PLANT BIOCHEMISTRY

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Lecture.1

Introduction to biochemistry, Carbohydrates-importance and classification

Introduction, Carbohydrates – importance &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

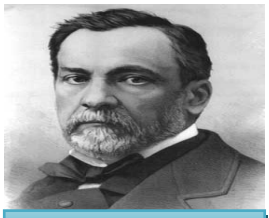
During 17th and 18th centuries, important foundations were laid in many fields of biology.

- 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.**

Mendel

- 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.

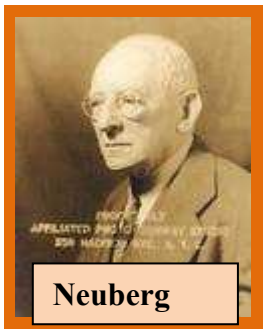
- **Louis Pasteur**, during 1857, did a great deal of *work on fermentations* and pointed out the *central importance of enzymes* in this process.



Louis Pasteur

- The breakthrough 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.

- **Neuberg** introduced the *term biochemistry* in 1903.


Neuberg

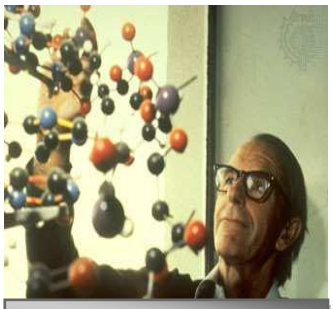
The early part of 20th century witnessed a sudden outburst of knowledge in **chemical analysis, separation methods, electronic instrumentation for biological studies (X-ray diffraction, electron microscope,** etc) which ultimately resulted in understanding the structure and function of several key molecules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


Watson and Crick

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

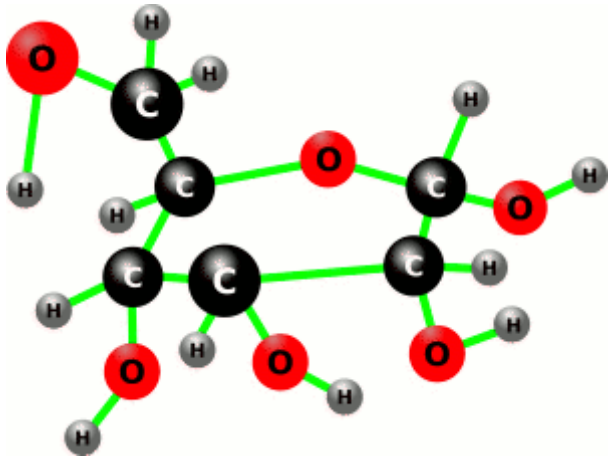
- **Frederick Sanger's** contributions in the *sequencing of protein* in 1953 and *nucleic acid* in 1977 were responsible for further developments in the field of protein and nucleic acid research.

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. They may be either aldoses or ketoses depending upon whether they contain a free aldehyde or ketone group, respectively	Classified into disaccharide, trisaccharide, tetrasaccharide and pentasaccharide depending upon the number of monosaccharides they contain.	Classified into homoglycans and heteroglycans depending upon the kind of monosaccharides present. Depending upon the function, they 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 metabolites 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 oligosaccharides
		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 acid	-	Constituent of pectin
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. Eg: Raffinose, Stachyose, Verbascose, Adjugose.

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

Questions

Choose the best answer

1. Carbohydrates are compounds with empirical formula

a. $(CH_2 O)_n$ b. $(C_2H_2O)_n$ c. $(CH_3O)_n$ d. $(C_3HO)_n$

Ans: $(CH_2 O)_n$

2. Pick out the structural polysaccharide / polysaccharides from the following

a. Cellulose b. Hemicellulose c. Starch d. a and b

Ans: Cellulose and Hemicellulose

3. ---------- is the storage polysaccharide in plants

a. starch b. pectin c. cellulose d. hemicelluloses

Ans: Starch

4. Chitin found in the exoskeleton of lower animals is a polymer of

a. N-acetyl glucosamine b.N-acetyl galactosamene

c. N-acetyl mannosameis d. N-acetyl fructosamine

Ans: N-acetyl glucosamine

5. Pick out the sugar in nuclei acids

a.Ribose b. Arabinose c.Raffinose d. Trehalose

Ans:Ribose

State True or False

6. Lactic acid with empirical formula $(CH_2O)_n$ is a carbohydrate.

Ans: False

7. ATP is a key compound in energy transfer and storage.

Ans: True

8. Monosaccharides are low molecular weight carbohydrates and can be hydrolyzed further.

Ans: False

9. Polysaccharides can be hydrolyzed by enzymes or acids.

Ans: True

10. Polysaccharides are soluble in water acid sweet in taste.

Ans: False

Answer in one sentence

11. Define carbohydrates.

12. What are monosaccharides?

13. How are polysaccharides classified?

14. Define reducing sugars.

Lecture.2

Occurrence and Structure of Monosaccharides and structures of disaccharides and polysaccharides

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*.

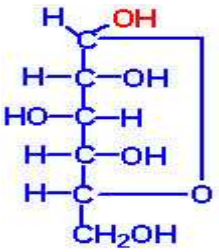
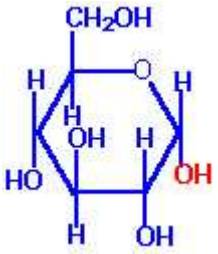
- For monosaccharides with *two or more asymmetric carbons*, the prefixes D or L refer to the *configuration of the penultimate carbon* (i.e, the asymmetric carbon farthest from the carbonyl carbon).
- If the *hydroxyl group on the **penultimate** carbon* is on the ***right-hand side*** of the carbon 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 whereas aldehydes are easily auto-oxidizable. 3. Glucose and other aldoses do not form bisulfite or aldehyde ammonia compound. 4. Glucose pentaacetate 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 α-D-Glucose	**Haworth Projection of α-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**.

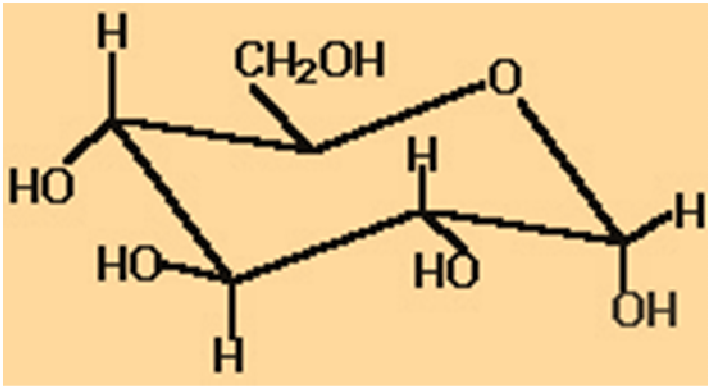
While writing the cyclic form (Haworth) of monosaccharides it is sometimes difficult to judge whether an OH group should be above or below the plane of the ring.

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 α-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

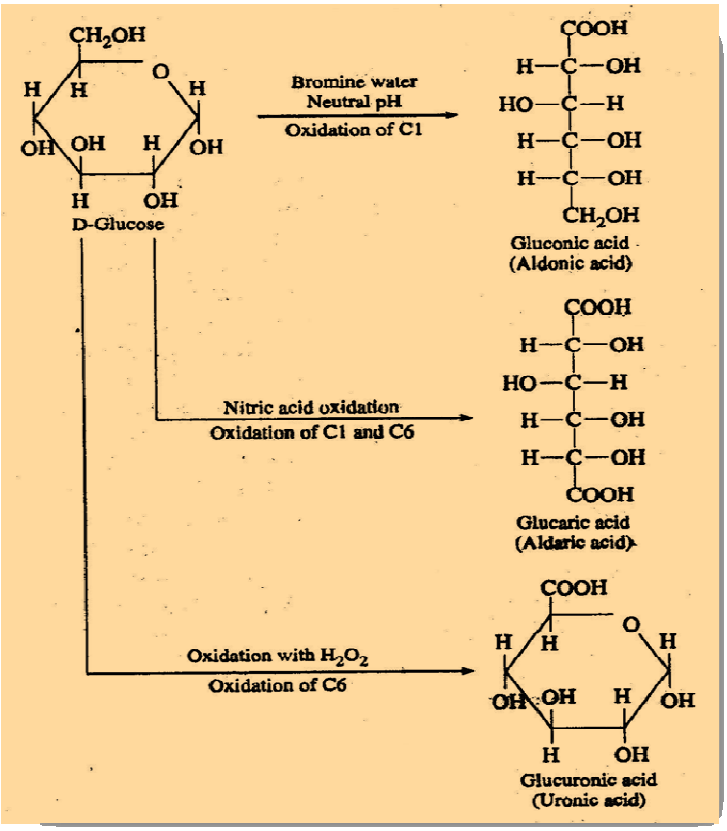
- Oxidation of an aldose with *bromine water* at neutral pH converts the aldehyde group (C_1) to a carboxyl group yields **Aldonic acid**.
- Hydrobromous acid formed by the reaction of water with bromine acts as an oxidizing agent.
- Ketoses are not readily oxidized by bromine water.
- Aldoses are not 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.
- *Glucose* on oxidation with nitric acid produces **glucaric or glucosaccharic acid**.
- The aldaric acid produced from *galactose* is called as **mucic acid**.



Oxidation products of glucose

Structure of Disaccharides & Polysaccharides

Composition, sources and properties of common disccharides

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-glucose+ α-D-glucose	β(1→4)	Milk. In 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.

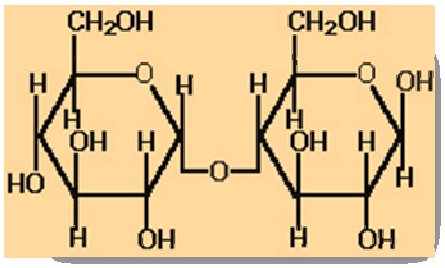
- The physiologically important disaccharides are maltose, lactose, trehalose and sucrose.
- Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond.
- The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups.
- 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 **α** and hence the glycosidic linkage is **α (1→ 4).**

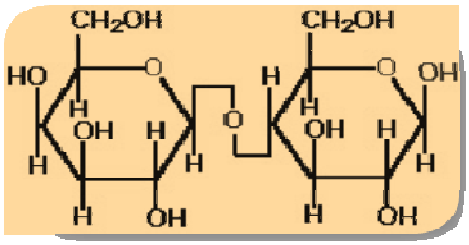
- *The anomeric carbon atom* of the second glucose is *free* and therefore maltose is a ***reducing sugar.***
- The second glucose residue is capable of existing in **α or β** configuration
- Maltose has been recorded occasionally in plants. It is usually obtained as a product of the ***enzyme hydrolysis 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

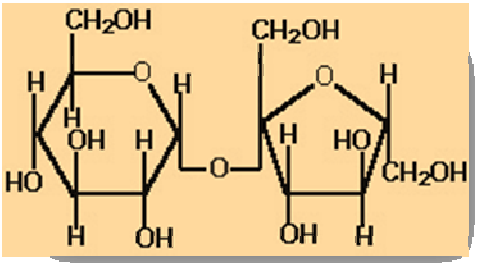
Non-reducing disaccharides

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°) than dextrorototary glucose (+52.2).
- 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 surosyl 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 gam 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 serve as reserve **material**.
- It also contributes to **frost resistance**

Polysaccharides

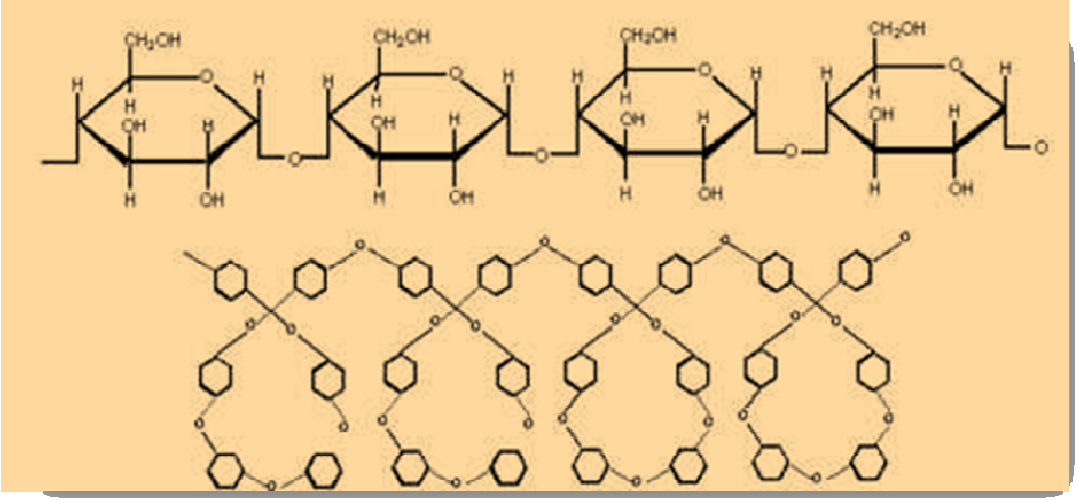
The polysaccharides found in nature either serve a structural function (structural polysaccharides) or play a role as a stored form of energy (storage polysaccharides).

Storage polysaccharides

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

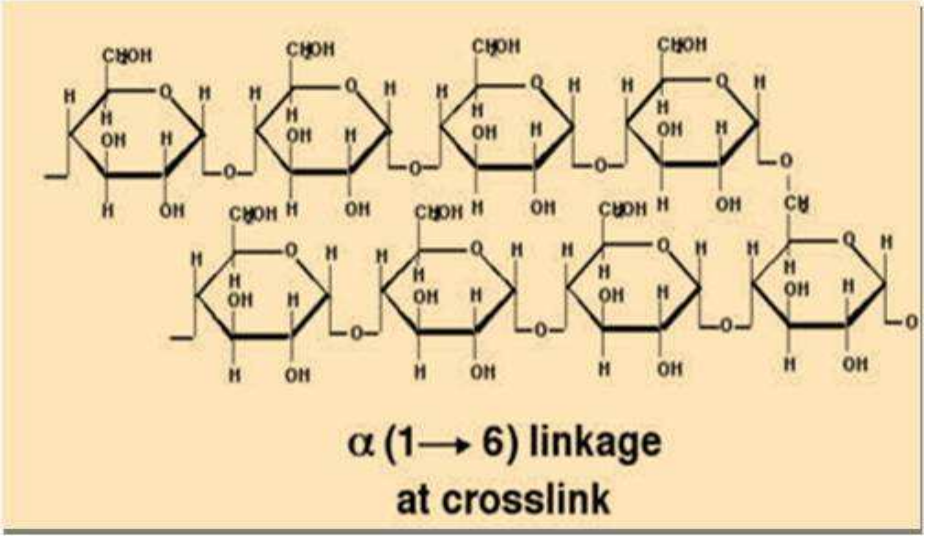
Starch

- 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 quasicrystalline macromolecular 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 synthesise 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 α 1 –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 α 1 - 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°C

Amylopectin



- 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% α 1–>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 form the backbone of the amylopectin molecule and extend over two or more clusters.

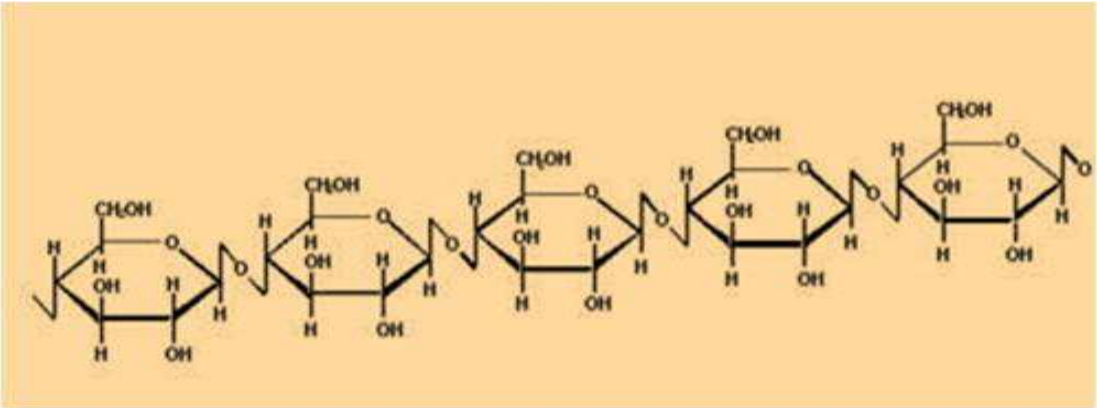
- Each cluster of a chain are primarily responsible for the crystalline regions within the granule.
- The intercrystalline regions occur at regular intervals (60 - 70 °A) containing the majority of α- 1 - 6 linkages.
- The amylopectin molecule is 100 - 150 A in diameter and 1200-4000 A long.
- Within 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 asoluble fibre that helps maintain normal bowel function,decreases constipation**,lowers cholesrerol 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 fibres 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 β-1∅ 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° relative to the adjacent glucose residues yielding a straight extended chain.
- Celluose 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 undergo 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 reducingproperty 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. H_2SO_4 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 which then react with α-naphthol to produce the pink color.

Questions

Choose the best answer

1. Pick out the aldo pentose from the following

a. D-arabinose b. D-erythrose c. D-glucose d. D-galactose

Ans: D-arabinose

2. Pick out the keto triose from the following

a. Acetayldehyde b. Dihydroxyacetore c. D-galactoe d. D-fructose

Ans: Dihydroxyacetore

3. Pyranose ring is the most common ring structure for

a. Aldo hexoses b. Aldopentoses c. Aldo tetroses d. Aldo heptoses.

Ans: Aldo heptoses.

4 ----- derivative is an important constituent of many structural polymers

a. Glucosamine b. Galactosamine c. Mannosamine d. Ribosamine

Ans: Glucosamine

5. ---------- are non-reducing disaccharides

a. Trehalose and Sucrose b. Trehalose and lactose

c. Sucrose and lactose d. Sucrose and Maltose

Ans: Trehalose and Sucrose

True or False

6. Carbon is said to be asymmetric if four different groups or atoms are attached to it. State True or False.

Ans: True

7. If the hydroxyl group on the penultimate carbon is on the right hand side of the carbon chain it belongs to the D family. State True or False.

Ans: True

8. All sucrosyl oligosaccharide are non-reducing in nature. State True or False.

Ans: True

9. ---------- was isolated from the berries of mountain ash

a. Sorbitol b. Mannitol c. Dulcitol d. Galactitol

10. Sucrose and trihalose are reducing disaccharides - State True or False.

Ans: False

Answer in one sentence

11. What are reducing non – reducing sugars?

12. What are sucrosyl oligosaccharides?

13. What is inulin?

14. Give two examples for structural polysaccharides

15. Write the molisch's test for identification of carbohydrates

Lecture.3

Mutarotation, optical activity and physical properties of sugars

Mutarotation, optical activity and physical properties of sugars

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**.**

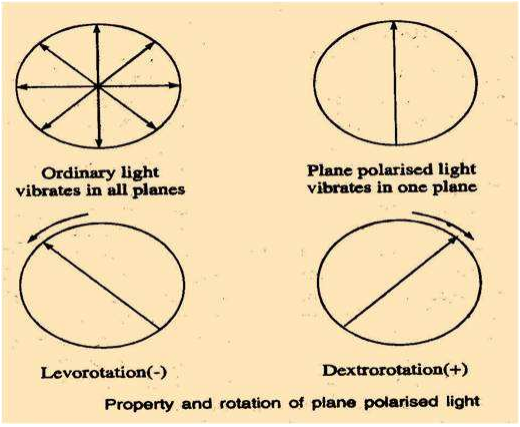
- **Diastereomers** are *stereoisomers that are not mirror images of each other*. **D-glucose, Dmannose, D-galactose** and other members of aldohexose are diastereoisomers.

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 $+52.2^o$.

Questions

Choose the best answer

1. ---------- isomers differ in arrangement of atoms around an asymmetric carbon atom.

a. Geometrical b. Optical c. Functional d. A and B

Ans: Optical

2. ---------- are epimers with respect to carbon 4.

a. Mannose and glucose b. Galactose and glucose c. Glucose and fructose d. fructose and galactose.

Ans: Galactose and glucose

3. ---------- are non-super imposable mirror images of each others.

a. Epimers b. anomers c. enantiomers d. diastereomers

Ans: enantiomers

4. Pick out the diasteromer from the following

a. D-galactose and D- glucose b. D-galactose and D-fructose

c. D-fructose and D-glucose d. D-glucose and D-rhamnose

Ans: D-galactose and D- glucose

5. Optical activity is measured using

a. Hygrometer b. Polarimeter c. Spirometer d. Pyrometer

Ans: Polarimeter

True or False

6. Geometrical isomers differ in the spatial arrangement of atoms across a double bond. State True or False.

Ans: True

7. Mannose and glucose are epimers with respect to carbons. State True or False

Ans: True

8. Diasteromers are mirror images of each other. State True or False

Ans: False

9. When the plane polarized light is rotated to the right, the compound is dextrorotatory. State True or False.

Ans: True

10. Sugars having potential free aldehyde or ketogroup exhibit mutarotation. State True or False.

Ans: True

Lecture.4

Chemical reactions of carbohydrates

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 undergo **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 reducingproperty 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. H_2SO_4 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.

Questions

Choose the best answer

1. Sugars under ---------- condition undergo caramelization

a. Dilute alkali b. Strong alkali c. Dilute acid d. Strong acid

Ans:Strong acid

2. Hydroxy methyl furfural is formed under ---------- conditions

a. Strong non-oxidising acidic b. weak non-oxidising acidic

c. Strong, oxidizing acidic d. weak, oxidizing acidic

Ans: Strong non-oxidising acidic

3. Sugars react with phenylhydrazone and the precipitate formed is called as

a. osazone b. glyzone c. Mannans d. Pentosans

Ans: osazone

4. ---------- test is used for detecting carbohydrate in solution

a. Bial's b. Osazone c. Molisch d. Iodine

Ans:Molisch

Write one sentence

5. How is hydroxyl methyl furfural formed?

6. Write the reaction of sugars with alkaline

7. Write about osazone formation.

True or False

8. Pentoses form, furfural derivative upon reach with strong, non-oxidizing acidic conditions.

Ans: True

9. Sugars may be reducing or non-reducing in nature.

Ans: True

10. Maltose and lactose are reducing sugars.

Ans: True

11. Reducing properly is the basis for Fehling's test and Benedict's test.

Ans: True

Lecture.5

Lipids- introduction, importance and classification

Occurrence and importance

1. The word lipid is derived from the *Greek* word **'lipos'** meaning fat.
2. Lipids are chemically heterogenous group of compounds that are ***insoluble in water but soluble in non-polar solvents such as chloroform.***
3. Lipids occur in plants and animals as **storage and structural components**
4. Structural lipids present in animals and plants are in the form of meat and vegetables respectively.
5. Storage fats occur in milk and adipose tissue of farm animals and in seed oils
6. Fats supply over **twice as much energy per unit weight** as proteins or carbohydrates.
7. Lipids are *anhydrous due to non-polar nature* and represent *more energy* than carbohydrates which are heavily hydrated due to polar nature.
8. The presence of lipids in diet contributes considerably *to palatability*.
9. Lipids contribute palatability in two ways. They *induce olfactory responses,* namely, taste in the mouth and aroma through nose.
10. Secondly, they contribute to the *texture of food* and is responsible for the mouth-feel.
11. Lipids also *supply the* **essential fatty acids** which are not synthesised in human beings but are essential for growth.
12. Lipids are ***essential for*** **the effective absorption of fatsoluble vitamins *A, D, E and K from intestine.***
13. Many ***enzymes require lipid molecules for maximal activity***. Examples are microsomal enzyme, glucose 6-phosphatase and mitochondrial enzyme, β-hydroxybutyrate dehydrogenase.

14. **Adrenal corticosteroids, sex hormones and vitamin D3 (Cholecalciferol)** are synthesized from lipid derivative- cholesterol.

15. Much of the lipid of mammals is located subcutaneously and **acts as insulation** against excessive heat loss to the environment.

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

17. **Classification**

    a. 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.	

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.	

Questions

Choose the best answer

1. ---------- are fat soluble vitamins

a. A_1D_1E x K b. A_1B x C c. BxC d. D_1E_1A and B

Ans: A1D1E x K

2. Vitamin 'D_3' adrenal corticosteroids are synthesized from

a. Phytosterol b. Eryosterol c. Cholesterol d. Stigmasterol

Ans: Cholesterol

3. ---------- are esters of fatty acids with glycerol and monohydric alcohols.

a. Simple lipids b. compound lipid c. derived lipids d. a and c

Ans: Simple lipids

4. ---------- are derived lipids

a. Fatty acids b. Glycolpids c. Phopholepide d. Sulfo lipids

Ans:Fatty acids

5. ---------- is animal wax

a. Carnauba b. bees wax c. Lanolein d. Glycerol

Ans:Lanolein

Write one word answer

6. Define essential fatty acids.

7. Define Simple lipids

8. Derived lipids

9. Two important functions of lipids

True or false

10. Lipids act as insulating agents. State True or False.

Ans: True

11. The word lipid is derived from 'lipos' a greek word. State True or False.

Ans: True

12. Glucose -6-phosphatase requires lipid molecules for maximal activity.

Ans: True

13. Ghee and butter are simple lipids of plants State True or False.

Ans: False

14 Fatty acids are derived lipids - State True or False.

Ans: True

Lecture.6 & 7

Structures of fatty acids and triacyl glycerol, essential fatty acids and Phosphplipids and their Importance

- 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 characterised 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 γlinolenic 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.

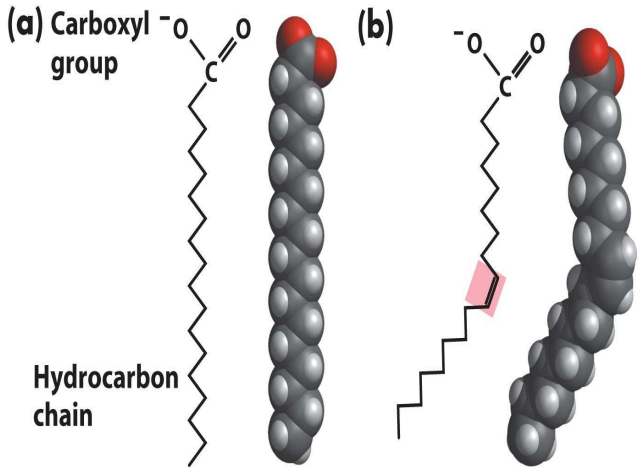
- The main source of these eicosanoids are the ***membrane phospholipids*** from which they are released by the action of phospholipase-A.

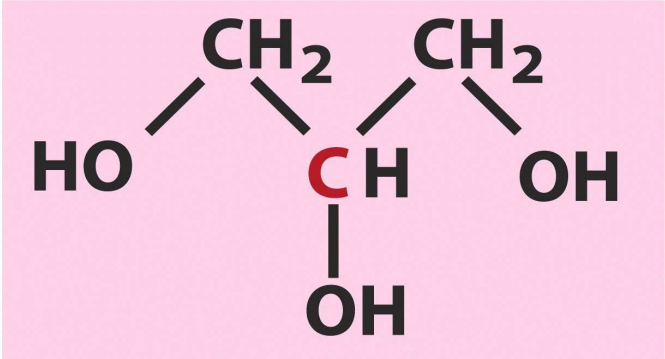
- **Phosphatidyl inositol** which contains a high concentration of arachidonic acid in carbon-2 of glycerol provides a major store of eicosanoid precursors.

- 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**.





Glycerol

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

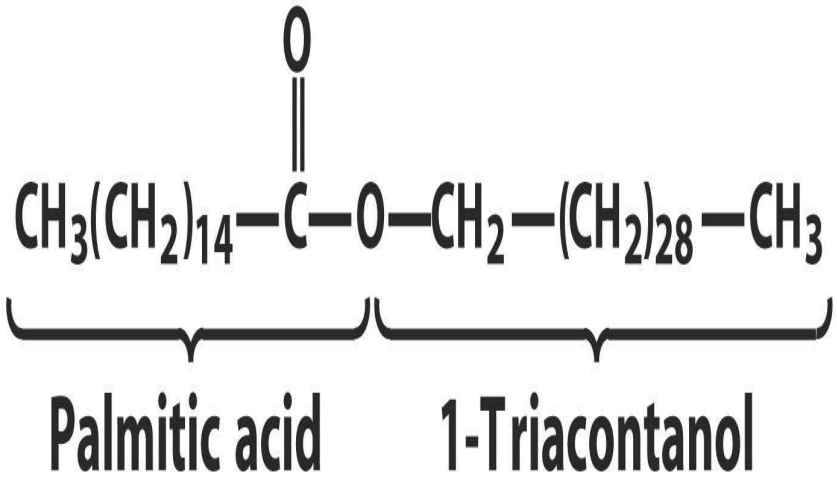
- Triacylglycerols are widely distributed in the plant kingdom. They are found both in *vegetative as well as reproductive tissues.*
- Triacylglycerols are normally stored in the **endosperm of the seed** although some plants store appreciable quantities of fat in the **fleshy fruit mesocarp**, for example, 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 □-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

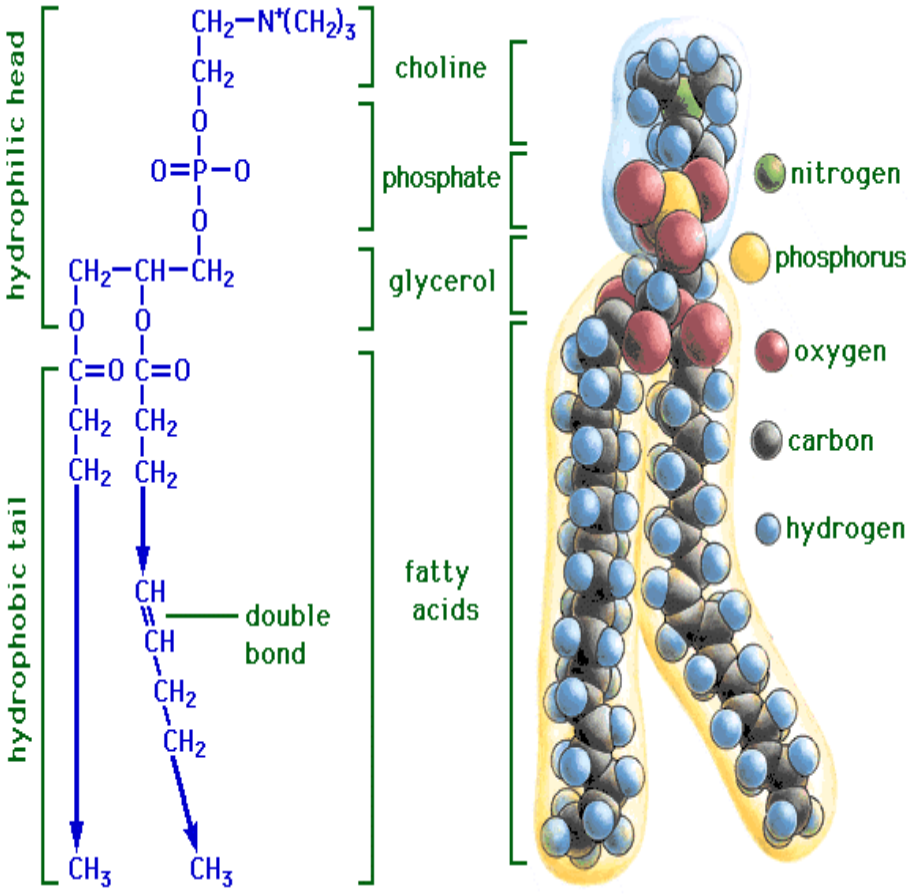
- The *outermost surface of the cell walls of epidermal cells* are covered with a **hydrophobic cuticle** which contains wax called cuticular wax.
- The main components of cuticular waxes are *hydrocarbon (odd chain alkanes) and its derivatives, wax esters, free aldehydes, free acids, free alcohols* 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.

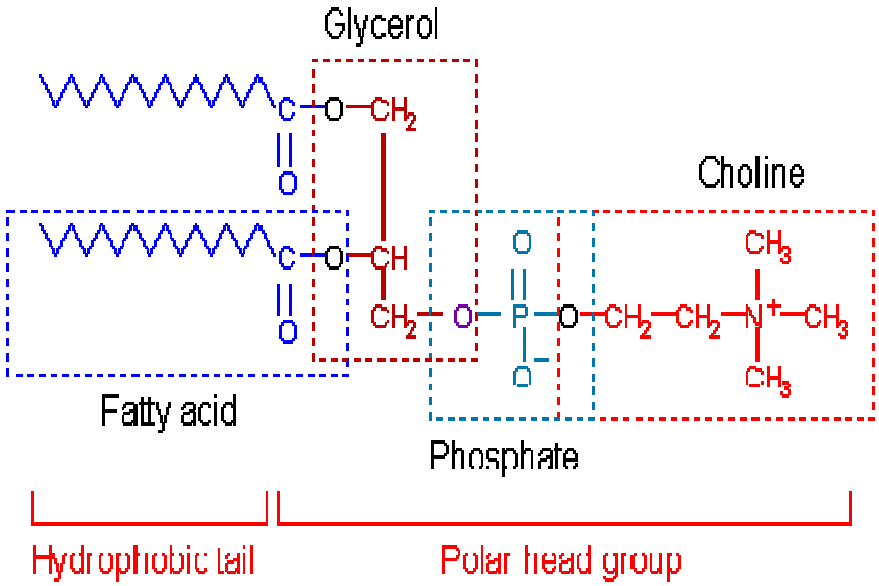
- It also helps in *protecting the tissues from chemical, physical and biological attack.*

Compound lipids

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

- These group 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

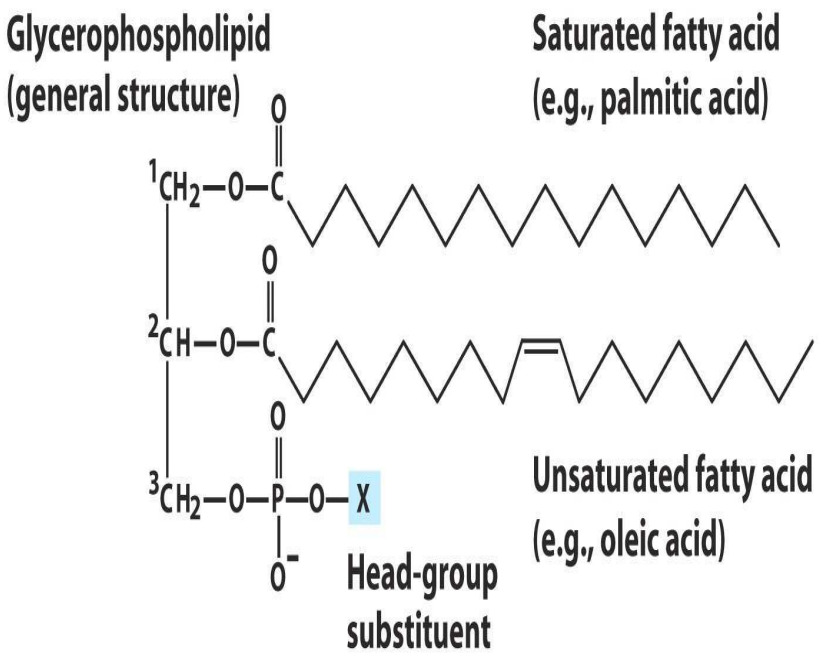
i. Phosphatidyl choline (lecithin)

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

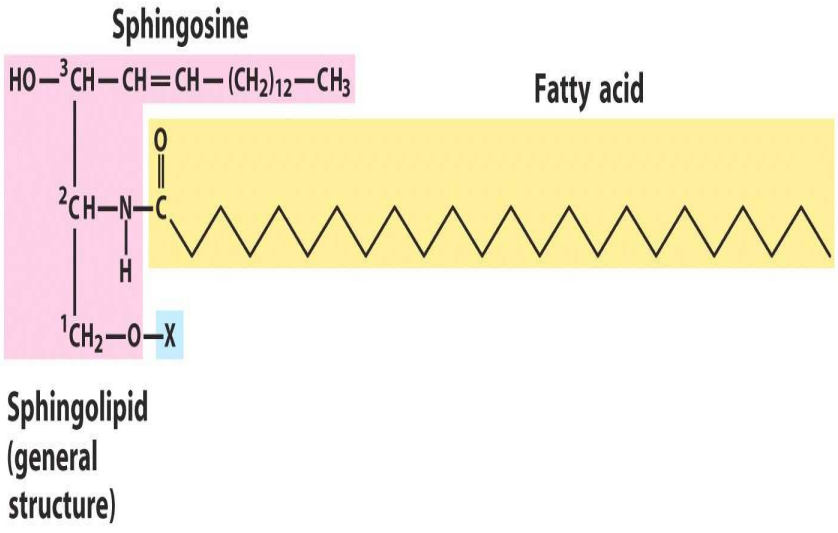
- Phosphatidyl inositol is an important **constituent of cell membrane phospholipids;**
- Upon stimulation by a suitable animal hormone it is cleaved into **diacylglycerol and inositol phosphate,** both of which act as **internal signals or second messengers.**

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 □***-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***

Properties of fat

Physical

- 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 □m.

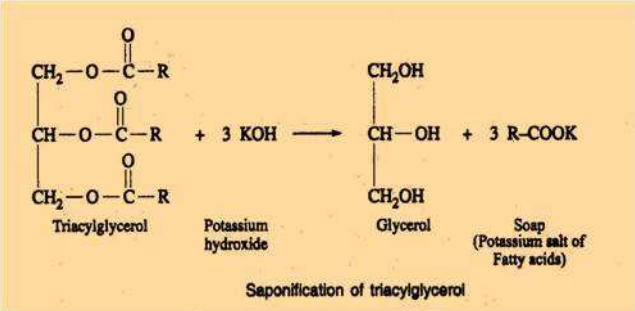
- Examples of ***naturally occurring emulsions are milk and yolk of egg***. But they are not mere fat droplets in water.

- 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

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



Saponification of triacylglycerol

- The **alkali salt of fatty acid** resulting from saponification is **soap**.

- The soaps we use for washing consists of Na or K salts of fatty acids like ***palmitic, stearic and oleic acid.***

- The potassium soaps are soft and soluble whereas the sodium soaps are hard and less soluble in water.

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 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.

Questions

Choose the best answers

1. Pick out the unsaturated fatty acid

a. Lauric b. myristic c. palmitic d. linoleic

Ans: linoleic

2. Castor bean seed oil is rich in

a. Ricinoleic b. esucic c. hydrocarpic d. chaulmoogric acid

Ans:Ricinoleic

3. ---------- can be synthesized from linolenic acid

a. Erucic acid b. Arachodonic c. Loproic d. Butysic

Ans: Ricinoleic

4. ---------- belongs to n-3 family and is an essential fatty acid

a.Lino b.Butrysic c.Arachidonics d.Capric

5. ----------serves as precursor for synthesis of prostaglandins X thromboxanes

a. Arachidonic b.linolenic c.Linoleic d.caproic

6. ---------- stones fat in the fruit mesocarp

a. Badam b. Cashew c. Avocado d. walnut

Ans: Avocado

7. ---------- Oil is used in treatment of leprocy

a. Hydnocarpic b. Chaulmoogric c. Rianioleic acid d. a and b

Ans: a and b

8. ---------- oil can be stored for years without becoming rancid

a. Coconut oil b. Palm oil c. Jatroba oil d. Safflower oil

Ans: Jatroba oil

9. Which is not a component of glycerophospholipids ?

a. Fatty acid b. Glycerol c. Sterol d. Tholine

Ans: Tholine

Short notes

10. What are glcerophospholipids?
11. Derived lipids
12. Simple lipids
13. Triacyl glycerol
14. ω- 3 and ω-6 fattyacids

Lecture.8

Physical constants and Chemical 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.***
- *S*ince 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

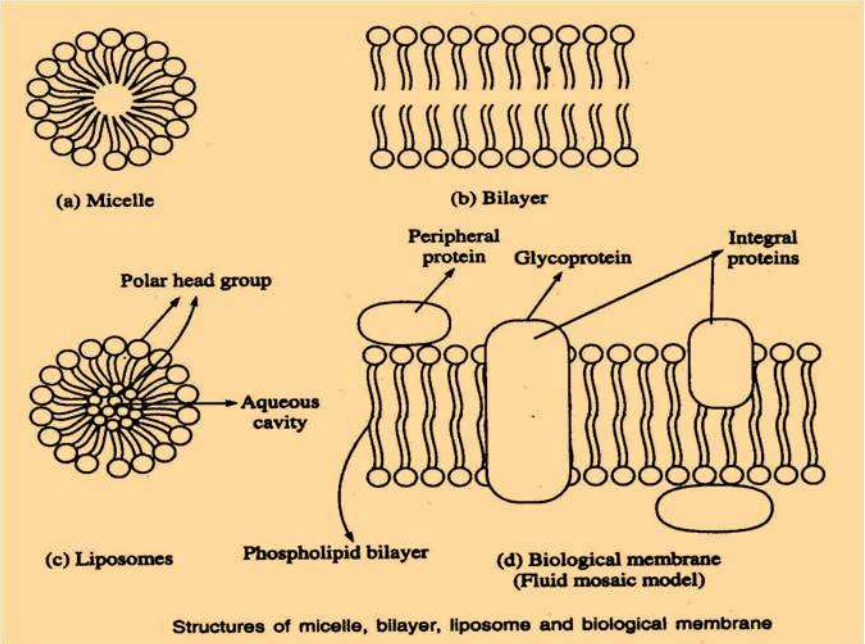
- Glycerophospholipids are virtually insoluble in water.
- Depending on the precise conditions and the nature of lipids used, ***three types of lipid aggregates can form when amphipathic lipids are mixed with water.***

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.

Questions

Choose the best answer

1. Saponification number is the number of milligrams of ---------- required to saponify 1gm of fat or oil.

a. KOH b. CaOH c.NaOH d. CuO

Ans: KOH

2. ---------- number is a measure of volatile soluble fatty acids.

a. Acid b. Iodine c. Reichert – Meisl d. Acetyl

3. Adulteration in ghee may be tested by finding --------- number.

a. Acetyl b. Polanski c. Acid d. Iodine

Ans: Polanski

4. ---------- of micelles

a. falty acids b. falty acids, lysophospholipids

c. fatty acids, lysaphospholipide x SDS d. SDS

Ans: fatty acids, lysaphospholipide x SDS

5. When a lipid bilayer folds back on itself to form a hollow sphere is called

a. Micelles b. Liposome c. Spheres d. Emulsion

Ans: Micelles

True or False

6. Lipid bilayers form the structural basis of biological membranes.

Ans: True

7. Hydrophilic head groups are at the surface.

Ans: True

8. Membrane cannot control the passage of substances across it.

Ans: **False**

9. Membrane is selectively impermeable.

Ans: **False**

10. Degree of unsaturation can be determined using Iodine number.

Ans: True

Write one word answer

1. Define saponification number.

2. Iodine number

3. Reicheet – Meisel number

4. Acid locum bet

5. PUFA

6. Acetyl number

Lecture.9

Plant pigments – Structure and Function of Chlorophyll and Carotenoids. Sterols basic structure, role of brassinosterols in plants

Chlorophylls

Chlorophylls occur in the photosynthetic pigment system as chromoproteins (pigment – protein complexes). In the case of chlorophylls and carotenoid, the pigment – protein linkage is relatively weak, being composed of non-covalent bonds. Those bonds are easily broken. Hence chlorophylls and carotenoids can be extracted simply by macerating the plant tissue with an organic solvent such as acetone. However, the linkage between phycobilins and protein is covalent and occurs as phycobiliproteins. The phycobiliproteins are water – soluble.

Four different chlorophylls have been extracted from plants. Chlorophyll b is almost as widespread but is absent in all the algae except few. Chlorophyll c_1 and c_2 are known. Chlorophyll d is also noticed.

Chlorophyll is a tetrapyrrole and the four atoms of the pyrroles are coordinated to a magnesium atom. Thus chlorophyll is a magnesium – porphyrin.

Absorption spectra of chlorophylls

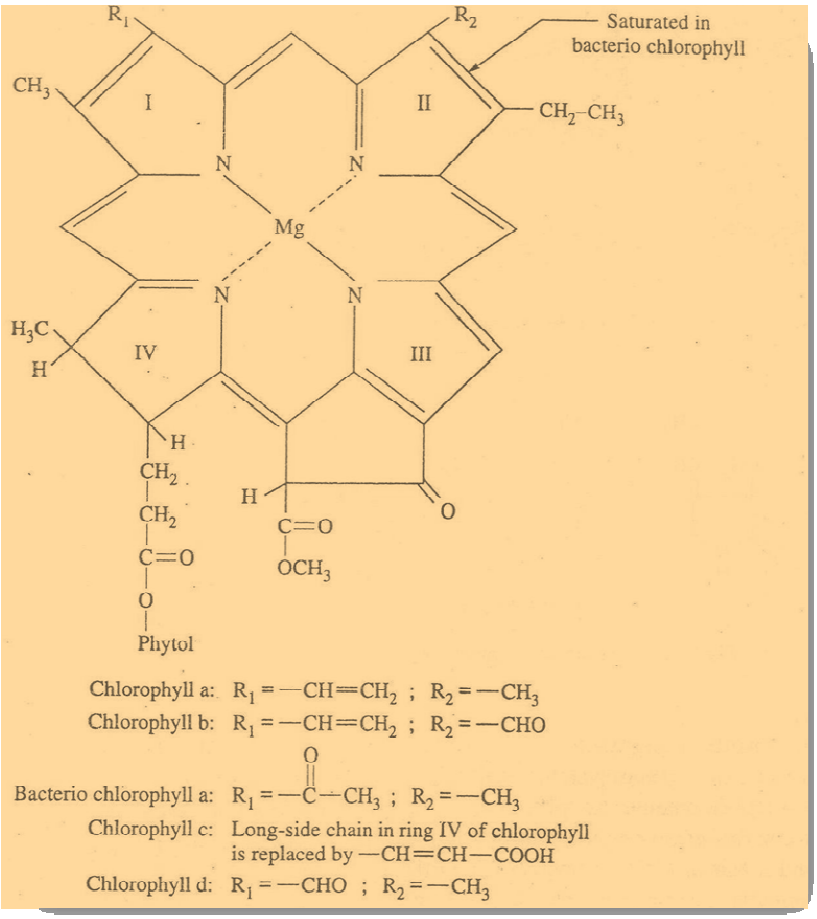
The absorption spectra of Chl a and b are different. Light that is not appreciably absorbed by chlorophyll a at 460 nm is absorbed by chlorophyll b. Thus these two kinds of chlorophylls complement each other in absorbing the incident sunlight. These chlorophylls are very effective photoreceptors because they contain networks of alternating single and double bonds (conjugated).

Accessory pigments

Carotenoids

The accessory pigments can also serve as receptors of light energy. The carotenoids are long polyisoprenoid (tetra – terpene) molecules having conjugated double bonds. Each end of the molecule contains an unsaturated substituted cyclohexane ring. There are two types of carotenoid pigments in chloroplasts, the carotenes which are

tetreterpene hydrocarbons without oxygen and the other is xanthophylls which are very similar in structure but contain oxygen atoms in their terminal rings.

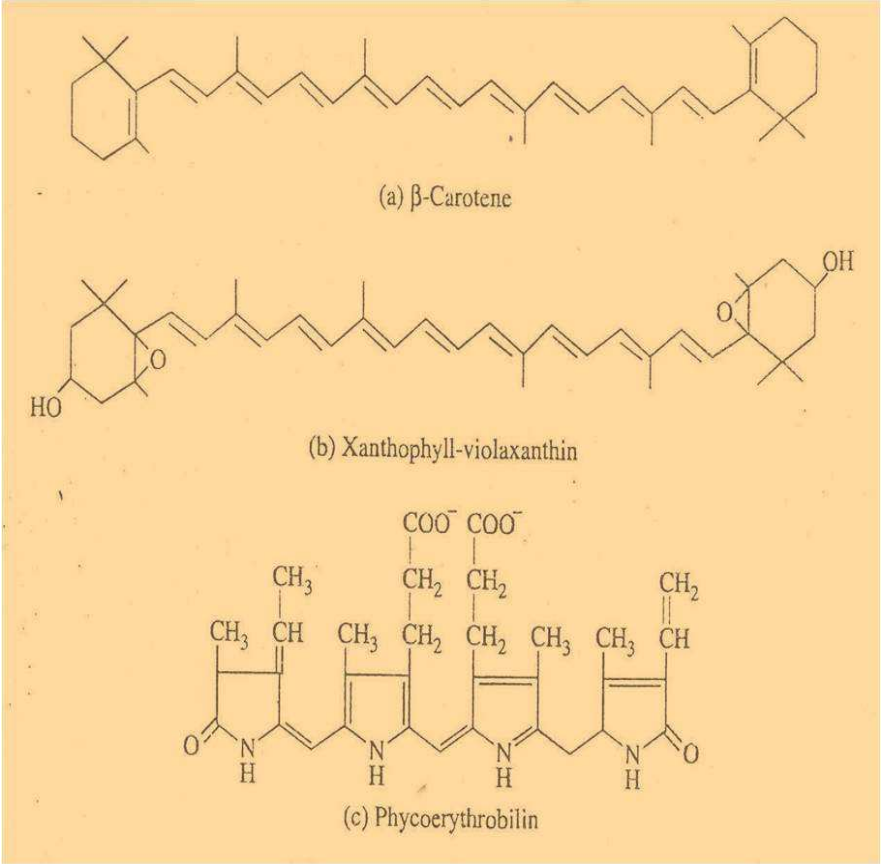


Structure of chlorophylls

The most abundant carotene is β – carotene and the xanthophylls is violaxanthin. The carotenoids absorb light in the range 400-500 nm.

Phycobilins

Phycobilin pigments occur in red and bule-green algae but not in higher plants. They are linear tetrapyrroles without bound Mg2+. Phycoerythrobilin is the major red pigment of red algae. The blue phycocyanobilin is the analogous conjugate of bule-green algae.



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 **perhydro cyclopentano 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 have been shown to interfere with ecdysteroids at their site of action, and are thus the first true ***antiecdysteroids.***

v. 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.

Questions

Choose the best answer

1. Chlorophylls and carotenoids can be extracted by macerating the tissue with ----------

a. Acetone b. Alcohol c. Ether d. Ethylacetate

Ans: Acetone

2. ---------- are accessory pigments

a. Chlorophyll -a b. Chlorophyll- b c. Carotenoids- d d. Chlorophyl -c

Ans: Carotenoids- d

3. ---------- occur in red x blue –green algae

a. Xanthophylls b. Phycobilins c. Carotenoids d. Antrocyanins

Ans: Phycobilins

4. The parent structure of sterols is known as

a. Perhydro cyclo pentano phenanthrene

b. Perhydro cyclo hexano phenanthrene

c. Perhydro heptano phenanthrene

d. Perhydro pyrolidene

Ans: Perhydro cyclo pentano phenanthrene

5. ---------- is an animal sterol

a. Cholesterol b. Stigma sterol c. Ergosterol d. Phytosterol

Ans: Cholesterol

True or False

6. Carotenoids are not accessory pigments.

Ans: False

7. Brassinosteriods promote ethylene biosynthesis.

Ans: True

8. Brassinosteriods to hot have any effect on stem elongation.

Ans: False

9. Cholesterol is precursor of vitamin D, bile acids, corticoteroids and female sex hormones.

Ans: True

10. Eryosterol is seen in yeast and fungus.

Ans: True

Write one word answer

11. Brassinosteroids
12. Physiological effects of brassinosteroids (any 2)
13. Cholesterol
14. Accessory pigments
15. Chlorophylls

Lecture.10

Amino acids – classification and properties, essential amino acids

Amino acids – classification and properties, essential amino acids

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.

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 □**-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

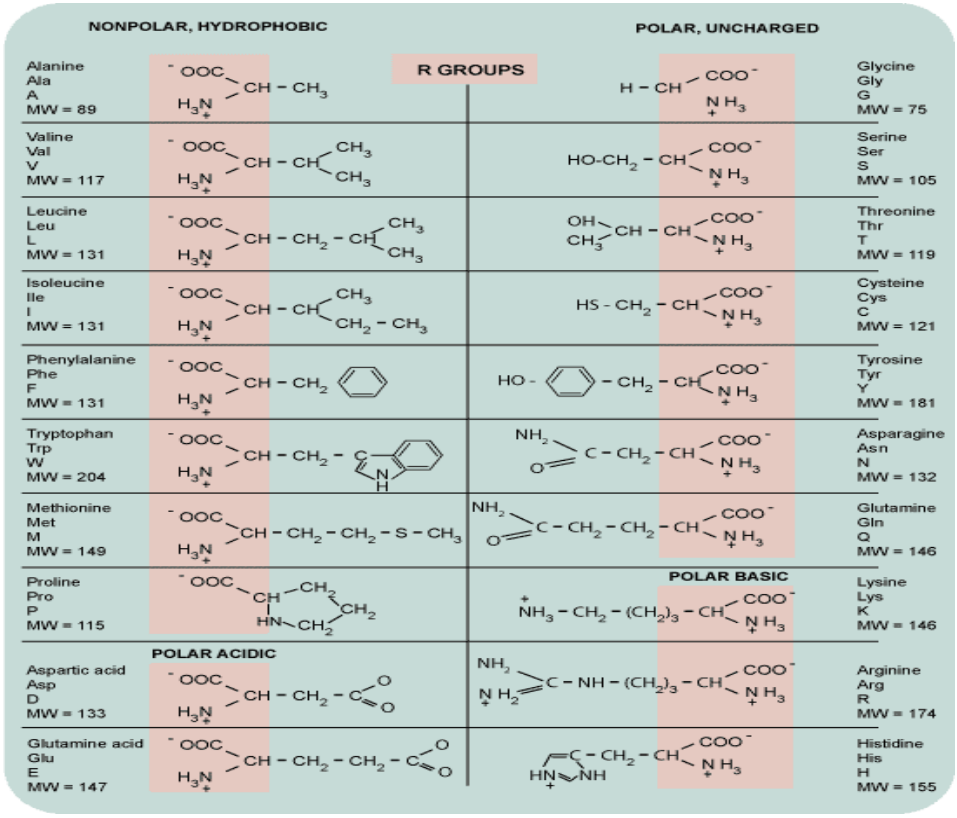
a) Amino acids with non-polar or hydrophobic, aliphatic R groups

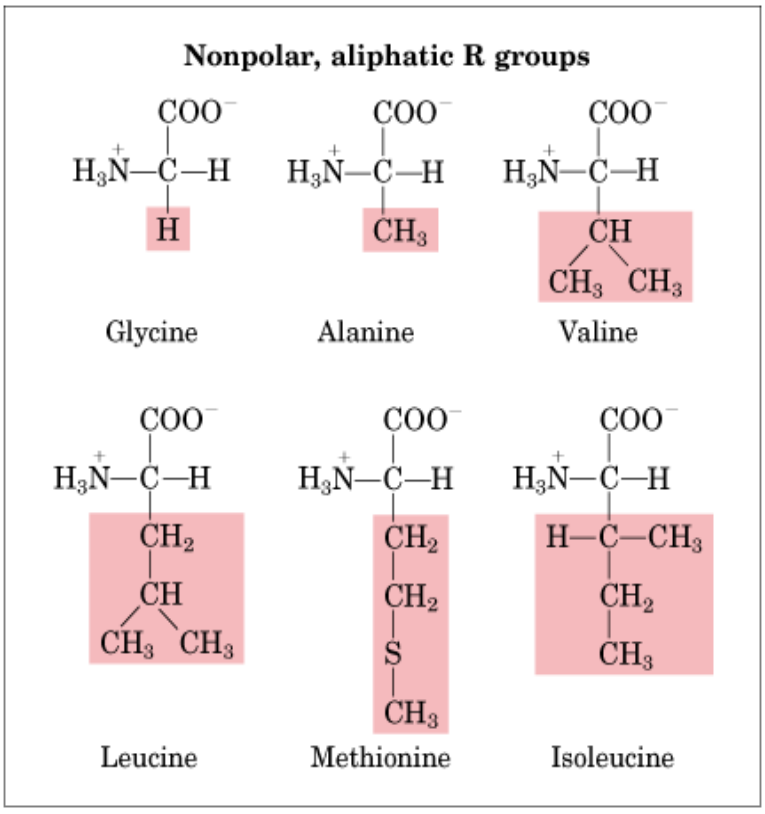
- This group of amino acids includes ***glycine, alanine, valine, leucine, isoleucine and proline.*** The hydrocarbon R groups are *non-polar and hydrophobic*.

- 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.

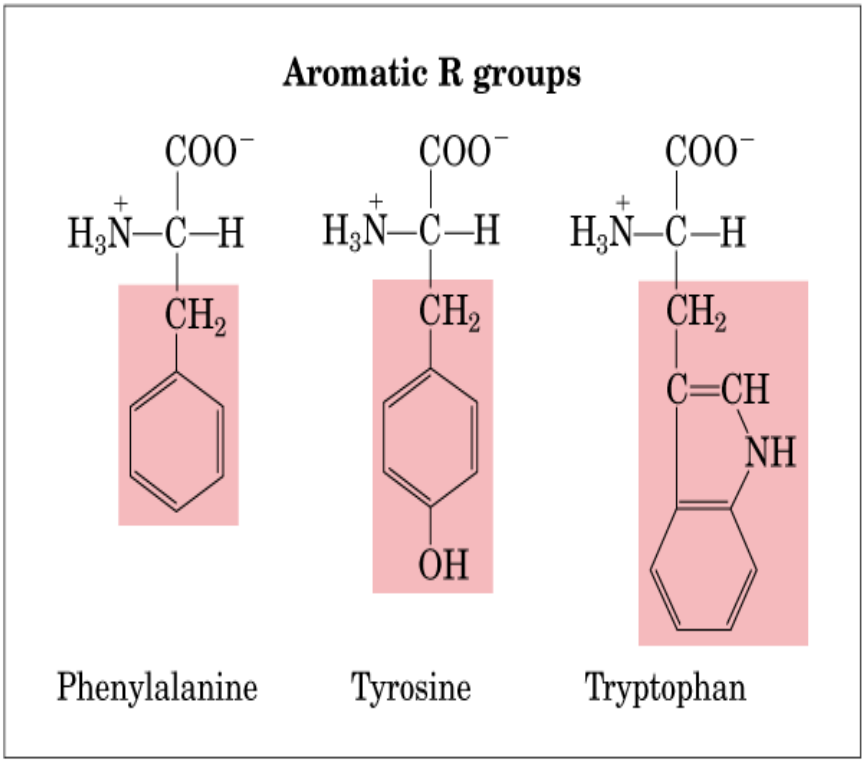
- On the other hand, the imino group of proline is held in a rigid conformation and reduces the structural flexibility of the protein.





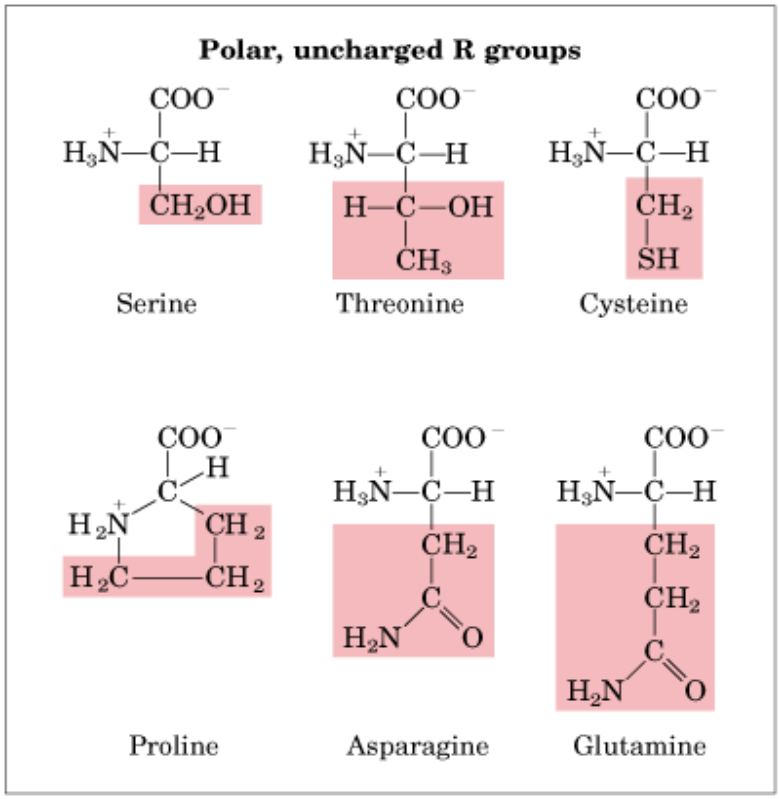
b) Amino acids with non-polar aromatic R groups

- 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

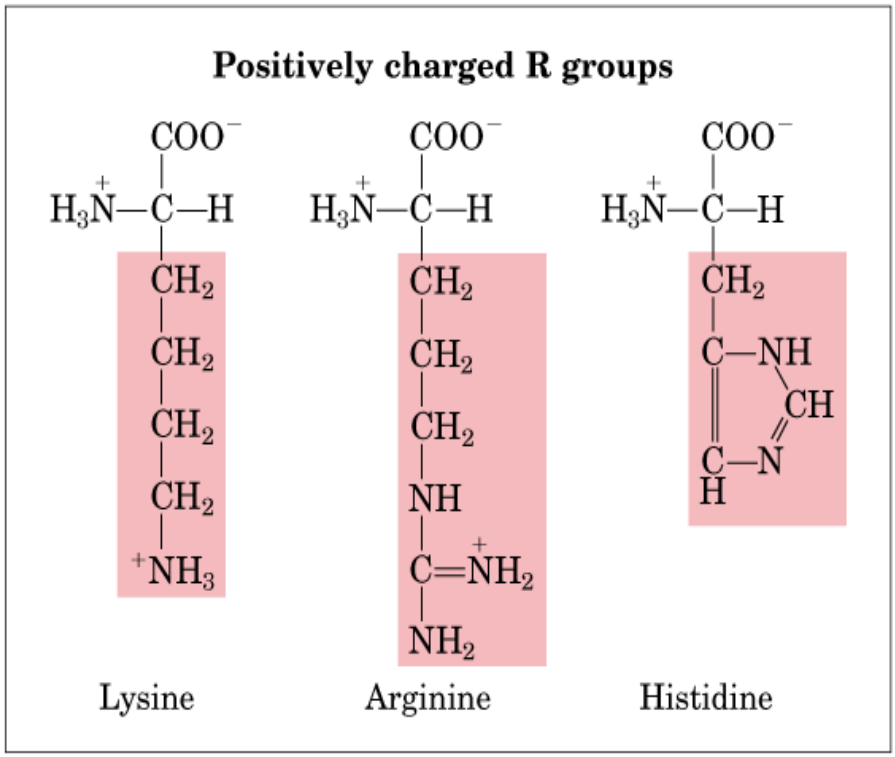
- This group of amino acids includes ***serine, threonine, cysteine, methionine, asparagine and glutamine.***
- The *hydroxyl group* of ***serine and threonine***, the *sulphur* atom of ***cysteine and methionine*** and the *amide group* of ***asparagine and glutamine***, contribute to the polarity.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

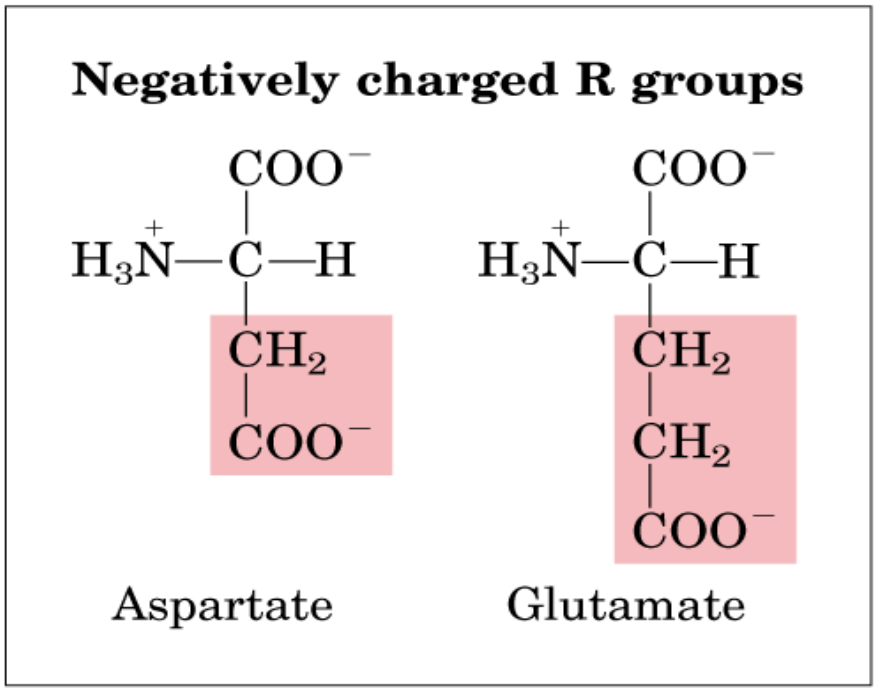


d) Amino acids with charged R groups

- **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 □-*amino group*; **arginine** has a positively charged *guanidino group*; and **histidine** has an *imidazole* group.


Positively charged R groups
Lysine
Arginine
Histidine


Negatively charged R groups
Aspartate
Glutamate

Properties of amino acids

Physical

- 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

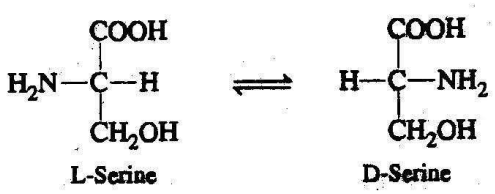
- Amino acids are ***amphoteric*** compounds, as they contain **both acidic (COOH)** and **basic (NH2) groups.**
- They can react with both alkalies and acids to form salts.
- In ***acid solution*** amino acids carry **positive charges** and hence they move towards cathode in an electric field.
- In ***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 □***-amino acids*** because NH2 group is attached to the □-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 □-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.*

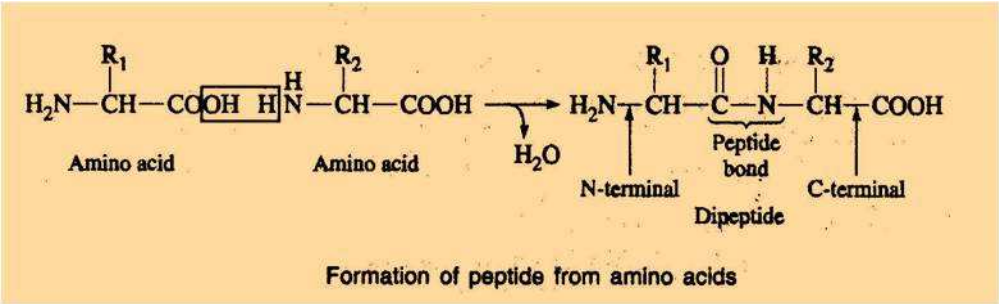
- ***Methionine, Arginine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Histidine, and Lysine are the essential amino acids*** (Remember **MATTVILPHLy**).

Peptide

- Amino acids are linked together by formation of ***covalent bonds.***
- The covalent bond is formed between the □***-carboxyl group of one amino acid and the*** □***-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.

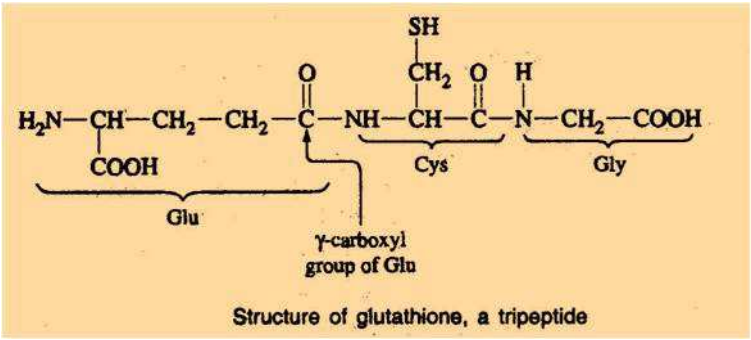
- In writing the peptide structure, the amino terminal (N-terminal) amino acid is written first and carboxyl terminal (C-terminal) amino acid written last.



Formation of peptide from amino acids

Peptides of physiological interest

- ***Glutathione*** is a commonly occurring tripeptide ***(□-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.***

Questions

Choose the best answer

1. ---------- are specialized proteins with catalytic activities.

a. Enzymes b. Peptides c. Glycoprotein's d. Proteoglycans

Ans: Enzymes

2. Glycine was derived from the greek word

a. Glykos b. Glucose c. Glycogen d. Glycosan

Ans:Glykos

3. ---------- is a non-polar hydrophobic amino acid

a. Gly b. Pbe c. Tyr d. Trp

Ans: Gly

4. ---------- are amino acids with polar uncharged R groups

a. Sereine x threoneine b. Serine x phenylalanine

c. Sereine x tyrosine d. Tyrosine x tryptophan

Ans: Sereine x threoneine

5. ---------- is a flowering agent

a. Potassium glutamate b. Sodium glutamate

c. Glutamic acid d. Sodium tartarate

Ans: Sodium glutamate

6. ---------- is an amino acid

a. Phe b. His c. Pro d. Tys

7. The aminogroup of amino acid combines with formaldehyde gives ---------- derivate

a. Dimethyol b. Dialkyl c. Trimethylol d. Pentamethylol

Ans: Dimethyol

8. Aminoacid +Ninhydrin forms ---------- colour complex

a. Green b. Blue c. Purple d. Yellow

Ans: Purple

9. Which one the following is a strong oxidizing agents

a. Val b. Ninhydrin c. Histidene d. Argurene

Ans: Ninhydrin

10. ---------- plays a role in control of blood pressure

a. Vasopressin b. Oxytocin c. Glutathione d. Aspartaone

Ans: Vasopressin

True or False

11. Histidine is a non-essential amino acid.

Ans: False

12. Amino acids are linked by peptide bonds in proteins.

Ans: True

13. Aspartame is an artificial sweetener.

Ans: True

Write one word answer

14. Define Glutathione

15. Define Vasopressin oxytocin

16. Define Essential amino acids

Lecture.11

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 -1.9oC 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

Questions

Choose the correct answer

1. Seed proteins contain ---------- in leiser quantities

(a) Albumins (b) Globulins (c) Prolamines (d) Glutelins

Ans : Albumins

2. Prolamines are deficient in ----------

(a) Lysine (b) Glycine(c) Tyrosine (d) Tryptophan

Ans : Lysine

3. Fishes are prevented from freezing by--------

(a) Antifreeze glycoprotein (b) Ricin (c) Lectin (d) Fibroin

Ans: Antifreeze glycoprotein

4. ---------- have protective defense function

(a) Antibodies (b) Albumin (c) Lectin (d) Myosin

Ans : Antibodies

True or False

5. Hemoglobin is a chromo protein.

Ans: True

6. Lipoproteins contain hyalusonic acid and Chontroitin sulphates.

Ans: False

7. Lectin it toxic protein in legumes agglutinates seed blood cells.

Ans: True

8. Snake venom is protein in nature.

Ans: True

Lecture.12

Colour reactions of Proteins, Hydrogen bond and Hydrophobic interactions.Structure 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.

