereas β-oxidation requires a Δ^2-*trans*-enoyl-CoA intermediate. Therefore, the enzyme ***Δ^3-cis, Δ^2-trans-enoyl-CoA isomerase*** converts $\Delta^{3,6}$-*cis*-dodecadienoyl-CoA into Δ^2-*trans*, Δ^6-cis-*dodecadienoyl-CoA*. This reaction bypasses the ***acyl-CoA dehydrogenase*** step, so $FADH_2$ is not produced in this round.



The newly formed Δ^2-*trans*, Δ^6-*cis*-dodecadienoyl-CoA immediately undergoes another round of β-oxidation to produce a shorter chain of 10-carbon molecule called Δ^2-*trans*, Δ^4-*cis-decadienoyl-CoA*. The Δ^2-*trans*, Δ^4-*cis*-decadienoyl-CoA is reduced by the enzyme ***Δ^2-trans, Δ^4-cis-decadienoyl-CoA reductase***, using NADPH + H^+, to produce Δ^3-*trans-decenoyl-CoA*, which is in turn isomerized into Δ^2-*trans-decenoyl-CoA* by the enzyme ***Δ^3-trans, Δ^2-trans-enoyl-CoA isomerase***.

After this correction, the molecule again follows the normal β-oxidation pathway, *i.e.*, four more β-oxidation cycles take place to produce five acetyl CoA molecule. Thus, complete β-oxidation of linoleic acid finally produces 9 molecules of acetyl-CoA. Compared with its saturated isoform, stearic acid, the β-oxidation of linoleic acid yields less energy because two FAD-dependent dehydrogenation steps are bypassed and one NADPH is consumed during reduction.

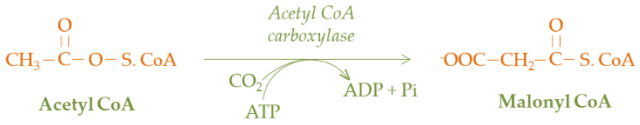
Q4. Discuss about the bio-synthesis of fatty acids with special reference to the role of fatty acid synthases. (6)

Ans: Biosynthesis of fatty acids is not just the reversal of fatty acid oxidation; but is a separate biochemical process. The default process of fatty acid biosynthesis can produce upto 16C chains, *i.e.*, palmitic acid. Therefore, the production of fatty acids with longer chain requires separate elongation process.

Site of fatty acid biosynthesis: Cytoplasm (*extramitochondrial system*) and microsomes of the different cells in body.

Initiation of biosynthesis of fatty acids: The raw materials for the biosynthesis of fatty acid are acetyl CoA (2C units). The first stage in fatty acid biosynthesis is production of malonyl CoA from carboxylation acetyl CoA. The reaction is a two-step process catalyzed by '***acetyl CoA carboxylase***' enzyme complex which requires biotin as cofactor. The acetyl CoA carboxylase system has two separate enzymes, *i.e.*, ***biotin carboxylase*** and ***carboxyl transferase;*** along with a peptide which carries biotin (***biotin carrier protein*** or ***BCP***).

- The ***biotin carboxylase*** fraction of the enzyme complex transfers one CO_2 from HCO_3^- to the biotin attached with BCP. The reaction is endergonic and requires energy released from hydrolysis of one molecule of ATP.
- The ***carboxyl transferase*** fraction then transfers one molecule of acetyl CoA to the carboxylated biotin and forms malonyl CoA which is released immediately from the enzyme complex.

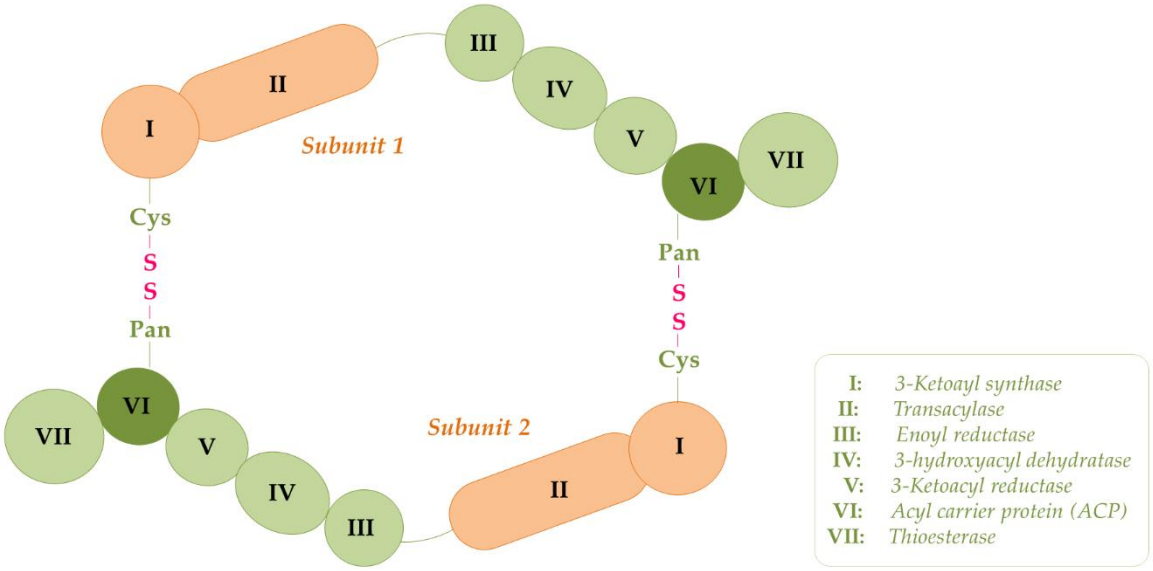


This reaction is completely irreversible and can be termed '***CO_2 fixation mechanism***' in animals. This is a rate limiting step in fatty acid biogenesis.

Different stages of extramitochondrial synthesis of fatty acids: All the biochemical reactions involved in fatty acid biosynthesis is carried out by a single '***multienzyme complex***' present in cytoplasm, known as '***fatty acid synthase***' which has different enzymatic fractions. The fatty acid synthase multienzyme complex is a homodimer of two separate subunit divisions. Both of these subunit divisions are composed of six enzymatic subunits and one non-enzymatic subunit. The names of different enzymatic subunits are as follows -

i) *Subunit I:* ***3-ketoacyl synthase***
ii) *Subunit II:* ***Transacylase***
iii) *Subunit III:* ***Enoyl reductase***
iv) *Subunit IV:* ***3-hydroxyacyl dehydratase***
v) *Subunit V:* ***3-ketoacyl reductase***
vi) *Subunit VII:* ***Thioesterase***

