Biochemistry – Introduction

Father of Biochemistry

Carl Neuberg

Biochemistry is the study of the chemistry of life, a bridge between biology and chemistry that studies how complex chemical reactions and chemical structures give rise to life.

It is a hybrid branch of chemistry which specializes in the chemical processes and chemical transformations in living organisms.

Biochemistry can be defined as:

- Study of the chemistry of living things
- The study of the chemical processes and compounds occurring in living organisms
- The chemical characteristics and reactions of a particular living system or biological substance
- The study of the chemical constituents of living matter and of their functions and transformations during life processes.
- Chemistry of life.

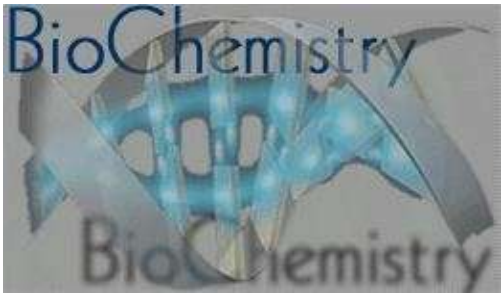
Development of biochemistry

Originally, it was thought that life was not subject to the laws of science the way nonlife was. It was thought that only living beings could produce the molecules of life

(from other, previously existing biomolecules). Then, in 1828, Friedrich Wöhler published a paper about the synthesis of urea, proving that organic compounds can be created artificially. The dawn of biochemistry may have been the discovery of the first enzyme, diastase, in 1833 by Anselme Payen. Although the term "biochemistry" seems to have been first used in 1881, it is generally accepted that the formal coinage biochemistry occurred in 1903 by Carl Neuberg, a German chemist. Since then, biochemistry has advanced, especially since the mid-20th century, with the development of new techniques such as chromatography, X-ray diffraction, NMR spectroscopy, radioisotopic labelling, electron microscopy and molecular dynamics simulations. These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle).

Today, the findings of biochemistry are used in many areas, from genetics to molecular biology and from agriculture to medicine. The first application of biochemistry was probably the making of bread using yeast, about 5000 years ago.

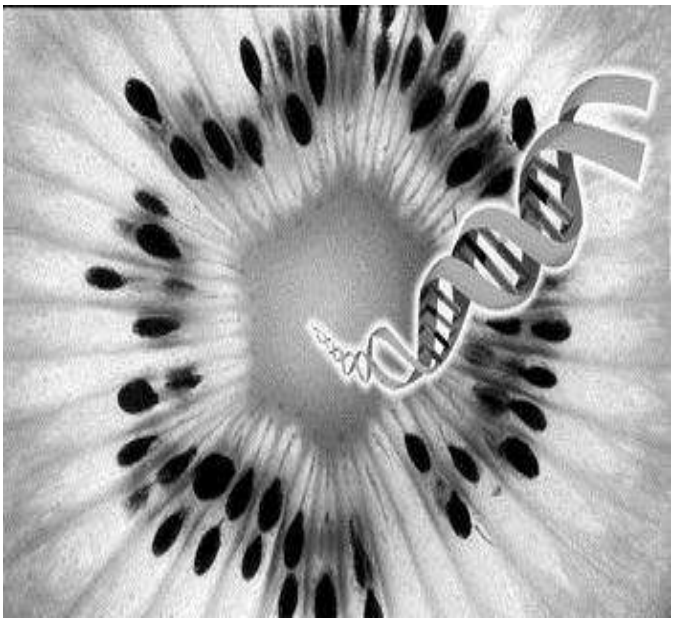
Evolution of Biochemistry

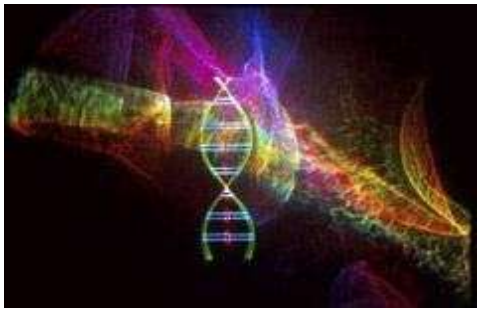


The idea of biochemistry started with the idea of chemical evolution developed by J.B.S. **Haldane,** a British scientist, and his theory of chemical evolution is life transpires as the natural process of the evolution of inorganic matter. Matter on the Earth, is the same as matter that appears throughout the vast Universe. Rules of chemistry show, matter naturally tends to arrange itself and transforms through time into larger macromolecules that are the ancestors to life, they tend to combine into the molecules that will eventually arrange themselves in living cells. That is what makes up the theory of chemical evolution. In this theory time

is the important factor in which the evolution process requires the most to occur. Billions of years have passed to progress us to where we are now.

In 1950s **Stanley Miller,** a student working in Harry Urey's lab, verified the authenticity of this theory. He chose some gases: methane, ammonia, water, and hydrogen; the four essential elements and compounds for life believed to be present on the newly developed Earth. These gases were added into a big container, and administered lighting-type energy. What was discovered at the end of the experiment was unparallel; about 12% of the carbon material in the "container" had converted into an organic acid, and about 9% of that was an alpha-amino-acid. What that tells us is that chemicals evolve in a direction; it is not random molecules that are produced, but a majority of the same molecules.





Alpha-amino-acids are the main component of a majority of the life on earth called "protein." Protein is made up of alpha amino acids. Thus the material we are all made up of is easily achieved from the conversion of these gases under conditions likely to have taken place in the early Earth.

There are errors with this theory. Organisms are also critically dependent upon nucleic acids, which run proteins. DNA stands for deoxyribonucleic acid, which is one of the most significant and mysterious things about the human body. DNA is how nucleic acids are put together in humans. Regrettably, precursors for DNA have not been established with this experiment. Nonetheless, tiny molecules for formaldehyde and cyanide have been exposed. These molecules can tolerate chemical processes to form the amino acids; they can also be recombined in faintly different ways to form other things, things that would supply the basis for nucleic acids; purine and pyrimidine bases, and sugars. Sugars can be formed from formaldehyde; purine and pyrimidine can be formed from cyanide-type molecules. So the simple substances resulting from this experiment can be subjected to other conditions, and altered to other molecules essential for life. This is how we now believe things to have happened.

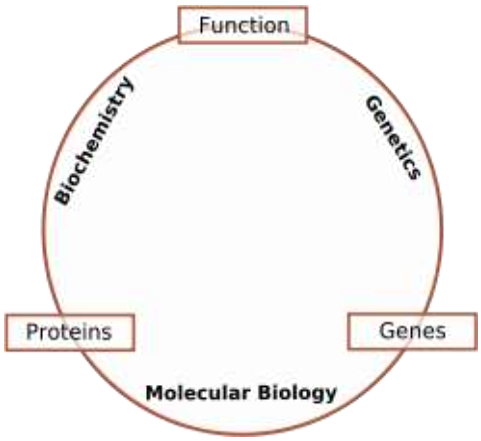
Important Scientists and their contribution to biochemistry

Date	Discovery
1835	Jons Berzelius writes a paper on chemical catalysis, uses amylase as an example.
1859	Charles Darwin publishes **On the Origin of Species**.
1860	Louis Pasteur recognizes that fermentation was catalyzed by enzymes, but he believes they are part of the "essence" of yeast.
1865	Gregor Mendel publishes his theory of genetics.
1869	Fredrick Meischer discovers DNA in cell nuclei.
1897	Edward and Hans Buchner extracts material from yeast that catalyzes the conversion of glucose to alcohol.
1900	Gregor Mendel's work on genetics is rediscovered.
1914	Fritz Lipmann elucidates the role of ATP in energy metabolism.
1926	James Sumner obtains crystalline jack bean urease and demostrates that it is a protein.
1926	Thomas Hunt Morgan writes **The Theory of the Gene**.
1934	Arnold Beckman develops the first pH meter.

1937	Hans Krebs discovers the citric acid cycle (TCA cycle).
1941	George Beadle & Edward Tatum propose the one-gene, one-enzyme hypothesis.
1944	Oswald Avery, Colin MacLeod, and Maclyn McCarthy use chemical methods to establish that DNA is the genetic material.
1950	Edwin Chargaff publishes observation that A=T, G=C (Chargaff's rules).
1952	Linus Pauling and Robert Corey propose the **α**-helix and the **β**-pleated sheet structures for proteins.
1952	Alfred Hershey and Martha Chase provide additional support for DNA as genetic material.
1953	James Watson and Frances Crick put forth the double helix model DNA.
1953	Fredrick Sanger determines the first amino acid sequence of a protein (insulin).
1956	Earl Sutherland isolates cyclic AMP.
1957	Matthew Meselson and Franklin Stahl carry out experiment to demonstrate semiconservative DNA replication.
1960	John Kendrew and Max Pertuz obtain the first three dimensional structure of proteins (hemoglobin and myoglobin).

1960	Jerald Huritz and Samuel Weiss discover RNA polymerase.
1961	Francois Jacob and Jaques Monod propound the operon model of gene control.
1963	Allosteric model for inhibition of enzymes (Jean-pierrre Changuex, F. jacob, and J. Monod).
1964	Acrylamide gel electrophoresis of proteins is developed.
1965	Marshal Nirenberg, H. Gobind Khorana, and severo Ochoa complete the elucidation of the genetic code.
1965	3-D model of first enzyme (lysozyme by David Phillips).
1965	Robert Holley determines the structure of a transfer-RNA.
1965	Jerome Vinograd discovers superhelical twisting.
1968	Mark Ptashne and Walter Gilbert identify the first repressor genes.
1969	Paula DeLucia and John Cairns isolate a mutant of E. coli called pol A1.
1969	First synthesis of an enzyme (Ribonuclease).
1970	Hamilton Smith discovers restriction endonucleases.
1970	Howard Temin and David Baltimore discover reverse transcriptase.
1973	Stanley Cohen and Herbert Boyer prepare recombinant DNA.

1974	Sung-Hou Kim, *et al.* produce the first X-ray structure of transfer RNA.
1977	Cesar Milstein discovers how to produce monoclonal antibodies.
1977	Allan Maxam and Walter Gilbert develop a chemistry for sequencing DNA.
1977	Fredrick Sanger, S. Nicklen and A.R. Coulson develop a chemistry for sequencing DNA.
1977	Phillip Sharp and Richard Roberts discover introns (intervening sequences).
1982	First x-ray structure of a membrane protein.



Schematic relationship between biochemistry, genetics and molecular biology.

Lecture-1

Introduction to Biochemistry, Carbohydrates – occurrence and classification

Biochemistry, as the name implies, is the ***chemistry of living organisms***. Living organisms, whether they are microorganisms, plants or animals are basically made up of the same chemical components. *Biochemistry is the study of the way in which these components are synthesized and utilized by the organisms in their life processes*. It bridges the gap between the conventional chemistry and biology.

In other words, life is nothing but thousands of ordered chemical reactions or chemistry is the logic of all biological phenomena.

History of biochemistry

During 17^{th} and 18^{th} centuries, important foundations were placed in many fields of biology.

- The 19th century observed the development of concepts - the cell theory by **Schleiden and Schwann**, **Mendel's study of inheritance and Darwin's theory of evolution.**
- The real push to biochemistry was given in 1828 when *total synthesis of urea* from lead cyanate and ammonia was achieved by **Wohler** who thus initiated the synthesis of organic compound from inorganic compound.
- **Louis Pasteur**, during 1857, did a great deal of *work on fermentations* and pointed out the *central importance of enzymes* in this process.
- The break through in enzyme research and hence, biochemistry was made in 1897 by **Edward Buchner** when he *extracted enzyme from yeast cells* in crude form which could ferment a sugar molecule into alcohol.
- **Carl Neuberg** coined the *term biochemistry* in 1903.

The early part of 20th century witnessed a sudden outburst of knowledge in **chemical analysis, separation methods, electronic instrumentation for biological studies (X-ray diffraction, electron microscope,** etc) which ultimately resulted in understanding the

structure and function of several key molecules involved in life processes such as proteins, enzymes, DNA and RNA.

- In 1926, **James Sumner** established the *protein nature of enzyme*. He was responsible for the isolation and crystallization of *urease*, which provided a break through in studying of the properties of specific enzymes.
- The first metabolic pathway elucidated was the *glycolytic pathway* during the first half of the 20th century by **Embden and Meyerhof. Otto Warburg, Cori and Parnas** also made very important contributions relating to glycolytic pathway.
- **Krebs** established the *citric acid and urea cycles* during 1930-40.
- In 1940, **Lipmann** described the *central role of ATP* in biological systems.
- The biochemistry of nucleic acids entered into a phase of exponential growth after the establishment of the *structure of DNA* in 1953 by **Watson and Crick** followed by the discovery of *DNA polymerase* by **Kornberg** in 1956.

From 1960 onwards, biochemistry plunged into an interdisciplinary phase sharing much in common with biology and molecular genetics.

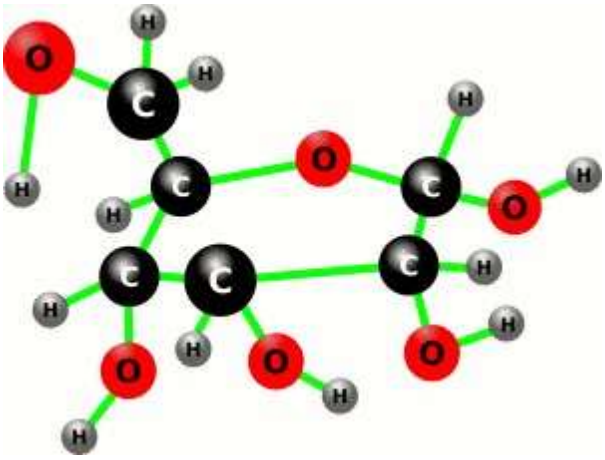
- **Frederick Sanger's** contributions in the *sequencing of protein* in 1953 and *nucleic acid* in 1977 were responsible for further developments in the field of protein and nucleic acid research.

The growth of biochemistry and molecular biology was phenomenal during the past two decades.

- The development of *recombinant DNA research* by **Snell** and coworkers during 1980 allowed for further growth and emergence of a new field, the genetic engineering.

Thus there was progressive evolution of biology to biochemistry and then to molecular biology, genetic engineering and biotechnology.

CARBOHYDRATES



Compounds with empirical formula, $(CH_2O)n$, were called as carbohydrates (*hydrates of carbons*). With the discoveries of many diverse carbohydrates it was noticed that many, but not all, carbohydrates have the above empirical formula; some also contain nitrogen, phosphorus or sulfur. There are some carbohydrates (derivatives) that do not possess (CH_2O) n. On the other hand, there are a few non-carbohydrate compounds like lactic acid with empirical formula (CH_2O) n. ***Hence, carbohydrates are chemically defined as polyhydroxy aldehydes or ketones, their derivatives and their polymers.***

Occurrence and importance

- The carbohydrates comprise one of the major groups of *naturally occurring biomolecules.* This is mainly because; the *light energy* from the sun is converted into *chemical energy* by plants through primary production and is transferred to sugars and carbohydrate derivatives.
- The dry substance of plants is composed of 50-80% of carbohydrates. The structural material in plants is mainly **cellulose** and related **hemicelluloses**.
- **Starch** is the important form of storage polysaccharide in plants.
- **Pectins** and sugars such as **sucrose and glucose** are also plant constituents.
- Many *non-carbohydrate* organic molecules are found conjugated with sugars in the form of **glycosides**.

- The carbohydrates in animals are mostly found in combination with proteins as **glycoproteins**, as well as other compounds.
- The storage form of carbohydrates, **glycogen,** found in liver and muscles, the **blood group substances, mucins, ground substance between cells in the form of mucopolysaccharides** are few examples of carbohydrates playing important roles in animals.
- **Chitin** found in the exo-skeleton of lower animals, is a polymer of N-acetyl glucosamine.

Carbohydrates are also universally found in other polymeric substances. For example,

- Fats are fatty acid esters of a sugar alcohol, **glycerol**.
- **Ribose and deoxyribose** are constituent of nucleic acids.

Moreover, in all living forms, the energy needed for mechanical work and chemical reactions are derived from carbohydrates.

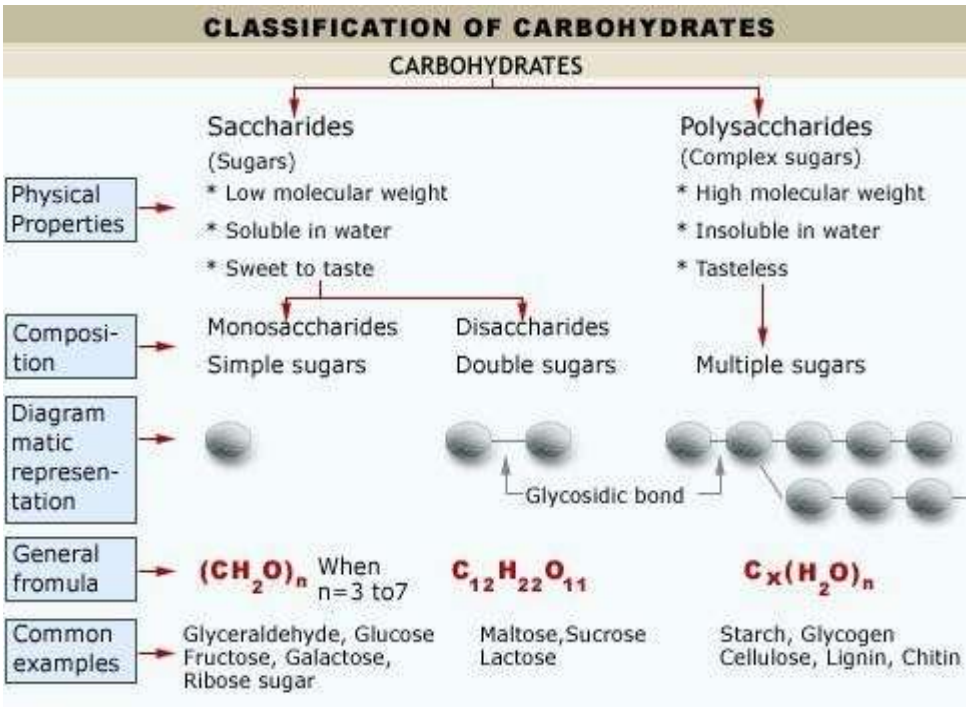
- **Adenosine triphosphate** and related substances that contain ribose as a constituent are key substances in energy storage and transfer.
- *The carbon skeletons* of almost **all organic molecules** are derived from carbohydrates.

Besides, the carbohydrates are the *basic raw material* of many important industries including **sugar and sugar products, starch products, paper and wood pulp, textiles, plastics, food processing and fermentation**.

CLASSIFICATION

Carbohydrates are classified into three major groups:

- Monosaccharides
- Oligosaccharides
- Polysaccharides



Classification of carbohydrates

Monosaccharides (Simple sugars)	**Oligosaccharides**	**Polysaccharides (Glycans)**
Low molecular weight carbohydrates and cannot be hydrolysed further	Contain 2-10 monosaccharides joined by glycosidic bonds. Low molecular weight carbohydrates which can be hydrolysed by enzymes or acids to yield monosaccharides	Contain many monosaccharides joined by glycosidic bonds. They can be hydrolysed by enzymes or acids.
Crystalline, soluble in water, and sweet in taste.	Powdery or crystalline, soluble in water and sweet in taste	Insoluble in water, tasteless, linear or branched
Classified into triose, tetrose, pentose, hexose and heptose depending upon the number of carbon atoms.	Classified into disaccharide, trisaccharide, tetrasaccharide and pentasaccharide depending upon the number of monosaccharides	Classified into homoglycans and heteroglycans depending upon the kind of monosaccharides present. Depending upon the function, they

They may be either aldoses or ketoses depending upon whether they contain a free aldehyde or ketone group, respectively	they contain.	are classified as storage and structural polysaccharides.
All monosaccharides are reducing in nature	Some of them are reducing and some of them are non-reducing in nature.	Non reducing in nature and give deep blue (amylose) or red colour (amylopectin) with iodine.

Monosaccharides:

Monosaccharides are the simplest form that cannot be hydrolyzed further into smaller units. They are classified into a) simple monosaccharides b) derived monosaccharides

Simple monosaccharides are further classified

- Based on the **type of functional group** and
- The **number of carbon atoms** they possess.

Derived monosaccharides include the *derivatives of simple monosaccharides* such as *oxidation products, reduction products, substitution products and esters*

Classification of monosaccharides

Monosaccharides	**No. of carbon atoms**	**Aldose**	**Ketose**	**Occurrence**
Simple				
Triose	3	D-Glycerose	Dihydroxy acetone	Intermediary meta-bolites in glucose metabolism
Tetrose	4	D-Erythrose	D-Erythrulose	

Pentose	5	D-Ribose	D-Ribulose	Ribose is a constituent of nucleic acid
		L-Arabinose	-	Occurs in oligosac-charides
		D-Xylose	D-Xylulose	Gum arabic, cherry gums, wood gums, proteoglycans
Hexose	6	D-Glucose	D-Fructose	Fruit juices and cane sugar
		D-Galactose	-	Lactose, constituent of lipids
		D-Mannose	-	Plant mannosans and glycoproteins
Heptose	7	-	D-Sedoheptulose	Intermediate in carbohydrate metabolism
Derived				
Deoxysugar	5	2-Deoxyribose	-	DNA
	6	L-Rhamnose	-	Component of cell wall
Aminosugar	6	D-Glucosamine	-	A major component of polysaccharide found in insects and crustaceans (chitin)
Polyol	6	Sorbitol	-	Berries
	6	Mannitol	-	Commercially prepared from mannose and fructose
Aldonic acid	6	Gluconic acid	-	-
Uronic acid	6	Glucuronic acid	-	Constituent of chondroitin sulfate
	6	Galacturonic	-	Constituent of pectin

		acid		
Aldaric acid (Saccharic acid)	6	Glucaric acid	-	Oxidation product of glucose
	6	Mucic acid	-	Oxidation product of galactose

Oligosaccharides:

They contain two to ten monosaccharide units joined by glycosidic linkages that can be easily hydrolyzed.

Polysaccharides:

They are high molecular weight polymers containing more than ten monosaccharides. They are either linear or branched in structure.

Polysaccharides are further classified based on

a) The kind of monosaccharides present as:

- **Homopolysaccharides** when made from a single kind of monosaccharide. Eg starch, cellulose, inulin, glycogen, chitin
- **Heteropolysaccharides** are made up of more than one type of monosaccharides. Eg. Hemicellulose, Mucopolysaccharides – Chondroitin sulphate, Hyaluronic acid Heparin and Keratan sulphate

b) Functional aspect as:

- **Storage Polysaccharide** eg. Starch, glycogen, inulin, Galactomannan
- **Structural Polysaccharide** eg**.** Cellulose, Chitin, Hemicellulose

Lecture: 2

Structure of Monosaccharides:

The *simplest* monosaccharide that possesses a hydroxyl group and a carbonyl group with an asymmetric carbon atom is the **aldotriose -glyceraldehyde**. (*A carbon is said to be asymmetric if four different groups or atoms are attached to it. The carbon is also called as a chiral center).*

- Glyceraldehyde is considered as a reference compound and it exists in two optically active forms, D and L

The two families of monosaccharides, D-and L occur based on the configuration of D and L glyceraldehydes. In general, the *D-family of sugars occur in nature*.

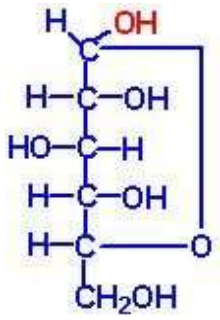
- For monosaccharides with *two or more asymmetric carbons*, the prefixes D or L refer to the *configuration of the penultimate carbon* (i.e, the asymmetric carbon farthest from the carbonyl carbon).
- If the *hydroxyl group on the **penultimate** carbon* is on the ***right-hand side*** of the carbon chain when the aldehyde or ketone group is written at the top of the formula it belongs to the **D family** and if on the ***left hand side*** it belongs to **L family**. The D or L has nothing to do with optical activity. D sugars may be dextro- or levorotatory.
- The important monosaccharides containing aldehyde group belonging to the D family are
  - the aldotetrose - D-erythrose
  - the aldopentoses - D-ribose, D-arabinose and D-xylose
  - the aldohexoses - D-glucose, D-mannose and D-galactose
- The important monosaccharide belonging to the L-family is L-arabinose.
- The important ketoses are
  - Ketotriose - dihydroxy acetone (*It is optically inactive since there is no asymmetric carbon*);
  - the ketotetrose - D-erythrulose;
  - the ketopentoses - D-ribulose and D-xylulose

- the ketohexose - D-fructose

Cyclic structure of Monosaccharides:

The monosaccharides exist either in cyclic or acyclic form. There are many evidences to show that the pentose and hexose monosaccharides are present in cyclic form. The evidences are 1. Glucose and other aldoses fail to give the Schiff's test for aldehydes. 2. Solid glucose is quite inert to oxygen where as aldehydes are easily auto-oxidizable. 3. Glucose and other aldoses do not form bisulfite or aldehyde ammonia compound. 4. Glucose penta acetate does not react with hydroxylamine. 5. Presence of two forms of glucose with different physical and chemical properties. 6. X-ray analysis definitely proves the existence of the ring structure and also the size of the ring. 7. Mutarotation.

- When an aldehyde or a ketone group is present in a molecule that also possesses hydroxyl groups, an ***intramolecular arrangement*** may occur to form a ***hemiacetal or a hemiketal***, respectively. This intramolecular hemiacetal or hemiketal is the basis for the cyclic structure of the sugars. Hence, **Haworth** (an English chemist) proposed a cyclic hemiacetal structure that accounts completely for its chemical properties

	
Cyclic Fischer Projection of □ -D-Glucose	**Haworth Projection of □ -D-Glucose**

- Two types of ring structures are possible, ***the five-membered furanose*** and ***the six-membered pyranose ring*** if the carbonyl group interact with hydroxyl group. These names are derived from the parent compounds 'furan' and 'pyran'.
- The most common ring structure for aldohexoses is the **pyranose ring** structure that involves the *first carbonyl carbon and the hydroxyl group attached to the fifth carbon.*
- The **furanose ring** structure is formed by *interaction of carbonyl carbon with the hydroxyl group attached to the fourth carbon*. This ***furanose*** *form is* ***less stable*** than the pyranose strucure and is not very common among aldohexoses.
- Very seldom is a seven-membered ring formed.
- *Fructose* exists in *solution* and in *compounds* as a *furanose*; however, in the *crystalline state* only the *pyranose ring* is believed to exist.
- **Ribose** occurs as the **furanose structure** in many important biological compounds.
- A **new asymmetric carbon** is introduced in the molecule due to this rearrangement. As a result of this new asymmetric centre, two isomers are formed.
- Isomeric forms of monosaccharides that *differ only in their configuration about the hemiacetal or hemiketal carbon atom* are called **anomers** and the carbon is referred as **anomeric carbon.**
- When the newly formed hydroxyl group in $C_1$and the ring are on the same orientation, it is α **- anomer**.
- When the newly formed hydroxyl group in C_1 and the ring are on opposite orientation, it is β **- anomer**.

While writing the cyclic form (Haworth) of monosaccharides it is sometimes difficult to judge whether an OH group should be above or below the plane of the ring.

A few rules can be followed for writing Haworth's structure for carbohydrates.

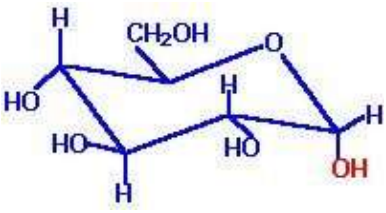
- Write the **oxygen** at the *upper right hand corner of the ring* structure (pyranose) and the carbons clockwise around the ring. At the fifth carbon it is necessary to

rotate the bond to 90^{o} to make the ring closure. For the D-family of sugars, it is customary to write the terminal CH_2OH above the plane of the ring.

- If the ***hydroxyl group or hydrogen atom occurs on the right-hand side*** of the carbon chain in the linear structure it is ***placed below the plane of the ring*** in the cyclic structure.
- Conversely, if the ***hydroxyl group or hydrogen atom is on the left-hand side*** of the carbon chain, it is placed ***above the plane of the ring*** in the structure formula

Conformational structure:

The six-membered pyranose ring is not actually planar, as suggested by Haworth, but assume usually the **stable chair conformation**.



Chair form of _ -D-Glucose

- The substituents are represented either axially or equatorially.
- The *axial substituents* project almost *parallel* with the vertical axis through the ring
- The *equatorial substituents* project roughly *perpendicular* to this axis.
- Substituents in the equatorial positions are less sterically hindered by neighbouring substituents. *Conformations with their bulky substituents in equatorial positions are favoured*.

Derived monosaccharides

The important functional groups present in monosaccharides are hydroxyl and carbonyl groups. The hydroxyl group forms esters, usually with phosphoric acid or is

replaced by a hydrogen or amino group. The carbonyl group undergoes reduction or oxidation to produce number of derived monosaccharides.

a) Deoxysugars

- In sugars, the *hydroxyl group is replaced by a hydrogen* to produce deoxy sugars (devoid of oxygen).
- The important deoxy sugar is **2-deoxy ribose** that occurs in *deoxy ribonucleic acid*.
- Other important deoxy sugars are *L-fucose and L. rhamnose*. The substitution of the hydroxyl group at C-6 of L. galactose or L.mannose with hydrogen produces fucose or rhamnose respectively.
- L-fucose occurs in the cell wall polysaccharides namely hemicelluloses and L-rhamnose occurs in pectic polysaccharides namely rhamnogalacturonan. These deoxy sugars are also found in the complex oligosaccharide components of glycoproteins and glycolipids.

b) Amino sugars

- The *hydroxyl group*, usually at **C-2**, is replaced by an *amino* group to produce aminosugars such as *glucosamine, galactosamine and mannosamine.*
- The amino group may be condensed with *acetic acid* to produce N-acetyl amino sugars, for example, **N-acetyl glucosamine.**
- This glucosamine derivative is important *constituent* of many *structural polymers* (**chitin, bacterial cell wall polysaccharides** etc.)

c) Polyols (alditols)

- Both aldoses and ketoses are *reduced* to **polyhydric alcohols (polyols)** when treated with *enzymes, sodium amalgam, and hydrogen under high pressure* with catalyst or sodium borohydride.
- Each aldose yields the corresponding alcohol upon reduction
- A ketose forms *two alcohols* because of the appearance of a new asymmetric carbon atom in the process.
- By this reduction process, the following sugars give rise to their respective alcohols under specified conditions.

Glucose	Sorbitol
Fructose	Sorbitol and mannitol
Mannose	Mannitol
Glyceraldehyde	Glycerol
Erythrose	Erythritol
Ribose	Ribitol
Galactose	Dulcitol

- Polyols occur in many plant products.
- Sorbitol was first isolated from the *berries* of mountain ash *(Sorbus aucuparia).*
- Commercially sorbitol is manufactured by the *hydrogenation of glucose*.
- Mannitol occurs in many terrestrial and marine plants.
- Potential food applications of polyols include **confectionery products, bakery products, deserts, jams and marmalade.**
- Sorbitol is an **excellent moisture conditioner** and is used in *pharmaceutical preparations* such as *elixirs and syrups*.
- Sorbitol, as a ***humectant*** in creams and lotions helps to stabilize the water content, providing better moisture control.
- The use of sorbitol or xylitol in *toothpaste and mouthwashes* is highly desirable.

d) Oxidation products

When aldoses are *oxidized* under proper conditions with different types of oxidizing agents, *three types of acids* are produced, namely **aldonic acids, uronic acids and aldaric acids or saccharic acids.**

Aldonic acid

- Oxidation of an aldose with *bromine water* at neutral pH converts the aldehyde group (C_1) to a carboxyl group yields **Aldonic acid**.

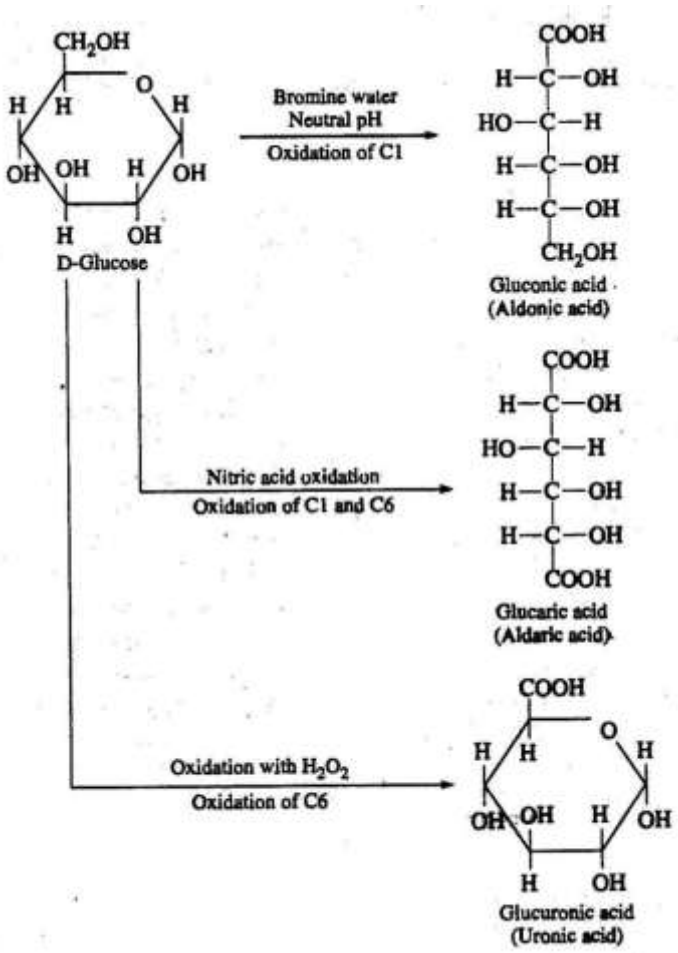
- Hydrobromous acid formed by the reaction of water with bromine acts as an oxidizing agent.
- Ketoses are not readily oxidized by bromine water.
- Aldoses are not only oxidized by bromine water but also by the alkaline iodine solution.

Uronic acid

- When aldoses are oxidised with *hydrogen peroxide* (H_2O_2) uronic acids are formed.
- In this reaction only primary alcohol group (C_6) is oxidized to carboxyl group, whereas the aldehyde group remains unchanged.
- Uronic acids are constituents of *pectic polysaccharides*.

Aldaric or saccharic acid

- When aldoses are oxidised with *nitric acid*, **saccharic acids** are formed.
- Both aldehyde (C_1) and primary alcohol groups (C_6) are oxidised to carboxyl groups.
- On oxidation with nitric acid, *Glucose* produces **glucaric or glucosaccharic acid**.
- The aldaric acid produced from *galactose* is called as **mucic acid**.



Oxidation products of glucose

Lecture3

Structure of Oligosaccharides & Polysaccharides

Composition, sources and properties of common disaccharides

Disaccharides	Constituent monosaccharides	Linkage	Source	Properties
Reducing disaccharides				
Maltose	α-D-glucose+ α-D-glucose	α(1→4)	Germinating cereal and malt	Forms osazone with phenylhydrazine. Fermentable by enzyme maltase present in yeast. Hydrolysed to two molecules of D-glucose. Undergoes mutarotation.
Lactose	β-D-galactose+ α-D-glucose	β(1→4)	In milk trace amounts it can be seen in urine during pregnancy	It shows reactions of reducing sugars including mutarotation. Decomposed by alkali. Not fermentable by yeast. Hydrolysed to one molecule of galactose and one molecule of glucose by acids and the enzyme lactase.
Non-reducing disaccharides				
Sucrose	α-D-glucose+ β-D-fructose	α,β(1→2)	Sugar beet, sugarcane, sorghum and carrot roots	Fermentable. Hydrolysed by dilute acids or enzyme invertase (sucrase) to one molecule of glucose and one molecule of fructose. Relatively stable to reaction with dilute alkali.

Trehalose	α-D-glucose+ α-D-glucose	α,α(1→1)	Fungi and yeast. It is stored as a reserve food supply in insect's hemolymph	It is hydrolysable by acids to glucose with difficulty. Not hydrolysed by enzymes.

The oligosaccharides commonly encountered in nature belong to disaccharides.

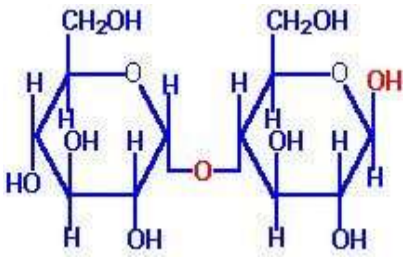
- The physiologically important disaccharides are maltose, lactose, trehalose and sucrose.
- Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond.
- The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups.
- This hydroxyl group present in one monosaccharide reacts with any one of the hydroxyl groups attached to C-1, C-2, C-3, C-4, or C-6 of another monosaccharide to produce 1→1, 1→2, 1→3, 1→4, and 1→6 linked disaccharides.
- When only one anomeric carbon is involved in glycosidic bond formation, reducing disaccharides are formed.
- If both anomeric carbon atoms of monosaccharides are involved in glycosidic bond formation that results in the formation of a non-reducing disaccharides such as trehalose (aldosyl-aldosyl disaccharide) or sucrose (aldosyl-ketosyl disaccharide)'.
- In the case of reducing disaccharides, one end of the molecule having free anomeric carbon is called reducing end and the other end, where the anomeric carbon is involved in glycosidic bond, is called as non-reducing end

Reducing disaccharides

Maltose

- Maltose is a disaccharide made up of **two glucose residue** joined by a ***glycosidic linkage*** between C-1 of one glucose residue and C-4 of the other.