$H_2N \longrightarrow COOH$

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

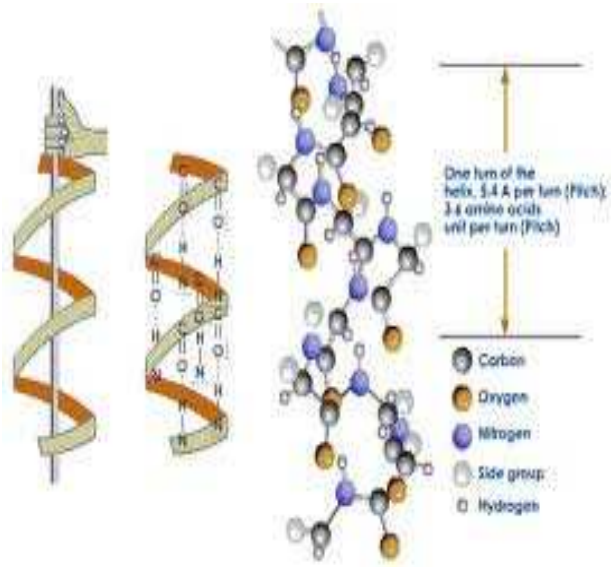
- The α-helix is the ***most stable*** arrangement of polypeptides

- 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.



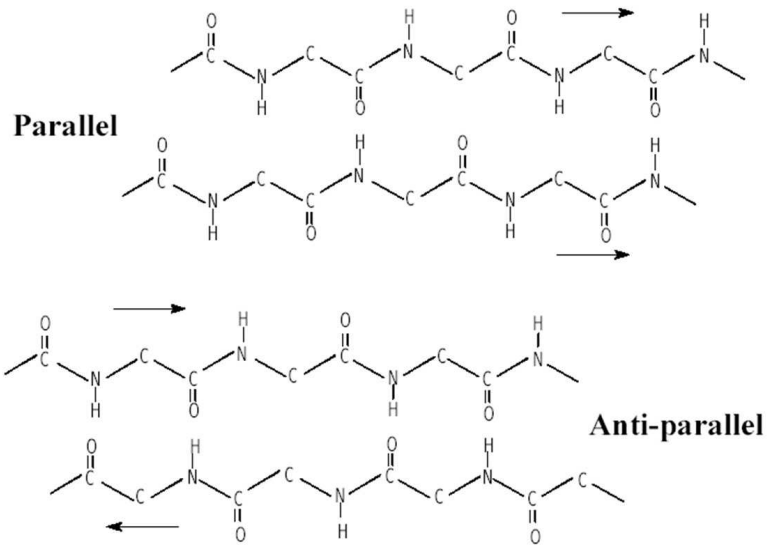
- Certain amino acids tend to ***disrupt the α-helix***. Among these are **proline** (the N atoms is part of the rigid ring and no rotation of the N-C bond can occur) and **amino acid with charged or bulk R groups** that either electrostatically or physically interferes with helix formation.

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.***

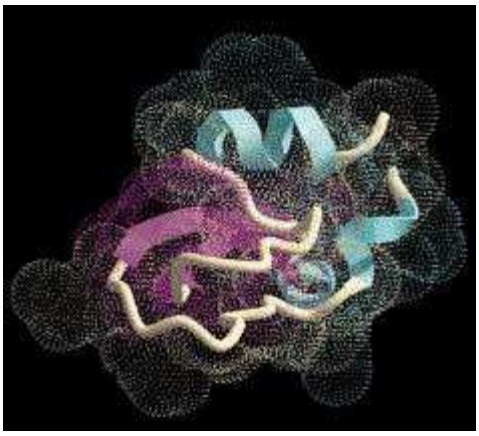
- In another type, known as ***antiparallel β-pleated sheet structure***, the polypeptide chains alternate in such a way that the -COOH end of the one polypeptide is next to the -NH_2 end of the other i.e. polypeptide chains run in opposite directions.

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.



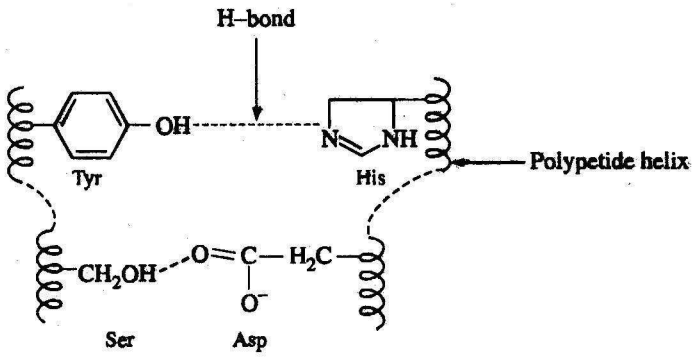
- But in terms of biological function, the regions of random coil are of equal importance to those of helix and pleated sheet.

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

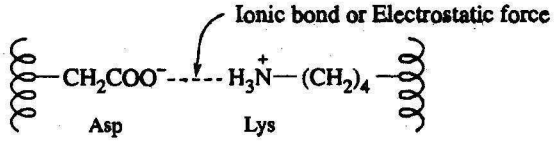
- Hydrogen bonds are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group



Hydrogen bonds in polypeptide chain

Salt-linkages (electrostatic forces; ionic bonds)

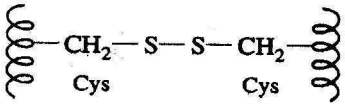
- Salt linkages are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group



Electrostatic forces in polypeptide chain

Disulfide bonds (S-S linkages)

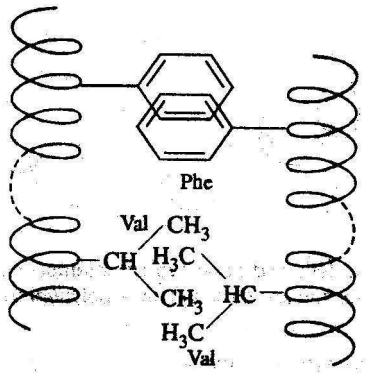
- The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains



S-S linkages in polypeptide chain

Hydrophobic bonds

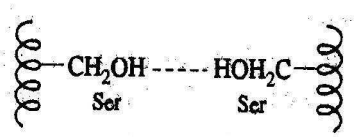
- Hydrophobic bonds are formed as a result of interaction between non-polar side chains



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**.



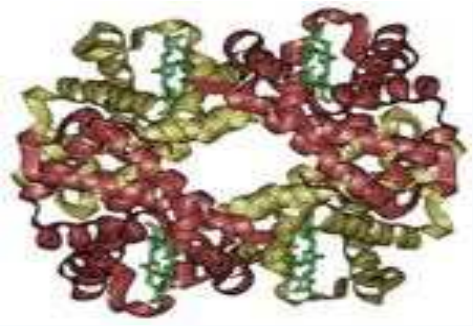
Dipole-dipole interaction in polypeptide chain

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 semiindependent 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 th 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.

b) **Acidic and basic reagents** cause changes in pH, which alter the charges present on the side chain of protein disrupting the salt linkages.

c) **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 βmercaptoethanol,** it is converted to an inactive, denatured molecule.
- When urea or mercaptoethanol is removed, it attains its native (active) conformation.

Chemical

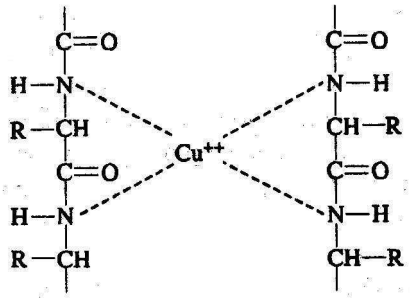
Colour reactions of proteins

- 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.

Questions

Choose the correct answer

1. **----------** refers to the number of amino acids and the order in which they are covalently linked.

(a**)** Primary structure **(**b) Secondary structure (c) Tertiary structure (d) Quartemary

Ans: Primary structure

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

(a) Hydrazine **(**b) Flucro dinitro benzene (c) PITC (d) 2 mercapto ethanol.

Ans: (a) Hydrazine

3. Biuret reaction is due to the formation of coordination complex between four nitrogen atoms of two polypeptide chains

(a) One copper atom (b) One ion atom (c) One sulphur atoms (d) One zinc atoms

Ans: One copper atom

4. Amino acid disrupts the α helix.

(a) Proline (b) Phenylalanine (c) Glycine (d) Hiztidine

Ans: Proline

True/False

1. Myoglobin has no quaternary structure since; it is composed of a single polypeptide chain.

Ans: True

2. Addition of nitric acid to protein produces yellow colour on heating – This is Hopkins's Cole reaction.

Ans: False

3. Trypsin hydrolyses the peptide bond on the carboxyl side of the basic amino acid residues of lysine or arginine.

Ans: True

Short notes

1. Xanthoproteic reaction

2. Hopkins- Cole reaction

3. Quaternary structure of proteins

4. Secondary Structure α helix

5. Secondary Structure α helix

Lecture.13

Enzymes – classification, Mechanism of enzyme action –active site

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.**

$$CO_2 + H_2O.\ H_2CO_3$$

- 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 feature 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.

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 catalysing 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**

Glucose + ATP ⟶ 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

Acetyl choline + H_2O ⟶ Acetic acid + Choline

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

$COOH - CH(OH) - CH_2\text{-}COOH \longrightarrow COOH - CH = CH - COOH + H_2O$
Malic acid Fumaric acid

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 $\longrightarrow$ 11 cis-retinal

Enzyme **Retinene isomerase**

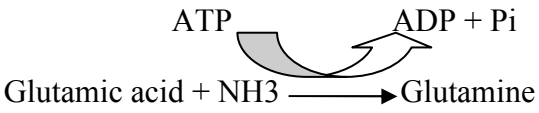
Systematic name: **All-trans retinene: 11-cis isomerase**

E.C.No. 5.2.1.3

VI. Ligases

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

Example



Enzyme: Glutamine synthetase

L.Glutamate: Ammonia ligase

E.C.6.3.1.2

Mechanism of enzyme action

- A chemical reaction such as A $\longrightarrow$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 uncatalysed reaction. Thus, they accelerate chemical reactions by **lowering the energy of activation**

Example

$H_2O_2 \rightarrow H_2O + (O)$

Catalase

Reaction condition	**Activation energy (KCal mol^{-1})**
Uncatalysed	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

$$\mathbf{[S] + [E] \longrightarrow [ES] \longrightarrow P + [E]}$$

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

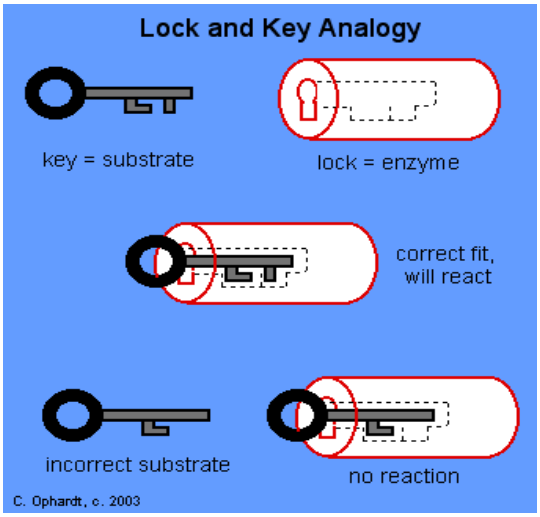
Active site

- **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 groupof 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

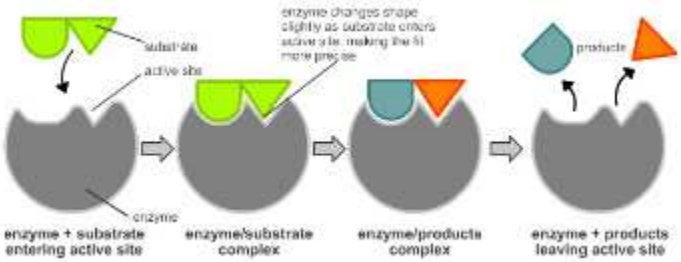


- An unfortunate feature of this model is the **implied rigidity of the catalytic site**.

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.



Questions

1. Km is expressed as

a. Mole/litre b. Mole c. Mg d. No units

Ans: Mole/litre

2. Give example for group specificity enzyme ----------

a. Acetyl choline esterase

b. Fumarase

c. Herco kinase

d. Retinene Isomerasl

Ans: Fumarase

3. Fischers lock and key theory was proposed by Emil Fischer.

Ans: True

4. The substrate binding site in the enzyme is referred as active site.

Ans: True

5. Urease acts only urea.

Ans: True

Lecture.14

Factors affecting enzyme action and Cofactors and coenzymes

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

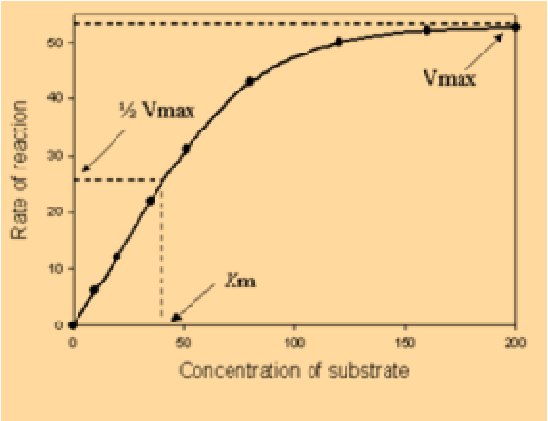
- Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the ***substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola.***
- At ***very low substrate concentration*** the *initial reaction velocity (v) is nearly proportional 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, eventhough 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.



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

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

v = Velocity of the reaction

At half maximal velocity [S] = K_m

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

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

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

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

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

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

  - 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 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^oC*** 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^oC.***
- 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.

$$E^- + SH^+ \longrightarrow ESH$$

acidic pH

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

alkaline pH

$SH^+ \longrightarrow S + H^+ + E^- \longrightarrow$ 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 ***possessess 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.

$$E + I \longrightarrow E I$$

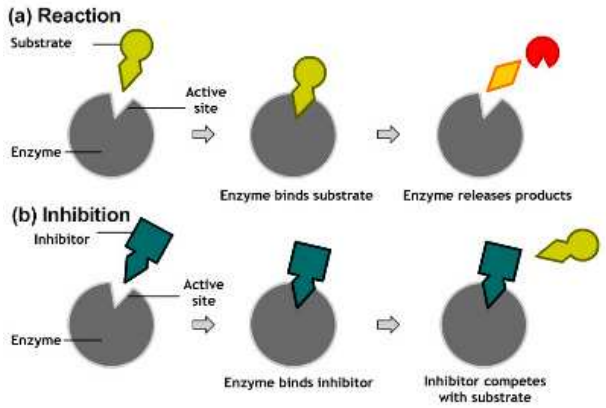
$\longleftarrow$

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.*

$$E + I \rightarrow EI$$

$$ES + I \rightarrow ESI$$

- 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.

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.
- ***Metalloenzymes*** 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 metalloenzymes 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.

$$\text{Lactate} + \text{NAD}^+ \longrightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+$$

- 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.**

Questions

Choose the best answer

1. The protein part of an enzyme is called

a. Apoenzyme b. 10 factor c. Coenzyme d. Prosthetic group

Ans: Apoenzyme

2. ---------- is an example for isozymes which occur as five different forms in the tissues of human and other vertebrates

a. LDH b. MDH c. SDH d. Isocitrate dehydrogenises

3. The two polypeptide chains in LDH are coded by ---------- different genes

a. Two b. Three c. Four d. Five

State True or False

4. Non-protein components are called as cofactors.

Ans: True

5. Coenzyme that is non-covalently bound to the enzyme protein is called prosthetic group.

Ans: False

6. Zn is lear acid in carbonic anhydrase.

Ans: True

Write short notes

7 Isoenzymes

8. LDH

9. Cofactors

10. Coenzymes

Lecture.15

Vitamins and minerals as coenzymes/co-factors

Introduction

Vitamins are low molecular weight organic compounds required in small amounts in the diet. Most of the vitamins are not synthesized in the human body but are synthesized by the plants. Hence these essential nutrients are mainly obtained through the food. Though most of them are present in the diet as such, some are present as precursors known as provitamins.

Vitamins are divided into two major categories. They are fat-soluble (A, D, E and K) and water-soluble vitamins (B-complex and vitamin C). B complex vitamins include thiamine (B_1), riboflavin (B_2), niacin, pyridoxine (B_6) biotin, folic acid, pantothenic acid and cobalamin (B_{12}). Inositol, choline and para-aminobenzoic acid are vitamin-like substances sometimes classified as part of the B complex, but no convincing evidence has been shown so far to be included as vitamins. All the fat-soluble vitamins and some B vitamins exist in multiple forms. The active forms of vitamin A are retinol, retinal and retinoic acid and vitamin D is available as ergocalciferol (D2) and cholecalciferol (D_3). He vitamin E family includes four tocopherols and four tocotrienols but α-tocopherol being the most abundant and active form. The multiple forms of vitamins are interconvertible and some are interchangeable.

Fat-soluble vitamins

The fat-soluble vitamins are soluble in fat and other nonpolar solvents. All are synthesized fully or partly from isoprene units and excess quantities are stored in fat containing cells. The fat-soluble vitamins appear not to function as components of coenzymes but to serve other important roles. The important dietary sources, functions and deficient diseases associated with fat-soluble vitamins are presented.

Fat-soluble vitamins

Vitamin	Functions	Some common dietary sources	Deficiency symptoms
Vitamin A	Visual cycle and maintaining epithelial cells	Fruits, vegetables, fish-liver oils	Night blindness and eventually total blindness, anorexia (appetite loss), dermatitis, recurrent infections; in children, cessation of skeletal growth and lesions in the central nervous system.
Vitamin D	Calcium metabolism	Fish-liver oil	Bone pain and skeletal deformities such as bowlegs (Rickets) and knock-knee in children. Osteomalacia in adults.
Vitamin E	Antioxidant	Plant oils, green leafy vegetables, milk, eggs, meat	Symptoms in humans, if any, are controversial; possibly anaemia
Vitamin K	Blood clotting	Leafy vegetables, soybeans, vegetable oils	Impaired blood clotting

Water-soluble vitamins

The water-soluble vitamins include B-complex group and vitamin C. The important dietary sources and deficient symptoms associated with them are presented in Table 8.5.

Coenzymes

Coenzyme action

Coenzyme accelerates the enzymatic reaction by helping the formation of the product (s) by action as acceptor for one of the products.

The substrate combines with the apoenzyme to form activated complex. But this combination takes place in the presence of coenzyme. The bond in the substrate is strained and ruptured when one of the cleavage products is directly transferred to the coenzyme, which has suitable receptor site in its structure. The other cleavage product now dissociates from the apoenzyme liberating the enzyme protein for fresh reaction.

The cleavage product attached to the coenzyme is next released from the surface of the coenzyme after the completion of enzyme action. Now both apoenzyme and coenzyme are regenerated to their original form and are ready for fresh reaction. A prosthetic group also acts in a similar fashion with the difference that the prosthetic group is firmly attached to the surface of the apoenzyme.

Water-soluble vitamins

Vitamin	Some common dietary sources	Deficiency symptoms in humans
1.Thiamine (Vitamin B_1)	Liver, meat, milk, vegetables, whole grains, nuts	Dry and wet beri-beri. Weight loss, muscle wasting, sensory changes, mental confusion, enlargement of heart, constipation
2.Riboflavin (Vitamin B_2)	Liver, wheat germ, eggs, milk, green leafy vegetables, meat	Magenta-coloured tongue, fissuring at the corners of mouth and lips, dermatitis
3.Niacin (nicotininc acid)	Meat, liver, cereals, legumes	Pellagra, Dermatitis when exposed to sunlight, weakness, insomnia, impaired digestion, diarrhea, dementia, irritability, memory loss, headaches
4.Pyridoxine (vitamin B_6 or pyridoxol)	Egg yolk, fish, meat, lentils, nuts, fruits, vegetables	Convulsions, dermatitis, weight loss, irritability, weakness in infants
5.PAntothenic acid	Eggs, peanuts, liver, meat, milk, cereals, vegetables	Vomiting, abdominal distress, cramps fatigue, insomnia
6.Biotin	Liver, yeast, meat, peanuts, eggs, chocolate, dairy products, grains fruits, vegetables	Dermatitis, skin dryness, depression, muscle pain, nausea, anorexia (appetite loss)
7.Folic acid	Yeast, liver green vegetables, some fruits	Anemia leading to weakness, tiredness, sore tongue, diarrhea, irritability headache, heart palpitations
8.Cobalamin (vitamin B_{12})	Meat, shellfish fish, milk, eggs	Neurological disorders anemia leading to tiredness, sore tongue, constipation, headache, heart palpitations
9.Ascorbic acid (vitamin C)	Vegetables and citrus fruits	Sore gums, loose teeth, joint pain, edema, anaemia, fatigue,

		depression, impaired iron absorption, and impaired wound healing.

The structure and coenzyme functions of B-complex group vitamins are described below.

Thiamine

The coenzyme form of thiamine, thiamine pyrophosphate (TPP).

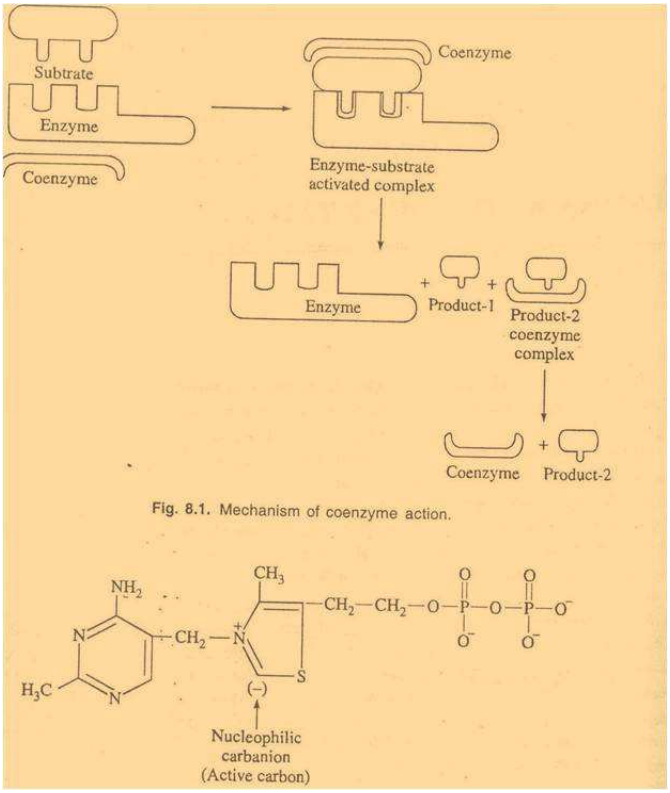


Fig. 8.1. Mechanism of coenzyme action.

Structure of thiamine pyrophosphate

The two important reactions in which TPP functions as coenzyme are

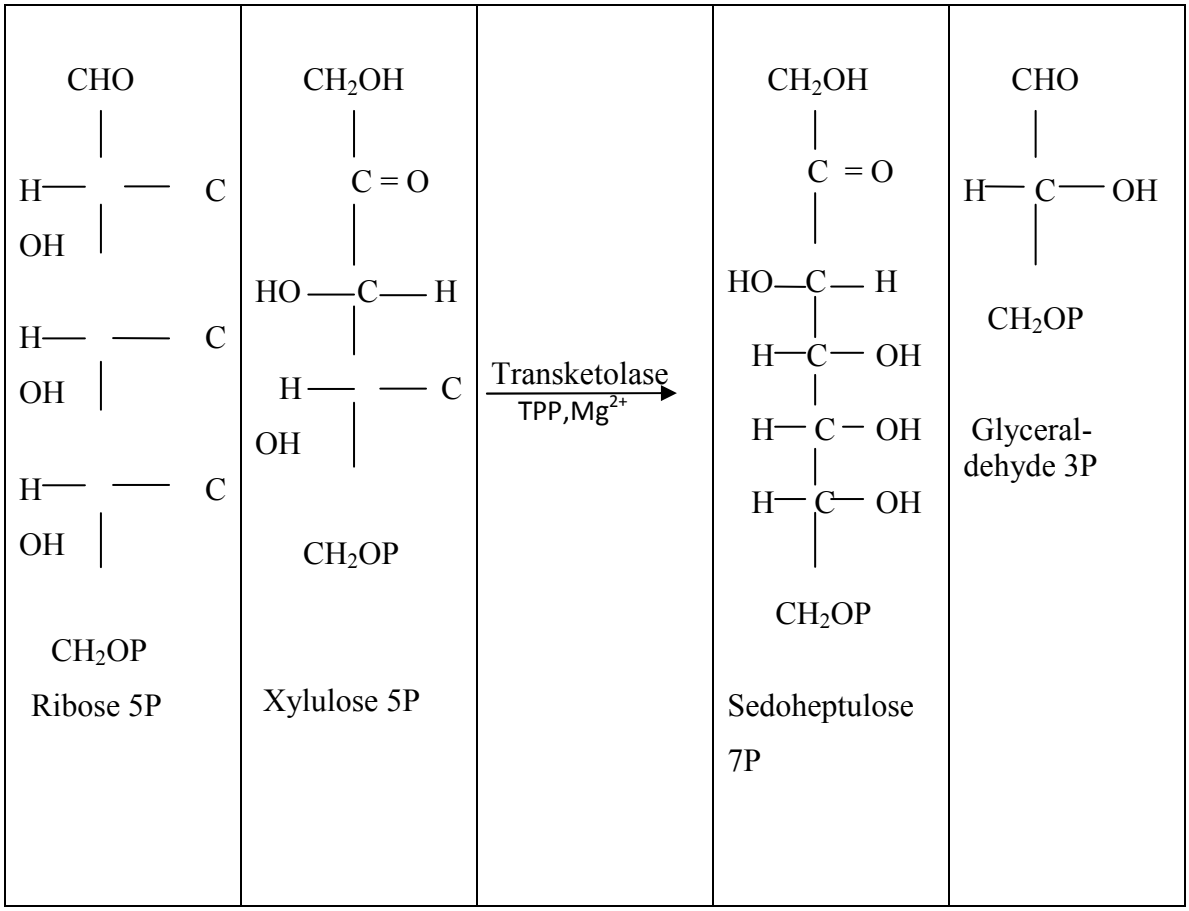
(i) Oxidative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate.

(ii) Transketolase reaction

TPP provides a reactive carbon on the thiazole ring and forms a carbanion stabilized by positively charged ring nitrogen. The carbanion is then free to add the carbonyl of pyruvate (or α-ketoglutarate). The addition compound is then decarboxylated eliminating CO_2 and generating hydroxyethyl-TPP. This reaction occurs

in a multienzyme complex known as pyruvate dehydrogenase complex (or α-ketoglutarate dehydrogenase complex). The acetaldehyde (decarboxylated product) moiety is then transferred to the lipoamide in the complex.

The role of TPP as a coenzyme in the transketolase reaction is very similar to that of oxidative decarboxylation. The carbanion of TPP combines with the carbonyl carbon of xylulose 5P. Carbon 1 and 2 of xylulose 5P are retained to form hydroxyethyl derivative of TPP. Then it is transferred to the carbonyl carbon of ribose 5P to form sedoheptulose 7P.



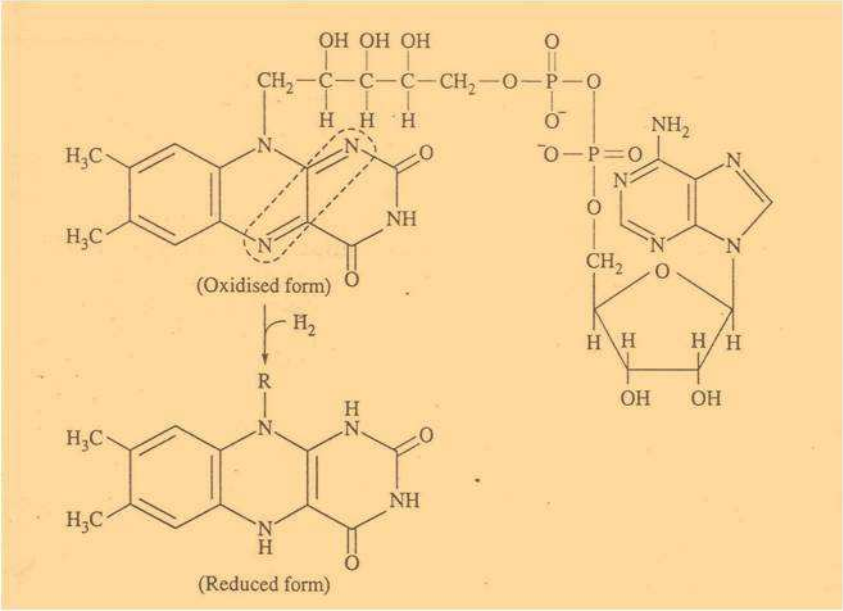
Riboflavin

The flavin monoucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two-coenzyme forms of riboflavin.

FMN and FAD serve as prosthetic groups of oxidation-reduction enzymes known as flavoenzymes or flavoproteins. They are usually tightly, but not covalently, bound to the protein. Many flavoproteins contain one or more metals as additional cofactors and are known as the metalloflavoproteins. In the catalytic cycle of flavoproteins the flavin moiety of the flavin nucleotides undergoes reversible reduction of the isoalloxazine ring to yield the reduced nucleotides $FMNH_2$ and $FADH_2$. L-amino acid oxidase contains tightly bound FMN as the prosthetic group. Succinate dehydrogenase and D-amino acid oxidase contain FAD as prosthetic group.

Niacin

The coenzyme forms of niacin are NAD and $NADP^+$ which function as the coenzymes of a large number of oxidoreductases collectively called as pyridine linked dehydrogenases.



These coenzymes are bound to the dehydrogenase protein relatively loosely during the catalytic cycle and therefore serve as substrate than as prosthetic group. They function as electron acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom from the substrate is transferred as a

hydride ion to the nicotinamide portion of the oxidized forms of these coenzymes. The other hydrogen atom from the substrate becomes a hydrogen ion. Pyridine linked dehydrogenases are specific for either NAD^+ or $NADP^+$, but a few will function with both. Isocitrate dehydrogenase and lactate dehydrogenase are NAD-specific. Glucose 6-P dehydrogenase is NADP specific. Glutamate dehydrogenase functions with NAD^+ or $NADP^+$.

Pyridoxine

The coenzyme form of pyridoxine is known as pyridoxal phosphate (PP).The most common type of reaction requiring PP as a coenzyme is transamination. Enzymes catalysing such reactions are known as transminases of aminotransferases. The coenzyme binds to its apoenzyme via Schiffs base between its aldehyde group and the epsilon

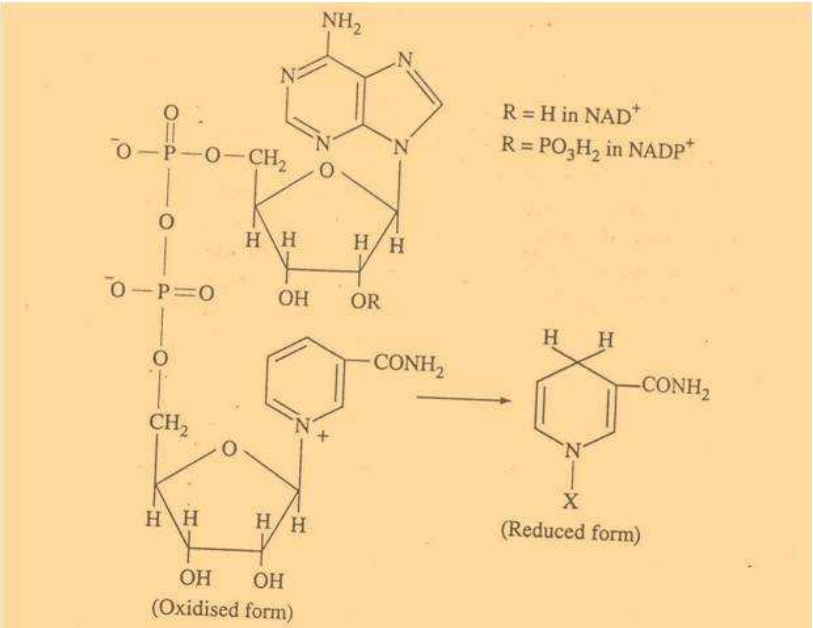
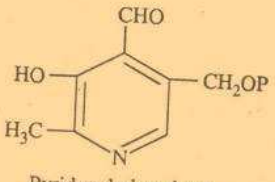


Fig. 8.5. Structure of nicotinamide adenine dinucleotide.

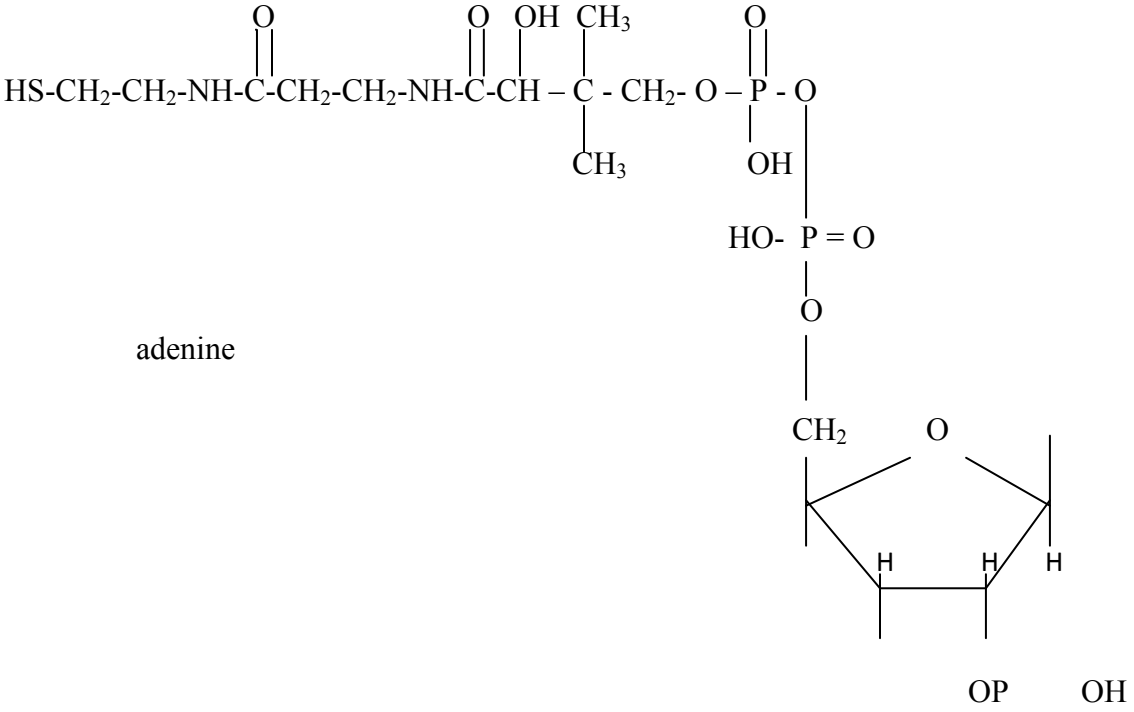


Pyridoxal phosphate

amino group of a lysine in the enzyme. Additional ionic bond is also formed between its phosphate and the enzyme. During reaction,α-amino group of amino acid displaces the epsilon amino group forming a new Schiff's base. By a series of electron shifts and rearrangements, the pyridoxal phosphate becomes pyridoxamine phosphate. The amino acid is oxidatively deaminated to form the corresponding α-keto acid to change it to an amino acid. PP also acts as coenzyme in the decarboxylation, desulfuration, transulfuration reactions associated with amino acid metabolism.

Pantothenic acid

The coenzyme form of pantothenic acid is coenzymes A and is represented as CoASH. The thiol group (-SH) acts as a carrier of acyl group. The acyl-sulphur bond formed between coenzyme A and the acyl moiety is a high energy bond, equivalent to the high energy bond of ATP. The function of coenzyme A is to serve as a carrier of acyl group in reactions associated with fatty acid oxidation, fatty acid synthesis, pyruvate oxidation and biological acetylations. It is also involved in many biosynthetic processes such as synthesis of cholesterol, terpenes and steroids.



Biotin

The important function of biotin is its role as coenzyme for carboxylase, which catalyses carbon dioxide fixation or carboxylation reaction. The epsilon amino group of lysine in carboxylase enzymes combines with the carbozyl group of biotin to form covalently linked biotinyl carboxyl carrier protein (BCCP or biocytin) . This serves as an intermediate carrier of carbon dioxide. The carboxylation of acetyl CoA to malonyl CoA in presence of acetyl CoA carboxylase requires biotin as coenzyme. Propionyl carboxylase and pyruvate carboxylase are also associated with biotin.

Folic acid

The coenzymes form of folic acid is tetrahydrofolic acid . Tetrahydrofolic acid is associated with one carbon metabolism.

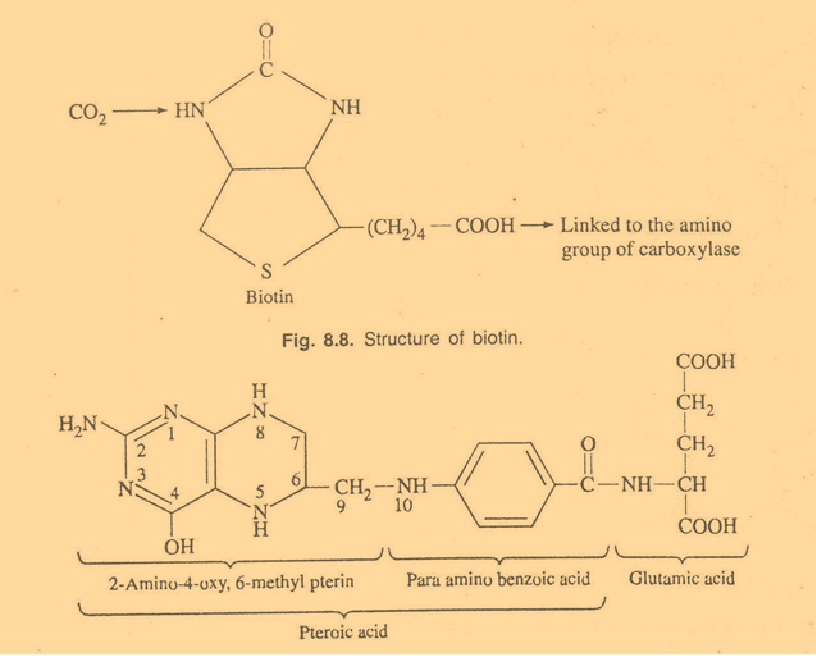
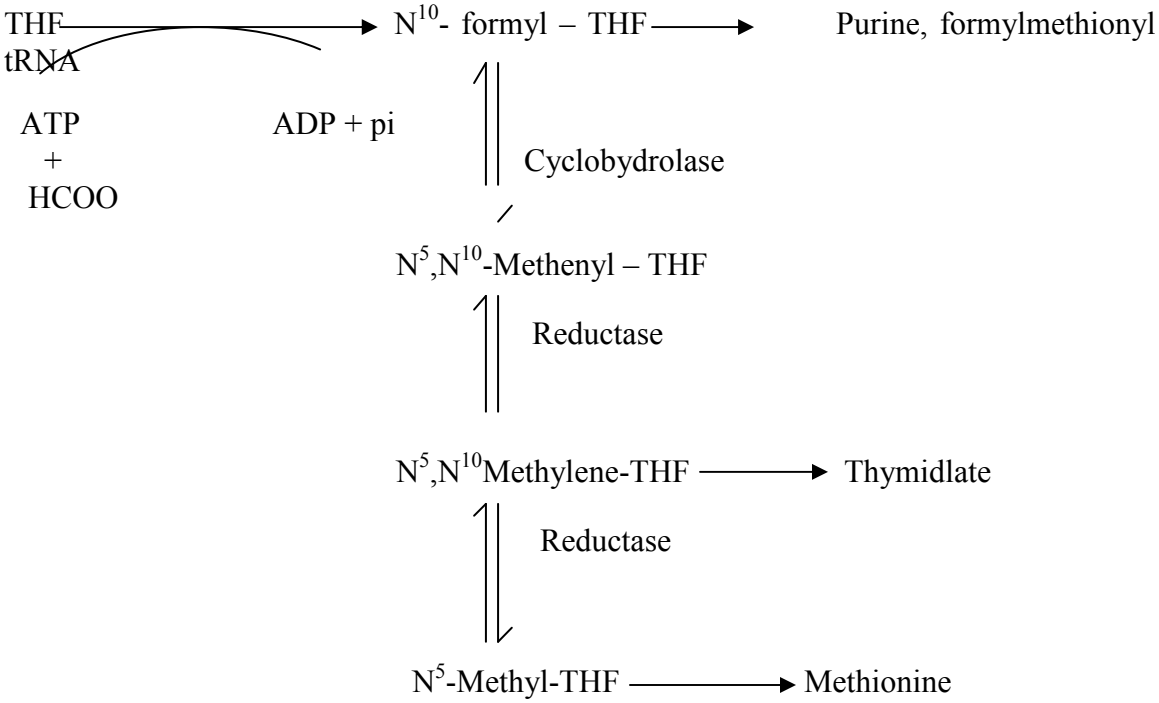


Fig. 8.8. Structure of biotin.

The tetrahydrofolic acid serves as a carrier of single carbon moieties such as formyl, methenyl, formyl or methyl group.

N^{10}-formyl-THF
synthotase

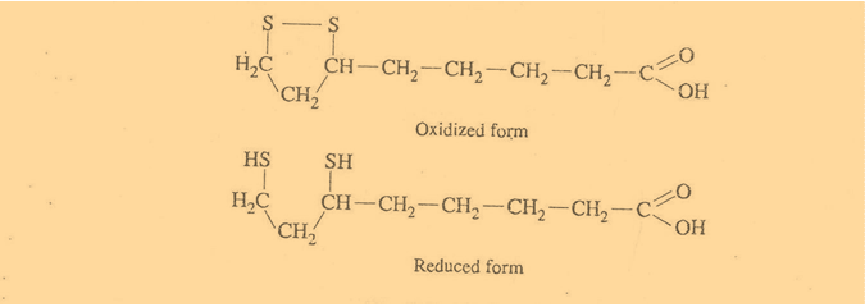


Formation and functions of one carbon derivatives of THF

involved in the biosynthesis of purine, pyrimidines, serine, methionine and glycine.

Lipoic acid

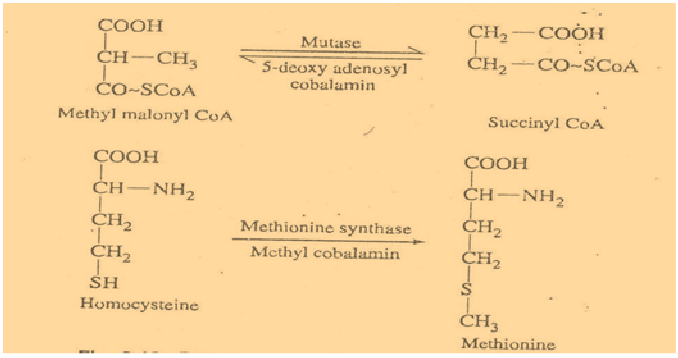
The oxidized and reduced forms of lipoic acid given in figure.Lipoic acid functions as a coenzyme in pyruvate and α - ketoglutarate dehydrogenase multienxyme complexes.



Vitamin B_{12} (cobalamin)

The 5-deoxyzdenosyl cobalamin and cobalamin function as coenzyme forms and are required for the action of several enzymes. Methyl malonyl CoA mutase uses 5-

deoxyadenosyl cobalamin as coenzyme.Methyl cobalamin functions as a carrier of methyl group to homocysteine and convert it to methionine (Figure)



Functions of important coenzymes and their precursors are presented in table

Coenzyme	**Short form (s)**	**Chemical groups transferred**	**Vitamin precursor**
Thiamine pyro-phosphate	TPP	Two carbon aldehydes	Thiamine
Flavin adenine dinucleotide	FAD	Electrons	Riboflavin
Flavin mono nucleotide Nicotinamide adenine dinucleotide	FMN NAD	Hydride ion	Nicotinic acid
Nicotinamide adenine dinucleotide phosphate Coenzyme A Pyridoxal phosphate	NADP CoASH PP	Acyl group Amino group	Pantothenic acid Pyridoxal (Pyridoxamine)
Coenzyme B_{12}	Cobamide	Hydrogen atoms and alkyl groups	Vitamin B_{12}
Biocytin	BCCP	Carbon dioxide	Biotin
Tetrahydrofolate	THF (FH4)	One-carbon groups	Folate
Lipoamide	-	Electrons and acyl groups	Lipoate

Questions

Choose the correct answer

1. ---------- and ---------- are the two coenzyme forms of riboflavin.

a. FMN x FAD b. NAD x NADP c. TPP x NAD d. Biotin x Ascorbic acid

Ans: FMN x FAD

2. Water soluble vitamin include ---------- x ----------

a. A x C b. B complex and Vitamin C c. D x B d. A x D

Ans: B complex and Vitamin

3. Vitamins that acts as antioxidant

a. K b. D c. E d. B

Ans: E

4. TPP functions as coenzyme of transketolase reaction.

Ans: True

5. PLP is involved in dehydrogenation.

Ans: False

6. Biotin acts as coenzyme for carboxylase.

Ans: True

7. Tetrahydro folic acid is involved in biosynthesis of purines and pyrimidines.

Ans: True

8. The active forms of vitamin A are retinol, retinal and retinoic acid. True or False

Ans: False

Lecture.16

Carbohydrate Metabolism - Glycolysis

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.

vi. Glycogenolysis

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.

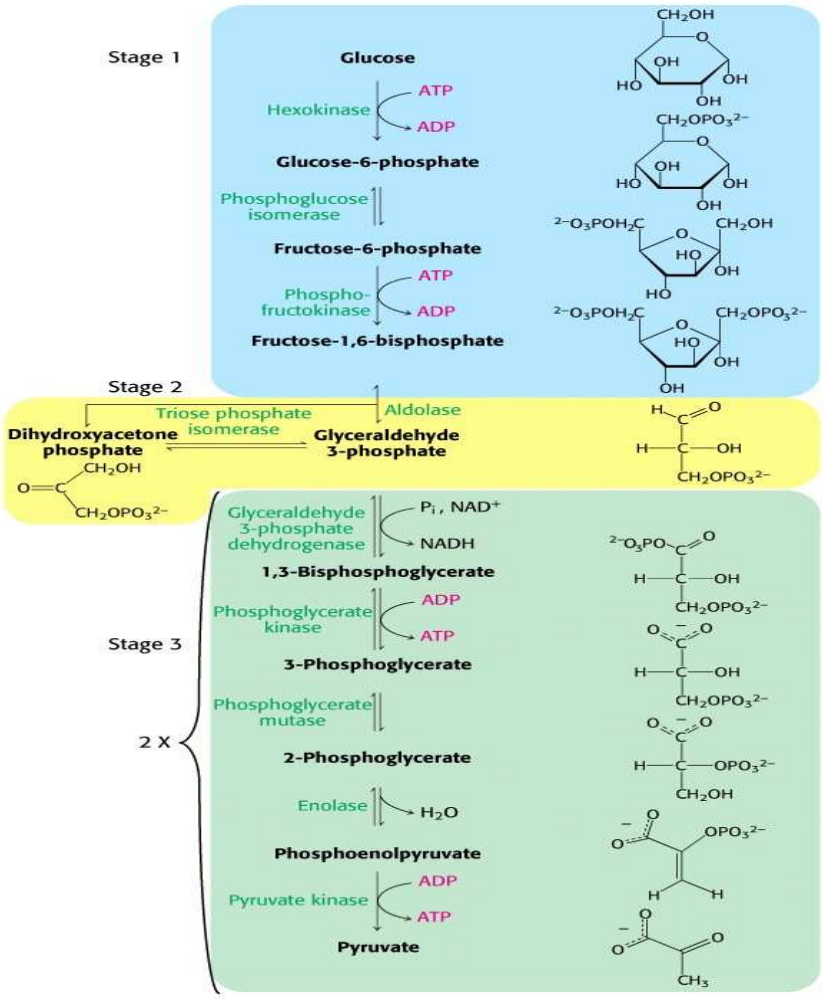
Stage 1: Conversion of glucose to fructose 1,6-bisphosphate

- 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^+ $\longrightarrow$ 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 Formed**
ATP			

Formation of 1,3-bisphospho glycerate from glyceraldehydes 3-phosphate	Glyceraldehyde 3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	**5**
Stage 2 Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate	Phosphoglycerate kinase	Phosphorylation at subtrate level	2
Stage 3 Formation of pyruvate from phosphoenol pyruvate	Pyruvate kinase	Phosphorylation at subrate level	2
Allowance for consumption of ATP by reactions catalysed	Hexokinase and phosphor fructose kinase		2
Number of ATP molecules enerated by catabolism of one molecule of glucose under aerobic conditions			**7**
Number of ATP molecules generated by the catabolism of one molecule of glucose under anaerobic conditions			**2**

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.

Questions

Choose the best answer

1. ---------- is the final common oxidative pathway for carbohydrates, fats and proteins.

a. TCA cycle b. HMP shunt c. Glycolysis d. Glyoxylate pathway

Ans: TCA cycle

2. ---------- is the alternative pathway to glycolysis and TCA cycle

a. Glyoxylate pathway b. HMP c. Gluconeogenesis d. Glycogenesis

Ans: HMP

3. The enzymes of glycolysis occur in

a. Mitochondria b. Golgi c. Cytoplasm d. Nucleus

Ans: Cytoplasam

State True or False

4. The phosphorylation of ADP to ATP is called substrate level phospboxylation.

Ans: True

5. Under anaerobic conditions, pyruvate undergoes oxidation yielding lactic acid.

Ans: False

6. Alcoholic fermentation is the basic of beer and wine making.

Ans: True

Write short notes

7. Brief about enzymes of TCA cycle

8. Substrate level phosphorylation

9. HMP shunts importance.

Lecture.18

TCA cycle. Bioenergetics of glucose

The tricarboxylic acid cycle

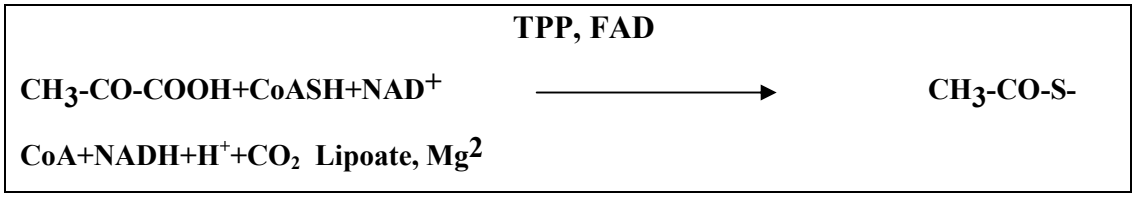
- In 1937, Sir Hans Krebs, an English biochemist proposed a pathway consisting of a cycle of reactions through which acetyl CoA is converted to carbon dioxide and water and hence the cycle was named as Kreb's cycle.
- All the enzymes catalyzing the reactions of this cycle occur inside **mitochondria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

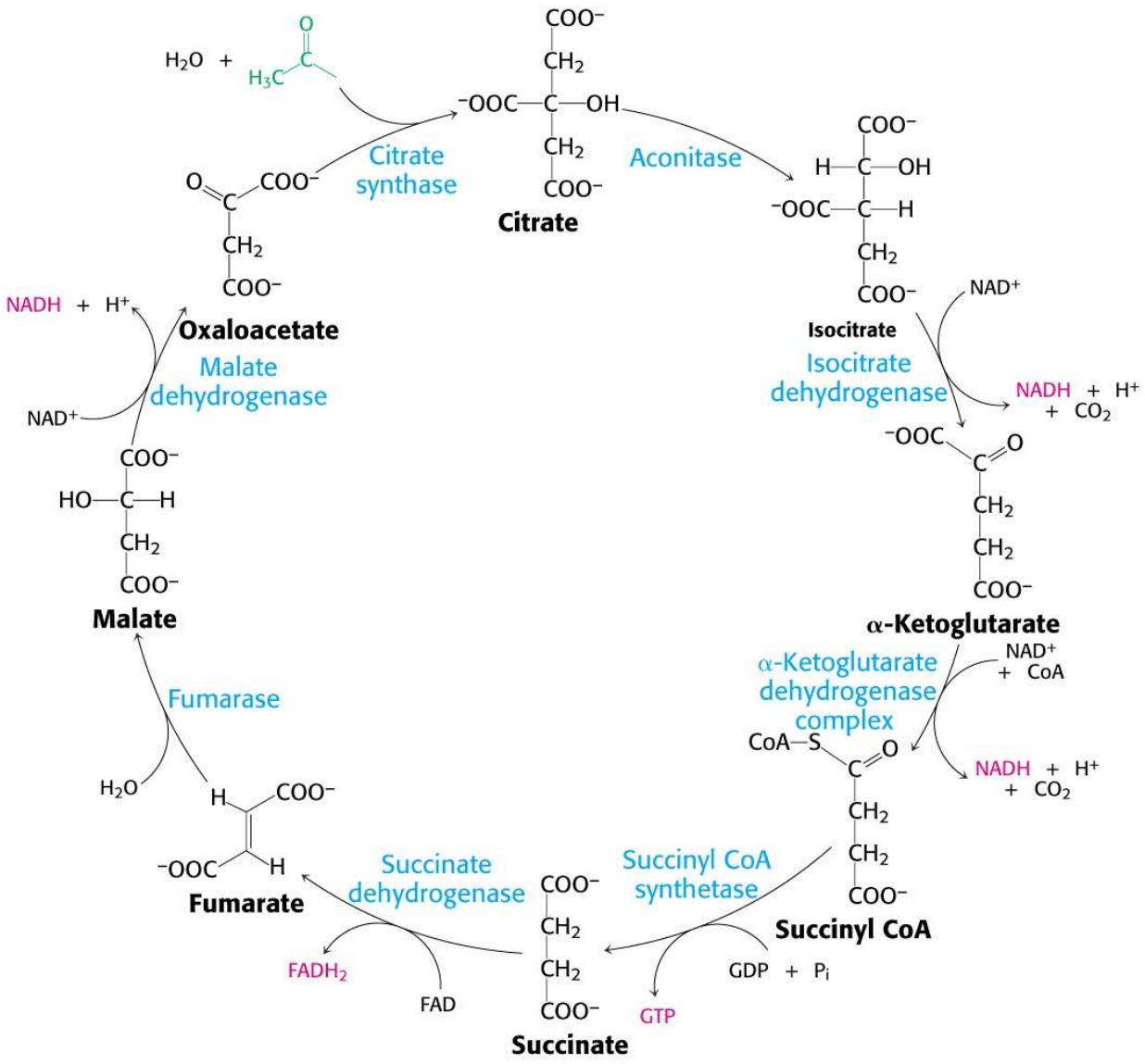
The reaction involves six cofactors: **coenzyme A, NAD^+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg^{2+}.**



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 isocitrate via cis-aconitate

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

iii. Oxidation of isocitrate to α-ketoglutarate

The enzyme, **isocitrate dehydrogenase** oxidatively decarboxylates isocitrate to α-ketoglutarate with simultaneous liberation of carbon dioxide. The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses carbon dioxide to form α-ketoglutarate. 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) is 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.

Glyoxylate cycle

- Plants, especially seedlings, can use acetate as the only source of carbon for all carbon compounds they produce.
- Acetyl CoA, which enters the TCA cycle, is completely oxidised to two molecules of CO_2. Thus it would not be possible for the cycle to produce the massive amounts biosynthetic precursors needed for acetate based growth unless alternative reactions were possible.
- Plants and bacteria employ a **modification of the TCA cycle** called the glyoxylate cycle to produce four **carbon dicarboxylic acids from acetyl CoA**. The glyoxylate cycle bypasses the decarboxylations of the TCA cycle.
- The enzymes of the glyoxylate cycle in plants are present in **glyoxysomes. Isocitrate lyase and malate synthase** are the additional enzymes required for this cycle in addition to TCA cycle enzymes.
- Glyoxysomes do not contain all the enzymes needed for the glyoxylate cycle. The enzymes succinate dehydrogenase, fumarase and malate dehydrogenase are absent.
- Hence glyoxysomes, with the help of mitochondria run their cycle Succinate molecules formed in glyoxysomes are transported to mitochondria where it is converted to oxaloacetate with the help of TCA cycle enzymes. The oxaloacetate is then converted to asparate and transported to glyoxysomes where it is transaminated to oxaloacetate.
- The oxaloacetate is converted to malate through glyoxylate cycle. The malate then enters the cytosol and converted into glucose via gluconeogenesis pathway.

The existence of glyoxylate cycle is important for the **germinating seeds** where photosynthesis is not possible. Triacylglycerols rich in oilseeds are degraded to acetyl CoA. Glyoxysomes formed during germination convert the acetyl CoA to oxaloacetate, which is then utilised for the conversion to glucose through gluconeogenesis. Once the growing seedling begins their photosynthesis to produce carbohydrates, the glyoxysomes disappear.

Questions

Choose the correct answer

1. Conversion of succinyl COA to succinate involves the formation of

a. GTP b. ATP c. CTP d. TTP

Ans: GTP

2. -------- is a strong competitive inhibitor of SDH, which blocks the citric acid cycle.

a. Fumarate b. Malonate c. Oxaloacetate d. Malate

Ans: Malonate

3. The ---------- inhibits the α-keloglutarate dehydrogenates complex

a. Arsenite b. Malonate c. Succinate d. Malate

Ans: Malonate

State True or False

4. The reversible hydration of fumarate to L-malate is catalyzed by fumarase.

Ans: True

5. Arsenite act as an inhibitor of TCA cycle.

Ans: True

6. Isocitrate lyase and fumarase are the additional enzymes required for glyoxylate cycle.

Ans: False

Write short notes

7. Glyoxylate cycle – significance

8. Enzymes of TCA cycle.

Lecture.19

Electron transport chain and oxidative phosphorylation

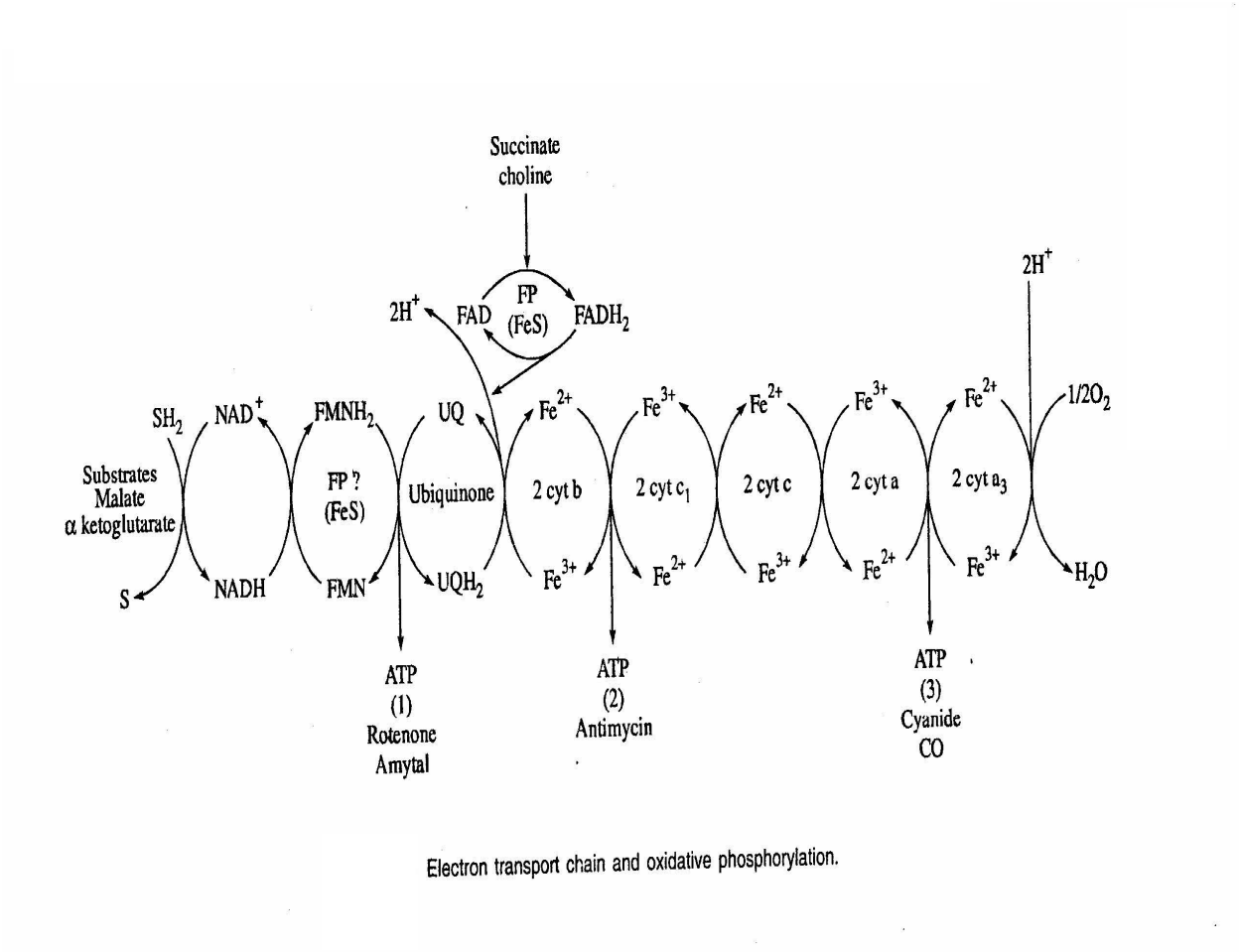
Electron transport chain and oxidative phosphorylation

- 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.



Electron transport chain and oxidative phosphorylation.

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.

Questions

Choose the best answer

1. Oxidation of one molecule of NADH results in generation of ---------- molecules of ATP

a. 2.0 b. 2.5 c. 1.5 d. 1.0

Ans: 2.5

2. ---------- inhibits transfer of reducing equivalents from SDH to ubiquinone

a. Carboxin b. Barbiturates c. Amytal d. Rotenone

Ans: Carboxin

3. ---------- inhibits cytochrome C – oxidase

a. CN b. Azide c. CO d. All the above

Ans: All the above

State True or False

4. Oxidative phosphorylation is synthesis of ATP driven by electron transfer.

Ans: True

5. Oxidation of one molecule of $FADH_2$ generates 3.0 molecules of ATP.

Ans: False

6. According to chemiosmotic theory the coupling of oxidation to phosphoxylation is direct.

Ans: False

Write Short notes

7. List the mechanism of ATP formation

8. Exhibitors of electron transfer

9. Uncouples of oxidative phosphoxylation

Lecture.20

Lipases and Phospholipases, β-Oxidation of fatty acids and energetics of β -Oxidation

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

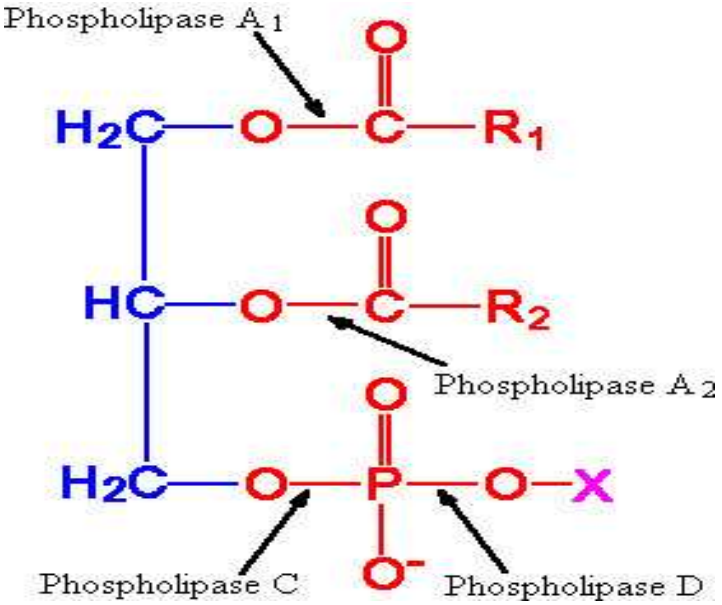
- They initiate digestion by hydrolyzing triacylglycerols to form free fatty acids and 1, 2-diacylglycerols.

- 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 A_1.**
- 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 **glycerophosphorylcholine** 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 phosphorylcholine from phosphatidylcholine.
- Phosphorylcholine is also liberated from sphingomyelin by this enzyme.

Phospholipase D

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

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.**

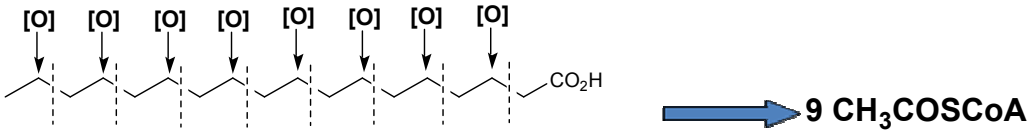
- A **thioester linkage** between the carboxyl group of a fatty acid and the sulfhydryl group of coenzyme A is formed with the hydrolysis of ATP.

- This activation reaction takes place on the **outer mitochondrial membrane** catalysed by **acyl CoA synthetase**.

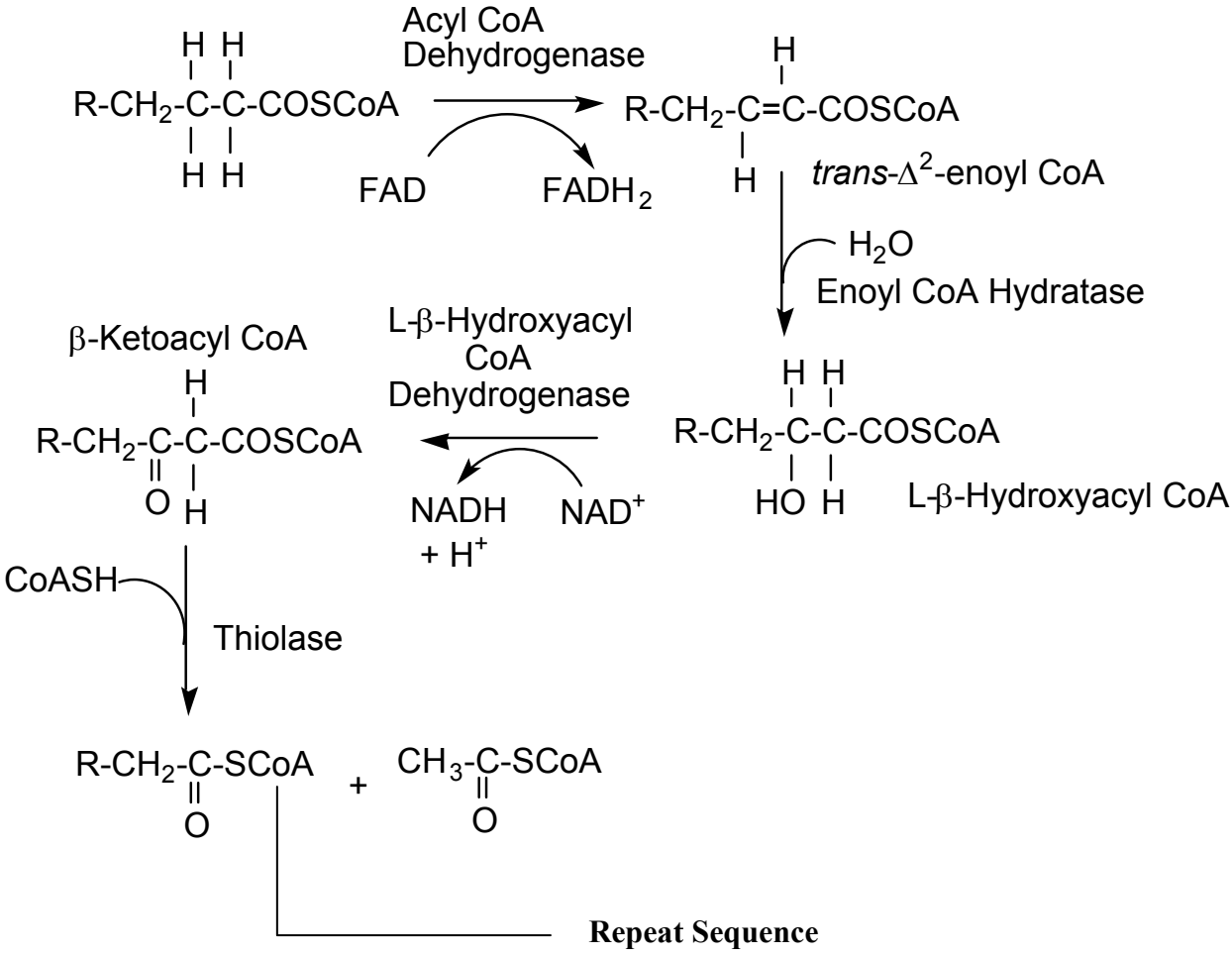
- Several **acyl CoA synthetases** each specific for fatty acids of different chain length are present in the membrane of mitochondria.

Beta Oxidation

- **Cleavage of fatty acids to acetate in tissues**
- **Occurs in mitochondria**

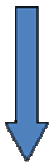


Beta Oxidation Reaction Sequence



Complete Beta Oxidation of Palmitoyl CoA

CH_3CH_2--CH_2CH_2--CH_2CH_2--CH_2CH_2--CH_2CH_2--CH_2CH_2--CH_2CH_2--$CH_2COSCoA$



7 Cycles

$$8\ CH_3COSCoA + 7\ FADH_2 + 7\ NADH + 7\ H^+$$

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

Questions

Choose the correct answer

1. Triglycerides undergo hydrolysis by

a. Protease b. fatty acylase c. Thiolase d. Lipases

Ans: Lipases

2. Phospholipase A is present in large amounts in

a Plants. b. Animal tissues c. Snake venom d. Insect

Ans: Snake venom

3. ---------- catalyzes the hydrolysis of choline

a. Phospholipase A b. Phospholipase B c. Phospholipase C d.Phospholipase D

Ans: Phospholipase D

4. Complete oxidation of palmeticacid yields ----------

a. 108 b. 106 c. 100 d.104

Ans: 106

State True or False

5. The products of lipid catabolism are transported via specialized structures called haustorium.

Ans: True

6. There is an increase in lipase activity following imbibitions in most cases of seeds.

Ans: True

7. Phospholipase B (Az) is otherwise termed as lyso phospbolipase.

Ans: True

Write short notes

8.α-oxidation

9. Phospholipids A

10. Activation of fatty acids

Lecture.21

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.