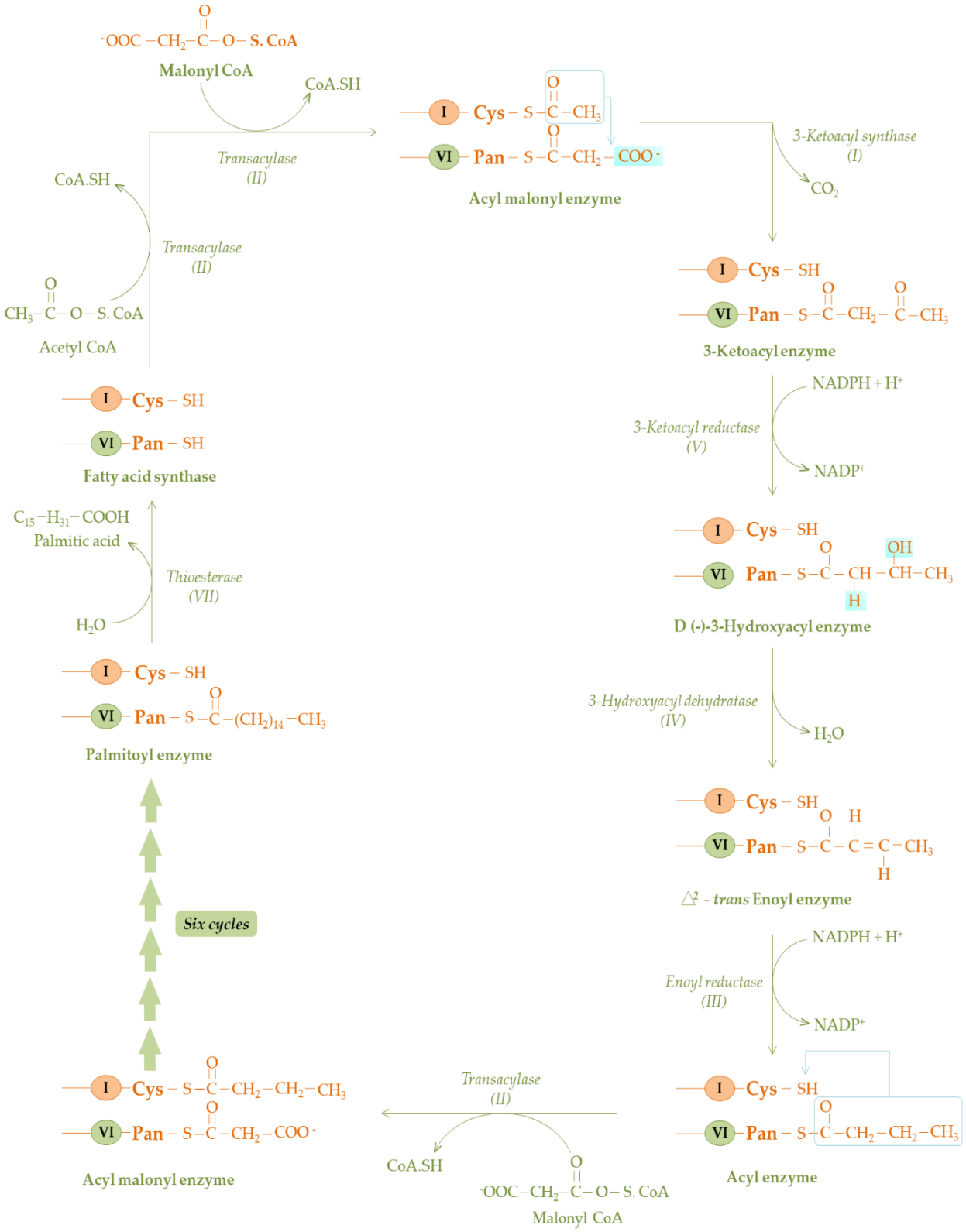
The subunit VI is the ***acyl carrier protein*** (***ACP***) which contains 4′-phosphopantetheine residues. The subunit I contains cysteine residue. Both of these 4′-phosphopantetheine and cysteine are thiol group (-SH) containing fractions. Formation of disulphide linkages between subunit I from one subunit division and subunit VI of the opposite subunit division stabilize the homodimer conformation of FA synthase.



The raw materials for FA synthase action are malonyl CoA and acetyl CoA molecules in cytoplasm.

- The enzyme ***transacylase*** (***subunit II***) transfers one acetyl group (-$COCH_3$) from acetyl CoA molecule to the cysteine residue of subunit I via releasing one CoASH. The same enzyme also transfers one malonyl group (-CO-CH_2-COO^-) to the 4′-phosphopantetheine of the ACP (subunit VI) with simultaneous release of one CoASH. The resultant product is called *acyl-malonyl enzyme*. This reaction regenerates the thiol (-SH) group at the cysteine residue attached to the subunit I.
- The next step is transfer of the acetyl group to the C^2 of the malonyl group with subsequent release of one CO_2 molecule. This decarboxylation is thermodynamically important as it favours the attachment of acetyl group to the C^2 of malonyl group. This reaction is catalyzed by the enzyme ***3-ketoacyl synthase*** (***subunit I***) and it forms *3-ketoacyl enzyme*.
- The 3-ketoacyl enzyme is then reduced at the C^2 and C^3 positions of ketoacyl moiety attached to the 4′-phosphopantetheine residue of ACP to form *D-(-)-3 hydroxyacyl enzyme* in a reaction catalyzed by the enzyme ***3-ketoacyl reductase*** (***subunit V***). The enzyme transfers two hydrogen atoms from NADPH + H^+ to its substrate.
- Thus produced D-(-)-3 hydroxyacyl enzyme is dehydrated with removal of one H from the C^2 and one –OH group from the C^3 position of the acyl moiety of the latter as H_2O in a reaction catalyzed by the enzyme ***3-hydroxyacyl dehydratase*** (***subunit IV***). This dehydration of the D (-) 3-hydroxyacyl enzyme generates a double bond between C^2 and C^3 positions of the acyl group giving rise to Δ^2-*transenoyl enzyme*.
- The Δ^2-transenoyl enzyme thus formed is reduced at its C^2 and C^3 positions and the acyl moiety is transferred to the –SH group of cysteine residue of subunit I in a reaction catalyzed by the enzyme ***enoyl reductase*** (***subunit III***) to generate *acyl enzyme*. The enzyme transfers two H atoms from NADPH + H^+ to reduce its substrate. This reaction regenerates the –SH group at 4′-phosphopantetheine residue of ACP.
- The ***transacylase*** fraction of FA synthase transfers malonyl (-CO-CH_2-COO^-) group again to the –SH group of 4′-phosphopantetheine residue attached to ACP with concomitant release of one coenzyme A molecule (CoASH) leading to formation of *acyl-malonyl enzyme* once again and ***3-ketoacyl synthase*** comes into action once more. This repeats the cycle again and again. At the end of each cycle the chain length attached to the 4′-phosphopantetheine residue of ACP increases by two carbon unit. The cycles continue until the chain length achieves sixteen carbons (palmitoyl moiety).
- The C^{16} acyl (palmitoyl) moiety from 4′-phosphopantetheine residue of ACP is then transferred to one water molecule instead of the cysteine residue of subunit I by the enzyme ***thioesterase*** (***subunit VII***) releasing palmitic acid and regenerating the FA synthase again.

Thus, fatty acid biosynthesis is a highly coordinated anabolic process that plays vital role in energy storage and cellular metabolism. Beginning with acetyl CoA and proceeding through a series of enzyme-catalyzed reactions, it leads to the formation of long-chain fatty acids essential for membrane structural integrity and metabolic functions.