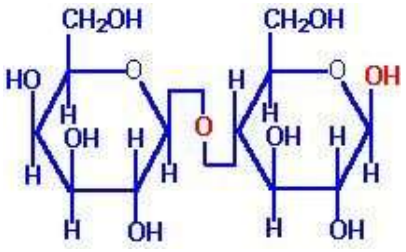
- The *configuration of the anomeric carbon* of glucose involved in the linkage is and hence the glycosidic linkage is **(1→ 4).**
- *The anomeric carbon atom* of the second glucose is *free* and therefore maltose is a ***reducing sugar.***
- Maltose has been recorded occasionally in plants. It is usually obtained as a product of the ***enzyme hydrolysis of starch*** during *germination or malting process*.



Maltose

Lactose

- Lactose is a *reducing disaccharide* found only in ***milk***.
- It is made up of *galactose at the non-reducing end* and *glucose at the reducing end.*
- They are connected by a **β (1→ 4) linkage**



Lactose

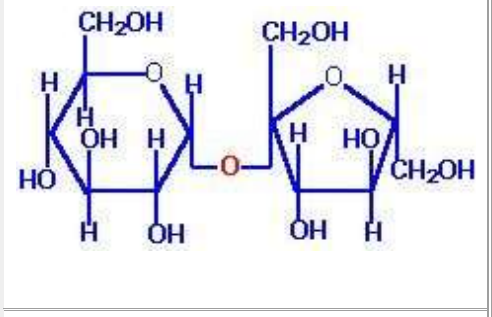
Non-reducing disaccharides

Trehalose

- Trehalose, a non-reducing disaccharide, occurs as a major constituent of the circulating fluid (hemolymph) of insects and serves as an energy storage compound.
- It is also present to a limited extent in the fat body of a variety of insects.
- It gives twice the amount of energy as that of glucose and at the same time maintains the osmotic balance.
- It has been described as an important adaptation of insects engaged in flight.
- The anomeric carbons of both glucose moieties are involved in the formation of glycosidic bond.

Sucrose

- Sucrose, a sugar of commercial importance, is widely distributed in higher plants.
- **Sugarcane and sugar beet** are the sole commercial sources.
- It is made up of **glucose and fructose**.
- The anomeric carbon atom of glucose (C-1) and fructose (C-2) are involved in linkage and is therefore a **non-reducing disaccharide**
- Sucrose is a major **intermediate product of photosynthesis** and it is the principal *form in which sugar is transported* from the leaves to other portions of plants via their vascular systems.



Sucrose

Invert sugar

- The *hydrolysis of sucrose* when followed polarimetrically the optical rotation changes from **positive (dextro-)** to **negative (levo**-).
- The dextrorotatory sucrose on hydrolysis yield levorotatory mixture of glucose and fructose.
- The levorotaion is due to the *presence of fructose* which is by itself more levorotatory (-92^0) than dextrorototary glucose ($+52.2^0$).
- This phenomenon is called inversion and the mixture of glucose and fructose is called invert sugar.
- This reaction is catalysed by the ***enzyme invertase.***
- Invert sugar is more sweeter than sucrose.
- *Honey* contains plenty of invert sugar and therefore is very sweet.

Sucrosyl oligosaccharides

- The **degree of polymerization (DP**) of sucrosyl oligosaccharides normally ranges from *3 to 9.*
- Though sucrose is found at higher concentration in all plants, members of the sucrosyl oligosaccharides occur at least in traces in each plant family.
- The main accumulation of sucrosyl oligosaccharides is found in ***storage organs such as roots, rhizomes and seeds.***
- The important members of sucrosyl oligosaccharides are **raffinose (DP-3), stachyose (DP-4), verbascose (DP-5) and ajugose (DP-6).**
- All sucrosyl oligosaccharides are **non-reducing** in nature.

Raffinose

- It occupies the second position next to sucrose in abundance in the plant kingdom.
- Raffinose occurs only at low concentration in the leaves of leguminous plants, but accumulates in the storage organs such as seeds and roots.
- Most of the leguminous seeds contain these oligosaccharides in large amounts.

- Bengal gram has higher amounts of raffinose.
- Red gram and Green gram have significantly high amounts of verbascose and stachyose than Bengal gram and Black gram.
- These sucrosyl oligosaccharides are responsible for **flatulence** following the consumption of these legumes.
- It serves as **reserve material**.
- It also contributes to **frost resistance**

Polysaccharides

The polysaccharides found in nature either serve a structural function (structural polysaccharides) or play a role as a stored form of energy (storage polysaccharides).

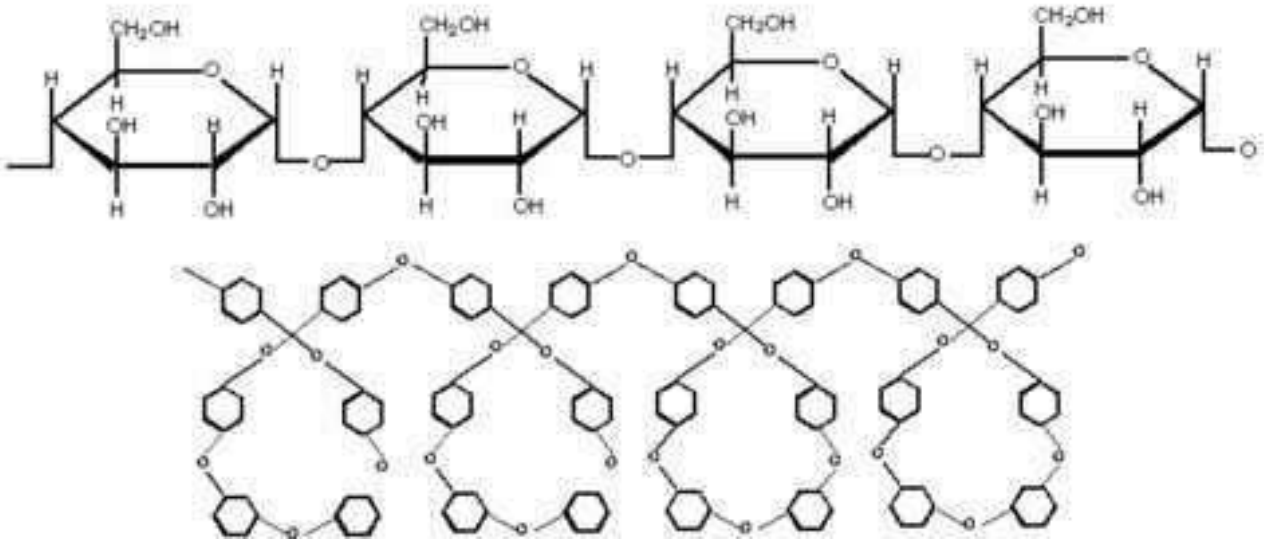
Storage polysaccharides

- Starch, galactomanans and inulin are important storage polysaccharides in plants.

Starch

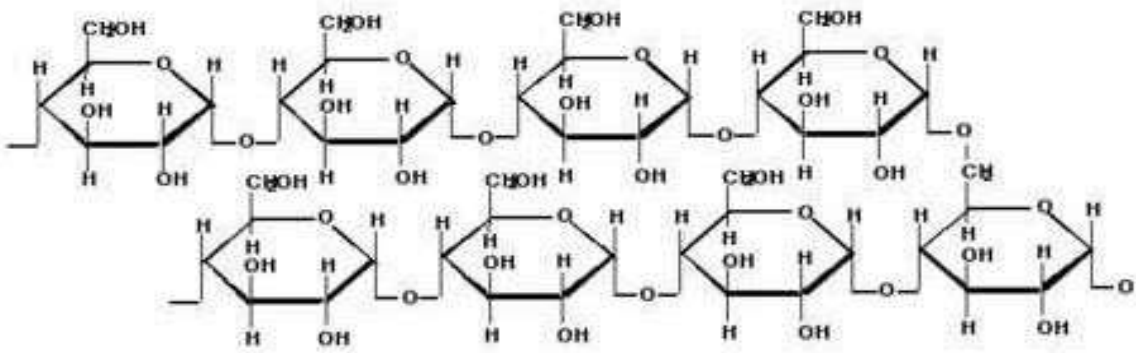
- The principal food-reserve polysaccharide in the plant kingdom is starch.
- It forms the major source of carbohydrate in the human diet.
- Starch has been found in some protozoa, bacteria and algae. But the major source is plants where it occurs in the seeds, fruits, leaves, tubers and bulbs in varying amount from a few percent to over 74%.
- Starch is an alpha-glucan that has structurally distinct components called amylose and amylopectin.
- A third component referred as the intermediate fraction has also been identified in some starches.
- Starch molecules are organized into quasi-crystalline macro molecular aggregates called granules.
- The shape of the granules are characteristics of the source of the starch.
- The two components, amylose and amylopectin, vary in amount among the different sources from less than 2% of amylose in waxy rice or waxy maize to about 80% amylose in amylomaize.
- The majority of starches contain 15 to 35% of amylose.

- The ratio of amylose and amylopectin is a function of the enzymes, **granulosis bound starch synthase (GBSS) and soluble starch synthase (SSS).**
- GBSS is able to synthesis amylose in a form that is not a substrate for branching enzyme to form amylopectin.
- Waxy mutants containing only amylopectin lack the GBSS but still contain soluble starch synthase.



- Amylose is made up of α- D-glucose units linked mostly in a linear way by α1-4 linkages
- It has a molecular weight of 150,000 to 1,000,000 depending on its biological origin.
- It has been shown that amylose has some elements of nonlinearity.
- It consists of a mixture of linear molecules with limited, long-chain branching involving α1-6 linkages.
- The branches contain several hundred glucose residues.
- Amylose gives a characteristic blue color with iodine due to the ability of the iodine to occupy a position in the interior of a helical coil of glucose units.
- Pure amylose binds 20% of iodine at 20^0C

Amylopectin



α (1→ 6) linkage
at crosslink

- Amylopectin is a **branched, water-insoluble polymer** comprised of thousands of D-glucose residues.
- The main chain of amylopectin consists of D-glucose residues joined by α (1- 4) glycosidic bonds.
- Side chains of glucose residues are attached to the main chain by α(1-6) glycosidic bonds.
- Each chain contains 15-25 glucose residues joined by α (1->4) bonds.
- It contains 94-96% α1-4 and 4-6% α 1-6 linkages.
- The molecular weight of amylopectin is in the order of 10^7 - 10^8.
- Robin and co-workers have proposed a model for amylopectin
- In this model, A and B chains are linear and have degree of polymerization as 15 and 45 respectively.
- The B chain forms the backbone of the amylopectin molecule and extend over two or more clusters.
- Each cluster of A chain are primarily responsible for the crystalline regions within the granule.
- The intercrystalline regions occur at regular intervals (60 - 70 ^{0}A) containing the majority of α- 1 - 6 linkages.

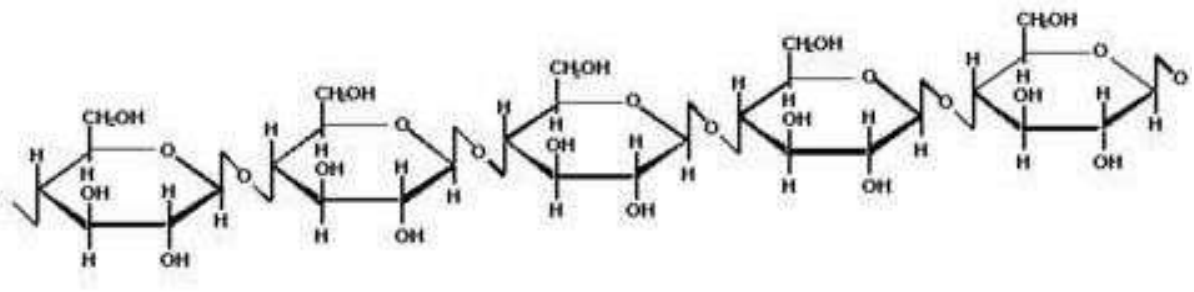
- The amylopectin molecule is 100 - 150 ^{0}A in diameter and 1200-4000 ^{0}A long.
- Within the granule, amylose may be located between amylopectin molecules and associated with the linear regions of the amylopectin molecule.
- Amylopectin produces a purple to red color with iodine.

Inulin

- Inulin is a non-digestible fructosyl oligosaccharide found naturally in more than 36000 types of plants.
- It is a storage polysaccharide found in onion, garlic, chicory, artichoke, asparagus, banana, wheat and rye.
- It consists of mainly, if not exclusively, of β 2-1 fructosyl-fructose links
- A starting glucose moiety can be present, but is not necessary.
- Inulin is a soluble fibre that helps maintain normal bowel function, decreases constipation**, lowers cholesterol and triglycerides**.
- It is used for **fat replacement and fibre enrichment** in processed foods.

Structural polysaccharides

Cellulose



- Cellulose is the most abundant organic substance found in nature.
- It is the principal constituent of cell walls in higher plants.
- It occurs in almost pure form (98%) in cotton fibers and to a lessor extent in flax (80%), jute (60-70%), wood (40-50%) and cereal straws (30-43%).
- It is linear, unbranched homoglycan of 10,000 to 15,000 D-glucose units joined by β1-4 linkages
- The structure of cellulose can be represented as a series of glucopyranose rings in the chair conformation.
- The most stable conformation for the polymer is the chair turned 180^0 relative to the adjacent glucose residues yielding a straight extended chain.
- Cellulose molecules within the plant cell walls are organized into biological units of structure known as microfibrils.
- A microfibril consists of a bundle of cellulose molecules arranged with its long axis parallel to that of the others.
- This arrangement permits the formation of intramolecular hydrogen bonding between the hydroxyl group of C-3 of one glucose residue and the pyranose ring oxygen atom of the next glucose residue.
- This hydrogen bond impart a double bond character to the glycosidic bond and impedes the rotation of adjacent glucose residues around the glycosidic bond.

- Within the microfibril, the adjacent cellulose molecules are linked by intermolecular hydrogen bond between C-6 hydroxyl group of one molecule and the glycosidic bond oxygen atom of adjacent cellulose molecule
- The cross section of the microfibril consists of a central crystalline core of about 5–30 nm short diameters.
- The central crystalline core contains around 50-100 cellulose molecules which are arranged in perfect three dimensional array and exhibits a crystalline structure.
- Surrounding this crystalline core is a region of paracrystalline matrix which contains about 100 polysaccharide molecules of cellulose and hemicellulose
- This region does not have perfect three-dimensional order and water molecules are able to penetrate the paracrystalline region but not the crystalline core.

Chemical properties of carbohydrates

Monosaccharides

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.

Reaction with alkali

Dilute alkali

- Sugars in weak alkaline solutions undergo isomerization to form 1, 2-enediol followed by the formation of a mixture of sugars.

Strong alkali

- Under strong alkaline conditions sugar undergoes caramelization reactions.

Reducing property of sugars

- Sugars are classified as either reducing or non-reducing depending upon the presence of potentially free aldehyde or keto groups.
- The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition.

- The aldehyde group of aldoses is oxidized to carboxylic acid. This reducing property is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions.
- All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose).
- If the reducing groups are involved in the formation of glycosodic linkage, the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).

Reaction with phenylhydrazine

- When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow precipitate is obtained.
- The precipitated compound is called as osazone. One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars.
- The osazone of D-galactose is different.
- Different sugars form osazone at different rates. For example, D-fructose forms osazone more readily than D-glucose.
- The osazones are crystalline solids with characteristic shapes, decomposition points and specific optical rotations.
- The time of formation and crystalline shape of osazone is utilized for identification of sugars.
- If methyl phenylhydrazine is used instead of phenylhydrazine in the preparation of osazone, only ketoses react.
- This reaction serves to distinguish between aldose and ketose sugars.

Reaction with acids

- Monosaccharides are generally stable to hot dilute mineral acids though ketoses are appreciably decomposed by prolonged action.

- Heating a solution of hexoses in a strong non-oxidising acidic conditions, hydroxyl methyl furfural is formed.
- The hydroxymethyl furfural from hexose is usually oxidized further to other products When phenolic compounds such as resorcinol, α-naphthol or anthrone are added, mixture of coloured compounds are formed
- The molisch test used for detecting carbohydrate in solution is based on this principle.
- When conc. H2SO4 is added slowly to a carbohydrate solution containing α-naphthol, a pink color is produced at the junction.
- The heat generated during the reaction hydrolyse and dehydrate it to produce furfural or hydroxymethyl furfural which then react with α-naphthol to produce the pink color.

Lecture 4

Physical properties of carbohydrates

A. Isomerism

In organic chemistry, isomerism is defined as the *existence of more than one compound with the same molecular formula.* A close observation of the structure of monosaccharides (hexoses) indicate that they possess same molecular formula ($C_6H_{12}O_6$) but with different physical and chemical properties. There are different types of isomerism

- **D-glucose and D-fructose** differ in the *position of carbonyl group* (aldehyde and ketone group). These two compounds are **functional isomers**.
- Another type of isomerism exhibited by *compounds possessing asymmetric carbon atom* like monosaccharides, is **stereoisomerism**. These stereoisomers *differ in the spatial arrangement of atoms or groups*. There are *two* types of stereoisomerisms - **geometrical and optical isomerism.**
  - **Geometrical isomers** (*cis-trans*) *differ in the spatial arrangement of atoms across a double bond*. Geometrical isomerism is not noticed among carbohydrates.
  - **Optical isomers** *differ in the arrangement of atoms around an asymmetric carbon atom.* The number of possible optical isomers can be calculated using the formula 2^n where n=number of asymmetric carbon atoms. For example, glucose contains *four* asymmetric carbon atoms and the possible optical isomers of glucose are $2^4 = 16$.

Epimers, enantiomers and diastereomers:

- **Epimers** are monosaccharides *differing in configuration around a single carbon atom other than the carbonyl carbon.* e.g. **Mannose and glucose** are epimers with respect to carbon 2. **Galactose and glucose** are epimers with respect to carbon 4.
- **Enantiomers** are *non- superimposable mirror images of each other*. They differ in the ability to rotate the plane polarized light. A solution of one

enantiomer rotates the plane of such light to the right, and a solution of the other to the left. **D-glucose and L-glucose** are examples of enantiomers**.**

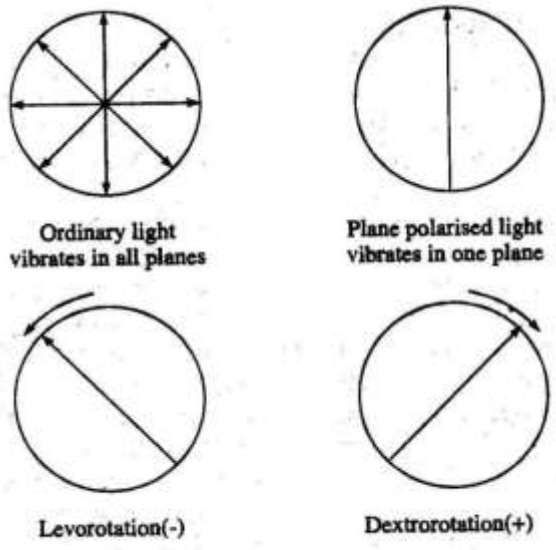
- **Diastereomers** are *stereoisomers that are not mirror images of each other*. **D-glucose, D-mannose, D-galactose** and other members of aldohexose are diastereoisomers.

B. Optical activity:

A ray of ordinary light vibrates in all directions at right angles to the direction in which the ray is travelling. When this light is passed through a Nicol prism, the emerged light *vibrates in only one direction* and such light is called as a **'plane polarized light'**

When a beam of plane polarized light is passed through a sugar solution, that is optically active, the plane-polarized light will be rotated either to the right (clockwise) or to the left (anticlockwise).

- When the plane polarized light is rotated to the **right**, the compound is **dextrorotatory** and is written as (+).
- If the plane polarized light is rotated to the **left**, the compound is **levorotatory** (-)



Property and rotation of plane polarised light

Optical activity is measured using polarimeter. Optical activity varies with the concentration of the sugar solution and length of the polarimeter tube where sugar solution is placed.

Specific rotation (α) of a sugar molecule is calculated by the formula:

$$(\alpha) = \frac{\text{Observed rotation}}{\text{Length of tube (dm)} \times \text{concentration}}$$

Where, T= temperature and D = D line of spectrum.

The specific rotation of some important sugars are given below:

D - glucose (dextrose) + 52.2 D - fructose (levulose) - 92.0 D - galactose + 80.5

D – mannose + 14.6 L - arabinose + 104.5 Sucrose + 66.5

C. Mutarotation:

- Mutarotation refers to the *change in optical rotation* when an aqueous sugar solution is allowed to stand.
- Sugars having potential *free aldehyde or keto group* exhibit mutarotation.
- Many sugars exist in two crystalline forms. For example, when D-glucose is dissolved in *water* and allowed to crystallize out by evaporation of water, one form of D-glucose is obtained. If D-glucose is crystallized from *acetic* acid or *pyridine,* another form of D-glucose is obtained. These two forms exhibit different physical and chemical properties.
- A freshly prepared aqueous solution of α-D glucose has a specific rotation of $+113^{o}$. If the solution of α- D-glucose is allowed to stand, the specific rotation changes to $+52.2^{o}$.

- Similarly, a fresh solution of β- D-glucose has a specific rotation of $+19^{o}$ which changes to $+52.2^{o}$ on standing.
- This change in optical rotation is called **mutarotation.** On standing in solution, the hemiacetal ring opens and reformed to give a mixture of α- and β-D-glucose having a specific rotation of $+52.2^{o}$.

Lecture 5

Chemical reactions of carbohydrates

Monosaccharides

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.

Reaction with alkali

Dilute alkali

- Sugars in weak alkaline solutions undergo isomerization to form **1, 2-enediol** followed by the formation of a mixture of sugars.

Strong alkali

Under strong alkaline conditions sugar undergoes **caramelization reactions.**

Reducing property of sugar

- Sugars are classified as either reducing or non-reducing depending upon **the presence of potentially free aldehyde or keto groups.**
- The reducing property is mainly due to the ability of these sugars to **reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition**.
- The aldehyde group of aldoses is oxidized to carboxylic acid.
- This reducing property is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions.
- All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose).
- If the reducing groups are **involved in the formation of glycosodic linkage**, the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).

Reaction with phenylhydrazine

- When reducing sugars are heated with **phenylhydrazine** at pH 4.7 a yellow precipitate is obtained.
- The precipitated compound is called as **osazone.**
- One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars.
- This reaction serves to distinguish between aldose and ketose sugars.

Reaction with acids

- Heating a solution of hexoses in a strong non-oxidising acidic conditions, **hydroxyl methyl furfural** is formed.
- The hydroxymethyl furfural from hexose is usually oxidized further to other products When **phenolic compounds such as resorcinol, α-naphthol or anthrone** are added, mixture of coloured compounds are formed
- The **molisch test** used for detecting carbohydrate in solution is based on this principle.
- When conc. H2SO4 is added slowly to a carbohydrate solution containing α-naphthol, a pink color is produced at the juncture.
- The heat generated during the reaction **hydrolyse and dehydrate** it to produce **furfural or hydroxymethyl furfural w**hich then react with α-naphthol to produce the pink color.

Lecture 7

LIPIDS - occurrence and classification

Occurrence and importance

- The word lipids is derived from the *Greek* word **'lipos'** meaning fat.
- Lipids are chemically heterogenous group of compounds that are ***insoluble in water but soluble in non-polar solvents such as chloroform.***
- Lipids occur in plants and animals as **storage and structural components**
- Structural lipids present in animals and plants are in the form of meat and vegetables respectively.
- Storage fats occur in milk and adipose tissue of farm animals and in seed oils
- Fats supply over **twice as much energy per unit weight** as proteins or carbohydrates.
- Lipids are *anhydrous due to non-polar nature* and represent *more energy* than carbohydrates which are heavily hydrated due to polar nature.
- The presence of lipids in diet contributes considerably *to palatability*.
- Lipids contribute palatability in two ways. They *induce olfactory responses*, namely, taste in the mouth and aroma through nose.
- Secondly, they contribute to the *texture of food* and is responsible for the mouth-feel.
- Lipids also *supply the* **essential fatty acids** which are not synthesised in human beings but are essential for growth.
- Lipids are ***essential for* the effective absorption of fat soluble vitamins *A, D, E and K from intestine.***
- Many ***enzymes require lipid molecules for maximal activity***. Examples are microsomal enzyme, glucose 6-phosphatase and mitochondrial enzyme, β-hydroxybutyrate dehydrogenase.
- **Adrenal corticosteroids, sex hormones and vitamin D3 (Cholecalciferol)** are synthesized from lipid derivative- cholesterol.
- Much of the lipid of mammals is located subcutaneously and **acts as insulation** against excessive heat loss to the environment.

- The subcutaneous lipid deposits also *insulate the important organs against mechanical trauma.*

Classification

Lipids are broadly classified into **simple, compound and derived lipids**

Classification of lipids

Lipids		
Simple lipids	*Compound lipids*	*Derived lipids*
Esters of fatty acids with glycerol and monohydric alcohols	Esters containing chemical groups in addition to alcohol and fatty acids	Substances derived form simple and compound lipids by hydrolysis. Alcohols, fatty acids, aldehydes, ketones, sterols and hydrocarbons
Depending upon the constituent alcohols they are further subdivided into fats or oils and waxes	Depending upon the chemical groups they are further subdivided into phospholipids, glycolipids, sulpholipids and lipoproteins	
Fats, also termed as triacylglycerols are esters of fatty acids with glycerol e.g. Plants-vegetable oils; Animals-ghee and butter	Phospholipids contain phosphate group. Phopholipids are further grouped as glycerophospholipids e.g., Lecithin if the constituting alcohol is glycerol or as sphingophospholipids if the alcohol is sphingosine e.g., sphingomyelin	
Waxes are esters of fatty acids and alcohols other than glycerol e.g., Plant wax-carnauba wax;	Glycolipids contain hexose units preferably galactose alongwith fatty acids and alcohol eg. cerebrosides	
Insect wax-beeswax;	Plant sulpholipids contain sulfated hexose with fatty acids and alcohol	
Animal wax -lanolin	Lipoproteins contain protein subunits along with lipids. Depending upon density and lipid compound they are further classified as VLDL. LDL and HDL.	

Lecture - 7 -9

Plant fatty acids

- Fatty acids are **carboxylic acids** with hydrocarbon chains of *2 to 36 carbons*.
- More than 200 fatty acids have been isolated from higher and lower plants.
- Of these, only a few are present in *large quantities* in most plant lipids. These are referred as **major fatty acids**.
- Fatty acids present in *smaller proportions* are called as **minor fatty acids.**
- Major and minor fatty acids are usually biosynthesised by analogous pathways.
- Fatty acids that occur *only in a few* plant species are called as **unusual fatty acids.**

Major fatty acids

- The major fatty acids are **saturated or unsaturated** with an unbranched carbon chain.
- The *saturated fatty acids* are ***lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acid***
- The ***unsaturated fatty acids are oleic (9-octadecenoic), linoleic (9, 12-octadecadienoic) and α-linolenic (9, 12, 15- octadecatrienoic) acid.***
- They are usually found in the lipids from all parts of plants
- The structure of fatty acids are written as *a symbol of two numbers separated by a colon:* the *first number* denotes the carbon atoms in the chain and the *second number denotes the number of unsaturation centres.*
- The *positions of double bonds* are specified *by superscript numbers* following (delta).
- Thus 18:2 ($\Delta^{9,\ 12}$) indicates an eighteen carbon fatty acid with two double bonds between C-9 and C-10, and between C-12 and C-13.
- The double bonds of all naturally occurring unsaturated fatty acids are in ***the cis configuration***.
- The *non-polar hydrocarbon chain* accounts for the *poor solubility of fatty acids* in water.

Minor fatty acids

The fatty acid composition of cow's and goat's milk are characterized by a high content of *short and medium chain saturated fatty acids*.

Some minor fatty acids

Common name	Carbon skeleton	Systematic name
Butyric	4:0	Butanoic
Caproic	6:0	Hexanoic
Caprylic	8:0	Octanoic
Capric	10:0	Decanoic

Unusual fatty acids

- The unusual fatty acids are found only in few individual species or genus or a whole family.
- Castor bean (*Ricinus communis*) seed oil is rich in **ricinoleic acid (**90%) which is *12- hydroxy oleic acid* $CH_3(CH_2)_5$-CH(OH)-CH_2-CH=CH-$(CH_2)_7$-COOH.
- Rape seed *(Brassica napus)* is rich in **erucic acid** *(cis-13-docosenoic acid* $CH_3(CH_2)_7$-CH=CH-$(CH_2)_{11}$-COOH).
- **Hydnocarpic and chaulmoogric acids** are found in chaulmoogra oil which is used in the *treatment of leprosy*.

Essential fatty acids

- Human body is unable to synthesise all fatty acids found in the body.
- Those fatty acids that **are not synthesised in the body** but required for normal body growth and maintenance are called as **essential fatty acids**.
- These fatty acids are to be *supplied through diet.*
- **Linoleic and linolenic acids** *are essential fatty acids*

- The longer chain fatty acids can be synthesised by the body from *dietary linoleic* and α-linolenic acids.
- **Arachidonic acid** is essential but it can be synthesised by the body from *linolenic acid*. It is also present in the meat
- Linoleic acid is grouped under **n-6 family** because the 6th carbon from methyl end possesses the double bond.
- Other fatty acids that are synthesised in the body from *linoleic acid* such as α-linolenic and arachidonic acids also belong to n-6 family.
- **α-Linolenic acid** belongs to n-3 family and is an essential fatty acid.
- The third carbon from the methyl end possess the double bond
- The organs and tissues that perform the more routine and generalized functions such as *adipose tissue, liver, muscle, kidney and the reproductive organs* tend to have *membranes in which n-6 family of polyunsaturated fatty acids predominate.*
- *Nervous tissue and retina of the eye* have a larger proportion of the longer chain acids with 5 or 6 double bonds predominantly of the n-3 family.
- Fish oils and spirulina are rich in fatty acids of n-3 family.
- **Arachidonic acid** serves as precursor for the **synthesis of prostaglandins,thrombaxanes *and* prostacyclins.**
- These fatty acid derivatives are called as 'eicosanoid' meaning 20 C compounds.
- The main source of these eicosanoids are the ***membrane phospholipids*** from which they are released by the action of phospholipase-A.
- **Phosphatidyl inositol** which contains a high concentration of arachidonic acid in carbon-2 of glycerol provides a major store of eicosanoid precursors.
- Phosphatidyl inositol is an important constituent of cell membrane phospholipids; upon stimulation by a suitable animal hormone it is cleaved into diacylglycerol and inositol phosphate, both of which act as ***internal signals or second messengers***

Simple lipids

- Lipids containing **only fatty acids and glycerol or long chain alcohols** (monohydric) are called as **simple lipids** which include **fats, oils and waxes**.

Fats and oils

- Triacylglycerols are the simplest lipids constructed from fatty acids and glycerol.
- They are also referred as *triglycerides, fats or neutral fats*.
- Triacylglycerols are composed of **three fatty acids esterified to the three hydroxyl groups of glycerol**
- When all the 3 fatty acid molecules are of *the same kind* the triacylglycerol is said to be **simple triacylglycerol.**
- **Mixed triacylglycerol** possesses two or more different fatty acids.
- Triacylglycerol that are solid at room temperature are called as **fats**
- Liquid triacylglycerols are called as oils.
- Neutral fats or oils are mostly composed of mixed triacyl glycerol.
- Fats are usually ***rich in saturated fatty acids*** and the *unsaturated fatty acids predominate in oils.*
- Most oil-producing plants store their lipids in the form of triacylglycerols.

Storage fats or oils

- Triacylglycerols are widely distributed in the plant kingdom. They are found both in *vegetative as well as reproductive tissues.*
- Triacylglycerols are normally stored in the **endosperm of the seed** although some plants store appreciable quantities of fat in the **fleshy fruit mesocarp**, for example, avocado.
- Some plants like the **oil palm**, store oils in both the **mesocarp (Palm oil)** and the **endosperm (Palm kernel oil).**
- The oil present **as droplets** in the cytoplasm of the **seed cells.**
- These droplets are called as **oil bodies** and are surrounded by a membrane composed of phospholipids and protein.
- Most of the common edible oils (groundnut, sunflower, gingelly, soybean, safflower, rice bran) contain limited number of the common fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids.
- Palm kernel and coconut oils contain higher amount of medium chain saturated fatty acids.

- Seed oils contain small amount of phospholipids, carotenoids, tocopherols, tocotrienols and plant sterols depending on the species of plant and degree of processing.

Structural or hidden fats in plants

- The leaves of higher plants contain upto 7% of their dry weight as fats;
- Some of them are present as surface lipids, the others as components of **leaf cells**, especially in the **chloroplast membrane**.
- The fatty acid composition of plant membrane lipids is very simple.
- Six fatty acids- palmitic, palmitoleic, stearic, oleic,linoleic and α-linolenic generally account for over 90% of the total fatty acids.

Waxes

- Waxes are **esters of long-chain saturated and unsaturated fatty acids with long chain alcohol.**
- The carbon number of fatty acids vary from 14 to 34 and that alcohol from 16 to 30. For example, beeswax is an ester of *palmitic acid* with a *30 carbon alcohol,* **triacontanol**

- Waxes are the **chief storage form of metabolic fuel** in marine phytoplanktons.
- Biological waxes find a variety of applications in the *pharmaceutical, cosmetic and other industries.*
- Lanolin from lamb's wool, beeswax, carnauba wax, spermaceti oil from whales are widely used in the **manufacture of lotions, ointments and polishes**.
- Waxes are *not easily hydrolysed like fats or digested by lipases*.

Liquid wax - Jojoba oil

- About 50% of the **seed dry weight of jojoba** consists of a liquid wax which is unique in the plant kingdom and is similar to sperm whale oil.
- The wax is made up of straight chain esters with an average total chain length of 42 carbons

- Jojoba wax has a wide range of industrial uses including *cosmetics, pharmaceuticals, extenders for plastics, printers ink, gear oil additives and various lubricants.*
- The oil is *highly stable* and can be stored for years *without becoming rancid.*

Cuticular wax

- The *outermost surface of the cell walls of epidermal cells* are covered with a **hydrophobic cuticle** which contains wax called cuticular wax.
- The main components of cuticular waxes are *hydrocarbon (odd chain alkanes) and its derivatives, wax esters, free aldehydes, free acids, free alcohols* and other components like *mono esters of phenolic acids and aliphatic alcohols.*
- The main function of the cuticular wax is to **reduce the excessive losses and gains of water** by the underlying tissue.
- It also helps in *protecting the tissues from chemical, physical and biological attack.*

Compound lipids

Compound lipids contain certain chemical groups *in addition to alcohol and fatty acids.*

- These groups of lipids include **glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins.**

Glycerophospholipids

- The important **structural lipid in biological membrane** is glycero phospholipid which contains **glycerol, fatty acids phosphoric acid and a nitrogenous base**.
- The general structure of a glycerophospholipid is given below
- Without alcoholic residue (X), it is called as **phosphatidic acid**
- Depending on the *alcoholic residue attached to phosphatidic acid,* they are named as

    i. Phosphatidyl choline (lecithin)
    ii. Phosphatidyl ethanolamine (cephalin)
    iii. Phosphatidyl serine
    iv. Phosphatidyl inositol

v. Phosphatidyl glycerol (which include monophosphatidyl glycerol and diphosphatidyl glycerol or cardiolipin).

Phosphatidyl choline (lecithin)

- Lecithin contains **glycerol, fatty acids, phosphoric acid and a nitrogenous base, choline**
- Lecithins are widely distributed in the *membranes of cells having both metabolic and structural functions.*
- **Dipalmityl lecithin** is a very effective ***surface active agent*** preventing adherence due to surface tension of the inner surfaces of the lungs.
- Most phospholipids have a *saturated fatty acid in the C1 position* but an *unsaturated fatty acid in the C2 position.*

Phosphatidyl ethanolamine (cephalin)

- The cephalin differs from lecithin *only in the nitrogenous group where ethanolamine is present instead of choline*

Phosphatidyl serine

- The hydroxyl group of the amino acid L-serine is esterified to the phosphatidic acid

Phosphatidyl inositol

- Phosphatidyl inositol is an important **constituent of cell membrane phospholipids;**
- Upon stimulation by a suitable animal hormone it is cleaved into **diacylglycerol and inositol phosphate,** both of which act as **internal signals or second messengers.**

Phosphatidyl glycerol and diphosphatidyl glycerol (Cardiolipin)

- **Cardiolipin** is a phospholipid that is found in **membranes of mitochondria**.
- It is formed from phosphatidylglycerol

Sphingophospholipids

- The phosphate and fatty acids are attached to the alcohol **sphingosine** instead of glycerol in sphingophospholipids.
- The fatty acids are attached through an **amide linkage** rather than the ester linkage.
- The base present is normally **choline.**
- The structure of the parent compound sphingosine and phytosphingosine are shown below
- C-1, C-2 and C-3 of the sphingosine or phytosphingosine bear functional groups,-OH, -NH2 and -OH respectively, which are structurally homologous with the three hydroxyl groups of glycerol.
- Carbon 4 to 18 in sphingosine and C-5 to 18 in phytosphinogsine resembles that of a fatty acid.
- When a fatty acid is attached by an amide linkage to the -NH_2, group the resulting compound is a **ceramide** which is similar to diacyl glycerol
- **Ceramide** is the fundamental structural unit common to all sphingophospholipids
- **Sphingophospholipids** are found in the seeds of several plant species.
- There is a range of molecular species among the phospholipid sub groups which differ from one another in the fatty acid composition
- All the sub groups of phospholipids are found in plant photosynthetic tissue
- Animal phospholipids contain mostly fatty acids with chain length between 16 and 20. The predominant fatty acids are palmitic, stearic, oleic, linoleic and arachidonic.
- Plant leaf phospholipids have a more limited range with very few fatty acids greater than C-18.
- The approximate proportion of each phospholipid expressed as a percentage of the total phospholipid present is phosphatidyl choline, 45%; phosphatidyl ethanolamine, 10%;
- Trace amounts of phosphatidyl serine, phosphatidyl inositol, 8%; monophosphatidyl glycerol, 35%, diphosphatidylglycerol, 2%.
- The **diphosphatidyl glycerol** is present in the ***inner mitochondrial membrane.***
- The phospholipids are only ***minor components of seed lipids*** in which triacylglycerol predominate.