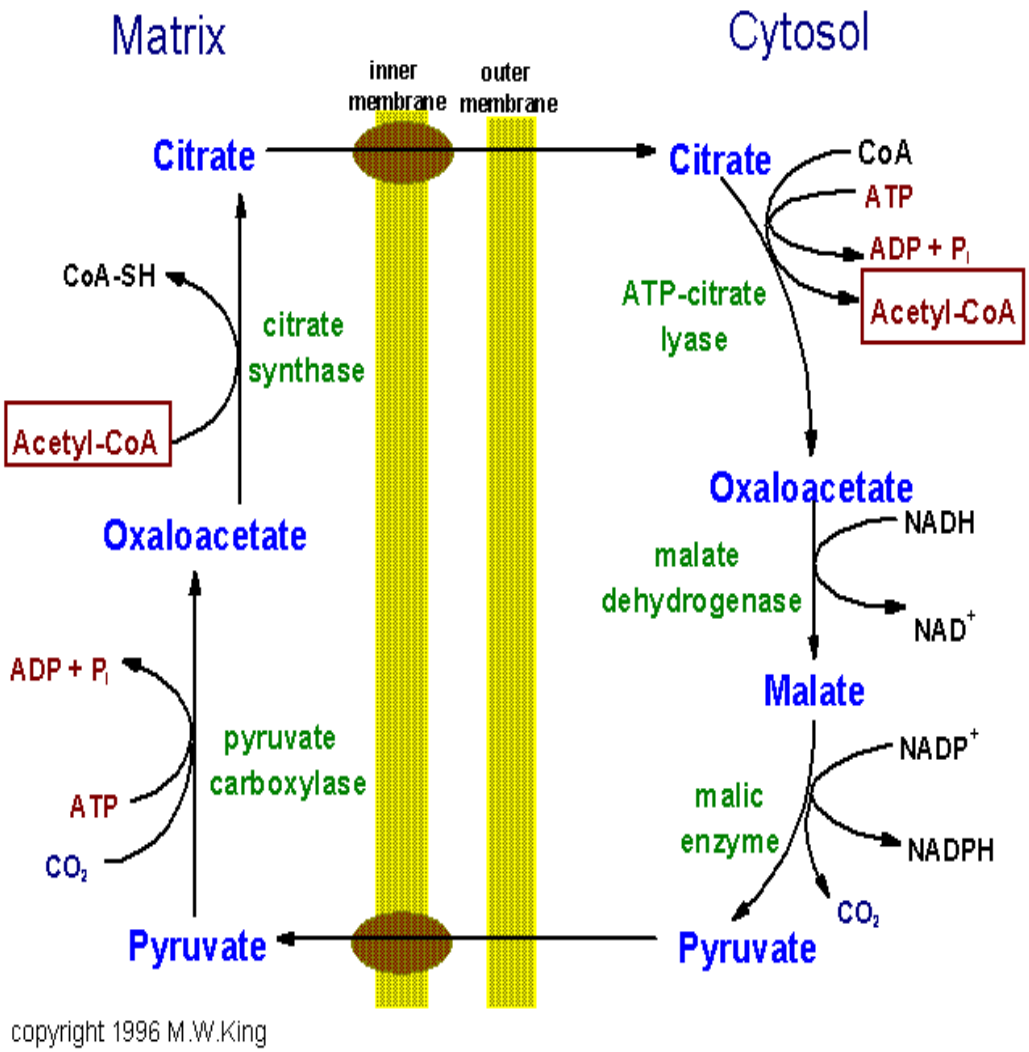
i. Synthesis takes place in the **cytosol,** in contrast with degradation or oxidation, which occurs in the **mitochondrial matrix**.

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.

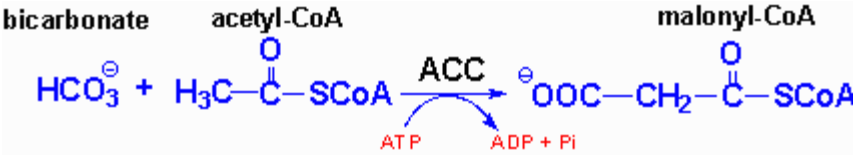
Pathway for the movement of acetyl-CoA units from within the mitochondrion to the cytoplasm for use in lipid and cholesterol biosynthesis.



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

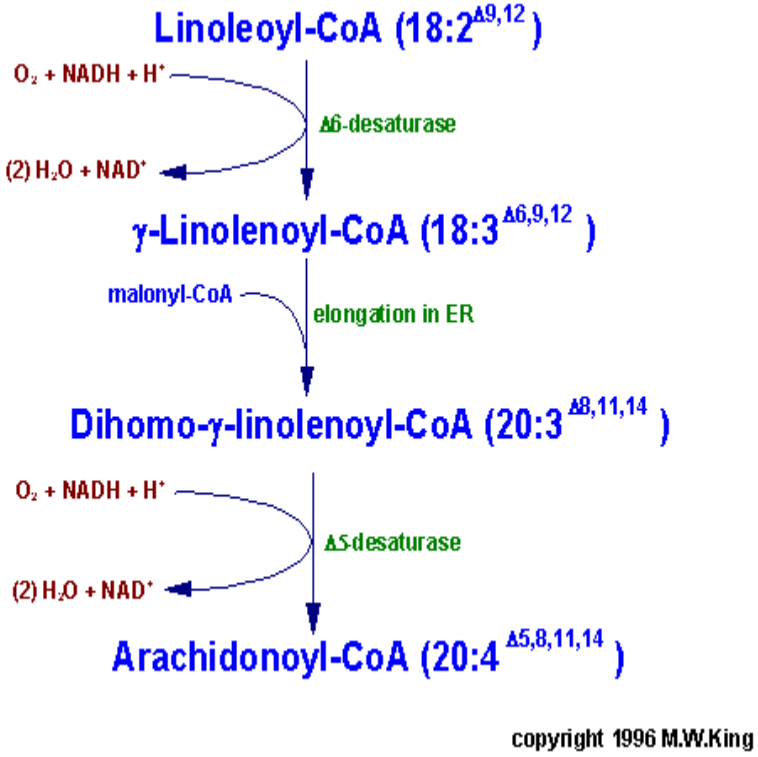
- Elongation by the **fatty acid synthase complex** stops upon formation of palmitate (16 C).

- 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.

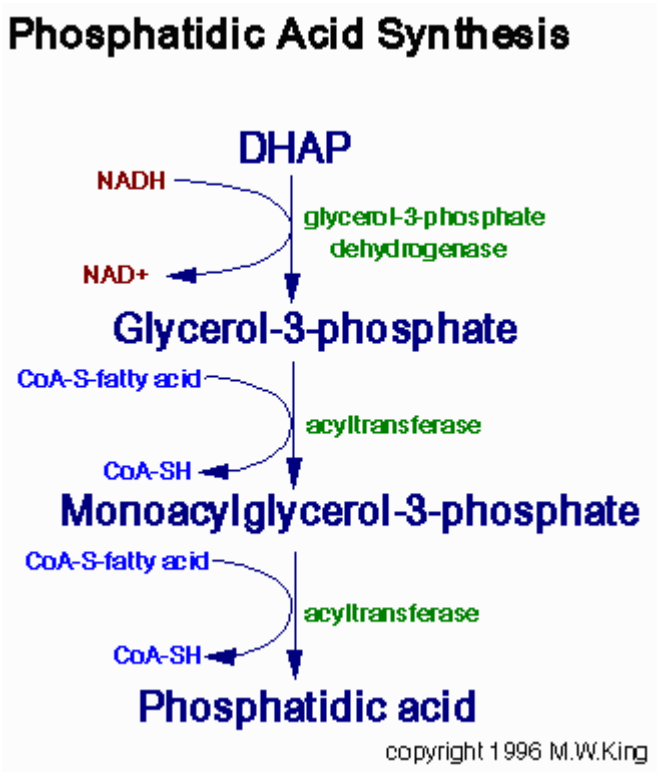
Biosynthesis of unsaturated fatty acids

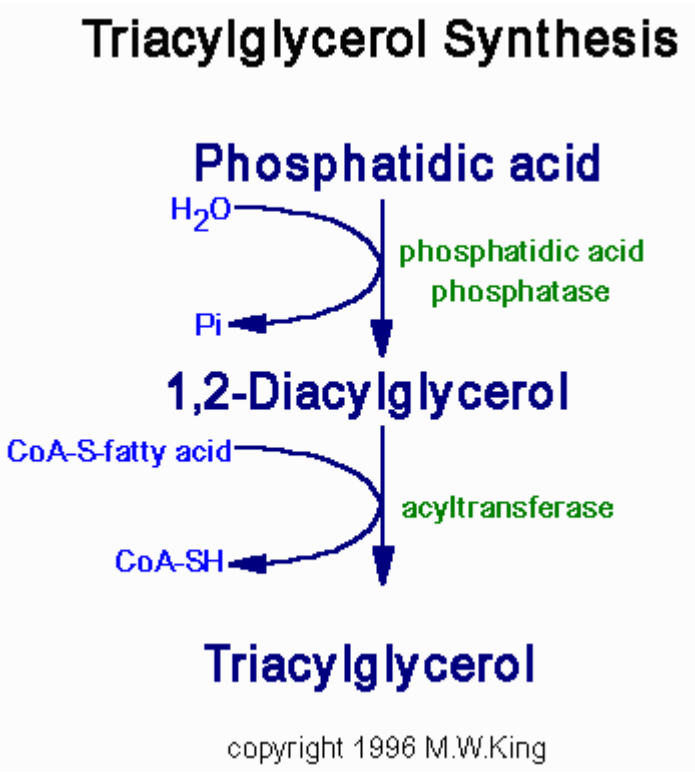
- Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids, **palmitoleate, 16:1, (Δ^9) and oleate, 18:1 (Δ^9)** respectively.
- Each of these fatty acids has a single double bond between C-9 and C-10.
- The **double bond is introduced into the fatty acid chain** 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 synthesise 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.

Questions

Choose the correct answer

1. ---------- is converted to other PUFA like arachidonic acid

   a.Oleate b. Lipoleate c. Stearate d. Palmitate

   Ans: Lipoleate

2. Fatty acids are activated to acyl CoA by the enzyme

   a. Acyl CoA synthetase b.Acyl CoA Dhase c. Glycerol 3 –(p) dehydrogenase d. Lipase

   An: Acyl CoA synthetase

3. ---------- Catalyzes the activation of glycerol to glycerol-3-(p)

   a. Glycerol dehydrogenase b. Glycerolkinase c.Phosphoglycerokinase d.Phosphoglycerate mutase

   Ans: Glycerolkinase

True or False

4. Fatty acid biosynthesis occurs by reversal of β-oxidation pathway.

   Ans: True

5. Fatty acid degradation occurs in cytosol.

   Ans: False

Short notes

6. Biosynthesis of TAG
7. Formation of acyl ACP
8. Fatty acid synthase complex.

Lecture.22

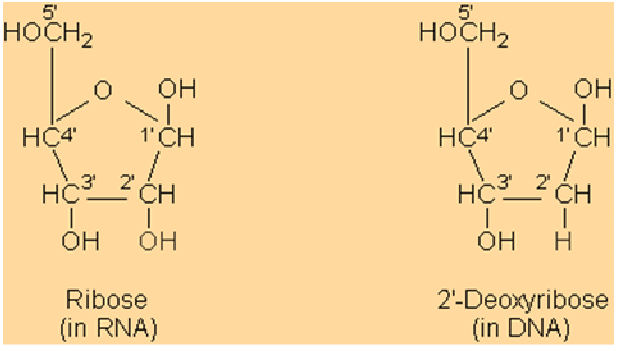
Nucleic acids: Structure and function

Macromolecular Structures: DNA

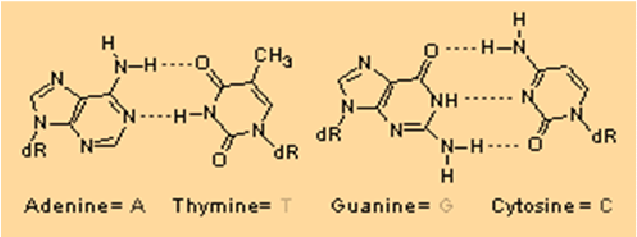
In general, a single cell contains ~ 10^4 – 10^5 different kinds of molecules. Roughly half of these molecules are small, whose molecular weights usually do not exceed several hundred (E.g. inorganic ions and organic compounds). The others are polymers that are so massive (molecular weights from 10^4 – 10^{12} Da) and are called as macromolecules. These molecules are of three classes: proteins, nucleic acids and polysaccharides, which are polymers of amino acids, nucleotides and sugars, respectively. There are also subclasses of these groups. E.g. glycoproteins (proteins carrying sugar groups), lipoproteins (proteins carrying lipids or fats), lipopolysaccharides etc. Knowledge of the properties of macromolecules is essential for understanding living process. In this lecture, the structure of nucleic acid will be studied in details because it is the molecule of live, which store and carry information of live which is the primary function of nucleic acid.

There are two kinds of nucleic acids- ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). A nucleic acid is a polynucleotide i.e. a polymer consisting of nucleotides. Each nucleotide has the three following components (Fig).

1. A cyclic five-carbon sugar: this is ribose, in the case of RNA and deoxyribose, in deoxyribonucleic acid DNA. The structure of ribose and 2'-deoxyribose differ only in the absence of a 2'-OH group in deoxyribose, *a difference that makes DNA chemically more stable than RNA.*



2. A purine or pyrimidine base attached to the 1'-carbon atom of the sugar by an N-glycosidic bond. The purines found in nucleic acids are adenine (A) and guanine (G) and the pyrimidines are cytosine (C), thymine (T) and Uracil (U). DNA and RNA both contain A, G and C. however, T is found only in DNA and U is found only in RNA. *There are exceptions to this rule- T is present in some tRNA molecules and there are few phages who's DNA exclusively contains U rather than T.*



3. A phosphate attached to the 5' carbon of the sugar by a phosphoester linkage. *This phosphate is responsible for the strong negative charge of both nucleotides and nucleic acids.*

A base linked to a sugar at position C1 is called as nucleoside. The sugar C1 carbon atom is joined to the N9 atom of purines and N1 atom of the pyrimidines by *β-N-glycosidic bond*. When a nucleoside linked with phosphate then it is called as nucleotide. The terminology used to describe nucleic acid components is listed in Table 1.

Table 1.Nucleotide nomenclature

Base	Nucleoside*	Nucleotides
Purine		
Adenine (A)	Adenosine (rA)	Adenylic acid or adenosine monophosphate (AMP)
	Deoxyadenosine (dA)	Deoxyadenylic acid or deoxyadenosine monophosphate (dAMP
Guanine (G)	Guanosine$ (rG)	Guanylic acid or guanosine monophosphate (GMP)
	Deoxyguanosine (dG)	Deoxyguanylic acid or deoxyguanosine monophosphate (dGMP)

Pyrimidine		
Cytosine (C)	Cytidine (rC)	Cytidylic acid or cytidine monophosphate (CMP)
	Deoxycytidine (dC)	Deoxycytidylic acid or deoxycytidine monophosphate (dCMP)
Tymine (T)	Thymidine[@] (dT)	Thymidylic acid or thymidine monophosphate (TMP)
Uracil (U)	Uridine[#] (rU)	Uridylic acid or uridine monophosphate (UMP)

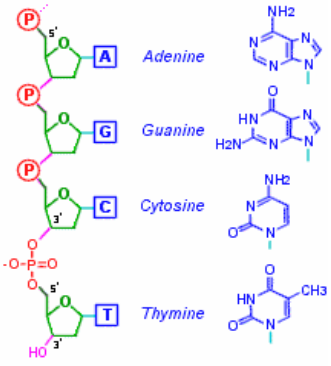
* Note that the names of purine nucleosides end in –osine and the names of pyrimidine nucleosides end in –idine. Note that in shorthand notation, nucleoside and nucleotide derivatives of deoxyribose are distinguished by the prefix'd' and 'r'. Only the second shorthand notation can discriminate between 5' and 3' phosphates, with 5' phosphate residues placed before the base (e.g. pA is adenosine-5'-monophosphate) and 3' phosphates placed after the base (e.g. Ap is adenosine-3'-monophosphate).

[$] Guanosine should not be confused with guanidine, which is not a nucleic acid base.

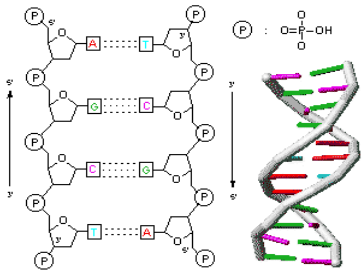
[@] Thymidine is the deoxy-form. The ribo form, ribosylthymine, is not generally found in nucleic acids

[#] Uridine is the ribo-form. Deoxyuridine is not commonly found, although deoxyuridylic acid is on the pathway for synthesis of thymidylic acid.

The nucleotides in the nucleic acids are covalently linked by a second *phosphoester bond* that joins the 5'phosphate of one nucleotide and 3' –OH group of the adjacent nucleotide (Fig). Thus, the phosphate is esterified to both 3' and 5' carbon atoms; this unit is often called a *phosphodiester group*. Thus a polynucleotide chain is formed.



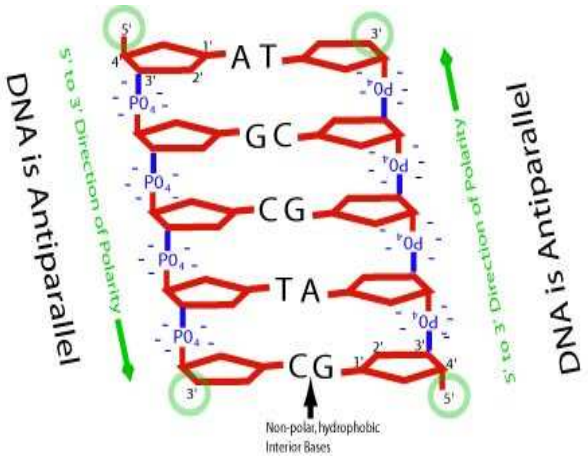
Two polynucleotides interact with one another and produce DNA double helix structure. This double helix structure was first proposed by James Watson and



Francis Crick in 1953 based on the X –ray diffraction studies of M. Wilkins and R. Franklin on DNA fibers (which revealed that the polynucleotide is helical and the bases of the nucleotides are stacked with their planes separated by a spacing of 3.4 A) and Chargraff's rule (the amounts of purine and pyrimidines present in the Organism are equal, i.e. A+T = G+C).

Watson and Crick combined chemical and physical data for DNA with a feature of the X-ray diffraction diagram that suggested that two helical strands are present in DNA and showed that the two strands are coiled about one another to form a double-stranded helix. The sugar phosphate backbones follow a helical path at the outer edge of the molecule and the bases are in a helical array in the central core. The bases of one strand are hydrogen bonded to those of other strand to form the purine-pyrimidine base pairs viz., A: T and G: C. Because each pair contains one two-ringed purine (A or G) and one single ringed pyrimidine (T or C, respectively), the length of each pair (in the sugar to sugar direction) is about the same and the helix can fit into a smooth cylinder.

The two polynucleotide strands of the DNA double helix are antiparellel i.e. the 3'-OH terminus of one strand is adjacent to the 5'-P (5' – phosphate) terminus of the other. The two bases in each base pair lie in the same plane and the plane of each pair is perpendicular to the helix axis. The base pairs are rotated 36° with respect to each adjacent pair, so there are 10 pairs per helical turn (Fig). The helix has two external helical grooves, a deep wide one (the major groove) and a shallow narrow one (the minor groove); both of these grooves are large enough to allow protein molecules to come in contact with the bases. Base pairing is one of the most important features of the DNA structure because it means that the base sequences of the two strands are complementary. In other words, purine in one strand is always pairs with pyrimidine in other strand i.e., if one strand has the base sequence AATGCT, the other strand has the sequence TTACGA, reading in the same direction. Specific pairing is achieved by reciprocal positioning of hydrogen bond acceptors and donors. Three



hydrogen bonds form in G:C base pairs and two in A:T (or A:U) base pairs. This has deep implications for the mechanism of DNAreplication because in this way, the replica of each strand is given the base sequence of its complementary strand. This form of DNA double helix, known as B-form, is prevalent *in vivo*. However, other forms of the helix (such as A-form, Z-form) with distinct structures also exist (Table 2). The A-form of DNA (which is prevalent *in vitro*) is less soluble than the B-form. That is why DNA, which is over dried during plasmid preparation, for example, is difficult to dissolve.

Table 2. Comparison of morphological features and helical parameters of the three major types of DNA helix.

Morphological Characteristics	Conformation		
	A	B	Z
Helical sense	Right	Right	Left
Pitch (base pairs per turn)	11	10	12
Major groove	Deep, narrow	Wide	Flat
Minor groove	Broad, shallow	Narrow	Narrow and very deep
Helix diameter	2.3 mm	1.9 mm	1.8 mm

Questions

1. The sugar residues are covalently joined by

a) 5' -> 3' phosphodiester bonds **b)** H_2 bonds **c)** phosphorylated ions **d)** covalent bonds

Ans: 5' -> 3' phosphodiester bonds

2. The bases in nucleic acids govern which one of the following process that underpins the essential biological process?

a) Replication **b)** recombination **c)** gene expression **d)** all the above

Ans: all the above

3. Nucleotide is made up of

a) Sugar **b)** phosphate **c)** nitrogenous base **d)** all the above

Ans: all the above

4. Which one of the following base is present both in DNA and RNA?

a) Adenine **b)** guanine **c)** cytosine **d)** all the above

Ans: all the above

5. The diameter of the double helix is

a) 20 Å **b)** 34 Å **c)** 3.4Å **d)** varies from one point to another

Ans: 20 Å

6. The number of bases in A form DNA per turn of the helix is

a) 20 **b)** 10 **c)** 34 **d)** 3.4

Ans: 10

7. Which one of the following phosphate moiety involved in 5' ->3' phosphodiester bond formation?

a) α **b)** β **c)** γ **d)** all the above

Ans: α

8. DNA or RNA polymerases are catalyze the 5' -> 3' phosphodiester bond formation and the byproduct of this reaction is

a) Pyrophosphate **b)** pyrophosphatase **c)** pyrosequences **d)** pyrates

Ans: pyrophosphate

9. Chargaff's rules state that

a) A=T **b)** G=C **c)** complementarity base paring **d)** all the above

Ans: all the above

10. The most stable nitrogenous bases in double helix DNA is

a) G: C **b)** A: T **c)** A: C **d)** T: G

Ans: G: C

11. Because of base interactions, the outside of the helix is not smooth and it is termed as

a) Major groove **b)** minor groove **c)** both a and b **d)** none of the above

Ans: both a and b

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Lecture.23

DNA Replication

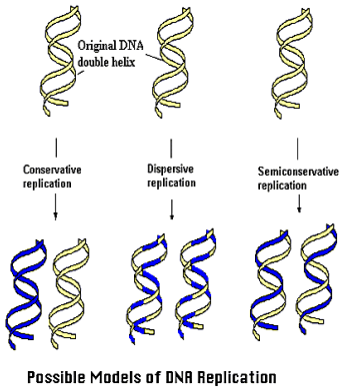
Genetic information is transferred from parent to progeny organisms by a faithful replication of the parental DNA molecules. At the biochemical level, replication is defined as a template-directed nucleic acid synthesis reaction where the template and nascent (growing) strand are the same type of nucleic acid. Replication is a polymerization reaction and can be divided into stages of initiation, elongation and termination.

Replication of dsDNA is a complicated process that is not completely understood due to the following facts:

1. A supply of energy is required to unwind the helix
2. The single strands resulting from the unwinding tend to form intrastrand base pairs
3. A single enzyme can catalyze only a limited number of physical and chemical reactions and many reactions are needed in replication.
4. Several safeguards have evolved that are designed both to prevent replication errors and to eliminate the rare errors that do occur
5. Both circularity and the enormous size of the DNA molecules impose geometric constraints on the replicative system and how this fit into the system has to be understood.

Models for the replication of DNA

The replication of cellular DNA was originally conceived as two models: conservative and dispersive. In conservative model, the parental DNA remains unchanged and gets passed to one daughter cell, whereas newly synthesized DNA gets passed to the other. But in dispersive replication, new DNA synthesis is interstitial (small openings), and each daughter cell receives a mixture of parental and newly synthesized DNA.



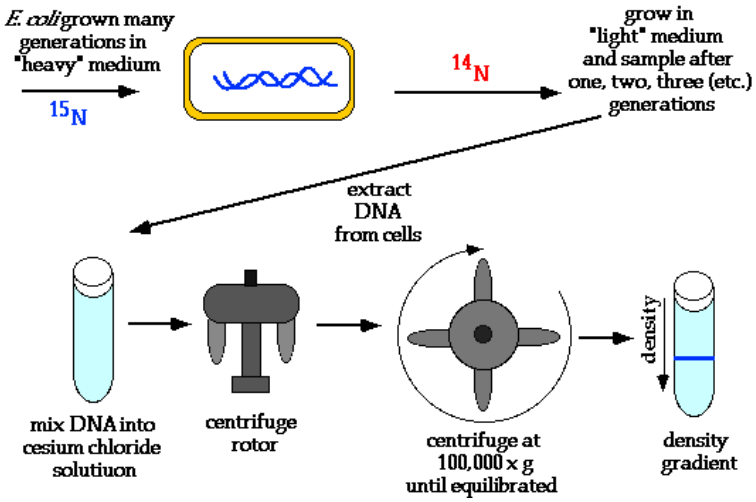
Possible Models of DNA Replication

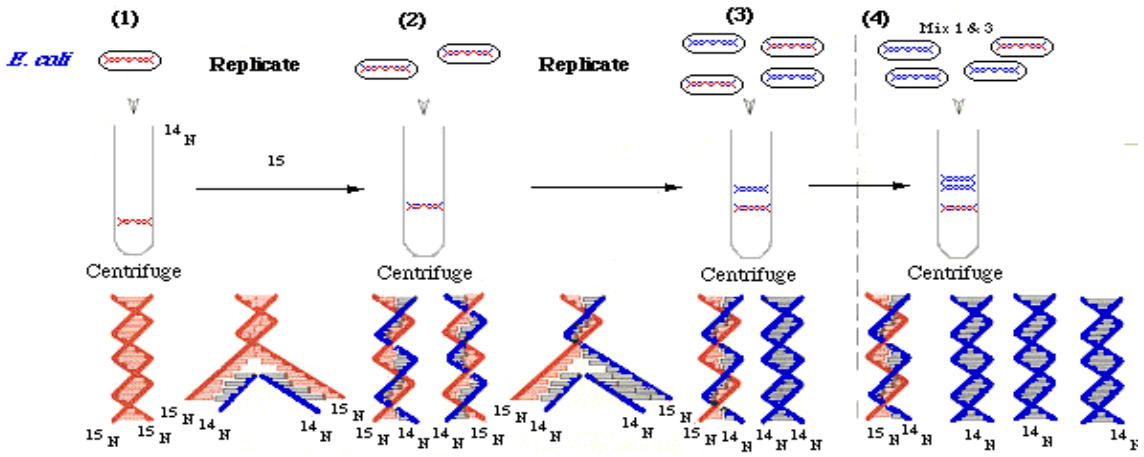
In a third, semi conservative model, proposed by Watson and Crick, the parental strands remain unchanged, but the duplex is separated into two halves. Each parental strand acts as a template for replication and the daughter duplexes have one parental strand and one daughter strand each.

The semi conservative model holds good for cellular DNA, but the single stranded genomes of viruses and some plasmids replicate conservatively - the structure of the single parental strand is conserved following replication.

Meselson-Stahl Experiment: Proof for Semiconservative DNA replication

In 1958, Mathew Meselson and Franklin Stahl showed that the replication of bacterial chromosomal DNA was semi conservative. *E. coli* were grown for many generations in a medium containing ^{15}N so that, their DNA became universally labeled with the isotope (heavy DNA). The cells were then shifted to a medium containing normal ^{14}N and DNA was isolated from cells after one and two rounds of replication. The DNA was analyzed by buoyant density centrifugation, which discriminates between heavy DNA and normal light DNA and intermediate DNA containing one heavy strand and one light strand. After one round of replication, the DNA was all of intermediate density and after two, there were equal amounts of intermediate density and light DNA (Fig). Thus, this experiment proved that cellular genomes replicate semi conservatively.





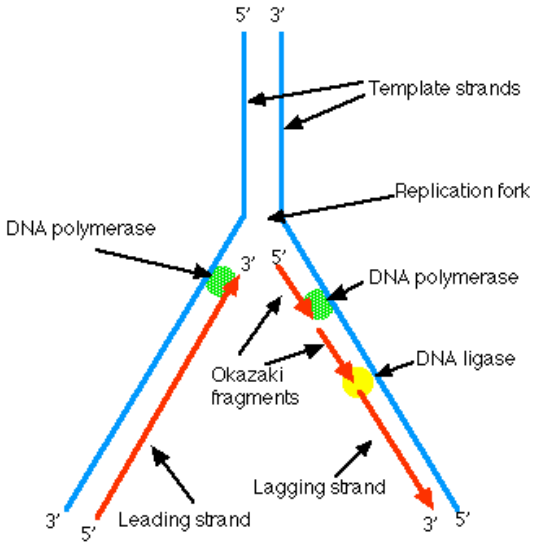
Semidiscontinuous replication

Watson and Crick's semi conservative model of DNA replication predicted the existence of a replication fork, a dynamic Y-shaped structure with a barrel composed of parental duplex DNA and arms composed of daughter duplex DNA, each daughter duplex consisting of one parental and one daughter strand (Fig). At the center of the fork, the parental duplex would be unwound and nucleotides would be added to the growing daughter strands. However, this model has a paradox, which can be summarized as follows:

1. cellular DNA replication is semi conservative
2. both daughter strands are extended simultaneously
3. the strands of the parental duplex are antiparellel
4. DNA polymerases extend DNA only in the 5' → 3' direction.

How can simultaneous 5'→ 3' elongation of both daughter strands occur at a replication fork when the parental templates have opposite polarity? This can be achieved by semidiscontinuous replication, where one strand is extended continuously and the other is synthesized discontinuously as a collection of short fragments. The mechanism of semidiscontinuous DNA replication can be formally expressed as leading strand – lagging strand model (Figure). The leading strand is the nascent strand, which is synthesized continuously in the direction of fork movement because its 3' end is exposed

to the DNA polymerase. The leading strand template is thus the *forward template*. The lagging strand is the nascent strand, which is synthesized discontinuously in the opposite direction to the fork movement because its 5' end, the end.

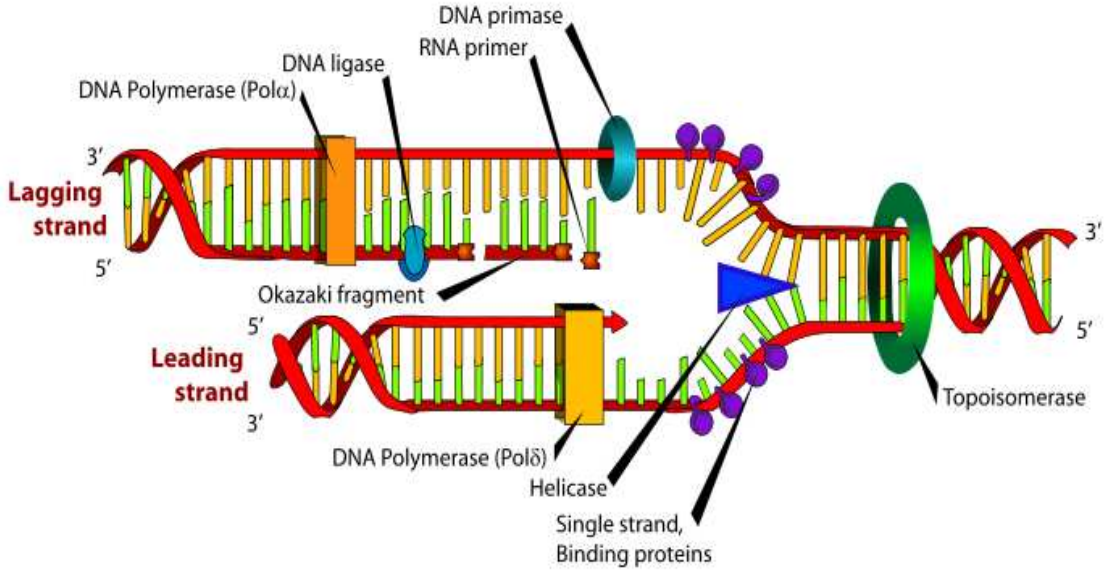


Leading strand – lagging strand model cannot be extended, is exposed to the DNA polymerase. The lagging strand is thus the *retrograde template.* The mechanism can be summarized as follows: as the replication fork moves forward and the leading strand is extended, a portion of retrograde template is exposed. DNA polymerase can then synthesize a small fragment of DNA, an *Okazaki fragment*, by moving backwards over the template in relation to the fork progression. The lagging strand is so called because the leading strand must be synthesized first to uncover the corresponding portion of lagging strand template. The enzyme dissociates from the template when it reaches the previously synthesized Okazaki fragment, by which time a further portion of retrograde template has been exposed. The enzyme can then reinitiate and synthesize a new Okazaki fragment. By repeating this back-stitching process over and over, the lagging strand would appear to grow in the 3'→5' direction.

Mechanism of DNA replication

Initiation

Most organisms contain one or more enzymes called topoisomerases, which can produce variety of topological changes in DNA. The brief outline of replication mechanism in *E. coli* is described here. An enzyme called as helicase binds with single strand binding (SSB) protein along with DnaB protein and unwinds the helix. The unpaired bases are coated with SSB. DNA gyrase (Eco topoisomerase II) has the ability to produce negative superhelicity generated during replication. That is, positive



superhelicity is removed by gyrase introducing negative twists by binding ahead of the advancing replication fork.

Elongation

The leading strand advances along one parental strand by nucleotide addition catalyzed by the pol III holoenzyme (DNA polymerase III). The term holoenzyme refers to an enzyme that contains several different subunits and retains some activity even when one or more subunits are missing. In *E. coli*, two types of DNA polymerases exist viz., pol I and pol III. They are able to synthesize DNA from four precursor molecules, four-deoxynucleoside 5'-triphosphates (dNTPs viz., dATP, dGTP, dTTP and dCTP), as long as a DNA molecule to be copied (template DNA) is provided. Neither 5'monophosphates nor 5' diphosphates, nor 3'-mono, di, tri-phosphates can be polymerased; only the

5'triphosphates are substrate for the polymerization reaction. In addition they require nucleic acid fragment to initiate the polymerization. The overall chemical reaction catalyzed by the DNA polymerase is:

Poly (nucleotide)$_n$-3'-OH + dNTP $\Leftrightarrow$ Poly (nucleotide)$_{n+1}$-3'-OH + PP$_i$

The polymerase also catalyzes depolymerization. In order to drive the reaction to the right, pyrophosphate must be removed, and this is done by a potent pyrophosphatase, a widely distributed enzyme that breaks down pyrophosphate to inorganic phosphate.

In addition, polymerization occurs only is the presence of primer- that is an oligonucleotide hydrogen bonded to the template strand and whose terminal 3'-OH group is available for reaction. Because polymerization consists of a reaction between a 3'OH group at the end of the growing strand and an incoming nucleoside-5' triphosphate. When the nucleotide is added it supplies another free 3'-OH group.

The primer for both leading and lagging strand synthesis is a short RNA oligonucleotide that consists of 1 to 60 bases; the exact number depends on the particular organism. This RNA primer is synthesized by copying a particular base sequence from one DNA strand and differs from a typical RNA molecule, in that after its synthesis the primer remains hydrogen bonded to the DNA template. In bacteria two different enzymes are known that synthesize primer RNA molecules – RNA polymerase and Primase. The DnaB protein complex moves along the other parental strand, prepriming it so that primase will synthesis a primer RNA. Pol III holoenzyme adds nucleotides to the primer, thereby synthesizing a precursor fragment. This synthesis continues up to the primer of the preceding precursor fragment.

Apart from this function, DNA polymerases also has 3'$\rightarrow$5' exonuclease, 5'$\rightarrow$3' exonuclease and endonuclease activity and so they can perform nick translation and strand displacement. By nick translation the RNA is removed and replaced by DNA. Once the RNA is gone, DNA ligase seals the nick, thereby joining the precursor fragment to the lagging strand. Pol II moves back along the DNA (in the direction of advancement of the fork) until it encounters the next primer and the process continue again and again.

Since each strand has 5'-P terminus and 3'-OH terminus, strand growth is said to proceed in the 5'→3' direction (Fig). The advance of the replication fork continues until replication is completed. An unsolved question is how the rates of growth of the leading and lagging strands are coordinated.

Termination

In a unidirectionally replicating molecule, replication terminates at the origin. In bidirectionally replicating molecule, it may be of two types: 1. there might be definite termination sequence. 2. Two growing points collide and termination occurs where ever the collision point happens to be.

Replication in Eukaryotes

The complete mechanism of initiation, elongation and termination of linear DNA molecule and chromatin replication has not yet been elucidated. However, it is believed that there might be multiple replication forks exist during replication. Similarly different isoforms of DNA polymerases have been identified in eukaryotes with specific functions.

Fidelity of DNA replication

There is no single molecule whose integrity is as vital to the cell as DNA. Thus, in the course of hundreds of millions of years there have evolved efficient systems for correcting the occasional mistakes that occur during replication. DNA repair/damage can occur as the result of exposure to environmental stimuli such as alkylating chemicals or ultraviolet or radioactive irradiation and free radicals generated spontaneously in the oxidizing environment of the cell. These phenomena can, and do, lead to the introduction of mutations in the coding capacity of the DNA. Mutations in DNA can also, but rarely, arise from the spontaneous tautomerization of the bases (the rare imino form of adenine can form a stable hydrogen bond with cytosine and the enol form of thymine can pair with guanine).

E. coli cells possess at least five distinct mechanisms for the repair of defects in DNA: 1) light-dependent repair or photoreactivation, 2) excision repair, 3) mismatch

repair, 4) post-replication repair and 5) error-prone repair. Mammals seem to possess all of the repair mechanisms found in *E. coli* except photoreactivation.

Questions

1. Who has shown that replication is semi-conservative event?

a) Mathew Meselson and Franklin Stahl b) Watson and Crick

c) Delbruck and Korenberg d) none of the above

Ans: Mathew Meselson and Franklin Stahl

2. The enzyme which unwinds DNA ahead of replication fork is

a) DNA helicase b) DNA ligase c) DNA polymerase d) DNA primase

Ans: DNA helicase

3. *Taq* DNA polymerase, widely used in PCR, is homologous to *E. coli* pol I but lacks

a) 3'→5' exonuclease activity b) 5'→3' exonuclease activity

c) 5'→3' polymerase activity d) 3'→5' polymerase activity.

Ans: 3'→5' exonuclease activity

4. During replication, the functional complex primosome is formed by

a) primase b) helicase c) both a and b d) α –polymerase.

Ans: both a and b

5. Enzymes which digest nucleic acid by hydrolyzing phosphodiester bonds are called as

a) nucleases b) deoxyribo nucleases (if substrate is DNA)

c) ribonucleases (if substrate is RNA) d) all the above

Ans: all the above

6. Which of the following is true?

a) DNA polymerase recognizes 'ori' in parental DNA and unwinds the double helix at these sites so that DNA replication can occur.

b) Helicases recognize replication fork and unwinds the double helix at replication fork so that DNA replication can occur

c) Helicases recognize origin of replication in parental DNA and unwinds the double helix at these sites so that DNA replication can occur

d) None of the above

Ans: Helicases recognize origin of replication in parental DNA and unwinds the double helix at these sites so that DNA replication can occur

7. If the parental DNA strand, 3' GGCATATTCGCTGCAGT 5', is used as a template DNA strand the newly synthesized, antiparallel strand would be as follows

8. The sequence of one strand of DNA is 5'-AGTCGACGA-3'. What would be the 5' to 3' sequence of the complementary strand?

a) 5' – TCAGCTGCT – 3' b) 5' – TCGTCGACT – 3'

c) 5' – AGTCGACGA - 3' d) too little information to predict

Ans: 5' – TCGTCGACT – 3'

9. In Eukaryotes, the DNA replication occurs during

a) S phase b) G1 phase c) G2 phase d) all phases

Ans: S phase

10. DNA ligase

a) Unwinds the helical DNA by breaking the hydrogen bonds between complementary bases

b) Adds DNA nucleotides to the RNA primer

c) Links the DNA fragments of the lagging strand together

d) Synthesizes a short RNA primer at the beginning of each origin of replication

Ans: Links the DNA fragments of the lagging strand together

11. The 5' end of the DNA is the one with

a) the terminal phosphate group on the 5' carbon of the deoxyribose

b) a terminal hydroxyl (OH) group on the deoxyribose of the 3' carbon of the deoxyribose

c) a terminal hydroxyl (OH) group on the deoxyribose of the 5' carbon of the deoxyribose

d) None of the above

Ans: the terminal phosphate group on the 5' carbon of the deoxyribose

12. _____ is one correct reason for why Okazaki fragments are created during lagging strand DNA synthesis

a) DNA is only polymerized in the 3' to 5' direction.

b) DNA polymerase requires RNA priming

c) DNA is only polymerized in the 5' to 3' direction

d) DNA polymerase requires a 5' -0H on the growing polymer

Ans: DNA is only polymerized in the 5' to 3' direction

13.At the completion of DNA replication, each newly synthesized DNA strand is

a) Identical in sequence to the strand opposite which it was synthesized
b) Complementary in sequence to the strand opposite which it was synthesized
c) A hybrid stand consisting both DNA and RNA strand
d) Oriented in the same direction as the strand which it was synthesized

Ans: Complementary in sequence to the strand opposite which it was synthesized

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Lecture.24
Transcription

The transfer of genetic information from DNA to RNA molecules and then from RNA to protein molecules accomplishes Gene expression. RNA molecules are synthesized by using a portion of one strand of DNA as a template in a polymerization reaction that is catalyzed by enzymes called RNA polymerases. The process by which RNA molecules are initiated, elongated and terminated is called transcription. Two aspects of transcription must be considered – 1. The enzymology and 2. The signals that determine where on a DNA molecule transcription begins and stops.

Gene

The term gene was coined by Wilhelm Johansen in 1909 to describe a heritable factor responsible for the transmission and expression of a given biological character. In 1911, T. H. Morgan showed that genes were located on chromosomes and were physically linked together and in 1944, O. Avery and his colleagues shown that DNA was the genetic material. Thus, a simple picture of a gene evolved - a length of DNA in a chromosome, which encoded the information for a protein. At any given locus, the DNA which is transcribed can be termed a transcription unit. In prokaryotes, a transcription unit may consists of several genes (constituting an operon) whereas in eukaryotes, transcription units are almost always equivalent to a single gene.

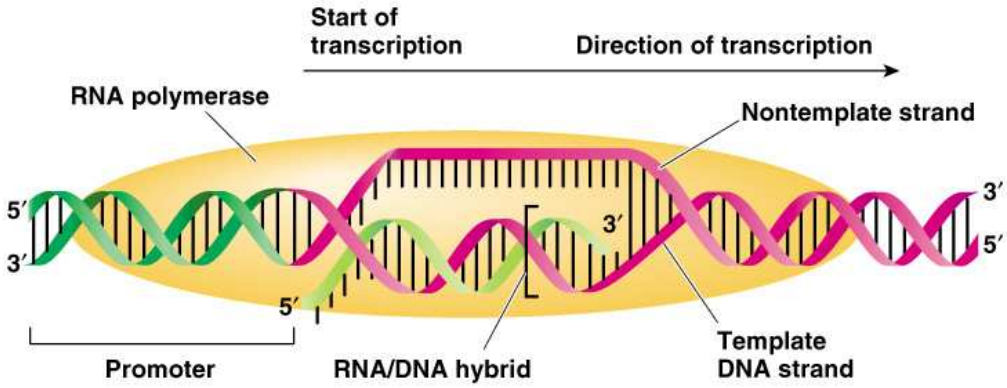
In short, Gene (Cistron) is the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer sequence) as well as intervening sequences (introns) between individual coding segments (exons).

Basic features of RNA synthesis

Thus, a gene, which code the genetic information first transcribed as RNA. The essential chemical characteristics of the synthesis of RNA are following:

1. The precursors in the synthesis of RNA are the four ribonucleoside 5' – triphosphates (NTP) ATP, GTP, CTP and UTP.

2. In the polymerization reaction a 3'OH group of one nucleotide reacts with the 5' – triphosphate of a second nucleotide; pyrophosphate is removed and a phosphodiester bond results by the activity of RNA polymerase.
3. The sequence of bases in an RNA molecule is determined by the base sequence of the DNA. Each base added to the growing end of the RNA chain is chosen by its ability to base – pair with the DNA strand used as a template; thus the bases C, T, G and A in a DNA strand cause G, A, C and U, respectively, to appear in the newly synthesized RNA molecule.
4. The DNA molecule being transcribed is double stranded, yet in any particular region only one strand serves as a template.



5. RNA chain grows in the 5'→3' direction (antiparellel) as that of DNA synthesis.
6. RNA polymerases, in contrast with DNA polymerases, are able to initiate chain growth; that is, no primer is needed.
7. Only ribonucleoside 5'- triphosphates participate in RNA synthesis and the first base to be laid down in the initiation event is a triphosphate (Fig).

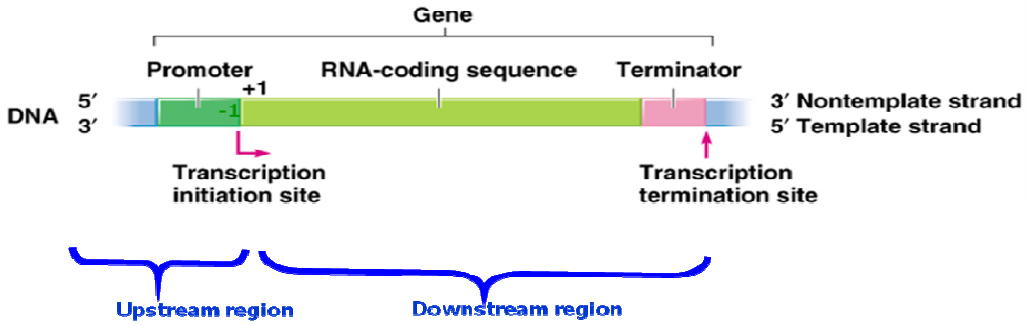
The synthesis of RNA consists of four discrete stages: 1. binding of RNA polymerase to a template at a specific site 2. initiation 3. chain elongation 4. Chain termination and release.

Binding of RNA polymerase

E. coli RNA polymerase consists of five subunits – two identical α subunits and one each types of β, β' and σ. The σ subunit dissociates from the enzyme during the elongation stage of RNA polymerization. The term core enzyme is used to describe the

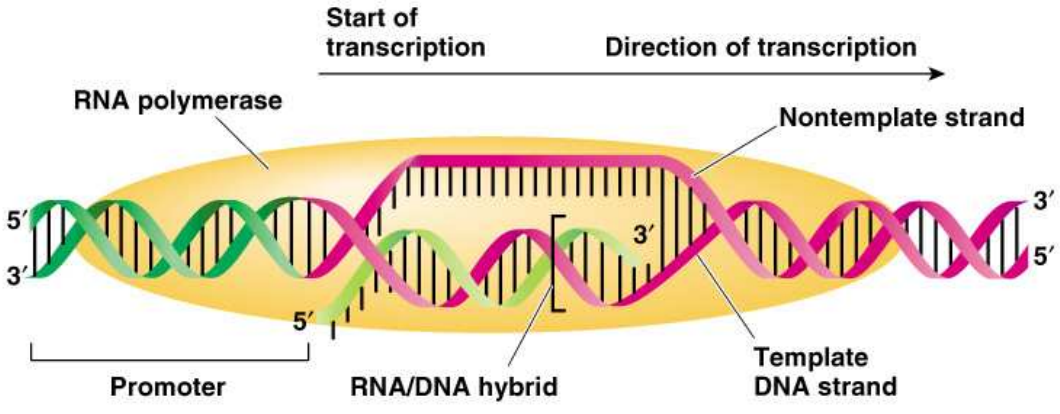
σ-free unit, $\alpha_2\beta\beta$. The complete enzyme, $\alpha_2\beta\beta\sigma$ is called the holoenzyme. RNA polymerase is sufficiently large that it can come into contact with many DNA bases simultaneously. The first step in transcription is the binding of RNA polymerase to a DNA molecule. Binding occurs in particular regions called promoters, which are sequences in which several interactions occur.

Initiation: RNA polymerase recognizes the promoter



Several events occur at a promoter: RNA polymerase recognize a specific DNA sequence, attach in a proper conformation, locally open the DNA strands in order to gain access to the bases to be copied and then initiate synthesis. These events are guided by the base sequence of the DNA, the polymerase σ subunit (without which the promoter is not recognized).

The specific binding region in DNA is in a region 5 –10 bases prior to the left of the first base copied into mRNA. This region is called as Pribnow box. All sequences found in Pribnow boxes are considered to be variants of a basic sequence TATAAT, hence they are called as TATA box. The first base transcribed was chosen as a reference point and numbered +1. The direction of transcription was called downstream; all upstream bases, which are not transcribed, were given negative numbers starting from the reference. Thus, the Pribnow box is enclosed between –21 and –4 depending on the particular promoter.



There is a second important region, to the left of the Pribnow box, whose sequences in different promoters have common features. This six base sequence which is called as the "–35 sequence "has a consensus TTGACA, may be initial site of binding of the enzyme, when the sequence is present.

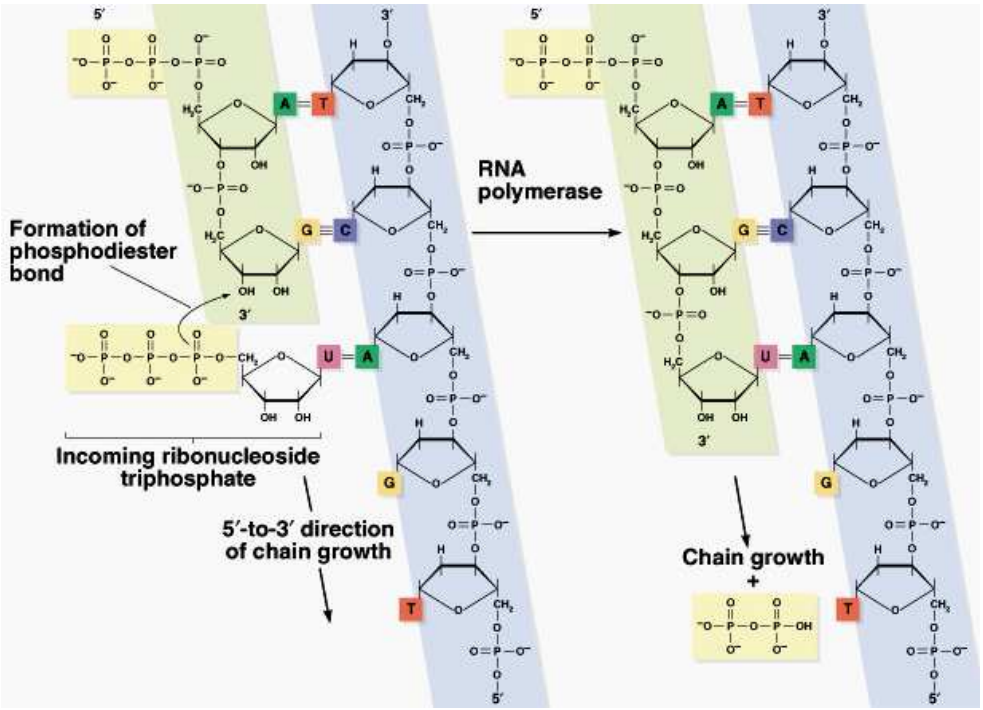
The Pribnow box is thought to orient RNA polymerase, such that synthesis proceeds from left to right and to be the region at which the double helix opens to form the open- promoter complex. This highly stable complex is the active intermediate in chain initiation. The DNA double helix in an open-promoter complex is locally unwound, starting about 10 bp from the left end of Pribnow box and extending about 20 bp past the position of the first transcribed base. It seens that RNA polymerase itself induces this unwinding and undergoes a conformational change itself in so doing. This melting is necessary for pairing of incoming ribonucleotides.

2. RNA chain initiation

Once the open promoter complex has formed, RNA polymerase is ready to initiate synthesis. RNA polymerase contains two nucleotide binding sites called the initiation site and the elongation site. The initiation site primarily binds to purine triphosphates, ATP or GTP. ATP is usually the first nucleotide in the chain. The initiating nucleoside triphosphate binds to the enzyme in the open-promoter complex and forma a hydrogen bond with the complementary DNA base. The elongation site (also called the catalytic site) is the filled with a nucleoside triphosphate that is selected by its ability to hydrogen-bond with the next base in the DNA strand. The two nucleotides are then joined together, the first base is released from the initiation site and initiation is complete. In some way, the details of which are not understood, the RNA polymerase and the template strand move relative to each other, so the binding sites and the catalytic sites are shifted by exactly one nucleotide. The drug, rifampicin is useful in studying initiation. It binds to the β subunit of RNA polymerase blocking the transition from the chain initiation phase to the elongation phase.

Chain elongation

After several nucleotides (~ 8) are added to the growing chain, RNA polymerase undergoes a conformational changes and loses the σ subunit. Thus now this process enters into elongation phase, and most elongation is carried out by the core enzyme of RNA polymerase. The core enzyme moves along the DNA, binding a nucleoside triphosphate that can pair with the next DNA base and opening the DNA helix as it moves. The DNA helix resumes its original shape as synthesis proceeds. The newly synthesized RNA is released from its hydrogen bonds with the DNA as the helix re-forms.

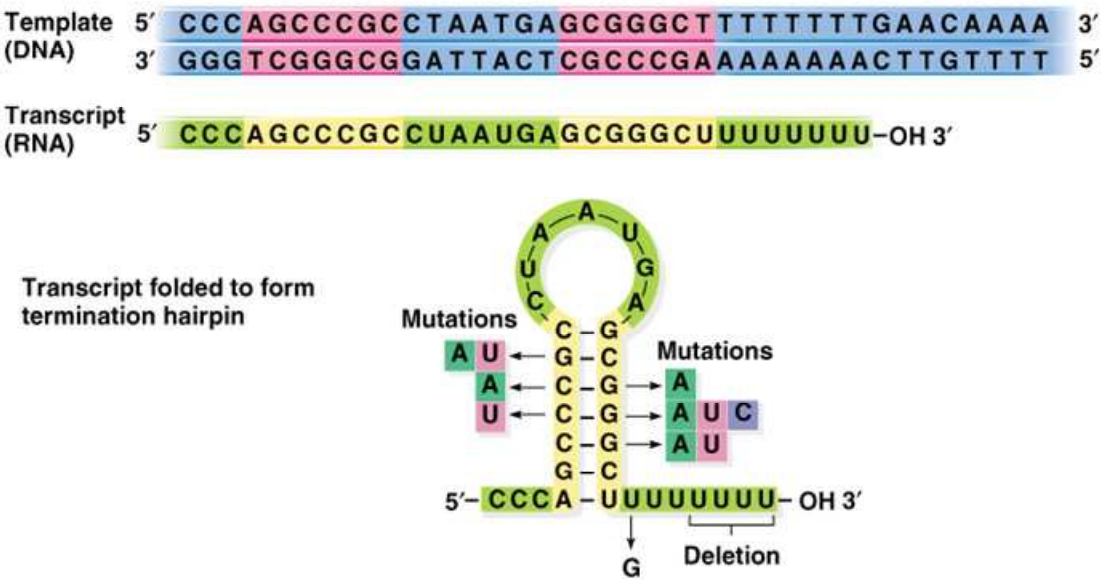


Termination and release of new RNA

Termination of RNA synthesis occurs at specific base sequences within the DNA molecule. These sequences are of two types, simple terminators and those that require auxiliary termination factors. At a particular sequence, if there is an inverted repeat containing a central nonrepeating segment, ie., the sequence in one DNA strand would read like TATAT- NNN – ATATA, then the RNA transcribed from this strand form a

intrastrand base paring and leads to stem and loop structure. Termination occurs at this hairpin region for unknown reasons.

Alternatively, termination also occurs due to auxiliary termination protein called – Rho protein. It binds tightly with RNA which has segment that are rich in C (especially repeating C's). The binding helps the protein to acquire a powerful ATP cleaving activity that is essential to its action in termination. This is because, nucleoside triphosphates cannot reach RNA polymerase since they are degraded by Rho protein. Significant differences exist between the structures and modes of synthesis of the RNA molecules of prokaryotes and eukaryotes, though the basic mechanisms of their functions are nearly the same.



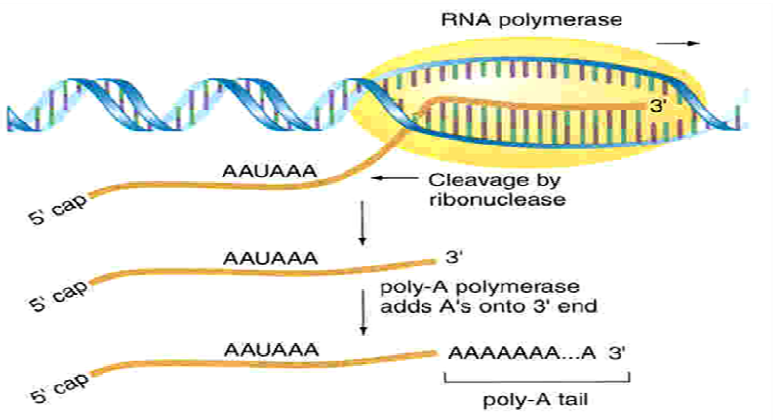
Classes of RNA molecules

There are three major classes of RNA molecules – messenger RNA (mRNA; an informational molecule), ribosomal RNA (rRNA; a structural molecule) and transfer RNA (tRNA; a structural and informational molecule).

mRNA

The base sequence of a DNA molecule determines the amino acid sequence of every polypeptide chain in cell, though amino acids have no affinity for DNA. Thus, instead of direct pairing between amino acids and DNA, a multistep process is used in

which the information contained in the DNA is converted to a form in which amino acids can be arranged in an order determined by the DNA base sequence. This process begins with the transcription of the base sequence of one of the DNA strands (the coding strand) into the base sequence of an RNA molecule (mRNA). The protein synthesizing machinery of the cell obtain the information, i.e., the amino acid sequence of a particular protein to be synthesized, from this RNA molecule. The nucleotide sequence of the mRNA is then read in groups of three bases (a group of three is called as Codon) from a start codon to stop codon, with each codon corresponding either to one amino acid or a stop signal.



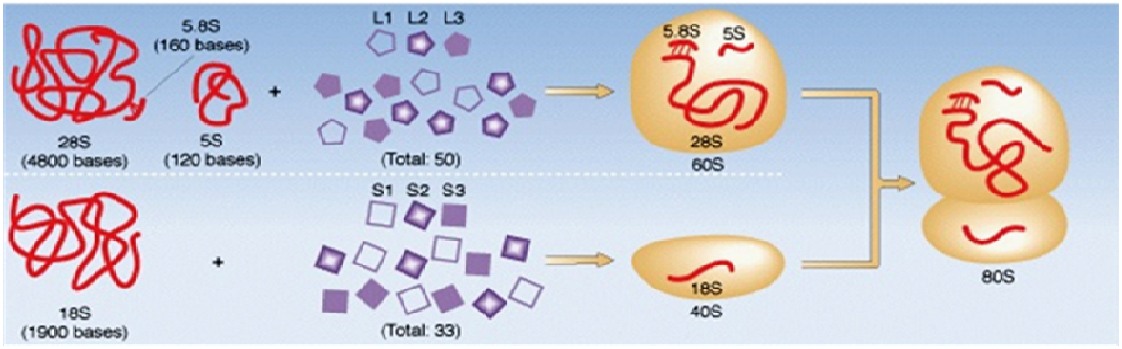
A DNA segment corresponding to one polypeptide chain plus the translational start and stop signals for protein synthesis is called a cistron and an mRNA encoding a single polypeptide is called monocistronic mRNA. In prokaryotes, it is very common for an mRNA molecule to encode several different polypeptide chains; in this case it is called as polycistronic mRNA. The segment of RNA corresponding to a DNA cistron is often called a reading frame, since the protein synthesizing system reads it.

In addition to reading frames and start and stop sequences for translation, other regions in mRNA are significant. Translation of an mRNA molecule rarely starts exactly at one end of the RNA and proceeds to the other end; instead, initiation of synthesis of the first polypeptide chain of a polycistronic mRNA may begin hundreds of nucleotides from the 5'- P terminus of the RNA. The section of untranslated RNA before the coding regions is called a leader. In some cases, the leader contains a regulatory region.

Untranslated sequences usually found at both the 5'-P and 3'-OH termini and a polycistronic mRNA molecule typically contain intercistronic sequences (spacers) usually tens of bases long. An important characteristic of prokaryotic mRNA is that its lifetime is short (only few minutes) compared to other types of RNA molecules.

Ribosomal RNA and transfer RNA

During the protein synthesis, genetic information is supplied by mRNA. Amino acids do not line up against the mRNA template independently during protein synthesis but are aligned by means of a set of about 50 adaptor RNA molecules called transfer RNA (tRNA) and this is occurred on the surface of an RNA-containing protein particle called as ribosome. These particles consist of several classes of ribosomal RNA (rRNA) and ribosomal proteins, which are stable molecules and having various functions. Whereas the transfer RNA molecule exist in the cell, has a capacity of 'reading' three adjacent mRNA bases and placing corresponding amino acid at a site on the ribosome at which a peptide bond is formed with an adjacent amino acid. Neither rRNA nor tRNA is translated into polypeptide chain.



Ribosome structure (eukaryotes)

23S & 5S rRNA 34 proteins (L1-L31) 30S& 50S 70S (prokaryotes)
16S rRNA 21 proteins (S1-S21)

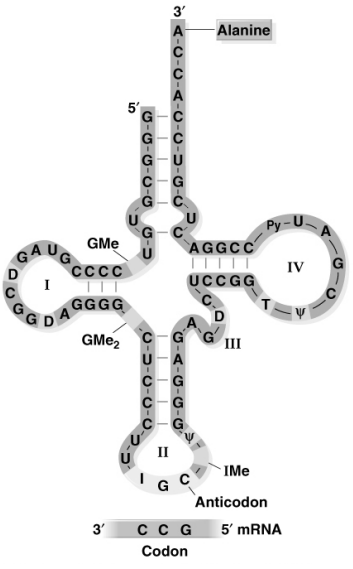
The synthesis of both rRNA and tRNA molecules is initiated at a promoter and completed at terminators; in this respect, their synthesis is no different from that of mRNA. However, the following three properties of these molecules indicate that neither rRNA nor tRNA molecules are the immediate products of transcription (called as primary transcripts):

1. The molecules are terminated by a 5' monophosphate rather than the expected triphosphate found at the ends of all primary transcripts.
2. Both rRNA and tRNA molecules are much smaller than the primary transcripts.
3. All tRNA molecules contain bases other than A, G, C and U and these unusual bases are not present in the original transcript.

All of these molecular changes are made after transcription by processes collectively called as posttranscriptional modification or more commonly, processing.

All ribosomes comprise two dissimilar sized subunits, the large and small subunits. Each subunits consists of several rRNA and numerous ribosomal proteins (r-proteins). In *E. coli*, the 70S ribosome is composed of a small 30S subunit and a large 50S subunit. The small subunit comprises 21 different proteins (named S1-S21) and the 16S RNA. The large subunit comprises 34 proteins (named L1-L34) and the 23S and 5S rRNAs. Some proteins are common to both subunits (e.g. L6, S20). Eukaryote ribosomes are larger (80S) and contain more components. The small (40S) subunit comprises 33 proteins and the 18S rRNA whilst the large (60S) subunit contains 50 proteins and three rRNAs of 28S, 5.8S and 5S. The spatial organization of the ribosome is complex. rRNA makes up 60-65 % pf the total mass and is essential for structural integrity and function, adopting complex tertiary and quaternary conformations by intra and inter molecular base pairing.

The tRNAs are relatively homogeneous family of RNA molecules, usually 75-100 nucleotides in length, which are extensively processed during their production. They possess a characteristic secondary and tertiary structure (Figure), most importantly the acceptor stem (to which the amino acid binds) and the anticodon loop (which carries the three nucleotide anticodon that forms complementary base pairs with codons in the mRNA). Bacterial cells contain up to 35 different tRNAs and eukaryotic cells up to 50. This number is lower than the number of possible codons in the genetic code, but greater than the number of amino acids specified by the code. This indicates that individual



tRNAs can recognize more than one codon (called as wobble pairing), but that different tRNAs may be charged with the same amino acids (these are called as isoaccepting tRNAs). The tRNAs are charged (conjugated to their corresponding amino acids) by enzymes termed amino acyl tRNA synthetases. There is one enzyme for each amino acid and therefore each synthetase recognizes all its cognate isoaccepting tRNAs.

Genetic Code

The genetic code is the collection of base sequences (called as codons) that corresponds to each amino acid and to stop signals for translation. Since there are 20 amino acids, there must be more than 20 codons to include signals for starting and stopping the synthesis of particular protein molecules. If one assumes that all codons have the same number of bases, then each codon must contain at least three bases. Because: A single base cannot be a codon since there are 20 amino acids and only 4 bases. Pairs of bases also cannot serve as codons because there are only $4^2 = 16$ possible pairs of four bases. Triplets of bases are possible because there are $4^3 = 64$ triplets, which is more than adequate. In many cases, several codons designate the same amino acid- that is the code is redundant or degenerate. In the in vitro system, protein synthesis can start at any base. However, in vivo it starts only at AUG codon. Similarly it stops at either UAA, UGA or UAG.

Tabel 1. The universal genetic code

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe (F)	UCU Ser (S)	UAU Tyr (Y)	UGU Cys (C)	U
	UUC Phe (F)	UCC Ser (S)	UAC Tyr (Y)	UGC Cys (C)	C
	UUA Leu (L)	UCA Ser (S)	UAA Stop	UGA Stop	A
	UUG Leu (L)	UCG Ser (S)	UAG Stop	UGG Trp (W)	G
C	CUU Leu (L)	CCU Pro (P)	CAU His (H)	CGU Arg (R)	U
	CUC Leu (L)	CCC Pro (P)	CAC His (H)	CGC Arg (R)	C
	CUA Leu (L)	CCA Pro (P)	CAA Gln (Q)	CGA Arg (R)	A
	CUG Leu (L)	CCG Pro (P)	CAG Gln (Q)	CGG Arg (R)	G
A	AUU Ile (I)	ACU Thr (T)	AAU Asn (N)	AGU Ser (S)	U
	AUC Ile (I)	ACC Thr (T)	AAC Asn (N)	AGC Ser (S)	C
	AUA Ile (I)	ACA Thr (T)	AAA Lys (K)	AGA Arg (R)	A
	AUG Met (M)	ACG Thr (T)	AAG Lys (K)	AGG Arg (R)	G
G	GUU Val (V)	GCU Ala (A)	GAU Asp (D)	GGU Gly (G)	U
	GUC Val (V)	GCC Ala (A)	GAC Asp (D)	GGC Gly (G)	C
	GUA Val (V)	GCA Ala (A)	GAA Glu (E)	GGA Gly (G)	A
	GUG Val (V)	GCG Ala (A)	GAG Glu (E)	GGG Gly (G)	G

Questions

1. Transcription, the synthesis of RNA using DNA as template, is required for

a) Gene expression b) DNA replication c) Translation d) all the above

Ans: all the above

2. Transcription is asymmetric *i.e.*,

a) both DNA strands are used as template b) only one of the strand of DNA is used as template c) combinations of different parts of DNA is used as template d) all the above

Ans: one of the strand of DNA is used as template

3. The nascent RNA strand synthesized as continuous strand during transcription and it is analogous to

a) Lagging strand b) leading strand c) antiparallel strands d) all the above

Ans: leading strand

4. The nucleotide immediately preceding the transcription unit on the DNA strand is defined as position

a) +1 b) 0 c) -1 d) 5' UTR

Ans: -1

5. RNA polymerases

a) Require RNA primers b) do not require primer and initiate strand synthesis *de novo*

c) do not proofread their transcripts d) both b and c

Ans: both b and c

6. Transcription factors recognize and binds to

a) Silencer b) enhancer c) promoter d) all the above

Ans: all the above

7. Roger Kornberg got Noble prize for the year 2006 for his work on

a) RNA Polymerase b) Histone c) DNA Polymerase d) Ribosome

Ans: RNA Polymerase

8. Which of the following features would you NOT expect to find in heterogeneous nuclear RNA (hnRNA)?

a) Intron b) polycistronic coding c) polyadenylation at 3'-end d) 5-' cap structure

Ans: polycistronic coding

9. The non-coding DNA that occurs within eukaryotic gene is referred to as

a) Spacer DNA b) Promoter c) terminator d) Intron DNA

Ans: Intron DNA

10. Which of the following statements about introns is incorrect? Introns are

a) Found in most eukaryotic genes and not translated during protein synthesis

b) Removed during RNA processing

c) Normally not transcribed

d) Responsible for the fact that the most eukaryotic mRNAs are much shorter than the genes from which they are derived.

Ans: Normally not transcribed

11. DNA is double stranded. One strand is called the coding strand and the other the non-coding strand. The non-coding strand is used as the template to make mRNA. The relationship between the base sequence of the coding strand and the base sequence of the mRNA (ignoring the fact that mRNA will contain uracil instead of thymine) is

a) Complementary b) Identical c) anti-parallel d) none of the above

Ans: Complementary

12. The RNA strand synthesized during transcription elongates until

a) The entire chromosome has been copied into RNA

b) The RNA polymerase runs into the next gene

c) An intron encountered on the DNA template strand

d) A specific termination sequence is reached on the DNA template strand

Ans: A specific termination sequence is reached on the DNA template strand

13. Transcriptional regulation is controlled by

a) methylation of DNA b) number of genes in the genome c) poly A tail d) introns

Ans: methylation of DNA

14. ESTs are obtained through

a) Genomic DNA library b) cDNA library c) RT-PCR d) all the above

Ans: cDNA library

15. The only one start codon is ----------

(a) GAT (b) ATG (c) TAG (d) GTA

Ans: ATG

16. In prokaryotes, the matured mRNA is

a) Identifcal to the initial mRNA b) Shorter than the initial mRNA c) Longer than the initial mRNA d) None of the above

Ans: Identifcal to the initial mRNA

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- Virtual Cell: http://www.life.uiuc.edu/plantbio/cell/
- www.kbiotech.com
- www.johnkyrk.com

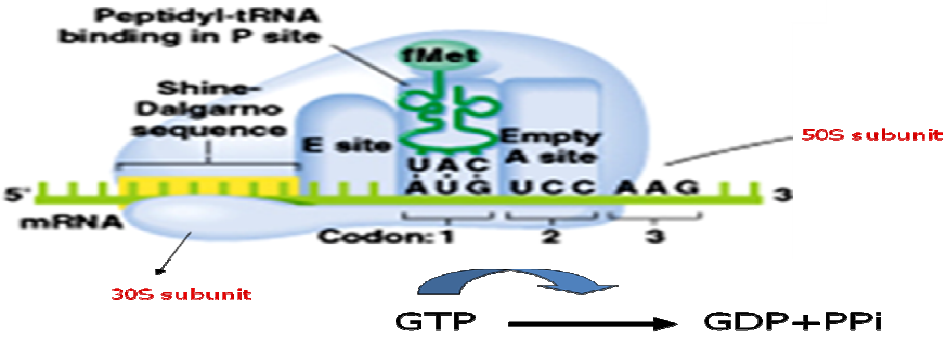
Lecture.25

Fatty acid and triacyl glycerol biosynthesis

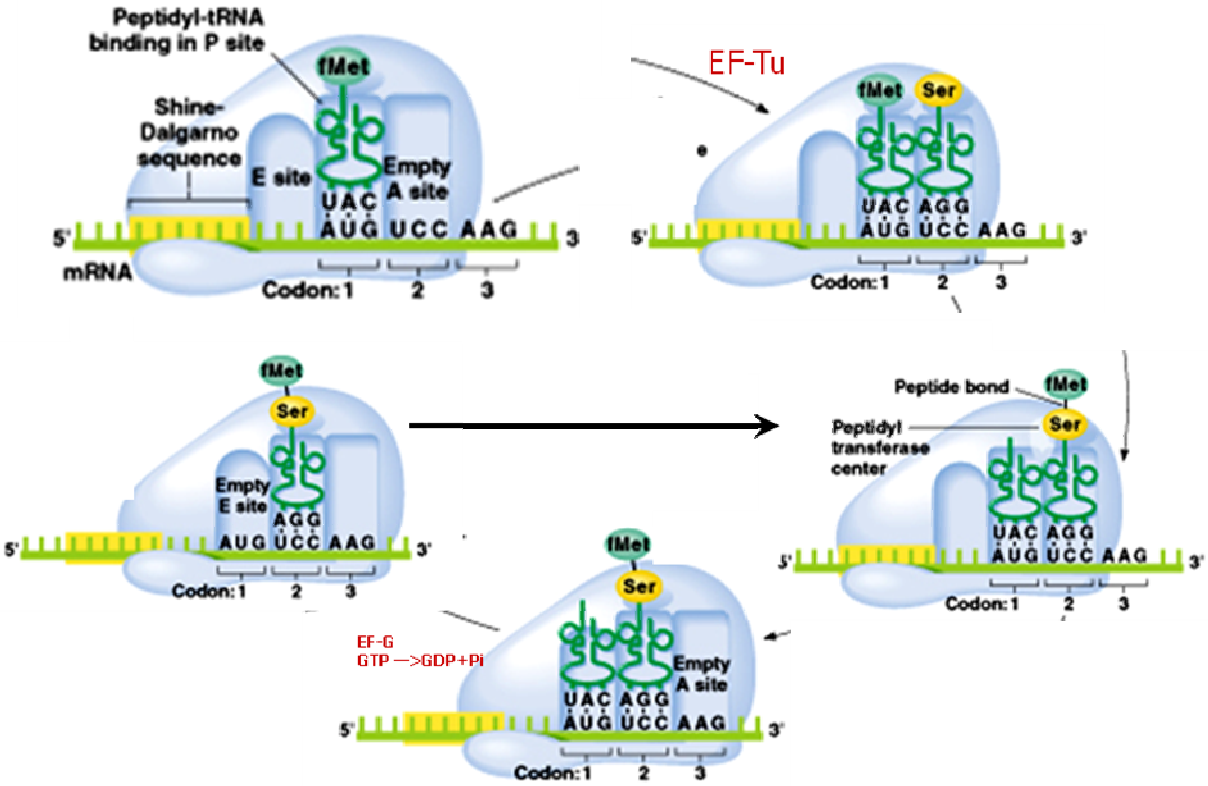
The synthesis of every protein molecule in a cell is directed by intracellular DNA. There are two aspects to understand how this is accomplished – Information aspect and Chemical aspect. Information aspect meant the mechanism by which a base sequence in a DNA molecule is translated into an amino acid sequence of a polypeptide chain. The chemical aspect refers to the actual process of synthesis of the protein: the means of initiating synthesis; linking together the amino acids in the correct order; terminating the chain; releasing the finished chain from the synthetic apparatus; folding the chain, and often postsynthetic modification of the newly synthesized chain. The overall process is called as Translation. Protein synthesis can be divided into three stages: 1. polypeptide chain initiation, 2. chain elongation and 3. Chain termination.