UNIT-8: NUCLEIC ACID METABOLISM

Degradation of purine; Purine Salvage pathway and significance.

Q1. Discuss how purines are degraded. (5)

Ans: Purine nucleotides are continuously synthesized and degraded into the body as part of regular *nucleic acid turnover*. This degradation of purines is an important metabolic process through which *adenine* and *guanine* nucleotides are broken down into simpler compounds that can be excreted from the body. In humans, the final product of purine catabolism is ***uric acid***, whereas in many lower animals, uric acid undergoes further degradation to ***allantoin***, ***allantoic acid***, ***urea***, or ***ammonia***.

Site of purine degradation: Purine degradation occurs mainly in the *liver* and *intestinal mucosa*.

Purine degradation pathway involves a sequence of dephosphorylation, deamination, phosphorolysis, and oxidation reactions catalyzed by specific enzymes. The principal purine nucleotides undergoing degradation are *adenosine monophosphate* (AMP) and *guanosine monophosphate* (GMP).

a) Degradation of adenosine monophosphate: The degradation of AMP may proceed into two alternative pathways.

i) In one pathway, AMP is first deaminated to *IMP*, whereas in the second pathway AMP is first converted to *adenosine*. The first step in this pathway is the deamination of AMP. A

ii) MP reacts with water in the presence of the enzyme ***AMP deaminase***, resulting in the removal of the amino group as ammonia and formation of *inosine monophosphate* (IMP). IMP formed in this reaction serves as an intermediate in both purine synthesis and degradation.

iii) IMP is then hydrolyzed by the enzyme ***5'-nucleotidase***. During this reaction, the phosphate group attached to IMP is removed, producing the nucleoside *inosine* and inorganic phosphate. This reaction represents the dephosphorylation stage of purine degradation.

iv) In an alternative pathway, AMP is first converted into *adenosine*. AMP is hydrolyzed by the enzyme ***5'-nucleotidase***. The phosphate group is removed to form adenosine. Adenosine is an important nucleoside involved in several physiological functions.

v) Adenosine undergoes deamination in the presence of ***adenosine deaminase*** (**ADA**) to produce inosine. The amino group is removed as ammonia, producing inosine. Adenosine deaminase is clinically important because deficiency of this enzyme leads to *severe combined immunodeficiency* (SCID).

vi) Inosine then undergoes hydrolytic cleavage catalyzed by ***nucleosidase***. Ribose is removed from inosine, producing the free purine base *hypoxanthine*. Hypoxanthine is an important intermediate that may either be salvaged back into IMP or undergo further degradation.

vii) Hypoxanthine is oxidized to *xanthine* by the enzyme ***xanthine oxidase***. Molecular oxygen acts as the electron acceptor in this reaction. Hydrogen peroxide is generated as a by-product during this oxidation reaction.

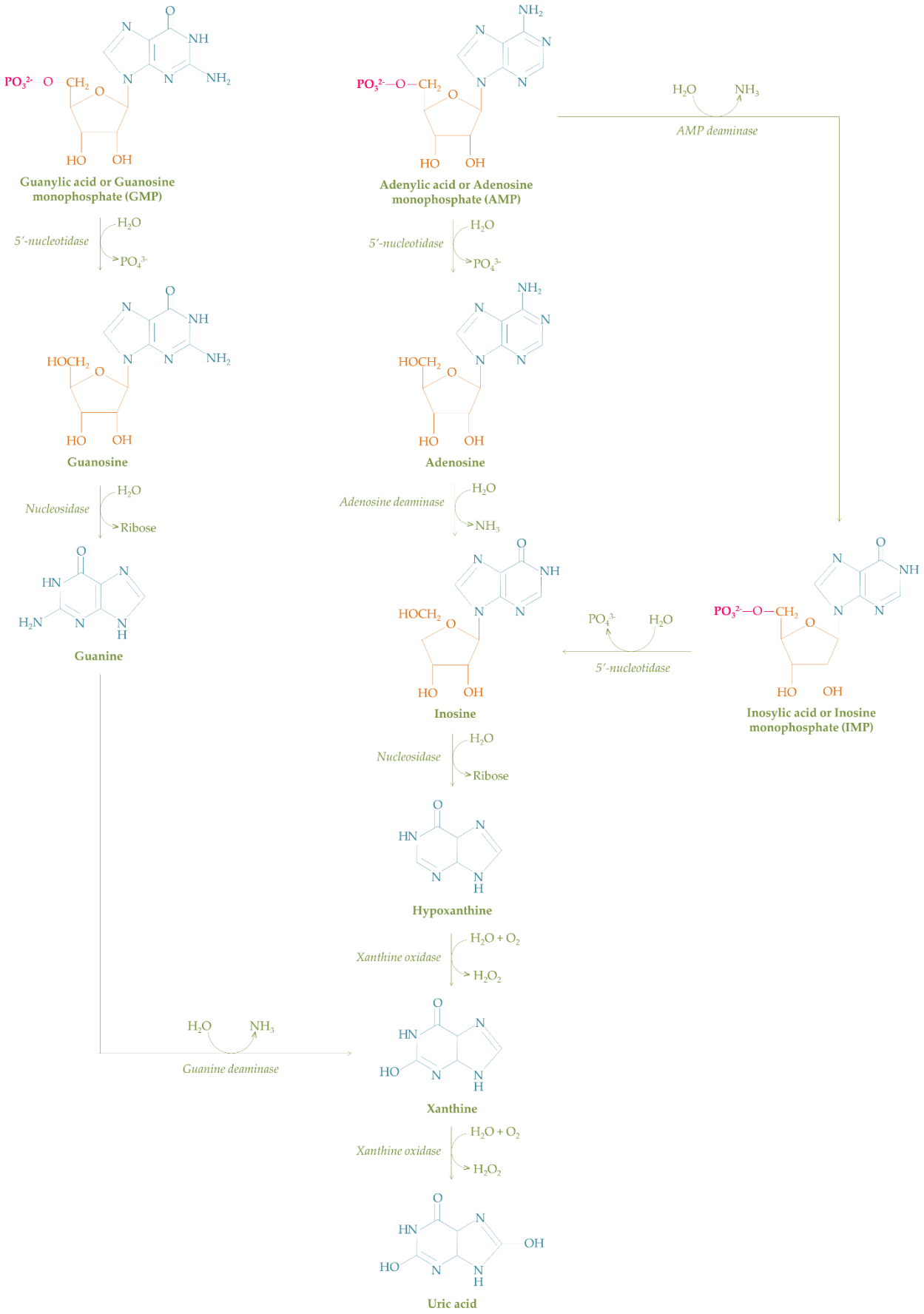
b) Degradation of guanosine monophosphate: The degradation of GMP proceeds through *guanosine* and *guanine* formation before formation of *uric acid*.

i) GMP undergoes dephosphorylation by the enzyme ***5'-nucleotidase***, producing *guanosine*.

ii) Guanosine is then cleaved by purine ***nucleosidase***. Ribose is removed and the free purine base *guanine* is produced.

iii) Guanine undergoes deamination reaction catalyzed by the enzyme ***guanine deaminase***, also known as ***guanase***, to produce *xanthine*.