- The ***most abundant*** mammalian phospholipid is ***phosphatidyl choline***.
- The phospholipids carry an electrical charge and interact with water. They are called as **polar or hydrophilic molecules and also as *amphiphilic* molecules**.
- The sphingomyelins, the main sphingophospholipids of animals, are not present in plants.

Glycolipids and sulpholipids

- Glycolipids are structurally characterised by the presence of one or more monosaccharide residues and the **absence of a phosphate.**
- They are O-glycoside of either sphingosine or glycerol derivative. The monosaccharides commonly attached are **D-glucose, D-galactose or N-acetyl D-galactosamine.**
- Monogalactosyl diglycerides and digalactosyl diglycerides have been shown to be present in a wide variety of higher plant tissues
- The 3 position of 1, 2-diacylglycerol is linked to 6- sulpho-6-deoxy D-glucose by an ***α-glycosidic bond in plant sulpholipid***
- The predominant fatty acid present in sulpholipid is ***linolenic acid.***
- The sulpholipid is mostly present in ***chloroplasts, predominantly in the membranes of thylakoid.***
- ***Cerebrosides*** are composed of a monosaccharide residue glycosidically linked to C-1 of an N-acylated sphingosine derivative.
- The monosaccharide is ***D-glucose in plants*** and ***D-galactose in animals***.

Lipoprotein

- Protein molecules associated with triacylglycerol, cholesterol or phospholipids are called lipoproteins.
- Triacylglycerols derived from intestinal absorption or from the liver are not transported in the free form in circulating blood plasma, but move as ***chylomicrons, as very low density lipoproteins (VLDL) or as free fatty acids (FFA) - albumin complexes.***
- Besides, two more physiologically important groups of lipoproteins are ***low density lipoprotein (LDL) and high density lipoprotein (HDL).***

- The major lipid components of **chylomicrons and VLDL are triacylglycerol,** whereas the predominant lipids in **LDL and HDL are cholesterol and phospholipid respectively.**
- The protein part of lipoprotein is known as **apoprotein.**
- Lipoproteins occur in milk, egg-yolk and also as ***components of cell membranes***

Sterols

- The characteristic structure of sterol is their **steroid nucleus** consisting of four fused rings, three with six carbons (Phenanthrene) and one with five carbons (cyclopentane).
- This parent structure is known as **cyclopentano perhydro phenanthrene**.
- The steroid nucleus is almost **planar** and relatively rigid.
- Steroids with methyl groups attached to carbons 10 and 13 and 8-10 carbon atoms in the side chain at position 17, ***an alcoholic group at position 3 and a double bond between carbons 5 and 6 are classified as sterols.***
- **Cholesterol** is the ***most abundant sterol*** in animals.
- Cholesterol is a ***major component of animal plasma membranes*** and occurs in lesser amounts in the membranes of their subcellular organelles.
- Its polar OH group gives it a ***weak amphiphilic character***, whereas its fused ring system provides it with greater stability than other membrane lipids.
- Cholesterol is therefore an ***important determinant of membrane properties***.
- It is also abundant in blood plasma lipoproteins where 70% of it is esterified to long chain fatty acids to form cholesteryl esters.
- Plants contain little cholesterol. Rather, the most common sterol components of their membranes are ***stigmasterol and β-sitosterol*** which differ from cholesterol only in their aliphatic side chains.
- Yeast and fungi have another sterol named ***ergosterol*** which has a double bond between C7 and C8.
- In animal system, cholesterol functions as a **precursor of various physiologically important compounds such as vitamin D, bile acids, female sex hormones and corticosteroids**.

- In plants, cholesterol functions as an intermediate compound in the ***synthesis of various phytosteroids*** such as ***saponins, cardiac glycosides, phytoecdysteroids and brassinosteroids.***

Brassinosteroids

- In 1979, a ***novel plant growth regulating steroidal substance*** called **brassinolide** was isolated from rape (*Brassica napus*) pollen
- More than 24 compounds are known (designated as BR1, BR2).
- Pollen is the richest source.
- Brassinosterols are active at concentration much lower (nM to pM range) than those of other types of hormones.
- Brassinosterols elicit a pronounced ***stem elongation response*** in dwarf pea epicotyls, mung bean epicotyls that are sensitive also to gibberellic acids but not auxins.
- Brassinosteroids are thought by some to be a new class of plant hormones.
- The evidences are
    i. They are widely distributed in the plant kingdom.
    ii. They have an ***effect at extremely low concentration.***
    iii. They have a range of effects which are different from the other classes of plant hormones.
    iv. They can be ***applied to one part of the plant and transported to another where in very low amounts elicit a biological response.***

- They are widely distributed including dicots, monocots, gymnosperms and algae, and in various plant parts such as pollen, leaves, flowers, seeds, shoots and stems.
- Among the naturally occurring **brassinosteroids, brassinolide and castasterone** are considered to be the most important because of their wide distribution as well as their potent physiological activity.

Physiological effects of brassinosteroids

i. Promotion of ethylene biosynthesis by ***stimulating ACC synthase activity***.
ii. Promote ***elongation of vegetative tissue*** in a wide variety of plants at very low concentration.
iii. They are powerful inhibitors of root growth and development (via ethylene).
iv. They enhance ***resistance to chilling, disease, herbicides*** and salt stress, promote germination and decrease fruit abortion and drop.

Practical application of BR

- Large scale field trials in China and Japan over a six year period have shown that **24-epibrassinolide**, an alternative to brassinolide, increased the production of agronomic and horticultural crops (***wheat, corn, tobacco, watermelon and cucumber).***
- **Environmental stresses** were also seem to be allievated by treatment with brassinolide.

Properties of fats

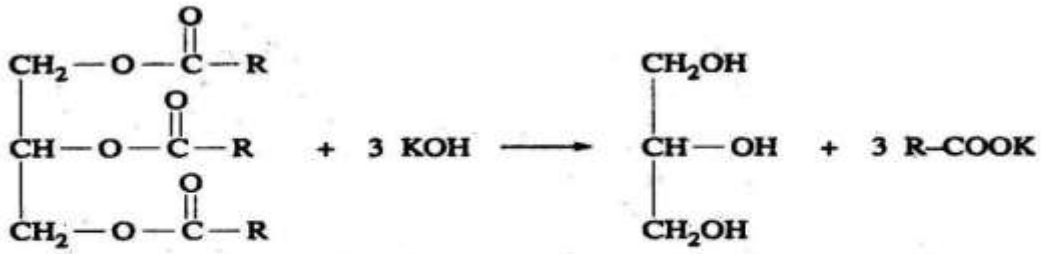
Physical properties:-

- Fats are greasy to touch and leave an oily impression on paper.
- They are **insoluble in water** and soluble in organic solvents.
- Pure triacylglycerols are tasteless, odourless, colourless and neutral in reaction.
- They have **lesser specific gravity** (density) than water and therefore float in water.
- Though fats are insoluble in water, they can be broken down into minute dropletsand dispersed in water. This is called ***emulsification***.
- A satisfactory emulsion is one highly stable and contains very minute droplets with diameter less than 0.5 µm.
- Examples of ***naturally occurring emulsions are milk and yolk of egg***. But they are not mere fat droplets in water.
- They contain hydrophilic colloidal particles such as proteins, carbohydrates and phospholipids which act as stabilizing agents.

- ***Emulsification greatly increases the surface area of the fat*** and this is an essential *requisite for digestion of fat* in the intestine.

Chemical properties:-

- The most important chemical reaction of neutral fat is their hydrolysis to yield three molecules Alkali hydrolysis (saponification)The process of alkali hydrolysis is



Saponification of triacylglycerol

Enzyme hydrolysis:-

- Hydrolysis of triacylglycerol may be accomplished enzymatically through the action of **lipases.**
- Lipases are widespread in both plants and animals.

Rancidity

- Development of disagreeable odour and taste in fat or oil upon storage is called ***rancidity.***
- Rancidity reactions may be due to **hydrolysis of ester bonds** (hydrolytic rancidity) or due to **oxidation of unsaturated fatty acids** (oxidative rancidity).

Hydrolytic rancidity

- This involves ***partial hydrolysis of the triacylglycerol*** to mono and diacylglycerol.
- The hydrolysis is hastened by the presence of ***moisture, warmth and lipases*** present in fats or air.
- In fats like butter which contains a high percentage of volatile fatty acids, hydrolytic rancidity produces disagreeable odour and taste due to the liberation of the ***volatile butyric acid***.

- Butter becomes rancid more easily in summer.

Oxidative rancidity

- The unsaturated fatty acids are oxidised at the double bonds to form **peroxides**, which then decompose to form aldehydes and acids of objectionable odour and taste.

Hydrogenation

- The ***degree of unsaturation*** of the fatty acids present in triacylglycerol determines whether a fat is liquid or solid at room temperature.
- The presence of *more unsaturated fatty acids* ***lower the melting point***.
- The presence of highly unsaturated fatty acids makes the oil more susceptible to ***oxidative deterioration***.
- The objective of hydrogenation is to ***reduce the degree of unsaturation and to increase the melting point of the oil.***
- The oil can be selectively hydrogenated by careful choice of ***catalyst and temperature.***
- ***Hydrogenation of unsaturated fats in the presence of a catalyst*** is known as **hardening**.
- Normally the process of hydrogenation is partial so as to get desired characteristics and to avoid products with high melting points.
- Hydrogenation is carried out in a closed container in the presence of **finely powdered catalyst (0.05 - 0.2% of nickel) at temperature as high as 180^{o}C**.
- The catalyst is usually removed by ***filtration.***
- During hydrogenation process a proportion of the ***cis double bonds are isomerized to trans double bonds*** and there is also ***migration of double bonds***.
- The hydrogenation process has made it possible to extend the food uses of a number of vegetable oils and marine oils whose melting points are too low.

Constants of fats and oils

- Since fats and oils form essential nutrient of human diet, it is necessary ***to identify a pure fat or to determine the proportion of different types of fat or oil mixed as adulterant in edible oils and fats like butter and ghee.***
- With an adequate knowledge of the characteristic composition of fats or oils, it is possible to identify the fat or oil under investigation.
- ***The chemical constants also give an idea about the nature of fatty acids present in fats or oils.***
- Even though ***gas chromatographic method*** is available to identify and quantify the fatty acids present in fat or oil, the physical and chemical constants are still used in routine public health laboratories where such sophisticated facilities are lacking.

Lecture 10

Physical constants

i. Specific gravity

- Since different oils have different specific gravity, any variation from normal value shows mixture of oils.

ii. Refractive index

- Fats have ***definite angles of refraction.***
- Variation from the normal value indicates adulteration of fats or oils.

iii. Solidification point or setting point

- Solidification point is ***the temperature at which the fat after being melted, sets back to solid or just solidifies.***
- Each fat has a specific solidification point.

Chemical constants

i. Saponification number

- It is defined as ***milligrams of KOH required to saponify 1 gm of fat or oil***.
- Saponification number is ***high for fat or oil containing low molecular weight*** or short chain fatty acids and vice versa.
- It gives a clue *about the molecular weight and size of the fatty acid in the fat or oil.*

ii. Iodine Number

- It is defined as the ***number of grams of iodine taken up by 100 grams of fat or oil.***
- Iodine number is a ***measure of the degree of unsaturation of the fatty acid.***
- Since the quantity of the iodine absorbed by the fat or oil can be measured accurately, it is possible to calculate the relative unsaturation of fats or oil.

iii. Reichert-Meisel number (R.M. number)

- This is a **measure of the volatile soluble fatty acids**.
- It is confined to butter and coconut oil.

- It is defined as ***the number of millilitres of 0.1 N alkali required to neutralise the soluble volatile fatty aicds contained in 5 gm of fat.***
- The determination of Reichert-Meisel number is important to the food chemist because it helps to ***detect the adulteration in butter and ghee***.
- Reichert-Meisel value is reduced when animal fat is used as adulterant in butter or ghee.

iv. Polanski number

- Ghee may be adulterated by the addition of ***insoluble, non-volatile fatty acids (by addition of animal fat).***
- This can be tested by finding out the Polanski number.
- It is defined as ***the number of millilitres of 0.1 N potassium hydroxide solution required to neutralise the insoluble fatty acids (not volatile with steam distillation) obtained from 5 gm of fat.***

v. Acetyl number

- It is defined as ***the amount in millilitres of potassium hydroxide solution required to neutralise the acetic acid obtained by saponification of 1 gm of fat or oil after acetylation.***
- Some fatty acids contain ***hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride***.
- This results in the introduction of acetyl groups in the place of free hydroxyl groups.
- The acetic acid in combination with fat can be determined by titration of the liberated acetic acid from acetylated fat or oil with standard alkali.
- Acetyl number is thus a ***measure of the number of hydroxyl groups present in fat or oil.***

vi. Acid number

- It is defined as ***the milligram of potassium hydroxide required to neutralise the free fatty acids present in one gram of fat or oil.***
- Acid number ***indicates the amount of free fatty acids present in fat or oil***.
- The ***free fatty acid content increases with age of the fat or oil.***

Molecular aggregation of phospholipids

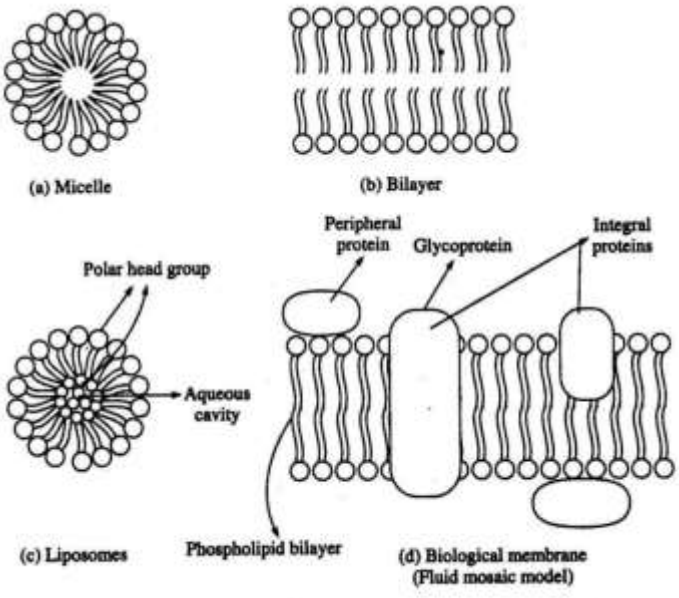
- Glycerophospholipids are virtually insoluble in water.
- Depending on the precise conditions and the nature of lipids used, ***three types of lipid aggregates can form when amphipathic lipids are mixed with water.***

Micelles

- Free fatty acids, lysophospholipids and sodium dodecyl sulphate (SDS) form micelle.
- **Micelles** are relatively small spherical structures involving a few dozen to few thousand molecules arranged so that their **hydrophobic regions** aggregate in the interior excluding water and their ***hydrophilic head groups are at the surface in contact with water.***
- This molecular arrangement eliminates unfavourable contacts between water and the hydrophobic tails

Bilayer

- A second type of lipid aggregate in water is the bilayer in which two lipid monolayers combine to form a ***two dimensional sheet.***
- The hydrophobic portions in each monolayer interact excluding water.
- The hydrophilic head groups interct with water at the two surfaces of the bilayer lipid bilayers form the structural basis of biological membranes



Structures of micelle, bilayer, liposome and biological membrane

Liposomes

- The third type of lipid aggregate is formed ***when a lipid bilayer folds back on itself to form a hollow sphere*** called a liposome or vesicle.
- These bilayer vesicles enclose water creating a separate aqueous compartment

Biological membranes

- Proteins and polar lipids account for mass of biological membranes.
- The relative proportions of protein and lipid differ in different membranes, reflecting the diversity of biological roles.
- ***Amphipathic molecules*** form a lipid bilayer with the ***non-polar region of lipids facing outward.***
- In this lipid bilayer, globular proteins are embedded at regular intervals held by hydrophobic interactions.
- Some proteins protrude from one or other face of the membrane ***(peripheral proteins); some span its entire width (integral proteins).***
- The individual lipid and protein subunits in a membrane form a ***fluid mosaic***
- The membrane is fluid because the interactions among lipids, between lipids and proteins are non-covalent, leaving individual lipid and protein molecules free to move laterally.
- One of the key functions of a membrane is to ***control the passage of substances across it.***
- They are said to be ***selectively permeable.*** The different membranes of the cell have different selective permeabilities.

Lecture 11

AMINO ACIDS AND PROTEIN

- The word "Protein" was coined by ***J.J. Berzelius*** in 1838 and was derived from the Greek word **"Proteios"** meaning the *'**first rank**'*.
- Proteins are macromolecular polymers composed of ***amino acids*** as the basic unit linked by peptide bonds.
- *Amino acids* are the **fundamental structural units of all proteins**.
- These biopolymers contain carbon, hydrogen, oxygen, nitrogen and sulphur.
- The elementary composition of most proteins is very similar; approximate percentages are C=50-55, H=6-8, O=20-23, N=15-18 and S=Traces

Occurrence:

- Proteins are found in all ***living cells***.
- They form essential ***constituent of protoplasm, cell membrane and nuclear material.***
- They may be present as *simple* proteins or *complexes with lipids or nucleic acids*.
- Proteins from different tissues such as ***muscle, bone, brain, blood and other biological fluids*** differ in composition and properties.
- In cereal and leguminous plants, ***seeds*** contain comparatively *higher* amounts of protein than stem, leaves and flowers.
- *Tuber crops* usually contain *less* amounts of protein in all parts.
- **Enzymes** are specialized proteins with ***catalytic activities*** and are present in all living organisms.
- Proteins serve as ***regulators of metabolic reactions***, directly as components of enzymes and indirectly in the form of chemical messengers known as **hormones** as well as *receptors for hormones*.
- They ***regulate and integrate*** the numerous physiological and metabolic processes in the body.
- Proteins are the **center of action** in many biological processes.

Amino acids

All proteins are formed from **20** different **amino acids**. All the amino acids have trivial or common names *based on the source* from which they were first isolated or based on their properties. For eg.

Asparagine was named so, as it was isolated from *asparagus* and **glycine** was so named because of its *sweet taste* (Greek:'glykos' meaning sweet).

All the 20 amino acids, except **proline**, found in proteins have an amino group and a carboxyl group attached to the same carbon atom, namely the **α-carbon**. They differ only in the *side chains* (R groups). The 20 amino acids found in proteins are referred as the ***standard or normal or protein amino acids***.

There are many other amino acids found in nature but do not occur in proteins. They are referred as ***non-protein amino acids***.

Classification of protein amino acids

The protein amino acids are classified according to the **chemical nature** of their R groups as ***aliphatic, aromatic, heterocyclic and sulphur containing amino acids***. More meaningful classification of amino acids is based on the ***polarity of the R groups***. The polarity of the R groups varies widely from totally non-polar to highly polar. The 20 amino acids are classified into four main classes whose structures, three-letter and one-letter symbols are given below

a) Amino acids with non-polar or hydrophobic, aliphatic R groups

- This group of amino acids includes ***glycine, alanine, valine, leucine, isoleucine and proline.*** The hydrocarbon R groups are *non-polar and hydrophobic*.
- The side chains of alanine, valine, leucine and isoleucine are important in *promoting hydrophobic interactions* within protein structures.
- The minimal steric hindrance of the glycine side chain (hydrogen) allows more flexibility than other amino acids.

- On the other hand, the imino group of proline is held in a rigid conformation and reduces the structural flexibility of the protein.

b) Amino acids with non-polar aromatic R groups

- This group includes ***phenylalanine, tyrosine and tryptophan***.
- All these amino acids participate in ***hydrophobic interactions***, which is stronger than aliphatic R groups because of stacking one another.
- Tyrosine and tryptophan are more polar than phenylalanine due to the presence of hydroxyl group in tyrosine and nitrogen in the indole ring of tryptophan.
- The absorption of ultraviolet (UV) light at 280 nm by tyrosine, tryptophan and to a lesser extent by phenylalanine is responsible for the characteristic strong absorbance of light by proteins. *This property* is exploited in the *characterization and quantification of proteins*.

c) Amino acids with polar, uncharged R groups

- This group of amino acids includes ***serine, threonine, cysteine, methionine, asparagine and glutamine.***
- The *hydroxyl group* of ***serine and threonine***, the *sulphur* atom of ***cysteine and methionine*** and the *amide group* of ***asparagine and glutamine***, contribute to the polarity.
- The R groups of these amino acids are more hydrophilic than the non-polar amino acids.

d) Amino acids with charged R groups

- **Acidic:** The two amino acids with acidic R groups are ***aspartic and glutamic acids.*** These amino acids have a net negative charge at pH 7.0.
- **Basic:** This group includes ***lysine, arginine and histidine***. The R groups have a net positive charge at pH 7.0. The **lysine** has a second *ε-amino group*; **arginine** has a positively charged *guanidino group*; and **histidine** has an *imidazole* group.

Properties of amino acids

Physical properties:-

- Amino acids are white crystalline substances.
- Most of them are ***soluble in water*** and insoluble in non-polar organic solvents (e.g., chloroform and ether).
- Aliphatic and aromatic amino acids particularly *those having several carbon atoms have limited solubility in water* but readily soluble in polar organic solvents.
- They have ***high melting points*** varying from 200-300^{o}C or even more.
- They are tasteless, sweet or bitter.
- Some are having good *flavour*. **Sodium glutamate** is a valuable *flavouring* agent and is used in the preparation of certain dishes and sauces.

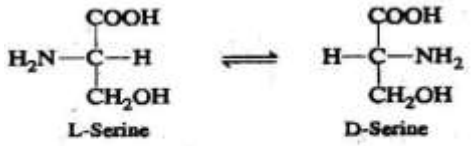
Amphoteric nature of amino acids

- Amino acids are ***amphoteric*** compounds, as they contain **both acidic (COOH)** and **basic (NH2) groups.**
- They can react with both alkalies and acids to form salts.
- In ***acid solution*** amino acids carry **positive charges** and hence they move towards cathode in an electric field.
- In ***alkaline solution***, the amino acids carry negative charges and therefore move towards anode.
- When an amino acid is dissolved in water, it exists as **inner salt** carrying both positive and negative charges. This occurs as a result of *dissociation of carboxyl* group to release the H+ ion, which passes from the carboxyl to the amino group. The amino acids possessing ***both positive and negative charges*** are called **zwitterions.**
- The zwitterion reacts as an **acid** with a base by ***liberating a proton (H+)*** from the NH3+ group and as a result possesses a ***net negative charge***.
- On the other hand, zwitterions reacts with an acid **as base**, ***combining with the proton (H+)*** of the acid resulting in the formation of a compound having a **net positive charge**. These reactions are reversible.

- The **pH** at which the amino acid has *no tendency to move either towards positive or negative* electrode is called **isoelectric pH or isoelectric point**.
- At *isoelectric pH*, the amino acid molecule bears a ***net charge of zero.***

Isomerism

- All amino acids except proline, found in protein are ***α-amino acids*** because NH2 group is attached to the α-carbon atom, which is next to the COOH group.
- Examination of the structure of amino acids reveals that ***except glycine***, all other amino acids possess ***asymmetric carbon*** atom at the alpha position.
- Because of the *presence of asymmetric carbon atom*, amino acids exist in ***optically active forms***.
- For example, in the steric configuration for serine, the *carboxyl group* is written on the ***top***, while the *amino group* is written to the ***left*** in the case of L-serine and to the *right* in the case of D-serine. This distinction will hold good for all the amino acids having asymmetric carbon atoms.



Structure of D- and L-Serine

- 'D' and 'L' do not refer to the optical rotation, but to the steric configuration of amino group to the right and left side of the carboxyl group.
- The ***direction of optical rotation*** of amino acid is indicated by the symbol **+ or -,** which follows the designation 'D' or 'L'.
- The steric configuration and optical rotation of an amino acid may be simultaneously expressed as D (+) or D (-) and L (+) or L (-).
- L-forms are more common than D-forms and most of the ***naturally occurring amino acids*** are ***L-amino acids.***

Chemical properties

a) Reactions due to amino group

Reaction with formaldehyde (Formal titration)

- Amino acid exists as zwitterion in aqueous medium. If an amino acid solution is treated with *excess* of ***neutralized formaldehyde solution***, the amino group combines with formaldehyde forming **dimethylol amino acid** which is an *amino acid formaldehyde complex*.
- Hence the ***amino group is protected*** and the proton released is titrated against alkali.
- This method is used to find out the ***amount of total free amino acids*** in plant samples.

Reaction with nitrous acid

- Nitrous acid reacts with the amino group of amino acids to form the corresponding hydroxyacids and liberate nitrogen gas.

Reaction with ninhydrin

- Ninhydrin is a ***strong oxidizing agent***.
- When a solution of amino acid is boiled with ninhydrin, the amino acid is ***oxidatively deaminated*** to produce ammonia and a ketoacid.
- The **keto acid** is decarboxylated to produce an ***aldehyde*** with one carbon atom less than the parent amino acid.
- The *net reaction is that ninhydrin oxidatively deaminates and decarboxylates* α*-amino acids to* CO_2*,* NH_3 *and an aldehyde.*
- The *reduced ninhydrin* then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple coloured compound known as **Ruhemann's purple**.
- This ninhydrin reaction is employed in the ***quantitative determination of amino acids.***

- Proteins and peptides that have free amino group(s) (in the side chain) will also react and give colour with ninhydrin.

b) **Reactions due to carboxyl group**

Decarboxylation

- The carboxyl group of amino acids is ***decarboxylated*** to yield the corresponding **amines.** Thus, the vasoconstrictor agent, ***histamine*** is produced from histidine.
- Histamine stimulates the ***flow of gastric juice into the stomach*** and the ***dilation and constriction of specific blood vessels.***
- Excess reaction to histamine causes the ***symptoms of asthma*** and various ***allergic reactions.***

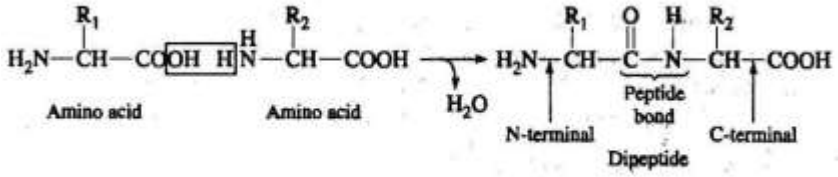
Essential amino acids

- Most of the prokaryotic and many eukaryotic organisms (plants) are capable of synthesizing all the amino acids present in the protein. But higher animals including man possess this ability only for certain amino acids.
- *The amino acids, which are needed for normal functioning of the body but* ***cannot be synthesized from metabolic intermediates,*** *are called* ***essential amino acids***.
- These must be obtained from the ***diet*** and a *deficiency* in any one of the amino acids *prevents growth and may even cause death.*
- ***Methionine, Arginine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Histidine, and Lysine are the essential amino acids*** (Remember **MATTVILPHLy**).

Peptide

- Amino acids are linked together by formation of ***covalent bonds.***
- The covalent bond is formed between the ***α-carboxyl group of one amino acid and the α-amino group of the next amino acid.***
- The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a **peptide bond** and the compound formed is a **peptide.**

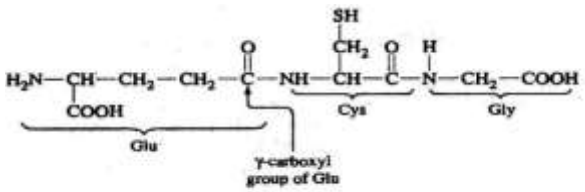
- The peptide formed between two amino acids is a dipeptide; three amino acids is a tripeptide; few amino acids are an oligopeptide and many amino acids is a polypeptide.
- In writing the peptide structure, the amino terminal (N-terminal) amino acid is written first and carboxyl terminal (C-terminal) amino acid written last.



Formation of peptide from amino acids

Peptides of physiological interest

- ***Glutathione*** is a commonly occurring tripeptide ***(γ-glutamyl cysteinyl glycine***) in many living organisms.



Structure of glutathione, a tripeptide

- It has a role in ***detoxification of toxic compounds*** in physiological system.
- The nanapeptides (nine amino acids), oxytocin and vasopressin are important animal peptide hormones.
- **Oxytocin** *induces labor in pregnant women* and controls contraction of uterine muscle.
- **Vasopressin** plays a role in *control of blood pressure* by regulating the contraction of smooth muscles.
- A dipeptide ***L-aspartyl-L-phenylalanine***, is of *commercial importance*. This dipeptide is about 200 times sweeter than cane sugar. The methyl ester of this dipeptide is called as ***aspartame*** and marketed as an *artificial sweetener* for ***diabetics.***

Lecture 12

Proteins – Importance and classification

Classification of protein

Proteins are classified based on their

- Solubility and composition
- Function
- Shape & size

A. Classification based on solubility and composition

According to this classification, proteins are divided into three main groups as **simple, conjugated and derived proteins**.

(i) Simple proteins

- Simple proteins yield *on hydrolysis*, **only amino acids.**
- These proteins are further classified *based on their solubility in different solvents as well as their heat coagulability.*

Albumins

- Albumins are readily *soluble in water, dilute acids and alkalies*
- coagulated by heat.
- Seed proteins contain albumin in lesser quantities.
- Albumins may be precipitated out from solution using high salt concentration, a process called **'salting out'**.
- They are deficient in **glycine.**
- *Serum albumin and ovalbumin (egg white)* are examples.

Globulins

- Globulins are ***insoluble or sparingly soluble in water***, but their solubility is greatly increased by the *addition of neutral salts such as sodium chloride*. These proteins are coagulated by heat.
- They are deficient in ***methionine***.

- *Serum globulin, fibrinogen, myosin of muscle and globulins of pulses* are examples.

Prolamins

- Prolamins are insoluble in water but soluble in 70-80% aqueous alcohol.
- Upon *hydrolysis* they yield much *proline* and amide nitrogen, hence the name prolamin.
- They are deficient in ***lysine***.
- *Gliadin of wheat and zein of corn* are examples of prolamins.

Glutelins

- Glutelins are ***insoluble in water*** and ***absolute alcohol but soluble in dilute alkalies and acids.***
- They are ***plant proteins*** e.g., *glutenin of wheat.*

Histones

- Histones are small and stable basic proteins
- They contain fairly large amounts of basic amino acid, ***histidine***.
- They are *soluble in water, but insoluble in ammonium hydroxide*.
- They are not readily coagulated by heat.
- They occur in ***globin of hemoglobin and nucleoproteins***.

Protamines

- Protamines are the simplest of the proteins.
- They are ***soluble in water and are not coagulated by heat***.
- They are basic in nature due to the presence of large quantities of ***arginine.***
- Protamines are found in association with nucleic acid in the *sperm cells of certain fish.*
- ***Tyrosine and tryptophan*** are usually *absent* in protamines.

Albuminoids

- These are ***characterized by great stability and insolubility in water and salt solutions.***
- These are called albuminoids because they are essentially *similar to albumin and globulins.*

- They are highly *resistant to proteolytic enzymes*.
- They are *fibrous in nature and form most of the supporting structures of animals*.
- They occur as *chief constituent of exoskeleton structure such as hair, horn and nails.*

ii. Conjugated or compound proteins

- These are ***simple proteins combined with some non-protein substances known as prosthetic groups.***
- The nature of the non-protein or prosthetic groups is the basis for the sub classification of conjugated proteins.

Nucleoproteins

- Nucleoproteins are simple basic proteins (protamines or histones) in salt combination with *nucleic acids as the prosthetic group*.
- They are the important *constituents of nuclei and chromatin*.

Mucoproteins

- These proteins are composed of ***simple proteins in combination with carbohydrates*** like ***mucopolysaccharides***, which include **hyaluronic acid and chondroitin sulphates.**
- On hydrolysis, mucopolysaccharides yield *more than 4% of amino-sugars*, hexosamine and uronic acid e.g., ***ovomucoid*** from egg white.
- Soluble mucoproteins are neither readily denatured by heat nor easily precipitated by common protein precipitants like trichloroacetic acid or picric acid.
- The term ***glycoproteins*** is restricted to those proteins that contain small amounts of carbohydrate usually *less than 4% hexosamine*.

Chromoproteins

- These are proteins containing ***coloured prosthetic groups*** e.g., haemoglobin, flavoprotein and cytochrome.

Lipoproteins

- These are proteins conjugated with ***lipids such as neutral fat, phospholipids and cholesterol***

Metalloproteins

- These are ***metal-binding proteins***.
- A β-globulin, termed ***transferrin*** is capable of combining with ***iron, copper and zinc.***
- This protein constitutes 3% of the total plasma protein.
- Another example is **ceruloplasmin**, which contains ***copper.***

Phosphoproteins

- These are proteins containing ***phosphoric acid***.
- Phosphoric acid is linked to the *hydroxyl group of certain amino acids like serine* in the protein *e.g., casein of milk.*

iii. Derived proteins

- These are proteins derived by *partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes*.
- They include two types of derivatives, ***primary-derived proteins and secondary-derived proteins.***

Primary-derived proteins

- These protein derivatives are formed by processes causing only *slight changes in the protein molecule and its properties.*
- There is *little or no hydrolytic cleavage of peptide bonds*.

Proteans

- Proteans are *insoluble products formed by the action of water, dilute acids and enzymes.*
- These are particularly formed from globulins but are insoluble in dilute salt solutions
- e.g., ***myosan from myosin, fibrin from fibrinogen***.

Metaproteins

- These are formed by the *action of acids and alkalies upon protein*.
- They are insoluble in neutral solvents.

Coagulated proteins

- Coagulated proteins are insoluble products formed by the *action of heat or alcohol* on natural proteins
- e.g., cooked meat and cooked albumin.

Secondary-derived proteins

- These proteins are formed in the *progressive hydrolytic cleavage of the peptide bonds of protein molecule.*
- They are roughly grouped into ***proteoses, peptones and peptides according to average molecular weight.***
- Proteoses are *hydrolytic products of proteins, which are soluble in water and are not coagulated by heat.*
- Peptones are hydrolytic products, which have simpler structure than proteoses.
- They are soluble in water and are not coagulated by heat.
- Peptides are composed of relatively few amino acids.
- They are water-soluble and not coagulated by heat.
- The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:

Protein ⟶ Protean ⟶ Metaprotein

Proteoses ⟶ Peptones ⟶ Peptides ⟶ amino acids

b. Classification of proteins based on function

Proteins are classified based on their functions as:

Catalytic proteins – Enzymes

- The most striking characteristic feature of these proteins is their ability to ***function within the living cells as biocatalysts.***
- These **biocatalysts** are called as enzymes.
- Enzymes represent the largest class.
- Nearly 2000 different kinds of enzymes are known, each catalyzing a different kind of reaction.

- They ***enhance the reaction rates*** a million fold.

Regulatory proteins - Hormones

- These are polypeptides and small proteins found in relatively *lower concentrations* in animal kingdom but ***play highly important regulatory role in maintaining order in complex metabolic reactions***
- e.g., *growth hormone, insulin* etc.

Protective proteins - Antibodies

- These proteins have ***protective defense function***.
- These proteins combine with foreign protein and other substances and fight against certain diseases.
- e.g., immunoglobulin.
- These proteins are produced in the *spleen and lymphatic cells in response to foreign substances called antigen.*
- The newly formed protein is called **antibody** which specifically combines with the antigen which triggered its synthesis thereby prevents the development of diseases.
- **Fibrin** present in the **blood** is also a protective protein.

Storage proteins

- It is a major class of proteins which has the function of ***storing amino acids as nutrients and as building blocks for the growing embryo***.
- Storage proteins are ***source of essential amino acids***, which cannot be synthesized by human beings.
- The major storage protein in pulses is ***globulins and prolamins in cereals.***
- In rice the major storage protein is glutelins.
- *Albumin of egg and casein of milk* are also storage proteins.

Transport proteins

- Some proteins are *capable of binding and transporting* specific types of molecules through blood.
- ***Haemoglobin*** is a conjugated protein composed of colourless basic protein, the ***globin*** and ***ferroprotoporphyrin or haem***.