1. Initiation

In bacteria protein synthesis begins by the association of one 30S subunit (not the 70S ribosome), one mRNA molecule, a charged $tRNA^{fMet,}$, three proteins known as initiation factors and guanosine 5'-triphosphate (GTP). These molecules comprise the 30S preinitiation complex. Following formation of the 30S preinitiation complex, a 50S subunit joins to the 30S subunit to form a 70S initiation complex. This joining process requires hydrolysis of the GTP contained in the 30S preinitiation complex. There are two tRNA binding sites which overlap the 30S and 50S subunits. These sites are called as aminoacyl or A site and the peptide or P site; each site consists of a collection of segments of S and L proteins and 23S rRNA. The 50S subunit is positioned in the 70S initiation complex such that the $tRNA^{fMet,}$ which was previously bound to the 30S preinitiation complex, occupies the P site of the 50S subunit. Positioning $tRNA^{fMet,}$ in the P site fixes the position of the anticodon of $tRNA^{fMet,}$ such that it can pair with the initiator codon in the mRNA. Thus, the reading frame is unambiguously defined upon completion of the 70S initiation complex.



The A site of the 70S initiation complex is available to any tRNA molecule whose antiocodon can pair with the codon adjacent to the initiation codon. However, entry to the A site by the tRNA requires a helper protein called an elongation factor (EF), specifically EF-Tu. After occupation of the A site a peptide bond between fMet and the adjacent amino acid can be formed. Once it was thought that the blockage of NH_2 group of fMet by the formyl group was responsible for peptide bond formation between the COOH group of fMet and the NH_2 group of the adjacent amino acid. However, in eukaryotes the starting amino acid is Met and not fMet and protein synthesis proceeds in the correct direction. Presumably, the relative orientation of the two amino acids in the A and P sites determines the linkage that is made.



The peptide bond is formed by an enzyme complex called peptidyl transferase. The active site of peptidyl transferase consists of portions of several proteins of the 50S subunit. As the peptide bond is formed, fMet is cleaved from the $tRNA^{fMet,}$ in the P site. After the peptide bond forms, an unchanged tRNA occupies the P site and a dipeptidyl-tRNA is in the A site. At this point three movements, which together comprise the translation step, occur:

1. The deacylated $tRNA^{fMet,}$ leaves the P site.

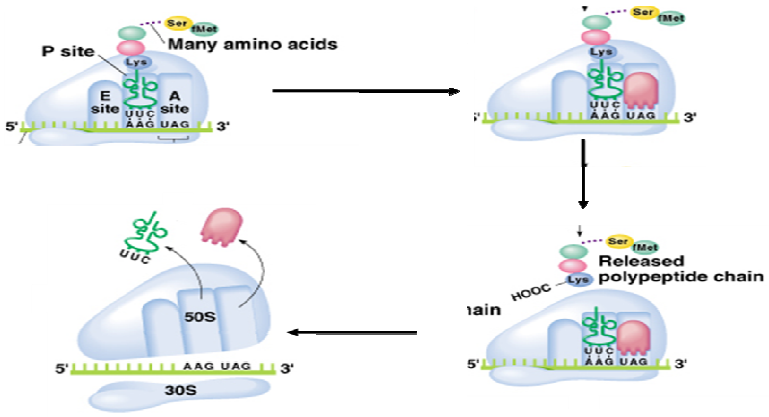
2. The peptidyl-tRNA moves from the A site to the P site and

3. The mRNA moves a distance of three bases in order to position the next codon at the A site. The translocation step requires the presence of another elongation protein EF-G and hydrolysis of GTP. The movement of the mRNA by three bases is probably dependant on the movement of tRNA from the A site to the P site and in fact, it is likely that mRNA translocation is a consequence of tRNA motion.

After translocation has occurred, the A site is again available to accept a charged tRNA molecule having a correct anticodon. If a $tRNA^{Met,}$ molecule, were to enter the A site (because an internal AUG site were present), protein synthesis would stop because a peptide bond cannot form with the blocked NH_2 group of fMet. However, in as much as the factor EF-Tu is needed to facilitate tRNA entry into the A site, this misadventure is prevented, since EF-Tu cannot bind to $tRNA^{fMet,}$.

When a chain termination codon is reached, there is no aminoacyl-tRNA that can fill the A site and chain elongation stops. However, the polypeptide chain is still attached to the tRNA occupying P site. Release of the protein is accomplished by release factors (RF), proteins that in part respond to chain termination codons. There are two such release factors in *E. coli* – RF1, which recognizes the UAA and UAG codons and RF2, which recognizes UAA and UGA. Why the number of release factor is not one (ie. useful to all 3 codons) or three (ie., one for each stop codon) is not known (in eukaryotes, there is only one release factor). Each release factor forms an activated complex with GTP; this complex binds to a termination codon and alters the specificity of peptidyl transferase. In the presence of release factors peptidyl transferase catalyses the reaction of the bound peptidyl moiety with water rather than with the free aminoacyl-tRNA. Thus the polypeptide chain, which has been held in the ribosome solely by the interaction with the

tRNA in the P site, is released from the ribosome. The 70S ribosome dissociates into 30S and 50S subunits and the system is ready to start synthesis of a second chain.



Differences between protein synthesis in eukaryotes and prokaryotes

1. In eukaryotes, the initiating amino acid is methionine (Met) and not fMet. The initiating tRNA, which responds only to AUG, is designated $tRNA^{Met}_{init}$, or $tRNA^{Met}_{f}$ to distinguish it from the $tRNA^{Met,}$ used in translating internal AUG codons.
2. At least nine initiation factors plus GTP are required for binding of $tRNA^{Met,}_{f}$, to the preinitiation complex. Two of these are similar to the factors in *E. coli* that prevent binding of the large subunit to the small subunit. One of these factors, eIF3, is a larger complex containing nine protein subunits and having a molecular weight of 7 X 10^5. An enzyme called initiating tRNA hydrolase, which cleaves the bond between methionine and $tRNA^{Met,}_{f}$ removes the initial tRNA molecule after the first peptide bond forms, is also present in the complex. In prokaryotes, the corresponding enzyme, tRNA deacylase, is a ribosomal component.
3. Binding of $tRNA^{Met,}_{f}$, must occur before mRNA can bind, whereas for prokaryotes the mRNA can bind either before or after binding of the initiator tRNA. For binding of mRNA two other initiation factors are needed and ATP must be cleaved to form ADP and P_i. The reason for the cleavage of ATP is unknown. Binding occurs initially at or near the 5' cap and is mediated by a cap binding factor (which is unnecessary for uncapped viral mRNA). The fact that the AUG codon nearest the 5'

terminus is almost always the initiating codon is a significant difference between prokaryotes and eukaryotes and plays important role in metabolic regulation.

4. More factors are needed for binding of the 60S subunit than for binding of the bacterial 50S subunit.
5. At least four elongation factors are needed by eukaryotes. These factors probably differ in structure and size in different tissues, often forming aggregates containing as many as 50 monomers.
6. Little is know about termination in eukaryotes, thoughs release factors have been purified. Surprisingly, release in *in vitro* systems requires the presence of one of four tetranucleotides – UAAA, UAGA, UGAA or UAGG.
7. The most striking difference is that transcription and translation is not couple in eukaryotes whereas it is coupled in prokaryotes. In eukaryotes, the mRNA is synthesized in nucleus and then transported to cytoplasm where the ribosome located.
8. In prokaryotes, degradation of mRNA occurs continuously and while translation is in process. The half-life of a typical bacterial mRNA is about 1.8 minutes. Eukaryotic mRNA is very stable- possibly because of the 5' cap. Degradation occurs very slowly and a typical half-life is several hours.

Questions

1. In eukaryotes, translation occurs in

a) Cytoplasm b) nucleus c) cellular compartment d) all the above

Ans: Cytoplasm

2. In eukaryotes, nascent mRNA is usually

a) polycistronic b) monocistronic c) dicistronic d) tricistronic

Ans: monocistronic

3. The templates used for protein synthesis is

a) genic DNA b) mRNA c) rRNA d) tRNA

Ans: mRNA

4. A sequence of translatable codons are referred as

a) ORF b) RBS c) promoter d) enhancer

Ans: ORF

5. The mRNA those are stable for hours or even days are synthesized in

a) Eggs b) muscles c) bones d) all the above

Ans: eggs

6. The role of modification of 5' end of mRNA with 7-methylguanosine cap is

a) Ribosome binding b) aligning P site c) aligning A site d) mRNA stability

Ans: ribosome binding

7. The maximum portion (60-65%) of total RNA consists of

a) mRNA b) tRNA c) rRNA d) hnRNA

Ans: rRNA

8. Which site of the ribosomes binds with incoming charged tRNAs during elongation?

a) A (aminoacyl) site b) P (peptidyl tRNA) site c) GTPase domain d) peptidyl transferase domain

Ans: A (aminoacyl) site

9. In eukaryotes, the small subunit of ribosome along with cap binding proteins (CBP) (eIF-4F) binds at

a) Shine-Dalgarno sequence b) Kozak sequence (ACCAUGG) c) promoter

d) Enhancer

Ans: Kozak sequence (ACCAUGG)

10. Examples of programmed misreading, where the normal interpretation of the sequence of codons is suppressed is

a) Read through b) selenocystein insertion (at termination codon- UGA; called as selenocystein insertion sequence) c) frame shifting d) all the above

Ans: all the above

11. A protein composed of 300 amino acids would be encoded by an mRNA of

a) 300 nucleotides b) 400 nucleotides c) 900 nucleotides d) 400 nucleotides

Ans: 900 nucleotides

12. The three types of RNA responsible for forming part of the structure upon which protein synthesis occurs, dictating the order of amino acids in the growing protein chain and transporting amino acids to their site of protein synthesis, respectively are

a) mRNA, rRNA, tRNA b) tRNA, mRNA, rRNA

c) rRNA , mRNA, tRNA d) rRNA, tRNA, mRNA

Ans: rRNA, mRNA, tRNA

13. Anticodon is a part of the tRNA molecule that is responsible for specific attachment to the

a) mRNA b) Amino acid c) Ribosomal complex d) rRNA

Ans: mRNA

14. Which of the following sentence is incorrect

a) Eukaryotic nuclear mRNAs are monocistronic

b) mRNA that carry the information for more than one type of protein is not found eukaryotic cells

c) A bacterial mRNA can carry the information for more than one type of protein

d) All the above

Ans: mRNA that carry the information for more than one type of protein is not found eukaryotic cells

15. In prokaryotes, many ribosomes translate an mRNA simultaneously, forming a structure called

a) polysome b) polycistron c) 30S initiation complex d) all the above

Ans: polysome

16. In Eukaryotes, to locate the initiator codon, the small ribosomal subunit has to first locate

a) The 5'-methyl guanosine cap b) Ribosome binding site c) Shine-Dalgarno sequence d) all the above

Ans: Shine-Dalgarno sequence

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- Virtual Cell: http://www.life.uiuc.edu/plantbio/cell/
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- www.johnkyrk.com

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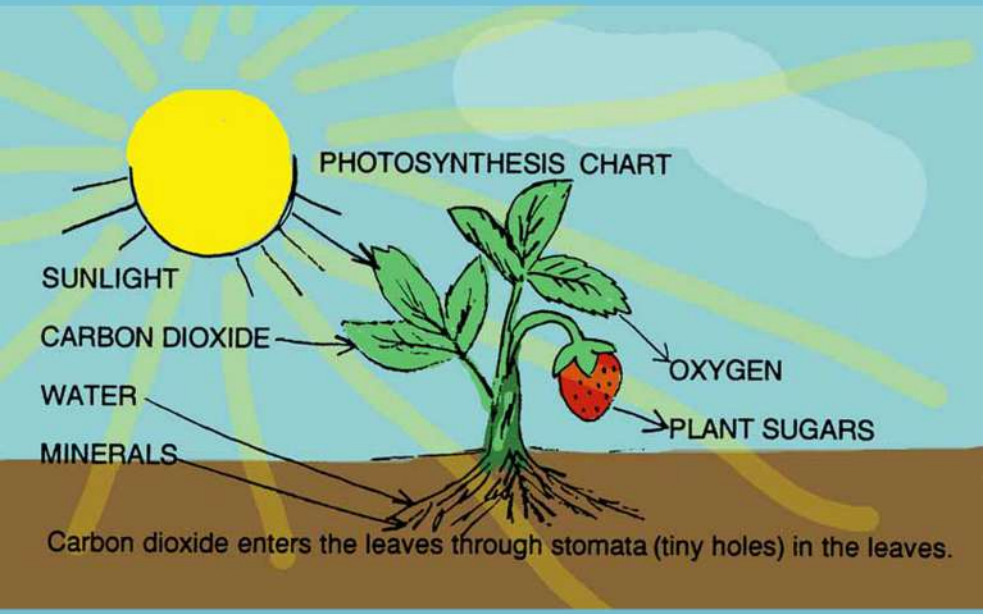

PPHY-261
Crop Physiology

PPHY261 CROP PHYSIOLOGY (2+1)

AIM

To expose the students to the basic concepts and underlying application of Crop Physiology

THEORY

UNIT I: PLANT WATER RELATIONS

Introduction – **review on plant anatomy** - Importance of crop physiology in agriculture, Role and significance of water - diffusion, imbibitions, osmosis and its significance, plasmolysis, Definitions - field capacity, water holding capacity of soil and permanent wilting point, Absorption of water - mode of water absorption – active and passive absorption and factors affecting absorption, Translocation of solutes - phloem and xylem transport, Transpiration - types - Steward's theory of mechanism - significance, factors affecting transpiration and guttation - antitranspirants.

UNIT II: NUTRIO PHYSIOLOGY

Mineral nutrition - introduction - criteria of essentiality of elements - macro, secondary and micronutrients - sand and soil less culture- hydroponics, Mechanism of uptake - physiological role of nutrients, Foliar diagnosis - nutritional and physiological disorders - foliar nutrition and fertigation **.**

UNIT III : PHOTOSYNTHESIS& RESPIRATION

Photosynthesis - requirements of photosynthesis - light, CO_2, pigments and water, Mechanism of photosynthesis - light reaction - cyclic and non cyclic photophosphorilation - Red drop - Emerson Enhancement Effect, Photosynthetic pathways - C3, C4 and CAM, Differences between C_3, C_4 and CAM pathways - Factors affecting photosynthesis, Photorespiration - photorespiration process and significance of photorespiration, Respiration - Glycolysis, TCA and Pentose Phosphate Pathway, Oxidative phosphorylation - differences between oxidative phosphorylation and photophosphorylation. Respiratory quotient and energy budgeting in respiration.

UNIT IV : GROWTH PHYSIOLOGY

Growth - growth curve, phases of growth and factors influencing growth, Growth analysis - LAI, LAD, SLW, SLA, LAR, NAR, RGR and CGR in relation to crop productivity,- Source sink relationship - Photoperiodism - Role of phytochrome in flowering and regulation of flowering. Transmission of stimulus - theories of flowering-Vernalisation – devernalisation- Protein and fat synthesis- Plant growth regulators - growth hormones - definition and classification - physiological role of auxins and GA, Physiological role of Cytokinin, Ethylene and ABA - synthetic growth regulators and their uses in crop productivity, Practical application of Plant Growth Regulators in crop productivity

UNIT V : STRESS PHYSIOLOGY

Environmental stresses - water stress - physiological changes - adaptation to drought and amelioration, Temperature stress - Physiological changes - low and high temperature - chilling injury - tolerance – alleviation, Low light and UV radiation stresses - salt stress - physiological changes and alleviation, Global warming – **Carbon Sequestration** physiological effects on crop productivity, Seed germination - physiological changes during seed germination,. Abscission – senescence- **ripening** - types, causes, physiological and biochemical changes and regulation.

PRACTICALS

UNIT I : PLANT WATER RELATIONS

Preparation of solutions – **Anatomical textures of plant body** - Measurement of plant water status - Relative Water Content - Measurement of transpiration - studying the structure of stomata - Stomatal Index.

UNIT II : NUTRIO PHYSIOLOGY

Identification of Physiological disorders - Nutritional disorders in crops plants - Rapid tissue testing methods - Field visit for foliar diagnosis

UNIT III : PHOTOSYNTHESIS& RESPIRATION

Estimation of plant pigments in crop plants - determination of photosynthetic efficiency - differences in C_3 and C_4 plants - estimation of soluble protein.

UNIT IV : GROWTH PHYSIOLOGY

Measurement of leaf area by different methods - Growth analysis Practical application of plant growth regulators. . -

UNIT V ; STRESS PHYSIOLOGY

Estimation of Chlorophyll Stability Index and proline content - Elevated CO_2 and crop productivity.

LECTURE SCHEDULE

1. Introduction - Importance of crop physiology in agriculture.
2. Role and significance of water - diffusion, imbibition, osmosis and its significance, plasmolysis.
3. Definition - field capacity, water holding capacity of soil and permanent wilting point.
4. Absorption of water - mode of water absorption – active and passive absorption and factors affecting absorption.
5. Translocation of solutes - phloem and xylem transport.
6. Transpiration - types - Steward's theory of mechanism - significance, factors affecting transpiration and guttation - antitranspirants.
7. Mineral nutrition - introduction - criteria of essentiality of elements - macro, secondary and micronutrients - soil less culture - sand and hydroponics.
8. Mechanism of uptake - physiological role of nutrients.
9. Foliar diagnosis - nutritional and physiological disorders - foliar nutrition- fertigation
10. Photosynthesis - requirements of photosynthesis - light, CO_2, pigments and H_20.
11. Mechanism of photosynthesis - light reaction - cyclic and non cyclic photophosphorylation - Red drop - Emerson Enhancement Effect.
12. Photosynthetic pathways - C_3, C_4 and CAM.
13. Differences between C_3, C_4 and CAM pathways - Factors affecting photosynthesis.
14. Photorespiration - photorespiration process and significance of photorespiration.
15. Respiration - Glycolysis, TCA and Pentose Phosphate Pathway.
16. Oxidative phosphorylation - differences between oxidative phosphorylation and photophosphorylation. Respiratory quotient and energy budgeting in respiration.
17. Factors affecting respiration - difference between photorespiration and dark respiration - role of respiration.
18. Protein and fat synthesis.
19. Photoperiodism - short day, long day and day neutral plants - phytochrome. Role of phytochrome in flowering and regulation of flowering.
20. Transmission of stimulus - theories of flowering.
21. Vernalisation - mechanism of vernalisation and its significance - devernalisation.

22. Source sink relationship - yield components - harvest index and its importance
23. Growth - growth curve, phases of growth and factors influencing growth
24. Growth analysis - LAI, LAD, SLW, SLA, LAR, NAR, RGR and CGR in relation to crop productivity.
25. Plant growth regulators - growth hormones - definition and classification - physiological role of auxins and GA.
26. Physiological role of Cytokinin, Ethylene and ABA - synthetic growth regulators and their uses in crop productivity.
27. Practical application of Plant Growth Regulators in crop productivity.
28. Environmental stresses - water stress - physiological changes - adaptation to drought and amelioration.
29. Temperature stress - Physiological changes - low and high temperature - chilling injury - tolerance - alleviation.
30. Low light and UV radiation stresses - salt stress - physiological changes and alleviation.
31. Global warming - **Carbon Sequestration** -physiological effects on crop productivity.
32. Seed germination - physiological changes during seed germination.
33. Abscission - senescence – **ripening**- types, causes, physiological and biochemical changes and regulation.

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01. INTRODUCTION

The spectacular diversity of plant size and form is familiar to everyone. In nature all plants carry out similar physiological processes. As primary producers, plants convert solar energy to chemical energy. Being non motile, plants must grow toward light, and they must have efficient vascular systems for movement of water, mineral nutrients, and photosynthetic products throughout the plant body. Green land plants must also have mechanisms for avoiding desiccation.

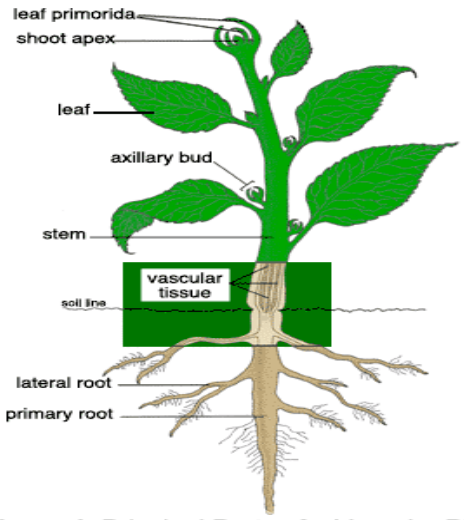


Figure 1. Principal Parts of a Vascular Plant

The meaning of Plant Physiology refers to "the science of properties and functions in normal conditions". The aim of the Plant Physiology has been described as early as the early 20th Century by the Russian Plant Physiologyist, V.I. Palladin as : "Which is to gain a complete and thorough knowledge of all the Phenomena occurring in plants, to analyse complex life processes. So as to interpret them in terms of simpler one and reduce them finally to the principles of physics and chemistry". Nevertheless, Noggle and fritz (1983) described the Plant Physiology as "the science concerned with processes and functions, the response of plants to changes in environment and the growth and development that results from responses

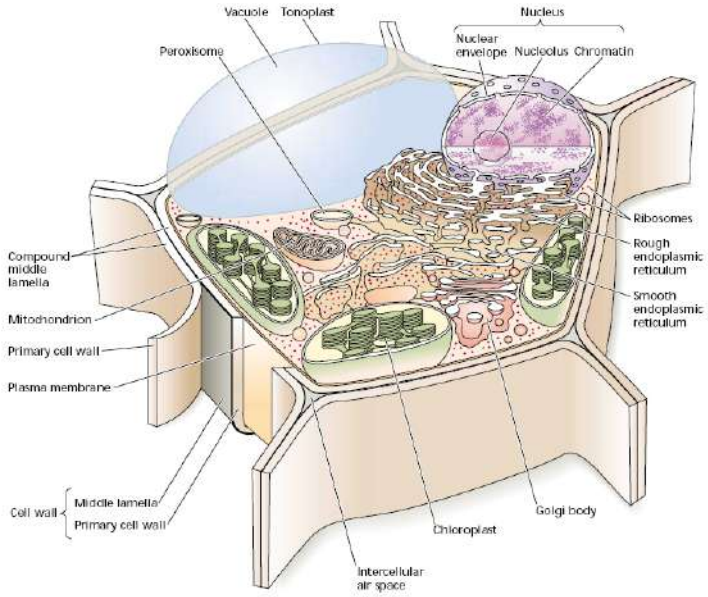
Crop physiology is concerned with the processes and functions of the crops at cellular, sub-cellular and whole plant levels in response to environmental variables and growth. In short, **physiology is the study of functional aspects of crop plants.**

Cell

Plants are multicellular organisms composed of millions of cells with specialized functions. At maturity, such specialized cells may differ greatly from one another in their structures. However, all plant cells have the same basic eukaryotic organization: They contain a nucleus, a cytoplasm, and sub cellular organelles, and they are enclosed in a membrane that defines their boundaries.

In plants, cell migrations are prevented because each walled cell and its neighbor are cemented together by a **middle lamella**. As a consequence, plant development unlike animal development, depends solely on patterns of cell division and cell enlargement.

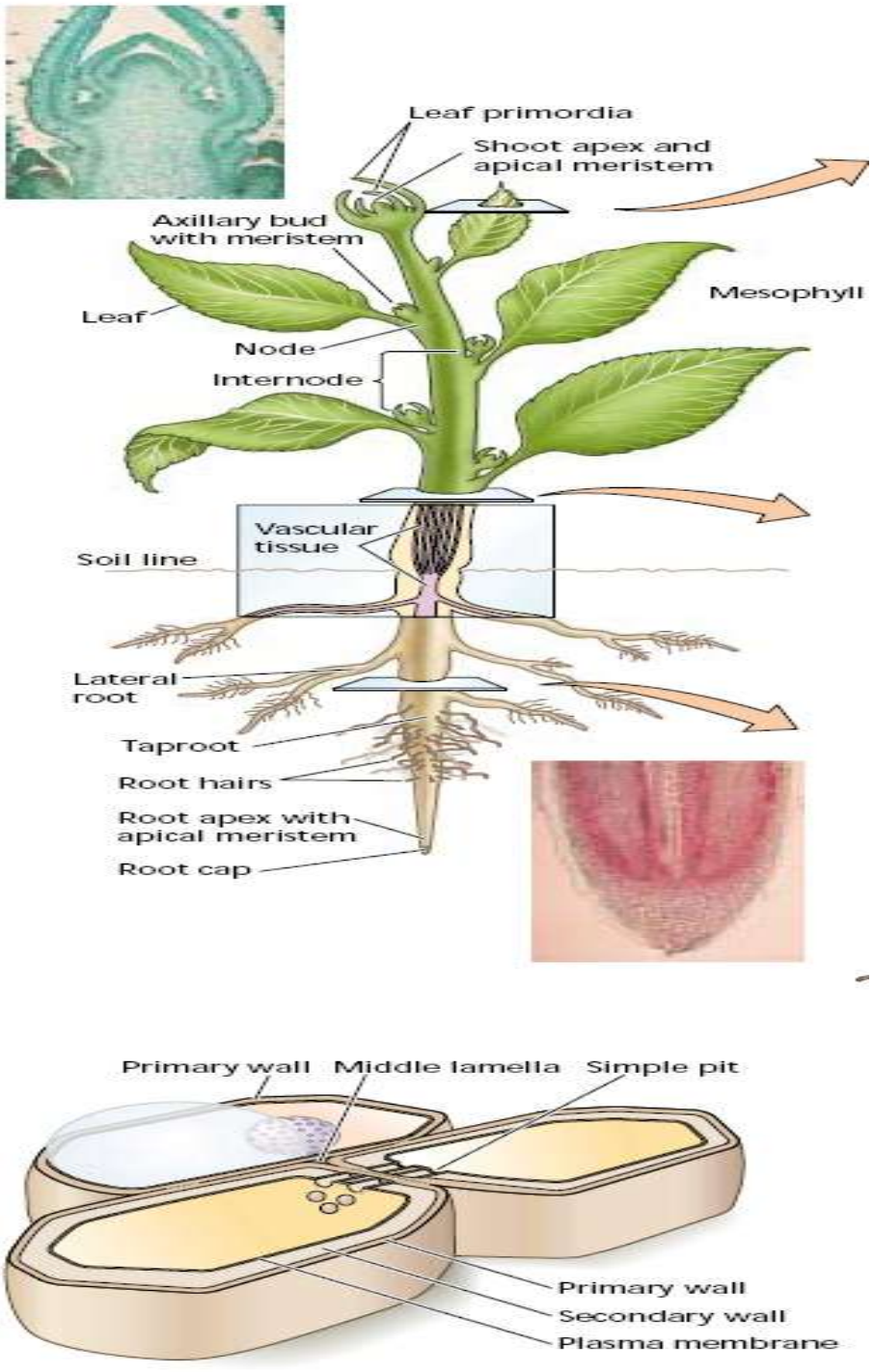
Plant cells have two types of walls: primary and secondary. **Primary cell walls** are typically thin and are characteristic of young, growing cells. **Secondary cell walls** are thicker and stronger than primary walls and are deposited when most cell enlargement has ended. Secondary cell walls owe their strength and toughness to **lignin**, a brittle, glue-like material. The evolution of lignified secondary cell walls provided plants with the structural reinforcement necessary to grow vertically above the soil and to colonize the land.



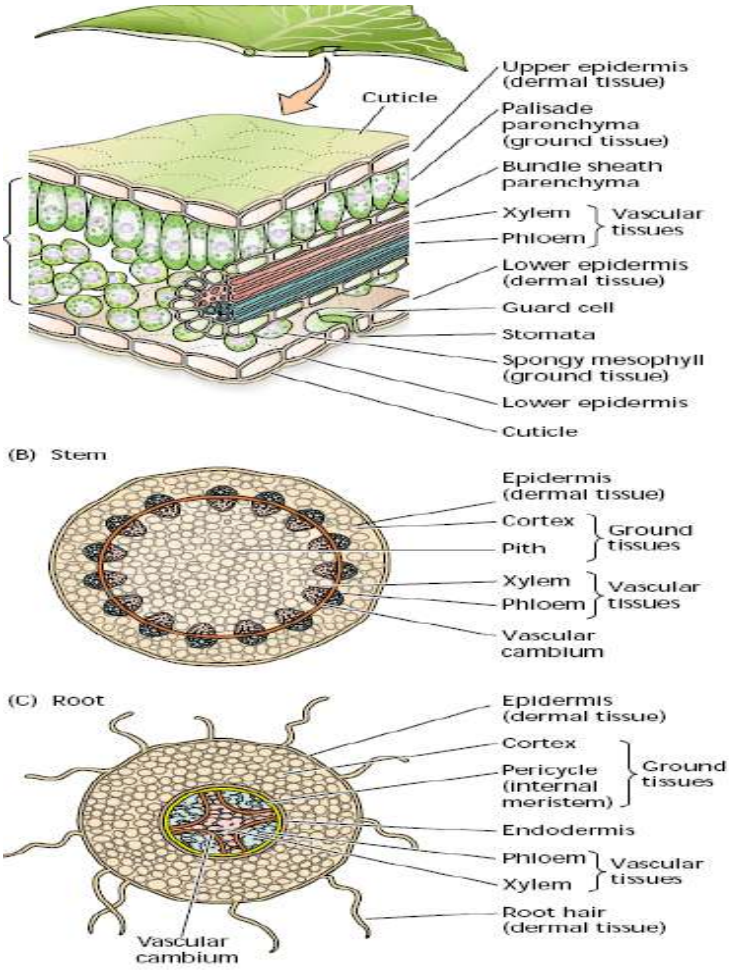
Plant anatomy

There are two categories of seed plants, gymnosperms and angiosperms. **Gymnosperms** are the less advanced type. **Angiosperms**, the more advanced type of seed plant which dominate the landscape. About 250,000 species are known, but many more remain to be characterized. The major innovation of the angiosperms is the flower; hence they are referred to as ***flowering plants***.

Three major tissue systems are found in ***flowering plants;*** in all plant organs contain dermal tissue, ground tissue, and vascular tissue. The vegetative body is composed of three organs: **leaf**, **stem**, and **root**. The primary function of a leaf is photosynthesis, that of the stem is support, and that of the root is anchorage and absorption of water and minerals. Leaves are attached to the stem at **nodes**, and the region of the stem between two nodes is termed the **internode**. The stem together with its leaves is commonly referred to as the **shoot**.



Plant parts (Source: Plant Physiology by Taiz and Zeiger)



Plant growth is concentrated in localized regions of cell division called **meristems**. Nearly all nuclear divisions (mitosis) and cell divisions (cytokinesis) occur in these meristematic regions. In a young plant, the most active meristems are called **apical meristems**; they are located at the tips of the stem and the root At the nodes, **axillary buds** contain the apical meristems for branch shoots. Lateral roots arise from the **pericycle**, an internal meristematic tissue Proximal and overlapping the meristematic regions are zones of cell elongation in which cells increase dramatically in length and width.

Cells usually differentiate into specialized types after they elongate.The phase of plant development that gives rise to new organs and to the basic plant form is called **primary growth**. Primary growth results from the activity of apical meristems, in which cell division is followed by progressive cell enlargement, typically elongation. After elongation in a given region is complete, **secondary growth** may occur. Secondary growth involves two lateral meristems: the **vascular cambium** (plural *cambia*) and the **cork cambium.** The vascular cambium gives rise to secondary xylem (wood) and secondary phloem. The cork cambium produces the periderm, consisting mainly of cork cells.

The architecture, mechanics, and function of plants depend crucially on the structure of the cell wall. The wall is secreted and assembled as a complex structure that varies in form and composition as the cell differentiates. Without a cell wall, plants would be very different organisms from what we know. Indeed, the plant cell wall is essential for many processes in plant growth, development, maintenance, and reproduction:

• Plant cell walls determine the mechanical strength of plant structures, allowing those structures to grow to great heights.

• Cell walls glue cells together, preventing them from sliding past one another. This constraint on cellular movement contrasts markedly to the situation in animal cells, and it dictates the way in which plants develop

• A tough outer coating enclosing the cell, the cell wall acts as a cellular "exoskeleton" that controls cell shape and allows high turgor pressures to develop.

• Plant morphogenesis depends largely on the control of cell wall properties because the expansive growth of plant cells is limited principally by the ability of the cell wall to expand.

• The cell wall is required for normal water relations of plants because the wall determines the relationship between the cell turgor pressure and cell volume

• The bulk flow of water in the xylem requires a mechanically tough wall that resists collapse by the negative pressure in the xylem.

• The wall acts as a diffusion barrier that limits the size of macromolecules that can reach the plasma membrane from outside, and it is a major structural barrier to pathogen invasion.

Much of the carbon that is assimilated in photosynthesis is channeled into polysaccharides in the wall. During specific phases of development, these polymers may be hydrolyzed into their constituent sugars, which may be scavenged by the cell and used to make new polymers

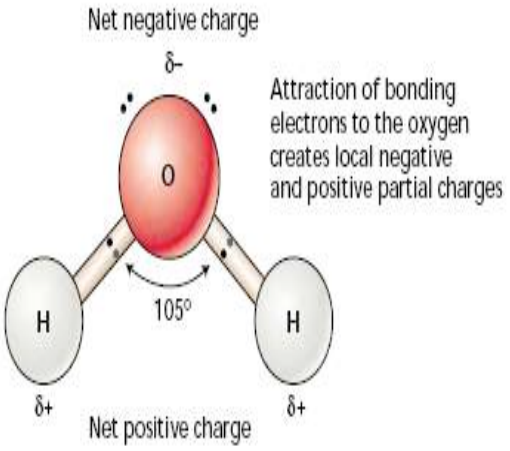
02. Role and significance of water

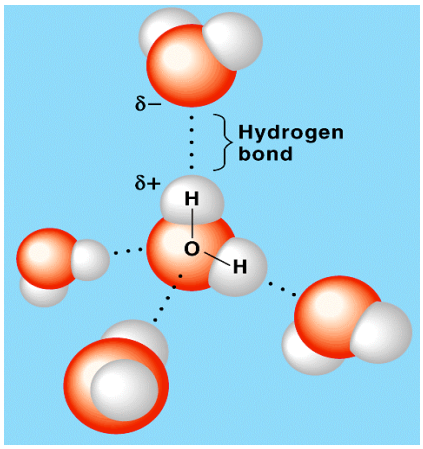
Water is said to be the liquid of life. Because, life is originated in organic environmental and in the course of evolution it became fully dependent upon water in a number of ways Water is one of the most plentiful chemicals available in the earth and the chemical formula is H_20. It is a tiny V-shaped molecule contains three atoms do not stay together as the hydrogen atoms are constantly exchanging between water molecules

The water molecule consists of an oxygen atom covalently bonded to two hydrogen atoms. The two O—H bonds form an angle of 105° (Figure). Because the oxygen atom is more **electronegative** than hydrogen, it tends to attract the electrons of the covalent bond. This attraction results in a partial negative charge at the oxygen end of the molecule and a partial positive charge at each hydrogen.

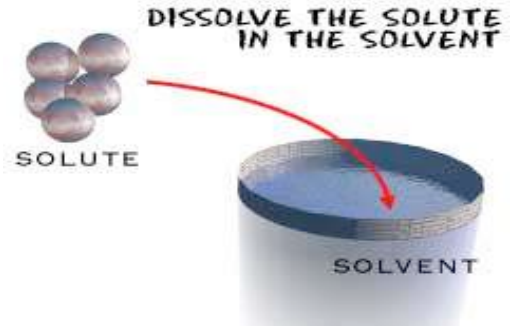
Water has special properties that enable it to act as a solvent and to be readily transported throughout the body of the plant. These properties derive primarily from the polar structure of the water molecule.

- The Polarity of water molecules gives rise to hydrogen bonds
- The Polarity of water makes an excellent solvent
- The Thermal properties of water result from hydrogen bonding
- The Cohesive and adhesive properties of water are due to hydrogen bonding





- Solute: type of molecule dissolved in another type of substance; that substance is called a...
- Solvent: substance that dissolves the solute



Importance of water to plants

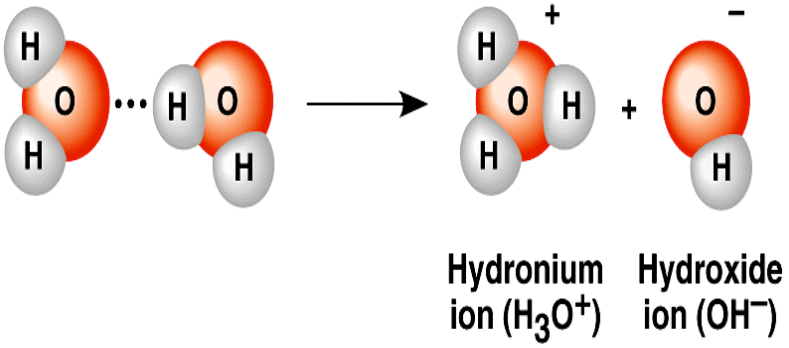
- Water typically constitutes 80 to 95% of the mass of growing plant tissues.
- Water is the main constituent of protoplasm comprising up to about 90-95 per cent of its total weight. In the absence of water, protoplasm becomes inactive and is even killed.
- Different organic constituents of plants such as carbohydrates proteins, nucleic acid and enzymes etc. Lose their physical and chemical properties in the absence of water.
- Water participates directly in many metabolic processes. Inter conversion of carbohydrates and organic acids depend upon hydrolysis and condensation reaction.
- Water increases the rate of respiration. Seeds respire fast in the presence of water.
- Water is the source of hydrogen atom for the reduction of CO_2 in the reaction of photosynthesis.

CONCENTRATION EXAMPLES

- High solute concentration: lots of sugar dissolved in a relatively small amount of water
- Low solute concentration: little sugar dissolved in a relatively high amount of water

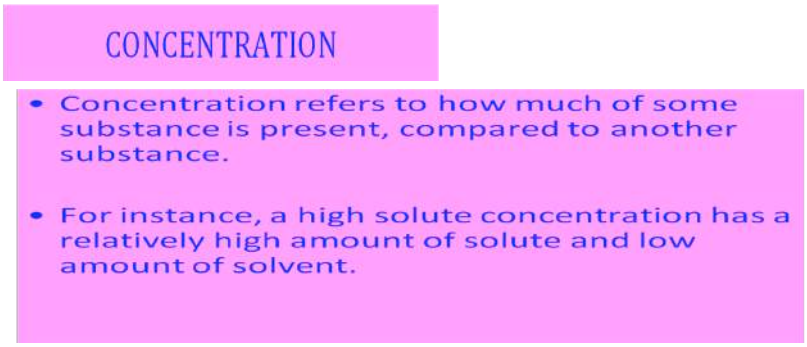
- Water acts as a solvent and acts as a carrier for many substance. If forms the medium in which several reactions take place.
- Water present in the vacuoles helps in maintaining the turgidity of the cells which is a must for proper activities of life and to maintain this from and structure.
- Water helps in translocation of solutes
- In tropical plants, water plays a very important role of thermal regulation against high temperature.
- The elongation phase of cell growth depends on absorption of water.

"Dissociation" of water



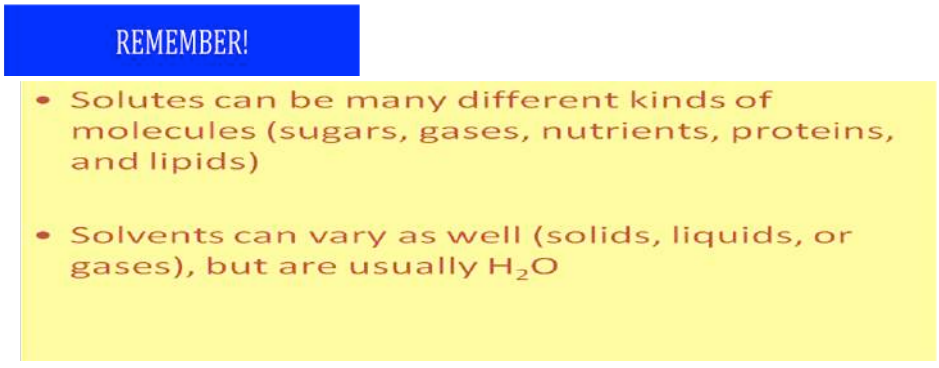
Properties of water

1. Solvent for electrolyte & non electrolyte
2. High specific heat
3. High latent heat of vaporization (540 cal g-1)
4. Cohesive and Adhesive Properties
5. High surface tension
6. High Tensile Strength
7. Stabilizes temperature
8. Transparent to visible radiation
9. Low viscosity

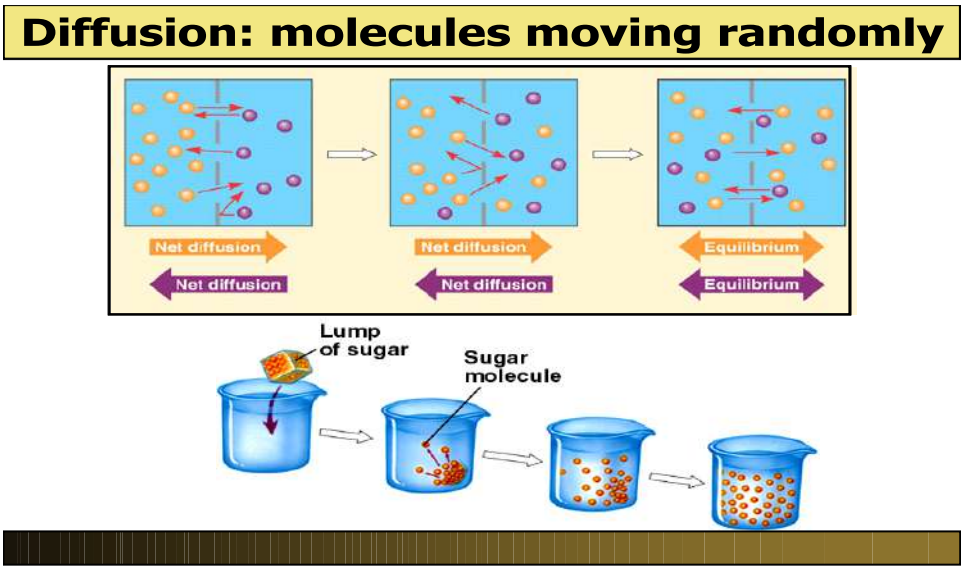


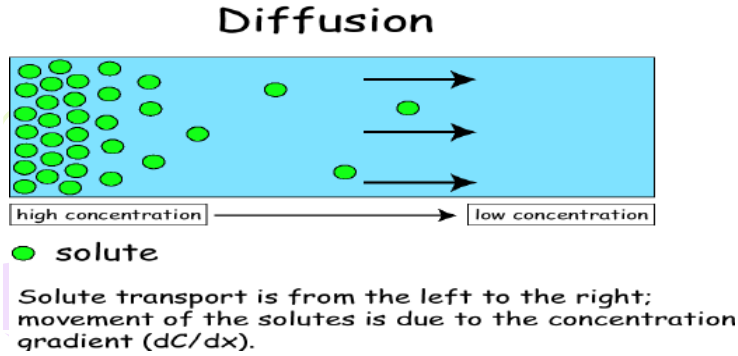
HOW IT HELPS IN PLANTS?

WATER PLAYS A CRUCIAL ROLE in the life of the plant. For every gram of organic matter made by the plant, approximately 500 g of water is absorbed by the roots, transported through the plant body and lost to the atmosphere. Even slight imbalances in this flow of water can cause water deficits and severe malfunctioning of many cellular processes. Thus, every plant must delicately balance its uptake and loss of water.



Diffusion, osmosis and imbibitions





The movement of materials in and out of the cells in plants taken place in a solution or gaseous form. Although the exert process of this is not very clear, three physical process are usually involved in it. They are diffusion, osmosis and imbibition.

The movement of particles or molecules from a region of higher concentrations to a region of lower concentration is called as diffusion. The rate of diffusion of gases is faster than liquids or solutes. The diffusion particles have a certain pressure called as the diffusion pressure which is directly proportional to the number as concentration of the diffusing particles. These forms the diffusion takes place always from a region of higher diffusion pressure to a region of lower diffusion pressure (i.e) along a diffusion pressure gradient. The rate of diffusion increases if,

i) Diffusion pressure gradient is steeper

ii) Temperature is increased

iii) Density of the differing particles is lesser

iv) Medium through which diffusion occurs is less concentrated.

Diffusion of more than one substance at the same time and place may be at different rates and in different direction, but is independent of each other. A very common example of this is the gaseous exchange in plants.

Beside osmotic diffusion the above mentioned simple diffusion also plays a very important role in the life of the plants.

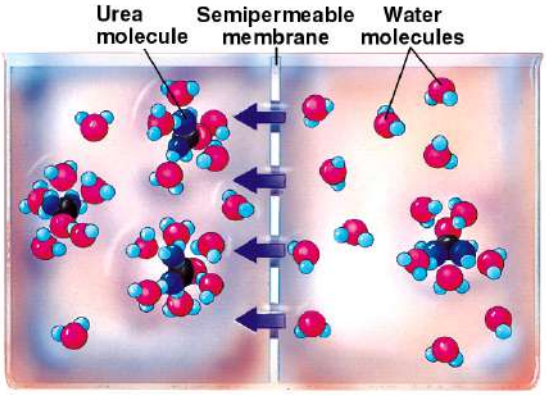
- It is an essential step in the exchange of gases during respiration and photosynthesis
- During passive salt uptake, the ions are absorbed by diffusion
- It is important in stomatal transpiration as the last step in the pollen, where diffusion of water vapour from the interrelation space into the outer atmosphere occurs through open stomata.

Osmosis

The diffusion of solvent molecules into the solution through a semi permeable membrane is called as osmosis (some times called as *Osmotic diffusion*). In case there are two solutions of different concentration separated by the semi permeable membrane, the diffusion of solvent will take place from the less concentrated suitable into the more concentrated solution till both the solutions attain equal concentration.

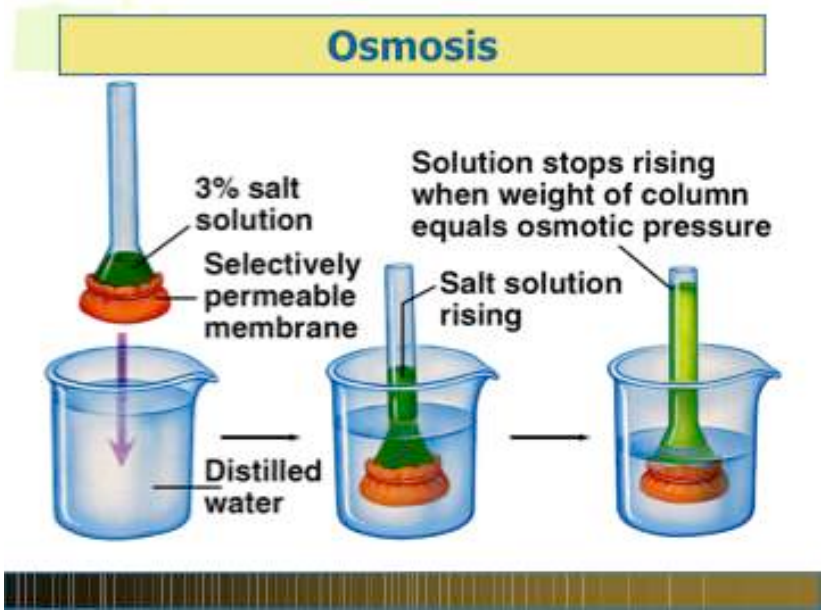
What happens in osmosis

- **Osmosis is the diffusion of water across selectively permeable membranes.**

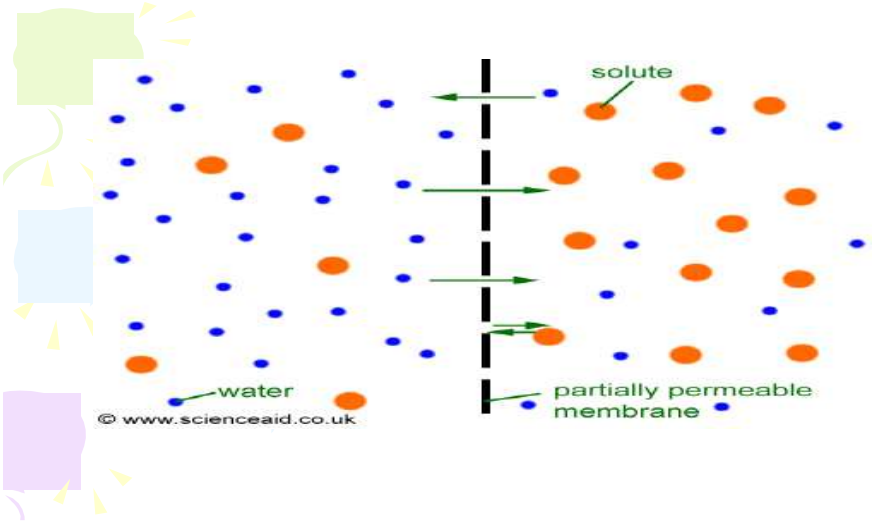


Osmotic pressure

As a result of the separation of solution from its solvent (or) the two solutions by the semi permeable membrane, a pressure is developed in solution to the pressure by dissolved solutes in it. This is called as osmotic pressure (O.P). OP is measured in-terms of atmospheres and is directly proportional to the concentration of dissolved solutes in the solution. More concentration solution has higher O.P. O.P of a solution is always higher than its pure solvent.



During osmosis, the movement of solvent molecules taken place form the solution whose osmotic pressure is lower (i.e less concentration as hypotonic) into the solution whose osmotic pressure is higher (i.e, more concentrated as hypertonic). Osmotic diffusion of solvent molecules will not take place if the two solutions separated by the semipermeable membrane are of equal concentration having equal *Osmotic pressures* (i.e., they are isotonic). In plant cells, plasma membrane and tonoplant act as selectively permeable or differentially permeable membrane.



End-osmosis

Of a living plant cell is placed in water or hypotonic solution whose O.P is lower than cell sap, water in-terms into the cell sap by osmosis and the process is called end osmosis. As a result of entry of water with the cell sap, a pressure is developed which press the protoplasm against the cell wall and become turgid. This pressure is called a turgor pressure.

Consequence of the turgor pressure is the wall pressure which is exerted by the elastic cell wall against the expanding protoplasm. At a given time, turgor pressure (T.P) equals the wall pressure (W.P).

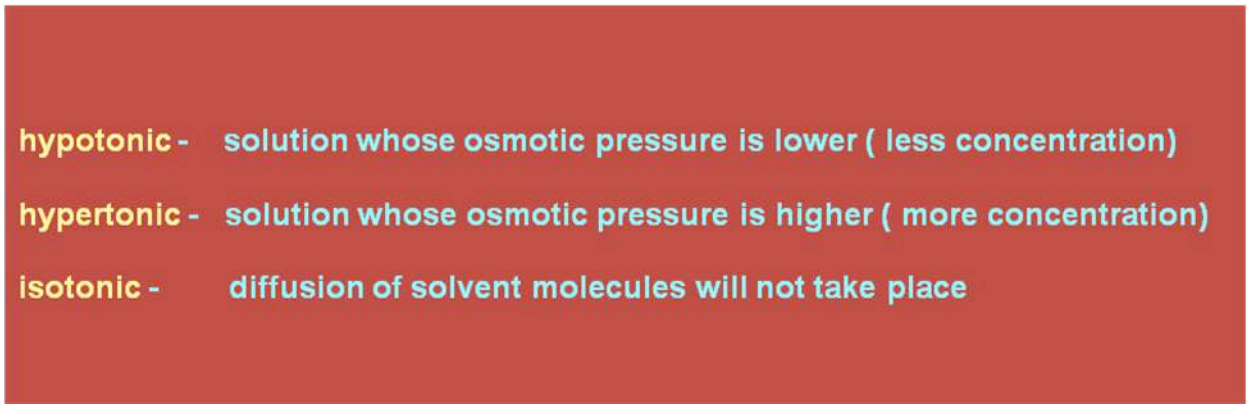
T.P = W.P

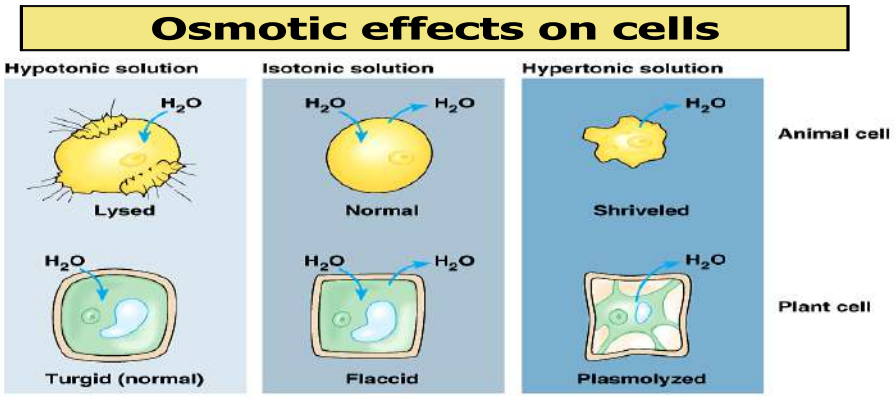
Exosmosis

If on the other hand, the plant cell is placed in hypertonic solution (whose O.P is higher than cell sap) the water cover out the cell sap into the outer solution and the cell becomes flaccid. This process is known as exosmosis. Cell (or) tissue will remain as such in isotonic solution.

Significance of osmosis in plants

1. Large quantities of water are absorbed by roots from the soil by osmosis
2. Cell to cell movement of water and other substances dissolve is involves osmosis
3. Opening and closing of stomata depend upon the turgor pressure of guard cells
4. Due to osmosis, the turgidity of the cells and hence the shape or from of them organs is maintained.
5. The resistance of plants to drought and frost increases with increase in osmotic pressure to later cells
6. Turgidity of the cells of the young seedling allows them to come out of the soil.





Imbibition

Certain substances if placed in a particular liquid absorb it and swell up. For example, when some pieces of grass or dry wood or dry seeds are placed in water they absorb the water quickly and swell up considerably so that their volume is increased. These substances are called as imbibants and the phenomenon as imbibition, certain force of attraction is existing between imbibants and the involved substance. In plants, the hydrophilic colloids *viz*., protein and carbohydrates such as starch, cellulose and pectic substance have strong altercation towards water.

Imbibition plays a very important role in the life of plants. The first step in the absorption of water by the roots of higher plants is the imbibition of water by the cell walls of the root hairs. Dry seeds require water by imbibition for germination.

As a result of imbibition, a pressure is developed which is called as imbibition pressure or matric potential (ψ_m). It is analogous to the osmotic potential of a solution. With reference to pure water, the values of ψ_m are always negative. The water potential of an imbibant is equal to its matric potential plus any turgor or other pressure (pressure potential) which may be imposed upon the imbibant.

$$\psi_w = \psi_m + \psi_P$$

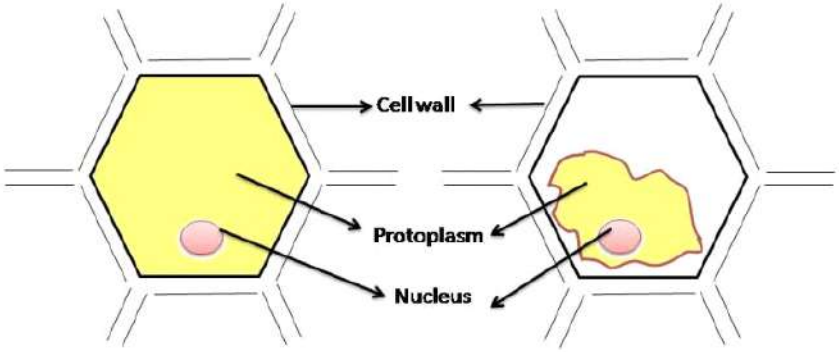
If the imbibant is unconfined to turgor or such pressure, the equation can be significant to

$$\psi_w = \psi_m$$

Plasmolysis

When a plant cell or tissue is placed in a hypertonic solution water cover out from the cell sap into the outer solution of exosmosis and the protoplasm begins to sprinkler or contract. The protoplasm separate from the cell wall and assures a spherical form and them phenomenon is called plasmolysis. Incipient plasmolysis is stage where protoplasm begins to contract from the cell wall. If a plasmolysed cell in tissue is placed in water, the process of endosmosis take place. Water enters into the cell sap, the cell becomes turgid and the protoplasm again assumes it normal shape and position. This phenomenon is called deplasmolysis.

Diagrammatic view of normal plant cell and plasmolysed plant cell



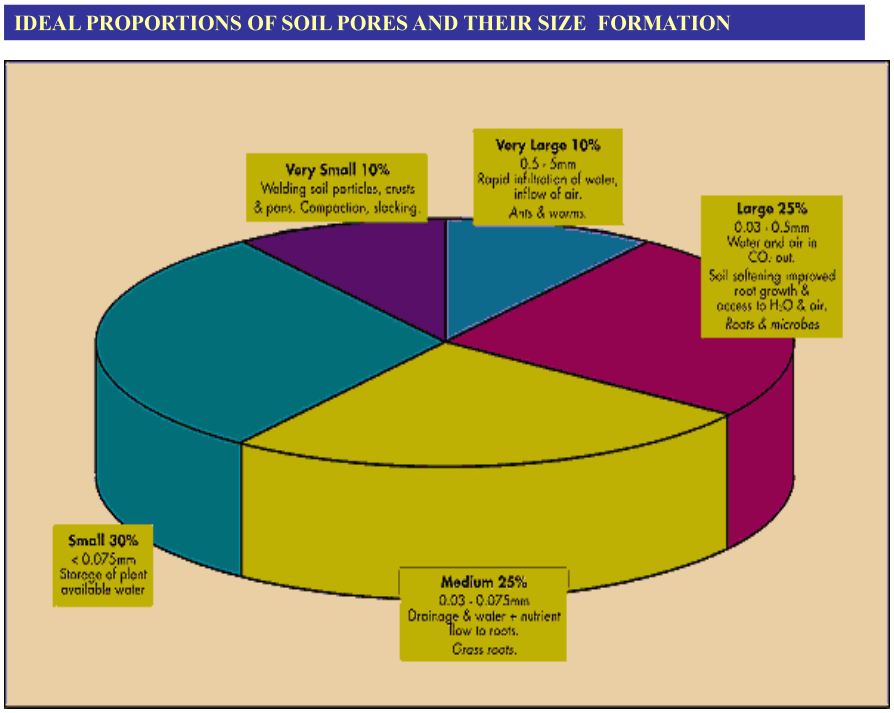
Advantages of plasmolysis

1. It indicates the semi permeable nature of the plasma membrane.
2. It is used in determine the osmotic pressure of the cell sap.
3. Plasmolysis is used in salting of meat and fishes. Addition of concentrated sugar solution to jam and jellies check the growth of fungi and bacteria which become plasmolysed in concentrated solution.

03. Field capacity, Available soil water and permanent wilting point

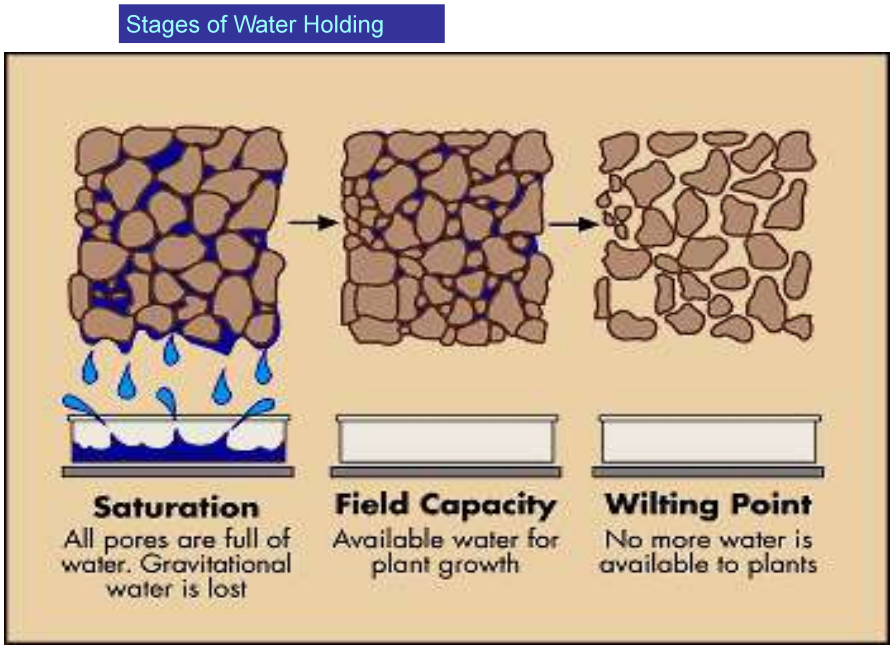
Field capacity or water holding capacity of the soil

After heavy rain fall or irrigation of the soil some water is drained off along the slopes while the rest percolates down in the soil. Out of this water, some amount of water gradually reaches the water table under the force of gravity (gravitational water) while the rest is retained by the soil. This amount of water retained by the soil is called as field capacity or water holding capacity of the soil.



Field capacity is affected by soil profiles soil structure and temperature. The effective depth of a soil, as determined by physical and chemical barriers, together with the clay content of the soil within that depth, determine the water holding capacity of the profile, and how much of the water is available to plants. Effective soil depth varies between plant species. Wheat is used as the benchmark plant in this assessment. Available water holding

capacity rankings are estimated from soil texture, structure and stone content within the potential root zone of a wheat plant.



Water-holding capacity is controlled primarily by soil texture and organic matter. Soils with smaller particles (silt and clay) have a larger surface area than those with larger sand particles, and a large surface area allows a soil to hold more water. In other words, a soil with a high percentage of silt and clay particles, which describes fine soil, has a higher water-holding capacity. The table illustrates water-holding-capacity differences as influenced by texture. Organic matter percentage also influences water-holding capacity. As the percentage increases, the water-holding capacity increases because of the affinity organic matter has for water.

It is the water content of the soil after downward drainage of gravitational water. It is the capillary capacity of a soil. It is the upper limit of soil water storage for the plant growth. At field capacity, the soil water potential is –0.1 to –0.3 bars.

Texture	Field Capacity	Wilting point	Available water
Coarse sand	0.6	0.2	**0.4**
Fine sand	1.0	0.4	0.6
Loamy sand	1.4	0.6	0.8
Sandy loam	2.0	0.8	1.2
Light sandy clay loam	2.3	1.0	1.3
Loam	2.7	1.2	1.5
Sandy clay loam	2.8	1.3	1.5
Clay loam	3.2	1.4	1.8
Clay	4.0	2.5	1.5
Self-mulching clay	4.5	2.5	2.0

Water potential

Every component of a system possesses free energy capable of doing work under constant temperature conditions. For non-electrolytes, free energy / mole is known as chemical potential. With refuse to water, the chemical potential of water is called as water potential. The chemical potential is denoted by a Greek letter Psi (ψ).

For pure water, the water potential is Zero. The presence of solute particles will reduce the free energy of water or decrease the water potential. Therefore it is expressed in vegetative value.

It is therefore, water potential of solution is always less than zero so in negative value.

For solutions, water potential is determined by three internal factors i.e.

ψ_w $= \psi_w + \psi_s + \psi_p$

ψ_S = is the solute potential or osmotic potential

ψ_p = pressure potential or turgor potential

ψ_w = is the matric potential. Matric potential can be measured for the water molecules adhering on the soil particles and cell wall.

In plant system, the matric potential is disregarded.

Therefore,

$\psi_w = \psi_s + \psi_p$

Osmotic pressure

Osmotic pressure is equivalent to osmotic potential but opposite in sign.Osmotic pressure in a solution results due to the presence of solutes and the solutes lower the water potential. Therefore osmotic pressure is a quantitative index of the lowering of water potential in a solution and using thermodynamic terminology is called as osmotic potential.

Osmotic pressure and osmotic potential are numerically equal but opposite in sign.

Osmotic pressure has positive sign

Osmotic potential has negative sign (ψ_s)

For eg.

I_A OP = 20 atm.

ψ_w = - 20 atm

Turgor pressure

In plant cell, the turgor pressure results due to the presence of water molecules is turgor pressure. The potential created by such pressures is called presure potential (ψ_p)

In a normal plant cell, the water potential

$\psi_w = \psi_s + \psi_p$ – partially turgid cell

(High)

ψ_w = Zero - Fully turgid cell

(Highest)

$\psi_w = \psi_s$ - Flaccid cell or plasmolysed cell

(Lowest)

Water relation

Water forms the major constituent of living (cells) things and the cells originated in a highly aqueous medium and all the vital processes of the life are carried out in it. Besides, water predominately arts as a source of hydrogen to plants and is released by the photolysis of water during photosynthesis.

In living tissue, water is the medium for many biochemical reactions and extraction process. Inorganic nutrients, photosynthesis, bases and hormones are all transported in aqueous solution. Evaporation of water can control the temperature of leaf on canopy soil nutrients are available to plant roots only when dissolved in water. In short, water is essential for life and plays a unique role in virtually all biological process.

Example:

There are 2 cells A and B in contact with each other, cell A has a pressure potential (turgor pressure) of 4 bars and certain sap with an osmotic potential of -12 bars.

Cell B has presume potential of 2 bars and certain sap with osmotic potential of -5 bars.

Then,

ψ_w of cell A $= \psi_s + \psi_p$

$= -12 + (+4)$

$= -8$ bars

ψ_w of cell B $= -5+(+2)$

-3 bars

Hence, water will move from cell B to cell A (i.e., towards lower or more negative water potential) with a form of (-8-(-3) = -5 bars.

Diffusion Pressure Déficit (DPD) (Suction pressure)

Diffusion pressure of a solution is always lower than its pure solvent. The difference between the diffusion pressure of the solution and its solvent at a particular temperate and

atmosphere conditions is called as diffusion pressure deficit (D.P.D). If the solution is more concentrated D.P.D increases but it decreases with the dilution of the solution,

D.P.D of the cell sap or the cells is a measure of the ability of the cells to absorb water and hence is often called as the suction pressure (S.P). It is related with osmotic pressure (O.P) and turgor pressure (T.P) of cell sap and also the wall pressure (W.P) as follows.

D.P.D. (S.P) = O.P – W.P

But

(W.P) = T.P

D.P.D = O.P – T.P

Due to the entry of the water the osmotic pressure of the cell sap decreases while its turgor pressure is increased so much so that in a fully turgid cell T.P equals the O.P

O.P = T.P = D.P.D = O

In fully plasmolysed cells: T.P = O

So D.P.D = O.P

D.P.D. incase of plant cells is not directly proportional to their osmotic pressure or the concentration of the cell sap but depend both on O.P and T.P. Higher osmotic pressure of the cell sap is usually accompanied by lower turgor pressure so that its D.P.D is greater and water enters into it. But sometimes it is possible that two cells are in contact with each other one having higher O.P and also higher turgor pressure than the other cell and still its does not draw water. It is because of its lower D.P.D., no matter is O.P is higher.

Cell a		Cell b	
O.P	= 25 atm. ⟶	O.P	= 30 atm
T.P	= 15 atm.	T.P	=10 atm. A
D.P.D	= 10 atm.	D.P.D	= 30 atm.

Cell a		Cell b		
O.P	= 35 atm. ←	O.P	= 40 atm	
T.P	= 10 atm.	T.P	= 20 atm.	B
D.P.D	= 25 atm.	D.P.D	= 20 atm.	

Entry of water into the cell depends on D.P.D and not on O.P only

ABSORPTION OF WATER – MODE OF WATER ABSORPTION – ACTIVE AND PASSIVE ABSORPTION AND FACTORS AFFECTING ABSORPTION.