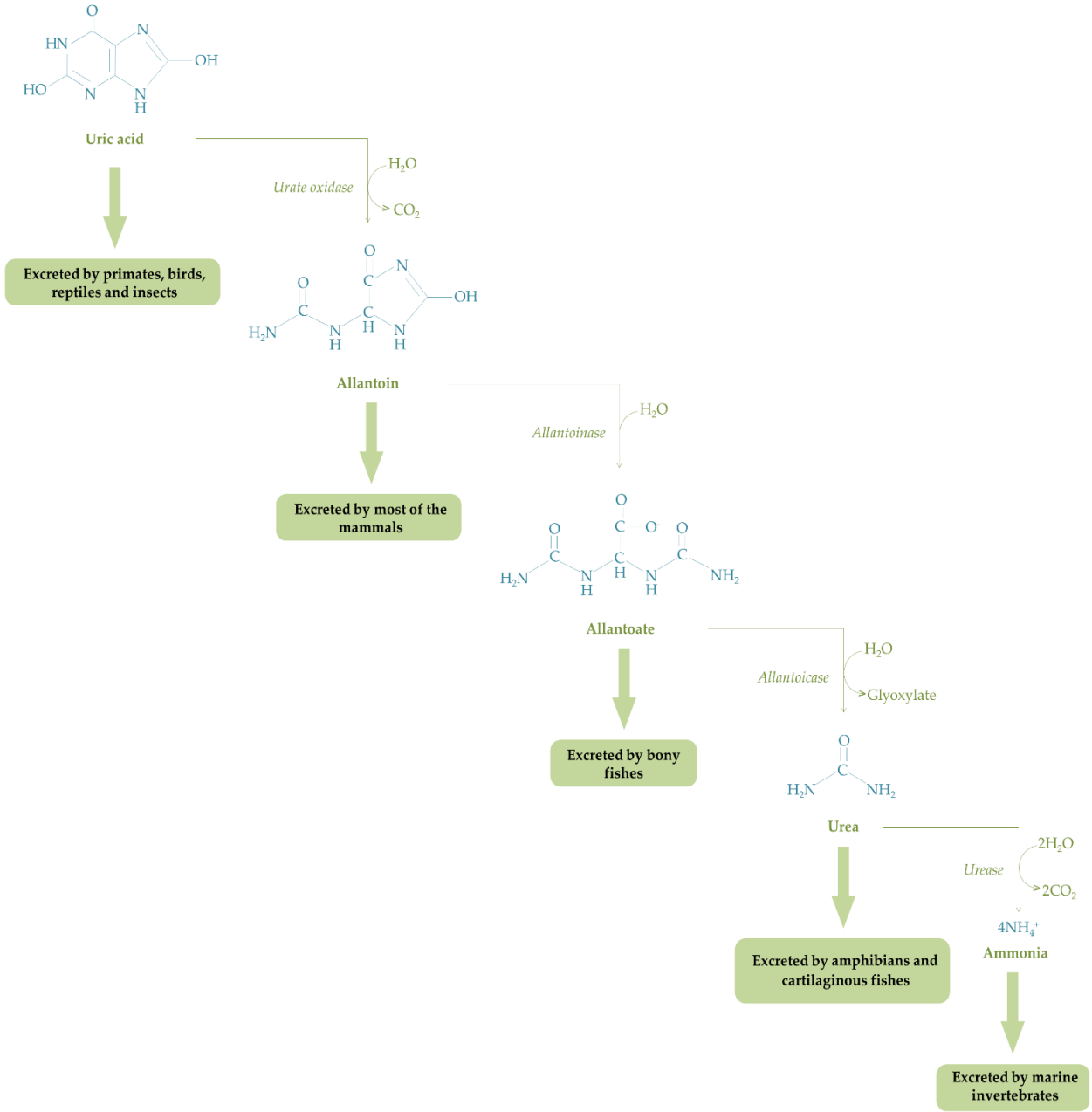
c) Formation of uric acid: Xanthine undergoes a second oxidation reaction catalyzed by ***xanthine oxidase*** to form *uric acid*. Uric acid is the final product of purine degradation in humans and higher primates because these organisms lack the enzyme *uricase*. However, other fates of uric acid into human are also reported, *i.e.*, uric acid may be converted into *allantoin*. Thus, both adenine and guanine nucleotides ultimately converge at xanthine and are finally converted into uric acid.



Q2. Discuss about the fate of uric acid produced during purine degradation. (4)

Ans: At the final stage of purine degradation xanthine is oxidized by ***xanthine oxidase*** to produce *uric acid*. In humans and higher primates, uric acid is excreted directly because ***uricase*** is absent. However, in many lower animals, uric acid undergoes further degradation.

i) ***Formation of allantoin:*** In most mammals, uric acid is oxidized by ***urate oxidase*** or ***uricase*** to produce *allantoin*. Allantoin is more soluble in water than uric acid and is readily excreted.
ii) ***Formation of allantoic acid or allantoate:*** In certain fishes, allantoin is hydrolyzed by ***allantoinase*** to form *allantoic acid*.
iii) ***Formation of urea:*** In amphibians and some fishes, allantoic acid is converted into *urea* in a reaction catalyzed by ***allantoicase*** which hydrolytically removes one molecule of *glyoxylate* from allantoic acid.
iv) ***Formation of ammonia:*** In marine invertebrates, urea may finally be hydrolyzed by ***urease*** to form ammonia and carbon dioxide.



Q3. State the differences between '*de novo*' and '*salvage*' pathway of purine or pyrimidine biosynthesis. (4)

Ans: Synthesis of purine and pyrimidine nucleotides are prerequisite for proper functioning of cells, and it occurs into two different biosynthetic anabolic pathways, *i.e.*, '*de novo*' and '*salvage*' pathways.

	De novo synthesis	Salvage synthesis
Definition	Synthesis of purine or pyrimidine from simple precursor molecules.	Reuse of purine or pyrimidines formed from degradation of nucleic acids to synthesize new nucleotides.
Starting materials	Amino acids, CO_2, formyl groups, ribose-5-phosphate.	Free purine bases like adenine, guanine and hypoxanthine.
Energy requirement	Requires large amount of energy (ATP).	Requires much lesser amount of energy.
Main purpose	Produces new purine or pyrimidine nucleotides when supply is insufficient.	Energy conservation via recycling of purine or pyrimidine nucleotides.
Initial substrate	5′-phosphoribosyl-1-pyrophosphate (PRPP).	5′-phosphoribosyl-1-pyrophosphate (PRPP), free purine and pyrimidine bases.
Major end product	Inosine monophosphate (IMP).	Adenosine monophosphate (AMP) or guanosine monophosphate (GMP).
Regulation	Highly regulated feedback inhibition by AMP or GMP.	Less complex regulation.
Site	Liver and proliferating cells.	Most of the tissues, especially brain.