- It has the *capacity to bind with oxygen and transport through blood* to various tissues.
- **Myoglobin,** a related protein, transports oxygen in muscle.
- **Lipids** bind to serum proteins like albumin and transported as *lipoproteins* in the blood.

Toxic proteins

- Some of the proteins are toxic in nature.
- **Ricin** present in **castor bean** is extremely toxic to higher animals in very small amounts.
- ***Enzyme inhibitors such as trypsin inhibitor*** bind to digestive enzyme and prevent the availability of the protein.
- **Lectin**, a toxic protein present in legumes, ***agglutinates red blood cells***.
- A bacterial toxin causes cholera, which is a protein.
- ***Snake venom*** is protein in nature.

Structural proteins

- These proteins serve as ***structural materials or as important components of extra cellular fluid.***
- Examples of structural proteins are ***myosin of muscles, keratin of skin and hair and collagen of connective tissue.***
- Carbohydrates, fats, minerals and other cellular components are organized around such structural proteins that form the molecular framework of living material.

Contractile proteins

- Proteins like **actin and myosin** function as *essential elements in contractile system* of skeletal muscle.

Secretary proteins

- **Fibroin** is a protein secreted by spiders and silkworms to form *webs and cocoons*.

Exotic proteins

- Antarctic fishes live in -1.9^{o}C waters, well below the temperature at which their blood is expected to freeze.

- These fishes are prevented from freezing by ***antifreeze glycoproteins*** present in their body.

C. Classification based on size and shape

Based on size and shape, the proteins are also subdivided into **globular and fibrous proteins.**

- **Globular proteins** are mostly *water-soluble and fragile in nature* e.g., ***enzymes, hormones and antibodies.***
- **Fibrous proteins** are *tough and water-insoluble*.
- They are used to build a variety of materials that support and protect specific tissues, e.g., skin, hair, fingernails and keratin

Lecture 13 - 14

Conformation of proteins

- ***Conformation of a protein*** refers to the ***three-dimensional structure*** in its ***native state.***
- There are many different possible conformations for a molecule as large as a protein.
- A protein can *perform its function* only when it is in its **native condition**.
- Due to the complexity of three-dimensional structures, the structure of protein is discussed at ***different levels of its organization***.

Four levels of structural organization can be distinguished in proteins:

1. Primary

2. Secondary

3. Tertiary

4. Quaternary

Primary structure

- Primary structure of protein refers to the ***number of amino acids and the order in which they are covalently linked together.***
- It also refers to the *location of disulfide bridges*, if there are any, in a polypeptide chain.
- The *peptide bond* is **covalent** in nature, ***quiet stable*** and referred as ***backbone of the protein.***
- They can be *disrupted by chemical or enzymatic hydrolysis but are not directly influenced by salt concentration, change in pH or solvent.*
- **Frederick Sanger** in 1953 determined the *complete amino acid sequence of insulin* for the first time.

The important steps involved in determining the primary structure of protein are

- *Determination of number of (chemically different) polypeptide chains or subunits in the protein.*
- *Separation of polypeptide chains if more than one are present in a protein.*
- *Determination of the amino acid sequence of the subunits.*
- *Elucidation of the position of the disulfide bonds, if any, between and within the subunits.*

1. Determination of number of polypeptides or subunits

Determination of the number of C-terminal or N-terminal amino acids will indicate the number of polypeptides in a protein.

H_2N.....> COOH

N-terminal C-terminal

N-terminal identification

- **Fluoro dinitro benzene (FDNB),** known as Sanger's reagent, was used to identify the N-terminal amino acid.
- This reagent was replaced by **dansyl chloride and Edman's reagent (phenyl isothiocyanate, PITC).**
- Edman's reagent is also used to determine the amino acid sequence of a polypeptide chain from the N-terminal by subjecting the polypeptide to repeated cycles of Edman degradation.
- After every cycle, the newly liberated **phenylthiohydantoin (PTH) amino acid** was identified
- The sequence of peptides containing *30-40 amino acids* can be determined using a sequencer by adopting the Edman's degradation method.

C-terminal identification

C-terminal amino acid can be determined by methods similar to those used for the N-terminal acid.

- **Hydrazine** is used to find out the *C-terminal amino acid*.

- It reacts with the ***carbonyl group of each peptide bond*** except C-terminal amino acid.
- The bond is cleaved and each amino acid derivative is released as the hydrazide derivative (hydrazinolysis).
- Since the carboxyl group of C-terminal amino acid is not involved in a peptide bond, it remains in the mixture as the only unmodified amino acid
- After chromatographic separation and comparison with the standards, the C-terminal amino acid can be identified.
- Carboxypeptidases are used for enzymic determination of the C-terminal amio acid.

Separation and purification of polypeptide chains

- Determination of C-terminal and/or N-terminal amino acids reveals the presence of one or more polypeptide chains in a protein.
- If the protein contains more than one polypeptide chain, separation of polypeptide chain is essential.
- If the polypeptide chains are connected by disulfide bond, they are cleaved to separate the individual peptide chains.
- The polypeptide is treated with **2-mercaptoethanol** ($HS\text{-}CH_2\text{-}CH_2OH$) so that reductive cleavage occurs and the polypeptide chains are separated.
- The resulting free-SH groups are usually alkylated by treatment with **iodoacetic acid**
- After cleaving the disulfide links using mercaptoethanol, subunits are dissociated using denaturing agents such **as urea or guinidinum ion or detergents such as sodium dodecyl sulphate (SDS).**
- The dissociated subunits are then separated using *ion exchange or gel filtration chromatographic method.*

Amino acid sequencing of polypeptides

- The amino acid sequence in polypeptides with 30-40 amino acids can be determined by Edman reaction.
- For polypeptides containing ***more than 40 amino acids***, both enzymatic and chemical methods are employed to break polypeptide chains into smaller peptides.

- The enzyme, **trypsin** hydrolyses the peptide bond on the ***carboxyl side of the basic amino acid residues of lysine or arginine***.
- The chemical reagent, ***cyanogens bromide*** cleaves peptide bond on the ***carboxyl side of methionine residues.***
- The hydrolyzed peptides are separated and the amino acid sequence is determined by Edman reaction.
- The hydrolysis of the original polypeptide by two different methods separately gives overlapping regions, from which the sequence is derived

Secondary structure

- Secondary structure refers to the ***steric relationship of amino acids that are close to one another in the linear sequence***.
- The folding of a linear polypeptide chain occurs to form a specific coiled structure.
- Such coiling or folding is maintained by **hydrogen bonds** and hydrogen bond is the only bond responsible for secondary structure.
- X-ray studies of several polypeptides by **Linus Pauling and Robert Corey** revealed that the peptide group has a **rigid, planar structure** which is a consequence of **resonance interactions** that give the peptide bond a 40% double bond character.
- Peptide groups mostly assume the **transconformation** in which successive C_2 atoms are on opposite sides of peptide bond joining them.
- The cis configuration creates steric interference.
- If a polypeptide chain is twisted by the same amount each of its C atoms, it assumes a helical conformation

Helix structure

- The α-helix is the ***most stable*** arrangement of polypeptides
- The helix structure of proteins is stabilized by ***intramolecular hydrogen bonding***.
- In this structure, ***hydrogen bonds are formed between the C=O group of one peptide bond and the N-H group of another after 3 amino acid units***.
- The polypeptide chain constituted by ***L-amino acids form a right-handed helix***, whereas the polypeptide chains made up of D-amino acids form a left-handed helix.

- In the α-helical conformation, ***all the side chains lie outside the helix*** whereas C, N, O and H of the peptide bond lie in the same plane.
- Certain amino acids tend to ***disrupt the α-helix***. Among these are **proline** (the N atoms is part of the rigid ring and no rotation of the N-C bond can occur) and **amino acid with charged or bulk R groups** that either electrostatically or physically interferes with helix formation.

The β-pleated sheet structure

- ***Pauling and Corey*** also proposed a second ordered structure, the β-pleated sheet for polypeptide.
- This structure is a ***result of intermolecular hydrogen bonding*** between the polypeptide chains to form a ***sheet like arrangement.***
- There are two ways in which proteins chains can form the pleated sheet structure.
- One is with the ***chains running in the same direction*** i.e. the -COOH or NH_2 ends of the polypeptide chains lying all at the top or all at the bottom of the sheet. This is called ***parallel pleated-sheet structure.***
- In another type, known as ***antiparallel β-pleated sheet structure***, the polypeptide chains alternate in such a way that the -COOH end of the one polypeptide is next to the -NH_2 end of the other i.e. polypeptide chains run in opposite directions.

The random coil

- Regions of proteins that are ***not identifiably organized as helices or pleated sheets*** are said to be present in ***random coil conformation***.
- Considerable portion of the protein may be present in this conformation.
- The term 'random' is unfortunate which imply less biological significance than more highly repeating regions.
- But in terms of biological function, the regions of random coil are of equal importance to those of helix and pleated sheet.

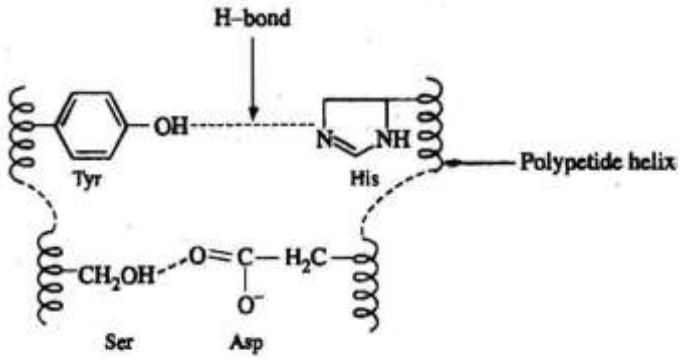
Tertiary structure

- Tertiary structure refers to the ***steric relationship of amino acid residues that are far apart in the linear sequence.***

- This leads to the twisting of polypeptide chains into **specific loops and bends** which are maintained chiefly by five kinds of bonds.

Hydrogen bonds

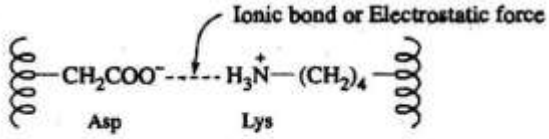
- Hydrogen bonds are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group



Hydrogen bonds in polypeptide chain

Salt-linkages (electrostatic forces; ionic bonds)

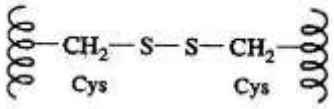
- Salt linkages are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group



Electrostatic forces in polypeptide chain

Disulfide bonds (S-S linkages)

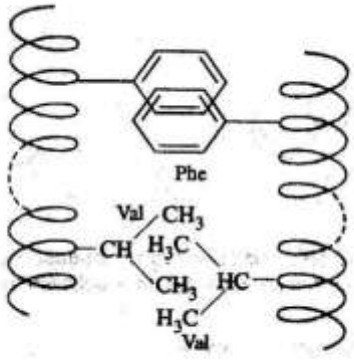
- The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains



S-S linkages in polypeptide chain

Hydrophobic bonds

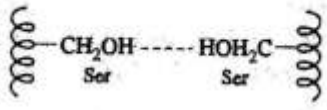
- Hydrophobic bonds are formed as a result of interaction between non-polar side chains



Hydrophobic bonds in polypeptide chain

Dipole-dipole interaction

- This interaction occurs between **polar unionized side chains**
- The folding of a polypeptide chain due to different covalent and non-covalent interactions is shown below.
- Out of the above bonds, ***the disulfide bond (covalent bond) is the strongest and cannot be affected by solvent, pH, temperature and salts*** whereas the above conditions.
- The disulfide bond can be split and reformed by oxidation/reduction respectively
- The tertiary structure gains special importance in the **case of enzymes**.



Dipole-dipole interaction in polypeptide chain

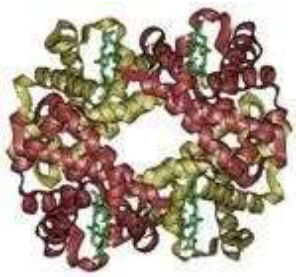
Domain

- Domains are ***structurally independent units*** that have the characteristics of a small globular protein.
- Domains often have a *specific function* such as the **binding of a small molecule.**

- A long peptide strand of a protein will often fold into ***multiple, compact semi-independent folded regions or domains.***
- Each domain having a ***characteristic spherical geometry*** with *a hydrophobic core and polar surface* very much like the tertiary structure of a whole globular protein
- The domains of a multidomain protein are often interconnected by a segment of polypeptide chain lacking regular secondary structure.
- In enzymes with more than one substrate or ***allosteric effector sites the different binding sites are often located in different domains.***
- In multifunctional proteins, the different domains perform different tasks.

Quaternary structure

- Proteins that have ***more than one subunit or polypeptide chains*** will exhibit quaternary structure.
- Quaternary structure refers to a ***functional protein aggregate (organization***) formed by interpolypeptide linkage of subunits or polypeptide chains.
- These subunits are held together by ***noncovalent surface interaction*** between the polar side chains.
- Proteins formed like above are termed ***oligomers*** and the individual polypeptide chains are variously termed protomers, monomers or subunits.
- The most common oligomeric proteins contain two or four protomers and are termed dimers or tetramers, respectively.
- ***Myoglobin*** has no quaternary structure since, it is composed of a single polypeptide chain.
- **Hemoglobin molecule**, which consists of ***four separate polypeptide chains***, exhibits quaternary structure.



A schematic of hemoglobin.

The ribbon parts represent the protein globin; the four green parts are the heme groups.

- Quaternary structure may influence the activity of enzymes.
- Some enzymes are *active only in their quaternary state* and become inactive when split into smaller units.
- Other enzymes are inactive in the quaternary state and are activated only when they are dissociated to form monomeric state.

Physical and chemical properties of proteins

Physical

- Pure proteins are generally tasteless, though the predominant taste of protein hydrolysates is bitter.
- Pure proteins are odourless.
- Because of the large size of the molecules, proteins exhibit many properties that are **colloidal in nature**.
- Proteins, like amino acids, are **amphoteric** and contain both acidic and basic groups.
- They possess **electrically charged groups** and hence ***migrate in an electric field***.
- Many proteins are **labile** and readily modified by alterations in **pH, UV radiation, heat and by many organic solvents.**
- The ***absorption spectrum of protein*** is maximum at 280 nm due to the presence of **tyrosine and tryptophan**, which are the strongest ***chromophores*** in that region.
- Hence the absorbance of the protein at this wavelength is adapted for its determination.

Denaturation of protein

- The comparatively weak forces responsible for maintaining secondary, tertiary and quaternary structure of proteins are readily disrupted with ***resulting loss of biological activity.***
- **This disruption of native structure is termed denaturation**.
- Physically, denaturation is viewed as ***randomizing the conformation of a polypeptide chain without affecting its primary structure***

- Physical and chemical factors are involved in the denaturation of protein

a. **Heat and UV radiation** supply kinetic energy to protein molecules causing the atoms to vibrate rapidly, thus disrupting the relatively weak hydrogen bonds and salt linkages. This results in denaturation of protein leading to coagulation. Enzymes easily digest denatured or coagulated proteins.
b. **Organic solvents** such as ethyl alcohol and acetone are capable of forming intermolecular hydrogen bonds with protein disrupting the intramolecular hydrogen bonding. This causes precipitation of protein.
c. **Acidic and basic reagents** cause changes in pH, which alter the charges present on the side chain of protein disrupting the salt linkages.
d. **Salts of heavy metal ions (Hg2+, Pb2+)** form very strong bonds with carboxylate anions of aspartate and glutamate thus disturbing the salt linkages. This property makes some of the heavy metal salts suitable for use as antiseptics.

Renaturation

- Renaturation refers to ***the attainment of an original, regular three-dimensional functional protein after its denaturation.***
- When active pancreatic ribonuclease A is treated with **8M urea or β-mercaptoethanol,** it is converted to an inactive, denatured molecule.
- When urea or mercaptoethanol is removed, it attains its native (active) conformation.

Chemical

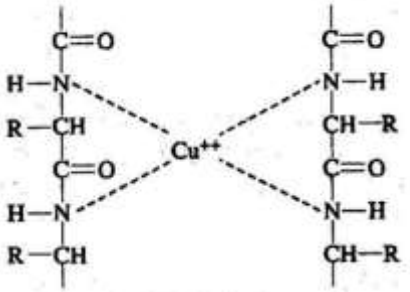
Colour reactions of proteins

- The colour reactions of proteins are of importance in the qualitative detection and quantitative estimation of proteins and their constituent amino acids.
- ***Biuret test*** is extensively used as a test to detect proteins in biological materials.

Biuret reaction

- A compound, which is having more than one ***peptide bond*** when treated with Biuret reagent, produces a **violet colour**. This is due to the formation of ***coordination***

complex between four nitrogen atoms of two polypeptide chains and one copper atom



Violet coloured coordination complex

Coordination complex with peptide bonds and copper

Xanthoproteic reaction

- Addition of **concentrated nitric acid** to protein produces **yellow colour** on heating, the colour changes to **orange** when the solution is made alkaline. The yellow stains upon the skin caused by nitric acid are the result of this xanthoproteic reaction. This is due to the ***nitration of the phenyl rings of aromatic amino acids.***

Hopkins-Cole reaction

- **Indole ring of tryptophan** reacts with glacial acetic acid in the presence of ***concentrated sulphuric acid*** and forms **a purple** coloured product. Glacial acetic acid reacts with concentrated sulphuric acid and forms glyoxalic acid, which in turn reacts with indole ring of tryptophan in the presence of sulphuric acid forming a **purple coloured** product.

Lecture 15

ENZYMES

One of the ***unique characteristics*** of a living cell is its ***ability to permit complex reactions*** to proceed *rapidly at the temperature of the surrounding environment.*

- The ***principal agents*** which participate in the remarkable transformations in the cell belong to ***a group of proteins named enzymes***. In the absence of enzymes in the cell, these reactions would proceed too slowly.
- ***Enzymes are proteins specialised to catalyse biological reactions*** with the following characteristics.

Characteristics of enzymes

- Enzymes being proteins ***exhibit all properties of proteins***.
- They have their ***specific isoelectric points*** at which they ***are least soluble***.
- Like proteins, they can be ***denatured by changes in pH and temperature***.
- The enzyme-catalysed reactions occur below $100^{o}C$, at atmospheric pressure and nearby ***neutral pH.***
- Enzymes ***undergo physical changes during the reaction*** but ***revert to their original form at the end of the reaction.***
- Enzymes exhibit ***enormous catalytic power***.The rates of enzymatically catalysed reactions are ***10^{6} - 10^{12} times greater*** than those of the corresponding uncatalysed reactions and several times greater than those of the corresponding chemically catalysed reactions.
- For example the ***carbonic anhydrase enzyme*** catalyses the conversion of ***carbondioxide to carbonic acid.***

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

- In this reaction, each enzyme molecule can hydrate 10^5 molecules of CO_2 per second.
- ***Enzyme activity is regulated*** in a variety of ways, ranging from controls over the ***amount of enzyme protein synthesised by the cell*** or ***modulation of activity***

through reversible interaction with ***metabolic inhibitors and activators*** or through ***isoenzymes.***

Specificity of the enzymes

- One of the characteristic features which distinguishes enzymes from catalysts is their **specificity.**
- Enzymes are specific in the ***reaction catalysed*** and in their ***choice of substrates***.
- It usually catalyses a single chemical reaction or a set of closely related reactions

Three kinds of specificities are observed.

i. Absolute specificity

- When enzymes ***catalyse only one particular reaction*** they are said to exhibit ***absolute specificity.***
- e.g. **Urease** acts only on urea.

ii. Group specificity

- Enzymes acting on a ***group of substances*** that ***possess a particular type of linkage*** common to that group of substances are said to exhibit group specificity.
- **Amylase** hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α1, 4).

iii. Optical specificity

- Almost all enzymes show a high degree of optical specificity.
- There are certain enzymes which catalyse the ***hydrolysis of same group of substances possessing same optical activity***
- ***Eg. D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid.***
- **Maltase** catalyses the **hydrolysis of α**-but not β- glycosides.

Classification of enzymes

- In ***olden days*** enzymes have been named by ***adding the suffix -ase*** to the name of the substrate (the molecule on which the enzyme acts).
- ***Ex. Urease (***Substrate urea***) Arginase (***Substrate arginine***)***

- Recent studies on the ***mechanism of enzyme catalysed reactions*** have led to a more rational classification of enzymes.
- ***The International Union of Biochemistry (IUB)*** established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes.
- This system is ***based on the substrate and reaction specificity***.
- Although, this ***International Union of Biochemistry*** system is *complex*, ***it is precise, descriptive and informative.***
- IUB system classifies enzymes into ***six major classes*** (should be written in specific order only)

  1. Oxidoreductases

  2. Transferases

  3. Hydrolases

  4. Lyases

  5. Isomerases

  6. Ligases

- Again each class is divided into subclasses according to the ***type of reaction catalysed.***
- Each enzyme is assigned a ***recommended name*** usually a short for everyday use, a ***systematic name*** which identify the reaction it catalyses and a ***classification number*** which is used where accurate and unambiguous identification of an enzyme is required **(OTHLIL).**

I. Oxidoreductases

- Enzymes ***catalysing oxido-reductions between two substrates, S and S'.***

$$S_{reduced} + S'_{oxidised} \rightarrow S_{oxidised} + S'_{reduced}$$

Example:

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \longrightarrow CH_3\text{-}CHO + NADH + H^+$$

(reduced) (oxidised) (oxidised) (reduced)

Enzyme: Recommended name ***Alcohol dehydrogenase***

Systematic name ***Alcohol: NAD^+ oxido-reductase***

Enzyme Commission number **E.C.1.1.1.1**

First digit 1 indicates ***oxido-reductase (Major class)***

Second digit 1 indicates ***enzymes acting on CH-OH group of donors (Sub-class)***

Third digit 1 indicates **NAD^+ as the electron acceptor (Sub-sub class)**

Fourth digit 1 indicates the ***specific enzyme***

II. Transferases

- Enzymes catalyzing the ***transfer of a functional group (G) other than hydrogen between substrates.***

$$S\text{-}\mathbf{G} + S' \longrightarrow S'\text{-}\mathbf{G} + S$$

Example: Phosphorylation of glucose by ***hexokinase***

$$\text{Glucose} + ATP \longrightarrow \text{Glucose} - 6\text{-Phosphate} + ADP$$

Enzyme: Recommended name: **Hexokinase**

Systematic name: ***ATP: D-hexose, 6- phosphotransferase***

Enzyme commission No: **2.7.1.1**

2 → *Transferase group (major class)*

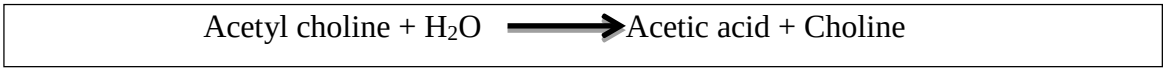
7 → *Transfer of phosphate group (sub-class)*

1 → *Alcohol group as acceptor of phosphate group (Sub-sub-class)*

1 → *Hexokinase*

III. Hydrolases

- Enzymes ***catalysing hydrolysis of ester, peptide or glycosidic bonds.***
- Example



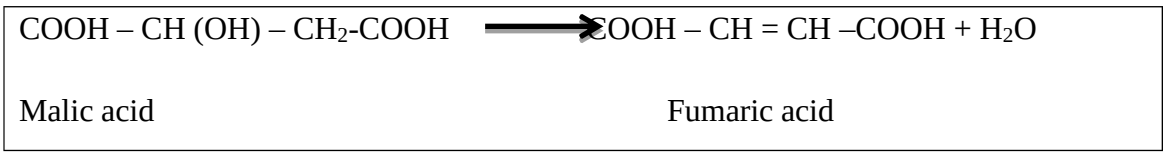
Enzyme: ***Acetyl choline esterase***

Systematic name: ***Choline:acetyl hydrolase***

E.C: 3.1.1.8

IV Lyases

- Enzymes catalysing the ***removal of groups from substrates by mechanism other than hydrolysis leaving a double bond in one of the products***.
- Example: convertion of malic acid to fumaric acid by fumarase



Enzyme: ***Fumarase (Fumarate hydratase***)

Systematic name: ***L. Malate hydrolyase***

E.C.No.4.2.1.2

V Isomerases

- Enzymes catalysing ***interconversion of optical, geometrical or positional isomers***

Example

All-*trans* retinal ⟶ 11 *cis*-retinal

Enzyme ***Retinene isomerase***

Systematic name: ***All-trans retinene: 11-cis isomerase***

E.C.No. 5.2.1.3

VI. Ligases

- Enzymes catalyzing ***the joining together of two compounds with the hydrolysis of a high energy compound.***
- Example

ATP ADP + Pi

Glutamic acid + NH3 ⟶ Glutamine

Enzyme: ***Glutamine synthetase***

L.Glutamate: Ammonia ligase

E.C.6.3.1.2

Lecture 16

Mechanism of enzyme action

- A chemical reaction such as A ⟶ P takes place because a certain fraction of the ***substrate possesses enough energy to attain an activated condition called the transition state.***
- This transition state is at the top of the energy barrier separating the reactants and products.
- ***The rate of a given chemical reaction is proportional to the concentration of this transition state species.***
- ***The energy of activation*** is the *amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state.*
- Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalyzed reaction. Thus, they accelerate chemical reactions by ***lowering the energy of activation***.

Example

H_2O_2 ⟶ $H_2O + (O)$

Catalase

Reaction condition	Activation energy (KCal mol^{-1})
Uncatalyzed	18
Catalysed by colloidal Pt	13
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in at least two steps.

Step 1: A molecule of enzyme (E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

[S] + [E]---------- > [ES] -------- > P+ [E]

The formation of an ES complex affords a lower activation energy.

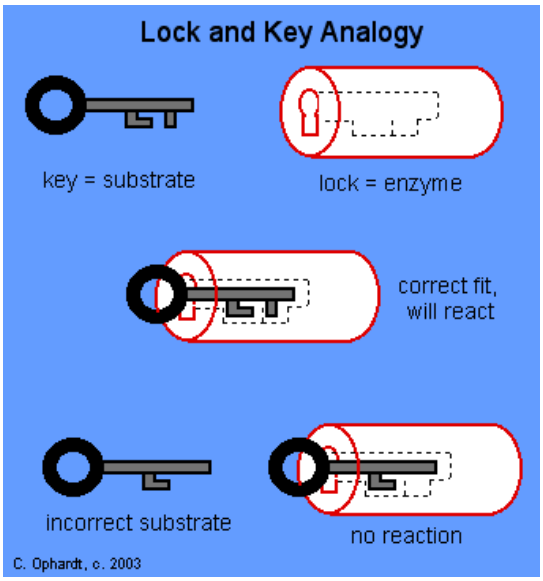
Active site

- ***The substrate binding site in the enzyme*** is referred as **active site.**
- The functional groups that are essential for the formation of ES complex occur at a specific location on the surface of the enzyme molecule.
- This section of enzyme ***where substrate binding and transformation of substrate to product*** occurs is called as **active site**.
- Many attempts have been made to implicate ***specific amino acid residues (side chain or R groups) as being part of the active site of various enzymes.***
- Some of the amino acids occurring at the active site of enzymes are *hydroxyl group of serine, sulfhydryl group of cysteine, imidazole group of histidine and carboxyl group* of aspartic acid.

Two theories were proposed to explain the mechanism of enzyme action.

1. Fischer's lock and key theory (Rigid template model)

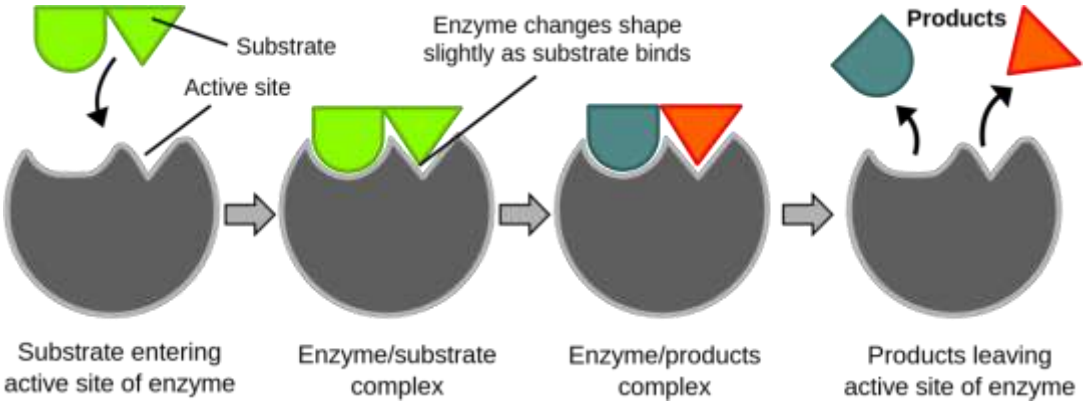
- During 1890, **Emil Fischer** proposed this theory
- According to this, the ***active site possesses a unique conformation which is complementary to the structure of the substrate*** thus *enabling the two molecules to fit together in much the same way as a key fits into a lock*.



- An unfortunate feature of this model is the ***implied rigidity of the catalytic site***.

2. Koshland's induced-fit theory

- Koshland had advocated a theory ***to account for the specificity of enzymes***.
- He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis.
- When the substrate molecule is bound by the enzyme, ***the catalytic groups assume favourable geometrical position to form the transition state.***
- The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate.
- In the induced fit model, the *substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both.*



Factors affecting enzymatic reaction

The factors that mainly influence any enzyme-catalysed reaction are:

1. Substrate concentration

2. Enzyme concentration

3. Temperature

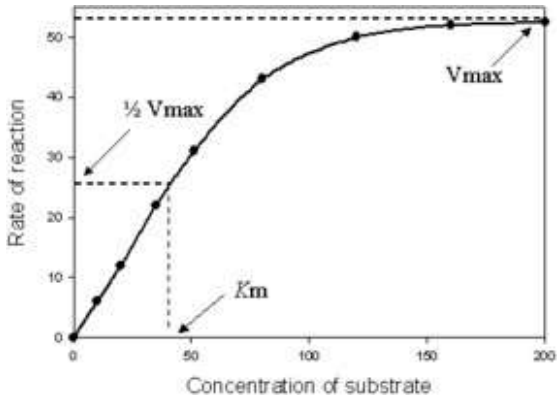
4. pH

5. Inhibitors

Other factors such as ***state of enzyme (oxidation), time and activators*** also affect enzyme-catalysed reaction to certain extent.

Substrate concentration

- Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the ***substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola.***
- At ***very low substrate concentration*** the *initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics).*
- However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics).
- With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).
- At initial point, even though the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate.
- Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S].
- At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate.
- Michaelis-Menten derived an equation to explain this type of behaviour.



$$v = \frac{V_{max}[S]}{\text{----------}}$$

[S] = Substrate concentration V_{max} = Maximum velocity

v = Velocity of the reaction

At half maximal velocity [S] = K_m

i.e $\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m+[S]}$

$\frac{K_m + [S]}{2} = \frac{V_{max}[S]}{V_{max}}$

$K_m + [S] = 2[S]$

$K_m = 2[S] - [S] = [S]$

Hence, Michaelis - Menten constant, ***Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre***.

- The Michaelis-Menten equation can be algebraically transformed into more useful way to plot the experimental data.
- Lineweaver and Burk have taken the reciprocal of both [S] and v of the Michaelis-Menten equation to give

$$\frac{1}{v} = \frac{Km}{Vmax[S]} + \frac{1}{Vmax}$$

- A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line.

- This line intercept X-axis at -1/Km and Y-axis at 1/Vmax.
- The slope of the line is **Km/Vmax.**
- The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km

Significance of Km

i. Km value may vary with substrate.
ii. An enzyme whose Km is very low will have a high degree of affinity for its substrate

Enzyme concentration

- When compared to substrate concentration, the concentration of enzyme is always ***very low*** on molar basis.
- Hence, ***increasing the enzyme concentration will always increase the reaction rate***

Temperature

- The ***velocity of enzyme-catalysed reactions*** roughly ***doubles with a 10^{o}C*** rise in temperature over a limited range of temperature
- Enzymes, being proteins, are ***denatured by heat*** and become ***inactive*** as the temperature increases beyond a certain point.
- Most of the enzymes are inactivated at temperatures ***above 60^{o}C.***
- The temperature at which the reaction rate is maximum is known as ***optimum temperature***

pH

- Most enzymes have a ***characteristic pH*** at which their activity is maximum; above or below this pH, the activity declines
- The *pH affects the ionic state of the enzyme* and frequently that of the substrate also.
- If a negatively charged enzyme (E^-) reacts with a positively charged substrate (SH^+), ESH is formed.
- At low pH values, E^- will be protonated and ESH is not formed.
- Similarly, at very high pH values SH^+ will ionize and lose its positive charge.

$E^- + SH^+$ ------ > ESH

acidic pH

$E^- + SH^+$ ------> $EH^+ + SH^+$ --------> No ESH formation

alkaline pH

SH^+ ----------> $S + H^+ + E^-$ --------> No ESH formation

- Another important factor is the ***change in conformation (denaturation) of enzyme at extreme pH values.***

Inhibitors

- Compounds that have the ***ability to combine with certain enzymes*** but ***do not serve as substrates*** and therefore ***block catalysis*** are called ***inhibitors***.
- The important type of inhibitors are ***competitive*** and ***noncompetitive inhibitors***.

Competitive inhibitor

- Any compound which ***possesses a close structural resemblance to a particular substrate*** and which ***competes with that of substrate for the same active site on the enzyme*** is called as **competitive inhibitor**.
- The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind.
- This is a **reversible process**.
- It ***depends upon the relative concentration of substrate and inhibitor***.
- Competitive inhibition can be completely reversed by addition of large excess of substrate

high inhibitor concn.

E + I ----------> E I

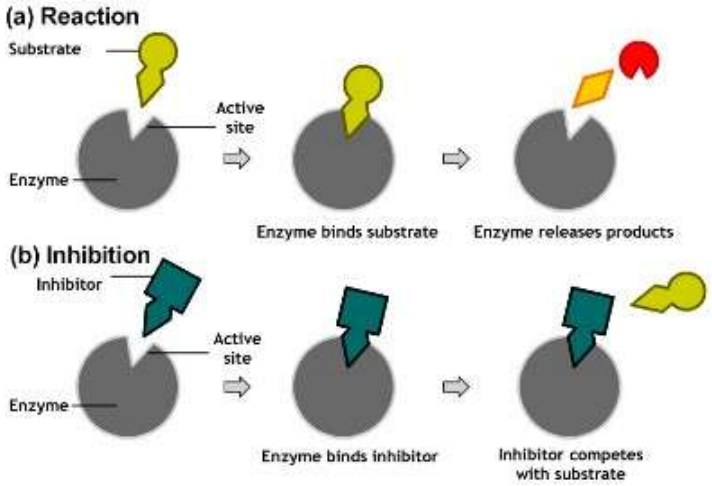
<----------

high substrate concn.

Eg. The enzyme, succinate dehydrogenase converts succinate to fumarate.

For this reaction, ***malonic acid*** is a ***competitive inhibitor*** as it structurally resembles that of succinate

- In case of competitive inhibition, **K_m is increased** but **V_{max} is not altered**.



Non-competitive inhibitor

- Non-competitive inhibitors ***bind to a site other than the active site on the enzyme*** often to ***deform the enzyme***, so that, it does not form the ES complex at its normal rate.
- Once formed, the ES complex does not decompose at the normal rate to yield products.
- These ***effects are not reversed*** by *increasing the substrate concentration.*

  E + I ------ > EI

  ES + I----- > ESI

- Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg^{2+}, Pb^{2+}).
- Some ***metalloenzymes*** are inhibited ***non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).***

- Inhibitors are used as ***tools to probe the mechanism of enzyme - catalysed reactions*** and *as* ***therapeutic agents***.
- In case of noncompetitive inhibition, Vmax is lowered but Km is not altered

Uncompetitive inhibitor:

- In case of uncompetitive inhibition, the inhibitor binds only to free enzyme and not to the enzyme substrate [ES] complex.

Lecture: 17

MIDSEMESTER EXAMINATION

Lecture 18

Enzymes – properties, classification and nomenclature

One of the ***unique characteristics*** of a living cell is its ***ability to permit complex reactions*** to proceed *rapidly at the temperature of the surrounding environment.*

- The ***principal agents*** which participate in the remarkable transformations in the cell belong to ***a group of proteins named enzymes***. In the absence of enzymes in the cell, these reactions would proceed too slowly.
- ***Enzymes are proteins specialised to catalyse biological reactions*** with the following characteristics.