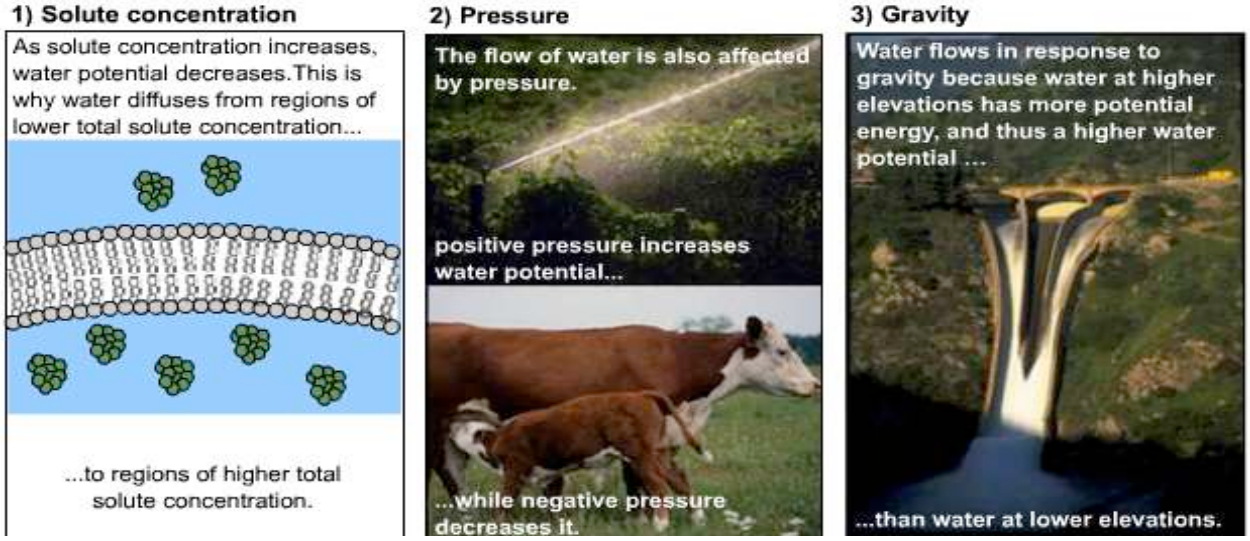
PRELUDE OF WATER POTENTIAL

Most organisms are comprised of at least 70% or more water. Some plants, like a head of lettuce, are made up of nearly 95% water. When organisms go dormant, they loose most of their water. For example, seeds and buds are typically less than 10% water, as are desiccated rotifers, nematodes and yeast cells. Earth is the water planet (that's why astronomers get so excited about finding water in space). Water is the limiting resource for crop productivity in most agricultural systems

LEARN MORE ABOUT WATER POTENTIAL

- In general, water always moves down its water potential gradient from areas of higher water potential to areas of lower water potential.
- Water potential is typically measured as the amount of pressure needed to stop the movement of water.
- The unit used to express this pressure is the megapascal (MPa).

The three factors that most commonly determine water potential are



WHAT IS WATER POTENTIAL?

Water potential is the potential energy of water relative to pure free water (e.g. deionized water) in reference conditions. It quantifies the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or matrix effects including surface tension. Water potential is measured in units of pressure and

is commonly represented by the Greek letter (Psi). This concept has proved especially useful in understanding water movement within plants, animals, and soil.

Components of water potential

Much different potential affect the total water potential and sum of these potentials determines the overall water potential and the direction of water flow:

$$\Psi = \Psi_0 + \Psi_\pi + \Psi_p + \Psi_s + \Psi_v + \Psi_m$$

Where:

- Ψ_0 is the reference correction,
- Ψ_π is the solute potential,
- Ψ_p is the pressure potential,
- Ψ_s is the gravimetric component,
- Ψ_v is the potential due to humidity, and
- Ψ_m is the potential due to matrix effects (e.g., fluid cohesion and surface tension.)

COMPONENT OF WATER POTENTIAL

1. Pressure potential

Pressure potential is based on mechanical pressure, and is an important component of the total water potential within plant cells. Pressure potential is increased as water enters a cell. As water passes through the cell wall and cell membrane, it increases the total amount of water present inside the cell, which exerts an outward pressure that is retained by the structural rigidity of the cell wall.

The pressure potential in a living plant cell is usually positive. In plasmolysed cells, pressure potential is almost zero. Negative pressure potentials occur when water is pulled through an open system such as a plant xylem vessel. Withstanding negative pressure potentials (frequently called tension) is an important adaptation of xylem vessels.

2.Solute potential

Pure water is usually defined as having a solute potential (Ψ_π) of zero, and in this case, solute potential can never be positive. The relationship of solute concentration (in molarity) to solute potential is given by the van 't Hoff equation:

$$\Psi_\pi = - miRT$$

Where

m - The concentration in molarity of the solute,

i - The van 't Hoff factor, the ratio of amount of particles in solution to amount of formula units dissolved,

R - The ideal gas constant, and T is the absolute temperature.

3. Matrix potential

When water is in contact with solid particles (e.g., clay or sand particles within soil) adhesive intermolecular forces between the water and the solid can be large and important. The forces between the water molecules and the solid particles in combination with attraction among water molecules promote surface tension and the formation of menisci within the solid matrix. Force is then required to break these menisci. The magnitude of matrix potential depends on the distances between solid particles--the width of the menisci and the chemical composition of the solid matrix. In many cases, matrix potential can be quite large and comparable to the other components of water potential discussed above.

It is worth noting that matrix potentials are very important for plant water relations. Strong (very negative) matrix potentials bind water to soil particles within very dry soils. Plants then create even more negative matrix potentials within tiny pores in the cell walls of their leaves to extract water from the soil and allow physiological activity to continue through dry periods.

4. Gravity (Ψ_g):

Contributions due to gravity which is usually ignored unless referring to the tops of tall trees.

ABSORPTION OF WATER

We know from a very early age that plants obtain water through their roots, though it is not perhaps until our school biology lessons that we learn of the important role that water plays in the process of photosynthesis. Most of the water absorption is carried out by the younger part of the roots. Just behind the growing tip of a young root is the piliferous region, made up of hundreds of projections of the epidermal tissue, the root hairs.

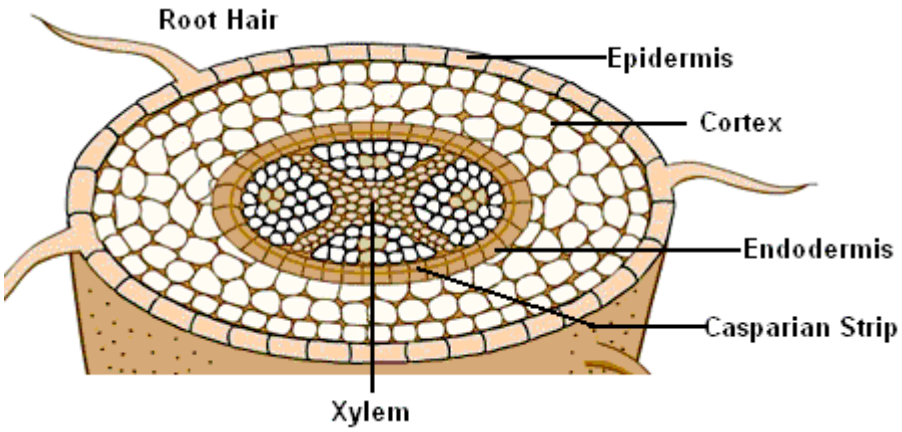
STRUCTURE INVOLVED IN WATER ABSORPTION

In higher plants water is absorbed through root hairs which are in contact with soil water and form a root hair zone a little behind the root tips. Root hairs are tubular hair like prolongations of the cells of the epidermal layer (when epidermis bears root hairs it is also known as pilloferous layer of the roots. The walls of root hairs are permeable and consist of pectic substances and cellulose which are strongly hydrophilic in nature root hairs contain vacuoles filled with cell sap. When roots elongate, the older root hairs die and new root hairs are developed so that they are in contact with fresh supplies of water in the soil. Lateral Movement of water is achieved through root. This can described as follows:

ROOTS

Often roots are overlooked, probably because they are less visible than the rest of the plant. However, it's important to understand plant root systems (Fig 1) because they have a pronounced effect on a plant's size and vigor, method of propagation, adaptation to soil types, and response to cultural practices and irrigation.

Fig 1. Diagrammatically the internal structure of a typical root



Roots typically originate from the lower portion of a plant or cutting. They have a root cap, but lack nodes and never bear leaves or flowers directly. Their principal functions are to absorb nutrients and moisture, anchor the plant in the soil, support the stem, and store food. In some plants, they can be used for propagation.

STRUCTURE OF ROOTS

Internally, there are three major parts of a root (Fig 2):

- The **meristem** is at the tip and manufactures new cells; it is an area of cell division and growth.
- Behind the meristem is the **zone of elongation**. In this area, cells increase in size through food and water absorption. As they grow, they push the root through the soil.
- The **zone of maturation** is directly beneath the stem. Here, cells become specific tissues such as epidermis, cortex, or vascular tissue.

A root's **epidermis** is its outermost layer of cells (Fig 2). These cells are responsible for absorbing water and minerals dissolved in water. **Cortex** cells are involved in moving water from the epidermis to the **vascular tissue** (xylem and phloem) and in storing food. Vascular tissue is located in the center of the root and conducts food and water.

Fig 2. Cross section of roots

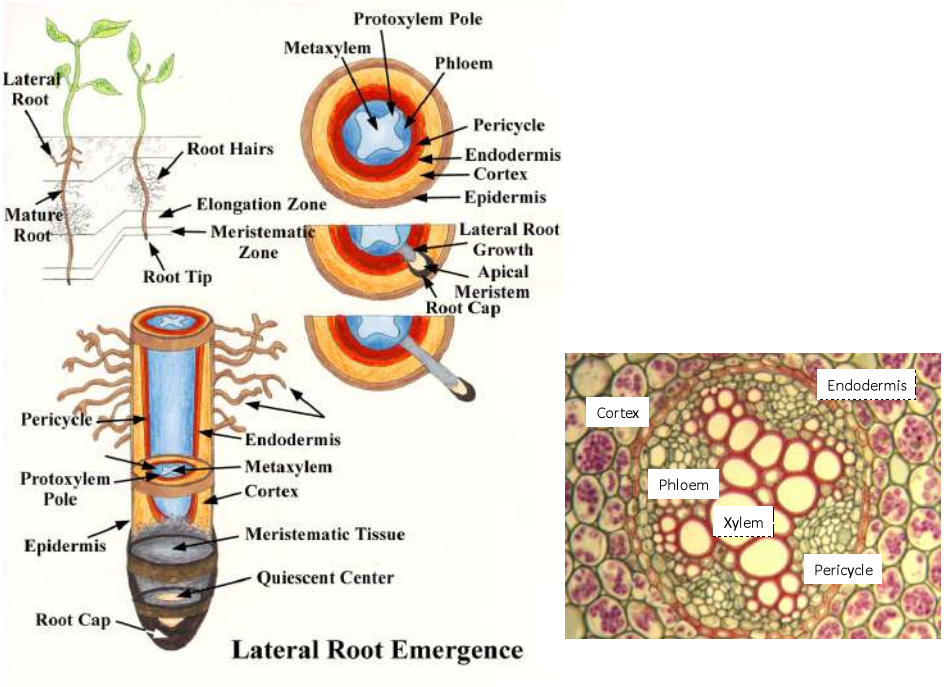
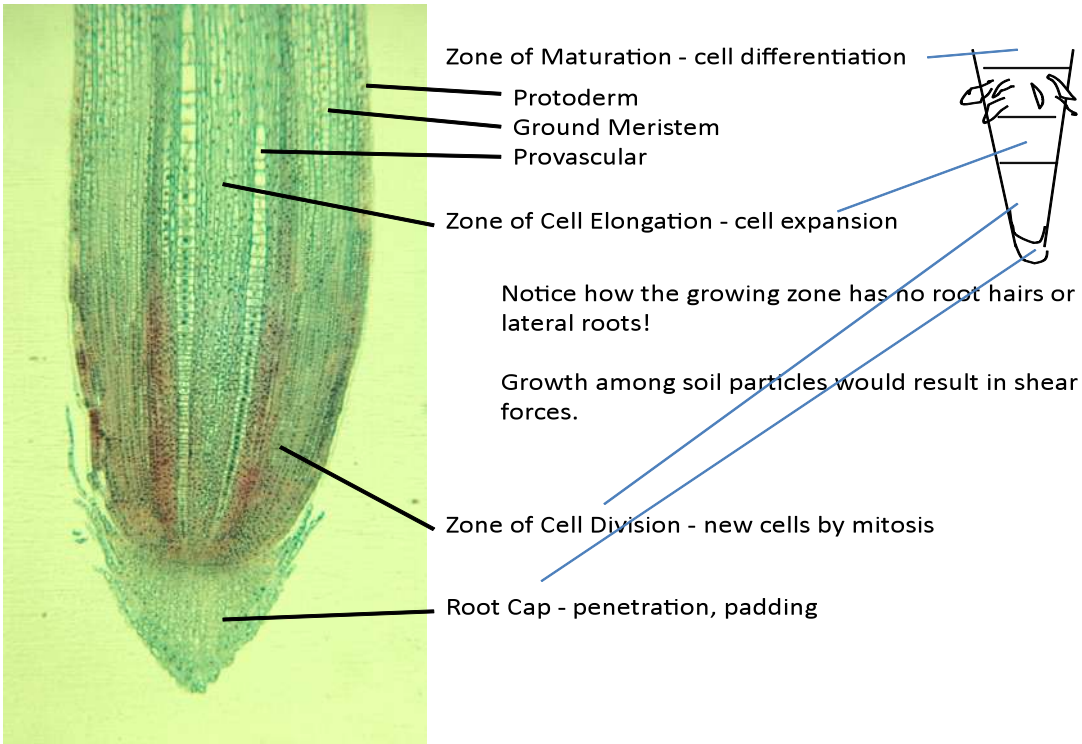


Fig 3. Structure of root hair



Externally, there are two areas of importance: the root cap and the root hairs (Figure 3). The **root cap** is the root's outermost tip. It consists of cells that are sloughed off as the root grows through the soil. Its function is to protect the root meristem.

Root hairs are delicate, elongated epidermal cells that occur in a small zone just behind the root's growing tip. They generally appear as fine down to the naked eye. Their function is to increase the root's surface area and absorptive capacity. Root hairs usually live 1 or 2 days. When a plant is transplanted, they are easily torn off or may dry out in the sun.

WATER MOVEMENT MECHANISM IN PLANTS

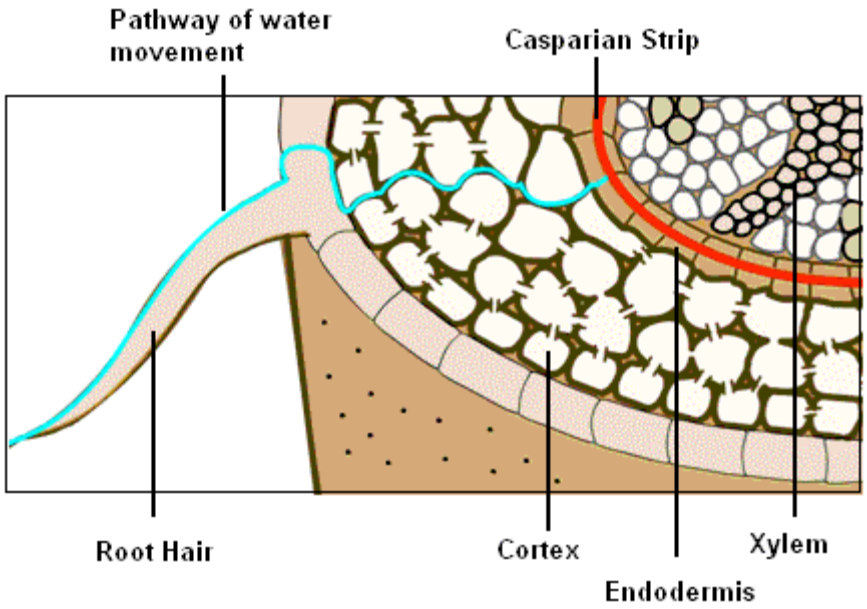
In plants, following two pathways are involved in the water movement. They are

(1) Apoplastic pathway
(2) Symplastic pathway
(3) Transmembrane pathway

1. Apoplastic pathway (Fig 4)

The apoplastic movement of water in plants occurs exclusively through the **cell wall** without crossing any membranes. The cortex receive majority of water through apoplastic way as loosely bound cortical cells do not offer any resistance. But the movement of water in root beyond cortex apoplastic pathway is blocked by casparian strip present in the endodermis.

Fig 4



2. Symplastic pathway (Fig 5)

The movement of water from one cell to other cell through the **plasmodesmata** is called the symplastic pathway of water movement. This pathway comprises the network of cytoplasm of all cells inter-connected by plasmodermata.

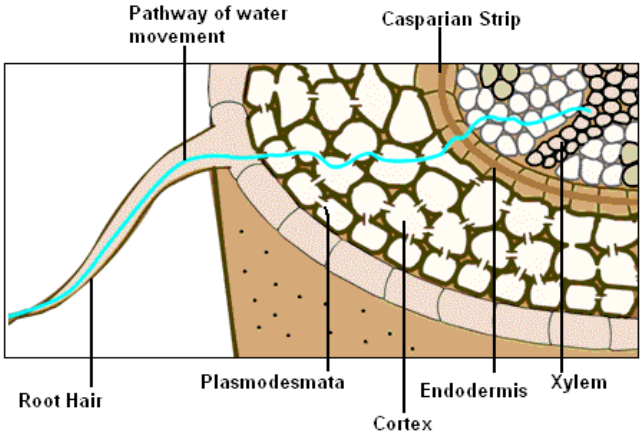
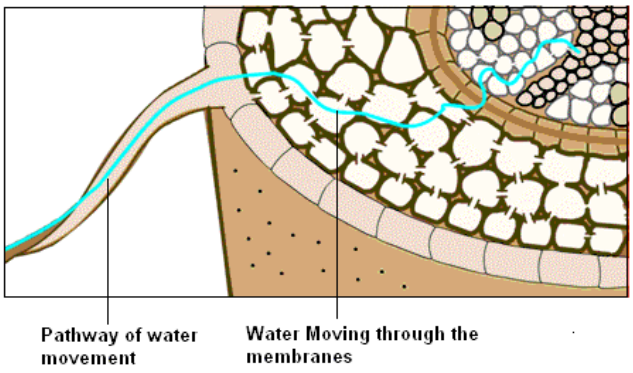


Fig 5

3. Transmembrane pathway (Fig 6)

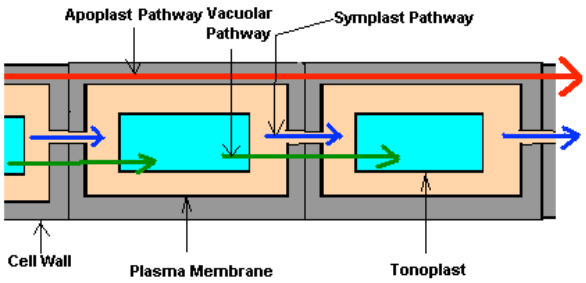
In plant roots, water movement from soil till the endodermis occurs through apoplastic pathway i.e. only through cell wall. The casparian strips in the endodermis are made-up of wax -like substance suberin which blocks water and solute movement through the cell wall of the endodermis. As a result water is forced to move through cell membranes and may cross the tonoplast of vacuole. This movement of water through cell membranes is called transmembrane pathway.

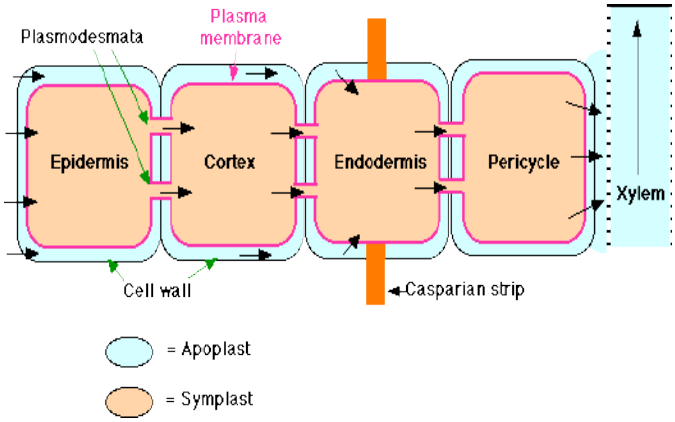
Fig 6.



Following schematic diagram showing the apoplastic and symplastic pathway of water movement through root (Fig 7)

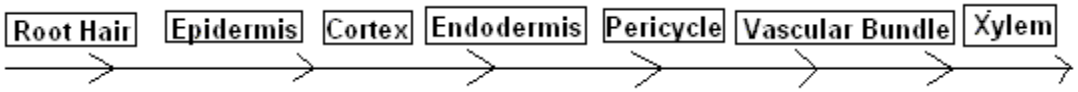
Fig 5





Apoplastic (Red) and symplastic (Blue) and transmembrane (green) pathways of movement of substances in a plant cell

With the help of the following schematic arrow flow chart, you can understand the path of water from soil to root xylem.



MECHANISM OF WATER ABSORPTION

1. Active absorption of water

In this process the root cells play active role in the absorption of water and metabolic energy released through respiration is consumed active absorption may be of two kinds.

Steps involved in the active osmotic absorption of water

First step in osmotic the osmotic absorption of water is the imbibition of soil water by the hydrophilic cell walls of root hairs. Osmotic pressure of the cell sap of root hairs is usually higher than the OP of the soil water. Therefore, the DPD and suction presume in the root hairs become higher and water from the cell walls enters into them through plasma membrane by osmotic diffusion. As a result, OP, suction pressure and DPD of root hairs how become lower, while their turgor pressure is increased.

Now the cortical cells adjacent to root hairs have high OP, SP & DPD in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from root hairs by osmotic diffusion. In the same way, by cell to cell osmotic diffusion gradually reaches the inner most cortical cells and the endodermis.

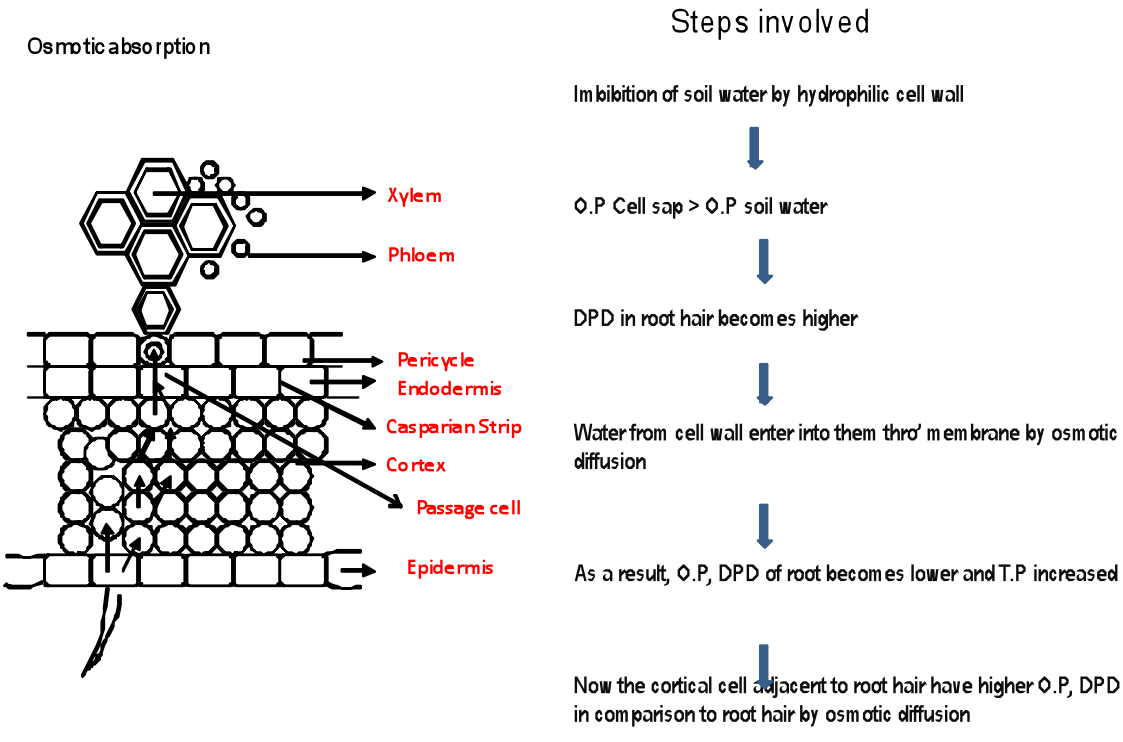
Osmotic diffusion of water into endodermis takes place through special thin walled passage cells because the other endodermis cells have casparian strips on thin walls which are impervious to water.

Water from endodermis cells is down into the cells of pericycle by osmotic diffusion which now become turgid and their suction pressure in decreased.

In the last step, water is drawn into xylem from turgid pericycle cells (In roots the vascular bundles are radical and protoxylem elements are in contact with pericycle). It is because in the absence of turgor presume of the xylem vessels, the SP of xylem vessels become higher than SP of the cells of the pericycle when water enters into xylem from pericycle a presume is developed in the xylem of roots which can raise the water to a certain height in the xylem. This pressure is called as root pressure.

(A) Osmotic absorption

Water is absorbed from the soil into the xylem of the roots according to osmotic gradient.



Likewise, water moves by osmotic diffusion and reaches endodermis

↓

Endodermis water moves thro' passage cell (because casparian cell)

↓

Now water reaches pericycle, pericylce becomes turgid and their DPD is decreased

↓

Last step, water is drawn into xylem from turgid pericycle cells (protoxylem in contact)

↓

Pressure is developed in the xylem of root by water entry – Root pressure

(B) Non-osmotic absorption

Water is absorbed against the osmotic gradient. Sometimes, it has been observed that absorption of water takes place even when OP of soil water is high than OP of cell sap. This type of absorption which is non-osmotic and against the osmotic gradient requires the expenditure of metabolic energy probably through respiration.

2. Passive absorption of water

It is mainly due to transpiration, the root cells do not play active role and remain passive.

STEPS:

Transpiration creates tension in water in the xylem of the leaves

↓

Tension is transmitted to water in xylem of root thro' xylem of stem and water rises upward to reach transpiring surface

↓

Hence soil water enters cortical cells thro' root hairs to reach xylem of roots to maintain the supply of water.

↓

The force for entry of water in leaves is due to rapid transpiration and root cells remain passive

2. Passive absorption of water

Passive absorption of water takes place when rate of transpiration is usually high. Rapid evaporation of water from the leaves during transpiration creates a tension in water in the xylem of the leaves. This tension is transmitted to water in xylem of roots through the xylem of stream and water rises upward to reach the transpiring surfaces. As the results soil water enters into the cortical cells through root hairs to reach the xylem of roots to maintain the supply of water. The force of this entry of water is created in leaves due to rapid transpiration and hence, the root cells remain passive during this process.

External factors affecting absorption of water

1. Available soil water

Sufficient amount of water should be present in the soil in such form which can easily be absorbed by the plants. Usually the plants absorb capillary water i.e water present in films in between soil particles other forms of water in the soil eg. Hygroscopic water, combined water, gravitational water etc. is not easily available to plants.

Increased amount of water in the soil beyond a certain limit results in poor aeration of the soil which retards metabolic activities of root cells like respiration and hence, the rate of water absorption is also retarded.

2. Concentration of soil solution

Increased concentration of soil solution (due to presence of more salts in the soil) results in higher OP. If OP of soil solution will become higher than the OP of cell sap in root cells, the water absorption particularly the osmotic absorption of water will be greatly suppressed. Therefore, absorption of water is poor in alkaline soils and marshes.

3. Soil air

Absorption of water is retarded in poorly aerated soils because in such soils deficiency of O_2 and consequently the accumulation of CO_2 will retard the metabolic activities of roots like respiration. This also inhibits

rapid growth and elongation of the roots so that they are deprived of fresh supply of water in the soil. Water logged soils are poorly aerated and hence, are physiologically dry. They are not good for absorption of water.

4. Soil temperature

Increase in soil temperature up to about 30°C favours water absorption. At higher temperature water absorption is decreased. At low temperature also water absorption decreased so much so that at about 0°C, it is almost decreased. This is probably because at low temperature.

1. The viscosity of water and protoplasm is increased
2. Permeability of cell membrane is decreased
3. Metabolic activity of root cells are decreased
4. Root growth and elongation of roots are checked.

Quiz

1. Roots have a **root cap**, but lack nodes and never bear leaves or flowers directly
2. The **meristem** is at the tip and manufactures new cells; it is an area of cell division and growth.
3. Behind the meristem is the **zone of elongation**
4. The **zone of maturation** is directly beneath the stem. Here, cells become specific tissues such as epidermis, cortex, or vascular tissue.
5. **Root hairs** are delicate, elongated epidermal cells that occur in a small zone just behind the root's growing tip.
6. The movement of water from one cell to other cell through the **plasmodesmata** is called the symplastic pathway of water movement.
7. The casparian strips in the endodermis are made-up of wax -like substance **suberin** which blocks water and solute movement through the cell wall of the endodermis.

05. TRANSLOCATION OF SOLUTES

Translocation of organic solutes

The movement of organic food materials or the solutes in soluble form one place to another in higher plants is called as translocation of organic solutes

Directions of translocation

Translocation of organic solutes may take place in the following directions.

1. Downward translocation

Mostly, the organic material is manufactured by leaves and translocated downward to stem and roots for consumption and storage.

2. Upward translocation

It takes place mainly during the germination of seeds, tubers etc. When stored food after being converted into soluble form is supplied to the upper growing part of the young seedling till it has developed green leaves.

Upward translocation of solutes also takes place through stem to young leaves, buds and flowers which are situated at the tip of the branch.

3. Lateral translocation

Radical translocation of organic solutes also takes place in plants from the cells of the pith to cortex.

Path of the translocation of organic solutes

1. Path of downward translocation

Downward translocation of the organic solutes takes place through phloem. This can be proved by the ringing experiment.

2. Path of upward translocation

Although translocation of organic solutes take place through phloem, but under certain conditions it may take place through xylem.

3. Path of lateral translocation

Lateral translocation from pith to cortex takes place through medullary rays.

Mechanism of translocation

Various theories have been put forward to explain the mechanism of phloem conduction. Among them Munchs' (1930) hypothesis is mot convincing.

Munchs mass flow on pressure flow hypothesis

According to this hypothesis put forward by Much (1930) and others, the translocation of organic solutes takes place though phloem along a gradient of turgor pressure from the region of higher concentration of soluble solutes (supply end) to the region of lower concentration (consumption end). The principle involved in this hypothesis can be explained by a simple physical system as shown in Fig.

Two members X and Y permeable only to water and dipping in water are connected by a tube T to form a closed system membrane X contains more concentrated sugar solution than in membrane Y.

Due to higher osmotic presence of the concentrated sugar solution in the membrane X, water enters into it so that its turgor pressure is increased. The increase in turgor pressure results in mass flow of sugar solution to membrane Y though the T till the concentration of sugar solution in both the membrane is equal.

In the above system it could be possible to maintain continuous supply of sugars in membrane X and its utilization on conversion into insoluble form in membrane Y, the flow of sugar solution from X to Y will continue indefinitely.

According to this theory, a similar analogous system for the translocation of organic solutes exists in plants. As a result of photosynthesis, the mesophyll cells in the leaves contain high concentration of organic food material in them in soluble form and correspond to membrane X or supply end.

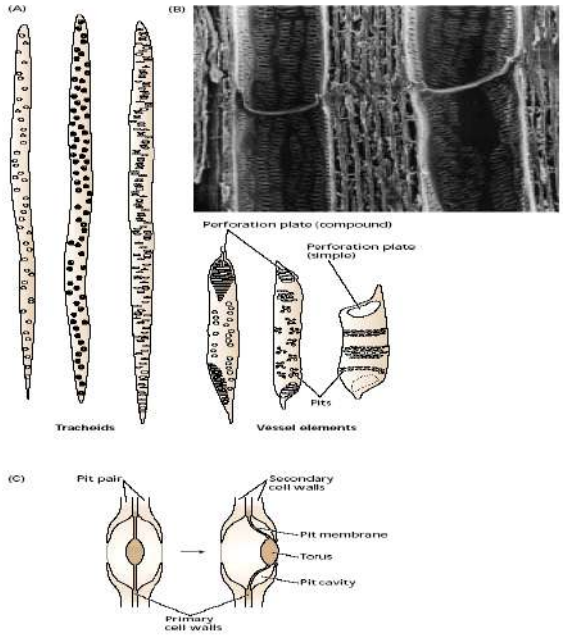
The cells of stem and roots where the food material is utilized or converted into insoluble form correspond to membrane Y or consumption end. While the sieve tubes in phloem which are placed and to end correspond to the tube T.

Mesophyll cells draw water from the xylem of the leaf due to higher osmotic pressure and suction presume of their sap so that their turgor pressure is increased. The turgor presume in the cells of stem and the roots is comparatively low and hence, the soluble organic solutes begin to flow en mass from mesophyll through phloem down to the cells of stem and the roots under the gradient of turgor presume. In the stem and the roots, the organic solutes are either consumed or converted into insoluble form and the excess water is released into xylem through cambium.

XYLEM TRANSPORT

ASCENT OF SAP

The water after being absorbed by the roots is distributed to all parts of the plants. In order to reach the topmost part of the plant, the water has to move upward through the stem. The upward movement of water is called as Ascent of sap.



Ascent of sap can be studied under the following two headings.

1. Path of ascent of sap
2. Mechanism of ascent of sap.

1. Path of ascent of sap

Ascent of sap takes place through xylem. It can be shown by the experiment.

A leafy twig of Balsam plant (it has semi transpiration stem) is cut under water (to avoid entry of air bubble through the cut end of the stem) and placed in a beaker containing water with some Eosine (a dye) dissolved in it.

After sometimes coloured lines will be seen moving upward in the stem. If sections of stem are cut at this time, only the xylem elements will appear to be filled with coloured water.

2. Ringing experiment

A leafy twig from a tree is cut under water and placed in a beaker filled with water. A ring of bark is removed from the stem. After sometime it is observed that the leaves above the ringing part of the stem remain fresh and green. It is because water is being continuously supplied to the upper part of the twig through xylem.

B. Mechanism of ascent of sap

In small trees and herbaceous plants, the ascent of sap can be explained easily, but in tall trees like Eucalyptus and conifers reaching a height of 300-400 feet), where water has to rise up to the height of several hundred feet, the ascent of sap, it feet, becomes a problem. To explain the mechanism of Ascent of sap, a number of theories have been put forward.

a. vital theory
b. root pressure theory
c. physical force theory
d. transpiration pull and cohesion of water theory

A. Vital theories

According to vital theories, the ascent of sap is under the control of vital activities in the stem.

1. According to Godlewski (1884) – Ascent of sap takes place due to the pumping activity xylem tissues which are living.
2. According to Bose (1923) – upward translocation of water takes place due to pulsatory activity of the living cells of the inner must cortical layer just outside the endodermis.

B. Root pressure theory

Although, root pressure which is developed in the xylem of the roots can raise water to a certain height but does not seem to be an effective force in ascent of sap due to the following reasons. Magnitude of root pressure is very low (about 2 atmos).

Even in the absence of root pressure, ascent of sap continues. For example, when leafy twig is cut under water and placed in a beaker full of water it remains fresh and green for sufficient long time.

C. Physical force theories

Various physical forces may be involved in ascent of sap.

1. Atmospheric pressure

This does not seem to be convincing because

it cannot act on water present in xylem in roots

Incase it is working, and then also it will not be able to raise water beyond 34.

2. Imbibition

Sachs (1878) supported the view that ascent of sap could take place by imbibition through the walls of xylem. But imbibitional force is insignificant in the A. of sap because it takes place through the lumen of xylem elements and not through walls.

3. Capillary force

In plants the xylem vessels are placed one above the other forming a sort of continuous channel which can be compared with long capillary tubes and it was thought that as water rises in capillary tube due to capillary force in the same manner ascent of sap takes place in the xylem.

D. Transpiration pull and cohesion of water theory

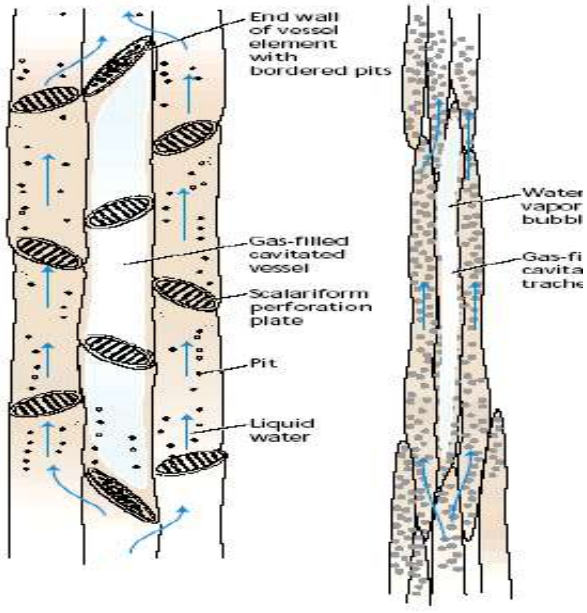
This theory was originally proposed by Dixon and Jolly (1894) later supported and elaborated by Dixon (1924). This theory is very convincing and has now been widely supported by many workers.

Although H- bond is very weak (Containing about 5 K –cal – energy) but they are present in enormous numbers as incase of water, a very strong mutual force of attraction or cohesive force develops between water molecules and hence they remain in the form of a continuous water column in the xylem. The magnitude of this force is very high (up to 350 atm), therefore the continuous water column in the xylem cannot be broken easily due to the

force of gravity or other abstractions offered by the internal tissues in the upward movement of water.

The adhesive properties of water i.e. attractions between the water molecules and the containers walls (here the walls of xylem) further ensure the continuity of water column in xylem.

When transpiration takes place in the leaves at the upper parts of the plant, water evaporates from the intercellular spaces of the leaves to the outer atmosphere through stomata. More water is released into the intercellular spaces from mesophyll cells. In turn, the mesophyll cells draw water from the xylem of the leaf. Due to all this, a tension is created in the xylem elements of the leaves. This tension is transmitted downward to water in xylem elements of the root through the xylem of petiole and stem and the water is pulled upward in the form of continuous unbroken water column to reach the transpiring surfaces up to the top of the plant



06. TRANSPIRATION

Although large quantities of water are absorbed by plant from the soil but only a small amount of it is utilized. The excess of water is lost from the aerial parts of plants in the form of water vapours. This is called as transpiration.

Transpiration is of three types

1. Stomatal transpiration

Most of the transpiration takes place through stomata. Stomata are usually confined in more numbers on the lower sides of the leaves. In monocots. Eg. Grasses they are equally distributed on both sides. While in aquatic plants with floating leaves they are present on the upper surface.

2. Cuticular transpiration

Cuticle is impervious to water, even though, some water may be lost through it. It may contribute a maximum of about 10% of the total transpiration.

3. Lenticular transpiration

Some water may be lost by woody stems through lenticells which is called as lenticular transpiration.

Mechanism of stomatal transpiration

The mechanism of stomatal transpiration which takes place during the day time can be studied in three steps.

i. Osmotic diffusion of water in the leaf from xylem to intercellular space above the stomata through the mesophyll cells.

ii. Opening and closing of stomata (stomatal movement)

iii. Simple diffusion of water vapours from intercellular spaces to other atmosphere through stomata.

- Inside the leaf the mesophyll cells are in contact
- With xylem, and on the other hand with intercellular space above the stomata

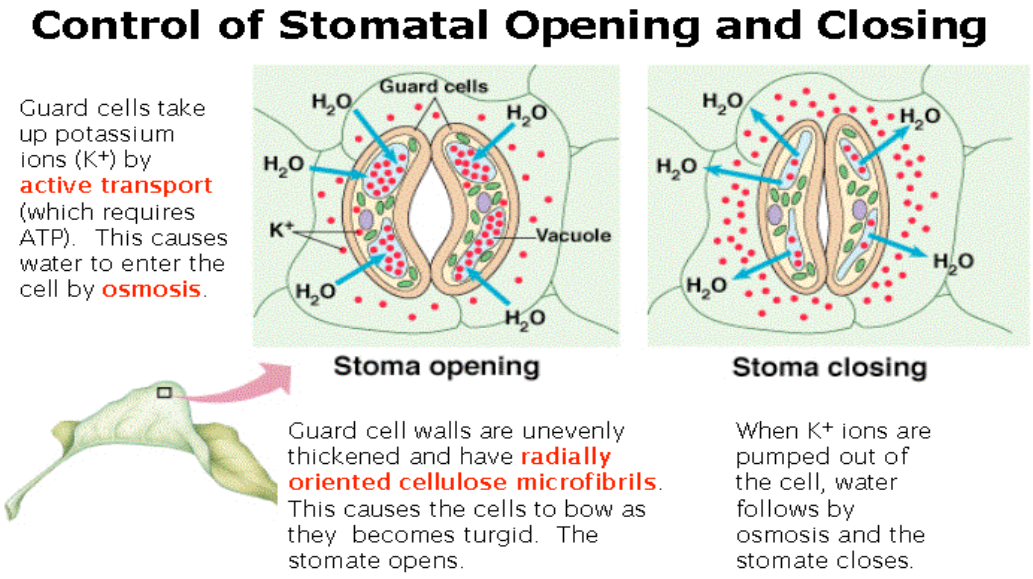
- When mesophyll cells draw water from the xylem they become turgid and their diffusion pressure deficit (DPD) and osmotic pressure (OP) decreases with the result that they release water in the form of vapour in intercellular spaces close to stomata by osmotic diffusion. Now in turn, the O.P and D.P.D of mesophyll cells become higher and hence, they draw water form xylem by osmotic diffusion.

Opening and closing of stomata (Stomatal movement)

The stomata are easily recognized from the surrounding epidermal cells by their peculiar shape. The epidermal cells that immediately surround the stomata may be similar to other epidermal cells or may be different and specialized. In the latter case, they are called as subsidiary cells.

The guard cells differ from other epidermal cells also in containing chloroplasts and peculiar thickening on their adjacent surface (in closed stomata) or on surfaces.

Consequent to an increase in the osmotic pressure (OP) and diffusion pressure deficit (DPD) of the guard cells (which is due to accumulation of osmotically active substances), osmotic diffusion of water from surrounding epidermal cells and mesophyll



cells into guard cells follows. This increase the turgor pressure (TP) of the guard cells and they become turgid. The guard cells swell, increase in length and their adjacent thickened surfaces starch forming a pore and thus the stomata open.

On the other hand, when OP and DPD of guard cells decrease (due to depletion of osmotically active substances) relative to surrounding epidermal and mesophyll cells, water is released back into the latter by osmotic diffusion and the guard cells become flaccid. The thickened surfaces of the guard cells come close to each other, thereby closing the stomatal pore and stomata.

Osmotic diffusion of water into guard cells occur when their osmotic pressure increases and water potential decreases (i.e become more negative) related to those of surrounding epidermal and mesophyll cells. The guard cells become flaccid when their osmotic pressure decreases relative to the surrounding cells (Movement of water takes place from a region of higher water potential to a region of lower water potential.

These may be several different agents or mechanisms which control stomatal movements.

Hydrolysis of starch into sugars in guard cells

Synthesis of sugars or organic acids in them

The active pumping of K^+ ions in the guard.

1. Hydrolysis of starch into sugars in guard cells

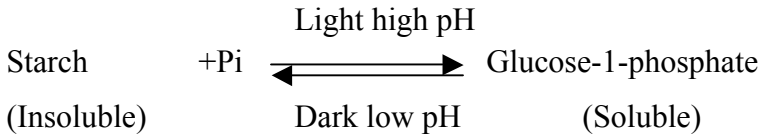
Starch – sugar Inter conversion theory

This classical theory is based on the effect of pH on starch phosphorylase enzyme which reversibly catalyses the conversion of starch + inorganic phosphate into glucose -1 phosphate.

During the day, pH is guard cells in high. This favours hydrolysis of starch (which is insoluble into glucose -1- phosphate (which is soluble) so that osmotic pressure is increased in guard cells.

Consequently water enters, into the guard cells by osmotic diffusion from the surrounding epidermal and mesophyll cells. Guard cells become turgid and the stomata open.

During dark, reverse process occurs. Glucose 1- phosphate is converted back into starch in the guard cells thereby decreasing osmotic pressure. The guard cell release water, become flaccid and stomata become closed.

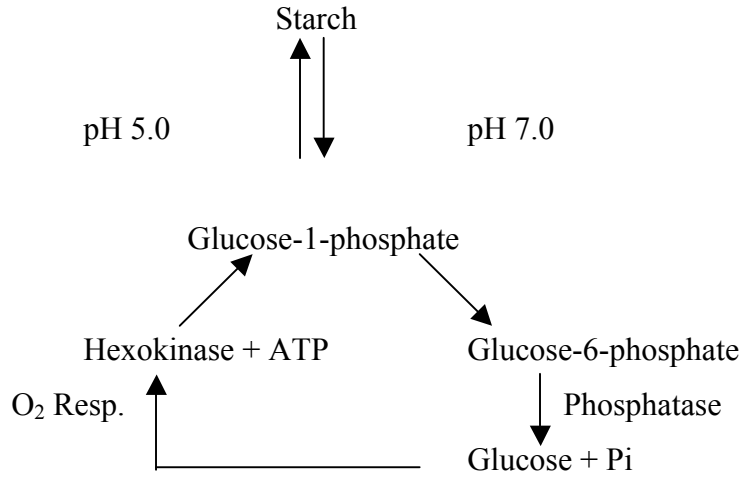


According to Steward 91964), the conversion of starch and inorganic phosphate into glucose-1-phosphate does not cause any appreciable change in the osmotic pressure because the inorganic phosphate and glucose-1-phosphate are equally active osmotically.

In this scheme he has suggested that,

Glucose-1-phosphate should be further converted into glucose and inorganic phosphate for the opening of stomata.

Metabolic energy in the form of ATP would be required for the closing of stomata which probably comes through respiration.



2. Synthesis of sugars or organic acids in Guard cells

During day light photosynthesis occurs in guard cells as they contain chloroplast. The soluble sugars formed in this process may contribute in increasing the osmotic potential of guard cells and hence resulting in stomatal opening. However, very small amounts of soluble sugars (osmotically active) have been extracted from the guard cells which are insufficient to affect water potential.

As a result of photosynthesis CO_2 concentration in guard cells decreases which leads to increased pH up of organic acids, chiefly malic acid during this period in guard cells. The formation of malic acid would produce proton that could operate in an ATP-driven proton K^+ exchange pump moving protons into the adjacent epidermal cells and K ions into guard cells and thus may contribute in increasing the osmotic pressure of the guard cells and leading to stomatal opening.

Reverse process would occur in darkness.

3. ATP –Driven proton (H^+) – K exchange pump mechanism in Guard cells

According to this mechanism, there is accumulation of K^+ ions in the guard cells during day light period. The protons (H^+) are 'pumped out' from the guard cells into the adjacent epidermal cells and in exchange K^+ ions are mediated through ATP and thus are an active process. ATP is generated in non-cyclic photophosphorylation in photosynthesis in the guard cells. The ATP required in ion exchange process may also come through respiration.

The accumulation of K ion is sufficient enough to significantly decrease the water potential of guard cells during day light. Consequently, water enters into them from the adjacent epidermal and mesophyll cells thereby increasing their turgor pressure and opening the stomatal pore.

Reverse situation prevails during dark when stomata are closed. There is no accumulation of 'K' in g cells in dark.

(iii) The last step in the mechanism of transpiration is the simple diffusion of water vapours from the intercellular spaces to the atmosphere through open stomata. This is because the intercellular spaces are more saturated with moisture is comparison to the outer atmosphere in the vicinity of stomata.

Significance of Transpiration

Plants waste much of their energy in absorbing large quantities of water and most of which is ultimately lost through transpiration.

Some people thin that – Transpiration as advantageous to plant.

Others regard it as an unavoidable process which is rather harmful.

Advances of transpiration

1. Role of movement of water

Plays an important role in upward movement of water i.e Ascent of sap in plants.

2. Role in absorption and translocation of mineral salts

Absorption of water and mineral salts are entirely independent process. Therefore transpiration has nothing to do with the absorption of mineral salts.

However, once mineral salts have been absorbed by the plants, their further translocation and distribution may be facilitated by transpiration through translocation of water in the xylem elements.

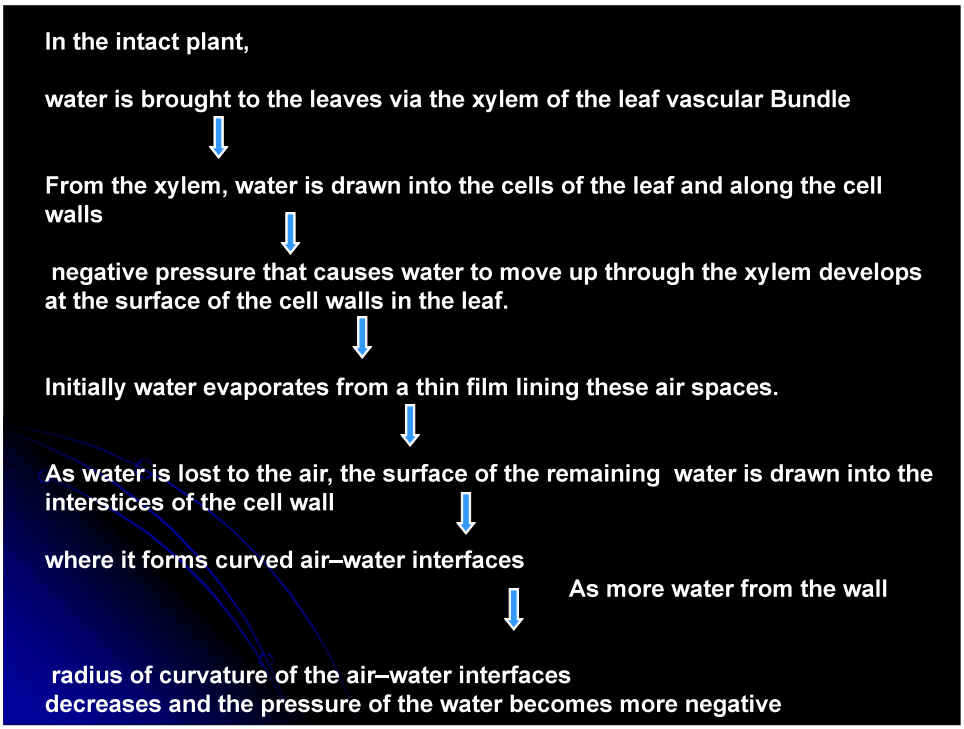
3. Role of regulation of temperature

Some light energy absorbed by the leaves is utilized in photosynthesis; rest is converted into heat energy

Transpiration from the leaf regulates by

1. **difference in water vapor concentration between the leaf air spaces and the external air**
2. **diffusional resistance (*r*) of this pathway**
3. **leaf stomatal resistance (*r*s)**
4. **leaf boundary layer resistance**
5. **control of stomatal apertures by the guard cells**

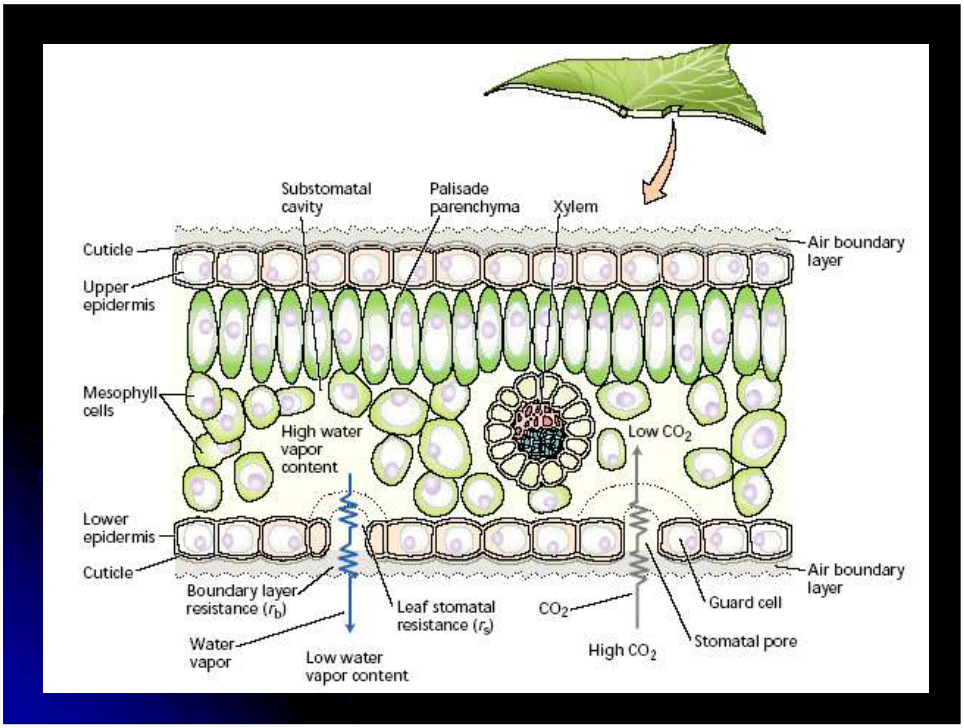
which raises their temperature. Transpiration plays an important role in controlling the temperature of the plants. Rapid evaporation of water from the aerial parts of the plant through transpiration brings down their temperature and thus prevents them from excessive heating.



Transpiration as a necessary evil

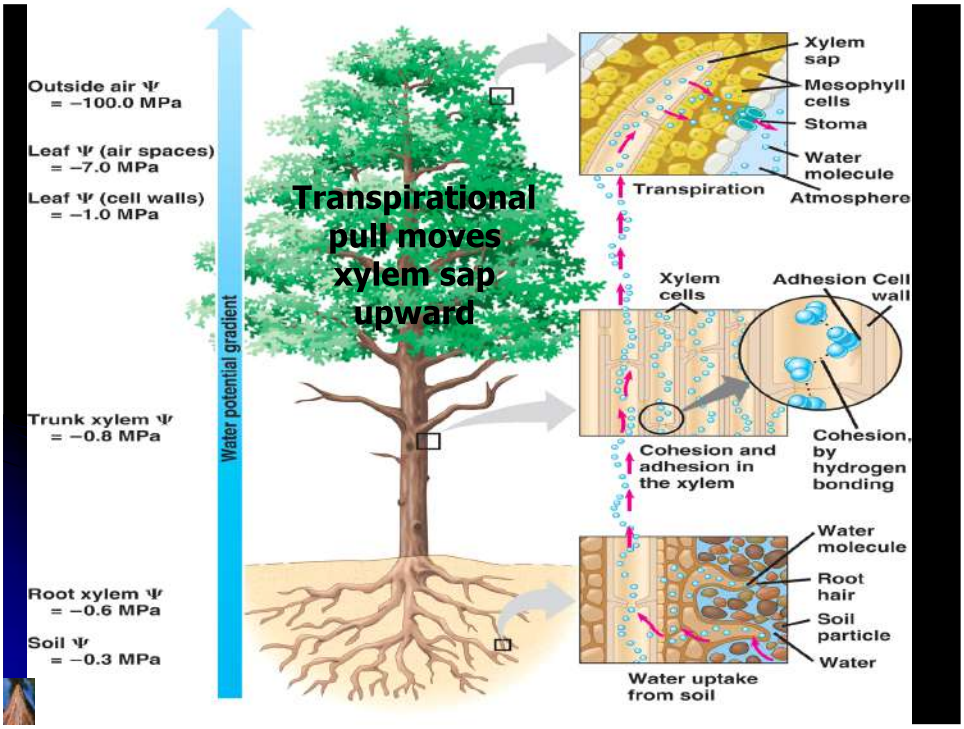
1. When the rate of transpiration is high and soil is deficient in water, an internal water deficit is created in the plants which may affect metabolic processes

2. Many xerophytes have to develop structural modification and adaptation to check transpiration.



3. Deciduous tress has to shed their leaves during autumn to check loss of water.

But, in spite of the various disadvantages, the plants cannot avoid transpiration due to their peculiar internal structure particularly those of leaves. Their internal structure although basically mean for gaseous exchange for respiration, P.S. etc. is such that it cannot check the evaporation of water. Therefore, many workers like Curtis (1926) have called transpiration as necessary evil.



Factors affecting transpiration rate

A. External factors

1. Atmospheric humidity

In humid atmosphere, (when relative humidity) is high), the rate of transpiration decreases. It is because atmosphere is more saturated with moisture and retards the diffusion of water vapour from the intercellular spaces of the leaves to the outer atmosphere through stomata.

In dry atmosphere, the RH is low and the air is not saturated with moisture and hence, the rate of transpiration increases.

2. Temperature

An increase in temperature brings about an increase in the rate of transpiration by

1. lowering the relative humidity
2. Opening of stomata widely

3. Wind

i. When wind is stagnant (not blowing), the rate of transpiration remains normal

ii. When the wind is blowing gently, the rate of transpiration increases because it removes moisture from the vicinity of the transpiration parts of the plant thus facilitating the diffusion of waster vapour from the intercellular spaces of the leaves to the outer atmosphere though stomata.

iii. When the wind is blowing violently, the rate of transpiration decreased because it creates hindrance in the outward diffusion of water vapours from the transpiring part and it may also close the stomata.

4. Light

Light increases the rate of transpiration because,

In light stomata open; It increases the temperature

In dark, due to closure of stomata, the stomatal transpiration is almost stopped.

5. Available soil water

Rate of transpiration will decrease if there is not enough water in the soil in such from which can be easily absorbed by the roots.

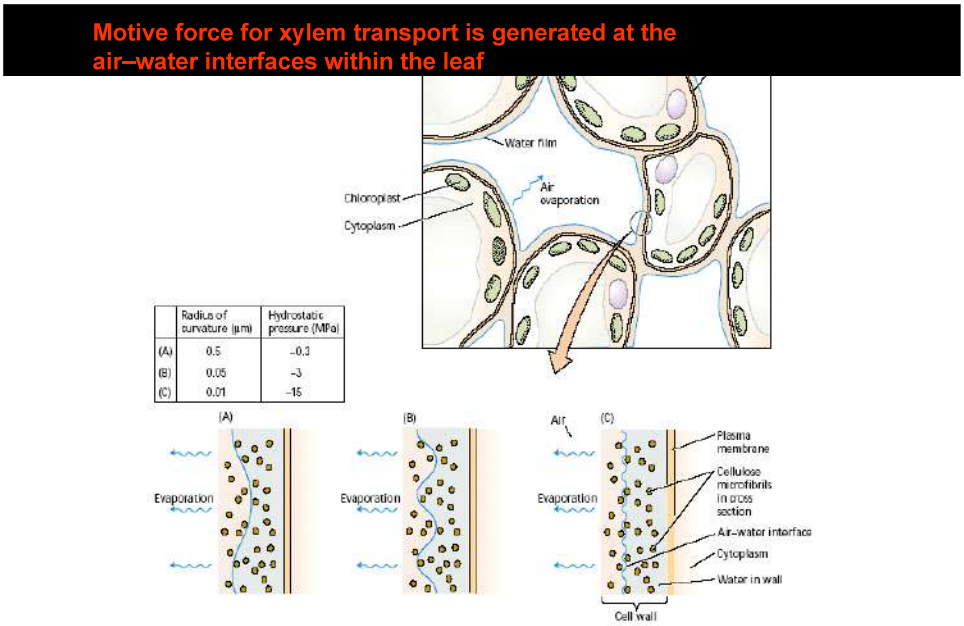
6. CO_2

An increase in CO_2 concentration in the atmosphere (Ova the usual concentration) more so inside the leaf, leads towards stomatal closure and hence it retards transpiration.

B. Internal factors

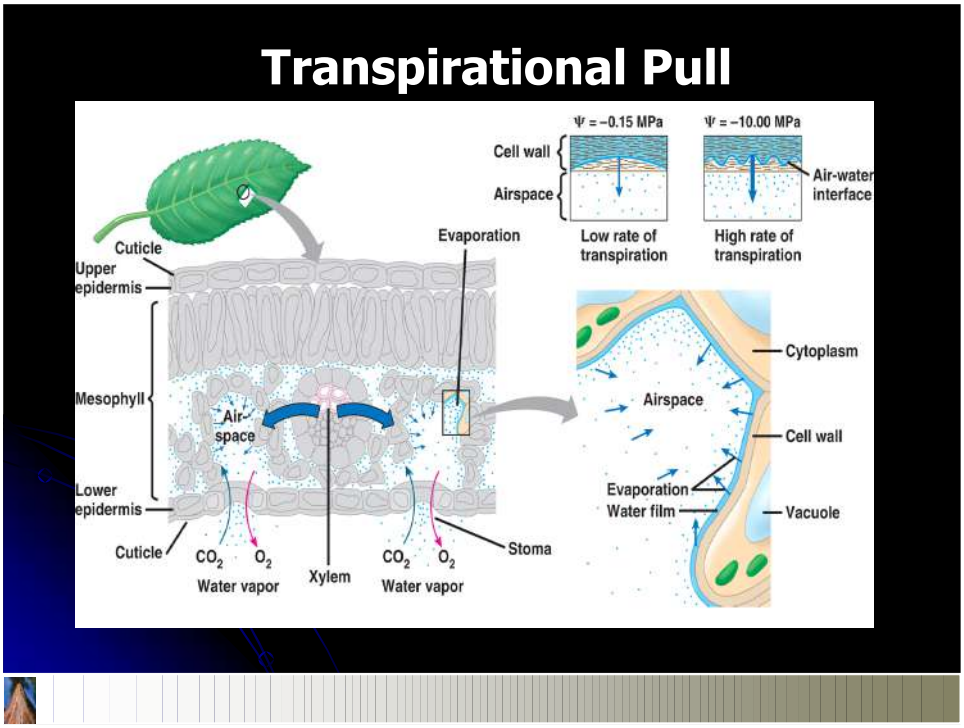
1. Internal water conditions

It is very essential for transpiration. Deficiency of water in the plants will result in decrease of transpiration rate. Increase rate of transpiration containing for longer periods often create internal water deficit in plants because absorption of water does not keep pace with it.



2. Structural features

The number, size, position and the movement of stomata affect rate of transpiration. In dark stomata are closed and stomatal transpiration is checked. Sunken stomata help in reducing the rate of stomatal transpiration. In xerophytes the leaves are reduced in size or may even fall to check transpiration. Thick cuticle on presence of wax coating on exposed parts reduces cuticles transpiration.

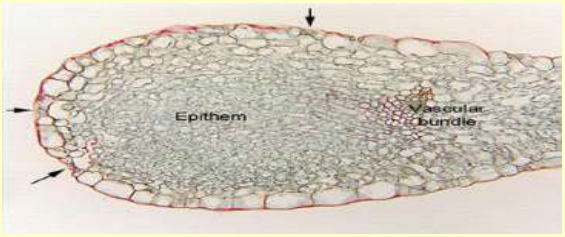


Antitranspirants

A number of substances are known which when applied to the plants retard their transpiration. Such substances are called as antitranspirants. Some examples of antitranspirants are colourless plastics, silicone, oils, low viscosity waxes, phenyl mercuric acetate, abscisic acid, CO_2, etc. Colourless plastic, silicone oils and low viscosity waxes belong to one group as these are sprayed on the leaves, form after film which is permeable to O_2 and CO_2 but not to water.

Fungicide phenyl mercuric acetate, when applied in low concentration (10^{-4} m), it exercised a very little toxic effect on leaves and resulted in partial closure of stomatal pores for a period of two weeks. Similarly ABA a plant hormone also induces stomatal closure. CO_2 is an effective antitranspirants. A little rise in CO_2 concentration from the natural 0.03% to 0.05% induces partial closure of stomata. Its higher concentration cannot be used which results in complete closure of stomata affecting adversely the photosynthesis and respiration.

GUTTATION



In some plants such as garden nasturtium, tomato, colocasia etc, water drops ooze out from the uninjured margins of the leaves where a main vein ends. This is called as guttation and takes place usually early in the morning when the rate of absorption and root pressure are high while the transpiration is very low.

The phenomenon of guttation is associated with the presence of special types of stomata at the margins of the leaves which are called as **water stomata or hydathodes**. Each hydathode consists of a water pore which remains permanently open.

Below this there is a small cavity followed by a loose tissue called as epithem. This epithem is in close association with the ends of the vascular elements of veins. Under high root pressure the water is given to the epithem by the xylem of the veins. From epithem

water is released into the cavity. When this cavity is completely filled with watery solution, the later begins to ooze out in the form of watery drops through the water pore.

Difference between transpiration and Guttation

Transpiration	Guttation
1. Water is lost from aerial parts of plants in the form of invisible water vapours	Watery solution oozes out from uninjured margins of aerial leaves only
2. Transpiration occurs mostly through stomata. It may also takes place through cuticle and lenticels	It occurs only through hydathodes (water stomata)
3. It takes place throughout the day, its rate being maximum at noon.	It takes place only early in the morning when root pressure and the rate of water absorption are higher

07. **MINERAL NUTRITION**

The term, *mineral nutrient* is generally used to refer to an inorganic ion obtained from the soil and required for plant growth. The chemical form in which elements are applied to plants is called as *nutrient*. Nutrition may be defined as the supply and absorption of chemical compounds needed for plant growth and metabolism

The nutrients indispensable for the growth and development of higher plants are obtained from three sources viz., atmosphere, water and soil. The atmosphere provides carbon and oxygen as carbon dioxide. Carbon is reduced during photosynthesis and oxygen is utilized during aerobic respiration. Soil provides the mineral ions.

Essential elements

The term essential mineral element was proposed by Arnon and Stout (1939). These are the composition of both macro and microelements, in the absence of any one of these elements the plant cannot maintain its normal growth and develops deficiency symptoms, affects metabolism and die prematurely. Of the many elements that have been detected in plant tissues, only 16 are essential for all higher plants. They are C, H, O, N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, B, Cl and Mo. In the absence of each of the essential elements, plants develop deficiency symptoms characteristic of the deficient element and die prematurely.

Macronutrients

The nutrient elements which are required for the growth of plants relatively in larger quantities are called as *major nutrients* or *macronutrients*. The major elements required for growth of plants are C, H, O, N, P, K, Ca, Mg and S. Among these nutrients, C, H and O are taken up by the plants from the atmosphere and water. The N, P, K, Ca, Mg and S are taken up by the plants from the soil and they are applied in the form of chemical fertilizers either through the soil or foliage.

Micronutrients

The nutrient elements which are required comparatively in small quantities are called as *minor* or *micro nutrients* or trace elements. The micronutrients required for the plant growth are Zn, Cu, Fe, Mn, Mo, B and Cl.

Tracer elements or labeled elements

The nutrient elements that are required for plants are some times labeled and used to study their movement or tracing out the involvement of such nutrients in metabolism in different organs of plants, are called as *tracer elements*. They may either be stable or radio active types and they are also called as *isotopic elements.*

E.g. Stable isotopes: ^{15}N, ^{12}C, ^{31}P

Radio active : ^{14}C, ^{32}P, ^{65}Zn, ^{56}Fe, ^{60}Co, etc.

Hidden hunger

When the plants are not able to meet their requirement either one or more of these essential elements, the plants will undergo starvation for such elements. At the initial stage of deficiency of such elements plants will not show any characteristic symptoms which could be exhibited morphologically and due to want of those elements some activities of plants would rather be affected and the internal deficiency is called as *Hidden hunger.*

General role of essential elements

In general, an element is essential to the life of a higher green plant for one or more of the following three reasons.

1. It may perform a nutritive role by being a component of one or more of the major classes of plant constituents.
2. It may be a catalytic role either as an action for of an enzyme or as an integral component of an enzyme.
3. It may function as a free ion and thereby exert a balancing role in maintaining electro-neutrality within plant cells (e.g. Potassium).

Criteria for essentiality of elements

The demonstration of the essentially several elements (macro and micronutrients), especially, micronutrients is rather very difficult. In view of the technical difficulties associated with demonstrating the essentiality of elements required in very small amounts, Arnon and Stout (1939) suggested the adoption of the following three criteria of essentiality for judging the exact status of a mineral in the nutrient of a plant.

1. The element must be essential for normal growth or reproduction and the plant processes cannot proceed without it.
2. The element cannot be replaced by another element.
3. The requirement must be direct i.e., not the result of some indirect effect such as relieving toxicity caused by some other substance.

Another recent suggestion to the criteria of essentiality is that some elements might better be called *functional or metabolic elements* rather than essential elements. This is intended to indicate that an element that is metabolically active, functional or metabolic may or may not be essential. For example in chlorine-bromine, chlorine is designated as a functional element rather than an essential element as chlorine can be substituted with bromine.

Based on the mobility in phloem, elements are also classified into three types.

1. Mobile elements : N, K, P, S and Mg
2. Immobile elements : Ca, Fe and B
3. Intermediate : Zn, Mn, Cu, Mo

Functions of elements

Protoplasmic elements : N, P, S

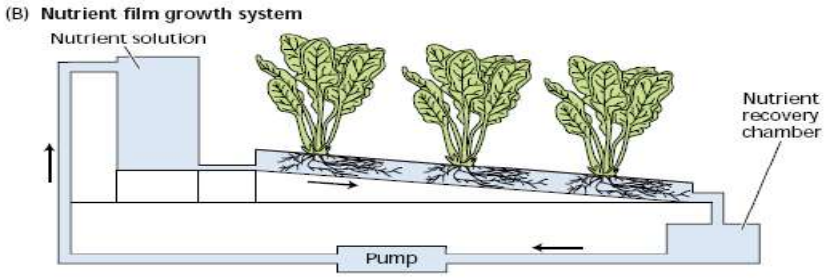
Balancing elements : Ca, Mg, K – counteract to toxic effects of other minerals by causing ionic balance.

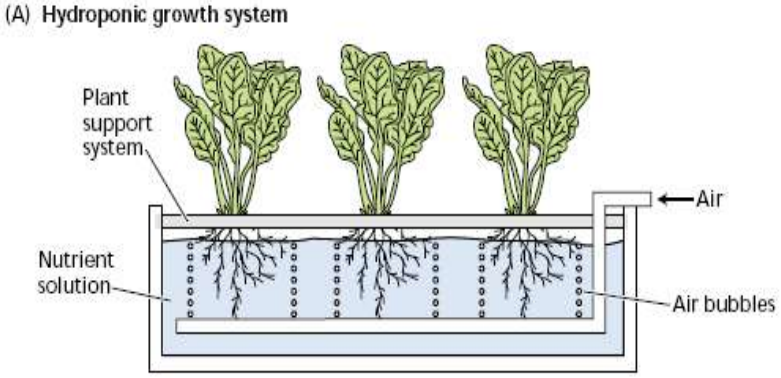
Frame work elements : C, H_2O – as they are the constituents of carbohydrates that form cell walls.

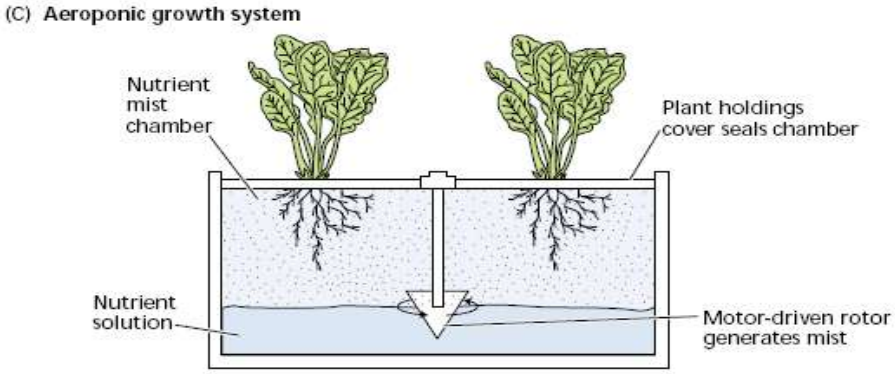
Catalytic elements : Mn, Cu, Mg, etc.

SOIL LESS GROWTH OR HYDROPONICS

The practice of growing plants in nutrient enriched water without soil is called as soil less growth or hydroponics. However, the term hydroponics is now being applied to plants rooted in sand, gravel or other similar matter which is soaked with a recycling flow of nutrient – enriched water.







According to a recent limited nations report on hydroponics: In area of tropics, where the water deficiency is the limiting factor in crop production, the soil less methods hold out much promise because of the more economical use of water.

The report also indicated that in some areas, lack of fertile soil or very thin soil layers may also move soil less methods worth serious consideration.

Besides these the other advantages of growing cucumbers, egg plants, peppers, lettuces, spinach and other vegetables hydroponically under controlled environment are

1. The regulation of nutrients

2. Control of pets and diseases
3. Reduction of labour cost
4. Sometimes quicker yield

But there is two main drawbacks of hydroponics farming.