Q4. Describe the '*salvage*' pathway of purine biosynthesis and state its significance. (4+2)

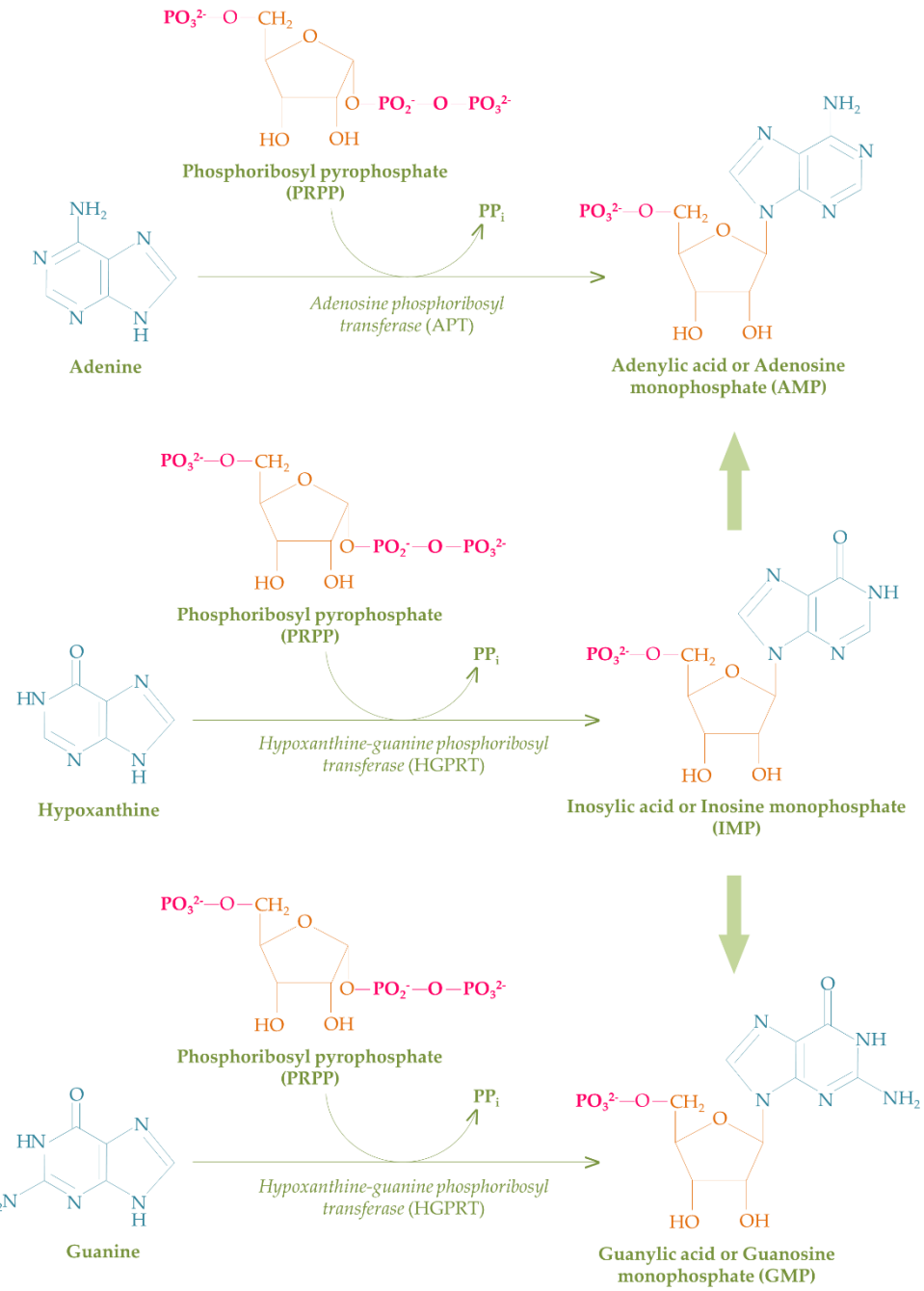
Ans: ***The salvage pathway of purine biosynthesis refers to the metabolic pathway by which free purine bases or purine nucleosides are converted back into purine nucleotides for reutilization by the cell.*** Although purines can be synthesized *de novo*, this process is energetically expensive because it requires several ATP-dependent reactions. To conserve energy, many cells utilize the salvage pathway, in which free purine bases and nucleosides formed during nucleic acid turnover are reutilized to synthesize purine nucleotides. The salvage pathway is particularly important in tissues that have limited capacity for de novo purine synthesis, such as *brain tissue*, *erythrocytes* (RBCs), *leukocytes* and *lymphoid tissue*. These tissues depend heavily on recycling preformed purine bases. Purine salvage occurs mainly by two mechanisms, *i.e.*, one-step salvage synthesis and two-step salvage synthesis.

a) One-step salvage synthesis: In the one-step salvage pathway, a free purine base directly combines with *phosphoribosyl pyrophosphate* (PRPP) to form the corresponding purine nucleotide. The reaction is catalyzed by enzymes known as ***phosphoribosyl transferases***. During this reaction, the *ribose-5-phosphate* moiety from PRPP is transferred to the purine base, while *pyrophosphate* (PP_i) is liberated. Hydrolysis of pyrophosphate makes the reaction essentially irreversible and energetically favorable. This pathway is the principal mechanism of purine salvage in mammalian tissues.

i) *Salvage of adenine:* Free *adenine* produced during nucleic acid turnover reacts with PRPP in the presence of the enzyme ***adenine phosphoribosyl transferase*** (**APRT**). In this reaction, adenine receives the activated ribose phosphate group from PRPP and is converted into *adenosine monophosphate* (AMP), also known as *adenylic acid*. APRT is highly specific for adenine and plays an important role in maintaining the intracellular AMP pool.

ii) *Salvage of hypoxanthine:* Hypoxanthine is salvaged by reacting with PRPP in the presence of the enzyme ***hypoxanthine-guanine phosphoribosyl transferase*** (**HGPRT**). The product formed is *inosine monophosphate* (IMP) or *inosylic acid*, which occupies a central position in purine metabolism. IMP subsequently serves as a precursor for the synthesis of both AMP and GMP according to cellular requirements.