Characteristics of enzymes

- Enzymes being proteins ***exhibit all properties of proteins***.
- They have their ***specific isoelectric points*** at which they ***are least soluble***.
- Like proteins, they can be ***denatured by changes in pH and temperature***.
- The enzyme-catalysed reactions occur below 100^{o}C, at atmospheric pressure and nearby ***neutral pH.***
- Enzymes ***undergo physical changes during the reaction*** but ***revert to their original form at the end of the reaction.***
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- For example the ***carbonic anhydrase enzyme*** catalyses the conversion of ***carbondioxide to carbonic acid.***

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

- In this reaction, each enzyme molecule can hydrate 10^5 molecules of CO_2 per second.
- ***Enzyme activity is regulated*** in a variety of ways, ranging from controls over the ***amount of enzyme protein synthesised by the cell*** or ***modulation of activity*** through reversible interaction with ***metabolic inhibitors and activators*** or through ***isoenzymes.***

Specificity of the enzymes

- One of the characteristic features which distinguishes enzymes from catalysts is their **specificity.**
- Enzymes are specific in the ***reaction catalysed*** and in their ***choice of substrates***.
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ii. Group specificity

- Enzymes acting on a ***group of substances*** that ***possess a particular type of linkage*** common to that group of substances are said to exhibit group specificity.
- **Amylase** hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α1, 4).

iii. Optical specificity

- Almost all enzymes show a high degree of optical specificity.
- There are certain enzymes which catalyse the ***hydrolysis of same group of substances possessing same optical activity***
- ***Eg. D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid.***

- **Maltase** catalyses the **hydrolysis of α**-but not β- glycosides.

Classification of enzymes

- In ***olden days*** enzymes have been named by ***adding the suffix -ase*** to the name of the substrate (the molecule on which the enzyme acts).
- ***Ex. Urease (***Substrate urea***) Arginase (***Substrate arginine***)***
- Recent studies on the ***mechanism of enzyme catalysed reactions*** have led to a more rational classification of enzymes.
- ***The International Union of Biochemistry (IUB)*** established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes.
- This system is ***based on the substrate and reaction specificity***.
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  1. Oxidoreductases

  2. Transferases

  3. Hydrolases

  4. Lyases

  5. Isomerases

  6. Ligases

- Again each class is divided into subclasses according to the ***type of reaction catalysed.***
- Each enzyme is assigned a ***recommended name*** usually a short for everyday use, a ***systematic name*** which identify the reaction it catalyses and a ***classification number*** which is used where accurate and unambiguous identification of an enzyme is required **(OTHLIL).**

I. Oxidoreductases

- Enzymes ***catalysing oxido-reductions between two substrates, S and S'.***

$$S_{reduced} + S'_{oxidised} \rightarrow S_{oxidised} + S'_{reduced}$$

Example:

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \longrightarrow CH_3\text{-}CHO + NADH + H^+$$

(reduced) (oxidised) (oxidised) (reduced)

Enzyme: Recommended name ***Alcohol dehydrogenase***

Systematic name ***Alcohol: NAD^+ oxido-reductase***

Enzyme Commission number **E.C.1.1.1.1**

First digit 1 indicates ***oxido-reductase (Major class)***

Second digit 1 indicates ***enzymes acting on CH-OH group of donors (Sub-class)***

Third digit 1 indicates **NAD^+ as the electron acceptor (Sub-sub class)**

Fourth digit 1 indicates the ***specific enzyme***

II. Transferases

- Enzymes catalyzing the ***transfer of a functional group (G) other than hydrogen between substrates.***

$$S\text{ - }\mathbf{G} + S' \longrightarrow S'\text{ - }\mathbf{G} + S$$

Example: Phosphorylation of glucose by ***hexokinase***

$$\text{Glucose} + ATP \longrightarrow \text{Glucose – 6- Phosphate} + ADP$$

Enzyme: Recommended name: **Hexokinase**

Systematic name: ***ATP: D-hexose, 6- phosphotransferase***

Enzyme commission No: **2.7.1.1**

2 → *Transferase group (major class)*

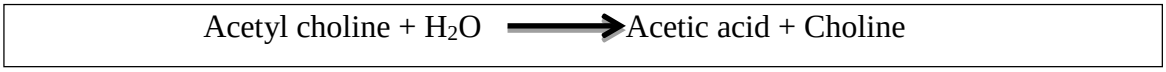
7 → *Transfer of phosphate group (sub-class)*

1 → *Alcohol group as acceptor of phosphate group (Sub-sub-class)*

1 → *Hexokinase*

III. Hydrolases

- Enzymes ***catalysing hydrolysis of ester, peptide or glycosidic bonds.***
- Example



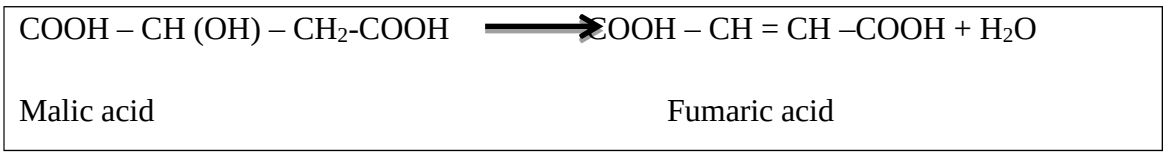
Enzyme: ***Acetyl choline esterase***

Systematic name: ***Choline:acetyl hydrolase***

E.C: 3.1.1.8

IV Lyases

- Enzymes catalysing the ***removal of groups from substrates by mechanism other than hydrolysis leaving a double bond in one of the products***.
- Example: convertion of malic acid to fumaric acid by fumarase



Enzyme: ***Fumarase (Fumarate hydratase***)

Systematic name: ***L. Malate hydrolyase***

E.C.No.4.2.1.2

V Isomerases

- Enzymes catalysing ***interconversion of optical, geometrical or positional isomers***

Example

All-*trans* retinal ⟶ 11 *cis*-retinal

Enzyme ***Retinene isomerase***

Systematic name: ***All-trans retinene: 11-cis isomerase***

E.C.No. 5.2.1.3

VI. Ligases

- Enzymes catalyzing ***the joining together of two compounds with the hydrolysis of a high energy compound.***
- Example

ATP ADP + Pi

Glutamic acid + NH3 ⟶ Glutamine

Enzyme: ***Glutamine synthetase***

L.Glutamate: Ammonia ligase

E.C.6.3.1.2

Lecture 19

Mechanism of enzyme action

- A chemical reaction such as A ⟶ P takes place because a certain fraction of the ***substrate possesses enough energy to attain an activated condition called the transition state.***
- This transition state is at the top of the energy barrier separating the reactants and products.
- ***The rate of a given chemical reaction is proportional to the concentration of this transition state species.***

- ***The energy of activation*** is the *amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state.*
- Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalyzed reaction. Thus, they accelerate chemical reactions by ***lowering the energy of activation***.

Example

$H_2O_2 \longrightarrow H_2O + (O)$

Catalase

Reaction condition	Activation energy (KCal mol^{-1})
Uncatalyzed	18
Catalysed by colloidal Pt	13
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in at least two steps.

Step 1: A molecule of enzyme (E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

[S] + [E]---------- > [ES] -------- > P+ [E]

The formation of an ES complex affords a lower activation energy.

Active site

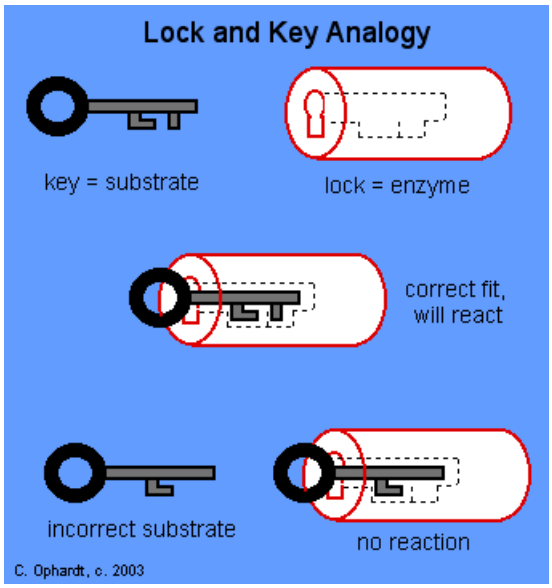
- ***The substrate binding site in the enzyme*** is referred as **active site.**
- The functional groups that are essential for the formation of ES complex occur at a specific location on the surface of the enzyme molecule.
- This section of enzyme ***where substrate binding and transformation of substrate to product*** occurs is called as **active site**.
- Many attempts have been made to implicate ***specific amino acid residues (side chain or R groups) as being part of the active site of various enzymes.***

- Some of the amino acids occurring at the active site of enzymes are *hydroxyl group of serine, sulfhydryl group of cysteine, imidazole group of histidine and carboxyl group* of aspartic acid.

Two theories were proposed to explain the mechanism of enzyme action.

1. Fischer's lock and key theory (Rigid template model)

- During 1890, **Emil Fischer** proposed this theory
- According to this, the ***active site possesses a unique conformation which is complementary to the structure of the substrate*** thus *enabling the two molecules to fit together in much the same way as a key fits into a lock.*

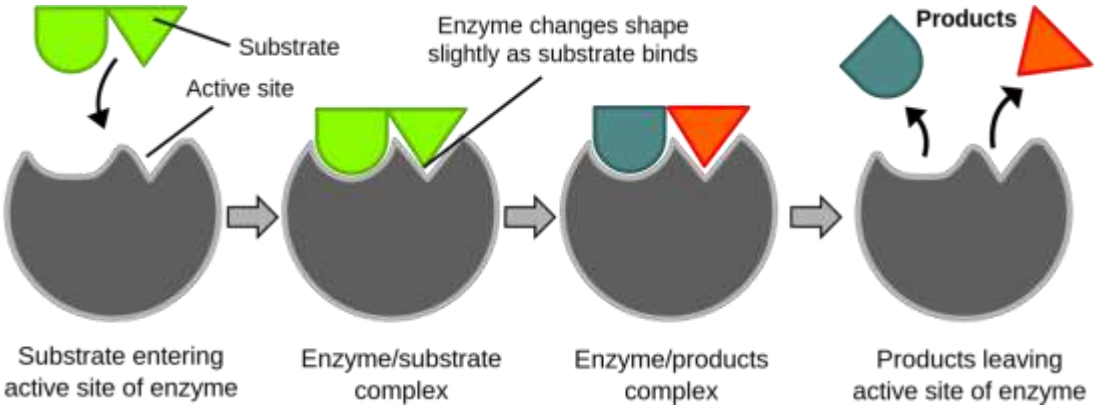


- An unfortunate feature of this model is the ***implied rigidity of the catalytic site***.

2. Koshland's induced-fit theory

- Koshland had advocated a theory ***to account for the specificity of enzymes***.
- He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis.
- When the substrate molecule is bound by the enzyme, ***the catalytic groups assume favourable geometrical position to form the transition state.***
- The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate.

- In the induced fit model, the *substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both.*



Lecture 20

Factors affecting enzymatic reaction

The factors that mainly influence any enzyme-catalysed reaction are:

1. Substrate concentration

2. Enzyme concentration

3. Temperature

4. pH

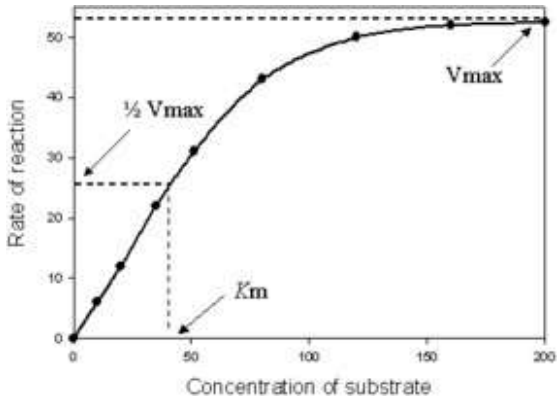
5. Inhibitors

Other factors such as ***state of enzyme (oxidation), time and activators*** also affect enzyme-catalysed reaction to certain extent.

Substrate concentration

- Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the ***substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola.***

- At ***very low substrate concentration*** the *initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics).*
- However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics).
- With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).
- At initial point, even though the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate.
- Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S].
- At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate.
- Michaelis-Menten derived an equation to explain this type of behaviour.



$$v = \frac{V_{max}[S]}{\text{----------}}$$

[S] = Substrate concentration V_{max} = Maximum velocity

v = Velocity of the reaction

At half maximal velocity [S] = K_m

$$\text{i.e} \quad \frac{Vmax}{2} = \frac{Vmax\,[S]}{Km+[S]}$$

$$\frac{Km + [S]}{2} = \frac{Vmax\,[S]}{Vmax}$$

$$Km + [S] = 2\,[S]$$

$$Km = 2\,[S] - [S] = [S]$$

Hence, Michaelis - Menten constant, ***Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre***.

- The Michaelis-Menten equation can be algebraically transformed into more useful way to plot the experimental data.
- Lineweaver and Burk have taken the reciprocal of both [S] and v of the Michaelis-Menten equation to give

$$\frac{1}{v} = \frac{Km}{Vmax\,[S]} + \frac{1}{Vmax}$$

  - A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line.
  - This line intercept X-axis at -1/Km and Y-axis at 1/Vmax.
  - The slope of the line is **Km/Vmax.**
  - The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km

Significance of Km

iii. Km value may vary with substrate.

iv. An enzyme whose Km is very low will have a high degree of affinity for its substrate

Enzyme concentration

- When compared to substrate concentration, the concentration of enzyme is always ***very low*** on molar basis.
- Hence, ***increasing the enzyme concentration will always increase the reaction rate***

Temperature

- The ***velocity of enzyme-catalysed reactions*** roughly ***doubles with a 10^{o}C*** rise in temperature over a limited range of temperature
- Enzymes, being proteins, are ***denatured by heat*** and become ***inactive*** as the temperature increases beyond a certain point.
- Most of the enzymes are inactivated at temperatures ***above 60^{o}C.***
- The temperature at which the reaction rate is maximum is known as ***optimum temperature***

pH

- Most enzymes have a ***characteristic pH*** at which their activity is maximum; above or below this pH, the activity declines
- The *pH affects the ionic state of the enzyme* and frequently that of the substrate also.
- If a negatively charged enzyme (E^-) reacts with a positively charged substrate (SH^+), ESH is formed.
- At low pH values, E^- will be protonated and ESH is not formed.
- Similarly, at very high pH values SH^+ will ionize and lose its positive charge.

$$E^- + SH^+ \xrightarrow{\text{------}} ESH$$

acidic pH

$$E^- + SH^+ \text{ ------> } EH^+ + SH^+ \text{ --------> No ESH formation}$$

alkaline pH

SH^+ ----------> S + H^+ + E^- --------> No ESH formation

- Another important factor is the ***change in conformation (denaturation) of enzyme at extreme pH values.***

Inhibitors

- Compounds that have the ***ability to combine with certain enzymes*** but ***do not serve as substrates*** and therefore ***block catalysis*** are called ***inhibitors***.
- The important type of inhibitors are ***competitive*** and ***noncompetitive inhibitors***.

Competitive inhibitor

- Any compound which ***possesses a close structural resemblance to a particular substrate*** and which ***competes with that of substrate for the same active site on the enzyme*** is called as **competitive inhibitor**.
- The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind.
- This is a **reversible process**.
- It ***depends upon the relative concentration of substrate and inhibitor***.
- Competitive inhibition can be completely reversed by addition of large excess of substrate

high inhibitor concn.

E + I ----------> E I

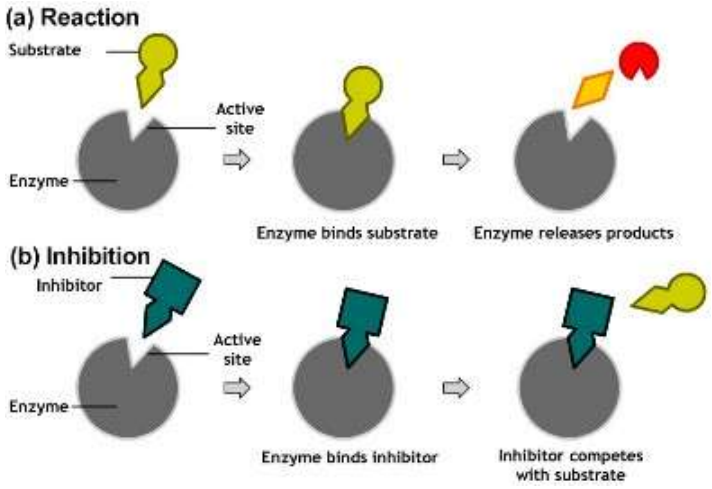
<----------

high substrate concn.

Eg. The enzyme, succinate dehydrogenase converts succinate to fumarate.

For this reaction, ***malonic acid*** is a ***competitive inhibitor*** as it structurally resembles that of succinate

- In case of competitive inhibition, **K_m is increased** but **V_{max} is not altered**.



Non-competitive inhibitor

- Non-competitive inhibitors ***bind to a site other than the active site on the enzyme*** often to ***deform the enzyme***, so that, it does not form the ES complex at its normal rate.
- Once formed, the ES complex does not decompose at the normal rate to yield products.
- These ***effects are not reversed*** by *increasing the substrate concentration.*

  E + I ------ > EI

  ES + I----- > ESI

- Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg^{2+}, Pb^{2+}).
- Some ***metalloenzymes*** are inhibited ***non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).***
- Inhibitors are used as ***tools to probe the mechanism of enzyme - catalysed reactions*** and *as* ***therapeutic agents***.
- In case of noncompetitive inhibition, Vmax is lowered but Km is not altered

Uncompetitive inhibitor:

- In case of uncompetitive inhibition, the inhibitor binds only to free enzyme and not to the enzyme substrate [ES] complex.

Lecture 21

Enzyme inhibition - competitive, non-competitive and uncompetitive inhibition

Enzyme Inhibition:-

Some of the substances reduces the catalytic activity of enzyme, the substance are called inhibitor .The process is called enzyme inhibition.

Inhibition can be divided into two types they are:-

1. Reversible inhibition
2. Irreversible inhibition

Reversible inhibition: - The inhibitor binds non covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. Reversible inhibition further divided into two:-

1. Competitive inhibition
2. Non- competitive inhibition

❖ **Competitive inhibition**

✓ The type of inhibitor (I) resembles closely related to real substrate (s) it is mentioned as substrate analogue.

✓ The inhibitors competes with substrate and binds at the active site of enzyme but does not undergo any catalysis, competitive inhibitors holds the active site so no place to bind with substrate to the enzyme.

✓ During this :- E +S→ES→ E+ P

E+I →EI

- ✓ In competitive inhibition the K_m value increases, V_{max} remains unchanged

❖ **Non- competitive inhibition :-**

- ✓ The inhibitor binds at a site other than the active site on the enzyme surface.
- ✓ This binding loss the enzyme function, no structural resemblance with the substrate .Inhibitor does not interfere with the enzyme substrate binding.

1. E+S→ ES →ES
2. E+I→EI+S
3. ES+I→EIS

In non-competitive inhibitor K_m value unchanged, Vmax is lowered.

Example:- heavy metals Ag^+ , Pb^{2+} etc…

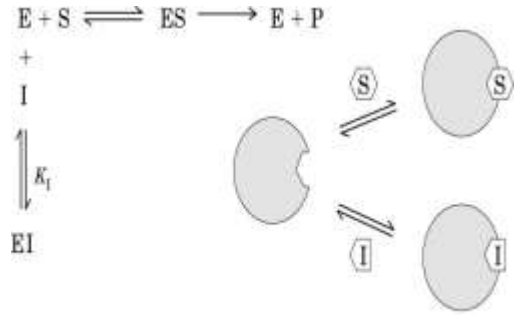
Irreversible inhibition:-

The inhibitors bind covalently with the enzymes and inactivate them these inhibitors are usually toxic poisonous substances.

Example – iodoacetate.

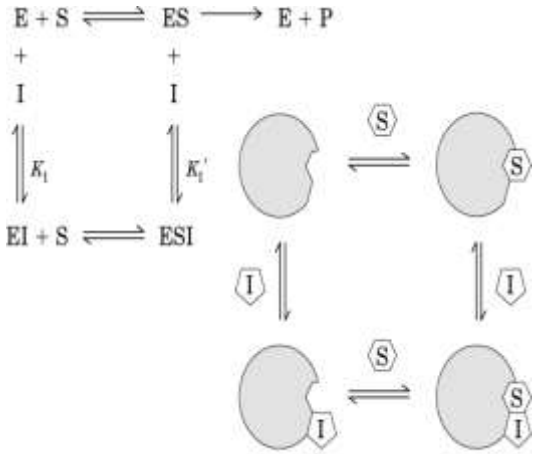
Irreversible inhibitors are frequently used to identify amino acid residues at the active site of the enzymes and also understand the mechanism of enzyme action.

Competitive Inhibition:-



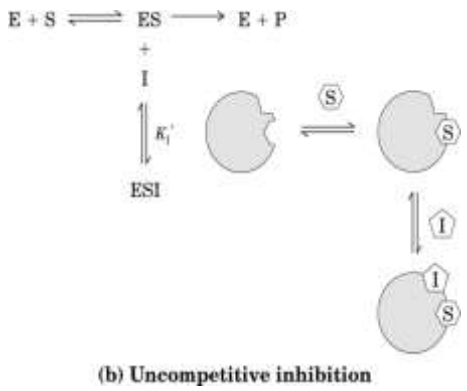
(a) Competitive inhibition

Noncompetitive Inhibition:-



(c) Mixed inhibition

Uncompetitive Inhibition:-



(b) Uncompetitive inhibition

Lecture 22

Apoenzymes, coenzymes and cofactors, Isozymes

A complete, catalytically active enzyme together with its coenzyme and/or metal ions is called holoenzyme.

- The ***protein part of an enzyme*** is called ***apoenzyme or apoprotein***.
- Enzymes require an additional non-protein component to carry out its catalytic functions.
- Generally these **non-protein components** are called as **cofactors.**
- The cofactors may be either one or more ***inorganic ions such as $Fe^{2}+$, $Mg^{2}+$, $Mn^{2}+$ and $Zn^{2}+$*** or a complex ***organic molecules called coenzymes***.
- A coenzyme or metal ion that is covalently bound to the enzyme protein is called ***prosthetic group.***
- Some enzymes require both coenzyme and one or more metal ions for their activity
- Coenzymes function as *transient carriers of specific functional groups*

Cofactors

- Metals are required as cofactors in approximately two thirds of all enzymes.
- ***Metallo enzymes*** contain a definite quantity of functional metal ion that is retained throughout whereas metal-activated enzymes bind metals less tightly but require added metals.
- The distinction between metallo enzymes and metal activated enzymes thus rests on the ***affinity of a particular enzyme for its metal ion.***
- The mechanisms whereby metal ions perform their function appear to be ***similar*** both in metalloenzymes and metal activated enzymes.
- Metals participate through their ***ability to act as Lewis acids and through chelate formation. Eg.*** For metal functioning as a Lewis acid is the **zinc** in **carbonic anhydrase.**
- The metal can also ***promote catalysis by binding substrate at the site of bond cleavage.*** In ***carboxypeptidase***, the carbonyl oxygen is chelated to the zinc. The ***iron-sulfur enzymes*** are unique class of metalloenzymes in which the active centre consists of one or more clusters of ***sulfur-bridged iron chelates***. These are of greater importance in plant systems

Isoenzymes

- **Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes.**
- Such multiple forms can be detected and separated by gel electrophoresis of cell extracts.
- Since they are ***coded by different genes***, they ***differ in amino acid composition and thus in their isoelectric pH values.***
- **Lactate dehydrogenase** is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates.
- All the five isozymes catalyze the same reaction.

Lactate + NAD+ --------- Pyruvate + NADH + H+

- They have the molecular weight of about 134,000 and contain four polypeptides.
- The five isozymes consist of five different combinations of two different kinds of polypeptides **M and H.**
- Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they **differ significantly in their Km values for their substrates as well as Vmax values.**
- The two polypeptide chains in LDH are coded by **two different genes.**
- Skeletal muscle contains four identical M chains and designated as M4; whereas heart muscle contains four identical H chains and designated as H4.
- LDH of other tissues are a mixture of the five possible forms H4, H3M, H2M2, HM3 and M4.
- A **determination of the relative amounts of the five LDH isozymes** and the total concentration of LDH in a serum sample can provide valuable **diagnostic information about which tissues have been damaged and the extent of the damage.**

Lecture 23

Metabolism of carbohydrate – Breakdown of starch by amylases , glycolysis and its energetics

Introduction

- Carbohydrates are major sources of energy for living organisms.
- The **chief source of carbohydrate** in human food is **starch**, which is the storage form of glucose in plants.

- Plants may store relatively large amounts of starch within their own cells in time of abundant supply, to be used later by the plant itself when there is a **demand for energy production.**
- **Glycogen** is the glucose storage polysaccharide of animals.
- It accounts for upto 10% of the mass of the liver and one percent of the mass of the muscle.
- Glycogen is larger and highly branched than amylopectin.
- By the action of several enzymes, such as **α-amylase, β-amylase, amylo α(1→6) glucosidase and ∝(1→4) glucosidase,** starch and glycogen from dietary intake are degraded finally to glucose.
- Carbohydrate is utilized by cells mainly in the form of glucose.
- The three principal monosaccharides resulting from the digestive processes are **glucose, fructose and galactose**.
- Both fructose and galactose are readily converted to glucose by the liver.
- Pentose sugars such as xylose, arabinose and ribose may be present in the diet, but their fate after absorption is obscure.
- Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

The major metabolic processes in carbohydrates are:

i. Glycolysis:

Glycolysis is the sequence of reactions that convert **glucose into pyruvate** with the concomitant trapping of the energy as ATP.

ii. The citric acid cycle:

It is the final **common oxidative pathway for carbohydrates, fats and proteins**. It is also a source of precursors for biosynthesis of various

biomolecules. The **acetyl CoA** that enters in this pathway is completely oxidised to **carbon dioxide and water** with concomitant production of reducing equivalents, namely **NADH and $FADH_2$.**

iii. The hexose monophosphate shunt:

It is an **alternative pathway** to the glycolytic pathway and the citric acid cycle for the oxidation of glucose to carbon dioxide and water with the **generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) molecules and ribose 5-phosphate.**

iv. Gluconeogenesis:

It is a biosynthetic pathway that generates **glucose from non-carbohydrate precursors.**

v. Glycogenesis:

It is a pathway by which **glycogen is synthesised** from glucose.

v. **Glycogenolysis**:

It is a pathway by which glycogen breakdown is takes place.

Glycolysis

- Glycolysis, also called as **Embden-Meyerhof-Parnas pathway (EMP pathway),** consists of a series of reactions through which glucose is converted to pyruvate with the concomitant production of relatively small amounts of adenosine triphosphate (ATP).
- It is derived from the Greek stem '**glykys'** meaning sweet and **'lysis'** meaning splitting.
- It is the primary pathway occurring in the cytoplasm of all the tissues of biological systems.

- All the enzymes responsible for the catalysis are found in the **extra-mitochondrial soluble fraction of the cells (cytoplasm).**

In plants, glucose and fructose are the main monosaccharides catabolised by glycolysis although others are also converted into these sugars.

- Glucose entering the glycolysis is derived from **starch or sucrose**, and fructose is derived from **sucrose.**
- The starch is either from seeds or chloroplasts of matured plants.
- Glycolysis normally takes place in the **presence of O_2** in higher plant cells.

The enzymes in the cytoplasm catalyse the reactions involved in the conversion of **glucose to pyruvate**.

The series of reactions indicated take place in 3 stages.

Stage 1: Conversion of glucose to fructose 1, 6-bisphosphate

- The formation of fructose 1, 6-bisphosphate takes place in three steps catalysed by enzymes.
- The purpose of these reactions is to form a compound that can be readily cleaved into phosphorylated three carbon units from which, through a series of reactions, ATP is formed.
- After the **first phosphorylation** reaction to form glucose 6-phosphate, isomerisation of glucose 6-phosphate to **fructose-6-phosphate** occurs which is conversion of an aldose into a ketose.
- A second phosphorylation reaction follows the isomerization, catalysed by **phosphofructokinase** resulting in the formation of fructose 1, 6-bisphosphate.

- **Phosphofructokinase** is the key enzyme in the control of glycolysis.

Stage 2: Conversion of fructose 1, 6-bisphosphate to 3-phosphoglycerate.

- The **splitting of fructose 1, 6-bisphosphate** occurs in the second stage of glycolysis resulting in the formation of a molecule of glyceraldehyde 3-phosphate and a molecule of dihydroxyacetone phosphate catalysed by **aldolase.**
- The dihydroxyacetone phosphate is isomerised to glyceraldehyde 3-phosphate by **phosphotriose isomerase.** The isomerisation reaction is rapid and reversible.
- In the next step, glyceraldehyde 3- phosphate is oxidised to 1,3-bisphosphoglycerate catalyzed by **glyceraldehyde 3-phosphate dehydrogenase.**
- The product is further converted into 3-phosphoglycerate and a molecule of ATP is formed. The phosphorylation of ADP to ATP is called **substrate level phosphorylation** since the phosphate group from a substrate molecule is transferred to ADP.

Stage 3: Formation of pyruvate

- An intramolecular rearrangement of the phosphoryl group occurs resulting in the formation of 2-**phosphoglycerate from 3-phosphoglycerate catalyzed by phosphoglycerate mutase.**
- The 2-phosphoglycerate formed undergoes **dehydration** forming phosphoenolpyruvate which gives rise to pyruvate and a molecule of ATP (substrate level phosphorylation).
- The reaction is irreversible and catalyzed by **pyruvate kinase.**

The net reaction in the transformation of glucose to pyruvate is

Glucose + 2 Pi + 2ADP + 2 NAD^+ ----→ 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + H_2O

- ✓ Once pyruvate is formed, further degradation is determined by the **presence or absence of oxygen.**
  - ✓ **Under anaerobic conditions**, in one of the pathways, pyruvate undergoes reduction yielding **lactic acid.** The formation of lactic acid is very rare in plants with exception of potato tubers maintained under anaerobic condition and some green algae.
  - ✓ In the second pathway, pyruvate is converted to **ethyl alcohol and carbon dioxide**.
  - ✓ The **alcoholic fermentation** is the basis of the beer and wine-making industries.
  - ✓ Under **aerobic conditions**, pyruvate is **oxidatively decarboxylated to acetyl CoA** which is then completely oxidised to CO_2 and water through the **citric acid cycle**

Energetics of glycolysis

From glucose, two molecules of glyceraldehyde 3-phosphate are formed in the second stage of glycolysis from which two molecules of pyruvate are obtained as end products of glycolysis. Hence energetic of glycolysis is calculated by taking into account two molecules of glyceraldehyde 3-phosphate.

Energetics of glycolysis

Stages/steps	Enzyme	Method of high energy bond formation	No. of ATP formed
Stage 1 Formation of 1, 3-bisphospho glycerate from glyceraldehyde 3-phosphate	Glyceraldehyde 3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
Stage 2 Formation of 3 ph from 1, 3 bisphospho from 1, 3 bisphospho glycerate	Phosphoglycerate kinase	Phosphorylation at subtrate level	2
Stage 3 Formation of pyr phosphoenol pyruvate	Pyruvate kinase	Phosphorylation at substrate level	2

- Allowance for consumption of ATP by reactions catalysed by hexokinase and 2 phosphofructokinase
- Number of ATP molecules generated by the catabolism of one molecule **7**of glucose under aerobic conditions
- Number of ATP molecules generated by the catabolism of one molecule 2 of glucose under anaerobic conditions

Significance of glycolysis

- Glycolysis is an almost **universal central pathway** of glucose catabolism occurring in the cytoplasm of all the tissues of biological systems leading to generation of energy in the form of ATP for vital activities.
- It is the pathway through which the **largest flux of carbon** occurs in most cells.
- Some plant tissues which are modified for the storage of starch such as potato tubers and some plants adapted to growth in inundated water such as water cress derive most of their energy from glycolysis.
- In plants, glycolysis is the **key metabolic component of the respiratory process,** which generates energy in the form of ATP in cells where photosynthesis is not taking place.
- Many types of **anaerobic microorganisms** are entirely dependent on glycolysis.
- Mammalian tissues such as renal medulla and brain solely dependent on glycolysis for major sources of metabolic energy.

Lecture 24

The tricarboxylic acid cycle reactions and bioenergetics

- In 1937, Sir Hans Krebs, an English biochemist proposed a pathway consisting of a cycle of reactions through which acetyl CoA is converted to carbon dioxide and water and hence the cycle was named as Kreb's cycle.
- All the enzymes catalyzing the reactions of this cycle occur inside **mitochondria (mitochondrial matrix**) in contrast with those of glycolysis, which occur in the cytosol.

Before pyruvate can enter the citric acid cycle, it must be oxidatively decarboxylated to acetyl CoA (active acetate).

Three different enzymes working sequentially in a multienzyme complex catalyse this reaction.

This formation of acetyl CoA from pyruvate **by alpha-oxidative decarboxylation** occurs in the mitochondrion following the formation of pyruvate in the cytosol during glycolysis.

The reaction involves six cofactors: **coenzyme A, NAD^+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg^{2+}.**

$$CH_3\text{-}CO\text{-}COOH + CoASH + NAD^+ \xrightarrow[\text{Lipoate, } Mg^{2}]{\text{TPP, FAD}} CH_3\text{-}CO\text{-}S\text{-}CoA + NADH + H^+ + CO_2$$

Reactions of the TCA cycle

Acetyl CoA, derived mainly from the oxidation of carbohydrates, lipids and proteins, combines with oxaloacetate to form **citrate** which is the first reaction of the citric acid cycle.

Subsequently, citrate is oxidised in a series of reactions liberating carbon dioxide and reducing equivalents (NADH, $FADH_2$).

The oxaloacetate is regenerated and functions therefore in a catalytic manner in the oxidation of acetyl CoA to two molecules of carbon dioxide.

The citric acid cycle has eight steps as described below:

i. Formation of citrate

The first step is the reaction between the four-carbon unit, oxaloacetate and the two-carbon unit, acetyl CoA resulting in the formation of citrate and coenzyme A catalysed **by citrate synthase**. The coenzyme A formed in this reaction is recycled.

ii. Formation of iso citrate via cis-aconitase

The isomerization of citrate to iso citrate catalysed by **aconitase** occurs in two steps with the formation of cis-aconitate as an intermediate. This formation of iso citrate involves both dehydration and hydration. The result is an interchange of hydrogen and a hydroxyl group. In this reaction, **fluoro acetate** acts as an inhibitor to the enzyme, aconitase.

iii. Oxidation of iso citrate to α-ketoglutarate

The enzyme, **iso citrate dehydrogenase** oxidatively decarboxylates iso citrate to α-keto glutarate with simultaneous liberation of carbon dioxide. The intermediate in this reaction is oxalo succinate, an unstable β-keto acid. While bound to the enzyme, it loses carbon dioxide to form α-keto glutarate. There are two different forms of isocitrate dehydrogenase (isozymes), one requiring NAD^+ and other requiring $NADP^+$.

iv. Oxidation of α-ketoglutarate to succinyl CoA

α-Ketoglutarate, undergoes oxidative decarboxylation forming succinyl-CoA and carbon dioxide in the presence of **α-ketoglutarate dehydrogenase complex,** an assembly consisting of three kinds of enzymes. The mechanism of this reaction is very similar to the reaction catalyzed by **pyruvate dehydrogenase complex**. This reaction is irreversible. **Arsenite** acts as an inhibitor of TCA cycle by inhibiting the action of α-ketoglutarate dehydrogenase complex.

v. Conversion of succinyl CoA to succinate

Succinate is formed in a reversible reaction from succinyl CoA catalysed by the enzyme, **succinyl CoA synthetase or succinate thiokinase** with the simultaneous formation of GTP and coenzyme A. Succinate thiokinase utilises GDP in animal tissues whereas it uses ADP predominantly in plants and bacteria. The **formation of GTP** in this reaction is a **substrate level phosphorylation reaction.**

vi. Formation of fumarate by oxidation of succinate

The succinate formed from succinyl CoA is oxidised to fumarate by **succinate dehydrogenase** with the participation of FAD. **Malonate,** an analogue of succinate being a strong competitive inhibitor of succinate dehydrogenase, blocks the citric acid cycle.

vii. Formation of malate by hydration of fumarate

The reversible hydration of fumarate to L-malate is catalysed by **fumarase.**

viii. Oxidation of malate to oxaloacetate

This reaction forms the last reaction of the citric acid cycle. NAD-linked malate dehydrogenase catalyses the oxidation of L-malate to oxaloacetate.

Energetics of tricarboxylic acid cycle

From one molecule of glucose, two molecules of pyruvate are formed which in turn give rise to two molecules of acetyl CoA. When two molecules of acetyl-CoA undergo oxidation through TCA cycle, the following number of high-energy bonds (ATPs) are produced.

Significance of the TCA cycle

i) The major significance of the citric acid cycle is to act as the **final common pathway for the oxidation of carbohydrates, lipids and proteins,** since glucose, fatty acids and many amino acids are all metabolised to acetyl CoA.

ii) This cycle serves as the mechanism by which much of **the free energy liberated during the oxidation of carbohydrate, lipids and amino acids is made available.**

iii) TCA cycle is of further significance since it has **dual or amphibolic role thus providing precursor compounds for biosynthesis of other biomolecules** (amino acids, fatty acids, and glucose.

Lecture – 25

The hexose monophosphate shunt (or) Pentose Phosphate Pathway

The hexose monophosphate shunt (HMP shunt), also called as pentose phosphate pathway (PPP) and phosphogluconate pathway is an alternate pathway for the oxidation of glucose. In 1930, Otto Warburg discovered the first evidence for the existence of this pathway, which was later, elucidated in 1950 by Frank Dickens group.