1. Firstly the cost of settling up the system is very high
2. Secondly it requires skills and knowledge its operation

08. MECHANISM OF UPTAKE - PHYSIOLOGICAL ROLE OF NUTRIENTS

Mechanism

Previously it was thought that absorption of mineral salts takes place along with water absorption. But it is now understood that mineral salt absorption and water absorption are two different processes.

Soil serves as a main source of mineral salts of ionic forms in clay crystals have a central nuclues called micelle

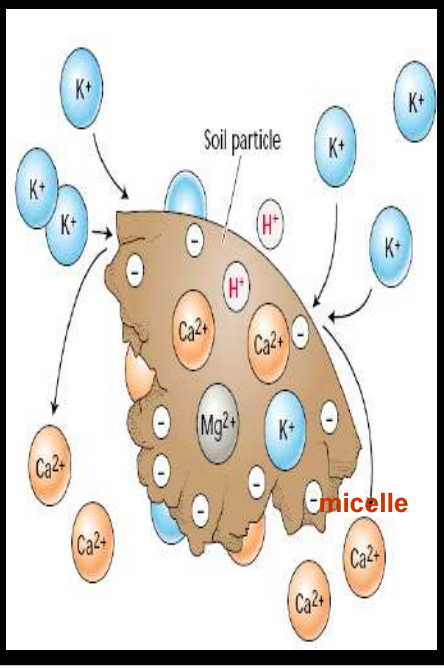
The micelles are negatively charged and maintain a balance, they attract and hold positively charged ions on the surface

cationic forms

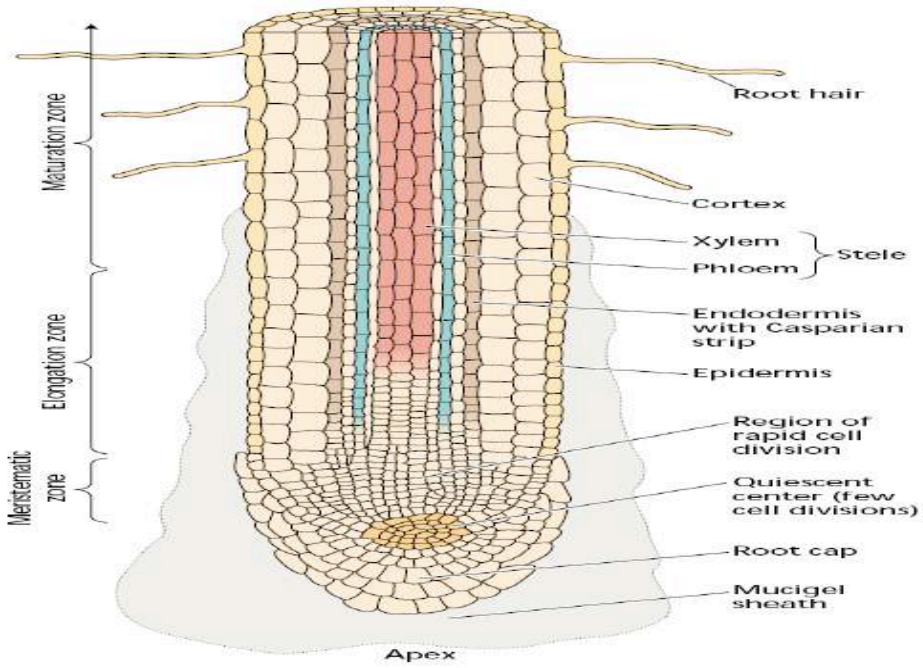
K, Mg, Ca, Fe, Mn, Cu, Zn and Co

anionic forms and N,P,B,S and Cl.

These ions are either in the form of loosely absorbed ions or firmly absorbed ions on the colloidal particles



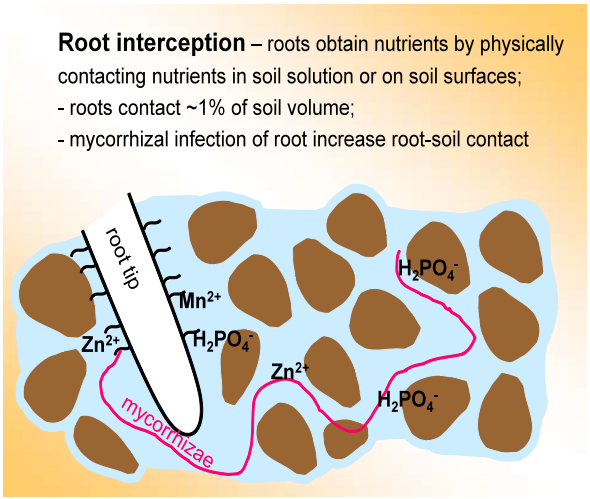
Mineral salts are absorbed from the soil solution in the form of ions. They are chiefly absorbed through the meristematic regions of the roots near the tips.



Plasma membrane of the root cells is not permeable to all the ions. It is selectivity permeable. All the ions of the same salt are not absorbed at equal rate but leads unequal absorption of ions. First step in the absorption of mineral salts is the process of Ion exchange which does not require metabolic energy.

The further processes of the absorption of mineral salts may be of two types.

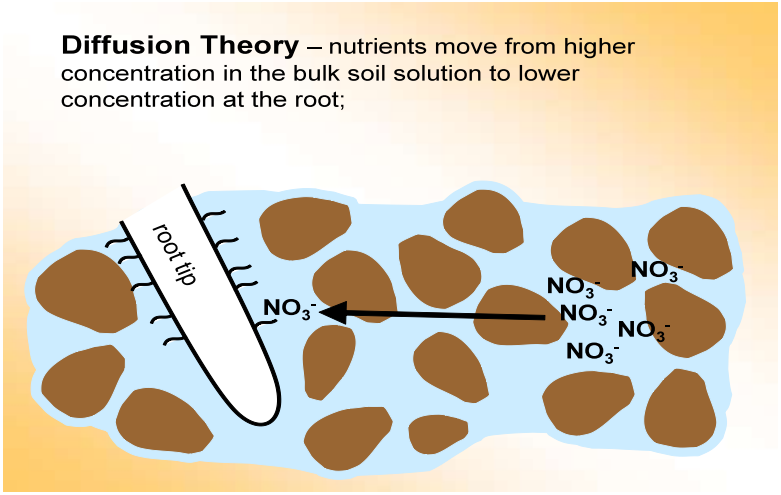
1. Passive and 2. Active



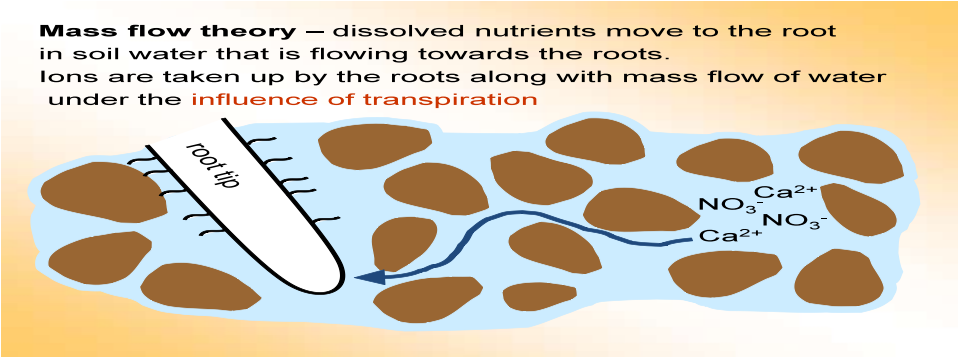
1. Passive absorption

When the concentration of mineral salts is higher in the outer solution than in the cell sap of the root cells, the mineral salts are absorbed according to the concentration gradient by simple process of diffusion. This is called as passive absorption because it does not require expenditure of metabolic energy.

Ion exchange

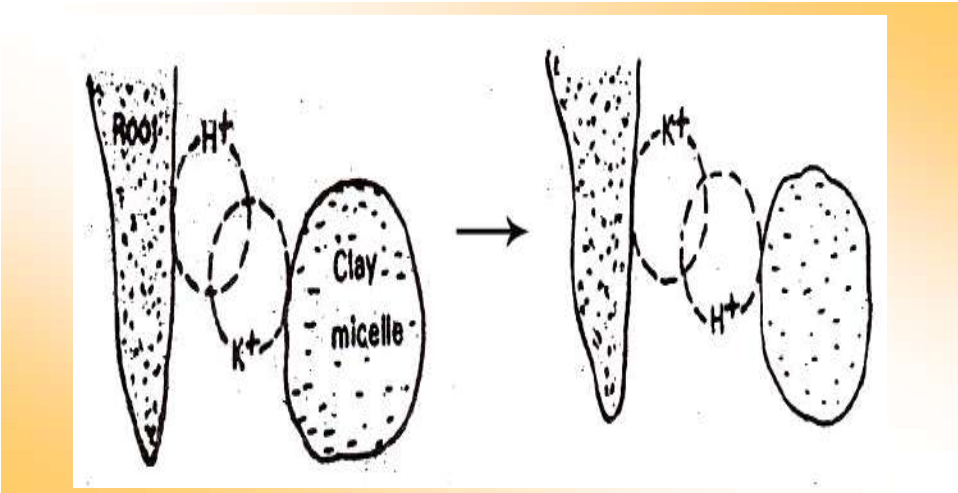


The ions adsorbed on the surface of the plasma membrane of the root cells may be exchanged with the ions of same sign from external solution for eg. The cation K^+ of the external soil solution may exchanged with H^+ ions adsorbed on the surface of the plasma membrane. Similarly anion may be exchanged with OH ions. There are two theories regarding the mechanism of ion exchange.



1. Contact exchange theory

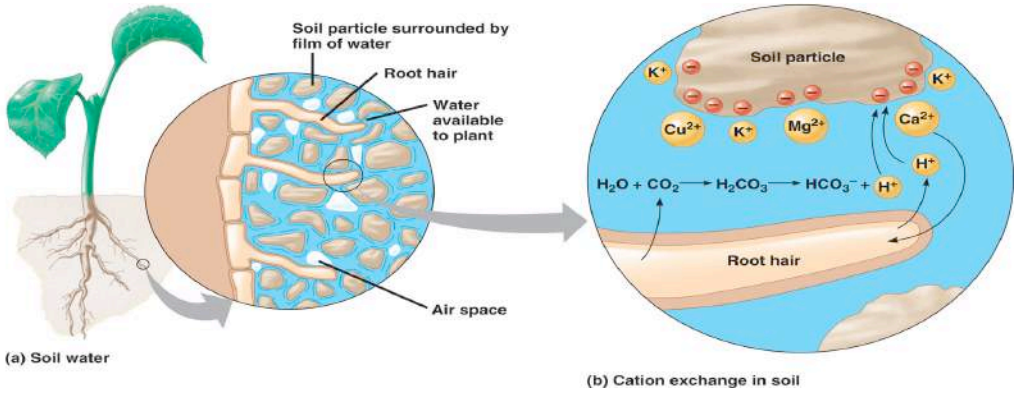
According to this theory the ions adsorbed or the surface of root cells and clay particles are not held tightly but oscillate within small volume of space. If the roots and clay particles are in close contact with each other, the oscillation volume of ions adsorbed on root surface may over by the oscillation volume of ions adsorbed on clay particles, and the ions adsorbed on clay particle may be exchanged with the ions adsorbed on root surface directly without first being dissolved in soil solution.



2. Carbonic acid exchange theory

According to this theory, the CO_2 released during respiration of root cells combines with water to form carbonic acid (H_2CO_3). Carbonic acid dissociates into H^+ and an anion HCO_3 in soil solution. These H^+ ions may be exchanged for cations adsorbed on the clay particles. The cations thus released into the soil solution from the clay particles, may be

adsorbed on root cells in exchange for H^+ ions or as in ion pairs with bicarbonate. Thus, the soil solution plays an important role in carbonic acid exchange theory.



2. Active absorption of mineral salts

It has been observed that the cell sap in plants accumulates large quantities of mineral slats ions against the concentration gradient. The accumulation of mineral salts against to concentration gradient is an active process which involves the expenditure of metabolic energy through respiration. The active absorption of mineral salts involves the operation of a carrier compound present in the plasma membrane of the cells.

The carrier concept

According to this theory, the plasma membrane is impermeable to free ions. But some compounds present in it acts as carrier and combines with ions to form carrier- ion-complex which can move across the membrane. On the inner side of the membrane this complex leaves releasing ions into the cell while the carrier goes back to the outer surface to pick up fresh ions. They are two hypotheses based on the carrier concept to explain the mechanism of active salt absorption. Although they are not universally accepted.

1. Lundegardhs cytochrome pump theory

Lundegardh and Burstrom (1933) believed that there was a definite correlation between respiration and anion absorption. Thus when a plant is transferred from water to a

salt solution the rate of respiration increases. This increase in rate of respiration over the normal respiration has been called as anion respiration or salt respiration.

Lundegardh (1954) proposed cytochrome pump theory which is based on the following assumptions.

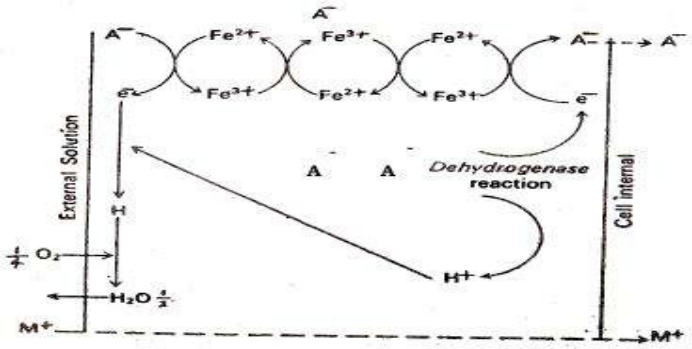
1. The mechanism of anion and cation absorption is different
2. Anions are absorbed through cytochrome chain by an active process.

(Cytochromes are ion – porphyrin proteins that act as enzymes and helps in election transfer during respiration).

3. Cations are absorbed passively.

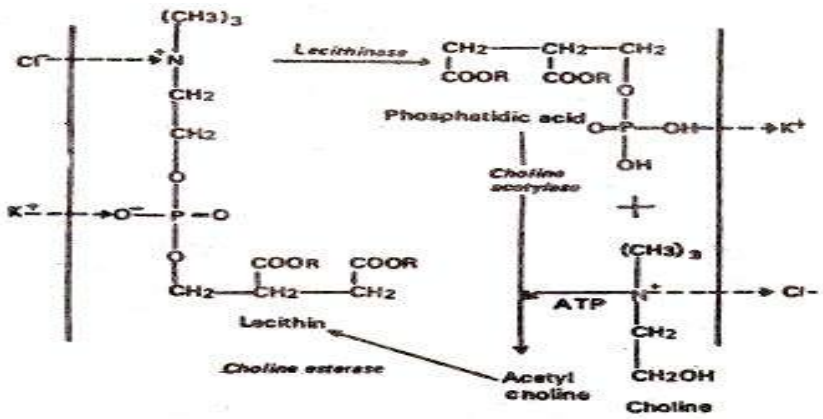
According to this theory

1) Dehydrogenase reactions on inner side of the membrane give rise to protons (H^+) and electrons (e^-).
2) The electrons travels over the cytochrome chain towards outside the membrane, so that the Fe of the cytochrome becomes reduced (Fe^{++}) on the outer surface and oxidized (Fe^{+++}) on the inner surface.
3) On the outer surface, the reduced cytochrome is oxidized by oxygen releasing the electron (e^-) and taking an anion (A^-).
4) The electron thus released unites with H^+ and oxygen to form water
5) The anion (A^-) travels over the cytochrome chain towards inside.
6) On the inner surface the oxidized cytochrome becomes reduced by taking an electron produced through the dehydrogenase reactions and the anion (A) is released.
7) As the result of anion absorption, a cation (M) moves passively from outside to inside to balance the anion.



2. Bennert – Clark's protein Lecithin Theory

In 1856, Bennet – Clark suggested that because the cell membranes chiefly consist of phospholipids and proteins and certain enzymes seem to be located on them, the carrier could be a protein associated with the phosphatide called as lecithin. He also assumed the presence of different phosphatides to correspond with the number of known competitive groups of cations and anions.



According to this theory

1. Phosphate group in the phosphatide is regarded as the active centre binding the cations and the basic choline group as the anion binding centre.
2. The ions are liberated on the inner surface of the membrane by decomposition of lecithin by the enzyme lecithinase.

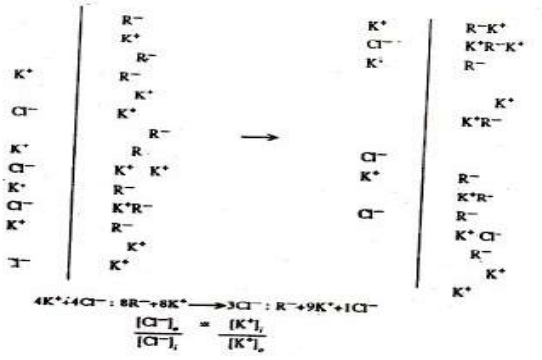
3. The regeneration of the carrier lecithin form phosphatidic acid and choline takes place in the presence of the enzyme choline acetylase and choline esterase and ATP. The latter acts as a source of energy.

Donnans' Equilibrium

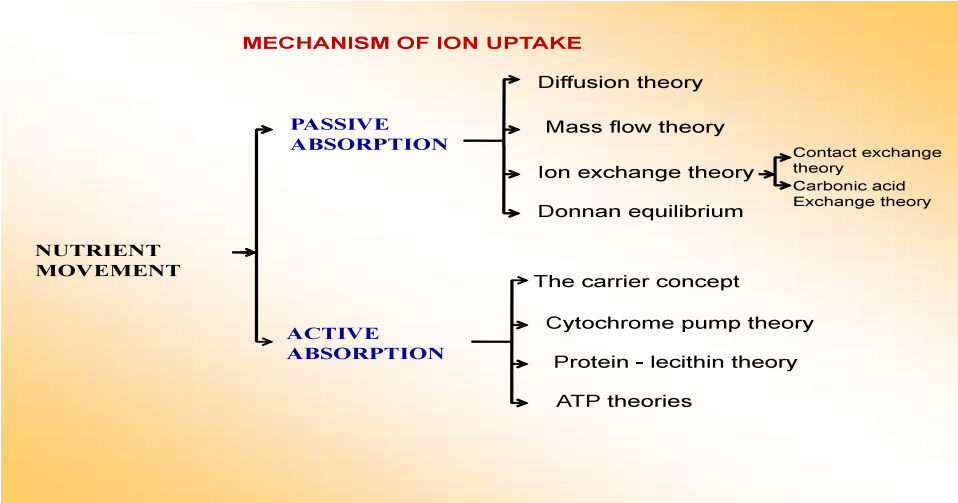
The accumulation of ions inside the cells without involving expenditure of the metabolic energy can be explained to some extent by Donnan's equilibrium theory.

According to this theory there are certain pre existing ions inside the cell which cannot diffuse outside through membrane. Such ions are called as in diffusible or fixed ions. However, the membrane is permeable to both anions and cations of the outer solutions.

Suppose there are certain fixed anions in the cell which is in contact with outer solution containing anions and cations. Normally equal number of anions and cations would have diffused into the cell through an electrical potential to balance each other, but to balance the fixed anions more cations will diffuse into the cell. This equilibrium is known as Donnan's equilibrium. In this particular case, there would be an accumulation of cations inside the cell.



If however, there are fixed cations inside the cell, the Donnan's equilibrium will result in the accumulation of anions inside the cell.



Specific roles of essential mineral elements

A. Macronutrients

1. Nitrogen

- Nitrogen is important constituent of proteins, nucleic acids, porphyries (chlorophylls & cytochromes) alkaloids, some vitamins, coenzymes etc
- Thus N plays very important role in metabolism, growth, reproduction and heredity.

2. Phosphorus

- It is important constituent of nucleic acids, phospholipids, coenzymes NADP, NADP H_2 and ATP
- Phospholipids along with proteins may be important constituents of cell membranes
- P plays important role in protein synthesis through nucleic acids and ATP
- Through coenzymes NAD, NADP and ATP, it plays important role in energy transfer reactions of cell metabolism eg. Photosynthesis, respiration and fat metabolism etc.

Potassium

- Although potassium is not a constituent of important organic compound in the cell, it is essential for the process of respiration and photosynthesis
- It acts as an activator of many enzymes involved in carbohydrate metabolism and protein synthesis
- It regulates stomatal movement

- Regulates water balance

Calcium

- It is important constituent of cell wall
- It is essential in the formation of cell membranes
- It helps to stabilize the structure of chromosome
- It may be an activation of may enzymes

Magnesium

- It is very important constituent of chlorophylls
- It acts as activation of many enzymes in nucleic acid synthesis and carbohydrate metabolism
- It plays important role in binding ribosomal particles during protein synthesis.

Sulphur

- It is important constituent of some amino acids (cystine, cysteine and methionine) with which other amino acids form the protein
- S helps to stabilize the protein structure
- It is also important constituent of vitamin i.e biotin, thiamine and coenzyme A
- Sulpho hydryl groups are necessary for the activity of many enzymes.

Iron

- Important constituent of iron porphyrin – proteins like cytochromes, peroxidase, catalases, etc.
- It is essential for chlorophyll synthesis
- It is very important constituent of ferredoxin which plays important role in photochemical reaction in photosynthesis and in biological nitrogen fixation.

Micro nutrients

Zinc

- It is involved in the biosynthesis of growth hormone auxin (indole 3 acetic acid)

- It acts activator of many enzymes like carbonic anhydrase and alcohol dehydrogenase, etc.

Manganese

- It is an activator of many respiratory enzymes
- It is also an activator of the enzyme nitrite reductase
- It is necessary for the evolution of oxygen (photolysis) during photosynthesis

Copper

- It is an important constituent of plastocyanin (copper containing protein)
- It is also a constituent of several oxidizing enzymes.

Boron

- Boron facilitates the translocation of sugars by forming sugar borate complex.
- It involves in cell differentiation and development since boron is essential for DNA synthesis
- Also involves in fertilization, hormone metabolism etc.

Molybdenum

- It is constituent of the enzyme nitrate reductase and thus plays an important role in nitrogen metabolism
- It is essential for flower formation and fruit set.

09. **Foliar diagnosis - Nutritional and Physiological disorders**

A. Foliar diagnosis - Symptoms

Nitrogen

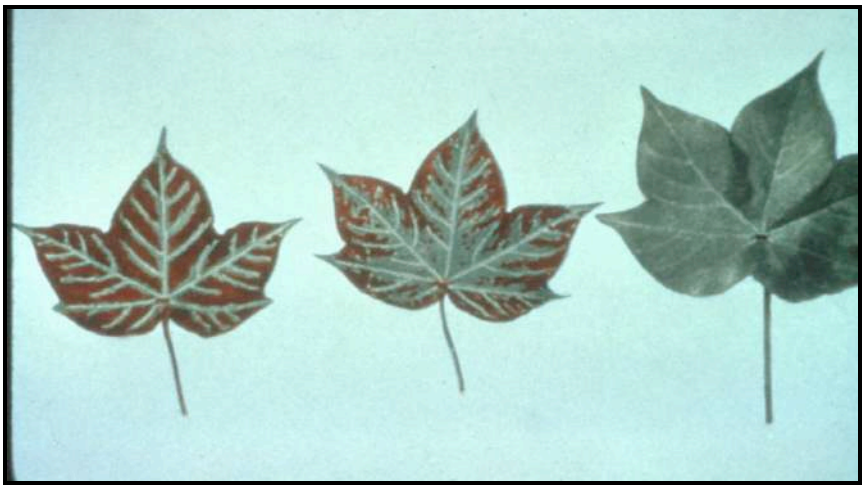
- Plant growth is stunted because protein content cell division and cell enlargement are decreased
- N deficiency causes chlorosis of the leave i.e yellowing older leaves are affected first
- In many plants eg. Tomato, the stem, petiole and the leaf veins become purple coloured due to the formation of anthocyanin pigments.

Phosphorus

- P deficiency may cause premature leaf fall
- Dead necrotic areas are developed on leave or fruits
- Leaves may turn to dark green to blue green colour. Sometimes turn to purplish colour due to the synthesis and accumulation of anthocyanin pigments.

Potassium

- Mottled chlorosis of leaves occurs
- Neurotic areas develop at the tip and margins of the leaf
- Plants growth remains stunted with shortening of internodes.



Calcium

- Calcium deficiency causes disintegration of growing meristematic regions of root, stem and leaves
- Chlorosis occurs along the margins of the younger leaves

- Malformation of young leaves takes place

Magnesium

- Mg deficiency causes mottled chlorosis with veins green and leaf tissues yellow or white appearing first on older leaves
- Dead neurotic patches appear on the leaves
- In cotton Mg deficiency leads o reddening of leaves and disorder is called as reddening in cotton.

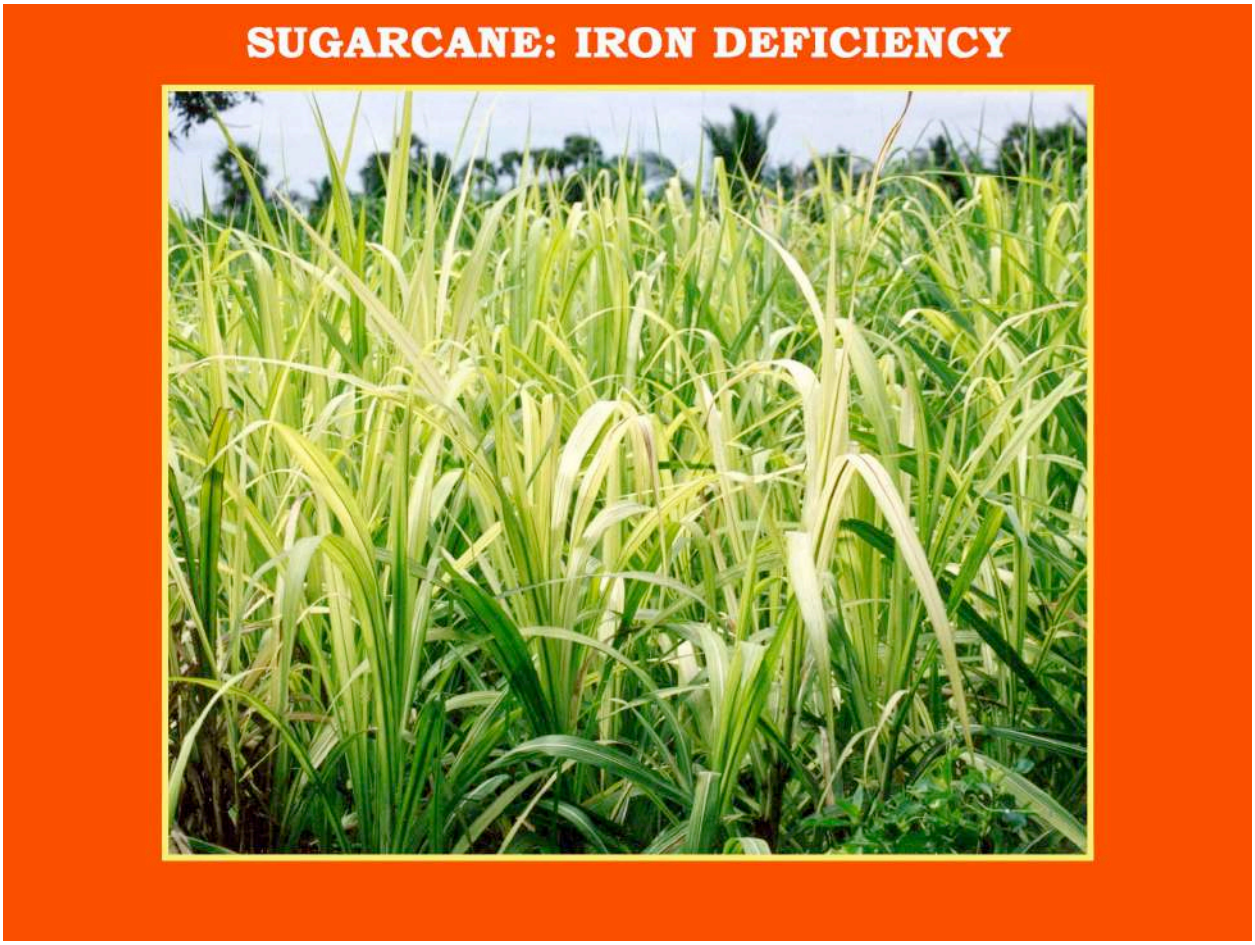
Sulphur

- Deficiency causes chlorosis of the leaves
- Tips and margins of the leaf roll in ward
- Stem becomes hard due to the development of sclerenchyma.

Micronutrients

Iron

Iron deficiency causes chlorosis of young leaves which is usually interveinal.



Zinc

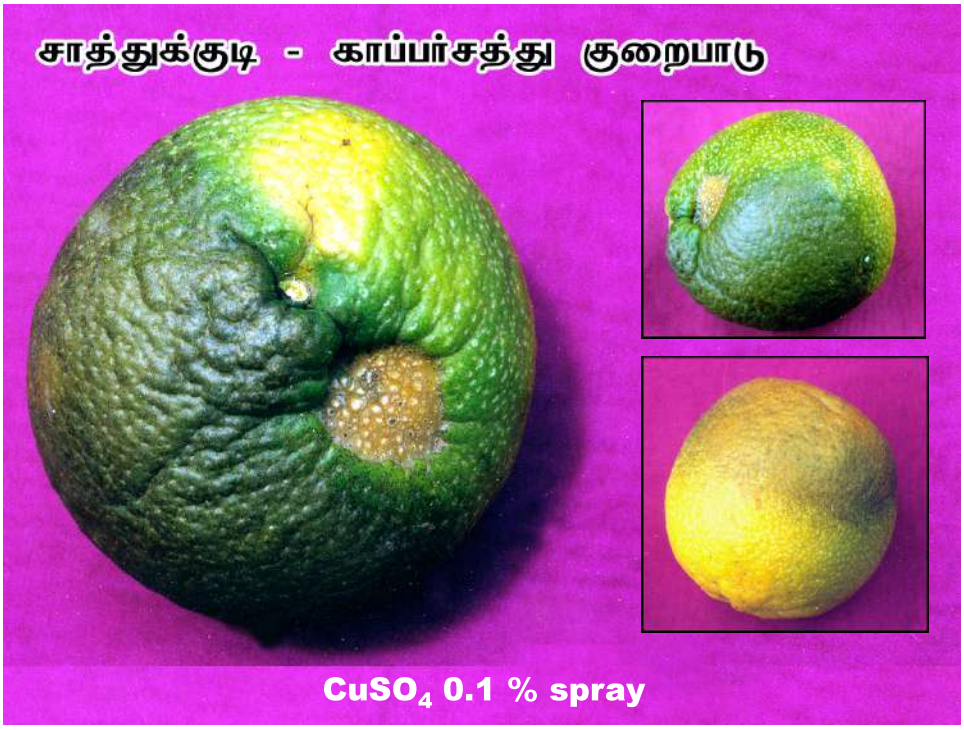
- Zinc deficiency causes chlorosis of the young leaves which starts from tips and the margins
- The size of the young leaves is very much reduced. This disorder is called as 'little leaf disease'
- Stalks will be very short.

Manganese

- The young leaves are affected by mottled chlorosis
- Veins remain green
- Small necrotic spots developed on the leaves with yellow strips

Copper

- Copper deficiency causes necrosis of the tip of the young leaves
- It also causes die-back of citrus and fruit trees
- Also causes reclamation disease or white tip disease of cereals and leguminous plants.



Boron

- Boron deficiency causes death of shoot tip
- Flower formation is suppressed
- Root growth is stunted
- The other diseases caused by B deficiency is
- Heart rot of beet
- Stem crack of celery
- Brown heart of cabbage
- Water core of turnip
- Internal cork formation in apple
- Hen and chicken in grapes

Molybdenum

- Molybdenum deficiency causes interveinal chlorosis of older leaves
- Flower formation is inhibited
- Causes whiptail disease in cauliflower plants.

Foliar Nutrition

Foliar nutrition is fertilizing certain crop plants through aerial spraying.

Mechanism

Penetration of the spray solution or nutrient solution occurs through cuticle the layer of polymerized wax which occurs on outer surface of the epidermal cells of leaves. After penetration in the cuticle, further penetration take place through fine, thread like semi-microscopic structure called ectodesmata. This extends through the outer epidermal cell wall, from the inner surface of the cuticle to the plasma membrane. When the substance reaches plasma membrane of an epidermal cell, it will be observed by mechanism similar to those which operate in root cells.

1. Foliar nutrition may serve as a mean of applying supplemental macronutrients during critical growth periods when it is impracticable to apply fertilizers to soil. Eg. Unusual period of dry weather.
2. Foliar nutrition may afford a remedy for the time lag between soil applied and plant absorbed. Time is too long because of fast growing rates.

NUTRITIONAL DISORDERS

When a nutrient element insufficiency (deficiency and/or toxicity) occurs, visual symptoms may or may not appear, although normal plant development will be slowed. When visual symptoms do occur, such symptoms can frequently be used to identify the source of the insufficiency.

Deficiency Symptoms

- Stunted or reduced growth of the entire plant with the plant itself either remaining green or lacking an over-all green color with either the older or younger leaves being light green to yellow in color.
- Chlorosis of leaves, either interveinal or of the whole leaf itself, with symptoms either on the younger and/or older leaves, or both (chlorosis due to the loss or lack of chlorophyll production).

- Necrosis or death of a portion (margins or interveinal areas) of a leaf, or the whole leaf, usually occurring on the older leaves.
- Slow or stunted growth of terminals (rosetting), the lack of terminal growth, or death of the terminal portions of the plant.
- A reddish purpling of leaves, frequently more intense on the under side of older leaves due to the accumulation of anthocyanin (Mottling)

Chlorosis is caused by the deficiency of mineral elements such as Mn, K, Zn, Fe, Mg, S and N. *Mottling* is caused due to the deficiencies of N, Mg, P, S and *Necrosis* due to the deficiency of Mg, K, Zn, Ca and Mo.

Toxicity Symptoms

Visual symptoms of toxicity may not always be the direct effect of the element in excess on the plant, but the effect of the excess element on one or more other elements. For example, an excessive level of potassium (K) in the plant can result in either magnesium (Mg) and/or calcium (Ca) deficiency, excess phosphorus (P) can result in a zinc (Zn) deficiency and excess Zn in an iron (Fe) deficiency.

These effects would compare to elements, such as boron (B), chlorine (Cl), copper (Cu), and manganese (Mn), which create visual symptoms that are the direct effect of an excess of that element present in the plant.

Some elements, such as aluminum (Al) and copper (Cu) can affect plant growth and development due to their toxic effect on root development and function.

Hidden Hunger

In some instances, a nutrient element insufficiency may be such that no symptoms of stress will visually appear with the plant seeming to be developing normally. This condition has been named hidden hunger, a condition that can be uncovered by means of either a plant analysis and/or tissue test.

A hidden hunger occurrence frequently affects the final yield and the quality of the product produced. For grain crops, the grain yield and quality may be less than expected; for fruit crops, abnormalities, such as blossomed rot and internal abnormalities may occur, and the post harvest characteristics of fruits and flowers will result in poor shipping quality and

reduced longevity. Another example is potassium (K) insufficiency in corn, a - deficiency that is not evident until at maturity when plants easily

PHYSIOLOGICAL DISORDERS

Physiological disorder is the abnormal growth pattern or abnormal external or internal conditions of fruits due to adverse environmental conditions such as deviation from normal state of temperature, light, moisture, nutrient, harmful gases and inadequate supply of growth regulators.

Disorders associated with low temperature

1. Leaf chlorosis and frost banding

Chlorosis was caused by a disruption of chloroplasts caused by winter cold. Green chlorophyll pigments are often converted in to yellow pigment. Leaf may appear with distinct bleached bands across the blade of young plants called frost banding e.g.: sugarcane, wheat and barley.

2. Leaf necrosis and malformations

Spring frost causes various types and degree of injury including cupping, crinkling finishing and curling of leaves of apple trees and stone fruits. The distortion is caused by death of the developed tissues before the expansion of leaves.

3. Stem disorders

Frost cracks develop when tree trunk or limps lost their heat too rapidly. The outer layer of bark and wood cool most rapidly and subjected to appreciable tension causing marked shrinkage and cracking following a sudden temperature drop. Affected timber is of poor quality.

Disorders associated with high temperature

1. Leaf scorch

High temperature causes leaf scorch directly or indirectly by stimulating excessive evaporation and transpiration. Tip burn of potato is a widespread example for this disorder.

2. Sunscald

In leaf vegetable crops like lettuce and cabbage, when leaves on the top of the head are exposed to intense heat, water soaked lesions or blistered appearance occur These irregular shaped areas become bleached and parched later.

3. Water core

In fruit crop like Tomato, exposure to high temperature causes death of the outer cells of fruit skin. Subsequently corky tissue occurs beneath the skin, with watery appearance of the flesh near the core of the fruits faster. Often light stress is coupled with heat stress e.g. sun scald of bean, sun burning of soybean and cowpea. In flower crop like chrysanthemum, increase in light intensity affects flower bud formation. Reproduction phase does not commence and modified into leaf like bracts.

Disorders caused by light stress

Adverse light intensity causes impaired growth and reduced vigour. Subsequently leaves gradually lose green colour, turning pale green to yellow, stems may dieback little every year. Insufficient light limits photosynthesis, causing food reserves to be depleted.

Identification of Physiological Disorders and Corrective Measures

Crop	Malady	Corrective measure
Rice	Severe chlorosis of leaves	1%super phosphate and 0.5% ferrous sulphate
Rice	Irregular flowering and chaffiness multiple deficiency of nutrients	1% super phosphate and magnesium sulphate.
Rice	Tip drying and marginal scoring and browning	1%super phosphate and 0.5% zinc sulphate.
Maize	Chlorosis	A spray solution containing 0.5% ferrous sulphate and 0.5% urea.
Maize	'White bud' yellowing in the bud leaves only	0.5% zinc sulphate spray with 1% urea.
Maize	Tip drying and marginal scoring pinkish colouration of lower leaves	1% super phosphate and 0.5% zinc sulphate.

Maize	Marginal scorching and yellowing.	0.5% ferrous sulphate and 1%urea
	Irregular drying of tips and margins	25 kg of zinc sulphate / ha
Sorghum	Chlorosis of younger leaves	Spray of 0.5% ferrous sulphate with 0.5%urea and 0.5% ammonium sulphate
Cowpea	Water soaked necrotic spots on leaf surface. Root growth very much restricted in 10-12 days old seedling	Spray containing sulphate and zinc sulphate 0.1% and 0.1% urea
Groundnut	Chlorosis of terminal leaves	0.5% ferrous sulphate and urea 1%

10. PHOTOSYNTHESIS

Photosynthesis is a vital physiological process where in the chloroplast of green plants synthesizes sugars by using water and carbon dioxide in the presence of light.

Photosynthesis literally means *synthesis with the help of light* i.e. plant synthesize organic matter (carbohydrates) in the presence of light.

Photosynthesis is sometimes called as carbon assimilation (assimilation: absorption into the system).This is represented by the following traditional equation.

$$6CO_2 + 12H_2O \xrightarrow[\text{Green pigments}]{\text{Light}} 6O_2 + C_6H_{12}O_6$$

carbohydrates

During the process of photosynthesis, the light energy is converted into chemical energy and is stored in the organic matter, which is usually the carbohydrate. One molecule of glucose for instance, contains about 686 K Calories energy. CO_2 and water constitute the raw material for this process and oxygen and water are formed as the by products during photosynthesis. *Stephen Hales* (1727) first explained the relationship between sunlight and leaves and *Sachs* (1887) established that starch was the visible product of photosynthesis.

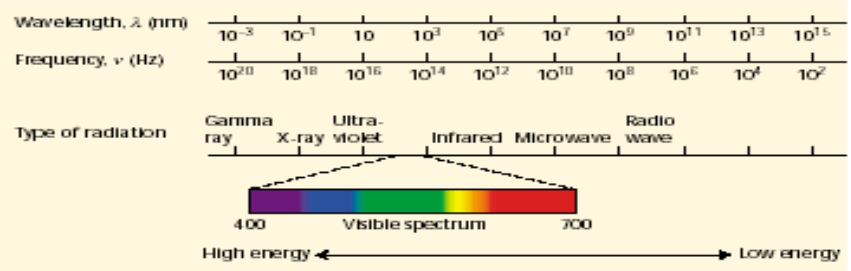


FIGURE 7.2 Electromagnetic spectrum. Wavelength (λ) and frequency (ν) are inversely related. Our eyes are sensitive to only a narrow range of wavelengths of radiation, the visible region, which extends from about 400 nm (violet) to about 700 nm (red). Short-wavelength (high-frequency) light has a high energy content; long-wavelength (low-frequency) light has a low energy content.

Photosynthetic apparatus

The chloroplast in green plants constitutes the photosynthetic apparatus. In higher plants, the chloroplast is discoid in shape, 4-6 μ in length and 1-2μ thick. The chloroplast is bounded by two unit membranes of approximately 50°A thickness and consists of lipids and

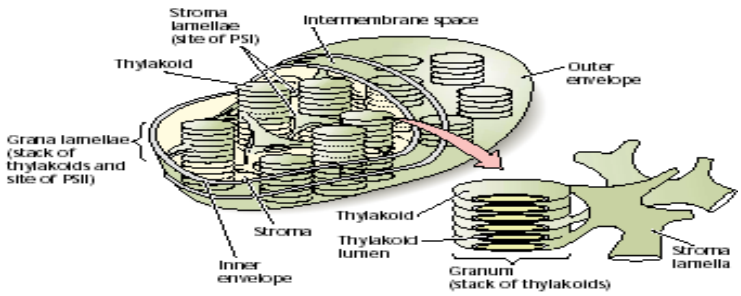
proteins. The thickness of the two membranes including the space enclosed by them is approximately 300°A (1 Angstrom: 0.1 cm).

Internally, the chloroplast is filled with a hydrophilic matrix called as *stroma* embedded with *grana.* Each grana consists of 5-25 disk shaped grana lamellae (thylakoid) placed one above the other like the stack of coins. Each grana lamella of thylakoid encloses a space called *loculus* and the thylakoid membrane consists of alternating layer of lipids and proteins. Some of the grana lamella of thylakoid of grana are connected with thylakoid of other grana by somewhat thinner *stroma lamella or fret membrane*. Chlorophyll and other photosynthetic pigments are confined to grana. The chlorophylls are the site of photochemical reactions.

Photosynthetic pigments

Photosynthetic pigments are of three types; Chlorophylls, Carotenoids and Phycobillins.

- Chlorophylls and Carotenoids are insoluble in water and can be extracted only with organic solvents such as acetone, petroleum ether and alcohol.
- Phycobillins are soluble in water
- Carotenoids include carotenes and xanthophylls. The xanthophylls are also called as *carotenols.*



Chlorophylls (green pigments)

Chlorophylls are magnesium porphyrin compounds. The porphyrin ring consists of four pyrrol rings joined together by CH bridges. Long chain C atoms called as phytol chain is attached to porphyrin ring at pyrrol ring IV.

The chemical structure of chlorophyll *a* and chlorophyll *b* are well established. The molecular formula for chlorophyll *a*: $C_{55}H_{72}O_5N_4$ Mg and chlorophyll *b*: $C_{55}H_{70}O_6N_4$ Mg. Both of them consist of Mg porphyrin head which is hydrophilic and a phytol tail which is

lipophilic. The two chlorophylls differ because in chlorophyll *b* there is a –CHO group instead of CH_3 group at the 3rd C atom in pyrrol ring II.

Chlorophyll is formed from protochlorophyll in light. The protochlorophyll lacks 2H atoms one each at 7th and 8th C atoms in pyrrol ring IV.

Carotenoids (yellow or orange pigments)

1. Carotenes: Carotenes are hydrocarbons with a molecular formula $C_{40}H_{56}$
2. Xanthophylls (carotenols)

They are similar to carotenes but differ in having two oxygen atoms in the form of hydroxyl or carboxyl group. The molecular formula is $C_{40}H_{56}O_2$.The role of Carotenoids is absorption of light energy and transfer the light energy to chlorophyll *a* molecules. They also play a very important role in preventing photodynamic damage within the photosynthetic apparatus. Photodynamic damage is caused by O_2 molecules which is very reactive and is capable of oxidizing whole range of organic compounds such as chlorophylls and there by making them unfit for their normal physiological function.

Phycobillins (red and blue pigments)

These also contain four pyrrol rings but lack Mg and the phytol chain.

Location of photosynthetic pigments in chloroplast

The photosynthetic pigments are located in grana portions of the chloroplast. They are present in the thylakoid membrane or membrane of grana lamella. The membrane of thylakoid is made up of proteins and lipids or the membrane consists of both lipid layer and protein layer. The hydrophilic *heads* of the chlorophyll molecules remain embedded in the protein layer while lipophilic phytol tail in the lipid layer. The other pigments are thought to be present along with chlorophyll molecules.

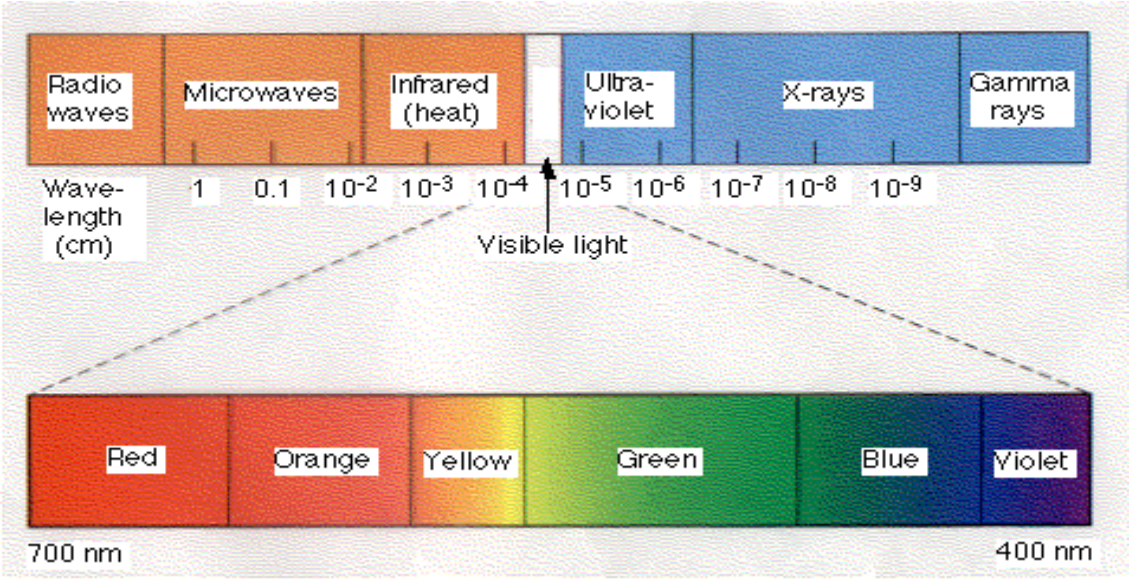
Distribution of photosynthetic pigments in plant kingdom

Pigments	**Distribution in plant kingdom**
Chlorophylls	

Chlorophyll *a*	All photosynthesizing plants except bacteria
Chlorophyll *b*	Higher plants and green algae
Chlorophyll *c*	Diatoms and brown algae
Chlorophyll *d*	Red algae
Bacteria chlorophylls *a, b, c, d & e*	Purple and green bacteria
Carotenoids	
Carotenes (α and β)	Higher plants and algae
Xanthophylls	Higher plants and algae
Lutein	Green leaves and Green and Red algae
Violaxanthin	Green leaves
Fucoxanthin	Brown algae
Phycobillins	
Phycocyanins	Blue green algae and red algae
Phycoerythrins	Blue green algae and Red algae
Allophycocyanin	Blue – green and Red algae

Light

The chief source of light energy for photosynthesis is sun. The solar radiation or solar energy passes through the space and reaches the earth in the form of *electromagnetic radiation* with waves of varying lengths. The various portions of electromagnetic spectrum are gamma rays, ultraviolet rays, visible rays and infrared rays. The wavelength of these rays ranges from 280 nm to 1000 nm.

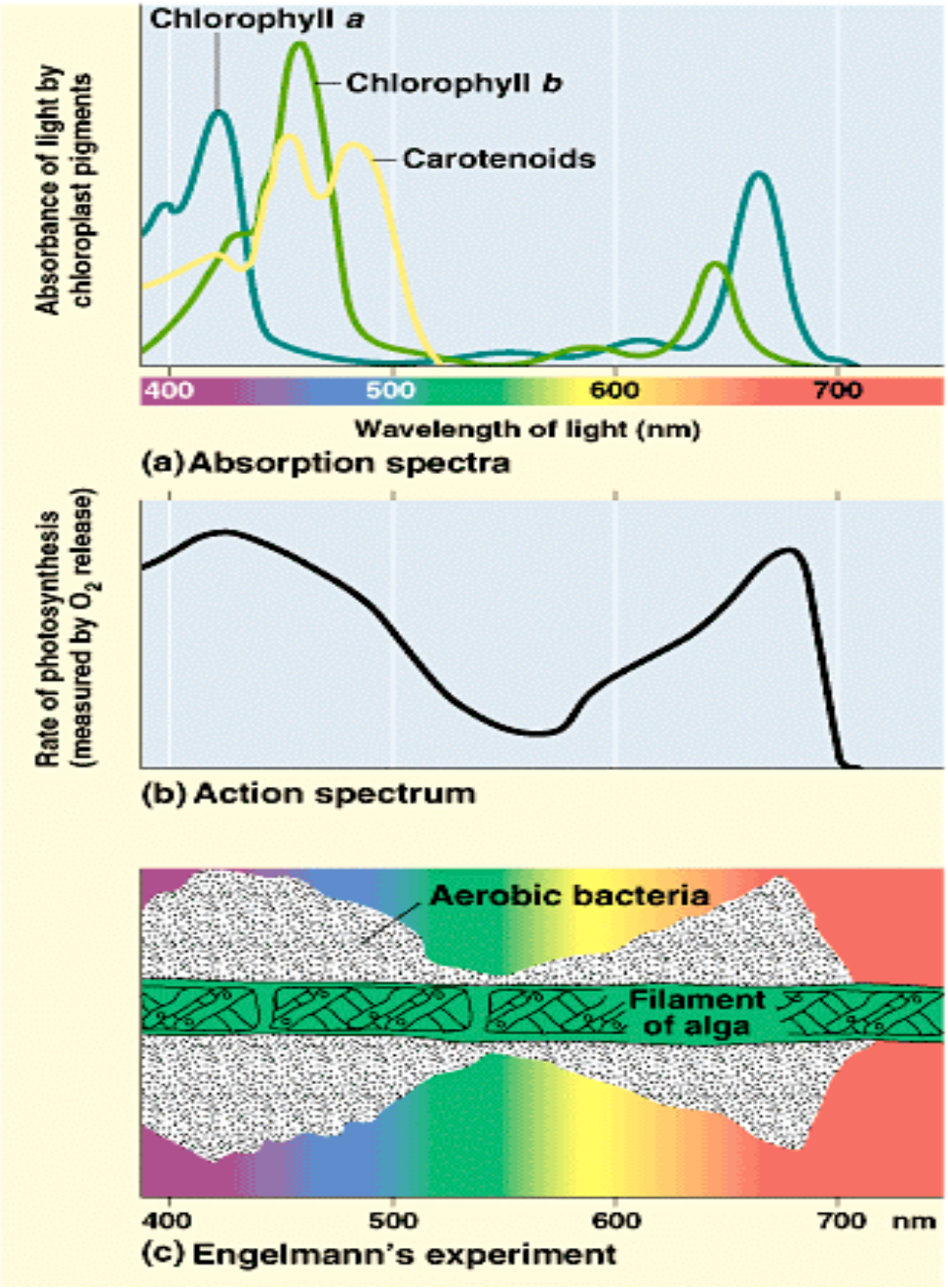


Below 280 nm	-	X rays, Gamma rays and Cosmic rays	
280-390 nm	-	Ultra violet radiation	
400-510 nm	-	Blue light	Visible light (PAR) (VIBGYOR)
510-610 nm	-	Green light	
610-700 nm	-	Red light	
700-1000 nm	-	Far red light (IR)	

Photosynthetic pigments absorb light energy only in the visible part of the spectrum. The earth receives only about 40% (or about $5x10^{20}$ K cal) of the total solar energy. The rest is either absorbed by the atmosphere or scattered into the space. Only about 1% of the total solar energy received by the earth is absorbed by the pigments and utilized in photosynthesis.

Absorption spectra of chlorophyll

The absorption of different wavelengths of light by a particular pigment is called *absorption spectrum*. Chlorophylls absorb maximum light in the violet blue and red part of the spectrum. The absorption peaks of chlorophyll *a* are 410 and 660; for chlorophyll *b* 452 and 642. Carotenoids absorb light energy in blue and blue green part of the spectrum.



Transfer of light energy absorbed by accessory pigments to chlorophyll *a*

All pigments except chlorophyll *a* are called as *accessory pigments or antenna pigments.* The light energy absorbed by accessory pigments is transferred to chlorophyll *a* molecule. The transfer of light energy from accessory pigments to chlorophyll *a* is called as *resonance or Forster transfer* and takes part in primary photochemical reaction in photosynthesis. Chlorophyll *a* molecules also absorb light energy directly. As a result of absorbing the light energy, the chlorophyll molecule gets *excited*.

Excited states of atoms or molecules (*fluorescence* and *phosphorescence*)

The normal state of the chlorophyll molecule or atom is called as *ground state or singlet state.* When an electron of a molecule or an atom absorbs a quantum of light, it is raised to a higher energy level which is called as *excited second singlet state*. This state is unstable and has a life time of 10^{-12} seconds.

The electron comes to the next higher energy level by the loss of some of its extra energy in the form of heat. This higher energy level is called as *excited first singlet state* and is also unstable with a half life of 10^{-9} seconds. From the first singlet state, the excited electron may return to the ground state in two ways viz., either losing its remaining extra energy in the form of heat or in the form of radiant energy. The second process is called *fluorescence*. The chlorophyll molecules exit the extra energy in the form of fluorescent light when they are exposed to incident light. Fluorescent light is of longer wavelength than the incident light.

The excited molecule or the atom may also lose its excitation energy by internal conversion and comes to another excited state called as *triplet state* which is meta stable with a half life of 10^{-3} seconds. From the triplet state, the excited molecule or the atom may return to the ground state in three ways.

(i) By losing its remaining extra energy in the form of heat

(ii) By losing extra energy in the form of radiant energy (*phosphorescence*) and the chlorophyll molecules emit phosphorescent light even after the incident radiant light is cut off. The phosphorescent light is of longer wavelength than incident light and also fluorescent light.

(iii) Electrons carrying the extra energy may be expelled from the molecule and is consumed in some further photochemical reaction and the fresh normal electron returns to the molecule.

Quantum requirement and quantum yield

Light rays consist of tiny particles called *photons* and the energy carried by a photon is called *quantum*. The number of photons (quantum) required to release one molecule of oxygen in photosynthesis is called *quantum requirement.* On the other hand, the number of oxygen molecules released per photon of light in photosynthesis is called as *quantum yield*. The quantum yield is always in fraction of one.

Warburg found minimum quantum requirement for photosynthesis as four. It is because the reduction of one molecule of CO_2 by two molecules of H_2O requires the transfer of 4H atoms. The transfer of each H atoms from H_2O to CO_2 requires one photon or quantum of light.

$$4H_2O \rightarrow 4\,OH^- + 4H^+$$

$$4OH^- \rightarrow 2H_2O + O_2 + 4e^-$$

$$4H^+ + CO_2 \rightarrow (CH_2O) + H_2O$$

$$2H_2O + CO_2 \rightarrow (CH_2O) + O_2 + H_2O$$

(CH_2O) in the above equation represent 1/6 of the carbohydrate molecule such as glucose. One molecule of glucose contains 686 K. cal of energy. Therefore, 1/6 glucose molecule contains 686/6 i.e., approximately 112 K.cal energy. It is also known that the rate of photosynthesis is maximum at red light and each photon of red light contains about 40 K cal. of energy. This would suggest that the efficiency with which the plants can convert light energy into chemical energy is 112 / 40 x 4: 70%, which indeed is very high.

According to Emerson and his coworkers, photosynthesis is a very complicated process and is not so efficient to convert all the light energy into chemical energy. There is a considerable loss of light energy absorbed during photosynthesis and therefore the minimum quantum requirement for photosynthesis as suggested by Emerson and coworkers are 8-10. Considering that the quantum requirement for photosynthesis is 8-10, the quantum yield would accordingly be 1/8 to 1/10 (0.125 to 0.10)

Mechanism of Photosynthesis

Photo systems (Two pigment systems)

The discovery of red drop and the Emerson's enhancement effect led the scientists to suggest that photosynthesis is driven by two photochemical processes. These processes are associated with two groups of photosynthetic pigments called as *pigment system I* and *pigment system II.* Wavelength of light shorter than 680 nm affect both the pigments systems while wavelength longer than 680 nm affect only pigment system I.

In green plants, pigment system I contains chlorophyll *a, b* and carotene. In this pigment system, a very small amount of chlorophyll *a* absorbing light at 700 nm, known as P700 however constitutes the reaction centre of *photosystem I*.

The pigment system II contains chlorophyll *b* and some forms of chlorophyll a (such as chlorophyll *a* 662, chlorophyll *a* 677 and chlorophyll *a* 679) and xanthophylls. A very small amount of special form of chlorophyll called P680 constitute the reaction centre of pigment system II. Carotenoids are present in both the pigment systems

The two pigment systems I and II are interconnected by a protein complex called cytochrome b_6–f complex. The other intermediate components of electron transport chain *viz*., plastoquinone (PQ) and plastocyanin (PC) act as mobile electron carriers between the complex and either of the two pigment systems. The light energy absorbed by other pigment is ultimately trapped by P700 and P680 forms of chlorophyll *a* which alone take part in further photochemical reaction.

Pigment system I (PSI) complex consists of 200 chlorophylls, 50 Carotenoids and a molecule of chlorophyll *a* absorbing light at 700 nm(P700) and this constitute the reaction centre of photosystem I. Pigment system II (PSII) complex consists of 200 chlorophylls, 50 Carotenoids and a mole of chlorophyll a absorbing light at 680 nm, called P 680 at the centre. This constitutes the reaction centre of pigment system II.

Photosynthetic units – The Quantasomes

Emerson and Arnold (1932) showed that about 2500 chlorophyll molecules are require fixing one molecule of CO_2 in photosynthesis. This number of chlorophyll molecules was called the *chlorophyll unit* but the name was subsequently changed to *photosynthetic*

unit. However, since the reduction or fixation of one CO_2 molecule requires about 10 quanta of light, it is assured that 10 flashes of light are required to yield one O_2 molecule or reduction of one molecule of CO_2. Thus each individual unit would contain $1/10^{th}$ of 2500 i.e., 250 molecules.

Action spectrum

The pigments present in plants or any living organism have the ability to absorb radiant energy to carry out photo physiological reactions. It is difficult to decide which specific pigment is actually associated with the particular photochemical reactions. Hence, a common procedure to identify the pigment involved in a particular photoreaction is to determine the action spectrum i.e. measuring the rate of the particular photoreaction.

Once the action spectrum for a photo physiological reaction is determined, the next step is to compare this action spectrum with absorption spectrum of a pigment.

Two pigments, A and B were isolated from the same plant and their absorption spectra were determined. Pigment A has a peak in absorption at 395 nm and the pigment B at 660 nm. The close correspondence between the absorption spectrum and the action spectrum of pigment B strongly supports that Pigment B is responsible for absorbing radiant energy to drive this photoreaction.

Mechanism of photosynthesis

The biosynthesis of glucose by the chloroplast of green plants using water and CO_2 in the presence of light is called photosynthesis. Photosynthesis is a complex process of synthesis of organic food materials. It is a complicated oxidation- reduction process where water is oxidized and CO_2 is reduced to carbohydrates. The mechanism of photosynthesis consists of two parts.

1. Light reaction / Primary photochemical reaction / Hill's reaction/ Arnon's cycle
2. Dark reaction / Black man's reaction / Path of carbon in photosynthesis.

1. Light reaction or Primary photochemical reaction or Hill's reaction

In light reaction, ATP and $NADPH_2$ are produced and in the dark reaction, CO_2 is reduced with the help of ATP and $NADPH_2$ to produce glucose. The light reaction is called primary photochemical reaction as it is induced by light Light reaction is also called as Hill's

reaction as Hill proved that chloroplast produce O_2 from water in the presence of light. It is also called as Arnon's cycle because Arnon showed that the H^+ ions released by the break down of water are used to reduce the coenzyme NADP to NADPH. Light reaction includes photophosphorylation as ATP is synthesized in the presence of light. The reaction takes place only in the presence of light in *grana* portion of the chloroplast and it is faster than dark reaction. The chlorophyll absorbs the light energy and hence the chlorophyll is called as *photosystem* or *pigment system.* Chlorophylls are of different types and they absorb different wavelengths of light. Accordingly, chlorophylls exist in two photo systems, Photosystem I (PSI) and Photosystem II (PS II). Both photo systems are affected by light with wavelengths shorter than 680nm, while PS I is affected by light with wavelengths longer than 680nm.

The components of photo systems

Photosystem I	Photosystem II
Chlorophyll *a* 670	Chlorophyll *a* 660
Chlorophyll *a* 680	Chlorophyll *a* 670
Chlorophyll *a* 695	Chlorophyll *a* 680 or P680
Chlorophyll *a* 700 or P700	Chlorophyll *b*
Chlorophyll *b*	Phycobillins
Carotenoids	Xanthophylls
P700 form of Chlorophyll *a*	P680 form of Chlorophyll *a*
is the active reaction centre	is the active reaction centre

The light reaction can be studied under the following headings.

i. Absorption of light energy by chloroplast pigments

Different chloroplast pigments absorb light in different regions of the visible part of the spectrum.

ii. Transfer of light energy from accessory pigments to chlorophyll *a*

All the photosynthetic pigments except chlorophyll *a* are called as accessory or antenna pigments. The light energy absorbed by the accessory pigments is transferred by resonance to chlorophyll *a* which alone can take part in photochemical reaction. Chlorophyll

a molecule can also absorb the light energy directly. In pigment system I, the photoreaction centre is P700 and in pigment system II, it is P680.

iii. Activation of chlorophyll molecule by photon of light

When P700 or P680 forms of chlorophyll *a* receives a photon (quantum) of light, becomes an excited molecule having more energy than the ground state energy. After passing through the unstable second singlet state and first singlet stage the chlorophyll molecules comes to the meta stable triplet state. This excited state of chlorophyll molecule takes part further in primary photochemical reaction i.e. the electron is expelled from the chlorophyll *a* molecule.

$$\text{Chlorophyll } a \xrightarrow{\text{Light}} \text{Excited triplet state of chlorophyll } a$$

$$\text{Excited triplet state of chlorophyll } a \longrightarrow \textit{Chlorophyll } a^{+} + e^{-}$$

iv. Photolysis of water and O_2 evolution (oxidation of water)

These processes are associated with pigment system II and are catalyzed by Mn^{++} and Cl^{-} ions. When pigment system II is active i.e it receives the light, the water molecules split into OH^{-} and H^{+} ions (*Photolysis of water*). The OH^{-} ions unite to form some water molecules again and release O_2 and electrons.

$$4H_2O \rightarrow 4H^{+} + 4\,(OH^{-})$$

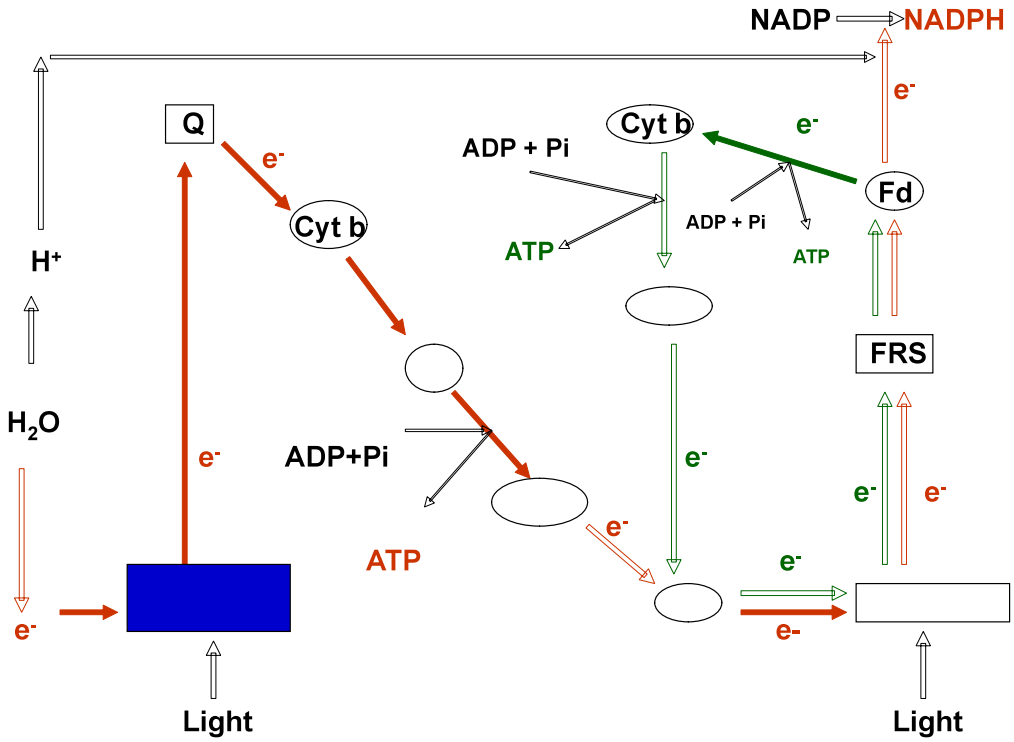
$$4(OH^{-}) \rightarrow 2H_2O + O_2 + 4e^{-}$$

$$2H_2O \rightarrow 4H^{+} + O_2 + 4e^{-}$$

v. Electron transport and production of assimilatory powers ($NADPH_2$ and ATP)

It has already been observed that when chlorophyll molecule receives the photon of light, an electron is expelled from the chlorophyll *a* molecule along with extra energy. This electron after traveling through a number of electron carriers is utilized for the production of $NADPH_2$ from NADP and also utilized for the formation of ATP molecules from ADP and inorganic phosphate (Pi). The transfer of electrons through a series of coenzymes is called *electron transport* and the process of formation of ATP from ADP and Pi using the energy of electron transport is called as *photosynthetic phosphorylation or photophosphorylation*. The types of Phosphorylation include *cyclic and non- cyclic.*

Cyclic electron transport and cyclic photophosphorylation



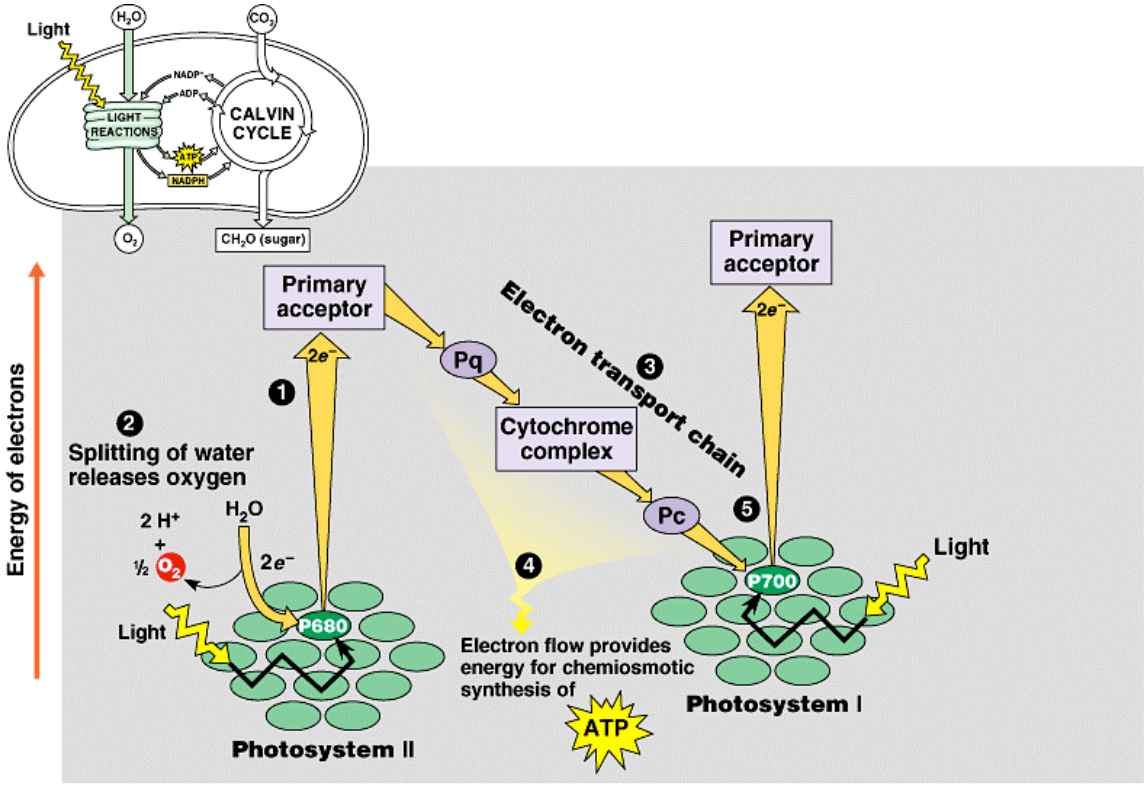
The electrons released from photosystem I goes through a series of coenzymes and returns back to the same photosystem I. This electron transport is called *cyclic electron transport.* The synthesis of ATP occurring in cyclic electron transport is called *cyclic photophosphorylation*. The cyclic electron transport involves only pigment system I. This situation is created when the activity of pigment system II is blocked. Under this condition,

1. Only pigment system I remain active
2. Photolysis of water does not take place
3. Blockage of noncyclic ATP formation and this causes a drop in CO_2 assimilation in dark reaction
4. There is a consequent shortage of oxidized NADP

Thus, when P700 molecule is excited in pigment system I by absorbing a photon (quantum) of light, the ejected electron is captured by ferredoxin *via* FRS. From ferredoxin, the electrons are not used up for reducing NADP to NADPH + H^+ but ultimately it falls back

to the P700 molecule via number of other intermediate electron carriers. The electron carriers are probably cytochrome b_6, cytochrome *f* and plastocyanin.

During this electron transport, phosphorylation of ADP molecule to form ATP molecule take place at two places i.e., between ferredoxin and cytochrome b_6 and between cytochrome b_6 and cytochrome *f*. Thus, two ATP molecules are produced in this cycle. Since the electron ejected from P700 molecule is cycled back, the process has been called as *cyclic electron transport* and the accompanying phosphorylation as the *cyclic photophosphorylation.*



Significance of cyclic photophosphorylation

1. During cyclic electron transport and phosphorylation, photolysis of water, O_2 evolution and reduction of NADP do not take place.
2. The electron returns or cycles back to original position in the P700 form of chlorophyll *a*. Here, chlorophyll molecule serves both as donor and acceptor of the electron.
3. It generates energy rich ATP molecules at two sites and as such cannot drive dark reactions of photosynthesis

On the other hand, non- cyclic photophosphorylation does not produce sufficient ATP in relation to NADPH to operate the dark phase of photosynthesis. Therefore, the deficiency of ATP molecule in non–cyclic photophosphorylation is made up by the operations of cyclic photophosphorylation.