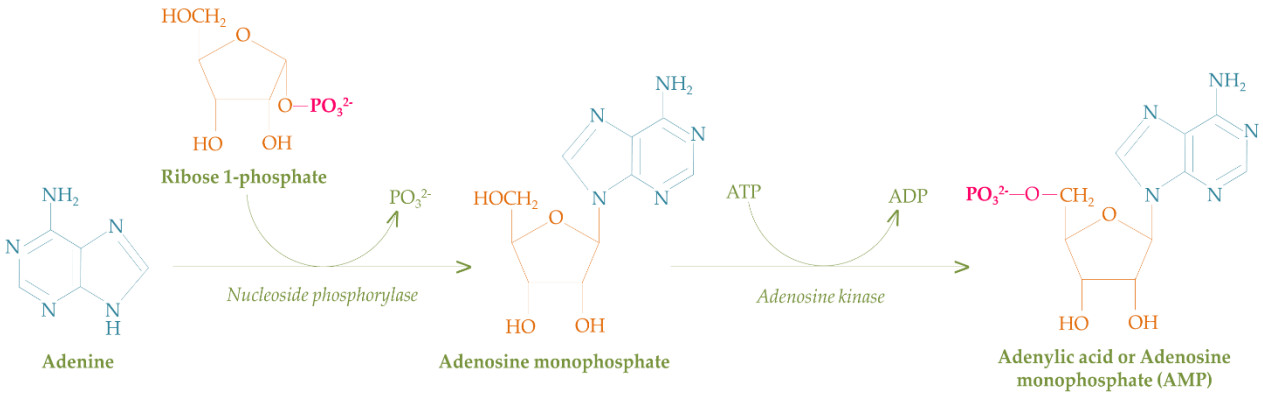
iii) *Salvage of guanine:* Guanine is also salvaged through the action of **HGPRT**. The nucleotide produced is *guanosine monophosphate* (GMP) or *guanylic acid*. Thus, HGPRT participates in the salvage of both hypoxanthine and guanine.



b) Two-step salvage synthesis: In certain tissues and under special metabolic conditions, purine nucleotides may also be synthesized from purine nucleosides through a two-step mechanism. This pathway involves conversion of nucleoside to free purine base and conversion of free base to nucleotide.

i) ***Step 1: Conversion of nucleoside to free base:*** Purine nucleosides are first ribosylated by the enzyme ***nucleoside phosphorylase*** in the presence of *ribose-1-phosphate*. For example, adenine undergoes ribosylation to yield *adenosine monophosphate* and *inorganic phosphate* (PO_4^{3-}) in a reversible reaction. However, under physiological conditions, the equilibrium usually favors nucleoside breakdown. The liberated *ribose-1-phosphate* can enter carbohydrate metabolic pathways.

ii) ***Step 2: Conversion of free base to nucleotide:*** The purine nucleosides formed in the first step are phosphorylated by kinases, *e.g.*, adenosine is phosphorylated by ***adenosine kinases*** to form *adenosine monophosphate* (AMP) or *adenylic acid*.



Significance of salvage pathway: Salvage synthesis of purines has profound physiological importances.

i) ***Energy conserving pathway:*** Recycling purines requires much less energy than de novo synthesis.
ii) ***Maintains nucleotide pool:*** Helps maintain adequate intracellular concentrations of AMP, GMP, and IMP.
iii) ***Essential in certain tissues:*** Some tissues lack sufficient *de novo* synthetic enzymes and therefore rely mainly on salvage reactions.
iv) ***Reduces purine degradation:*** Salvaging purines prevents excessive formation of uric acid.
v) ***Rapid supply of nucelotides:*** Supplies nucleotides rapidly to tissues with limited de novo synthesis.

Q5. State the role of PRPP in salvage pathways. (2)

Ans: The *5′-phosphoribosyl-1-pyrophopshate* or PRPP acts as the '*activated ribose phosphate donor*' in all salvage reactions. It is synthesized from ribose-5-phosphate derived from the hexose monophosphate (HMP) shunt pathway. The high-energy pyrophosphate bond present in PRPP provides the driving force necessary for nucleotide formation.

UNIT-9: FREE RADICALS AND ANTIOXIDANTS

Concepts of free radicals and antioxidants with examples.

Q1. What are free radicals? (2)

Ans: ***A free radical is an atom or molecule containing one or more unpaired electrons in its outer orbital.*** Because of the presence of unpaired electrons, free radicals are highly unstable, reactive, and short-lived. Examples include superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (OH•), hydroperoxy radical (HOO•), nitric oxide (NO•) etc. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) together constitute the major free radicals in biological systems.

Q2. What are reactive oxygen species? (2)

Ans: ***Reactive oxygen species (ROS) may be defined as chemically reactive derivatives of oxygen that are capable of oxidizing cellular components and producing oxidative damage***. These species are characterized by high chemical reactivity due to the presence of unpaired electrons or their ability to generate free radicals. Reactive oxygen species are broadly classified into *free radical ROS* (*e.g.*, $O_2^{\bullet -}$, OH• etc.) and *non-radical ROS* (*e.g.*, H_2O_2, $^1O_2$, HOCl and O_3).

Q3. Describe the sequential formation of free radicals? (4)

Ans: Free radicals are formed in a sequence of reactions.

i) ***Formation of superoxide anion:*** Molecular oxygen undergoes univalent reduction by accepting one electron to form superoxide anion. *Superoxide anion* is one of the primary free radicals produced in cells.

$$O_2 + e^- = O_2^{\bullet -}$$

ii) ***Formation of hydrogen peroxide:*** *Superoxide anion* can accept electrons and hydrogen ions to produce hydrogen peroxide. Hydrogen peroxide itself is not a free radical, but it is highly reactive and toxic.

$$2O_2^{\bullet -} + 2H^+ = H_2O_2 + O_2$$

iii) ***Formation of hydroxyl radical (Haber–Weiss/Fenton reaction):*** *Hydrogen peroxide* reacts with superoxide anion in the presence of Fe^{2+} to generate *hydroxyl radicals*. This reaction is known as the ***Haber–Weiss reaction*** or ***Haber–Weiss–Fenton reaction***. The hydroxyl radical is the most reactive and destructive oxygen radical known in biological systems.

$$O_2^{\bullet -} + H_2O_2 = OH^{\bullet} + OH^- + {}^1O_2$$

iv) ***Formation of hydroperoxy radical:*** *Superoxide anion* can accept a proton to form hydroperoxy radical. Hydroperoxy radicals can initiate lipid peroxidation in membranes.

$$O_2^{\bullet -} + H^+ = HOO^{\bullet}$$

v) ***Nitric oxide and peroxynitrite formation:*** *Nitric oxide* (NO•), produced from arginine by ***nitric oxide synthase***, reacts with *superoxide anion* to form peroxynitrite. Peroxynitrite decomposes to highly toxic radicals capable of damaging biomolecules.

$$O_2^{\bullet -} + NO^{\bullet} = ONOO^-$$

Q4. Why oxygen readily forms free radicals? (2)

Ans: Oxygen is essential for the survival of aerobic organisms because it participates in cellular respiration and energy production. However, during normal metabolic processes, oxygen may undergo partial reduction leading to

the formation of highly reactive chemical species known as free radicals. These reactive molecules can damage cellular structures such as lipids, proteins, carbohydrates, and nucleic acids. To protect the body from such injury, organisms possess a defense system composed of antioxidants and antioxidant enzymes. Molecular oxygen is paramagnetic because it possesses two unpaired electrons with parallel spins. Reduction of oxygen therefore occurs by the addition of single electrons one at a time rather than by simultaneous transfer of electron pairs. This favors formation of free radical intermediates.