The pathway is important during the hours of darkness and in non-photosynthetic tissues such as differentiating tissues and germinating seeds. In animal system, it occurs in certain tissues, notably liver, lactating mammary gland and adipose tissue in addition to the Embden - Meyerhof pathway. The enzymes of the shunt pathway are found in the extra mitochondrial soluble portion of the cell. It is in effect, a multicyclic process whereby three molecules of glucose 6-phosphate give rise to three molecules of CO_2 and three 5-carbon residues. The latter are rearranged to regenerate two molecules of glucose 6-phosphate and one molecule of glyceraldehyde-3-phosphate. Since two molecules of glyceraldehyde 3-phosphate can regenerate a molecule of glucose 6-phosphate by reactions, which are essentially a reversal of glycolysis, the pathway can account for the complete oxidation of glucose. Here oxidation is achieved by dehydrogenation using NADP and not NAD as in Embden-Meyerhof's glycolytic pathway. This pathway consists of a series of reactions taking place in three stages

Stage I. Formation of NADPH and ribulose 5-phosphate

The first three reactions of the pathway, catalysed by glucose-6-phosphate dehydrogenase, phosphogluconolactonase and

phosphogluconate dehydrogenase ultimately result in **the formation of ribulose 5-phosphate and NADPH.**

Stage II. Ribulose 5-phosphate is converted to ribose 5-phosphate

In this stage, the ribulose 5-phosphate is converted to ribose 5-phosphate by ribulose 5-phosphate isomerase and then to xylulose-5 phosphate by ribulose 5-phosphate epimerase. The ribose 5-phosphate is essential precursor in the biosynthesis of nucleotides.

Stage III. Formation of glyceraldehyde 3- phosphate

In the third stage, three molecules of the 5-carbon sugars are converted to two molecules of 6-carbon sugars and one molecule of 3-carbon sugar, glyceraldehyde 3- phosphate catalysed **by two enzymes, transaldolase and transketolase.**

Transketolase catalyses the transfer of a C_2 unit from xylulose 5-phosphate to ribose 5-phosphate yielding glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate.

Transaldolase catalyses the transfer of a three carbon unit from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate yielding erythrose 4-phosphate and fructose 6-phosphate.

Control of the HMP shunt

Ribose 5-phosphate and NADPH are the principal products of the HMP shunt. In this pathway, excess amount of ribose 5-phosphate is converted into glycolytic intermediates when the need for NADPH exceeds that of ribose 5-phosphate in nucleotide biosynthesis.

If ribose 5-phosphate is needed more than NADPH, fructose 6-phosphate and glyceraldehyde 3-phosphate are used for the synthesis of

ribose 5-phosphate by reversal of the transaldolase and transketolase reactions.

The rate of NADPH formation in the pathway is controlled by the rate of the glucose 6-phosphate dehydrogenase reaction.

Metabolic significance of the HMP Shunt

i. Major function of HMP shunt appears to be the production of reduced NADP (NADPH) required by anabolic (synthetic) processes such as fatty acid synthesis outside the mitochondria.
ii. The pathway provides ribose for nucleotide and nucleic acid synthesis.
iii. It also provides erythrose required for the synthesis of phenolics and other aromatic compounds through shikimate pathway.

Glucose 6-phosphate can be used as a substrate either for glycolysis or for the pentose phosphate pathway. On the basis of the cell's needs, it makes this choice for biosynthesis and for energy from catabolism. If glucose 6-phosphate is channeled into glycolysis, ATP is produced in abundance; but if it is channeled into pentose phosphate pathway. NADPH and ribose 5-phosphate are produced. The fate of glucose 6-phosphate is determined to a large extent of phosphofructokinase and glucose-6 P. There are four principal possibilities in which, depending upon the cell's need, HMP shunt operates.

i. More ribose 5-phosphate than NADPH is required

Most of the glucose 6-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by the glycolytic pathway. Two molecules of fructose 6-phosphate and one molecule of glyceraldehyde

3-phosphate are converted into three molecules of ribose 5-phosphate by a reversal of reactions catalysed by transaldolase and transketolase reactions.

ii. Both ribose 5-phosphate and NADPH are needed by the cell

In this, the first four reactions of the pentose phosphate pathway predominate. Ribose 5-phosphate is the principal product of the metabolism and NADPH is also produced. The net reaction for these processes is

Glucose 6 P + 2 $NADP^+$ + H_2O --------> Ribose 5-Phosphate + CO_2 + 2 NADPH + $2H^+$

3. More NADPH than ribose 5-phosphate is needed by the cell

Under this situation, glucose 6-phosphate is completely oxidized to carbon dioxide. Three reactions are active. First, two NADPH and one ribose 5-phosphate are formed by the oxidative branch of the pentose phosphate pathway. Then, ribose 5-phosphate is converted into fructose 6-phosphate and glyceraldehyde 3-phosphate by transketolase and transaldolase. In the final reaction, glucose 6-phosphate is resynthesised from fructose 6-phosphate and glyceraldehyde 3-phosphate by the gluconeogenic pathway. The sum of these reactions is

Glucose 6-phosphate + 12 $NADPH^+$ + 7H_2O -------> 6 CO_2 + 12 NADPH + 12H^+ + Pi

iv. Both NADPH and ATP are needed by the cell.

In this, fructose 6-phosphate and glyceraldehyde 3-phosphate derived from ribose 5-phosphate enter the glycolytic pathway and form pyruvate.

ATP and NADPH are concomitantly generated and five of the six carbons of glucose 6-phosphate emerge in pyruvate.

3 Glucose 6-phosphate + 6 $NADP^+$ + 5NAD^+ + 5 Pi + 10ADP ------> 5 pyruvate + 3 CO_2 + 6NADPH + 5NADH + 10ATP + 2H_2O + 10H^+

Comparative account of glycolysis and HMP shunt

These two major pathways are meant for the catabolism of glucose. They have little in common, e.g. the presence of metabolites like glucose 6-phosphate. The major differences are

i. ATP is not generated in the HMP pathway, whereas in glycolysis, ATP molecules are generated.

ii. Pentose phosphates are generated in the HMP pathway but not in glycolysis.

iv. NADH is produced in glycolytic pathway whereas NADPH is produced in HMP shunt.

Lecture 26

Respiration -Electron transport chain and oxidative phosphorylation

- The mitochondrion is the aerobic organelle in which the final stage of the oxidation of food occurs.
- It is the site of the citric acid cycle, fatty acid oxidation and oxidative phosphorylation, processes that are responsible for the formation of ATP under aerobic condition.
- The two most important energy transductions in the biological systems are the **oxidative phosphorylation (ATP synthesis driven by electron transfer** to oxygen) and photophosphorylation (ATP synthesis driven by light).
- Oxidative phosphorylation is the process in which ATP molecules are formed as a result of the transfer of electrons from the reducing equivalents, NADH or $FADH_2$ (produced by glycolysis, the citric acid cycle and fatty acid oxidation) to oxygen by a series of electron carriers in the form of a chain located in the inner membrane of mitochondria. This is the final reaction sequence of respiration.
- Since the electrons are transferred by a series of electron carriers in the form of a chain, it is known as electron transport chain (ETC).
- In plants, ATP is mainly derived through photosynthesis utilizing the energy derived from the sun. In non-photosynthetic tissues, ATPs are derived through respiration.

The electrons are transferred along a set of cytochromes in the form of a chain in steps from the **more electronegative components (NADH/$FADH_2$) to the more electropositive oxygen.**

The respiratory chain consists of a number of protein complexes that are remarkably complicated in nature. They are known as **NADH-**

ubiquinone reductase, succinate-ubiquinone reductase, ubiquinone-cytochrome c reductase and cytochrome c oxidase. These complexes are also called as **NADH dehydrogenase, succinate dehydrogenase, cytochrome b-c complex and cytochrome c oxidase respectively or as complexes I - IV.**

All the three reductases are also known as iron-sulphur proteins since they contain Fe-S centres as their critical components. Iron in these enzyme complexes can exist in two forms as Fe^{2+} and Fe^{3+}. Each cytochrome in its oxidised form (Fe^{3+}) accepts one electron and becomes reduced to Fe^{2+} form. Fe^{2+} donates electron to the next carrier.

Oxidation of one molecule of NADH results in generation of 2.5 molecules of ATP whereas oxidation of one molecule of $FADH_2$ generates 1.5 molecules of ATP.

Sites of ATP formation

When electrons are transported along the respiratory chain, due to high amount of energy released, ATP molecules are synthesised at the following three sites.

i. **Transfer of electrons from NADH to ubiquinone via flavoprotein (FMN).**
ii. **Transfer of electrons from cyt b to cyt c.**
iii. **Transfer of electrons from cyt a to cyt a_3**

Mechanism of ATP formation

Two principal hypotheses have been proposed for the mechanism of oxidative phosphorylation.

i. Chemical hypothesis

ii. Chemiosmotic theory

Chemical hypothesis

Many attempts have been made since 1920 to identify an energy-rich metabolite linking oxidation and phosphorylation. No such intermediates was isolated and in 1960, Peter Mitchell suggested that no possibility of existence of such an intermediate compound. So, the chemical hypothesis has become discredited.

Chemiosmotic theory

The chemiosmotic theory states that the coupling of oxidation to phosphorylation is indirect. According to this, the hydrogen ions (protons) generated by the oxidation of components in the respiratory chain are ejected to the outside (matrix) of the inner membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions (protons or H^+) is used to drive a membrane-located ATP synthase which in the presence of Pi + ADP forms ATP.

Inhibitors of respiratory chain

Inhibitors, which inhibit respiratory chain, may be grouped as follows:

i. **Inhibitors of electron transfer**
ii. **Inhibitors of ATP synthase**
iii. **Uncouplers of oxidative phosphorylation**

Inhibitors that arrest respiration by blocking the respiratory chain act at three sites.

Compounds such as barbiturates, amytal, rotenone prevent the transfer of electron from FeS centre to ubiquinone. Carboxin specifically inhibits transfer of reducing equivalents from succinate dehydrogenase to ubiquinone.

Antimycin A blocks electron transfer from cytochrome b to cytochrome c_1. Substances such as cyanide (CN^-), azide (N_3^-) and carbon monoxide inhibit cytochrome c oxidase by binding to heme group and are extremely poisonous. Oligomycin inhibits ATP synthase.

In the presence of the uncouplers such as dicoumarol and 2,4-dinitrophenol, oxidation proceeds without phosphorylation (dissociation of oxidation in the respiratory chain from phosphorylation) releasing energy in the form of heat rather than in the form of ATP.

Lecture: 27

Lipid metabolism - Lipases and Phospholipases

Lipids constitute one of the four major classes of compounds that are found in living systems. The lipids of metabolic significance include triacylglycerol, phospholipids and the products of lipid metabolism such as free fatty acids and glycerol.

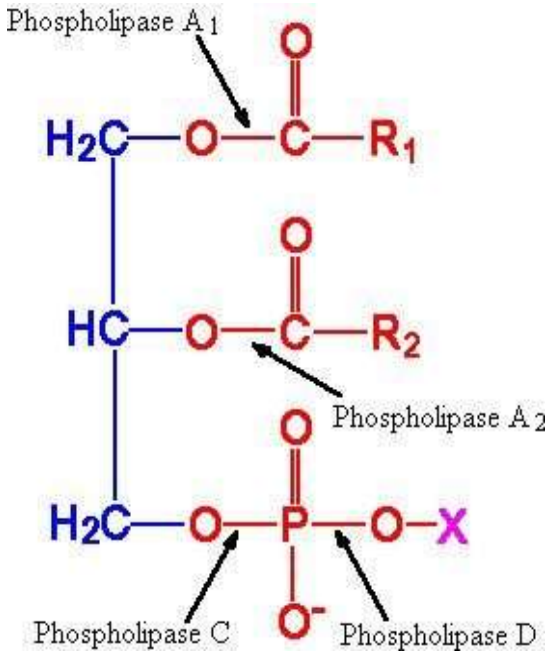
Lipases

- Triacylglycerols or triglycerides undergo hydrolysis by **lipases** to form glycerol and fatty acids, which undergo further oxidation generating energy.
- Lipases have been reported to be present in **dry seeds of some species, e.g. castor bean, Scots pine and Douglas fir** but at a low level, or absent in others e.g. apple.
- In most cases of seeds, **following imbibitions**, there appears to be a **rise in lipase activity** but whether this increase is due to the *de novo* synthesis of the enzyme or activation of existing lipases has not been determined.

- A **decline in lipase activity** is always associated with decline in acylglycerol reserves.
- In castor bean, as in many other fat-storing seeds, free fatty acids do not accumulate, but are rapidly degraded and converted to carbohydrate within the endosperm.
- In other seeds such as **germinating seeds of oil palm** (*Elaeis guineensis*), a different pattern of fat mobilization can be observed.
- The products of lipid catabolism are transported via specialized structures called **haustorium** through its vascular system.
- Lipases are generally **non-specific** and can hydrolyse a wide variety of triacylglycerols
- They initiate digestion by hydrolyzing triacylglycerols to form free fatty acids and 1, 2-diacylglycerols.
- Complete hydrolysis of triacylglycerols produces **glycerol and fatty acids.**
- Lipase hydrolyses easily the terminal fatty acids to produce 2-monoacyl glycerol as major

Phospholipases

- Phospholipases are the hydrolytic enzymes acting on phospholipids and splitting into different products.
- There are four types of phospholipases known as **phospholipase A_1, phospholipase A_2 or B_1, phospholipase C and phospholipase D.**



Phospholipase A

- Phospholipase A is present in large amounts in **snake venom and human pancreas.**
- It is also designated as **phospholipase A1.**
- It catalyses the hydrolysis of the fatty acids in the 2 or β-position of the phospholipids.
- Though this enzyme attacks on glycerophosphatides, it is fairly specific for phosphatidyl choline (lecithin).
- The enzyme is relatively stable to heat (below pH 7.0).
- The product of the hydrolysis, a **lysolecithin**,(monoacylphosphoryl choline) has a powerful **hemolytic activity**.

Phospholipase B (A_2)

- It is otherwise termed as **lysophospholipase** and widely distributed in nature often in association with phospholipase A.

- Phospholipase B is also designated as **phospholipase A_2** since it acts on the lysolecithin (the product obtained from phospholipid by the action of phospholipase A_1).
- The action of this enzyme following that of phospholipase A yields **glycerophosphoryl choline** as the final product.

Phospholipase C

- Phospholipase C is mostly found in the plant kingdom but it may also be present in some animal tissues and venoms.
- It catalyses the liberation of a 1,2-diacylglycerol and phosphoryl choline from phosphatidyl choline.
- Phosphoryl choline is also liberated from sphingomyelin by this enzyme.

Phospholipase D

- Phospholipase D, an enzyme described mainly in plants catalyses the **hydrolysis of choline** from phosphatidyl choline leaving phosphatidic acid.

Lecture: 28

Oxidation of fatty acids

Fatty acids obtained by hydrolysis of fats undergo different oxidative pathways designated as alpha (α), beta (β) and omega (ω) pathways.

α-oxidation

- α-Oxidation of fatty acids has been found in certain tissues especially in **brain tissue of mammals and plant systems.**

- It does not require CoA intermediates and no high-energy phosphates are generated.
- This type of oxidation results in the **removal of one carbon** at a time from the **carboxyl end of the fatty acid**.
- The physiological role of α-oxidation in plants is not yet fully established but it has been suggested that it may be **involved in the degradation of long chain fatty acids** as observed in many animal tissues.
- α-Oxidation is clearly the **main source of the odd-carbon fatty acids** and their derivatives that occur in some plant lipids.
- In this process, sequential removal of one carbon at a time from free fatty acids of chain length ranging from C_{13} to C_{18} occur.

ω-Oxidation

- ω-Oxidation is normally a very minor pathway brought about by **hydroxylase enzymes** involving **cytochrome P-450** in the **endoplasmic reticulum.**
- Fatty acids with oxygen function (alcoholic or carboxyl) at the methyl terminal end (ω-end) are formed by ω-oxidation and frequently occur as constituents of **cutin and suberin.**
- The requirements for the oxygenase-mediated conversion of a ω-methyl fatty acyl CoA into a ω-hydroxymethyl fatty acyl CoA are **molecular oxygen,** reduced pyridine nucleotide and **a non-heme iron protein** in higher plants.

β-Oxidation of fatty acids

In 1904, Franz Knoop made a critical contribution to the elucidation of the mechanism of fatty acid oxidation and demonstrated that most of the fatty acids are degraded by oxidation at the **β-carbon.**

- β-Oxidation of fatty acids takes place in **mitochondria.**
- Fatty acids are activated before they enter into mitochondria for oxidation.

Activation of fatty acids

- Fatty acids are converted into active intermediate in a reaction with **ATP and coenzyme A.**
- A **thioester linkage** between the carboxyl group of a fatty acid and the sulfhydryl group of coenzyme A is formed with the hydrolysis of ATP.
- This activation reaction takes place on the **outer mitochondrial membrane** catalysed by **acyl CoA synthetase**.
- Several **acyl CoA synthetases** each specific for fatty acids of different chain length are present in the membrane of mitochondria.

Penetration of long chain fatty acids into mitochondria

- Long chain acyl-CoA molecules do not readily get into the inner mitochondrial membrane and are carried across the inner membrane by conjugating with **carnitine** (β-hydroxy γ-trimethyl ammonium butyrate), a zwitterionic compound formed from lysine.
- Activation of lower fatty acids and their oxidation within the mitochondria occur independently of carnitine, but long-chain acyl CoA will become oxidised unless they form acylcarnitines.
- The acyl CoA combines with carnitine in the presence of **carnitine acyltransferase I,** which is bound to the outer mitochondrial membrane.
- **Acylcarnitine** is transported in, coupled with the transport out of one molecule of carnitine.

- ❖ The acylcarnitine then reacts with coenzyme A catalyzed by **carnitine palmitoyl transferase II,** located on the inside of the inner membrane.
- ❖ Acyl CoA is reformed in the mitochondrial matrix and carnitine is liberated.

Oxidation

A saturated acyl CoA is oxidised by a **recurring sequence of four reactions**

- ❖ Oxidation in presence of FAD, hydration, oxidation in presence of NAD^+, and thiolysis by CoASH.
- ❖ In β-oxidation, **2 carbons are cleaved** at a time from acyl CoA molecules, starting from the carboxyl end.
- ❖ The chain is **broken** between the α**-and** β-carbon atoms.
- ❖ The two-carbon units formed are acetyl CoA.

i. The first reaction in β-oxidation of acyl CoA is the formation of *trans* Δ^2- enoyl CoA or α, β-unsaturated acyl CoA in presence of acyl-CoA dehydrogenase and the coenzyme, FAD.

ii. The next step is the **hydration of the double bond** between C-2 and C-3 by enoyl CoA hydratase with the formation of β-hydroxy acyl CoA.

iii. In the third step, the β-hydroxy acyl CoA is **dehydrogenated** in the presence of β-**hydroxy acyl CoA dehydrogenase** and NAD^+ forming β-ketoacyl CoA.

iv. In the last step of β-oxidation, β-ketoacyl CoA reacts with coenzyme A in the presence of the enzyme, **thiolase.**

The products of this reaction are acetyl CoA and an acyl CoA containing **two carbons less than the original acyl CoA molecule** that underwent oxidation.

By the above steps of β-oxidation fatty acids are completely degraded to acetyl CoA units. The acetyl CoA formed from fatty acids can be oxidised to carbon dioxide and water via citric acid cycle.

Energetics of β oxidation

The energetics or the energy conserved in terms of ATP by oxidation of a molecule of palmitic acid is given below:

- Palmitic acid (16 carbons) undergoes β-oxidation forming eight molecules of acetyl CoA by undergoing **seven β-oxidation spirals.**
- When one cycle of β-oxidation takes place, one molecule of $FADH_2$, one molecule of NADH and one molecule of acetyl CoA are produced.
- Electrons from these reducing equivalents ($FADH_2$ and NADH) are transported through the **respiratory chain in mitochondria** with simultaneous regeneration of high-energy phosphate bonds.
- Mitochondrial oxidation of $FADH_2$ eventually results in the net formation of about 1.5 ATP.
- Likewise, oxidation of electrons from NADH yields 2.5 molecules of ATP. Hence, a total of **four ATP molecules** are formed per cycle and **ten molecules of ATP** are formed through Krebs's cycle from each molecule of acetyl CoA.

8 Acetyl CoA through TCA cycle yield (8x10) = 80 ATP

7 β-oxidation spiral reactions yield (7x4) = 28 ATP

Total **108 ATP**

ATP utilized in the initial step = 2 ATP

Hence, **complete oxidation of palmitic acid** yields **106 ATP.**

Oxidation of monounsaturated fatty acids

- Oxidation of monounsaturated fatty acids follows many of the reactions of saturated fatty acids except the requirement of **two additional enzymes, an isomerase and a novel reductase.**
- Reactions of monounsaturated fatty acid are explained by considering the oxidation of a C-16 unsaturated fatty acid, palmitoleic acid, having a single double bond between C-9 and C-10.
- Palmitoleic acid is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids.
- Palmitoleoyl CoA undergoes three cycles of degradation as in β oxidation. But the *cis* Δ^3 decenoyl CoA formed after the third cycle does not serve as a substrate for acyl CoA dehydrogenase.
- The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3.
- **An isomerase** converts the *cis* double bond into a *trans* double bond and shifts the position of double bond between C-2 and C-3.

- The subsequent or follow up reactions are those of the β oxidation pathway in which the *trans* Δ^2 decenoyl CoA is a regular substrate.

Oxidation of polyunsaturated fatty acids

The oxidation of a polyunsaturated fatty acid, linoleic acid, with *cis*-Δ^9 and *cis*-Δ^{12} double bonds, is considered.

- The *cis*-Δ^3 double bond formed after three rounds of β-oxidation is converted into a trans double bond by the **isomerase.**
- This permits one more round of β-oxidation.
- The acyl CoA produced by four rounds of β-oxidation of linoleic acid contains a *cis*-Δ^4 double bond, which undergoes dehydrogenation by **acyl CoA dehydrogenase** yielding *trans* Δ^2, *cis*-Δ^4 dienoyl intermediate.

- This intermediate is not a substrate for the next enzyme in the β-oxidation pathway.
- This intermediate is converted into a ***trans*** Δ^3 **enoyl CoA** to the trans Δ^2 form, an intermediate generally found in β-oxidation pathway and results in complete oxidation of the fatty acid.

Lecture: 29

Fatty acid and triacyl glycerol biosynthesis

Biosynthesis of fatty acids

- It was thought that fatty acid biosynthesis occurred by **reversal of the β-oxidation pathway.**
- On the contrary, it occurs by a separate pathway that differs from β-oxidation in several ways.

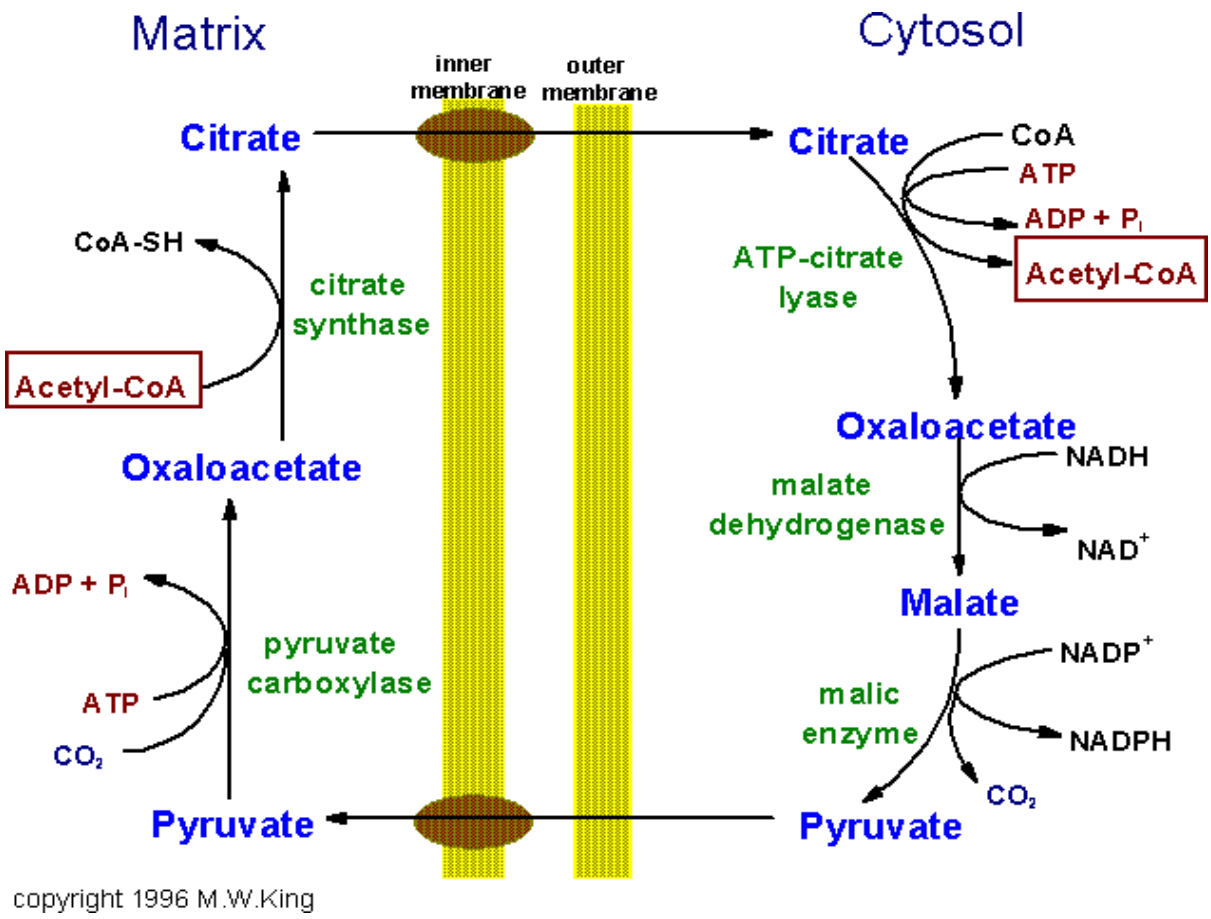
i. Synthesis takes place in the **cytosol,** in contrast with degradation or oxidation, which occurs in the **mitochondrial matrix**.

ii. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl group of an **acyl carrier protein (ACP)** whereas intermediates in fatty acid breakdown are bonded to coenzyme A.

iii. The enzymes of fatty acid synthesis in animals are **joined in a single polypeptide chain called fatty acid synthase.** In contrast, the degradative enzymes do not seem to be associated. Plants employ separate enzymes to carry out the biosynthetic reactions.

iv. The reductant in fatty acid synthesis is **NADPH,** whereas the oxidants in fatty acid oxidation are NAD^+ and FAD.

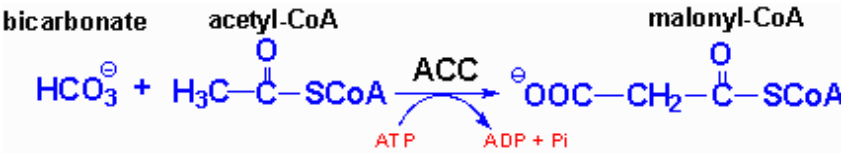
Pathway for the movement of acetyl-CoA units from within the mitochondrion to the cytoplasm for use in lipid and cholesterol biosynthesis.



The following seven steps are involved in fatty acid biosynthesis.

Formation of malonyl CoA

The synthesis of malonyl CoA from acetyl CoA is catalyzed by acetyl CoA carboxylase having biotin as prosthetic group. The production of malonyl CoA is the initial and controlling step in fatty acid synthesis. In this reaction, bicarbonate serves as a source of CO_2. The reaction takes place in two steps, namely carboxylation of biotin involving ATP and transfer of the carboxyl group to acetyl CoA resulting in malonyl CoA.



- **Acetyl CoA carboxylase** plays a key role in regulating fatty acid metabolism and the same is inactivated by phosphorylation.

ii) Formation acetyl and malonyl ACP

Acetyl transacylase and malonyl transacylase catalyze the formation of acetyl ACP and malonyl ACP respectively. Acetyl transacylase can transfer acetyl as well acyl groups whereas malonyl transacylase is highly specific.

Acetyl transacylase
Acetyl CoA + ACP ----------→ acetyl - ACP + COASH

Malonyl transacylase
Malonyl CoA + ACP ----------→ Malonyl - ACP + COASH

iii) Formation of acetoacetyl - ACP (β-ketoacyl ACP)

- Acetyl ACP condenses with malonyl ACP to form acetoacetyl ACP.
- Carbondioxide is eliminated from malonyl ACP.

iv) Reduction of β-ketoacyl ACP to β-hydroxyl acyl ACP.

- The β- keto group in acetoacetyl ACP is reduced by **NADPH-dependent β-ketoacyl reductase.**

v) Formation of unsaturated acyl ACP.

The β-hydroxyl group combines with the hydrogen atom attached to the γ-carbon and a water molecule is removed to form α, β-unsaturated acyl ACP.

vi) Formation of Acyl ACP

- The unsaturated acyl ACP is converted in the next step to a saturated acyl ACP by the enzyme **α, β-unsaturated acyl ACP reductase** using NADPH as the coenzyme.
- The resultant product contains two carbon atoms more than the starting material.
- Addition of subsequent acetyl units through malonyl ACP leads to the formation of 16-carbon palmitate.

Stoichiometry of fatty acid synthesis

The stoichiometry of the synthesis of palmitate is given below:

Acetyl CoA + 7 malonyl CoA + 14 NADPH + 20 H^+ ⟶

Palmitate + 7 CO_2 + 14 $NADP^+$ + 8 CoASH + 6 H_2O

The equation for the synthesis of the malonyl CoA used in the above reaction is

7 Acetyl CoA + 7 CO_2 + 7 ATP ⟶ 7 malonyl CoA + 7ADP + 7 Pi + 14 H^+

The overall stoichiometry for the synthesis of palmitate is

8 Acetyl CoA + 7 ATP + 14 NADPH + 6H^+ ⟶ Palmitate +

14 NADP + 8 CoASH + 6 H_2O + 7 ADP + 7 Pi

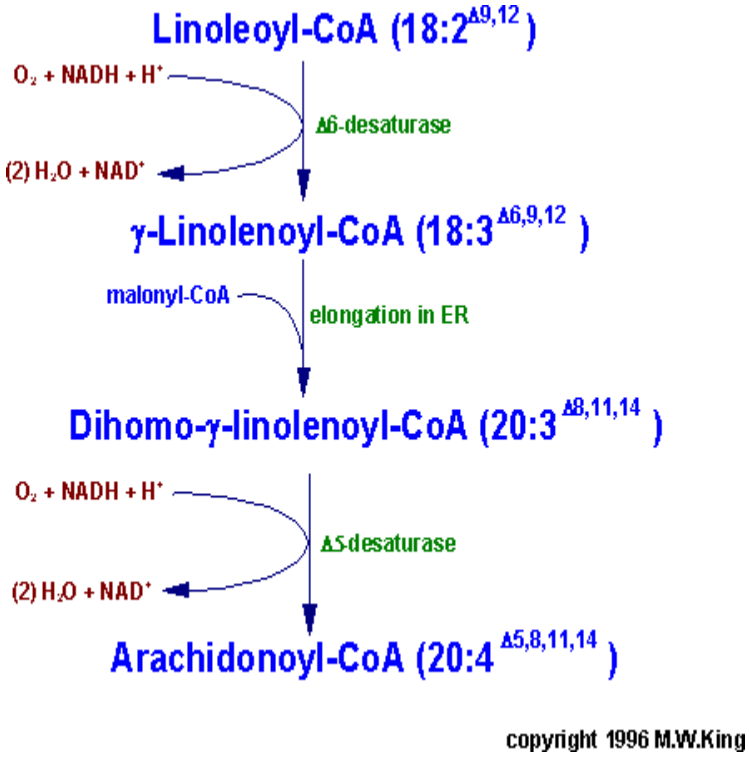
Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active

Elongation of fatty acids or synthesis of long chain fatty acids

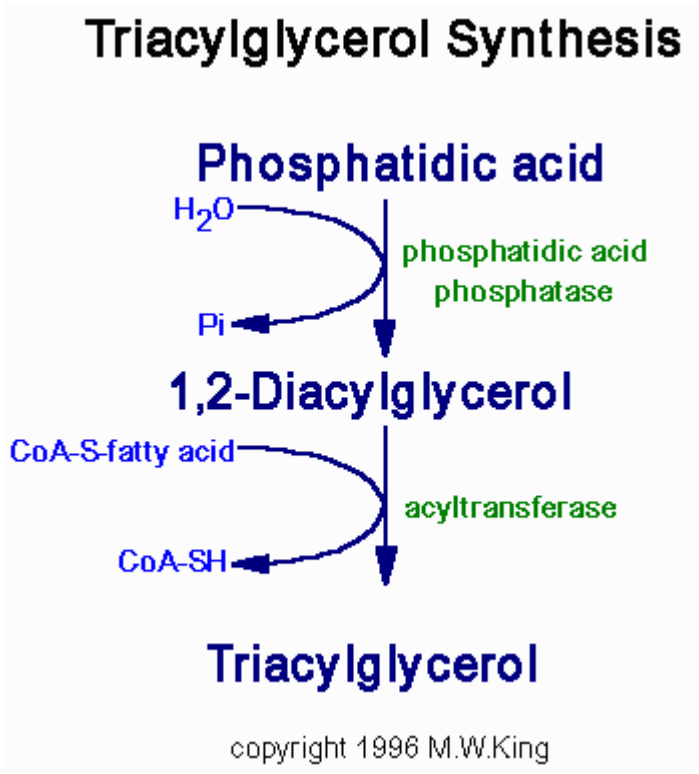
- Elongation by the **fatty acid synthase complex** stops upon formation of palmitate (16 C).
- Further elongation and the formation of double bonds are carried out by other enzyme systems**.**
- The major product of fatty acid biosynthesis is the 16-carbon fatty acid, palmitate.
- Additional enzymes are required to synthesise longer chain fatty acids.
- Chain elongation reactions occur both in **mitochondria and in microsomes.** Microsomes are small membrane-enclosed vesicles derived from the endoplasmic reticulum of cells.
- Mitochondria and microsomes carry out chain elongation by adding two-carbon units to fatty acids.
- The microsomal system has great physiological significance in that it provides the long chain fatty acids (18-24C) required for the **myelination of nerve cells in animal system.**
- Chain elongation occurs by a cycle **of condensation, reduction, dehydration followed by another reduction** that parallels cytosolic fatty acid biosynthesis.
- The more active elongation system adds **two carbons to palmitoyl-CoA to make it steroyl CoA.**
- The mechanism of elongation is identical with that known in the synthesis of palmitate except the enzyme systems and the acyl carrier protein.

Biosynthesis of unsaturated fatty acids

- Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids, **palmitoleate, 16:1, (Δ^{9}) and oleate, 18:1 (Δ^{9})** respectively.
- Each of these fatty acids has a single double bond between C-9 and C-10.
- The **double bond is introduced into the fatty acid chain** by an oxidative reaction catalysed by **fatty acyl-CoA desaturase**, which is **NADPH-dependent enzyme.**
- The unsaturated fatty acids, linoleate, 18:2 ($\Delta^{9,12}$) and α-linolenate, 18:3 ($\Delta^{9,12,15}$) cannot be synthesised by mammals; but plants can synthesize both.
- The **desaturases** responsible for synthesis of both the above fatty acids are present in **endoplasmic reticulum of plants**.
- The plant **desaturases** oxidise phosphatidylcholine-bound oleate and produce polyunsaturated fatty acids and do not directly add double bonds to the fatty acids.
- Once ingested, the linoleate are readily converted to other **polyunsaturated fatty acids like γ-linolenate, arachidonic acid** etc. in animals and human beings.



Biosynthesis of triacylglycerols



- Triacylglycerols are not synthesised by reversal of lipolysis.
- They are synthesisd by a different mechanism in which both glycerol and fatty acids are activated by ATP before they are incorporated into acylglycerols.

i) Activation of glycerol

- *Glycerol kinase* catalyses the activation of glycerol to glycerol 3-phosphate.
- If glycerol kinase is found in low quantity or absent, glycerol 3-phosphate will be formed from dihydroxyacetone phosphate obtained from glycolysis and this reaction is catalysed by the enzyme ***glycerol 3-phosphate dehydrogenase.***

ii) Activation of fatty acids

- Fatty acids are activated to acyl CoA by the enzyme **acyl CoA synthetase**, utilizing ATP and CoASH.
- Two molecules of acyl CoA combine with glycerol 3-phosphate to form 1,2-diacylglycerol phosphate.
- Formation of 1,2-diacyl glycerol phosphate takes place in two stages, catalysed by **glycerol 3-phosphate acyl transferase** and then by **1-acyl glycerol 3- phosphate acyl transferase.**
- The phosphate group is removed from 1,2-diacyl glycerol phosphate by **phosphatidate phosphatase** to form 1,2-diacyl glycerol.
- Triacylglycerols are finally formed by esterification of one or more molecule of acyl CoA with the diacylglycerol.

Alternative pathway for triacylglycerol biosynthesis

- In this pathway, dihydroxyacetone phosphate from glycolysis is reduced by NADPH, acylated and converted to lysophosphatidate.