Secondly, the cyclic photophosphorylation may be an important process in providing ATP for photosynthesis and other processes such as synthesis of starch, proteins, lipids, nucleic acids and pigments within the chloroplast.

Non cyclic photophosphorylation

The electron released from photosystem II goes through a series of enzymes and Co-enzymes to photosystem I. This is called non cyclic electron transport and the Synthesis of ATP in non cyclic electron transport is called non- cyclic photo phosphorylation. The main function of non cyclic electron transport is to produce the assimilatory powers such as $NADPH_2$ and ATP and the process occurs in photosystem I
and II.

This process of electron transport is initiated by the absorption of a photon (quantum) of light by P680 form of chlorophyll *a* molecule in the pigment system II, which gets excited and an electron is ejected from it so that an electron deficiency or a hole is left behind in the P680 molecule.

The ejected electron is trapped by an unknown compound known as Q. From Q, the electron passes downhill along a series of compounds or intermediated electron carriers such as cytochrome b_6, plastoquinone, cytochrome *f* and a copper containing plastocyanin and ultimately received by pigment system I. At one place during electron transport i.e.

between plastoquinone and cytochrome *f,* one molecule of ATP is formed from ADP and inorganic phosphate.

Now, when a photon of light is absorbed by P700 form of chlorophyll molecule in the pigment system I, this gets excited and an electron is ejected from it. This ejected electron is trapped by FRS (Ferredoxin Reducing Substance) and it is then transferred to a non-heme iron protein called ferredoxin. From ferredoxin, electron is transferred to NADP so that NADP is reduced to NADPH + H^+

The hole in pigment system I has been filled by electron coming from pigment system II. But, the hole or an electron deficiency in pigment system II is filled up by the electron coming from photolysis of water where, water acts as electron donor.

In this scheme of electron transport, the electron ejected from pigment system II did not return to its place of origin, instead it is taken up by pigment system I. Similarly, the electron ejected from pigment system I did not cycle back and was consumed in reducing NADP. Therefore, this electron transport has been called as *non–cyclic electron transport* and accompanying phosphorylation as *non–cyclic photophosphorylation.*

The non cyclic electron transport (photophosphorylation) takes the shape of Z and hence it is called by the name Z–scheme. Non cyclic photophosphorylation and O_2 evolution are inhibited by CMU (3-(4'-Chlorophyl) – 1-1 dimethylurea and 3-(3-4-dichlorophenyl)-1, 1-dimethylurea (DCMU).

Significance of non cyclic electron transport

1. It involves PS I and PSII
2. The electron expelled from P680 of PSII is transferred to PS I and hence it is a non cyclic electron transport
3. In non cyclic electron transport, photolysis of water (Hill's reaction and evolution of O_2) takes place.
4. Phosphorylation (synthesis of ATP molecules) takes place at only one place.
5. The electron released during photolysis of water is transferred to PS II.
6. The hydrogen ions (H^+) released from water are accepted by NADP and it becomes $NADPH_2$

7. At the end of non cyclic electron transport, energy rich ATP, assimilatory power $NADPH_2$ and oxygen from photolysis of water are observed.
8. The ATP and $NADPH_2$ are essential for the dark reaction wherein, reduction of CO_2 to carbohydrate takes place.

Comparison of cyclic and non cyclic electron transport and photophosphorylation in chloroplasts

	Cyclic electron transport and photo phosphorylation	Non cyclic electron transport and photo phosphorylation
1	Associated with pigment system I	Associated with pigment system I and II
2	The electron expelled from chlorophyll molecule is cycled back	The electron expelled from chlorophyll molecule is not cycled back. But, its loss is compensated by electron coming from photolysis of water
3	Photolysis of water and evolution of O_2 do not take place	Photolysis of water and evolution of O_2 take place
4	Phosphorylation takes place at two places	Phosphorylation takes place at only one place
5	$NADP^+$ is not reduced	$NADP^+$ is reduced to $NADPH^+ + H^+$

Significance of light reaction

1. Light reaction takes place in chlorophyll in the presence of light.
2. During light reaction, the assimilatory powers ATP and $NADPH_2$ are synthesized.
3. The assimilatory powers are used in dark reaction for the conversion of CO_2 into sugars.
4. Photolysis of water occurs in light reaction. The H^+ ions released from water are used for the synthesis of $NADPH_2$
5. Plants release O_2 during light reaction

Red drop and Emerson's enhancement effect

Robert Emerson noticed a sharp decrease in quantum yield at wavelength greater than 680 nm, while determining the quantum yield of photosynthesis in *chlorella* using

monochromatic light of different wavelengths. Since this decrease in quantum yield took place in the red part of the spectrum, the phenomenon was called as *red drop.*

Later, they found that the inefficient far-red light beyond 680 nm could be made fully efficient if supplemented with light of shorter wavelength (blue light). The quantum yield from the two combined beams of light was found to be greater than the sum effects of both beams used separately. This enhancement of photosynthesis is called as *Emerson's Enhancement.*

11. PHOTOSYNTHETIC PATHWAYS - C_3, C_4 AND CAM

Dark reaction or Blackman's reaction or Path of carbon in photosynthesis

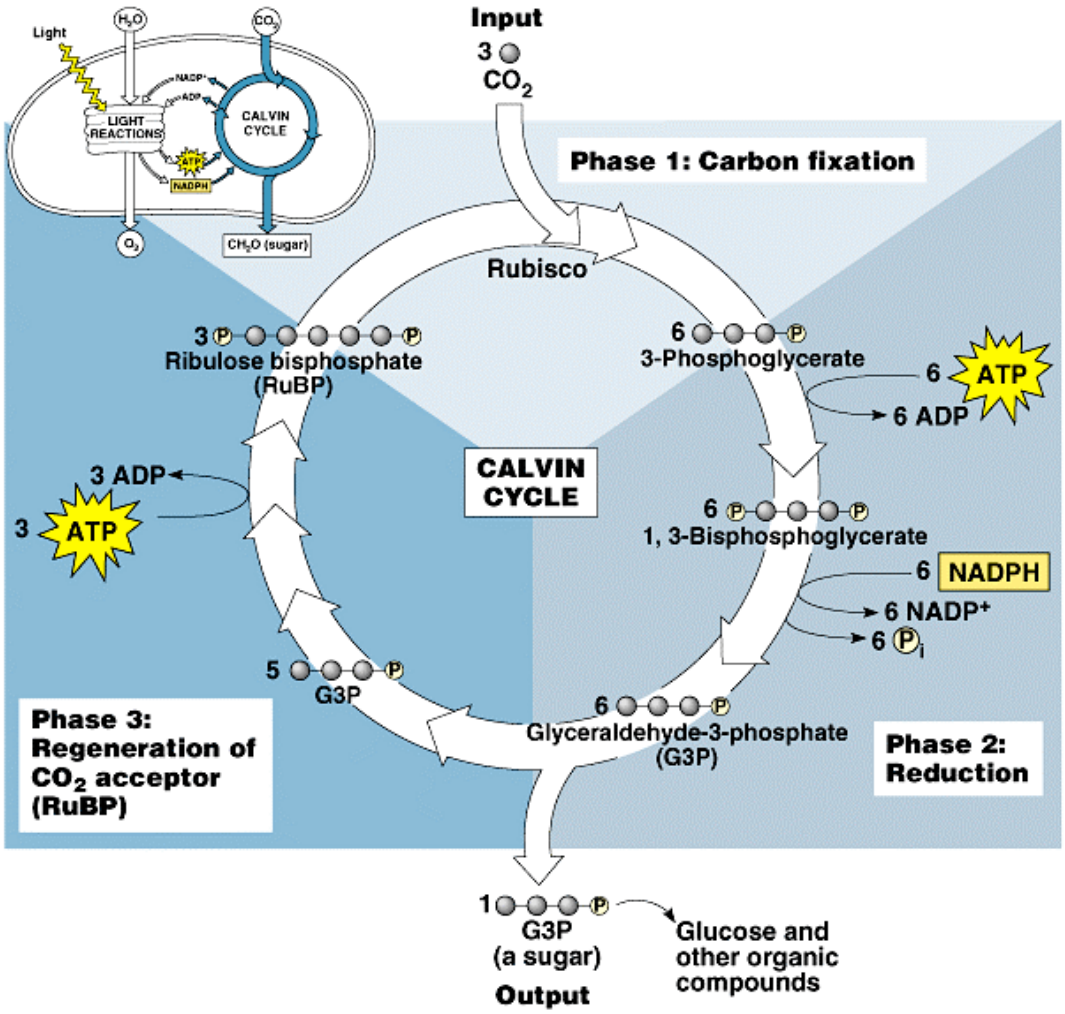
This is the second step in the mechanism of photosynthesis. The chemical processes of photosynthesis occurring independent of light is called *dark reaction*. It takes place in the stroma of chloroplast. The dark reaction is purely enzymatic and it is slower than the light reaction. The dark reactions occur also in the presence of light. In dark reaction, the sugars are synthesized from CO_2. The energy poor CO_2 is fixed to energy rich carbohydrates using the energy rich compound, ATP and the assimilatory power, $NADPH_2$ of light reaction. The process is called carbon fixation or carbon assimilation. Since Blackman demonstrated the existence of dark reaction, the reaction is also called as *Blackman's reaction*. In dark reaction two types of cyclic reactions occur

1. Calvin cycle or C3 cycle
2. Hatch and Slack pathway or C4 cycle

Calvin cycle or C3 cycle

It is a cyclic reaction occurring in the dark phase of photosynthesis. In this reaction, CO_2 is converted into sugars and hence it is a process of carbon fixation. The Calvin cycle was first observed by Melvin Calvin in chlorella, unicellular green algae. Calvin was awarded Nobel Prize for this work in 1961. Since the first stable compound in Calvin cycle is a 3 carbon compound (3 phosphoglyceric acid), the cycle is also called as C3 cycle. The reactions of Calvin's cycle occur in three phases.

1. Carboxylative phase
2. Reductive phase
3. Regenerative phase



1. Carboxylative phase

Three molecules of CO_2 are accepted by 3 molecules of 5C compound viz., ribulose diphosphate to form three molecules of an unstable intermediate 6C compound. This reaction is catalyzed by the enzyme, carboxy dismutase

$3\ CO_2$ + 3 Ribulose diphosphate $\xrightarrow{\text{Carboxy dismutase}}$ 3 unstable intermediate 6 carbon compound

The three molecules of the unstable 6 carbon compound are converted by the addition of 3 molecules of water into six molecules of 3 phosphoglyceric acid. This reaction is also catalyzed by the enzyme carboxy mutase.

3 unstable intermediate 6 C compound + $3\ H_2O$ $\xrightarrow{\text{Carboxy dismutase}}$ 3 phosphoglyceric acid

3 phosphoglyceric acid (PGA) is the first stable product of dark reaction of photosynthesis and since it is a 3 carbon compound, this cycle is known as C3 cycle.

2. Reductive phase

Six molecules of 3PGA are phosphorylated by 6 molecules of ATP (produced in the light reaction) to yield 6 molecules of 1-3 diphospho glyceric acid and 6 molecules of ADP. This reaction is catalyzed by the enzyme, Kinase

3 Phospho glyceric acid + ATP $\xrightarrow{\text{Kinase}}$ 1,3 diphospho glyceric acid + ADP

Six molecules of 1, 3 diphosphoglyceric acid are reduced with the use of 6 molecules of $NADPH_2$ (produced in light reaction) to form 6 molecules of 3 phospho glyceraldehyde. This reaction is catalysed by the enzyme, triose phosphate dehydrogenase.

1,3 diphospho glyceric acid + $NADPH_2$ $\xrightarrow{\text{Triose phosphate Dehydrogenase}}$ 3 phospho glyceraldehyde + NADP + H_3PO_4

3. Regenerative phase

In the regenerative phase, the ribose diphosphate is regenerated. The regenerative phase is called as *pentose phosphate pathway* or *hexose monophophate shunt*. It involves the following steps.

1. Some of the molecules of 3 phospho glyceraldehyde into dihydroxy acetone phosphate. Both 3 phospho glyceraldehyde and dihydroxy acetone phosphate then unite in the presence of the enzyme, aldolase to form fructose, 1-6 diphosphate.

   3 phospho glyceraldehyde $\xrightarrow{\text{Triose phosphate isomerase}}$ Dihydroxy acetone PO_4 (DHAP)

   3 phospho glyceraldehyde + DHAP $\xrightarrow{\text{Aldolase}}$ Fructose 1,6 diphosphate

2. Fructose 6 phosphate is converted into fructose 6 phosphate in the presence of phosphorylase

   Fructose 1,6 diphosphate $\xrightarrow{\text{Phosphorylase}}$ Fructose 6 phosphate

3. Some of the molecules of 3 phospho glyceraldehyde instead of forming hexose sugars are diverted to regenerate ribulose 1-5 diphosphate

   3 phospho glyceraldehyde $\longrightarrow$ Ribulose 1,5 diphosphate

4. 3 phospho glyceraldehyde reacts with fructose 6 phosphate in the presence of enzyme transketolase to form erythrose 4 phosphate (4C sugar) and xylulose 5 phosphate(5C sugar)

   3 phospho glyceraldehyde + Fructose 6 phosphate $\xrightarrow{\text{Transketolase}}$ Erythrose 4 phosphate + Xylulose 5 phosphate

5. Erythrose 4 phosphate combines with dihydroxy acetone phosphate in the presence of the enzyme aldolase to form sedoheptulose 1,7 diphosphate(7C sugar)

Erythrose 4 phosphate + DHAP —Aldolase→ Sedoheptulose 1 ,7 diphosphate

6. Sedoheptulose 1, 7 diphosphate loses one phosphate group in the presence of the enzyme phosphatase to form sedoheptulose 7 phosphate.

Sedoheptulose 1 ,7 diphosphate + ADP —Phosphatase→ Sedoheptulose 7 phosphate + ATP

7. Sedoheptulose phosphate reacts with 3 phospho glyceraldehyde in the presence of transketolase to form xylulose 5 phosphate and ribose 5 phosphate (both % c sugars)

Sedoheptulose 7 phosphate + 3 phospho glyceraldehyde —Transketolase→ Xylulose 5 phosphate + Ribose 5 phosphate

8. Ribose 5 phosphate is converted into ribulose 1, 5 diphosphate in the presence of enzyme, phosphopentose kinase and ATP. Two molecules of xylulose phosphate are also converted into one molecule of ribulose monophosphate. The ribulose monophosphate is phosphorylated by ATP to form ribulose diphosphate and ADP, thus completing Calvin cycle.

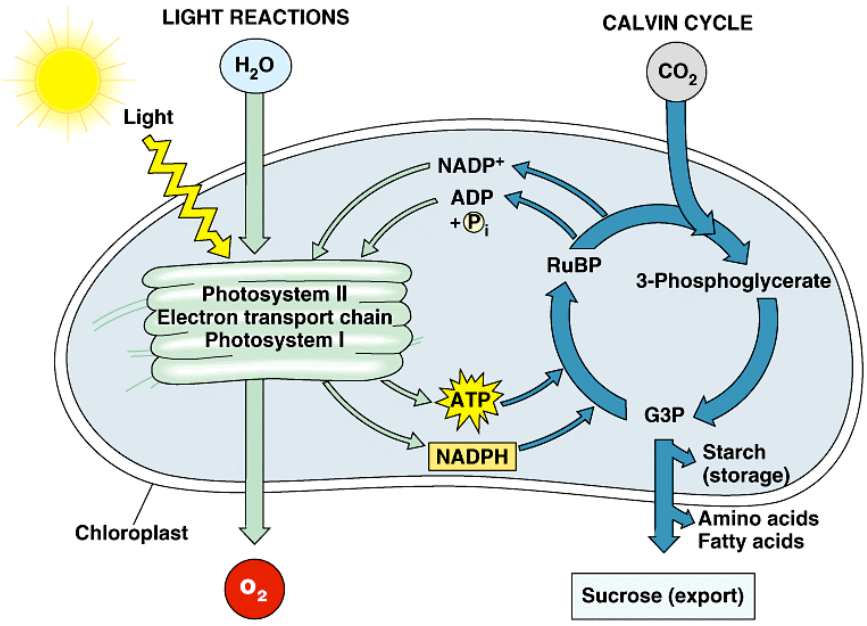
Ribose 5 phosphate + ATP —Phophopentokinase→ Ribulose 1,5 diphosphate + ADP

2 mols of Xylulose phosphate —Phophopentokinase→ 1 mol of Ribulose mono phosphate

Ribulose mono phosphate + ATP —Phophopentokinase→ Ribulose diphosphate + ADP

In the dark reaction, CO_2 is fixed to carbohydrates and the CO_2 acceptor ribulose diphosphate is regenerated. In Calvin cycle, 12 $NADPH_2$ and 18 ATPs are required to fix 6 CO_2 molecules into one hexose sugar molecule (fructose 6 phosphate).

6 CO_2 + 12 NADPH2 + 18 ATP → Fructose 6 phosphate + 12 NADP+ 18 ADP+ 17 Pi



Schematic diagram of light reaction and Calvin cycle

C4 cycle or Hatch and Slack pathway

It is the alternate pathway of C3 cycle to fix CO_2. In this cycle, the first formed stable compound is a 4 carbon compound viz., oxaloacetic acid. Hence it is called C4 cycle. The path way is also called as Hatch and Slack as they worked out the pathway in 1966 and it is also called as C4 dicarboxylic acid pathway. This pathway is commonly seen in many grasses, sugar cane, maize, sorghum and amaranthus.

The C4 plants show a different type of leaf anatomy. The chloroplasts are dimorphic in nature. In the leaves of these plants, the vascular bundles are surrounded by bundle sheath of larger parenchymatous cells. These bundle sheath cells have chloroplasts. These chloroplasts of bundle sheath are larger, lack grana and contain starch grains. The chloroplasts in mesophyll cells are smaller and always contain grana. This peculiar anatomy of leaves of C4 plants is called Kranz anatomy. The bundle sheath cells are bigger and look like a ring or wreath. Kranz in German means wreath and hence it is called Kranz anatomy. The C4 cycle involves two carboxylation reactions, one taking place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells. There are four steps in Hatch and Slack cycle:

1. Carboxylation
2. Breakdown

3. Splitting
4. Phosphorylation

1. Carboxylation

It takes place in the chloroplasts of mesophyll cells. Phosphoenolpyruvate, a 3 carbon compound picks up CO_2 and changes into 4 carbon oxaloacetate in the presence of water. This reaction is catalysed by the enzyme, phosphoenol pyruvate carboxylase.

Phosphoenol Pyruvate (3C) + CO_2 + H_2O —PEP carboxylase→ Oxaloacetate (4C) + H_3PO_4

2. Breakdown

Oxaloacetate breaks down readily into 4 carbon malate and aspartate in the presence of the enzyme, transaminase and malate dehydrogenase.

Oxaloacetate (4C) —Transaminase / Malate dehydrogenase→ Aspartate (4C) + Malate (4C)

These compounds diffuse from the mesophyll cells into sheath cells.

3. Splitting

In the sheath cells, malate and aspartate split enzymatically to yield free CO_2 and 3 carbon pyruvate. The CO_2 is used in Calvin's cycle in the sheath cell.

Malate —Decarboxylation→ CO_2 + Pyruvate

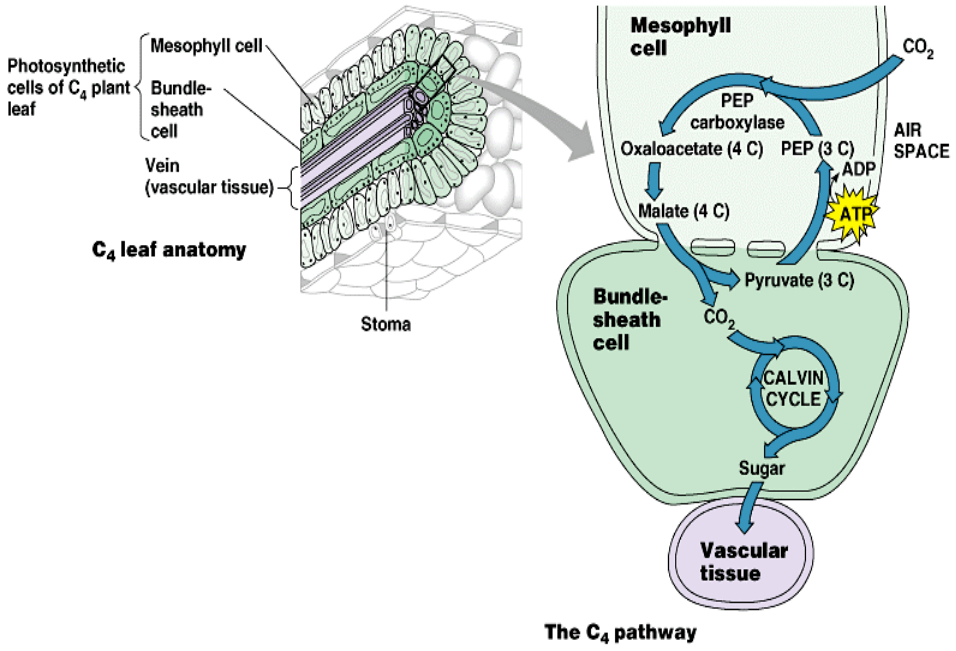
The second Carboxylation occurs in the chloroplast of bundle sheath cells. The CO_2 is accepted by 5 carbon compound ribulose diphosphate in the presence of the enzyme, carboxy dismutase and ultimately yields 3 phosphoglyceric acid. Some of the 3 phosphoglyceric acid is utilized in the formation of sugars and the rest regenerate ribulose diphosphate.

4. Phosphorylation

The pyruvate molecule is transferred to chloroplasts of mesophyll cells where, it is phosphorylated to regenerate phosphoenol pyruvate in the presence of ATP. This reaction is catalysed by pyruvate phosphokinase and the phophoenol pyruvate is regenerated.

Pyruvate + ATP + Pi —Pyruvate phosphokinase→ Phosphoenol pyruvate + AMP + Pyrophosphate

In Hatch and Slack pathway, the C3 and C4 cycles of carboxylation are linked and this is due to the Kranz anatomy of the leaves. The C4 plants are more efficient in photosynthesis than the C3 plants. The enzyme, phosphoenol pyruvate carboxylase of the C4 cycle is found to have more affinity for CO2 than the ribulose diphosphate carboxylase of the C3 cycle in fixing the molecular CO2 in organic compound during Carboxylation.



Crassulacean Acid Metabolism (CAM) cycle or the dark fixation of CO_2 in succulents

CAM is a cyclic reaction occurring in the dark phase of photosynthesis in the plants of Crassulaceae. It is a CO_2 fixation process wherein, the first product is malic acid. It is the third alternate pathway of Calvin cycle, occurring in mesophyll cells. The plants exhibiting CAM cycle are called CAM plants. Most of the CAM plants are succulents e.g., Bryophyllum, Kalanchoe, Crassula, Sedium, Kleinia etc. It is also seen in certain plants of Cactus e.g. Opuntia, Orchid and Pine apple families.

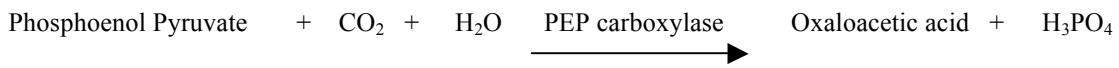
CAM plants are usually succulents and they grow under extremely xeric conditions. In these plants, the leaves are succulent or fleshy. The mesophyll cells have larger number of chloroplasts and the vascular bundles are not surrounded by well defined bundle sheath cells. In these plants, the stomata remain open during night and closed during day time. The CAM plants are adapted to photosynthesis and survival under adverse xeric conditions. CAM plants are not as efficient as C4 plants in photosynthesis. But they are better suited to conditions of extreme desiccation.

CAM involves two steps:

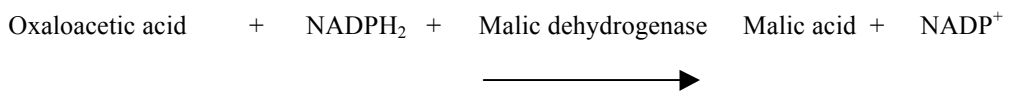
1. Acidification
2. Deacidification

Acidification

In darkness, the stored carbohydrates are converted into phophoenol pyruvic acid by the process of Glycolysis. The stomata in CAM plants are open in dark and they allow free diffusion of CO_2 from the atmosphere into the leaf. Now, the phosphoenolpyruvic acid carboxylated by the enzyme phosphoenol pyruvic acid carboxylase and is converted in to oxalaoacetic acid.



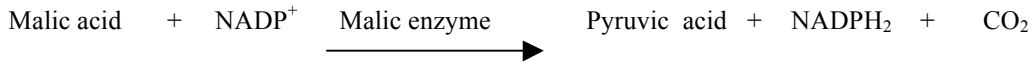
The oxaloacetic acid is then reduced to malic acid in the presence of the enzyme malic dehydrogenase. The reaction requires NADPH2 produced in Glycolysis.



The malic acid produced in dark is stored in the vacuole. The malic acid increases the acidity of the tissues.

Deacidification

During day time, when the stomata are closed, the malic acid is decarboxylated to produce pyruvic acid and evolve carbon dioxide in the presence of the malic enzyme. When the malic acid is removed, the acidity decreases the cells. This is called deacidification. One molecule of $NADP^+$ is reduced in this reaction.



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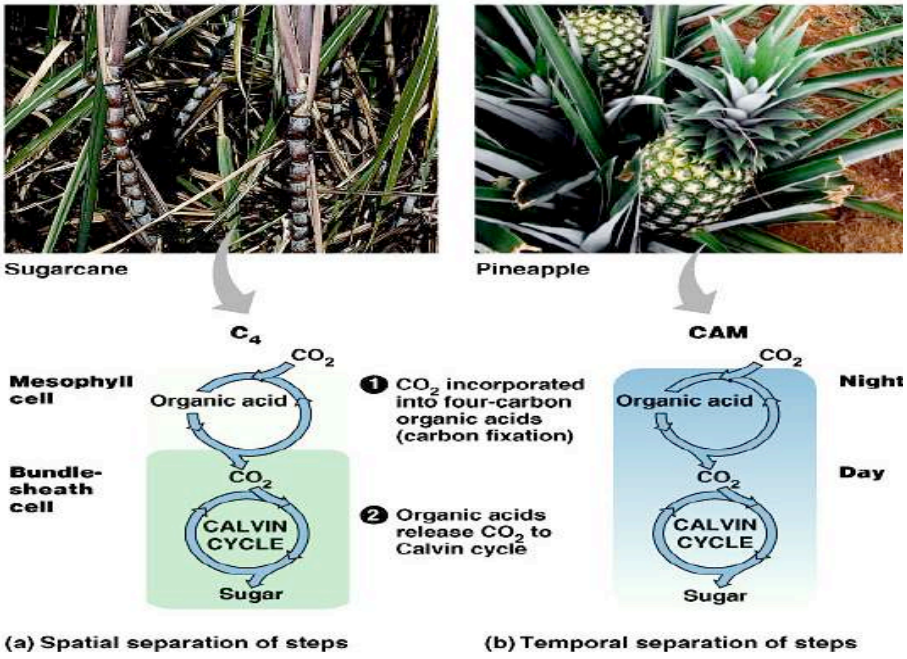
he pyruvic acid may be oxidized to CO_2 by the pathway of Kreb's cycle or it may be reconverted to phosphoenol pyruvic acid and synthesize sugar by C3 cycle. The CO_2 released by deacidification of malic acid is accepted by ribulose diphosphate and is fixed to carbohydrate by C3 cycle.

CAM is a most significant pathway in succulent plants. The stomata are closed during day time to avoid transpiration loss of water. As the stomata are closed, CO_2 cannot enter into the leaves from the atmosphere. However, they can carry out photosynthesis during the day time with the help of CO_2 released from organic acids. During night time, organic acids are synthesized in plenty with the help of CO_2 released in respiration and the CO_2 entering from

the atmosphere through the open stomata. Thus, the CO_2 in dark acts as survival value to these plants.



Comparison of the plants of C3 and C4 cycle

	C_3 Plant	**C_4 Plant**
1.	Only C_3 cycle is found	Both C_4 and C_3 cycles are found.
2.	The efficiency of CO_2 absorption at low concentration is far less and hence, they are less efficient.	The efficiency of CO_2 absorption at low concentration is quite high and hence, they are more efficient plants.
3.	The CO_2 acceptor is Ribulose-1, 5-diphosphate.	The CO_2 acceptor is phospho enol pyruvate.
4.	The first stable product is phospho glyceric acid (PGA).	Oxaloacetate (OAA) is the first stable product.
5.	Plants show one type of chloroplast (monomorphic type).	Plants show dimorphic type of chloroplast. The chloroplast of parenchymatous bundle sheath is different from that of mesophyll cells (dimorphic type). The chloroplasts in bundle sheath cell are centripetally

		arranged and lack grana. Leaves show *Kranz type* of anatomy.
6.	In each chloroplast, two pigment systems (Photosystem I and II) are present.	In the chloroplasts of bundle sheath cells, the photosystem II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of $NADPH + H^+$.
7.	The Calvin cycle enzymes are present in mesophyll chloroplast. Thus, the Calvin cycle occurs.	Calvin cycle enzymes are absent in mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
8.	The CO_2 compensation point is 50-150 ppm CO_2.	The CO_2 compensation point is 0-10 ppm CO_2.
9.	Photorespiration is present and easily detectable.	Photorespiration is present only to a slight degree or absent.
10.	The CO_2 concentration inside leaf remains high (about 200 ppm).	The CO_2 concentration inside the leaf remains low (about 100 ppm).
11.	The $^{13}C/^{12}C$ ratio in C-containing compounds remains relatively low (both $^{13}CO_2$ and $^{12}CO_2$ are present in air).	The ratio is relatively high, *i.e.* C_4 plants are more enriched with ^{13}C than C_3 plants.
12.	Net rate of photosynthesis in full sunlight (10,000 – 12,000 ft. c.) is 15-25 mg. of CO_2 per dm^2 of leaf area per hour.	It is 40-80 mg. of CO_2 per dm^2 of leaf area per hour. That is, photosynthetic rate is quite high. The plants are efficient.
13.	The light saturation intensity reaches in the range of 1000-4000 ft. c.	It is difficult to reach saturation even in full sunlight.
14.	Bundle sheath cells are unspecialized.	The bundle sheath cells are highly developed with unusual construction of organelles.
15.	The optimum temperature for the process is 10-25°C.	In these plants, it is 30-45°C and hence, they are warm climate plants. At this temperature, the rate of photosynthesis is double than that is in C_3 plants.

16.	18 ATPs are required to synthesize one glucose molecule.	30 ATPs are required to synthesize one glucose molecule.

Factors affecting photosynthesis

I. External factors

1. Light

It is the most important factor of photosynthesis. Any kind of artificial light such as electric light can induce photosynthesis. Out of the total solar energy, only 1-2 % is used for photosynthesis and the rest is used for other metabolic activities. The effect of light on photosynthesis can be studied under three categories.

a. Light intensity

Wolkoff (1966) found that the arte of photosynthesis is directly proportional to light intensity. But the extremely high light intensities do not favor for higher photosynthetic rates. The high light intensity which fails to accelerate photosynthesis is called light saturation intensity. Of the light falling on a leaf, about 80 per cent is absorbed, 10 per cent is reflected and 10 % is transmitted. The rate of photosynthesis is greater in intense light than in diffused light. The plants are grouped into two types on the basis of light requirement.

i. Heliophytes (Sun plants)

ii. Sciophytes (Shade plants)

At a specific light intensity, the amount of CO_2 used in photosynthesis and the amount of CO_2 released in respiration are volumetrically equal. This specific light intensity is known as *light compensation point.*

At very high light intensity, beyond a certain point, the photosynthetic cells exhibit *photo oxidation.* This phenomenon is called *solarisation* and a result of this, inactivation of chlorophyll molecules, bleaching of chlorophyll molecules and even inactivation of some enzymes take place resulting in the destruction of whole photosynthetic apparatus. In general, low light intensity favours stomatal closure and in turn reduced rate of photosynthesis.

b. Light quality (wavelength)

Photosynthesis occurs only in the visible part of the light spectrum i.e., between 400 and 700 nm. The maximum rate of photosynthesis occurs at red light followed by blue light.

The green light has minimum effect and photosynthesis cannot take place either in the infrared or in the ultraviolet light.

c. Light duration

In general tropical plants get 10-12 hours of light per day and this longer period of light favours photosynthesis.

2. Carbon dioxide

CO_2 is one of the raw materials required for photosynthesis. If the CO_2 concentration is increased at optimum temperature and light intensity, the rate of photosynthesis increases. But, it is also reported that very high concentration of CO_2 is toxic to plants inhibiting photosynthesis.

3. Temperature

The rate of photosynthesis increases by increase in temperature up to 40 ºC and after this, there is reduction in photosynthesis. High temperature results in the denaturation of enzymes and thus, the dark reaction is affected. The temperature requirement for optimum photosynthesis varies with the plant species. For example, photosynthesis stops in many plants at 0 ºC but in some conifers, it can occur even at -35 ºC. Similarly photosynthesis stops beyond 40-50 ºC in certain plants; but certain bacteria and blue green algae can perform photosynthesis even at 70 ºC.

4. Water

Water has indirect effect on the rate of photosynthesis although it is one of the raw materials for the process. The amount of water utilized in photosynthesis is quite small and even less than 1 per cent of the water absorbed by a plant. Water rarely acts as a limiting factor for photosynthesis. During water scarcity, the cells become flaccid and the rate of photosynthesis might go down.

5. Oxygen

Oxygen is a byproduct of photosynthesis and an increase in the O_2 concentration in many plants results in a decrease in the rate of photosynthesis. The phenomenon of inhibition of photosynthesis by o2 was first discovered by Warburg (1920) in green alga Chlorella and this effect is known as Warburg's effect. This is commonly observed in C3 plants.

In plants, there is a close relationship between Warburg's effect and photorespiration. The substrate of photorespiration is glycolate and it is synthesized from some intermediates of Calvin's cycle. In plants that show Warburg's effect, increased O_2 concentration result in diversion of these intermediates of Calvin cycle into the synthesis of glycolate, thereby showing higher rate of photorespiration and lower photosynthetic productivity.

6. Mineral elements

The elements like Mg. Fe, Cu, Cl, Mn, P etc are involved in the key reactions of photosynthesis and hence, the deficiency of any of these nutrients caused reduction in photosynthesis.

7. Chlorophyll content

It is very much essential to tarp the light energy. In 1929, Emerson found direct relationship between the chlorophyll content and rate of photosynthesis. In general, the chlorophyll sufficient plants are green in colour showing efficient photosynthesis. The chlorotic leaves due to irregular synthesis of chlorophyll or breakdown of chlorophyll pigment exhibit inefficient photosynthesis.

8. Leaf

The leaf characters such as leaf size, chlorophyll content, number of stomata. Leaf orientation and leaf age are some of the factors that are responsible for photosynthesis. The maximum photosynthetic activity is usually seen in the physiologically functional and full size leaves (usually third/fourth leaf from the tip of the shoot system).

9. Carbohydrates

If the accumulated carbohydrates are not translocated, the photosynthetic rate is reduced and respiration is increased. Sugar is converted into starch and gets accumulated in the chloroplasts. This reduces the effective surface in the chloroplast and the rate of photosynthesis is decreased.

10. Phytohormones

Treharne (1970) reported first that photosynthesis may be regulated by plant hormone system. He found that gibberellic acid and cytokinin increase the carboxylating activity and photosynthetic rates. Meidner (1967) also reported that kinetin @ 3µm causes 12 per cent increase in photosynthesis within one hour of the treatment.

PHOTORESPIRATION

The excessive respiration that takes place in green cells in the presence of light is called as photorespiration. Decker (1955) discovered the process and it is also called as C2 cycle as the 2 carbon compound glycolic acid acts as the substrate in photorespiration. In general, respiration takes place under both light and dark conditions. However in some plants, the respiration is more in light than in dark. It is 3-5 times higher than the rate of respiration in dark. Photorespiration is carried out only in the presence of light. But the normal respiration is not light dependent and it is called dark respiration.

In photorespiration, temperature and oxygen concentration play an important role. Photorespiration is very high when the temperature is between 25 and 30 ºC. The rate of photorespiration increases with the increase in the concentration of oxygen. Three cell organelles namely chloroplast, peroxisome and mitochondria are involved in the photorespiration. This kind of respiration is seen in plants like cotton, pulses, capsicum, peas, tomato, petunia soybean, wheat, oats, paddy, chlorella etc and it is absent in grasses.

Mechanism

1. In the presence of excess oxygen and low CO_2 , ribulose 1,5 diphosphate produced in the chloroplast during photosynthesis is split into 2 phospho glycolic acid and 3 phospho glyceric acid by the enzyme, ribulose 1,5 diphosphate oxygenase
2. The 3 phospho glyceric acid enters the Calvin cycle.
3. In the next step, phosphate group is removed from 2 phosphoglycolic acid to produce glycolic acid by the enzyme, phosphatase.
4. Glycolic acid then it come out of chloroplast and enter the peroxisome. Here, it combines with oxygen to form glyoxylic acid and hydrogen peroxide. This reaction is catalyzed by the enzyme, glycolic acid oxidase. Hydrogen peroxide is toxic and it is broken down into water and oxygen by the enzyme, Catalase. Photorespiration is an oxidation process. In this process, glycolic acid is converted into carbohydrate and CO_2 is released as the by product. As glycolic acid is oxidized in photorespiration, it is also called as glycolate metabolism.
5. The glyoxylic acid converted into glycine by the addition of one amino group with the help of the enzyme, amino transferase.

6. Now, the glycine is transported from the peroxisome into the mitochondria. In the mitochondria, two molecules of glycine condense to form serine and liberate carbon dioxide and ammonia.

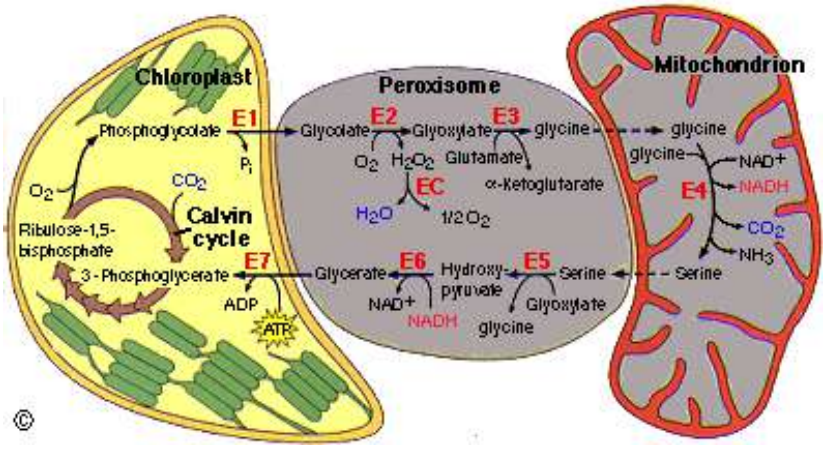
7. Amino group is removed from serine to form hydroxyl pyruvic acid in the presence of the enzyme, transaminase.

8. Hydroxy pyruvic acid undergoes reduction with the help of NADH to form glyceric acid in the presence of enzyme alpha hydroxyl acid reductase.

9. Finally, regeneration of 3 phosphoglyceric acid occurs by the phosphorylation of glyceric acid with ATP. This reaction is catalyzed by the enzyme, Kinase.

10. The 3 phosphoglyceric acid is an intermediate product of Calvin cycle. If it enters the chloroplast, it is converted into carbohydrate by photosynthesis and it is suppressed nowadays with the increased CO_2 content in the atmosphere.

PHOTORESPIRATION



Significance of photorespiration

1.Photorespiration helps in classifying the plants

Generally, photorespiration is found in C3 plants and absent in C4 plants.

2. Carbon dioxide is evolved during the process and it prevents the total depletion of CO_2 in the vicinity of chloroplasts.

2. The process causes oxidation of glycolic acid which arises as an unwanted byproduct of photosynthesis. The glycolic acid after oxidation is converted into carbohydrate but the remainder is converted into CO_2.

3. Photorespiration uses energy in the form of ATP and reduced nucleotides, but normal respiration yields ATP and reduced nucleotides.

4. It is believed that photorespiration was common in earlier days when CO_2 content was too low to allow higher rates.

12. **RESPIRATION**

The cellular oxidation or break down of carbohydrates into CO_2 and H_2O, and release of energy is called as *respiration*. It is a reverse process of photosynthesis. In respiration, the oxidation of various organic food substances like carbohydrates, fats, proteins etc, may take place. Among these, glucose is the commonest.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy (686 kcal)}$$

This oxidation process in not so simple and does not take place in one step. Breakdown of glucose involves many steps releasing energy in the form of ATP molecules and also forming a number of carbon compounds (intermediates). Respiration is a vital process that occurs in all living cells of the plant and the most actively respiring regions are floral buds, vegetative buds, germinating seedlings, stem and root apices.

Types of respiration

Degradation of organic food for the purpose of releasing energy can occur with or without the participation of oxygen. Hence, respiration can be classified into two types; aerobic and anaerobic respiration.

Aerobic respiration

Aerobic respiration takes place in the presence of oxygen and the respiratory substrate gets completely oxidized to carbon dioxide and water as end products.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy (686 kcal)}$$

(Glucose)

This type of respiration is of common occurrence and it is often used as a synonym of respiration.

Anaerobic respiration

It takes place in the absence of oxygen and the respiratory substrate is incompletely oxidized. Some other compounds are also formed in addition to carbon dioxide. This type of respiration is of rare occurrence but, common among microorganisms like yeasts.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 56 \text{ kcal}$$

Glucose Ethanol

Respiratory substrate

A respiratory substrate is an organic substance which can be degraded to produce energy which is required for various activities of the cell. The respiratory substrates include carbohydrates, fats, organic acids, protein etc.

Carbohydrates

The carbohydrates constitute the most important respiratory substrate and the common amongst them are starch, sucrose, glucose and fructose. The complex carbohydrates are first hydrolyzed to simple sugars and then they are utilized.

Starch → Disaccharides → Hexoses

Fats

The fats are important storage food in seeds. Nearly 80 per cent of the angiosperms have fats as the main storage food in their seeds. At the time of seed germination, large amount of fats are converted into carbohydrates while the remaining fats are utilized in respiration. Fats are first broken down to glycerol and fatty acids. The fatty acids are broken down to acetyl coenzyme by β-oxidation. The acetyl coenzyme enters Kreb's cycle for further degradation and releases energy. Glycerol can directly enter the respiratory channel via glyceraldehyde.

Organic acids

Organic acids normally do not accumulate in plants to any appreciable extent except in the members of the family, Crassulaceae. Organic acids are oxidized under aerobic conditions to carbon dioxide and water.

Proteins

Under normal conditions, proteins are used up as respiratory substrate only in seeds rich in storage proteins. In vegetative tissues, proteins are consumed only under starvation. The proteins are hydrolyzed to form amino acids. Later, the amino acids undergo deamination forming organic acids and the organic acids can enter Kreb's cycle directly.

Mechanism of Respiration

1. Glycolysis
2. Aerobic breakdown of pyruvic acid (Kreb's cycle)
3. Electron Transport System/ Terminal oxidation / oxidative phosphorylation
5. Pentose phosphate pathway

A. GLYCOLYSIS / EMBDEN – MEYER HOF – PARANAS (EMP) PATHWAY

Glycolysis can take place even in the absence of O_2. One molecule of the 6 carbon compound, glucose is broken down through a series of enzyme reactions into two 3-carbon compounds, the pyruvic acid. Glycolysis takes place in the cytoplasm and it does not require oxygen. Hence it is an anaerobic process.

1. Glucose molecules react with ATP molecules in the presence of the enzyme hexokinase to form glucose -6- phosphate.
   Glucose + ATP → Glucose -6- phosphate + ADP

2. Glucose-6-phosphate is isomerised into fructose-6-phosphate in the presence of phospho hexose isomerase.
   Fructose + ATP → Fructose -6- phosphate + ADP

3. Fructose-6-phosphate reacts with one molecule of ATP in the presence of phospho hexo kinase forming fructose 1, 6-disphosphate.
   Fructose – 6- phosphate + ATP → Fructose -1,6- biphosphate + ADP

4. Fructose 1, 6 diphosphate is converted into two trioses, 3-phospho glyceraldehyde and dihydroxy acetone phosphate in the presence of aldolase.
   Fructose -1,6- biphosphate → 3-phospho glyceraldehyde+ DHAP

5. 3-phosphoglyceraldehyde reacts with H_3PO_4 and forms 1,3-diphosphoglyceraldehyde where, the reaction is non –enzymatic.

6. 1, 3-Diphosphoglyceraldehyde is oxidized to form 1,3- diphosphoglycerate in the presence of triose-phosphate dehydrogenase and coenzyme NAD^+. The NAD^+ acts as hydrogen acceptor and reduced to $NADH^+ + H^+$ in the reaction.
   Glyceraldehde -3- phosphate + NAD + Pi → 1,3- diphosphoglycerate + NADH

6. 1, 3-Diphosphoglycerate reacts with ADP in the presence of phosphoglyceric transphorylase (kinase) to form 3 phosphoglyceric acid and ATP.

1,3- diphosphoglycerate + ADP → 3, Phosphoglycerate + ATP

7. 3, Phosphoglycerate → 2, Phosphoglycerate acid is isomerized into 2 phosphoglyceric acid in the presence of the enzyme, phospho glycero mutase
   3, Phosphoglycerate → 2, Phosphoglycerate

8. 2 phosphoglyceric acid is converted into 2-phosphoenolpyruvic acid in the presence of enolase.
   2, Phosphoglycerate → Phosphoenol pyruvate + H_2O

9. 2 phospho enol pyruvic acid reacts with ADP to form one molecule each of pyruvic acid and ATP in the presence of pyruvate kinase.
   Phosphoenol pyruvate + ADP → Pyruvate + ATP

Glycolysis or EMP pathway is common in both aerobic and anaerobic respiration.

The overall glycolytic process can be summarized as follows

$$C_6H_{12}O_6 + 2ATP + 2NAD + 4ADP + 2H_3PO_4$$

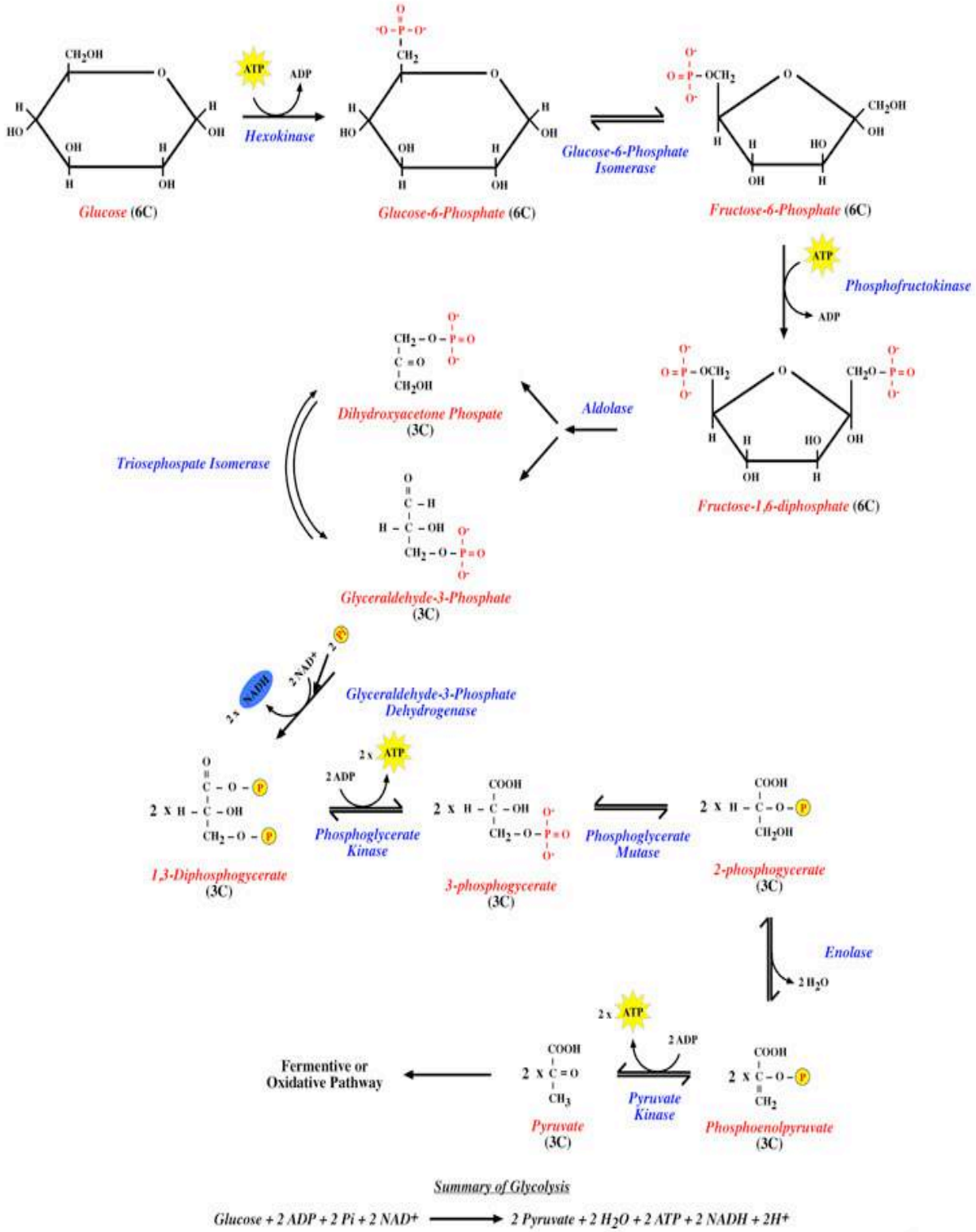
$$\downarrow$$

$$2\ CH_3COCOOH + 2ADP + 2NADH_2 + 4\ ATP$$

Pyruvic acid

- Thus there is a gain of 4-2 = 2 ATP molecules per hexose sugar molecule oxidized during this process.
- Besides this, 2 molecules of reduced coenzyme $NADH_2$ are also produced per molecule of hexose sugar in glycolysis.
- During aerobic respiration, these two $NADH_2$ are oxidized via the electron transport chain to yield 3 ATP molecules each. Thus 6 ATP molecules are formed.

Glycolysis



Summary of Glycolysis

$$Glucose + 2\,ADP + 2\,Pi + 2\,NAD^{+} \longrightarrow 2\,Pyruvate + 2\,H_2O + 2\,ATP + 2\,NADH + 2H^{+}$$

13. KREBS' CYCLE / CITRIC ACID CYCLE /TCA CYCLE

The pyruvic acid produced in glycolysis enters into Krebs' cycle for further oxidation. Krebs' cycle is also known as citric acid cycle or Tri carboxylic acid (TCA) cycle. This aerobic process takes place in mitochondria where necessary enzymes are present in matrix.

11. Pyruvic acid reacts with CoA and NAD and is oxidatively decarboxylated. One molecule of CO_2 is released and NAD is reduced. Pyruvic acid is converted into acetyl CoA.

$$\text{Pyruvic acid} + \text{CoA} + \text{NAD} \xrightarrow{\text{Pyruvate dehydrogenase}} \text{Acetyl} - \text{COA} + CO_2 + NADH_2$$

12. Acetyl-CoA condenses with oxaloacetic acid in the presence of condensing enzyme and water molecule to form citric acid. CoA becomes free.

$$\text{Acetyl CoA} + \text{Oxaloacetic acid} \xrightarrow[+ H_2O]{\text{Condensing enzyme}} \text{Citric acid} + \text{CoA}$$

13. Citric acid is dehydrated in the presence of aconitase to form cis – aconitic acid

$$\text{Citricacid} \xrightarrow[- H_2O]{\text{Aconitase}} \text{Cis} - \text{Aconitic acid}$$

14. Cis-aconitic acid reacts with one molecule of water to form Isocitric acid

$$\text{Cis-aconitic acid} + H_2O \longrightarrow \text{Isocitric acid}$$

15. Iso-citric acid is oxidized to oxalo succinic acid in the presence of Isocitric dehydrogenase. NADP is reduced to $NADPH_2$ in the reaction.

$$\xrightarrow{\text{IC dehydrogenase}} \text{Oxalo succinic acid} + NADPH_2$$

Isocitric acid + NADP

16. Oxalo succinic acid is decarboxylated in the presence of oxalo succinic decarboxylase to form α - ketoglutaric acid and a second molecule of CO_2 is released.

$$\text{Oxalosuccinic acid} \xrightarrow[\text{Decarboxylase}]{\text{Oxalosuccinic}} \alpha\text{-ketoglutaric acid} + CO_2$$

17. α - ketoglutaric acid reacts with CoA and NAD in the presence of α - ketoglutaric acid dehydrogenase complex and is oxidatively decarboxylated to form succinyl CoA and a third mole of CO_2 is released. NAD is reduced in the reaction.

$$\alpha\text{-keto glutaric acid} + CoA \xrightarrow[NAD \quad NADH_2]{} \text{Succinyl-CoA} + CO_2 + NADH_2$$

18. Succinyl CoA reacts with water molecule to form succinic acid. CoA becomes free and one molecule of GDP (Guanosine diphosphate) is phosphorylated in presence of inorganic phosphate to form one molecule of GTP.

$$\text{Succinyl-CoA} + \text{GDP} + \text{ip} \xrightarrow{H_2O} \text{Succinic acid} + \text{GTP}$$

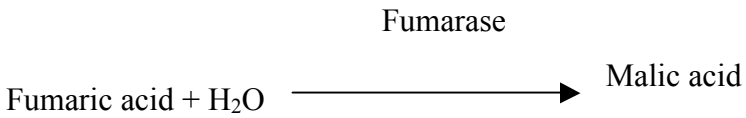
GTP may react with ADP to form one molecule of ATP

$GTP + ADP \rightarrow ATP + GDP$

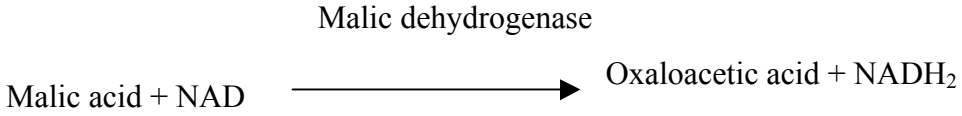
19. Succinic acid is oxidized to fumaric acid in the presence of succinic dehydrogenase and co enzyme FAD is reduced in this reaction.

$$\text{Succinic acid} + FAD \xrightarrow{\text{Succinic acid dehydrogenase}} \text{Fumaric acid} + FADH_2$$

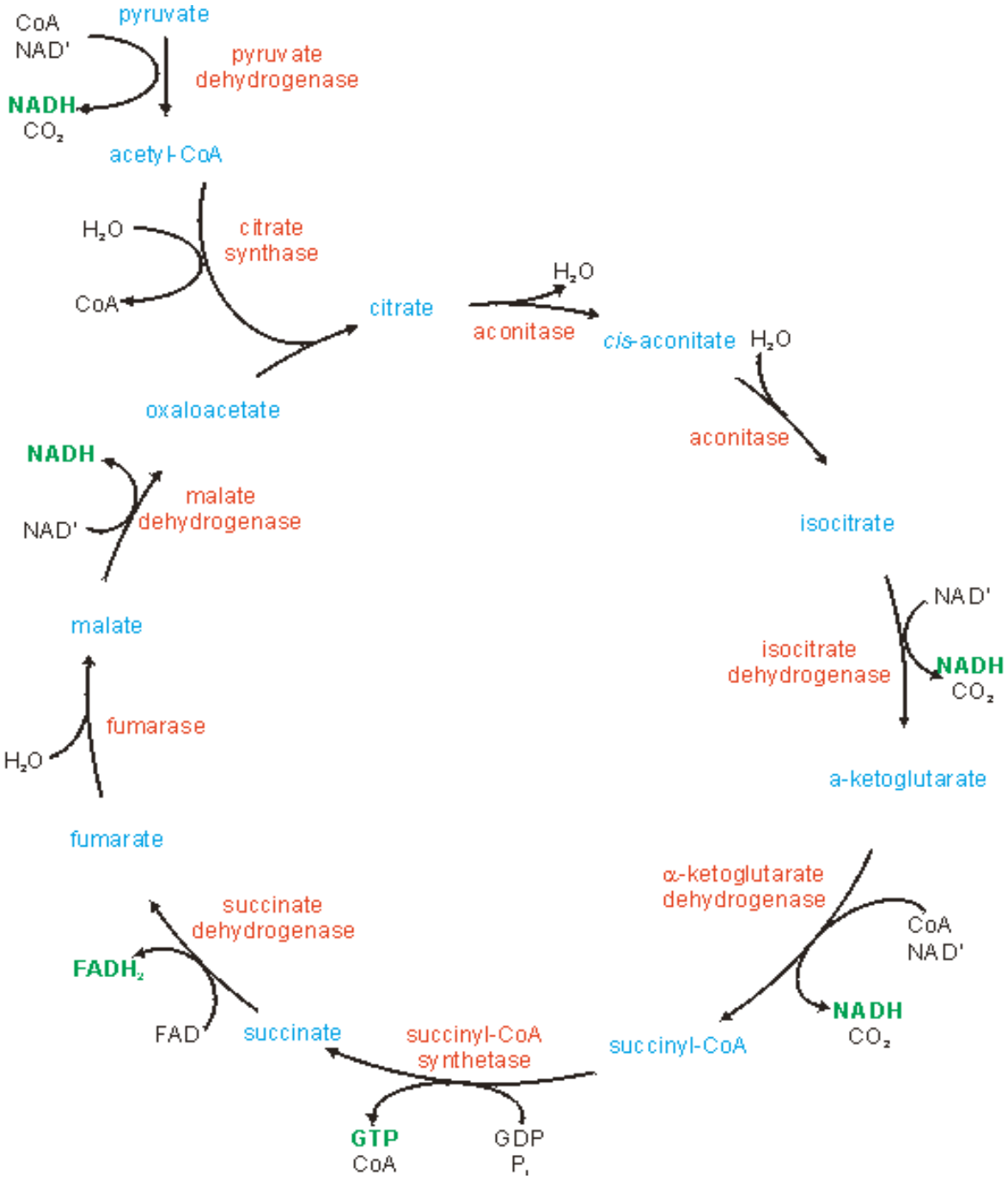
20. One mole of H_2O is added to Fumaric acid in the presence of fumarase to form malic acid.



21. In the last step, malic acid is oxidized to oxaloacetic acid in the presence of malic dehydrogenase and one molecule of coenzyme i.e. NAD is reduced.



KREBS CYCLE or TCA CYCLE



Pentose phosphate pathway (ppp) / Hexose mono phosphate (hmp) shunt/ Phosphogluconate pathway / Warburg and Dicken's pathway

The pentose phosphate pathway occurs in the cytoplasm outside the mitochondria and it is an alternative pathway to glycolysis and Kreb's cycle. The presence of some compounds like iodoacetate, fluorides, arsenates etc. inhibit some steps in glycolysis and that leads to the alternate pathway. This pathway was discovered by Warburg and Dicken (1938). This pathway does not produce ATP but it produces another form of energy called reducing power in the form of NADPH. It is not oxidized in the electron transport system but, it serves as hydrogen and electron donor in the biosynthesis of fatty acids and steroids. The pentose phosphate pathway consists of two distinct phases. In the first phase, hexose is converted into pentose and in the second phase, pentose is reconverted in to hexose.

In the process, oxidation of glucose 6 phosphate leads to the formation of 6 phosphogluconic acid (pentose phosphate). Since glucose is directly oxidized without entering glycolysis, it is called as *direct oxidation.*

6 Glucose 6 phosphate +12 NADP $\longrightarrow$ 5 Glucose 6 Phosphate + 12 $NADPH_2$+ 6 CO_2

It provides ribose sugars for the synthesis of nucleic acids and is also required for shikimic acid pathway. Although ATP is not produced, NADPH is produced and serves as hydrogen and electron donor in the biosynthesis of fatty acids and steroids. The pathway is also called as *phosphogluconate pathway* as the first product in this pathway is phosphogluconate.

OXIDATIVE PHOSPHORYLATION

C. TERMINAL OXIDATION OF THE REDUCED COENZYMES / ELECTRON TRANSPORT SYSTEM AND OXIDATIVE PHOSPHORYLATION

The last step in aerobic respiration is the oxidation of reduced coenzymes produced in glycolysis and Krebs' cycle by molecular oxygen through FAD, UQ (ubiquinone), cytochrome b, cytochrome c, cytochrome a and cytochrome a_3 (cytochrome oxidase).

Two hydrogen atoms or electrons from the reduced coenzyme ($NADH_2$ or $NADPH_2$) travel through FAD and the cytochromes and ultimately combines with $1/2O_2$ molecule to produce one molecule of H_2O. This is called as *terminal oxidation*.

The terminal oxidation of each reduced coenzyme requires $1/2O_2$ molecule and 2H atoms (i.e. 2 e^- + $2H^+$) to produce one H_2O molecule. Except for flavoproteins (like FAD)

and ubiquinone (UQ) which are hydrogen carriers, the other components of electron transport chain (cytochromes) are only electron carriers i.e. they cannot give or take protons (H^+)

During the electron transport, FAD and the iron atom of different cytochromes get successively reduced (Fe^{++}) and oxidized (Fe^{+++}) and enough energy is released in some places which is utilized in the photophosphorylation of ADP molecules in the presence of inorganic phosphate to generate energy rich ATP molecules. Since, this oxidation accompanies phosphorylation; it is called as *oxidative phosphorylation*.

One molecule of ATP with 7.6 Kcal.energy is synthesized at each place when electrons are transferred from

1. Reduced $NADH_2$ or $NADPH_2$ to FAD
2. Reduced cytochrome b to cytochrome c
3. Reduced cytochrome a to cytochrome a_3

Thus, oxidation of one molecule of reduced $NADH_2$ or $NADPH_2$ will result in the formation of 3 ATP molecules while the oxidation of $FADH_2$ lead to the synthesis of 2 ATP molecules.

According to the most recent findings, although in eukaryotes terminal oxidation of mitochondrial NADH / NADPH results in the production of 3 ATP molecules but that of extra mitochondrial NADH / NADPH yields only 2 ATP molecules. Therefore, the two reduced coenzyme molecules (NADH) produced per hexose sugar molecule during Glycolysis will yield only 2x2:4 ATP molecules instead of 6 ATP molecules. Complete oxidation of a glucose molecule (hexose sugar) in aerobic respiration results in the net gain of 36 ATP molecules in most eukaryotes.

One glucose molecule contains about 686 Kcal. Energy and 38 ATP molecules will have 273.6 Kcal energy. Therefore about 40% (273.6/686) energy of the glucose molecule is utilized during aerobic breakdown and the rest is lost as heat. Since huge amount of energy is generated in mitochondria in the form of ATP molecules, they are called as *Power Houses of the cell*.

ATP molecules contain energy in terminal pyrophosphate bonds. When these energy rich bonds break, energy is released and utilized in driving various other metabolic processes of the cell.

Differences between oxidative phosphorylation and Photophosphorylation

	Oxidative phosphorylation	**Photophosphorylation**

1	It occurs during respiration	Occurs during photosynthesis
2	Occurs inside the mitochondria (inner membrane of cristae)	Occurs inside the chloroplast (in the thylakoid membrane)
3	Molecular O_2 is required for terminal oxidation	Molecular O_2 is not required
4	Pigment systems are not involved	Pigment systems, PSI and PSII are involved
5	It occurs in electron transport system	Occurs during cyclic and non cyclic electron transport
6	ATP molecules are released to cytoplasm and used in various metabolic reactions of the cell	ATP molecules produced are utilized for CO_2 assimilation in the dark reaction of photosynthesis

Efficiency of respiration

The total energy content of one molecule of glucose is 686 Kcal. Out of this energy, available free energy is 673.6 Kcal and the energy content of ATP molecule is calculated as 7.3 Kcal. The efficiency of respiration may be expressed as follows.

$$\text{Efficiency of respiration: } \frac{\text{Kcal of energy conserved in ATP}}{\text{Total free energy available}} \times 100$$

$$\text{Efficiency of aerobic respiration} : \frac{38 \times 7.3}{673.6} \times 100 : 41\ \%$$

$$\text{Efficiency of anaerobic respiration: } \frac{2 \times 7.3}{47} \times 100 : 31\ \%$$

$$\text{Efficiency of fermentation} : \frac{2 \times 7.3}{40} \times 100 : 36.5\ \%$$

Respiratory quotient

The ratio of the volume of CO_2 released to the volume of O_2 taken during respiration is called as respiratory quotient and is denoted as RQ

$$RQ = \frac{\text{Volume of } CO_2}{\text{Volume of } O_2}$$

Value of RQ

The value of RQ depends upon the nature of the respiratory substrate and the amount of O_2 present in respiratory substrate.

1. When **carbohydrates** such as hexose sugars are oxidized in respiration, the value of RQ is 1 or unity because volume of CO_2 evolved equals to the volume of O_2 absorbed.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

Glucose

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{6}{6} = 1 \text{ or unity}$$

2. When **fats** are the respiratory substrate, the value of RQ becomes less than one because fats are poorer in O_2 in comparison to carbon and they require more O_2 for their oxidation,

$$2C_{51}H_{98}O_6 + 145O_2 \longrightarrow 102CO_2 + 98H_2O$$

Tripalmitin

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{102}{145} = 0.7$$

(Fats are oxidized in respiration usually during the germination of fatty seeds).

3. When **organic acids** are oxidized in respiration, the value of RQ becomes more than one. It is because organic acids are rich in O_2 and require less O_2 for their oxidation.

$$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$$

Malic acid

$$RQ = \frac{\text{volume of } CO_2}{\text{volume of } O_2} = \frac{4}{3} = 1.3$$

Energy budgeting

Stages	Gain of	Consumption	Net gain of

	ATP	of ATP	ATP
Glycolysis			
1) Glucose → Glucose 6 PO4		1	
2) Fructose 6 PO4 → Fructose 1,6 di PO4		1	
3) 1,3 diphosphoglyceraldehyde → 1,3 diphospho glyceric acid	6		
4) 1,3 diphospho glyceric acid → 3 phosphoglyceric acid	2		
5) 2 phosphoenol pyruvic acid → Pyruvic acid	2		
Total	10	-2	8
Kreb's cycle			
6) Pyruvic acid → Acetyl CoA	3		
7) Isocitric acid → Oxalosuccinic acid	3		
8) ketoglutaric acid → Succinyl CoA	3		
9) Succinyl Co A → Succinic Acid	1		
10) Succinic acid → Fumaric Acid	2		
11) Malic acid → Oxaloacetic acid	3		
Total ATP mol. produced per Pyruvic acid	15		15
Total ATP mol. produced for 2 Pyruvic acids	15 x 2:30		30
Grand Total	40	-2	8+ 30 = 38

FACTORS AFFECTING RESPIRATION

A. External factors

1. Temperature

Temperature has profound influence on the rate of respiration. Optimum temperature for respiration is about 30°C, minimum 0°C and maximum about 45°C. At low temperature, the respiratory enzymes becomes inactive, consequently the rate of respiration falls. It is due to this fact that the quality of fruits and vegetables stored at low temperature does not deteriorate. At very high temperature, respiration slows down and may even be stopped due to denaturation of the respiratory enzymes.

2. Oxygen

In complete absence of O_2, anaerobic respiration takes place while aerobic respiration stops. In higher plants, the anaerobiosis produces large amount of alcohol which is toxic to plants. If some amount of O_2 is available, anaerobic respiration slows down and aerobic respiration starts. The concentration of O_2 at which aerobic respiration is optimum and anaerobic respiration is stopped, is called as *extinction point.*

It is observed that under anaerobic conditions, much more sugar is taken up per quantity of yeast present than it is consumed in the presence of oxygen. The inhibition on the rate of carbohydrate breakdown by oxygen is called as *Pasteur's effect*.

3. Carbon dioxide

Higher concentration of CO_2 in the atmosphere especially in the poorly aerated soil has retarding effect on the rate of respiration.

4. Inorganic salts

If a plant or tissue is transferred from water to salt solution, the rate of respiration increases (called as *salt respiration*).

5. Water

Proper hydration of cells is essential for respiration. Rate of respiration decreases with decreased amount of water, so much so, that in dry seeds, the respiration is at its minimum. It is because in the absence of a medium, the respiratory enzymes become inactive.

6. Light

The effect of light is indirect on the rate of respiration through the synthesis of organic food matter in photosynthesis.

7. Wound or injury

Injury or wounds result in increased respiration as the plants in such a state require more energy which comes from respiration. The wounded cells become more meristematic to form new cells for healing the wound.

Internal factors

1. Protoplasmic factors

The amount of protoplasm in the cell and its state of activity influence the rate of respiration.

- The rate of respiration is higher in young meristematic cells which divide actively and requires more energy. Such cells have greater amount of protoplasm and no vacuoles.

- In old mature tissues, the rate of respiration is lower because of lesser amount of active protoplasm

2. Concentration of respiratory substrate

Increased concentration of respirable food material brings about an increase in the rate of respiration.

Under starvation conditions, such as in etiolated leaves, the rate of respiration slows down considerably. If such etiolated leaves are supplied with sucrose solution for few days even in dark conditions, the rate of respiration increases.

Differences between Photorespiration and Dark respiration

	Photorespiration	Dark /Mitochondrial respiration
1	It occurs in the presence of light	It occurs in the presence of both light and dark.
2	The substrate is glycolate	The respiratory substrate may be carbohydrate, fat or protein.
3	It occurs in chloroplast, peroxisome and mitochondria	The process occurs in the cytoplasm and mitochondria
4	It occurs in temperate plants like, wheat and cotton (mainly in C3 plants)	It occurs in C4 plants (maize and sugar cane)
5	It occurs in the green tissues of plants	It occurs in all the living plants(both green and non green)
6	The optimum temperature is 25- 35ºC	It is not temperature sensitive
7	This process increases with increased CO_2 concentration.	This process saturate at 2-3 percent O_2 in the atmosphere and beyond this concentration there is no increase.
8	Hydrogen peroxide is formed during the reaction	Hydrogen peroxide is not formed.
9	ATP molecules are not produced,	Several ATP molecules are produced.
10	Reduced coenzymes such as $NADPH_2$, $NADH_2$ and $FADH_2$ are not produced.	Reduced coenzymes such as $NADPH_2$, $NADH_2$ and $FADH_2$ are produced.
11	One molecule of ammonia is released	No ammonia is produced

	per molecule of CO_2 released.	
12	Phosphorylation does not occur	Oxidative phosphorylation occurs.