Q5. Discuss about the sources of free radicals. (3)

Ans: Free radicals are generated both physiologically and pathologically.

i) ***Mitochondrial respiratory chain:*** Leakage of electrons from the electron transport chain during oxidative phosphorylation leads to superoxide formation.
ii) ***Enzymatic oxidation reactions:*** Several oxidase enzymes produce ROS during metabolism, including xanthine oxidase, aldehyde oxidase, L-amino acid oxidase
iii) ***Cytochrome P-450 system:*** Hydroxylation reactions involved in metabolism of drugs, steroids, and xenobiotics generate free radicals.
iv) ***Respiratory burst in phagocytes:*** Activated neutrophils and macrophages generate superoxide radicals through NADPH oxidase during microbial killing.
v) ***Ionizing radiation:*** Radiation causes splitting of water molecules producing hydroxyl radicals.
vi) ***Autooxidation reactions:*** Autooxidation of hemoglobin, catecholamines, and unsaturated lipids generates reactive oxygen species.

Q6. Discuss about the biological effects of free radicals. (3)

Ans: Free radicals have profound physiological and pathophysiological importance, because they can attack almost all cellular components.

i) ***Lipid peroxidation:*** Polyunsaturated fatty acids of cell membranes are highly susceptible to oxidative attack. The process occurs in three stages, *i.e.*, a radical abstracts hydrogen from polyunsaturated fatty acids, the lipid radical reacts with oxygen and the peroxy radicals attack neighboring fatty acids producing a chain reaction and finally two radicals combine to form stable non-radical products. Consequences of lipid peroxidation includes loss of membrane fluidity, increased membrane permeability, inactivation of membrane enzymes, cell injury and death.
ii) ***Protein oxidation:*** Free radicals oxidize sulfhydryl (-SH) groups of proteins forming disulfide bonds, thereby altering protein structure and enzyme activity. Methionine residues may be converted to sulphoxides.
iii) ***DNA damage:*** Hydroxyl radicals can cause DNA strand breaks, base modifications, and mutagenesis.
iv) ***Cellular injury and aging:*** Oxidative damage accumulates with age and contributes to degenerative diseases. Free radical accumulation contributes to cellular senescence and aging.
v) ***Atherosclerosis:*** Oxidation of LDL cholesterol promotes plaque formation in blood vessels.
vi) ***Rheumatoid arthritis:*** Activated neutrophils release free radicals causing joint inflammation and tissue destruction.
vii) ***Ischemia-reperfusion injury:*** Restoration of blood supply to ischemic tissues generates ROS leading to tissue damage.
viii) ***Diabetes mellitus:*** Oxidative stress contributes to vascular and metabolic complications.
ix) ***Neonatal oxygen toxicity:*** Premature infants exposed to high oxygen concentrations are vulnerable to oxidative damage causing retinopathy of prematurity, bronchopulmonary dysplasia, necrotizing enterocolitis etc.

Q7. Describe the Haber-Weiss-Fenton reaction. (4)

Ans: The ***Haber–Weiss–Fenton reaction*** refers to a series of reactions involving superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and transition metal ions such as iron (Fe^{2+}), leading to the formation of the highly reactive hydroxyl radical ($OH^{\bullet}$). This reaction is of great biological importance because the hydroxyl radical produced is one of the most destructive reactive oxygen species responsible for oxidative damage to lipids, proteins, nucleic acids, and biological membranes.

a) Fenton reaction: The Fenton reaction was first described by **H.J.H. Fenton**. In this reaction, hydrogen peroxide reacts with ferrous iron (Fe^{2+}) to generate hydroxyl radical. Ferrous iron (Fe^{2+}) donates an electron to hydrogen peroxide. Hydrogen peroxide undergoes cleavage producing hydroxyl radical ($OH\bullet$), hydroxide ion (OH^-) with simultaneous oxidation of Fe^{2+} into Fe^{3+}. The hydroxyl radical formed is extremely reactive and can rapidly attack nearby biomolecules.

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH\bullet + OH^-$$

b) Haber–Weiss Reaction: In the Haber–Weiss reaction, superoxide anion reacts with hydrogen peroxide to generate hydroxyl radical. However, this reaction proceeds very slowly under physiological conditions unless catalyzed by transition metals such as iron or copper.

$$O_2\bullet^- + H_2O_2 = OH\bullet + OH^- + {}^1O_2$$

Combined Haber–Weiss–Fenton reaction: Biologically, the Haber–Weiss reaction is coupled with the Fenton reaction through cycling of iron between Fe^{2+} and Fe^{3+} states.

i) ***Reduction of ferric iron:*** Superoxide anion reduces ferric iron to ferrous iron:
ii) ***Fenton reaction:*** The regenerated Fe^{2+} reacts with hydrogen peroxide. Thus, iron continuously cycles between Fe^{2+} and Fe^{3+} forms, catalyzing continuous hydroxyl radical production.

The overall ***Haber–Weiss–Fenton reaction*** can therefore be represented as:

$$Fe^{3+} + O_2\bullet^- = Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH\bullet + OH^-$$

Physiological significance: The hydroxyl radical produced through Haber–Weiss–Fenton reaction is highly toxic because:

i) It initiates lipid peroxidation.
ii) It damages proteins and enzymes.
iii) It causes DNA strand breaks and mutations.
iv) It increases membrane permeability.
v) It contributes to oxidative stress and cellular injury.

During normal aerobic metabolism the superoxide radicals are generated into mitochondria. However, superoxide dismutase converts them into hydrogen peroxide. In presence of free iron (Fe^{2+}), hydrogen peroxide undergoes Fenton reaction producing hydroxyl radicals. For example, in ***ischemia-reperfusion injury***, tissue iron catalyzes Haber–Weiss–Fenton reactions leading to extensive oxidative tissue damage. Ceruloplasmin acts as an antioxidant by oxidizing Fe^{2+} to Fe^{3+}, thereby reducing availability of ferrous iron needed for Fenton reaction. This helps prevent hydroxyl radical generation. Excessive Haber–Weiss–Fenton reactions contribute to atherosclerosis, rheumatoid arthritis, neurodegenerative diseases, accelerates aging.

Q8. Write a short note on oxidative stress. (5)

Ans: ***Oxidative stress may be defined as a condition resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body, leading to excessive oxidative damage to cellular components.*** The aerobic organisms continuously utilize oxygen for the production of energy through oxidative metabolism. During these metabolic processes, *reactive oxygen species* (ROS) and *reactive nitrogen species* (RNS) are generated as by-products. Under normal physiological conditions, these reactive molecules are effectively neutralized by the antioxidant defense system of the body. However, when the generation of free radicals exceeds the capacity of antioxidant defense, a state known as oxidative stress develops. Oxidative stress plays an important role in cellular injury, aging, and the pathogenesis of numerous diseases including atherosclerosis, diabetes mellitus, cancer, rheumatoid arthritis, neurodegenerative disorders, and ischemia-reperfusion injury.