- This pathway accounts for less than 10% of total triacylglycerol synthesis.

Lecture 30

Transamination, deamination and decarboxylation

- Protein metabolism is a key physiological process in all forms of life.
- Proteins are converted to amino acids and then catabolised.
- The complete hydrolysis of a polypeptide requires mixture of peptidases because individual peptidases do not cleave all peptide bonds.
- Both **exopeptidases and endopeptidases** are required for complete conversion of protein to amino acids.

Amino acid metabolism

- The amino acids not only function as **energy metabolites** but also used as **precursors** of many physiologically important compounds such as **heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes.**
- In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of amino acids.
- Since the 20 common protein amino acids are distinctive in terms of their carbon skeletons, amino acids require unique degradative pathway.
- The degradation of the carbon skeletons of 20 amino acids converges to just ***seven metabolic intermediates*** namely.
    i. **Pyruvate**
    ii. **Acetyl CoA**

iii. **Acetoacetyl CoA**
iv. **α-Ketoglutarate**
v. **Succinyl CoA**
vi. **Fumarate**
vii. **Oxaloacetate**

- Pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as **precursors for glucose synthesis** through gluconeogenesis. Amino acids giving rise to these intermediates are termed as **glucogenic.**
- Those amino acids degraded to yield **acetyl CoA** or acetoacetate are termed **ketogenic.** Since these compounds are used to synthesize ketone bodies.
- Some amino acids are **both glucogenic and ketogenic** (For example, phenylalanine, tyrosine, tryptophan and threonine.

Catabolism of amino acids

The ***important reaction*** commonly employed in the *breakdown of an amino acid* is always the **removal of its α-amino group**. The product **ammonia** is excreted after conversion to **urea** or other products and the carbon skeleton is degraded to CO_2 releasing energy. The important reaction involved in the deamination of amino acids is

i. Transamination

ii. Oxidative deamination

iii. Non oxidative deamination

Transamination

- Most amino acids are deaminated by transamination reaction catalysed by **aminotransferases or transaminases.**

- The **α-amino group** present in an amino acid is transferred to an α-keto acid to yield a new amino acid and the α-keto acid of the original amino acid.
- The predominant amino group acceptor is **α-keto glutarate**. Glutamate's amino group is then transferred to oxaloacetate in a second transamination reaction yielding aspartate.

Glutamate + oxaloacetate ----------→ α-ketoglutarate + aspartate

pyridoxal phosphate

- **Pyridoxal phosphate**, the coenzyme of pyridoxine (vitamin B6) plays an important role in these reactions.
- Amino transferase reactions occur in two stages.
    - Pyridoxal phosphate is covalently attached to the amino transferases via a **Schiff's base linkage** formed between the aldehyde group of pyridoxal phosphate and the ***epsilon amino group of lysine*** residue of the enzyme. Pyridoxal phosphate is converted to pyridoxamine phosphate.
    - In the second stage, the amino group attached to pyridoxamine phosphate is transferred to a different keto acid to yield a new amino acid and releases pyridoxal phosphate

Oxidative deamination

- Transamination does not result in net deamination, since one amino acid is replaced by another amino acid.
- The **function of transamination is to funnel the amino nitrogen** into one or a few amino acids.

- For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is needed so that α-ketoglutarate can be regenerated for further transamination.
- The generation is accomplished by the oxidative deamination of glutamate by glutamate dehydrogenase.
- Glutamate is oxidatively deaminated in the mitochondrion by glutamate dehydrogenase**. NAD^+ or $NADP^+$** functions as the coenzyme.
- Oxidation is thought to occur with the transfer of a hydride ion from glutamate's α carbon to NAD $(P)^+$ to form α-iminoglutarate, which is then hydrolysed to α-ketoglutarate and ammonia.
- The ammonia produced is then converted to urea in mammals

Two non-specific amino acid oxidases namely, **L-amino acid and D-amino acid oxidases** catalyse the oxidation of L and D-amino acids utilizing **FAD as their coenzymes.**

$$\text{Amino acid} + \text{FAD} + H_2O \dashrightarrow \alpha\text{-Keto acid} + NH_3 + FADH_2$$

Non-oxidative deamination

- Amino acids such as serine and histidine are deaminated non-oxidatively

The other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration, dehydration etc.

Decarboxylation

- The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.
- The enzymes, **amino acid decarboxylases** are **pyridoxal phosphate-dependent enzymes.**

- Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the α-carbanion formed by the cleavage of bond between carboxyl and α-carbon atom.
- The physiologically active amines **epinephrine, nor-epinephrine, dopamine, serotonin, γ-amino butyrate and histamine** are formed through decarboxylation of the corresponding precursor amino acids.

Lecture 31

Ammonia assimilating enzymes, GDH, GS and GOGAT

Biosynthesis of ammonia

Ammonia is produced from **the catabolic pathways of amino acids**. Some of the ammonia that is generated is recycled and used in a variety of biosynthetic processes. The excess ammonia is excreted directly or converted to **uric acid or urea** for excretion depending on the organism.

- Many aquatic organisms simply excrete ammonia as NH_4^+ into the surrounding medium.
- Most terrestrial vertebrates convert the ammonia into urea (humans, other mammals and adult amphibians) or uric acid (birds, reptiles).
- In plants **ammonia is also derived from nitrate absorbed from the soil.** Nitrate is first converted to nitrite and then to ammonia.
- The major route for the **assimilation of ammonia into organic nitrogen** is the result of the **collaborative activity of glutamine synthetase (GS) and glutamate synthase** (also called as Glutamine oxoglutarate aminotransferase or GOGAT).
- Ammonia is fixed with the help of **glutamine synthetase** which catalyses the joining of ammonia to glutamic acid.

- The enzyme **GOGAT** is dependent either on NADPH (bacteria, roots and developing seeds but not in leaves) or ferredoxin (leaves, legume nodules, roots and legume seeds) to transfer the amino nitrogen from glutamine to oxoglutarate.
- The **net reaction** is the production of one molecule of glutamate from one molecule of oxoglutarate and one molecule of NH_4^+
- An additional enzyme **glutamate dehydrogenase (GDH)** is widely distributed but is not significantly involved in ammonia assimilation because of high Km value.
- All the 20 protein amino acids are synthesised by plants and microorganisms.
- Human beings are able to synthesise only 10 amino acids, which are called as **non-essential amino acids.**
- The synthesis of non essential amino acids require only one or two step reactions where as the synthesis of essential amino acids require multi step reactions.
- The synthesis of 20 amino acids is grouped into families where the precursor compounds are same for one family .

Lecture 32

Metabolic Interrelationship

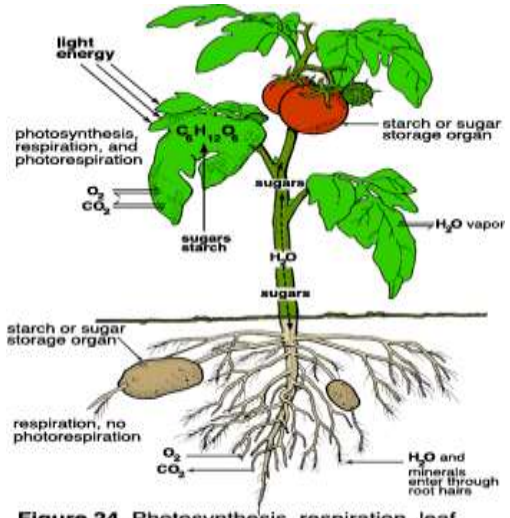
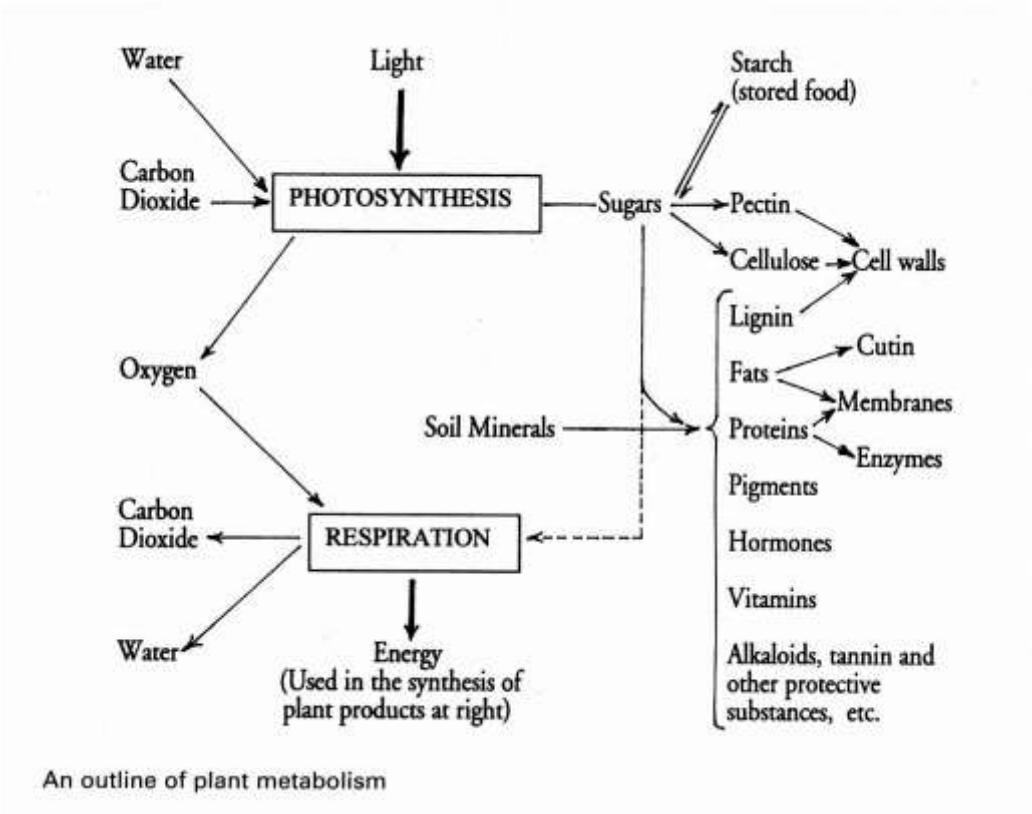


Figure 24. Photosynthesis, respiration, leaf water exchange, and translocation of sugar (photosynthate) in a plant.



An outline of plant metabolism

Primary metabolites:-

- Primary metabolites are compounds that are commonly produced by all plants and that are directly used in plant growth and development.
- The main primary metabolites are **carbohydrates, proteins, nucleic acids**, and **lipids**.

Carbohydrates

- Carbohydrates are the sugars made up of glucose and its isomers
- Carbohydrates come in many different sizes:
- Monosaccharides made up of one sugar unit (glucose or fructose)
- Disaccharides made up of two sugar units (sucrose is a glucose and a fructose)
- Polysaccharides are polymers made up of more than two sugar units

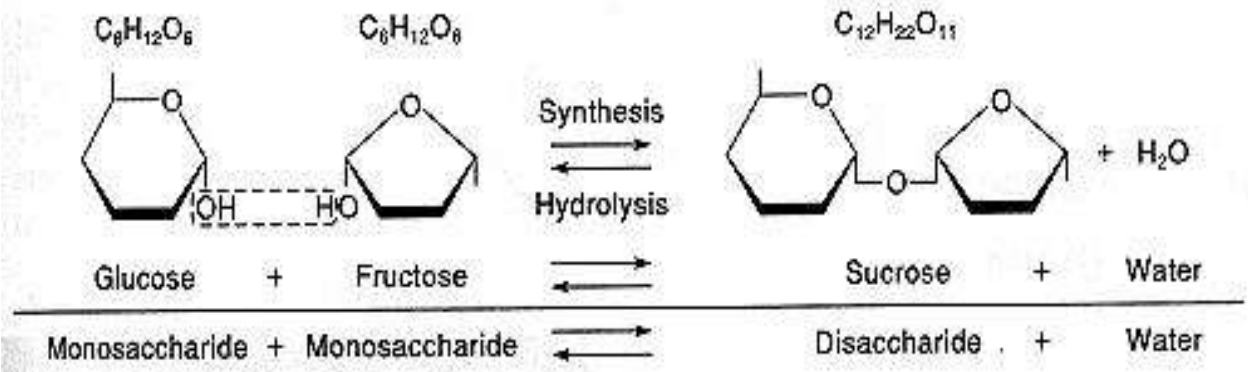
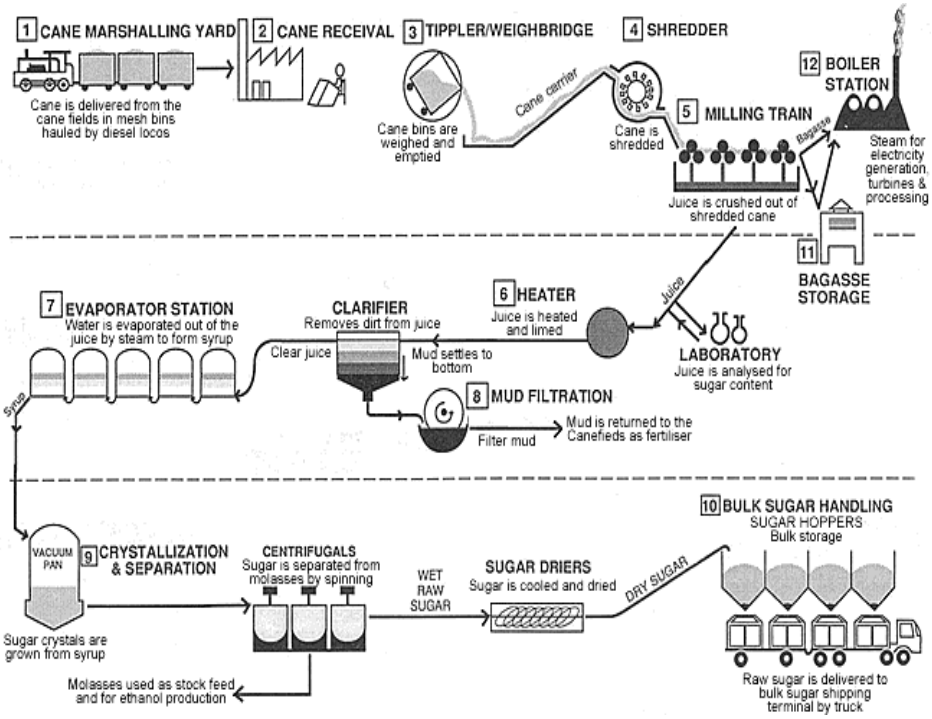


FIGURE 2.1

Diagram showing the synthesis and hydrolysis of sucrose. During synthesis, a bond forms between glucose and fructose, and a molecule of water is removed. Hydrolysis occurs as a molecule of water is added and the bond between glucose and fructose is broken. The enzyme that drives hydrolysis is sucrase; the synthesis of sucrose is actually a multistep reaction that involves several enzymes.



Polysaccharides:-

- Structural polysaccharides are used to support plants
- Storage polysaccharides are used to store energy for later use by the plant

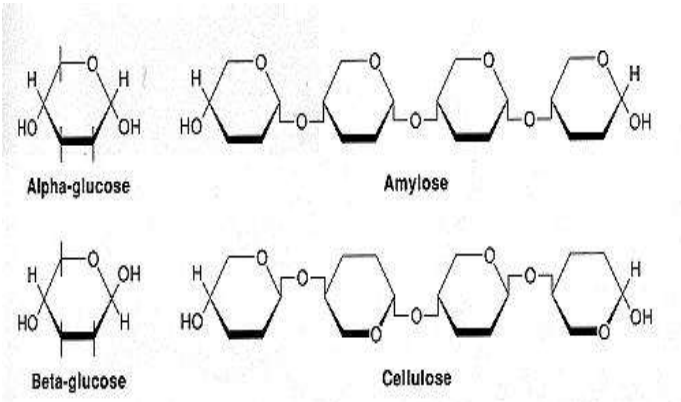


FIGURE 2.2

Line diagrams of alpha-glucose and beta-glucose, plus primary structures of amylose and cellulose. The main difference is that amylose is made of alpha-glucose, and cellulose is made of beta-glucose.

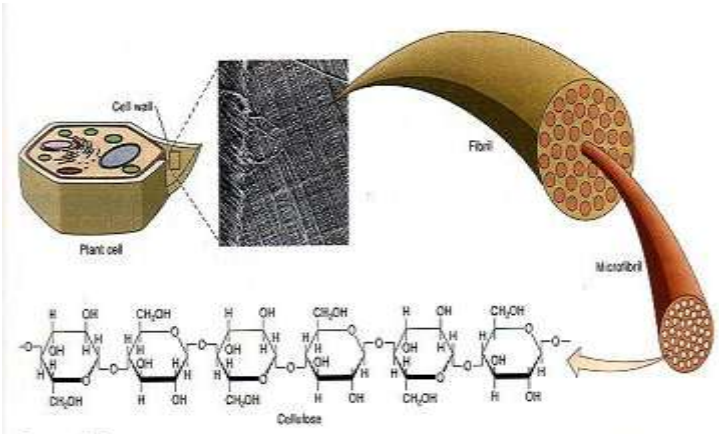
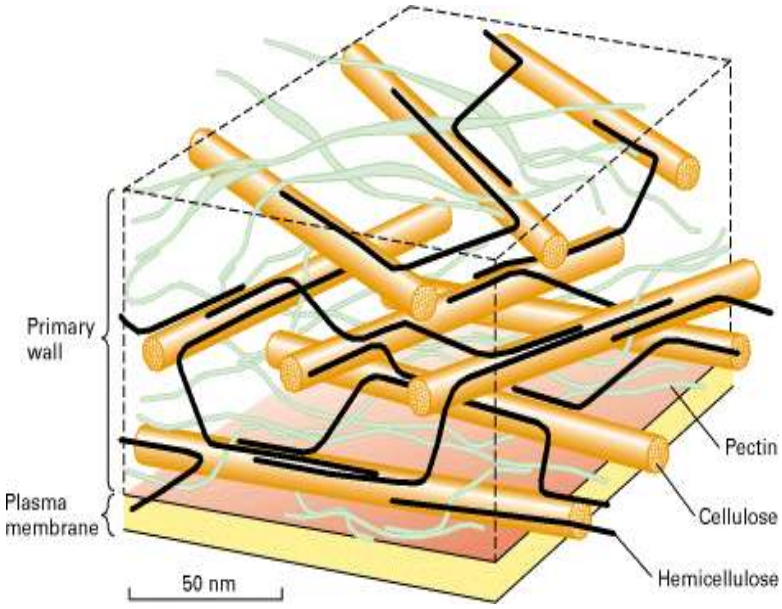


FIGURE 2.3

Model for the arrangement of fibrils, microfibrils, and cellulose in cell walls. The scanning electron micrograph shows the fibrils in a cell wall of the green alga Chaetomorpha. ×30,000.

- Pectins are mainly polymers of galacturonic acid.
- Hemicelluloses are highly variable and are not related to cellulose.
- Grass hemicelluloses are high in xylose, with small amounts of arabinose, galactose, and urionic acids. But pea family (Fabaceae) are high in arabinose, galactose and urionic acid, but low in xylose.
- Some of the most interesting hemicelluloses are not actually used structurally, but rather are exuded from stems, leaves, roots, or fruits in a sticky mixture called a gum.



Storage polysaccharides:-

- The most important storage polysaccharides are amylose and amylopectin. Amylose is a long chain of alpha-glucose, several hundred to several thousand molecules long. Amylopectin is more complex, often made up of 50,000 molecules.

- These two polymers are both used in making starch grains. Most starch grains are about 20% amylose and 80% amylopectin, but this varies with the plant

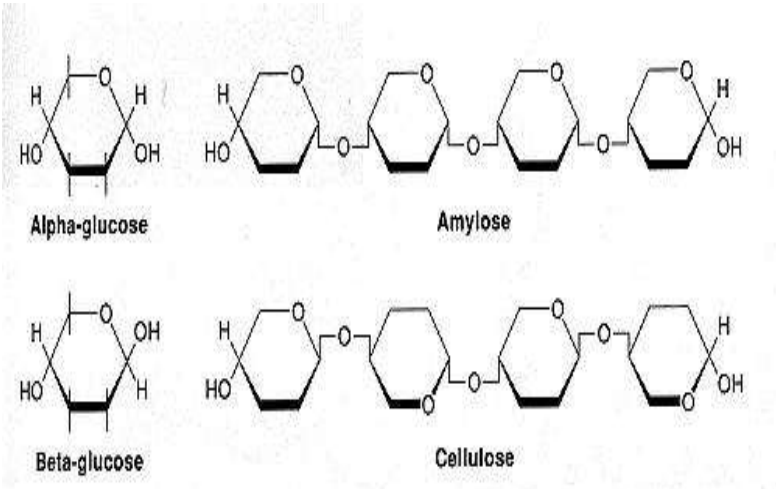


FIGURE 2.2

Line diagrams of alpha-glucose and beta-glucose, plus primary structures of amylose and cellulose. The main difference is that amylose is made of alpha-glucose, and cellulose is made of beta-glucose.

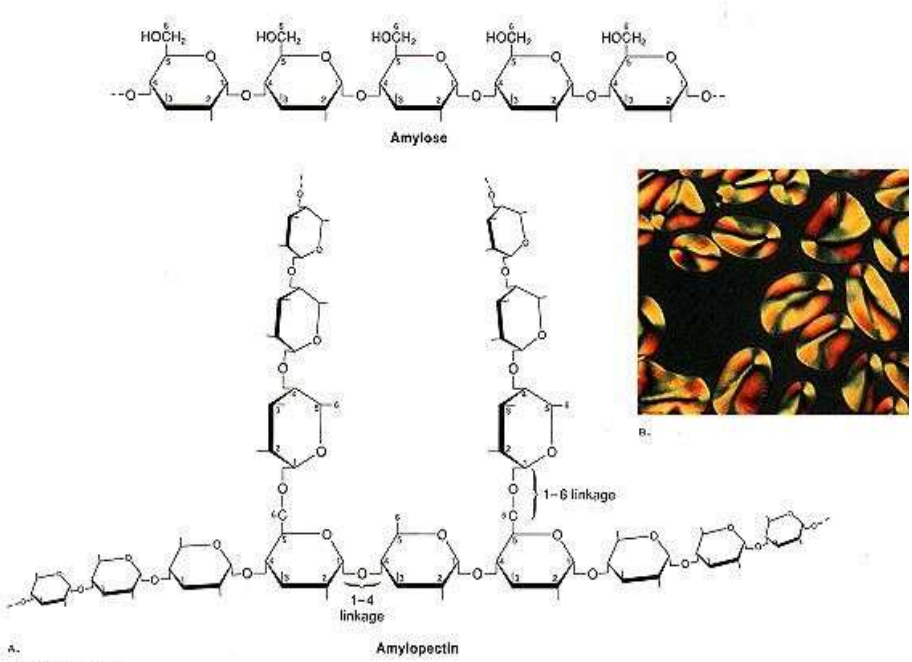
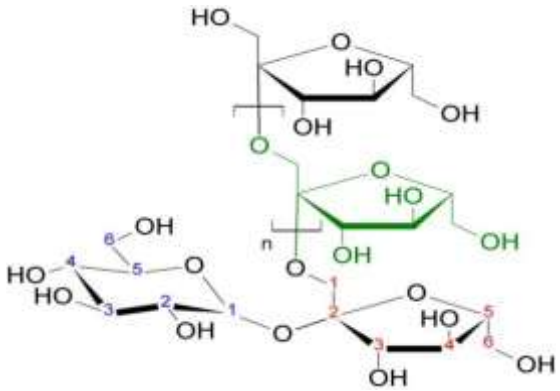


FIGURE 2.4

Forms of glucose-containing storage polymers in plants. (a) Amylopectin is made of short, helical chains that consist of branched amylose molecules. (b) Molecules of amylopectin aggregate into starch grains, as shown here from potato tubers, ×83.

Inulin- another storage polysaccharides:-



Proteins:-

- Proteins make up most of the remaining biomass of living plant cells.
- A protein consists of one or more polypeptides made up of amino acids. Plants make amino acids from the products of photosynthesis through a very complex process involving the acquisition of N, usually in the form of NH_4, and involving the use of large amounts of energy, in the form of ATP and NADPH.

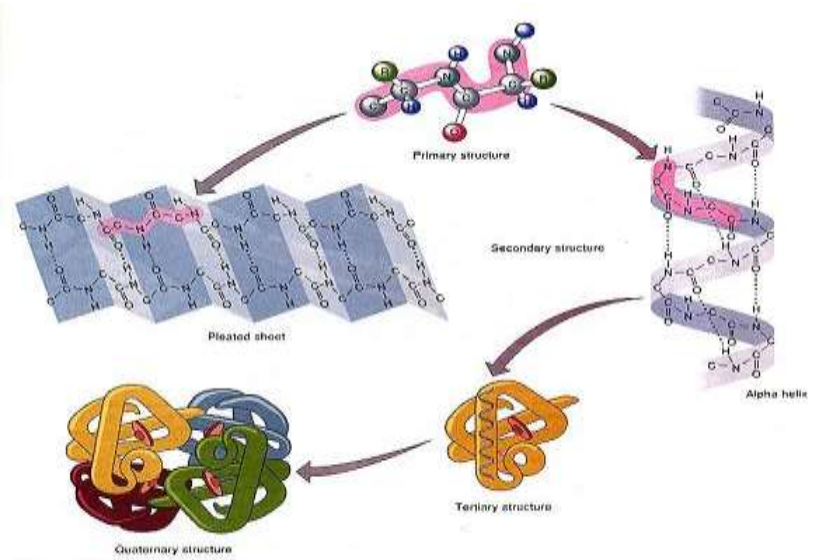
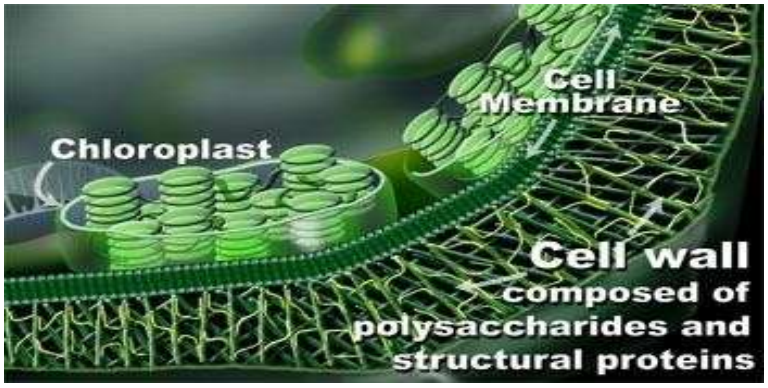


FIGURE 2.7
Models of the primary, secondary, tertiary, and quaternary structures of a protein. The secondary structure may consist of coils (alpha helixes) or folds (pleated sheets), depending on which amino acids form hydrogen bonds with each other.

Structural proteins:-

- Structural proteins make up 2 to 10% of the cell wall in plants. Expansions help increase the surface area of cell walls. Extensions help protect or repair damaged cell walls. The plant cell membrane is about 50% structural proteins.

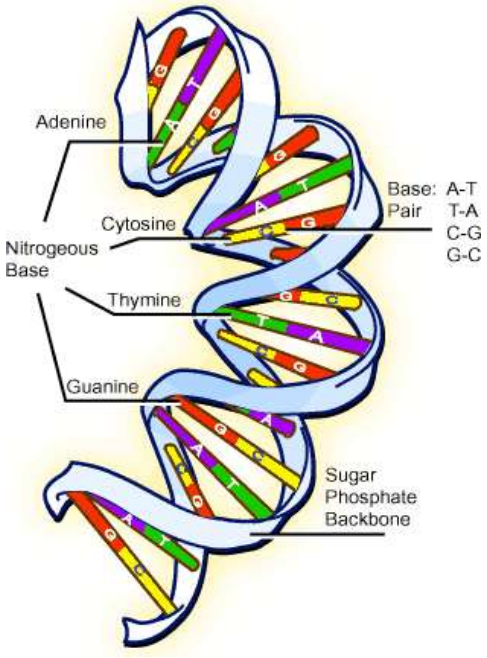


Storage proteins:-

- Storage proteins are used mostly in seeds and are used as source of nutrition for the early development of seedlings.
- Storage proteins used in seeds vary considerably between plant species.
- Corn produces a storage protein called **ZEIN**. Wheat produces a storage protein called **GLIADIN**

Nucleic acids:-

- The most complex biological polymers are the nucleic acids that make up RNA and DNA.
- The basic content of bases (adenine, thymine, gaunine and cytosine) are similar in all plants.



Lipids:-

- Unlike other biological polymers, lipids are not defined by specific, repeating monomeric units. Rather they are defined by their water-repelling properties.
- The only structure they share is that they mostly are made up of nonpolar hydrocarbon groups (CH_3, CH_2, and CH).
- Oils are fats that are liquid at room temperature.

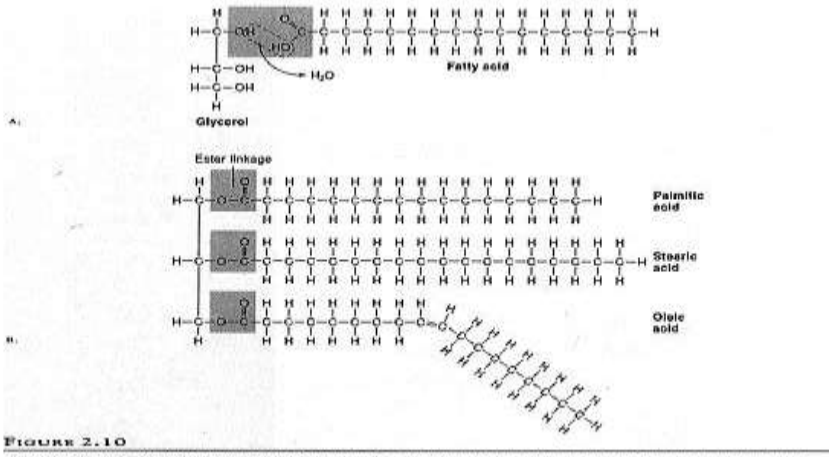


FIGURE 2.10
The structure of a fat. (a) An acylglyceride bond forms when the carboxyl group of a fatty acid links to the hydroxyl group of a glycerol, with the removal of a water molecule. (b) Fats are triacylglycerides whose fatty acids vary in length and also in the presence and location of carbon-carbon double bonds.

Oils:-

- Oils occur in all parts of a plant, but are most common in seeds. Some seeds have so much oil that it can be commercially harvested.
- The most commonly used oils are cotton, sesame, safflower, sunflower, olive, coconut, peanut, corn, castor bean, and soybean oils.
- The most common seed oil fatty acids are oleic acid (one double bond), linoleic acid (two double bonds), and linolenic acid (three double bonds).
- Linoleic and linolenic are essential fatty acids – we can't make them ourselves.

Olive oil

Waxes:-

- Waxes are complex mixtures of fatty acids linked to long-chain alcohols.
- Waxes comprise the outermost layer of leaves, fruits, and herbaceous stems and are called EPICUTICULAR waxes.
- Waxes embedded in the cuticle of the plant are cuticular waxes.
- Cutin is another wax in the cuticle and it makes up most of the cuticle.
- Suberin is a similar wax that is found in cork cells in bark and in plant roots. Both help prevent water loss by the plant.
- Structures of waxes vary depending on which plant produced them.
- Waxes are usually harder and more water repellant than other fats.

Jojoba Wax

Secondary metabolites:-

- Plants make a variety of less widely distributed compounds such as morphine, caffeine, nicotine, menthol, and rubber.
- These compounds are the products of secondary metabolism, which is the metabolism of chemicals that occurs irregularly or rarely among plants, and that have no known general metabolic role in plants.
- Secondary metabolites or secondary compounds are compounds that are not required for normal growth and development, and are not made through metabolic pathways common to all plants.
- Most plants have not been examined for secondary compounds and new compounds are discovered almost daily.

Lecture 33

Secondary metabolites - occurrence, classification and functions of phenolics

Secondary metabolites

- ✓ **Organic compounds** produced by the plants which have **no direct role in the growth and development** are called as **secondary metabolites**.
- ✓ There are about 100,000 secondary compounds that are produced by the plants and the structures of more than 15000 alkaloids, 30000 terpenes, several thousand phenyl propanoids, 1000 flavoniods, 500 quinones, 700 polyacetylenes and 800 non-protein amino acids have already been characterised.
- ✓ These secondary compounds produced by plants are grouped into **five** major groups.

1. Phenolics

2. Terpenoids

3. Alkaloids

4. Special nitrogen metabolites

5. Cuticular compounds

Phenolics

- ✓ **8,000 Phenolic structures known**
- ✓ **Account for 40% of organic carbon circulating in the biosphere**
- ✓ **Evolution of vascular plants: in cell wall structures, plant defense, features of woods and barks, flower colour, and flavor.**
  - ✓ Phenolics are a group of compounds characterized by at least **one aromatic ring bearing one or more hydroxyl groups.**
  - ✓ Most of the thousands of phenolics known to date are of plant origin.
  - ✓ These phenolic compounds are biosynthesised through **shikimate pathway.**
  - ➢ **Shikimate pathway**

Shikimate pathway is an important pathway in plants through which many secondary plant products are synthesised.

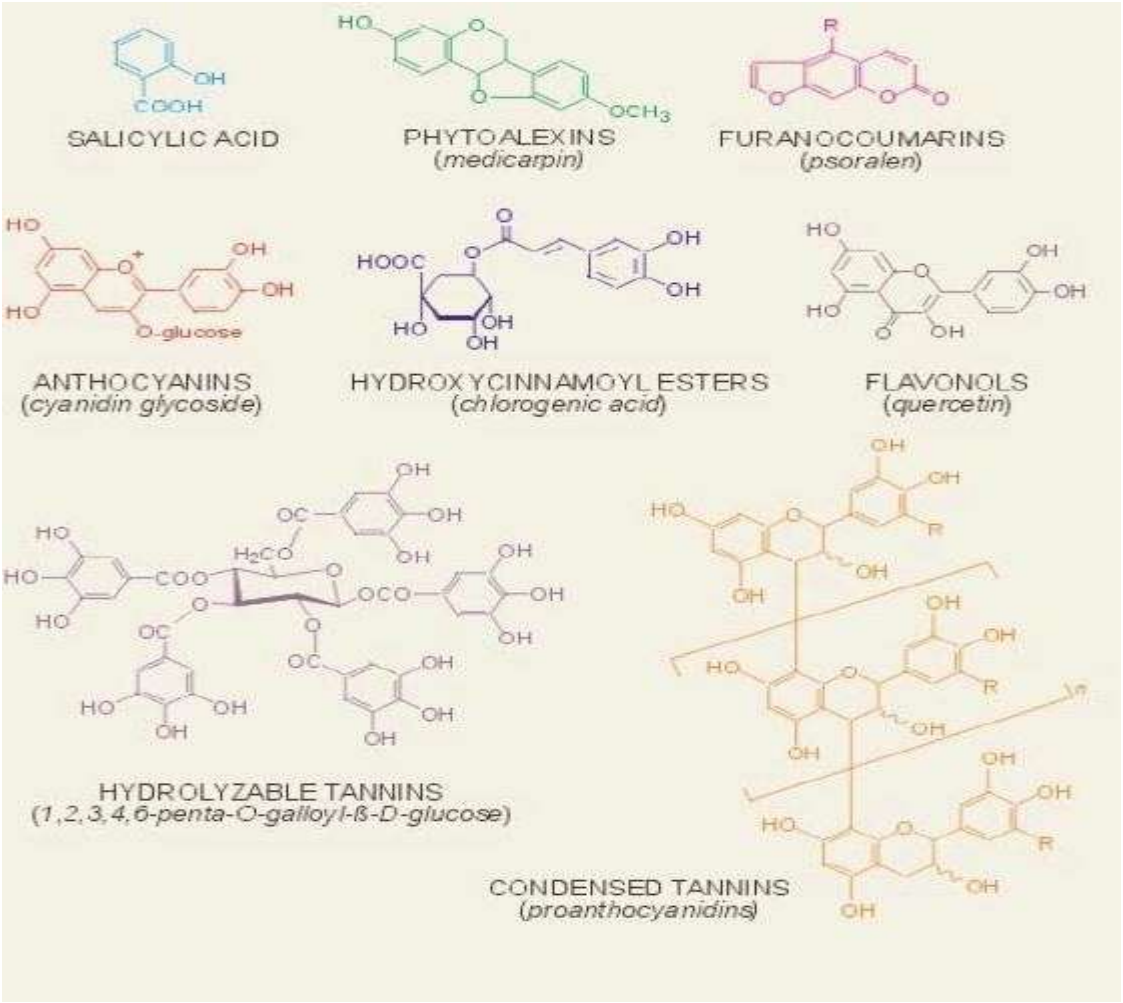
The **key starting materials** are **phosphoenolpyruvate (PEP) and erythrose 4P** derived from glycolysis and pentose phosphate pathways, respectively.