During oxidative stress, free radical generation exceeds the neutralizing capacity of antioxidants, resulting in damage to lipids, proteins, carbohydrates, nucleic acids, and biological membranes. The major reactive oxygen species associated with oxidative stress include ***superoxide anion*** ($O_2\bullet^-$), ***hydroxyl radical*** ($OH\bullet$), ***hydroperoxy radical*** ($HOO\bullet$), ***hydrogen peroxide*** (H_2O_2), ***singlet oxygen*** ($^1O_2$) and ***peroxynitrite*** ($ONOO^-$). Among these, hydroxyl radical is considered the most reactive and damaging free radical in biological systems. Reactive oxygen species are generated continuously during normal metabolism and also under pathological conditions. Oxidative stress may result from either increased free radical production or decreased antioxidant defense, *i.e.*, during inflammation, after an infection, smoking, environmental pollution, exposure to radiation, heavy metals, during hyperoxia, strenuous exercise, ischemia-reperfusion injury decreased antioxidant production, nutritional deficiency of vitamins E, C, and selenium, glutathione depletion, deficiency of antioxidant enzymes (*e.g.*, superoxide dismutase, catalase), aging and chronic diseases. Free radicals produced during oxidative stress attack cellular constituents through chain reactions. Oxidative stress represents a pathological state arising from imbalance between free radical generation and antioxidant defense mechanisms. Excessive reactive oxygen species attack lipids, proteins, nucleic acids, and biological membranes leading to cellular dysfunction and tissue injury. Although free radicals are continuously generated during normal metabolism, the body is normally protected by elaborate antioxidant systems. Failure of these protective mechanisms results in oxidative stress, which contributes to aging and numerous pathological conditions.

Therefore, maintenance of redox balance through effective antioxidant defense is essential for normal cellular function and overall health.

Q9. Describe the major antioxidant systems. (5)

Ans: Enzymatic antioxidants constitute the most important intracellular defense mechanism against free radical–mediated oxidative damage. These enzymes act catalytically to neutralize *reactive oxygen species* (ROS) and *reactive nitrogen species* (RNS), thereby protecting cellular lipids, proteins, carbohydrates, nucleic acids, and biological membranes from oxidative injury. Unlike non-enzymatic antioxidants, enzymatic antioxidants are not consumed during the reaction and can function repeatedly. The major enzymatic antioxidants present in biological systems are superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and ceruloplasmin. These enzymes work in a coordinated manner to detoxify free radicals and maintain intracellular redox balance.

a) Superoxide dismutase (SOD): ***Superoxide dismutase*** is the first line of defense against oxygen-derived free radicals. During aerobic metabolism, especially in the mitochondrial respiratory chain, molecular oxygen undergoes univalent reduction producing *superoxide anion radical* ($O_2\bullet^-$). This radical is highly reactive and can initiate destructive chain reactions in cells. Superoxide dismutase catalyzes the conversion of two molecules of superoxide anion into hydrogen peroxide and molecular oxygen. In this reaction, one superoxide molecule undergoes oxidation while the other undergoes reduction. Thus, the enzyme rapidly removes superoxide radicals before they interact with membrane lipids, proteins, or nucleic acids.

$$2O_2\bullet^- + 2H^+ = H_2O_2 + O_2$$

Superoxide dismutase is present in almost all aerobic cells. Different forms of the enzyme occur in different cellular compartments:

- ***Copper-zinc SOD*** (**Cu-Zn SOD**) is present in the cytosol.
- ***Manganese SOD*** (**Mn-SOD**) is present in mitochondria.
- ***Extracellular SOD*** is found in plasma and extracellular fluid.

By removing superoxide radicals, SOD prevents the formation of more toxic radicals such as hydroxyl radicals through ***Haber–Weiss reactions***.

b) Catalase: Catalase is an important antioxidant enzyme responsible for decomposition of hydrogen peroxide (H_2O_2). Hydrogen peroxide is continuously generated during metabolic reactions and by the action of superoxide dismutase. Although hydrogen peroxide itself is not a free radical, it is highly reactive and can generate hydroxyl

radicals in the presence of transition metals such as iron. Catalase converts hydrogen peroxide into water and molecular oxygen.

$$2H_2O_2 = 2H_2O + O_2$$

Catalase contains a heme iron center which participates in the catalytic breakdown of hydrogen peroxide. The enzyme is mainly localized in peroxisomes and is particularly abundant in liver cells, kidney tissues, and erythrocytes where oxidative reactions are highly active. The major function of catalase is to prevent accumulation of hydrogen peroxide within cells and thereby protect tissues from oxidative injury, lipid peroxidation, and membrane damage.

c) Glutathione peroxidase: Glutathione peroxidase is a selenium-containing antioxidant enzyme that detoxifies hydrogen peroxide and lipid peroxides using reduced glutathione (GSH) as a reducing agent. In this process, reduced glutathione donates hydrogen atoms to hydrogen peroxide, converting it into water.

$$H_2O_2 + 2GSH = GSSG + 2H_2O$$

Simultaneously, glutathione becomes oxidized to glutathione disulfide (GSSG). Glutathione peroxidase also destroys lipid peroxides formed during lipid peroxidation.

$$ROOH + 2GSH = ROH + GSSG + H_2O$$

Thus, the enzyme protects cellular and subcellular membranes from oxidative destruction caused by peroxidation of polyunsaturated fatty acids. Glutathione peroxidase is present in cytosol as well as mitochondria and is especially important in tissues where catalase activity is relatively low.

d) Glutathione reductase: The antioxidant activity of glutathione peroxidase depends upon continuous availability of reduced glutathione (GSH). Glutathione reductase regenerates reduced glutathione from oxidized glutathione (GSSG) using NADPH as a source of reducing power.

$$GSSG + NADPH + H^+ = 2GSH + NADP^+$$

This enzyme therefore maintains intracellular glutathione concentration and sustains antioxidant defense mechanisms. NADPH required for this reaction is mainly generated through the pentose phosphate pathway. Deficiency of NADPH production, as observed in glucose-6-phosphate dehydrogenase deficiency, impairs regeneration of glutathione and predisposes erythrocytes to oxidative hemolysis.

e) Ceruloplasmin: Ceruloplasmin is a copper-containing plasma protein possessing ferroxidase activity. It acts indirectly as an antioxidant by oxidizing ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Ferrous iron participates in Fenton and Haber–Weiss reactions leading to formation of highly reactive hydroxyl radicals. By converting Fe^{2+} into Fe^{3+}, ceruloplasmin prevents generation of hydroxyl radicals and thus inhibits oxidative tissue damage. Therefore, ceruloplasmin serves as an important extracellular antioxidant defense mechanism.

f) Ferricytochrome system: Ferricytochrome can oxidize superoxide radicals back to molecular oxygen, thereby reducing oxidative stress within cells. This mechanism acts as an auxiliary antioxidant pathway, especially in mitochondria where electron transport reactions continuously generate reactive oxygen species.

The enzymatic antioxidant system functions in a sequential and highly coordinated manner. Initially, superoxide radicals generated during metabolism are converted into hydrogen peroxide by superoxide dismutase. The hydrogen peroxide thus formed is subsequently detoxified either by catalase or by glutathione peroxidase. Glutathione reductase regenerates reduced glutathione required for glutathione peroxidase activity, while ceruloplasmin prevents hydroxyl radical formation by controlling free iron availability. Through this integrated defense system, enzymatic antioxidants prevent oxidative injury to biomolecules and maintain normal cellular homeostasis.