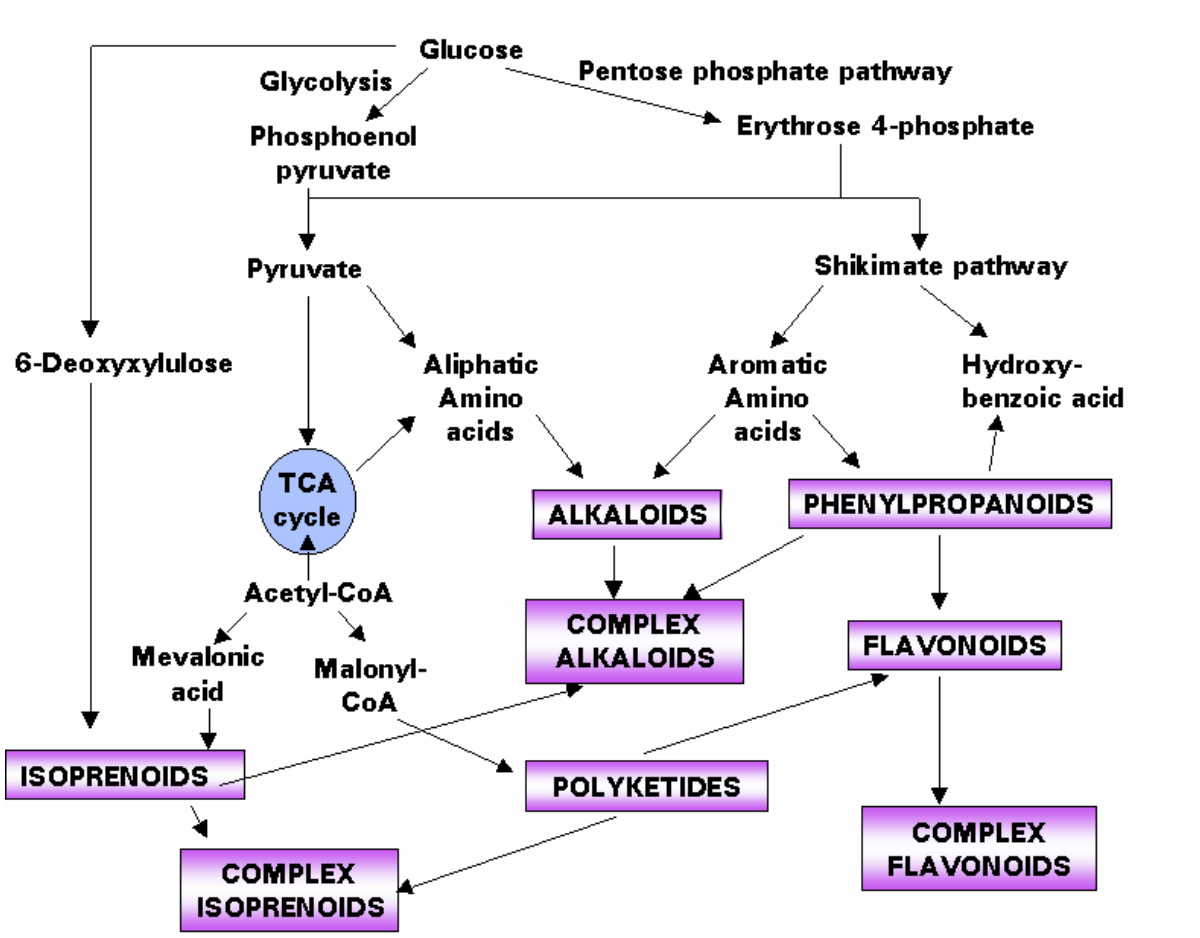
These two compounds condense to produce a six carbon cyclic compound with one carbon (COOH) side chain namely **shikimate.**

Then shikimate is phosphorylated and condensed with another molecule of PEP to produce a cyclic compound containing a three carbon and one carbon side chains.

This is finally converted to aromatic amino acids **phenylalanine and tyrosine.**

- ✓ These amino acids are **deaminated** followed by **hydroxylation** at different carbon atoms in the aromatic ring to form **cinnamic acid derivatives**.These cinnamic acid derivatives are utilised for the synthesis of different phenolic compounds.


SALICYLIC ACID
PHYTOALEXINS
(medicarpin)
FURANOCOUMARINS
(psoralen)
ANTHOCYANINS
(cyanidin glycoside)
HYDROXYCINNAMOYL ESTERS
(chlorogenic acid)
FLAVONOLS
(quercetin)
HYDROLYZABLE TANNINS
(1,2,3,4,6-penta-O-galloyl-ß-D-glucose)
CONDENSED TANNINS
(proanthocyanidins)



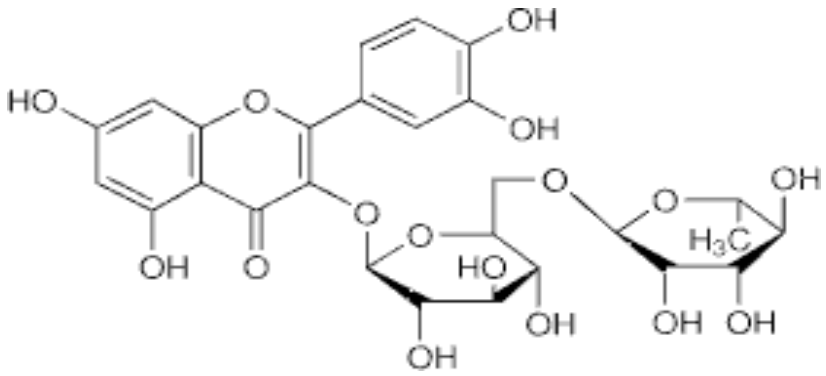
Phenolics can be classified into 2 groups:-

(i) Flavonoids (ii) Non-flavonoids.

FLAVONOIDS

- ✓ Largest group of phenols:4500
- ✓ Very often in epidermis of leaves and fruit skin.
- ✓ Major role in plants: color, pathogens, light stress
- ✓ Potential health promoting compounds-antioxidants

- ✓ A large number of genes known.
- ✓ The basic flavonoid skeleton can have a large number of substitutions on it:- Hydroxylgroups-Sugars-e.g. glucose, galactose, rhamnose. most structures are glycosylated-Methylated-Prenylated (farnesylated)-Acylated



Anthocyanins, Carotenoids, Chlorophylls

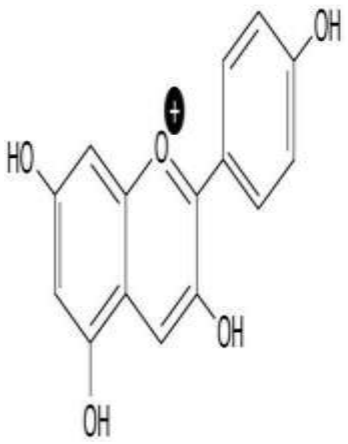
Anthocya

nidins

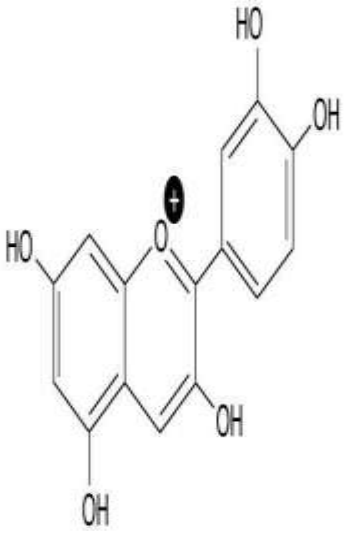
-A positive charge the C- ring

- Two double bonds in the C- ring

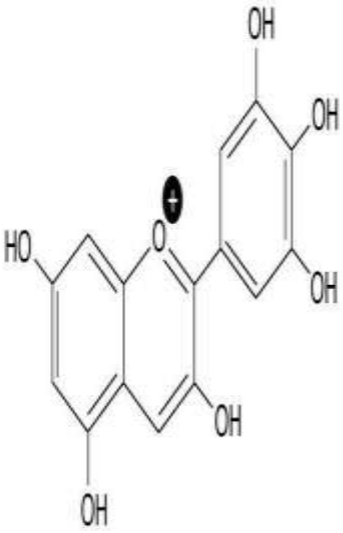
Anthocyanin



Pelargonidin



Cyanidin



Delphinidin

Pelargonium
(Geranium)

Rosa
(Rose)

Delphinium
(Larkspur)

anthocyanin fruit colour

Anthocyanin flower colour

Anthocyanin – leaf and root colour

The shikimate pathway

The shikimate pathway is defined as seven metabolic steps beginning with the condensation of phosphoenolpyruvate (PEP) and erythrose 4-phosphate (Ery4P) and ending with the synthesis of chorismate. It is the common route leading to the production of three aromatic amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). Higher plants use these amino acids not only as protein building blocks, but also as precursors for a large number of secondary metabolites, among them plant pigments, flavonoids, auxins, phytoalexins, lignin and tannins.

All pathway intermediates can also be considered as branch point compounds that may serve as substrates for other metabolic pathways. Under normal growth conditions, 20% of the carbon fixed by plants is directed towards the shikimate pathway. The shikimate pathway is restricted to plants, fungi and bacteria, making aromatic amino acids essential in the diets of animal. On the other hand, the pathway is an important target for herbicides antibiotics and live vaccines because chemicals that interfere with any enzyme activity in this pathway are safe for humans when handled in reasonable concentration.

✪ **Functions of phenolics**

1. Phenolics are of great importance as **cell wall components.**
   They form **part of cell wall structures** such as **lignins, cutins and suberins**, which provide **mechanical support and function as barriers against microbial attack**.
2. The **flavonoids and anthocyanins** contribute to flower and fruit colours. This is important for attracting insects and animals to the plant for pollination and seed dispersal.
3. Phenolics also play a **defensive role** in plants by protecting against predators. Simple phenolic acids, polyphenolics like tannins and phenolic resins at the plant surface are **effective feeding deterrents.(want to do something)**
4. Phenolics are accumulated as **post-infectional low molecular compounds** called **phytoalexins** as a result of microbial attack. Among the phenolic phytoalexins, hydroxycoumarins and hydroxycinnamate conjugates contribute to **disease resistance mechanism** in plants.
5. Phenolic compounds also produce **allelopathic effect**. A well known compound from Juglans species is juglone which is highly toxic for a wide range of plants. It occurs in the plant as a non-toxic glucoside

and is made active by deglucosylation and oxidation after leaching from the leaves into the soil.

6. Phenolics also function as **signal molecules** in the interaction between **nitrogen fixing bacteria and leguminous plants.**
7. These plants exude **flavonoids** which act selectively in Rhizobia as **inducers of nodulation gene transcription.**
8. Salicylic acid is strongly implicated as a **signal molecule** which induces active defense responses in several plant species against many types of pathogens. Recently, it has been shown that phenolic compounds function as **effective antioxidants.** Polyphenolics are important in **foodstuffs, wines and herbal teas** because of their **astringent taste.**
9. Plants rich in polyphenolics were used as **tanning agents** in leather industries.
10. **Phenolic pigments** (anthocyanins, flavones etc) of fruits are most widespread food colours occurring in fruit juices, wines and jams. Anthocyanins have considerable potential in the food industry as **safe and effective food additives.**

Lecture 34

Occurrence, classification and functions of terpenes and alkaloids

What are Terpenes?

- ✓ Naturally occurring hydrocarbons produced by plants, insects, and animals
- ✓ Emitted by the leaves of plants as a natural bi-product of metabolism
- ✓ Lots of different types.
- ✓ Terpenoids have been known since antiquity as ingredients of flavoring, perfumes and food colorants.
- ✓ Plants produce a great variety of products based on a branched C5 building block.
- ✓ The five carbon unit is synthesised by the condensation of three molecules of acetyl CoA. Some of these are primary metabolites such as plant hormones, carotenoids and steroids.
- ✓ However, majority of compounds are secondary metabolites called as terpenes.

Terpenes are classified according to the number of 5 carbon.

Class	Number of carbons
Hemiterpene	**5**
Monoterpene	**10**
Sesquiterpene	**15**
Diterpene	**20**
Sesterterpene	**25**
Triterpene	**30**
Tetraterpene	**40**

- **Polyterpene 4000**

General pathway of terpenoid biosynthesis:-

- Terpenoids are biosynthesised through acetate-mevalonate pathway.
- The precursor compound used is acetyl CoA. Initially two molecules of acetyl CoA condenses in presence of acetoacetyl CoA thiolase to form acetoacetyl CoA.
- Then one more molecule of acetyl CoA is added to acetoacetyl CoA toform hydroxymethyl glutaryl CoA (HMG CoA) with the help of HMG CoA synthase.
- The HMG CoA is then reduced to form mevalonic acid (MVA) in presence of NADPH and HMG CoA reductase.
- The conversion of MVA to isopentenyl pyrophosphate (IPP) occurs in three steps, each requiring one molecule of ATP.
- The IPP is then isomerised to form imethylallylpyrophosphate (DMAPP). DMAPP condenses with a molecule of IPP to produce geranyl pyrophosphate (GPP).
- GPP in turn acts as a prenyl donor to another IPP molecule to form farnesyl pyrophosphate (FPP).
- Dimerisation of GPP and FPP gives rise to diterpenes and triterpenes, respectively.
- Tetraterpenes (carotenoids) are produced by condensation of two molecules of geranyl geranyl pyrophosphate.

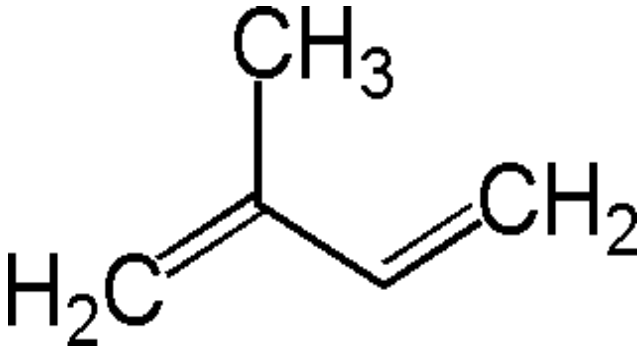
Bioactivities of terpenoids:-

- Monoterpenoids interfere with basic metabolic, physiological and behavioural functions of insects.
- Some exhibit acute toxicity, whereas others function as repellents, attractants, antifeedants, or affect
- Responsible for growth, development and reproduction.
- Monoterpenoids are cytotoxic to plants and play an important role in plant-plant interactions.
- Pinene, limonene and citronellol inhibit the growth of *Amaranthus retroflexus near the orange tree, Citrus aurantium. A number of monoterpenoids possess antimicrobial activity. The therapeutic properties of* essential oils (the major constituents are monoterpenoids) and their individual compounds are well known.
- Sesquiterpenoids function as insect antifeeding substances, insect juvenile hormones, pheromones and plant growth regulators (abscisic acid).

Terpene Structure:-

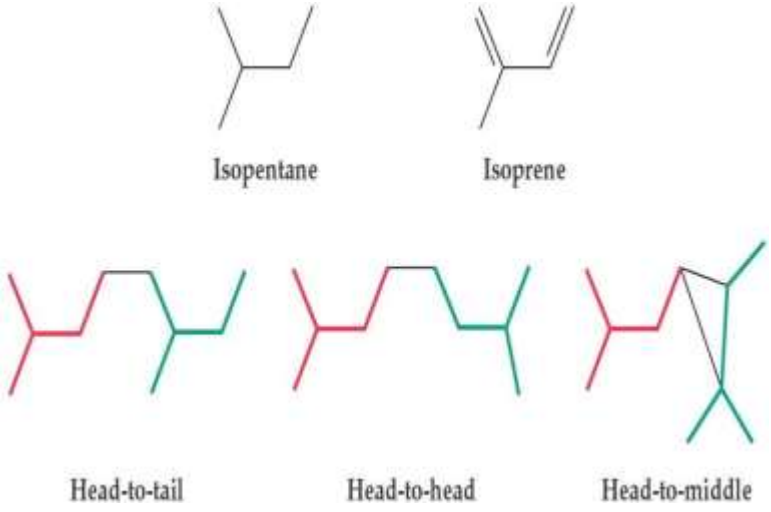
- Basic formula C_5H_8
- Can be linked head to tail in linear chains or rings

- $(C_5H_8)_n$ C_5 Rule N= number of linked isoprene units.



Terpene type	**Number of isoprene units**	**Carbon atoms**	**Example**
Monoterpene	2	10	menthol, camphor, limonene, pinene, thujone, nerol
Sesquiterpene	3	15	farnesol, carophyllene
Diterpene	4	20	phytol, Vitamin A
Sesterpene	5	25	-
Triterpene	6	30	squalene, lanosterol
Tetraterpene	8	40	β-carotene (provitamin A)

- **Terpenes are made from C5 units.**



Types

- Monoterpenes
- 10 carbons
- Used as topical pain and itch reliever
- Menthol (from Mint family of plants)
- Used as disinfectent and deodorant
- Borneol (from Pine oil)

Sesquiterpene

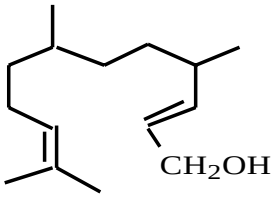
- ✪ 15 Carbons
- ✪ Santonin
- ✪ Photosensitizer from Wormwood
- ✪ Gossypol
- ✪ Male contraceptive from Cotton seeds

- Diterpenes
- 20 Carbons
- Taxol- an anti-cancer drug
- From bark of Pacific Yew
- Phorbol- Considered a cocarcinogen
  - From many of the Euphorbs in their latex
  - Contains potent skin irritants.

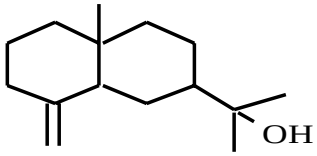
- Triterpenes
  - ✓ 30 Carbons
  - ✓ Bruceantin- occurs in plants as glycosides
  - ✓ Have been investigated for the treatment of cancers
  - ✓ Steroids are modified triterpenes
  - ✓ Testosterone, Progesterone, and Cortisone.
- Polyterpenes
  - Many Carbons
  - Found in the latex of the Rubber Tree
  - Produces Rubber

  - ✓ Tetraterpenes
  - ✓ 40 Carbons
  - ✓ Found in the red pigments of tomatos
  - ✓ Lycopene- antioxidant.

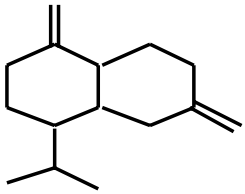
Sesquiterpene Examples:-



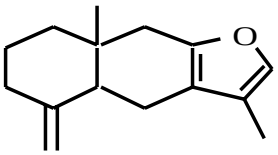
Farnesol



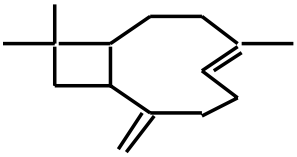
Eudesmol



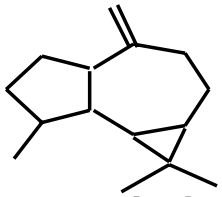
Bulgarene



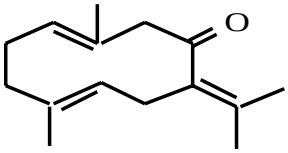
Atractylone



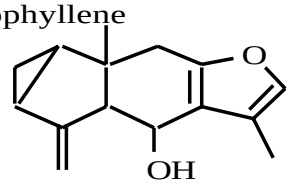
Caryophyllene



Aromadendrene

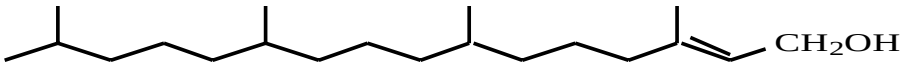


Germacrone

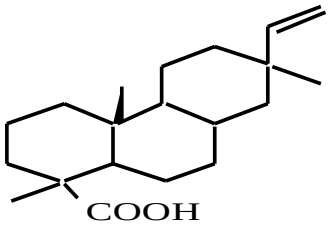


Linderene

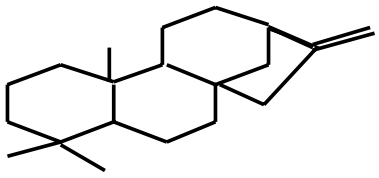
Diterpenes:-



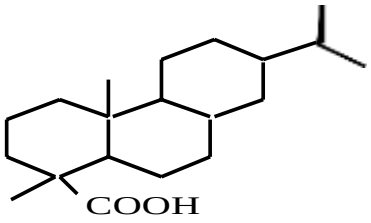
Phytol



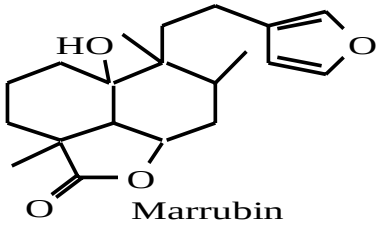
Pimeric acid



Kaurene

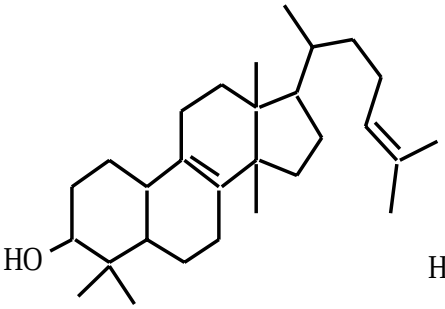


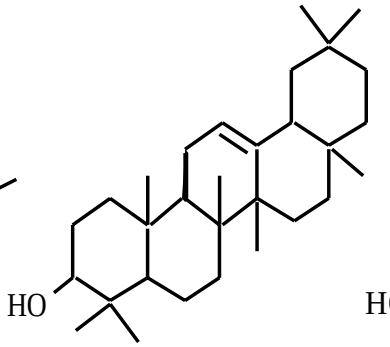
Abietic acid

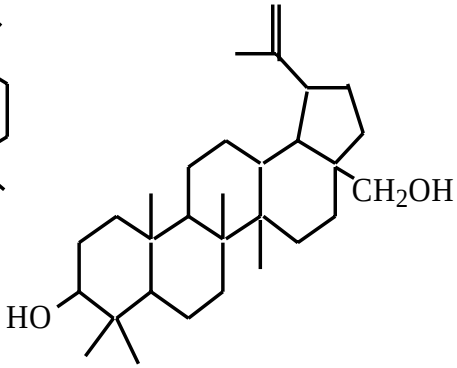


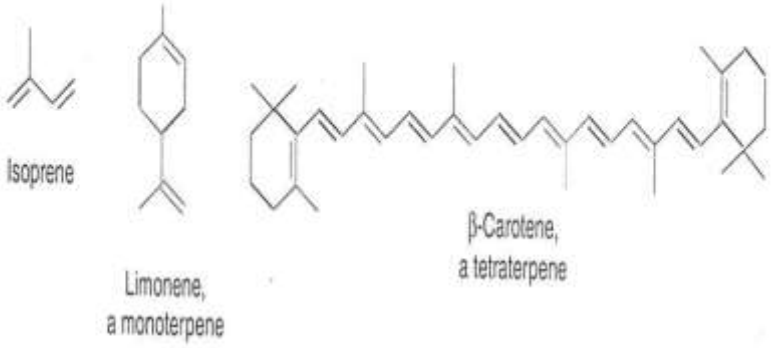
Marrubin

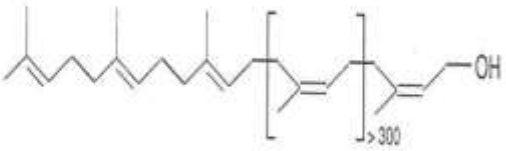
Triterpenes:-











Rubber,
a polyterpene

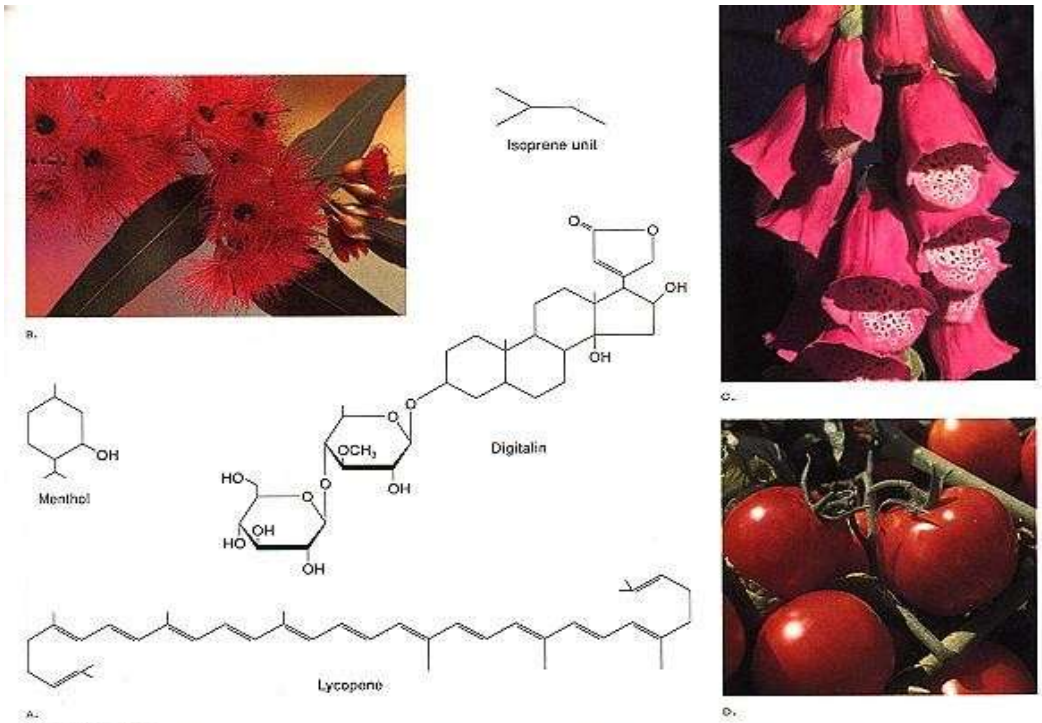


FIGURE 2.14

(a) Structures of three terpenoids. (b) *Eucalyptus* species produce menthol. (c) The purple foxglove (*Digitalis purpurea*) is a source of digitalin. (d) Lycopene is the main red pigment of tomatoes.

Uses of Terpenes:- (key points)….

- Insecticides
- Fragrances and Perfumes
- Food Additives
- Aroma therapy
- Cleaning Products (Pinesol)

Occurrence, classification and functions of alkaloids; Applications of secondary metabolites in food and pharma industries:-

✪ Alkaloids are nitrogen containing compounds having at least some basicity. They are usually heterocyclic and occur primarily in higher plants and some microorganisms.

✪ They usually exhibit significant physiological activity in humans and animals and represent one of the largest and most diverse groups of secondary products.

✪ Totally more than 7000 compounds are known in only 5% of the plant species. Ninety five percent of plant species are still remain to be examined for alkaloids.

✪ Alkaloids- 12000 structures known.

- **Poisons – Conine**
- **Narcotics – morphine**
- **Stimulants – caffeine**
- **Medicine – Taxol.**

- Alkaloids generally include alkaline substances that have nitrogen as part of a ring structure. More than 6500 alkaloids are known and are the largest class of secondary compounds. They are very common in certain plant families, especially:
- Peas – Fabaceae

- sunflower – Asteraceae
- poppy – Papaveraceae
- tomato – Solanaceae
- dogbanes – Apocynaceae
- milkweeds - Asclepiadaceae
- Citrus – Rutaceae.
- 1806 – Pharmacy assistant Friedrich Wilhelm Serturner isolated morphine from poppy seeds.
- Its structure was resolved in 1952. Socrates died when he was forced to drink extract of hemlock containing coniine.
- Nicotine (tobacco) is used as very potent insecticide for fumigating green houses.
- Cleopatra used extracts containing atropine to dilate her pupil in order to appear more attractive; atropine in low doses dilates pupils of the eye.
- Alkaloids are nitrogen containing natural products which are not otherwise classified as peptides, non-protein amino acids, amines, cyanogenic glucosides, glucosinolates, primary metabolites, purines, pyrimidines, cofactors, phytohormones.

Alkaloids are broadly classified into

- True alkaloids (possessing heterocyclic ring and derived from amino acids).
- (ii) Protoalkaloids (No heterocyclic ring but derived from amino acids)
- (iii) Pseudoalkaloids (Not derived from amino acids).

There are several methods for the classification of alkaloids.

1. Taxonomic (according to the family):

Here alkaloids may be classified as Solanaceous or Palilionaceous without reference to the chemical type of alkaloid present. It is more useful to describe alkaloids according to the genus in which they occur. E.g. Ephedra,Cinchona etc.,

2. Pharmacological:

This classification lists alkaloids according to their use or physiological activity. E.g. Analgesic alkaloids, cardioactive alkaloids etc.,

Mechanism of action of alkaloids at molecular level.

Alkaloids	**Target**
Berberine, quinine, β-carbolines	DNA/RNA intercalation
Pyrrolizidines	Alkylation of DNA/RNA
Coniine	Mutations
Lycorine, Vincristine	Inhibition of DNA/RNA polymerases
Vinblastine	Microtubules/Cytoskeleton
Quinolizidines	Inhibition of protein biosynthesis
Steroidal alkaloids	Membrane stability
Quinine, aconitine	Inhibition of ion channels, carrier
Nicotine, heliotrine	Acetylcholine receptors
Cocaine	Dopamine receptors
β-Carbolines	Serotomine receptors, GABA receptors
Cocaine, β-carbolines	Transport or degradution of neurotransmitters
Tetrahydroberberine	Adenyl cyclase inhibition
Polyhydroxyalkaloids	Inhibitors of hydrolases

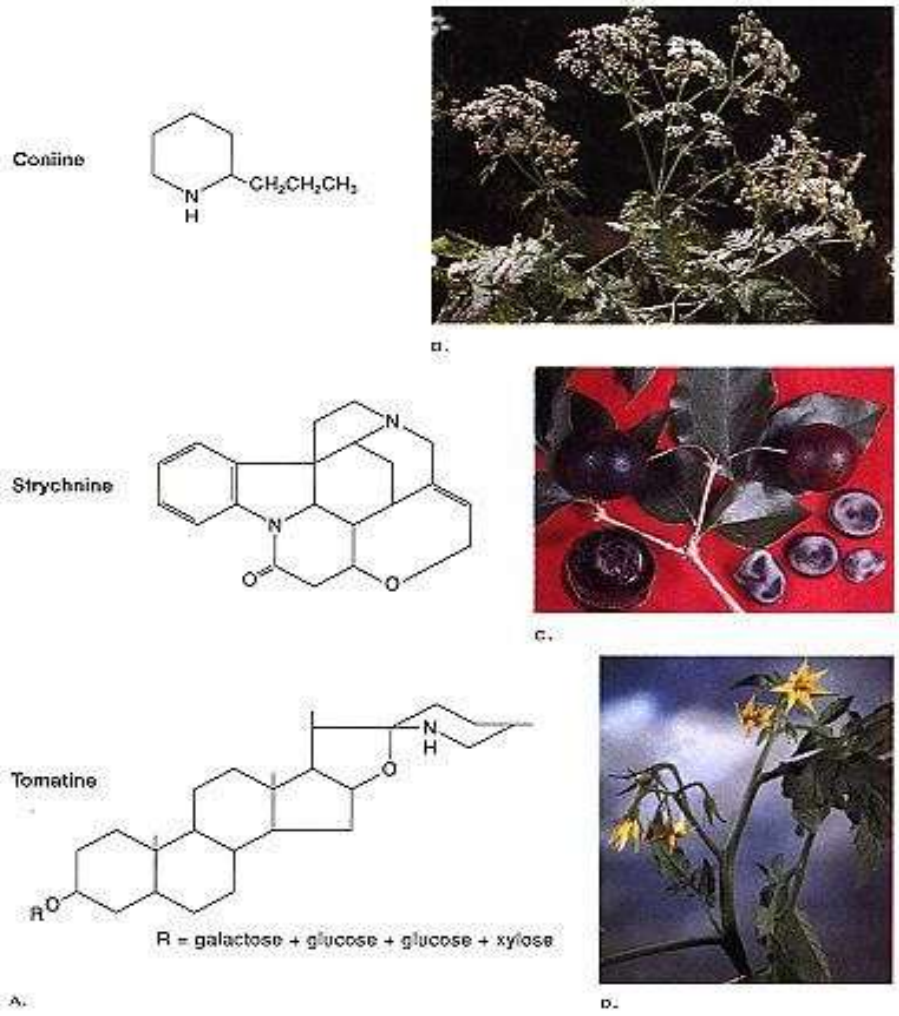


FIGURE 2.13

(a) Structures of three alkaloids. (b) Poison hemlock (*Conium maculatum*) produces coniine in its leaves. (c) Strychnine plant (*Strychnos nux-vomica*) produces strychnine in seed coats. (d) Tomato leaves (*Lycopersicon esculentum*) are a source of tomatine.

Alkaloids of medicinal importance:-

Ephedrine:

- Ephedrine and related alkaloids occur in the genus Ephedra which are small bush or shurb like primitive plants belonging to the Gnetaceae family.
- There are about 45 species and of these 96 some 25 are known to contain the alkaloid.

- The best known are *E. sinica, E. nebroidensis and E. elata. They contain about 1-2% alkaloid chiefly in the leaves.*

Uses: Ephedrine is a sympathomimitic amine i.e. it acts at the nerve - nerve spaces (synapses).

- ❖ These nerves are adrenergic i.e. the chemical transmitter between them is nor-adrenalin.
- ❖ Since it causes peripheral contraction of arterioles it is used to correct low blood pressure conditions and also to dilate the pupil of the eye (2-5% solution).
- ❖ As a broncho-dilator it is used in allergic conditions like asthma.

Atropine:

Many species of Solanaceae family contain a series of therapeutically important alkaloids known as the tropane alkaloids. Datura, Atropa, Hyoscyamus species contain tropane alkaloids. Atropane, hyosacyamine and hyoscine are the most important alkaloids of these plants. Atropine is (±) hyoscyamine and found in all Datura species.

Uses:

1. To relieve tremor, rigidity and salivation in Parkinsonism.

2. As an antidote.

3. It produces dilation of the pupil, which is useful in ophthalmology. 5. For the relief of bronchial spasm in asthma.

4. Relief of spasm of smooth muscles.

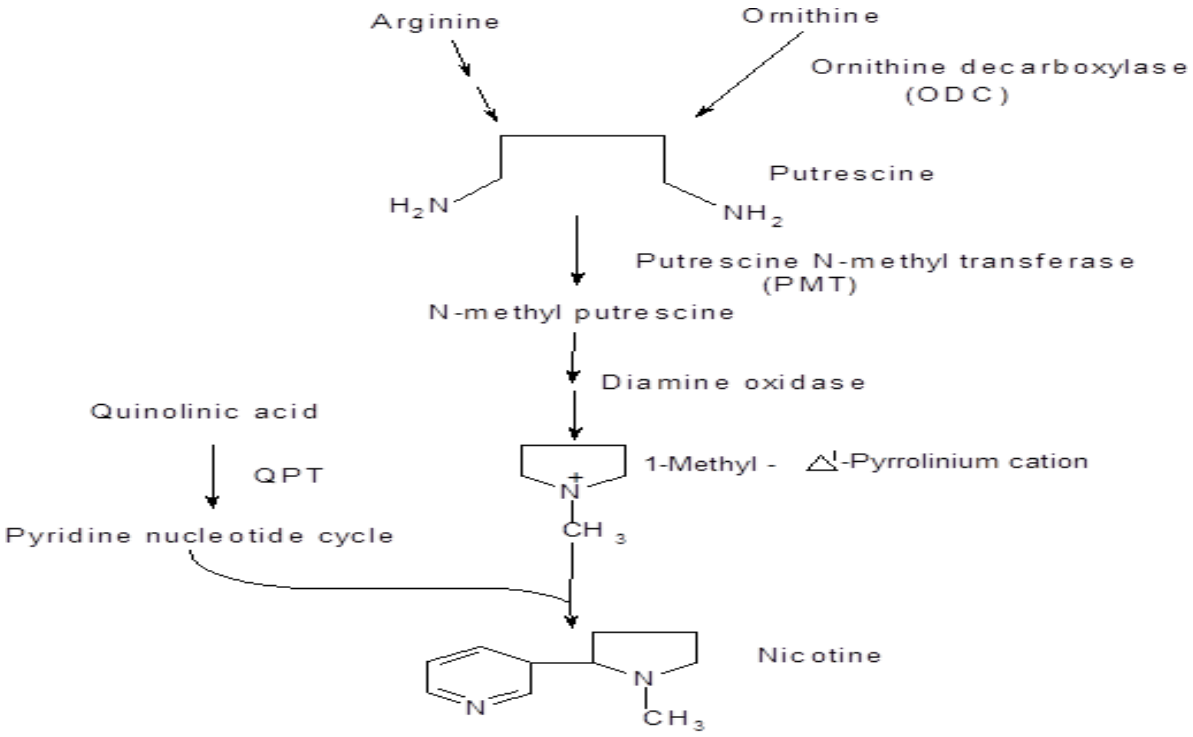
5. In small does as a gentle respiratory stimulant.

Biosynthesis of alkaloids

- ❖ Most of the alkaloids are derived from amino acids such as lysine, phenylalanine, tryptophan, aspartic acid, arginine and histidine. Although the basic skeletons of most alkaloids are derived from

amino acids, moieties derived from other pathways such as terpenoids are often attached to the basic skeleton.

- Biosynthetic pathways have been worked out in detail for a few alkaloids so far.
- Since there is no common pathway, the biosynthesis of individual alkaloids are not presented.



Scheme 8.1 Biosynthesis of nicotine