Differences between respiration and photosynthesis

	Respiration	Photosynthesis
1	It is catabolic process resulting in the destruction of stored food	It is an anabolic process resulting in the manufacture of food.
2	Light is not essential for the process	Light is very much essential
3	Oxygen is absorbed in the process	Oxygen is liberated
4	Carbon dioxide and water are produced	Carbon dioxide is fixed to form carbon containing compound
5	Potential energy is converted into Kinetic energy	Light energy is converted into chemical energy (potential energy)
6	Glucose and oxygen are the raw materials	Carbon dioxide and water are the raw materials
7	Energy is released during respiration and hence it is an exothermic process.	Energy is stored during photosynthesis and hence it is an endothermic process
8	Reduction in the dry weight	Gain in the dry weight
9	Chlorophyllous tissues are not necessary	Chlorophyllous tissues are essential for the process

Differences between aerobic respiration and fermentation

	Aerobic respiration	Fermentation
1	It occurs in all living cells of the plants throughout the day and night	Occurs outside the plant cells and in certain microorganisms
2	It takes place in the presence of oxygen	Absence of oxygen
3	The end products are CO_2 and H_2O	End products are CO_2 and alcohol or other organic acids
4	It is not toxic to plants	It is toxic to plants
5	Complete oxidation is food material is	Incomplete oxidation is observed

	observed	
6	Large amount of energy (673 kCal) is released per glucose molecule	Very small amount of energy (21 kCal) is released per glucose molecule
7	The complete oxidation yields 38 ATP molecules	The incomplete oxidation in fermentation yields only two ATP molecules
8	The enzyme, zymase is not required but many other enzymes and coenzymes are required	Zymase is required in the case of carbohydrates

14. PROTEIN AND FAT SYNTHESIS

Biosynthesis of protein

Protein is a complex organic nitrogenous substance found in all living tissues of plants and animals. They are polymer of amino acids in linear order. Synthesis of protein may take place from amino acids produced by direct amination of organic acids or by degradation of protein. Former is known as *primary protein synthesis* while the latter is called *secondary protein synthesis*. Protein synthesis occurs in pre – DNA synthesis phase (G_1 phase) of cell cycle.

Biosynthesis of protein takes place in prokaryotes as well as in eukaryotes. Kinds of protein to be synthesized depend upon the gene (DNA segment). Gene is continuous uninterrupted sequence of nucleotides which codes for a single polypeptide chain. Now it is believed that the sequence of some eukaryotic genes is found to be interrupted by nucleotides that are not represented with the amino acid sequence of protein. They are non-coding (silent). Genes control all metabolic processes by synthesizing proteins (enzymes).

Structure of an eukaryotic gene showing Exon (coding part) and Intron (non-coding part).
Mechanism of protein synthesis: Protein synthesis takes place in following two stages:

I. Transcription
II. Translation

Transcription: Transcription occurs throughout inter phase and continues up to early prophase of cell division. It is primary stage of protein synthesis. When DNA produces DNA the process is called replication but when DNA produces RNA the process is called transcription. In the former case DNA is duplicated while in the latter case protein is synthesized. During transcription RNA is synthesized on DNA template. Here, information or order contained in DNA is passed on the mRNA for synthesis of particular protein. The information is in coded form and consists of three nitrogenous bases (triplet codons). The part of DNA responsible for synthesis of mRNA is which leads to one polypeptide chain is

called cistron (functional gene). New strands of mRNA are synthesized on DNA template making use of RNA nucleotides present in surroundings in 5' 3' director just like DNA chains.

Single DNA strand serves as template for RNA polymerase and synthesizes RNA. DNA strand which serves as template for transcription is called the sense strand. The complementary strand is antisense strand. In SV – 40 viruses both strands of DNA are transcribed and it is called symmetrical transcription, but when only one strand is transcribed it is called asymmetrical transcription. Former type of transcription also occurs in Polymer virus DNA and in the mitochondria genome.

Mechanism of transcription

Transcription involves following events:

I. Uncoiling of DNA molecule
II. Synthesis and action of enzyme RNA polymerase
III. Synthesis of hn RNA / mRAN.

Uncoiling of DNA molecule: As per "nucleosome model" of chromosome. Chromosomes are 'matarmala' like beaded structure. Beads are separated by string and are making up of DNA and histone protein. Histone proteins are of 5 kinds: H2A, H2B, H3, H4 and H5 or H1. First four constitute the bead core and H1 links two beads. The DNA molecules are wrapped on histone protein cores and linker protein core in beaded and linker regions respectively.

Structure and function of enzyme RNA polymerase: RNA polymerase is a holoenzyme. Core particle consists of sub units α'β, β' and ψ. Cofactor consists of sigma factor. For functional RNA polymerase formation the two (core enzyme and sigma factor) gene united. Sigma factor recognized correct start signal at DNA template and core enzyme continues transcription. Sigma factor dissociates after initiation of transcription to adjure with other core enzyme of RNA polymerase. In prokaryotes RNA polymerase is only one type while in eukaryotes.

Production of mRNA / hnRNA: In prokaryotes where nucleus is not well organized mRNA is the direct product of transcription, while in eukaryotes the direct product of transcription is hnRNA (heteronucleic RNA) and mRNA is derived from hnRNA by cutting and splicing. HnRNA has coding and non-coding sequences. Coding sequences are interrupted by non-coding sequences. Non coding sequences are removed by splicing (cutting) by endonuclease enzyme and coding sequences are ligased together to from mRNA. The spliced non-coding sequences are degraded within nucleus. It never goes out of nucleus. Thus, only fraction of hnRNA is translocated to cytoplasm from nucleus via nuclear pore.

In eukaryotes migration of mRNA from nucleus to cytoplasm via nuclear pore occurs through poly a tail. According to another view, ribosome's pull the mRNA from nucleus to cytoplasm. Now mRNA gets established in cytoplasm.

Translation: Translation is a process in which order (message) given by DNA to mRNA for synthesis of particular protein is implemented (conveyed). Genetic information concealed in mRNA directs the synthesis of particular protein. These orders are in coded form. This coded information (expressed through codons) is recognized by tRNA having anticodons. Anticodons are opposite to codons (codons and anticodons are complementary to each other).

Mechanism of translation: It involves following events:

I. Activation and selection of amino acids
II. Transfer of amino acids to tRNA molecules
III. Formation of protein synthesizing apparatus and chain initiation
IV. Chain elongation
V. Chain termination

FAT SYNTHESIS

Fat synthesis can be studied under the following heads: -

1. Fat synthesis of Glycerol

There may be different methods of the formation of glycerol in plants, but one of the very common methods is from dihydroxy acetone phosphate which is an intermediate of glycolysis. Dihydroxyacetone phosphate is first reduced to α–glycerophosphate by the enzyme glycerol – 3 – phosphate dehydrogenase. Co-enzyme $NADH_2$ is oxidized in this reaction. α–glycerophosphate is then hydrolysed by *glycerol phosphatase* to liberate phosphoric acid and forming glycerol.

2. Synthesis of Fatty acids

Long chain saturated fatty acids* are synthesized in plants from active two carbon units, the acetyl – CoA ($CH_3CO.CoA$). Although the reactions of β – oxidation of fatty acids are reversible, the fatty acids are not formed simply by the reverse reactions of β – oxidation. Synthesis of fatty acids from $CH_3CO.CoA$ takes place step by step. In each step the fatty acid chain is increased by two carbon atoms. Each step involves two reactions –

(i) In the first reaction which takes place in the presence of acetyl – CoA carboxylase, acetyl – CoA combines with CO_2 to form malonyl – CoA (malonic acid is 3 – C compound). ATP provides energy while Mn^{++} and biotin are required as co-factors.

(ii) Malonyl CoA reacts with another molecule of $CH_3CO.CoA$ in the presence of fatty acid synthetase and Coenzyme $NADPH_2$ to form Coenzyme – A derivative butric acid (butyric acid contains 4 – atoms). One mol. Of CO_2, H_2O and CoA are released while $NADPH_2$ oxidised in the reaction.

Butyryl CoA, in the next step will combine with malonyl CoA to form CoA derivative of fatty acid containing 6-C atoms. This process is repeated till Coenzymes-A derivative of long chain fatty acid (which may contain up to 16-18C atoms) is produced.

(As a matter of fact the enzyme fatty acid synthetase is not simple but a complex of many enzymes (multienzyme complex) and an acyl carrier protein called as ACP**. And actually the reaction (ii) described above only summarises a number of reactions involved in the synthesis of fatty acid from acetyl – CoA which can be grouped under 3 categories

Initiation reaction: In this reaction acetyl CoA transfers its acetyl group to one of the – SH groups of multienzyme complex i.e. fatty acid synthatase.

Unsaturated fatty acids are synthesized by denaturation of saturated fatty acid. ACP is similar to CoA in having phosphopantetheine as the functional unit in their structures. In CoA, it is esterified to Adenosine 3, 5 – bisphosphate but in ACP, it is esterified to serine of a protein chain consisting of 81 amino acids.

Chain elongation reactions

Six different reactions involved here are (i) malonyl transfer, (ii) condensation, (iii) reduction, (iv) dehydration, (v) reduction and (vi) acyl transfer. Chain elongation starts with the transfer of malonyl group from malonyl – CoA to second – SH group of the multienzyme complex. Then, there is a condensation of the latter so that a 4 – C unit is produced. This unit by next three reactions i.e. reduction, dehydration and reduction is converted into saturated 4 – C unit (i.e. butyryl – CoA). In acyl transfer reaction the fatty acid residue is transferred back to the – SH group to which the acetyl group was transferred in initiation reaction. The cycle is repeated again and again with malonyl transfer, condensation etc. till the fatty acid residue consists of up to 16 - 18 C atoms. Each such turn elongates fatty acid chain by 2 – C atoms. Details of chain elongation reactions are given below

Termination reaction

When the fatty acid residue has attained a desired length the chain elongation stops at reaction (v) and the cycle is not repeated. The acyl group instead of being transferred to the – SH of the enzyme is transferred to – SH group of Co-enzyme A (CoASH) molecule. Thus, CoA derivate of the fatty acid is produced which can then be utilized in fat synthesis. The enzyme becomes free.

It is believed that during this process of fatty acid synthesis, the acyl group of fatty acid bound to the – SH group of ACP. The latter then passes it from one enzyme of the complex to the other.

(3) Condensation of fatty acids and Glycerol

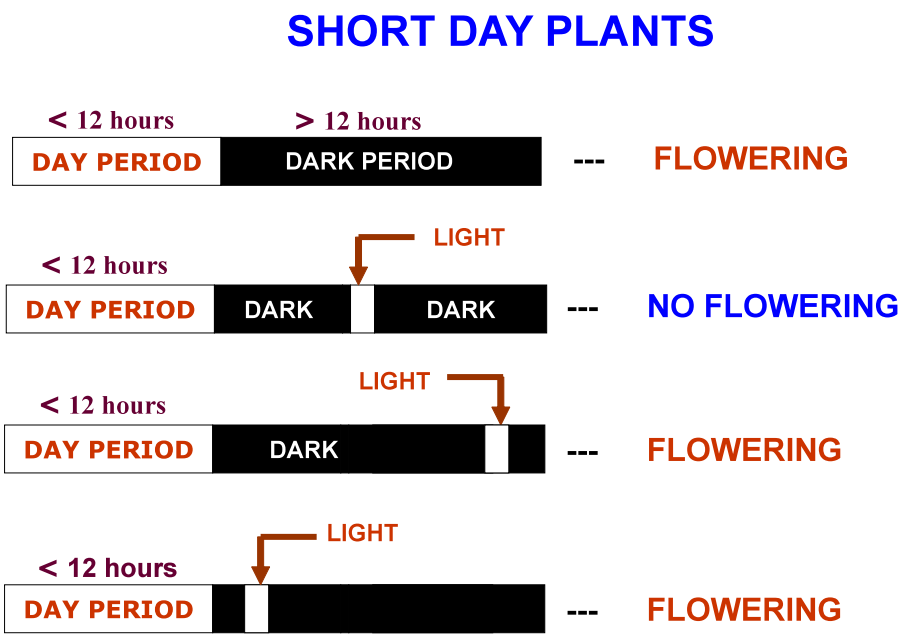
The fats or triglycerides are synthesized not from glycerol and free fatty acids but from α – glycerophosphate and CoA derivatives of fatty acid, i.e. fatty acyl CoA residues. First, there is acylation of α – glycerophosphate by two fatty acyl – CoA molecule to from phosphatidic acid. Now dephosphorylation occurs in the presence of phosphatase and a deglyceride is formed. The acylation of the free – OH groups of diglyceride completes the biosynthesis of triglyceride or fat.

15. **PHOTOPERIODISM**

Photoperiodism is the phenomenon of physiological changes that occur in plants in response to relative length of day and night (i.e. photoperiod). The response of the plants to the photoperiod, expressed in the form of flowering is also called as photoperiodism. The phenomenon of photoperiodism was first discovered by Garner and Allard (1920).Depending upon the duration of photoperiod, the plants are classified into three categories.

1. Short day plants (SDP)
2. Long day plants (LDP)
3. Day neutral plants (DNP)

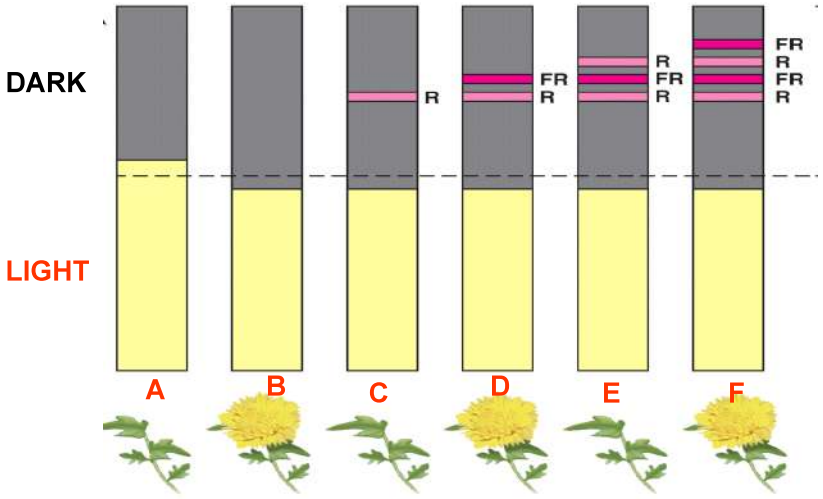
1. Short day plants



These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for subsequent flowering. These plants are also known as long-night plants

E.g. Rice, coffee, soybean, tobacco and chrysanthemum

- In short day plants, the dark period is critical and must be continuous. If this dark period is interrupted with a brief exposure of red light (660-665 nm wavelength), the short day plant will not flower.
- Maximum inhibition of flowering with red light occurs at about the middle of critical dark period.
- However, the inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 mm wavelength)
- Interruption of the light period with red light does not have inhibitory effect on flowering in short day plants.
- Prolongation of the continuous dark period initiates early flowering.



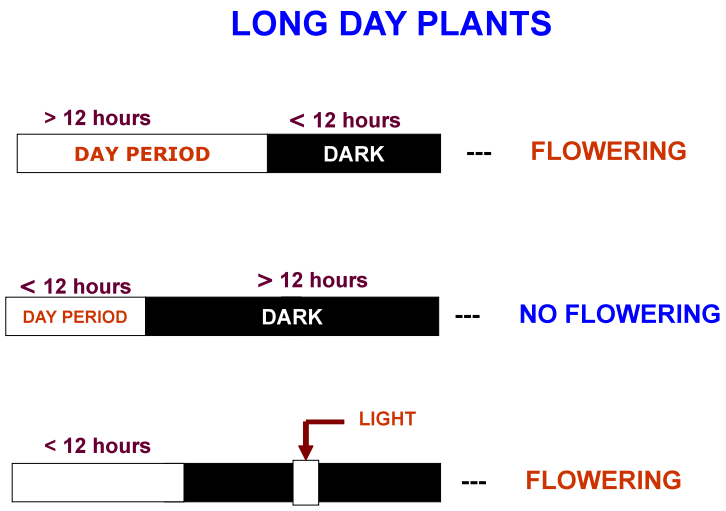
SHORT DAY PLANTS - FLOWERING

2. Long day plants

These plants require longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. These plants are also called as short night plants.

E.g. Wheat, radish, cabbage, sugar beet and spinach.

- In long day plants, light period is critical
- A brief exposure of red light in the dark period or the prolongation of light period stimulates flowering in long day plants.



3. Day neutral plants

These plants flower in all photoperiod ranging from 5 hours to 24 hours continuous exposure.

E.g. Tomato, cotton, sunflower, cucumber, peas and certain varieties of tobacco.

During recent years, intermediate categories of plants such as *long short day plants* and *short long day plants* have also been recognized.

i. Long short day plants

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. E.g. Bryophyllum.

ii. Short –long day plants

These are long day plants but must be exposed to short day during early periods of growth for subsequent flowering. E.g. certain varieties of wheat and rye.

Differences between short day and long day plants

	Short day plant	Long day plant
1	Plants flower when photoperiod is less than the critical day length	Plants flower when photoperiod is more than the critical day length
2	Interruption during light period with darkness does not inhibit flowering	Interruption during light period with darkness inhibit flowering
3	Flowering is inhibited if the long dark period is interrupted by a flash of light	Flowering occurs if the long dark period is interrupted by a flash of light
4	Long continuous and uninterrupted dark period is critical for flowering	Dark period is not critical for flowering
5	Flowering does not occur under alternating cycles of short day and short light period.	Flowering occurs under alternating cycles of short day followed by still shorter dark periods

Phytochrome

It is observed that that a brief exposure with red light during critical dark period inhibits flowering in a short day plant and this inhibitory effect can be reversed by a subsequent exposure with far-red light. Similarly, prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants.

This inhibition of flowering in short day plant and stimulation of flowering in long day plants involves the operation of a proteinaceous pigment called *phytochrome*. It is present in the plasma membrane of cells and it has two components, chromophore and protein. Phytochrome is present in roots, coleoptiles, stems, hypocotyls, cotyledons, petioles, leaf blades, vegetative buds, flower tissues, seeds and developing fruits of higher plants.

The pigment, phytochrome exists in two different forms i.e., red light absorbing form which is designated as *Pr* and far red light absorbing form which is designated as *Pfr*. These two forms of the pigment are photo chemically inter convertible. When *Pr* form of the pigment absorbs red light (660-665 nm), it is converted into *Pfr* form. When *Pfr* form of the pigment absorbs far red light (730-735 nm), it is converted into *Pr* form. The *Pfr* form of pigment gradually changes into *Pr* form in dark.

It is considered that during day time, the *Pfr* form of the pigment is accumulated in the plants which are inhibitory to flowering in short day plants but is stimulatory in long day plants. During critical dark period in short day plants, this form gradually changes into *Pr* form resulting in flowering. A brief exposure with red light will convert this form again into *Pfr* form thus inhibiting flowering.

Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because, the *Pfr* form after absorbing far-red light (730-354 nm) will again be converted back into *Pr* form.

Prolongation of critical light period or the interruption of the dark period by red- light in long day plants will result in further accumulation of the *Pfr* form of the pigment, thus stimulating flowering in long-day plants.

Differences between Pr and Pfr forms of phytochrome

	Pr form	**Pfr form**
1	It is blue green in colour	It is light green in colour
2	It is an inactive form of phytochrome and it does not show phytochrome mediated responses	It is an active form of phytochrome and hence shows phytochrome mediated responses
3	It has maximum absorption in red region (about 680nm)	It has maximum absorption in far-red region (about 730nm)
4	It can be converted into Pfr form in red region (660-665nm)	It can be converted into Pr form in far red region (730-735nm)
5	It is found diffused throughout the cytosol	It is found in discrete areas of cytosol
6	The Pr form contains many double bonds in pyrrole rings	The Pfr form contains rearranged double bonds in all pyrrole rings

Significance of photoperiodism

Photoperiodism is an example for *physiological preconditioning*. The stimulus is given at one time and the response is observed after months. Exposure to longer photoperiods hastens flowering (E.g). In wheat, the earing is hastened. During long light exposure, *Pr* form

is converted into *Pfr* form and flowering is initiated. If dark period is greater, *Pfr* is converted into *Pfr* form that inhibits flowering.

The important phytochrome mediated photo responses in plants include photoperiodism, seed germination, sex expression, bud dormancy, rhizome formation, leaf abscission, epinasty, flower induction, protein synthesis, pigment synthesis, auxin catabolism, respiration and stomatal differentiation.

16. TRANSMISSION OF STIMULUS - THEORIES OF FLOWERING.

Photoperiodic Induction

The influence of the length of day and night on the initiation of flowering is called *photoperiodic induction* or *photo induction*.

Plants may require one or more inductive cycle for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycle is subsequently placed under unfavourable photoperiod, it will still flower.

Flowering will also occur if a plant receives inductive cycles after intervals of unfavourable photoperiods (i.e. discontinuous inductive cycle). This persistence of photoperiodic after effect is called as photoperiodic induction.

- An increase in the number of inductive cycles results in early flowering of the plant. For instance, xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64 days. It can be made to flower even after 13 days if it has received 4-8 inductive cycle. In such case number of flowers is also increased.
- Continuous inductive cycles promote early flowering than discontinuous inductive cycle.

  Some of the examples of plants which requires more than one inductive cycle for subsequent flowering are,

  Biloxi soybean (SDP) - 2 inductive cycles
  Salvia (SDP) - 17 Inductive cycles
  Plantago (LDP) - 25 Inductive cycles

Critical day length

Maryland mammoth tobacco and xanthium are short day plants, but the *Maryland mammoth* tobacco is induced to flower when the photoperiod is shorter than 12 hours (12L /12D) whereas, xanthium is induced to flower when the photoperiod is shorter than 15.5 hours (15.5L /8.5D). The photoperiod required to induce flowering is referred to as the *critical day length*. Hence, the critical day length for *Maryland mammoth* tobacco and

xanthium are 12 and 15.5 hours respectively. A short day plant is one that flowers on photoperiods shorter than the critical day length.

Long day plants, on the other land, are induced to flower on photoperiods longer than critical day length. For example, the critical day length for *Hyoscyamus niger* is 11 hours (11L /13D) and it is induced to flower on photoperiods longer than 11 hours.

Suppose, xanthium and *Hyoscyamus niger* are exposed to a photoperiod of 14 hours of light and 10 hours of darkness (14L/10D), flowering will be induced in both plants. Xanthium, a short-day plant, will flower because 14L /10D photoperiod is shorter than critical day length of 15.5 hours. Hyoscyamus, a long-day plant, will flower because 14L/10D is longer than the critical day length of 11 hours.

Perception of photoperiodic stimulus and presence of a floral hormone

- Photoperiodic stimulus is perceived by the leaves and a floral hormone is produced in the leaves which are then translocated to the apical tip, subsequently causing initiation of floral primordia.
- Photoperiodic stimulus perceived by the leaves can be shown by a simple experiment on cocklebur (xanthium), a short day plant. Cocklebur plant will flower if it has previously been kept under short day conditions. If the plant is defoliated and kept under short day condition, it will not flower. Flowering will also occur if all the leaves of the plant except one leaf have been removed.
- If the cocklebur plant whether intact or defoliated is kept under long day condition it will not flower. But if even one of its leaves is exposed to short day condition and the rest are under long day condition, flowering will occur.
- The photoperiodic stimulus is transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day and the other to long day photoperiod, flowering occurs on both the branches.
- Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition. However, if one branch is exposed to long photoperiod and the other

has been defoliated, under short day conditions, flowering will not occur in any of the branches.

Flowering stimulus: Florigen

The flowering stimulus is produced in leaves and translocated to apical and lateral meristems where flower formation is initiated. Chailakhyan (1937) called the flowering stimulus or flowering hormone as *Florigen.*

Flowering stimulus is similar in long day plants and short day plants. This can be proved by a grafting experiment and can be translocated from one plant to another.

Maryland mammoth tobacco, a short day plant and *Hyoscyamus niger*, a long day plant, are grafted so that the leafy shoots of both the species are available for experiment. If the grafted plants are exposed to either long day a short day conditions, both partners flower. If grafting union is not formed, the flowering stimulus is not translocated from one partner to another partner.

Theories of Flowering

1. Bunning's hypothesis
2. Chailakhyan's hypothesis

Bunning's hypothesis:

Bunning (1958) assumes the presence of endogenous rhythms (Oscillator which consist of two half cycles. The first half cycle occurs in day and is called *photophilous phase*. During this, anabolic process predominates including flowering in plants. The other half cycle is dark, sensitive and is called *skotophilous phase*. In this, catabolic process (dehydration of starch) predominates.

SD plants have a critical day length of 9 hours. This period falls within the photophilous phase. Light during scotophil phase will inhibit photo process initiated during photophase. The L.D. plants have a critical day length of 15 hours and some light falls in the skoto philous phase. Under these conditions in L.D. plants will flower. In S.D. plants

oscillator is present close to skoto philous phase, while in L.D. plants it is close to *photo philous phase*.

Chilakhyan's hypothesis:

This hypothesis assumes that flowering hormone – florigen is a complex of two types of substances – gibberellin and anthesins. Gibberellin is essential for growth of the plant stems and anthesins are required for flower formation.

According to him, flowering in all annual seed plants requires two phases: (i) *Floral stem formation phase* (ii) *Flower formation phase*. First phase involves increased carbohydrate metabolism and respiration with increased content of GA in leaves. Second phase requires intensive nitrogen metabolism, higher content of anthesins in leaves and nucleic acid metabolites in stem buds.

Long day conditions favour the first phase while short day conditions favour second phase. In long day plants gibberellins are critical, while anthesins are critical in short day plants. However, anthesin is hypothetical; it has not been isolated as yet.

VERNALISATION

The cold treatment given to plant buds, seeds or seedlings for promoting early flowering is known as *Vernalisation*. In short, the chilling treatment for induction of early flowering is called Vernalisation.

Besides an appropriate photoperiod, certain plants require a low temperature treatment during their early stages of the life for subsequent flowering in the later stages. This low temperature treatment requirement was termed vernalization by Lysenko (1928). Due to vernalization, the vegetative period of the plant is cut short resulting in an early flowering. In nature, vernalisation takes place in the seed stage in annuals like winter rye (*Secale cereale*). The biennials and many perennials respond to cold treatment at a very late stage. E.g. Henbane, apples etc.

Perception of cold stimulus and presence of floral hormone

The cold stimulus is perceived by the apical meristems. The perception of the cold stimulus results in the formation of a floral hormone which is transmitted to other parts of the plant. In certain cases, the cold stimulus may even be transmitted to another plant across a graft union.

For instance, if a vernalized henbane plant is grafted to an unvernalized henbane plant, the later also flowers. This is due to the induction of the plant to produce a hormone named as *Vernalin* by Melchers (1939).

Conditions necessary for vernalization

1. Age of the plant

The age of the plant is an important factor in determining the responsiveness of the plant to the cold stimulus and it differs in different species. In cereals like winter wheat, the vernalization is effective only if the germinating seeds have received cold temperature treatment for sufficient time.

While in the case of biennial variety of henbane (*Hyoscyamus niger*), the plant will respond to the cold treatment, only if they are at rosette stage and completed at least 10 days of growth.

2. Appropriate low temperature and duration of the exposure

Most suitable temperature for vernalizing the plants ranges between 1-6°C. The effectiveness of low temperature treatment decreases from 0 to 4°C. Low temperature at about -6°C is completely ineffective. Similarly at high temperatures from 7°C onwards, the response of the plants is decreased. Temperature of about 12-14°C is almost effective in vernalizing the plant. Besides an appropriate low temperature, a suitable duration of the cold treatment is essential for vernalization. Depending upon the degree of temperature and in different species this period may vary, but usually the duration of the chilling treatment is about one and half months or more.

3. Oxygen

The vernalization is an aerobic process and requires metabolic energy. In the absence of O_2, cold treatment becomes completely ineffective.

4. Water

Sufficient amount of water is also essential for vernalization. Vernalization of the dry seed is not possible.

Mechanism of Vernalization

There are two main theories to explain the mechanism of vernalisation.

1. Phasic developmental theory

This theory was proposed by Lysenko (1934) as follows.

(i) The growth (increase in size) and development (i.e. progressive change in the characteristic of the new organs) are two distinct phenomenons.

(ii) According to this theory, the process of the development of an annual seed plant consists of a series of phases which must occur in some predetermined sequence.

(iii) Commencement of any of these phases will take place only when the preceding phase has been completed.

(iv) The phases require different external conditions for the completion such as light and temperature.

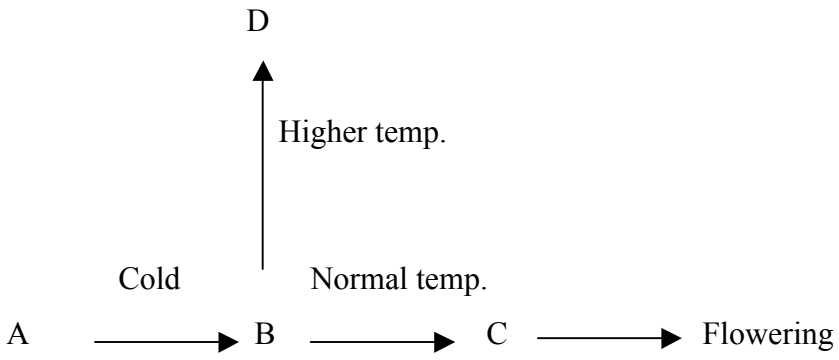
(v) Vernalization accelerates the thermo phase i.e. that phase of development which is dependent upon temperature.

Thus, in winter wheat, low temperature is required for the completion of first thermo phase. After this, the next phase that is dependent upon light (photo phase) starts. Vernalization of winter wheat accelerates the first thermo phase so that there is an early swing from vegetative to reproductive phase or flowering.

2. Hormonal theories

It has already been described that vernalization probably involves the formation of a floral hormone called as *vernalin*. Based on this fact, many hypothetical schemes have been

proposed by different workers from time to time. The first hormonal theory proposed by Long and Melchers (1947) is schematically shown below.



According to this scheme, the precursor A is converted into a thermo labile compound B during cold treatment. Under normal conditions B changes into C which ultimately causes flowering. But at higher temperature B is converted into D and flowering does not take place (devernalization).

Devernalization

The positive effect of the low temperature treatment on the vernalization of the plant can be counteracted by subsequent high temperature. This is called devernalization. The devernalized plant can again be vernalized by subsequent low temperature treatment.

Vernalization and Gibberellins

The gibberellins are known to replace the low temperature requirement in certain biennial plants such as henbane, where the plant normally remains vegetative and retains its rosette habit during the first growing season and after passing through the winter period flowers in the next season. The gibberellins cause such plants to flower even during the first year.

Significance of vernalization

1. Vernalization shortens the vegetative period of the plant
2. It increases cold resistance of the plants
3. Vernalization increases the resistance of plants to fungal diseases.
4. It is a physiological process that substitutes or compensates the effect of thermo phase.
5. In biennials, vernalisation induces early flowering and early fruit setting.
6. A non vernalised shoot apex can be induced to flower by grafting the plant with a vernalised plant.

17. SOURCE SINK RELATIONSHIP

Source

1. It is the regions of photoassimilates production
2. Export photoassimilates
3. Chlorophyllous tissues
4. Leaves, stipules, fruit wall, young stem, pedicel, awns, peduncle, calyx, bract etc

Sink

1. Regions of photoassimilates consumption
2. Import photoassimilates
3. Growing regions
4. Storage organs – Fruit and Seed

Source strength

1. Source Size x Source activity
2. Differences in CO_2 fixation (Rubisco & PEP Case)
3. Leaf characters – size, thickness, mesophyll size, compaction, vascular bundle
4. Carrying capacity of sieve element (temp., H_2O, nutrients, hormone)

Sink strength

1. Sink size x Sink activity
2. Potential capacity of the sink to accumulate assimilates
3. Competition among different sink

Source sink interaction

1. Source sink equilibrium
2. Small surplus source for stress
3. High source size during sink differentiation
4. Improve strength by activity
5. Synchrony of sink organ development

6. Increased HI is reached – increase DMA
7. Reduce photorespiration in C3 plants

Evans (1983)

Reduced growth of non harvestable organ

Prolonged faster storage

Enhanced competition of storage organ

Enhanced competition of regulatory process

Reduced stem weight and height

Reduced root weight with adequate nutrient and H2O

Improved agronomic support (avoid biotic & abiotic stress)

Hormonal regulation

Developmental plasticity (small surplus source for stress)

Efficient system

1. Quick export of photoassimilates to avoid end product inhibition
2. Efficient root system
3. More photosynthetic rate
4. Optimum LAI (4 to 6)
5. High photosynthetic rate & high DMA

Blackman's law of limiting factor

1. A process is controlled by several factors
2. The phase of the process is limited by slowest factor
3. Compensation mechanism working under canopy level

Dry matter accumulation (DMA)

G x E interaction; nutrients; CO2 fixation rate (path way); photorespiration; vascular network; LAI & LAD; source-sink limiting condition; root-shoot balance

HI

$Ye = Yb \times h$

/ $HI = \{Yield_{(Eco)} / Yield_{(Biol)}\} \times 100$

Improve Harvest index (HI)

Increase biomass production (DMA)

Synchronized development of reproductive organ

Reproductively determinate

High source strength at the time of sink differentiation

Reduced growth of non harvestable organ

Reduced leaf growth at reproductive stage with high LAD

Optimum LAI and early peak LAI

More prolonged and faster storage, enhanced competitiveness among of the storage organ

High photosynthetic rate

Improved HI by increased size and number of sink organ

Decline in duration of Vegetative growth and increased duration of Reproductive growth.

Limitations

Source: wheat, rice, pulses, oilseeds

Sink: bajra, ragi

Transport: sorghum, maize (green leaf at harvest; senescence of phloem Parenchyma)

Sink limitation:

Late anthesis (Long Vegetative phase)

Indeterminate (Vegetative & Reproductive growth)

Vegetative growth at Reproductive phase

Less sink number and size

Hormonal imbalance

Any Stress

Multi-sink demand (nodules supply 25 – 75 % of N demand)

Source limitation:

Low canopy photosynthesis

Low optimum LAI

Slow peak LAI (lag vegetative growth)

Low LAD at filling

Early leaf senescence

Stress – nutrients, water

Plant Growth Regulators (PGRs)

ABA inhibit sucrose uptake in source (Loading)

Auxin promotes source uptake

Starch accumulation in chloroplast inhibit photosynthesis

ABA in leaves causes closer of stomata (Inhibit CO_2 fixation)

Cytokinin delays senescence of source and sink

Cytokinin in sink increases photoassimilates import

Ethylene induces senescence process.

18. **PLANT GROWTH**

Growth is defined as a vital process that brings about a permanent and irreversible change in any plant or its part in respect to its size, form, weight and volume. Growth is restricted only to living cells and is accomplished by metabolic processes involving synthesize of macromolecules, such as nucleic acids, proteins, lipids and polysaccharides at the expense of metabolic energy.

Growth at cellular level is also accompanied by the organization of macromolecules into assemblages of membranes, plastids, mitochondria, ribosome and other cell organelles. Cells do not definitely increase in size but divide, giving rise to daughter cells. An important process during cell division is synthesis and replication of nuclear DNA in the chromosomes, which is then passed into the daughter cells. Therefore, the term growth is used to denote an increase in size by cell division and cell enlargement, together with the synthesis of new cellulose materials and the organization of cellular organelles.

Growth regions

Typical growth regions in plants are the apices of shoot and root. Such growing regions are known as apical meristems, primary meristems or regions of primary growth. These apical meristems are responsible for the increase in length, differentiation of various appendages and formation of plant tissues.

Phases of growth

Growth is not a simple process. It occurs in meristematic regions where the meristematic cell has to pass through the following 3 phases.

1. Cell formation phase
2. Cell elongation phase
3. Cell differentiation (cell maturation)

The cell formation phase is represented by meristematic zone and cell enlargement phase by cell elongation zone.

The dividing meristematic cells are thin walled and have dense protoplasm with a large nucleus and with or without very small vacuoles. The intercellular spaces are also absent. The newly formed cells after the first phase of cell division have to pass through the second phase of cell enlargement. During the second phase of cell elongation on account of large quantities of solutes inside the growing cell, water enters the cell due to osmotic effect

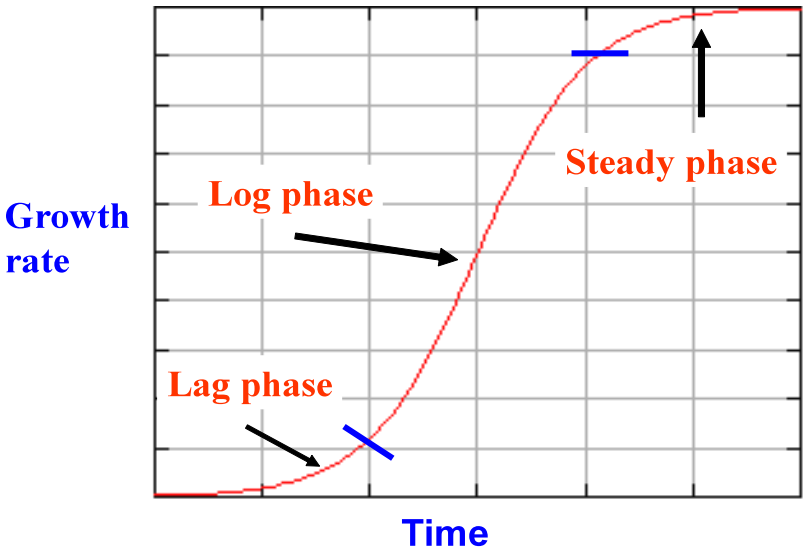
resulting in the increased turgidity and expansion and dilation of the thin and elastic cell wall. This phase also results in appearance of large vacuoles.

In the last phase or cell maturation, the secondary walls are laid down and cell matures and gets differentiated into permanent tissue.

Growth curve

Growth curve is a graph obtained by plotting the growth rate of a plant against time factor. The growth rate of a cell, a plant organ, a whole plant or the whole life cycle of plant is measured in terms of length, size, area, volume or weight. It has been found that different growth phases result in 'S' shaped curve or sigmoid curve. In initial stages during the phase of cell formation, the growth rate increases slowly while it increases rapidly during the phase of cell elongation or cell enlargement and again slows down during the phase of cell maturation.

GROWTH RATE PHASES - GROWTH CURVE



The period during which the course of growth takes place is known as grand period of growth. Thus, in a standard growth curve, three well marked regions can be observed, the initial growth stage (lag phase), the grand period of growth (exponential or log phase) and the steady stage (maturity stage or senescence or stationary phase). The overall growth may be affected by external or internal factors but the S- shaped curve of grand period of growth is

never influenced. This growth curve suits well to the entire life of an annual plant when measured in terms of dry weight against time.

Early growth of the plant is limited by the amount of food reserves in the seed. When the emerged seedlings develop an adequate root system and enough leaf surfaces to support vigorous photosynthesis and anabolism, a period of rapid increase in size is possible.

High metabolic rates are not maintained indefinitely and eventually processes are set in motion that leads to cessation of growth. The factors responsible for the decrease in growth are competition for essential metabolites, growth substances, water, light or the accumulation of inhibitors, toxic substances or waste materials.

Blackman (1919) suggested that the growth of the plants can be represented by equation.

$$W_1 = W_o\, e^{rt}$$

Where, W_1 is the final size (Wt, ht etc) after time t. W_o is the initial size at the beginning of the time period. r is the rate at which plant substance is laid down during time t and e is the base of natural logarithm. Blackman pointed out that equation describes the way in which money placed at compound interest increases with time; the term compound interest law is used to describe such phenomenon. In banks, compound interest is usually applied quarterly or annually so that the increase in amount occurs as a jump. With plant system, compound interest is applied continuously and size increase follows a smooth curve.

From the equation, the final size of an organism (W_1) depends on the initial size (W_o). Larger seed give a larger plant.

In addition, equation shows that plant size also depends on the magnitude of r, the relative growth rate. Blackman suggested that r might be used as a measure of the ability of the plant to produce new plant material and called r as the *efficiency index*. The plants with high efficiency index could be expected to outperform plants with low efficiency index.

Measurement of growth

The measurement of growth is possible in terms of either increase in weight or increase in volume or area. The common and simplest method for the measurement of

growth can be a direct method by which the growth is measured by a scale at regular intervals from beginning to end. The other methods that can be used are horizontal microscope, auxanometers.

Factors influencing growth

Growth is affected by all factors that affect the activity of protoplasm. Both physiological and environmental factors such as water, minerals, photosynthesis, respiration, climate and edaphic factors significantly influence the growth. In general, factors can be grouped into external and internal factors.

External factors

1. Light

It has direct effect on photosynthesis and transpiration. Light in terms of intensity, quality and periodicity influence the growth very much.

Light intensity: A weak light promotes shortening of internodes and affects expansion of leaf. Very weak light reduces the rate of over all growth and also photosynthesis due to poor development of chlorophyll and higher rate of water loss from the plant.

Light quality: The different wavelengths of light have different responses to growth. In blue violet radiation, the internodal growth is pronounced while green colour light promotes the expansion of leaves as compared to complete spectrum of visible light. The red light favours the growth while infra red and UV is detrimental to growth.

Light duration: The re is remarkable effect of the duration of light on the growth. The induction and suppression of flowering depend on duration

2. Temperature

The plants have different temperature requirements based on the region where they are grown. In general, best growth takes place between 28 and 33 C. and it varies from temperate to tropical conditions. The optimum temperature requirement is essential for seed germination, growth, metabolic activities, flowering and yield.

3. Oxygen

The growth of the plant is directly proportional to the amount of oxygen which is essential for respiration during which the food materials are oxidized to release energy.

4. Carbon dioxide

It is one of the major factors that influence the photosynthesis. The rate of photosynthesis increases as the availability of CO2 increases while other factors are not limiting.

5. Water

Water is an essential factor for growth. It is essential for uptake of nutrients, translocation of nutrients and food materials, regulating transpiration and for various physiological processes like photosynthesis, respiration and enzymatic activities.

6. Nutrients and food materials

The rate of growth is directly proportional to the availability of nutrients and food materials. The shortage of food supply affects the growth as it provides the growth material to the growing region and also it provides the potential energy to the growing region.

Internal factors

1. Growth hormones and their availability
2. Resistance to climatic, edaphic and biological stresses
3. Photosynthetic rate and respiration
4. Assimilate partitioning and nitrogen content
5. Chlorophyll and other pigments
6. Source-sink relationship and enzyme activities

19. GROWTH ANALYSIS

Growth analysis can be used to account for growth in terms that have functional or structural significance. The type of growth analysis requires measurement of plant biomass and assimilatory area (leaf area) and methods of computing certain parameters that describe growth. The growth parameters that are commonly used in agricultural research and the name of the scientists who proposed the parameters are given below.

LAI	-	Williams (1946)
LAR	-	Radford (1967)
LAD	-	Power *et al*. (1967)
SLA	-	Kvet *et al*. (1971)
SLW	-	Pearce *et al*. (1968)
NAR	-	Williams (1946)
CGR	-	Watson (1956)
RGR	-	Williams (1946)
HI	-	Nichiporovich (1951)

i. Leaf Area

This is the area of photosynthetic surface produced by the individual plant over a period of interval of time and expressed in cm^2 $plant^{-1}$.

ii. Leaf Area Index (LAI)

Williams (1946) proposed the term, Leaf Area Index (LAI). It is the ratio of the leaf of the crop to the ground area over a period of interval of time. The value of LAI should be optimum at the maximum ground cover area at which crop canopy receives maximum solar radiation and hence, the TDMA will be high.

$$LAI = \frac{\text{Total leaf area of a plant}}{\text{Ground area occupied by the plant}}$$

iii. Leaf Area Ratio (LAR)

The term, Leaf Area Ratio (LAR) was suggested by Radford (1967), expresses the ratio between the area of leaf lamina to the total plant biomass or the LAR reflects the

leafiness of a plant or amount of leaf area formed per unit of biomass and expressed in cm^{-2} g^{-1} of plant dry weight.

$$LAR = \frac{\text{Leaf area per plant}}{\text{Plant dry weight}}$$

iv. Leaf Weight Ratio (LWR)

It was coined by (Kvet *et al.,* 1971) Leaf weight ratio is expressed as the dry weight of leaves to whole plant dry weight and is expressed in g g^{-1}.

$$LWR = \frac{\text{Leaf dry weight}}{\text{Plant dry weight}}$$

v. Leaf Area Duration (LAD)

To correlate dry matter yield with LAI, Power *et al.* (1967) integrated the LAI with time and called as Leaf Area Duration. LAD takes into account, both the duration and extent of photosynthetic tissue of the crop canopy. The LAD is expressed in days.

$$LAD = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

L_1 = LAI at the first stage

L_2 = LAI at the second stage, $(t_2 - t_1)$ = Time interval in days

vi. Specific Leaf Area (SLA)

Specific leaf area is a measure of the leaf area of the plant to leaf dry weight and expressed in cm^2g^{-1} as proposed by Kvet *et al.* (1971).

$$SLA = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

Hence, if the SLA is high, the photosynthesizing surface will be high. However no relationship with yield could be expected.

vii. Specific Leaf Weight (SLW)

It is a measure of leaf weight per unit leaf area. Hence, it is a ratio expressed as g cm^{-2} and the term was suggested by Pearce *et al.* (1968). More SLW/unit leaf area indicates more biomass and a positive relationship with yield can be expected.

$$SLW = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

viii. Absolute Growth Rate (AGR)

AGR is the function of amount of growing material present and is influenced by the environment. It gives Absolute values of biomass between two intervals. It is mainly used for a single plant or single plant organ e.g. Leaf growth, plant weight etc.

$$AGR = \frac{h_2 - h_1}{t_2 - t_1} \quad cm\ day^{-1}$$

Where, h1 and h2 are the plant height at t_1 and t_2 times respectively.

ix. Net Assimilation Rate (NAR)

The term, NAR was used by Williams (1946). NAR is defined as dry matter increment per unit leaf area or per unit leaf dry weight per unit of time. The NAR is a measure of the average photosynthetic efficiency of leaves in a crop community.

$$NAR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where, $W_1$and W_2 is dry weight of whole plant at time t_1 and t_2 respectively

L_1 and L_2 are leaf weights or leaf area at t_1 and t_2 respectively

$t_1 - t_2$ are time interval in days

NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time (g $g^{-1}day^{-1}$)

x. Relative Growth Rate (RGR)

The term was coined by Williams (1946). Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval in relation to the initial weight or Dry matter increment per unit biomass per unit time or grams of dry weight increase per gram of dry weight and expressed as unit dry weight / unit dry weight / unit time (g $g^{-1}day^{-1}$)

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W1 and W_2 are whole plant dry weight at t_1 and t_2 respectively

t_1 and t_2 are time interval in days

xi. Crop Growth Rate (CGR)

The method was suggested by Watson (1956). The CGR explains the dry matter accumulated per unit land area per unit time (g m^{-2} day^{-1})

$$CGR = \frac{(W_2 - W_1)}{\rho\,(t_2 - t_1)}$$

Where, W1 and W_2 are whole plant dry weight at time $t_1 - t_2$ respectively

ρ is the ground area on which W_1 and W_2 are recorded.

CGR of a species are usually closely related to interception of solar radiation

xii. Total dry matter production (TDMP) and its distribution

The TDMP is the biomass accumulated by the whole plant over a period of interval of time and its distribution (allocation) to different parts of the plant such as roots, stems, leaves and the economic parts which controls the sink potential.

xiii. Translocation percentage (TP)

The term translocation percentage indicates the quantum of photosynthates translocated from source (straw) to the grain (panicle/grains) from flowering to harvest.

$$TP = \frac{\text{Straw weight at flowering – straw weight at harvest}}{\text{Panicle weight at flowering – panicle weight at harvest}}$$

xiv. Light extinction coefficient

It is the ratio of light intercepted by crop between the top and bottom of crop canopy to the LAI.

$$K = \frac{\log_e I / I_o}{LAI}$$

Where, I_o and I are the light intensity at top and bottom of a population with LAI

xv. Light Transmission Ratio (LTR)

It is expressed as the ratio of quantum of light intercepted by crop canopy at top to the bottom. Light intensity is expressed in K lux or W m^{-2}

$$LTR = I / I_o$$

Where, I : light intercepted at the bottom of the crop canopy

I_o: light intercepted at the top of the crop canopy

xvi. Dry Matter Efficiency (DME)

It is defined as the percent of dry matter accumulated in the grain from the total dry matter produced over the crop growth period.

$$DME = \frac{\text{Grain yield}}{\text{TDMP}} \times \frac{100}{\text{Duration of crop}}$$

xvii. Unit area efficiency (UAE)

It is expressed as the quantum of grain yield produced over a unit land area for a specified crop growth period.

$$UAE = \frac{\text{Grain yield}}{\text{Land area}} \times \frac{1}{\text{Duration of crop}}$$

xviii) Harvest Index

The harvest index is expressed as the percent ratio between the economic yield and total biological yield and was suggested by Nichiporovich (1951).

$$HI = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

20. PLANT GROWTH REGULATORS

Plant growth regulators or phytohormones are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. Thimmann (1948) proposed the term *Phyto hormone* as these hormones are synthesized in plants. *Plant growth regulators* include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Auxins are the hormones first discovered in plants and later gibberellins and cytokinins were also discovered.

Hormone

An endogenous compound, which is synthesized at one site and transported to another site where it exerts a physiological effect in very low concentration. But ethylene (gaseous nature), exert a physiological effect only at a near a site where it is synthesized.

Classified definition of a hormone does not apply to ethylene.

Plant growth regulators

- Defined as organic compounds other than nutrients, that affects the physiological processes of growth and development in plants when applied in low concentrations.
- Defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting.

Plant Hormone

When correctly used, is restricted to naturally occurring plant substances, there fall into five classes. Auxin, Gibberellins, Cytokinin, ABA and ethylene. Plant growth regulator includes synthetic compounds as well as naturally occurring hormones.

Plant Growth Hormone

The primary site of action of plant growth hormones at the molecular level remains unresolved.

Reasons

- Each hormone produces a great variety of physiological responses.
- Several of these responses to different hormones frequently are similar.
- The response of a plant or a plant part to plant growth regulators may vary with the variety of the plant.
- Even a single variety may respond differently depending on its age, environmental conditions and physiological state of development (especially its natural hormone content) and state of nutrition. There are always exceptions for a general rule suggesting the action of a specific growth regulator on plants.
- There are several proposed modes of action in each class of plant hormone, with substantial arguments for and against each mode.

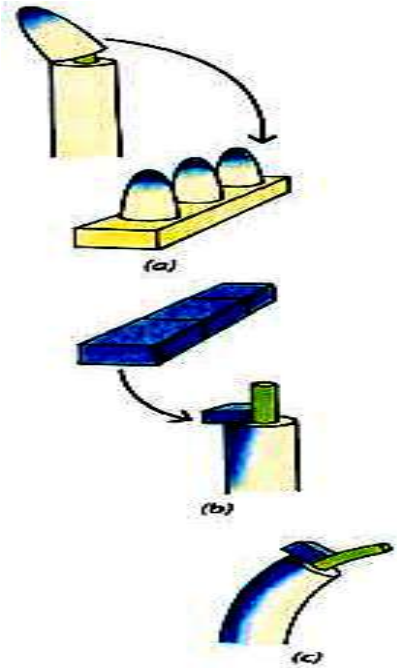
Hormone groups

Auxin	-	Substances generally resembles IAA and has the ability to stimulate the elongation of coleoptiles.
Gibberellins	-	are diterpenoids, which have the ability to elongate the stem of green seedlings especially certain dwarf and rosette types.
Cytokinin	-	Usually substituted Adenines, which resembles zeatin (Naturally occurring cytokinin in *Zea mays*) and have the ability to stimulate cytokinensis in cultures of tobacco cells.
Ethylene	-	Gaseous regulator that stimulate is diametric growth in the apices of dicot seedlings.
Inhibitors	-	are regulators of growth, which originally depress the

Auxins

Auxins are a group of phytohormones produced in the shoot and root apices and they migrate from the apex to the zone of elongation. Auxins promote the growth along the longitudinal axis of the plant and hence the name (auxeing : to grow). The term, auxin was introduced by Kogl and Haagen- Smit (1931). Went (1928) isolated auxin from the Avena coleoptile tips by a method called *Avena coleoptile or curvature test* and concluded that no growth can occur without auxin. Auxins are widely distributed through out the plant however, abundant in the growing tips such as coleoptile tip, buds, root tips and leaves. Indole Acetic Acid (IAA) is the only naturally occurring auxin in plants. The synthetic auxins include,

Avena Curvature Test



Cut off the tips of Avena coleoptile

↓

Placed on agar block

↓

Auxin diffuses into agar block

↓

Placed the agar block Asymmetrically On cut coleoptile stump

↓

Coleoptile showed typical curvature

IBA : Indole Butyric Acid

NAA : Naphthalene Acetic acid

MENA: Methyl ester of Naphthalene acetic acid

MCPA: 2 Methyl 4 chloro phenoxy acetic acid

TIBA : 2, 3, 5 Tri iodo benzoic acid

2, 4-D : 2, 4 dichloro phenoxy acetic acid

2, 4, 5-T: 2, 4, 5 – Trichloro phenoxy acetic acid

Natural auxins may occur in the form of either *free auxins*- which freely move or diffuse out of the plant tissues readily or *bound auxins*- which are released from plant tissues only after hydrolysis, autolysis or enzymolysis.

Physiological effects of auxin

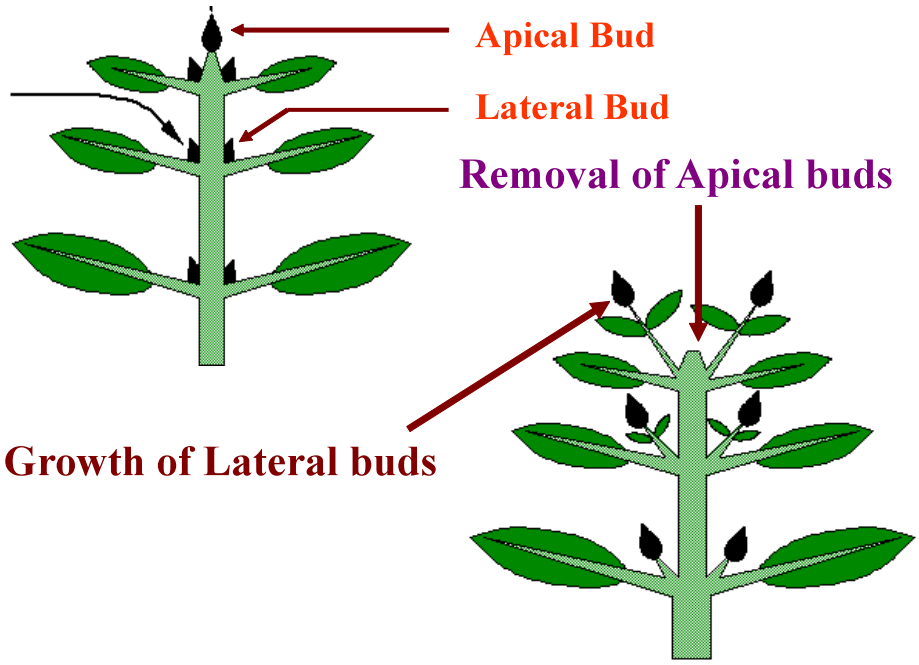
1. Cell division and elongation

The primary physiological effects of auxin are cell division and cell elongation in the shoots. It is important in the secondary growth of stem and differentiation of xylem and phloem tissues.

2. Apical dominance

In many plants, if the terminal bud is intact and growing, the growth of lateral buds just below it remains suppressed. Removal of the apical bud results in the rapid growth of lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the lateral buds to grow is known as *apical dominance*.

Skoog and Thimmann (1948) pointed out that the apical dominance might be under the control of auxin produced at the terminal bud and which is transported downward through the stem to the lateral buds and hinders the growth. They removed the apical bud and replaced it with *agar* block. This resulted in rapid growth of lateral buds. But when they replaced the apical bud with agar block containing auxin, the lateral buds remained suppressed and did not grow.



3. Root initiation

In contrast to stem, the higher concentration of auxin inhibits the elongation of roots but the number of lateral roots is considerably increased i.e., higher concentration of auxin induces more lateral branch roots. Application of IAA in lanolin paste (lanolin is a soft fat prepared from wool and is good solvent for auxin) to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of abscission

Natural auxins prevent the formation of abscission layer which may otherwise result in the fall of leaves, flowers and fruits.

5. Parthenocarpy

Auxin can induce the formation of parthenocarpic fruits (fruit formation without pollination and fertilization). In parthenocarpic fruits, the concentration of auxin in the ovaries is higher than in the ovaries of plants which produce fruits only after fertilization. In

the later cases, the concentration of the auxin in ovaries increases after pollination and fertilization.

6. Respiration

Auxin stimulates respiration and there is a correlation between auxin induced growth and respiration. Auxin may increase the rate of respiration indirectly through increased supply of ADP by rapidly utilizing ATP in the expanding cells.

7. Callus formation

Besides cell elongation, auxin may also be active in cell division. In many tissue cultures, where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Eradication of weeds

Some synthetic auxins especially 2, 4- D and 2, 4, 5-T are useful in eradication of weeds at higher concentrations.

9. Flowering and sex expression

Auxins generally inhibit flowering but in pine apple and lettuce it promotes uniform flowering.

Distribution of auxin in plants

In plants, auxin (IAA) is synthesized in growing tips or meristematic regions from where; it is transported to other plant parts. Hence, the highest concentration of IAA is found in growing shoot tips, young leaves and developing auxiliary shoots. In monocot seedling, the highest concentration of auxin is found in coleoptile tip which decreases progressively towards its base.

In dicot seedlings, the highest concentration is found in growing regions of shoot, young leaves and developing auxiliary shoots. Within the plants, auxin may present in two forms. i.e., *free auxins* and *bound auxins*. Free auxins are those which are easily extracted by various organic solvents such as diethyl ether. Bound auxins on the other hand, need more drastic methods such as hydrolysis, autolysis, enzymolysis etc. for extraction of auxin. Bound

auxins occur in plants as complexes with carbohydrates such as glucose, arabionse or sugar alcohols or proteins or amino acids such as aspartate, glutamate or with inositol.

Biosynthesis of auxin (IAA) in plants

Thimann (1935) found that an amino acid, tryptophan is converted into Indole 3 acetic acid. Tryptophan is the primary precursor of IAA in plants. IAA can be formed from tryptophan by two different pathways.

1. By deamination of tryptophan to form indole-3-pyruvic acid followed by decarboxylation to from indole-3-acetaldehyde. The enzymes involved are tryptophan deamintion and indole pyruvate decarboxylase respectively.
2. By decarboxylation of tryptophan to form tryptamine followed by deamination to form indole-3-acetaldehyde and the enzymes involved are tryptophan decarboxylase and tryptamine oxidase respectively. Indole 3-acetaldehyde can readily be oxidized to indole 3-acetic acid (IAA) in the presence of indole 3-acetaldehyde dehydrogenase.

Transport of auxin in plant

The transport of auxin is predominantly polar. In stems, polar transport of auxin is basipetal i.e., it takes place from apex towards base. Polar transport of auxin is inhibited by 2, 3, 5 Triiodobenzoic acid (TIBA) and Naphthyl thalamic acid (NPA). The substances are called as antiauxins.

Destruction / Inactivation of auxin in plants

Auxin is destructed by the enzyme IAA oxidase in the presence of O_2 by oxidation.

$$IAA + H_2O_2 + O_2 \xrightarrow{\text{IAA Oxidase}} \text{3-methylene oxindole} + H_2O + CO_2$$

Rapid inactivation may also occur by irradiation with x-rays and gamma rays. UV light also reduces auxin levels in plants. Inactivation or decomposition of IAA by light has been called as photo oxidation.

Mechanism of Action

IAA increases the plasticity of cell walls so that the cells stretch easily in response to turgor pressure. It has been suggested that IAA acts upon DNA to influence the production of mRNA. The mRNA codes for specific enzymes responsible for expansion of cell walls. Recent evidences indicate that IAA increases oxidative phosphorylation in respiration and enhanced oxygen uptake. The growth stimulation might be due to increased energy supply and it is also demonstrated that auxin induces production of ethylene in plants.

Gibberellins

Discovery

A Japanese scientist Kurosawa found that the rice seedlings infected by the fungus *Gibberella fujikuroi* grow taller and turned very thin and pale. An active substance was isolated from the infected seedlings and named as Gibberellin.

Biosynthesis of gibberellins in plants

The primary precursor for the formation of gibberellins is acetate.

Acetate + COA → Acetyl COA → Mevalonic acid → MA pyrophosphate → Isopentanyl pyrophosphate → Geranyl pyrophosphate → GGPP → Kaurene → Gibberellins.

Physiological effects of gibberellins

1. Seed germination

Certain light sensitive seeds eg. Lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of buds

In temperature regions the buds formed in autumn remain dormant until next spring due to severe cold. This dormancy of buds can be broken by gibberellin treatments. In potato also, there is a dormant period after harvest, but the application of gibberellin sprouts the refer vigorously.

3. Root growth

Gibberellins have little or no effect on root growth. At higher concentration, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of internodes

The most pronounced effect of gibberellins on the plant growth is the elongation of the internodes. Therefore in many plants such as dwarf pea, dwarf maize etc gibberellins overcome the genetic dwarfism.

5. Bolting and flowering

In many herbaceous plants, the early period of growth shows rosette habit with short stem and small leaves. Under short days, the rosette habit is retained while under long days bolting occurs i.e. the stem elongates rapidly and is converted into polar axis bearing flower primordia. This bolting can also be induced in such plants by the application of gibberellins even under non-inductive short days.

In *Hyoscyamus niger* (a long day plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in long day plants the gibberellin treatment usually results in early flowering. In short day plants, its effects are quite variable. It may either have no effect or inhibit or may activate flowering.

6. Parthenocarpy

Germination of the pollen grains is stimulated by gibberellins; likewise, the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases, eg. pome and stone fruits where auxins have failed to induce parthenocarpy, the gibberellins have proven to be successful. Seedless and fleshly tomatoes and large sized seedless grapes are produced by gibberellin treatments on commercial scale.

7. Synthesis of the enzyme α - amylase

One important function of gibberellins is to cause the synthesis of the enzyme α - amylase in the aleurone layer of the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Distribution of gibberellins in plant

Gibberellins are found in all parts of higher plants including shoots, roots, leaves, flower, petals, anthers and seeds. In general, reproductive parts contain much higher concentrations of gibberellins than the negative parts. Immature seeds are especially rich in gibberellins (10-100 mg per g fresh weight).

In plants, gibberellins occur in two forms free gibberellins and bound gibberellins. Bound gibberellins usually occur as gibberellin – glycosides.

CYTOKININS (Kinetin)

Kinetin was discovered by Skoog and Miller (1950) from the tobacco pith callus and the chemical substance was identified as 6-furfuryl aminopurine. Because of its specific effect on *cytokinesis* (cell division), it was called as cytokinins or kinetin. The term, cytokinin was proposed by Letham (1963). Fairley and Kingour (1966) used the term, *phytokinins* for cytokinins because of their plant origin. Chemically cytokinins are kinins and they are purine derivatives.

Cytokinins, besides their main effect on cell division, also regulate growth and hence they are considered as natural plant growth hormones. Some of the very important and commonly known naturally occurring cytokinins are Coconut milk factor and Zeatin. It was also identified that cytokinin as a constituent of t-RNA.

Naturally occurring cytokinins

Cytokinins can be extracted from coconut milk (liquid endosperm of coconut), tomato juice, flowers and fruits of *Pyrus malus*; fruits of *Pyrus communis* (Pear), *Prunus cerasiferae* (plum) and *Lycopersicum esculentum* (bhendi); Cambial tissues of *Pinus radiata*, *Eucalyptus regnans* and *Nicotiana tabacum*; immature fruits of *Zea mays*, *Juglans* sp. and *Musa* sp; female gametophytes of *Ginkgo biloba*; fruitlets, embryo and endosperms of *Prunus persica*; seedling of *Pisum sativum*; root exudates of *Helianthus annuus* and tumour tissues of tobacco. According to Skoog and Armstrong (1970), at least seven well established types of cytokinins have been reported from the plants.

Biosynthesis

It is assumed that cytokinins are synthesised as in the case of purines in plants (nucleic acid synthesis). Root tip is an important site of its synthesis. However, developing seeds and cambial tissues are also the site of cytokinin biosynthesis. Kende (1965) reported that cytokinins move upwards perhaps in the xylem stream. However, basipetal movement in petiole and isolated stems are also observed. Seth *et al* (1966) found that auxin enhances kinetin movement (translocation) in bean stems.

Physiological effects of cytokinins

1. Cell division

The most important biological effect of kinetin on plants is to induce cell division especially in tobacco pith callus, carrot root tissue, soybean cotyledon, pea callus etc.

2. Cell enlargement

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Significant cell enlargement has been observed in the leaves of *Phaseolus vulgaris*, pumpkin cotyledons, tobacco pith culture, cortical cells of tobacco roots etc.

3. Concentration of apical dominance

External application of cytokinin promotes the growth of lateral buds and hence counteracts the effect of apical dominance

4. Dormancy of seeds

Like gibberellins, the dormancy of certain light sensitive seeds such as lettuce and tobacco can also be broken by kinetin treatment.

5. Delay of senescence (Richmand - Lang effect)

The senescence of leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. Senescence can be postponed to several days by kinetin treatment by improving RNA synthesis followed by protein synthesis.

Richmand and Lang (1957) while working on detached leaves of *Xanthium* found that kinetin was able to postpone the senescence for a number of days.

6. Flower induction

Cytokinins can be employed successfully to induce flowering in short day plants.

7. Morphogenesis

It has been shown that high auxin and low kinetin produced only roots whereas high kinetin and low auxin could promote formation of shoot buds.

8. Accumulation and translocation of solutes

Plants accumulate solutes very actively with the help of Cytokinin and also help in solute translocation in phloem.

9. Protein synthesis

Osborne (1962) demonstrated the increased rate of protein synthesis due to translocation bys kinetin treatment.

10. Other effects

Cytokinins provide resistance to high temperature, cold and diseases in some plants. They also help in flowering by substituting the photoperiodic requirements. In some cases, they stimulate synthesis of several enzymes involved in photosynthesis.

11. Commercial applications

Cytokinins have been used for increasing shelf life of fruits, quickening of root induction and producing efficient root system, increasing yield and oil contents of oil seeds like ground nut.

Ethylene

Ethylene is the only natural plant growth hormone exists in gaseous form.

Important physiological elects

1. The main role of ethylene is it hastens the ripening of fleshy fruits eg. Banana, apples, pears, tomatoes, citrus etc.
2. It stimulates senescence and abscission of leaves
3. It is effective in inducing flowering in pine apple
4. It causes inhibition of root growth
5. It stimulates the formation of adventitious roots
6. It stimulates fading of flowers
7. It stimulates epinasty of leaves.

Abscisic acid

Addicott (1963) isolated a substance strongly antagonistic to growth from young cotton fruits and named Abscissin II. Later on this name was changed to Abscisic acid. This substance also induces dormancy of buds therefore it also named as Dormin.

Abscisic acid is a naturally occurring growth inhibitor.

Physiological effects

The two main physiological effects are

1. Geotropism in roots
2. Stomatal closing
3. Besides other effects

1. Geotropism in roots

Geotropic curvature of root is mainly due to translocation of ABA in basipetal direction towards the root tip.

2. Stomatal closing

ABA is synthesized and stored in mesophyll chloroplast. In respond to water stress, the permeability of chloroplast membrane is lost which resulted is diffusion of ABA out of chloroplast into the cytoplasm of the mesophyll cells. From mesophyll cells it diffuses into guard cells where it causes closing of stomata.

3. Other effects

i. Including bud dormancy and seed dormancy

ii. Includes tuberisation

iii. Induces senescence of leaves fruit ripening, abscission of leaves, flowers and fruits

iv. Increasing the resistance of temperate zone plants to frost injury.

Growth retardants

There is no. of synthesis compounds which prevent the gibberellins from exhibiting their usual responses in plants such as cell enlargement or stem elongation. So they are called as anti gibberellins or growth retardants. They are

1. Cycocel (2- chloroethyl trimethyl ammonium chloride (CCC)
2. Phosphon D – (2, 4 – dichlorobenzyl – tributyl phosphonium chloride)
3. AMO – 1618
4. Morphactins

5. Maleic hydrazide

21. PRACTICAL APPLICATION OF PLANT GROWTH REGULATORS IN CROP PRODUCTIVITY

Commercial uses of growth regulators

1. Rooting and plant propagation

a) Auxin compound like IBA NAA, 2,4-D, 2, 4,5-T

b) IBA produces strong fibrous root system

2. Germination and dormancy

a) Gibberellin is a potent germination promoter

b) Abscissic acid – germination inhibitor (Anti – Gibberellin)

c) Induce Dormancy - ABA

d) Breaking of dormancy - Auxins and Gibberellin

3. Fruit set and Development

a) Fruit setting → 2, 4, 5 – T

b) Fruit size increment in grapes → Gibberellic acid

c) Shelf life increment in fruits and flowers → Cytokinin

d) Good fruit shape ------ Gibberellic acid + Cytokinin

e) Parthenocarpic fruit – Gibberellins, IAA and PAA

4. Sex expression

Production of male flowers → Gibberellins (cucumber)

Production of female flowers → Auxins and Gibberellins

↓ ↓

Cucumber maize

5. Abiscission

Control of abscission → NAA and IAA

Induce Abscission → Ethrel

6. Morphogenesis

Auxin and Cytokinin

7. Weed control

2, 4-D and 2, 4, 5-T

8. Plant organ size

Increases plant height → GA

Shorten the plant height → TIBA

↓

Tri iodo benzoic acid

Increases Tillering → Cytokinin

Ex: BAP (Benzyl amino purine) and TIBA

9. Antitranspirants → ABA and PMA

↓

Phenyl mercury acetate

10. Papaya Later flow → Ethephon

11. Rubber latex flow → 2, 4 – D and 2, 4,5 – T

12. Fruit ripening → Ethrel

13. Sugarcane ripeners → Glyphosphate and CCC

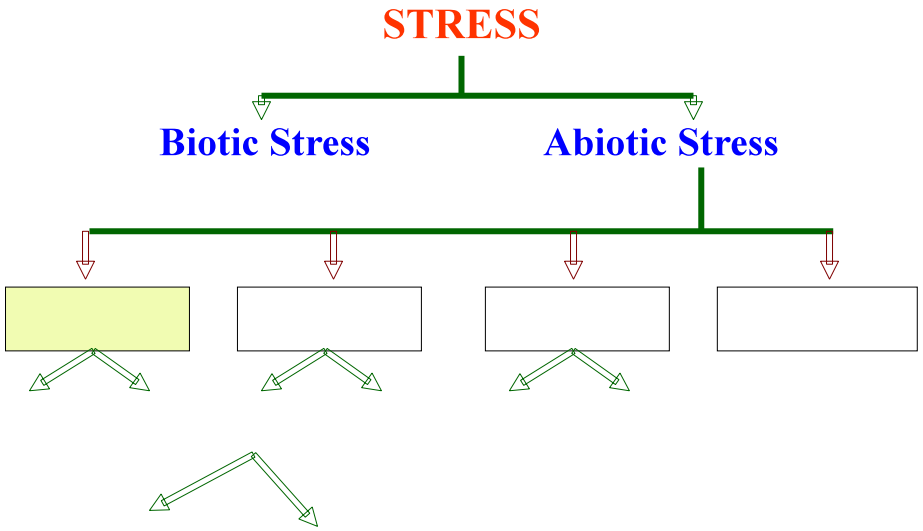
Hormone	Major Functions	Where Produced
Auxin	Stimulates cell elongation; involved in phototropism, gravitropism, apical dominance, and vascular differentiation; stimulates ethylene synthesis and induces adventitious roots on cuttings	Meristems of apical buds, embryo of seed, young leaves
Cytokinin	Stimulates cell division, reverses apical dominance, involved in shoot growth, delays leaf sequence	Synthesized in roots and transported to other organs
Ethylene	Stimulates fruit ripening, leaf and flower senescence, and abscission	Tissues of ripening fruits, nodes of stems, senescent leaves and flowers
Abscisic Acid	Inhibits growth, stimulates stomatal closure, maintains dormancy	Leaves, stems, green fruit
Gibberellin	Stimulates shoot elongation, stimulates bolting and flowering in biennials, regulates production of hydrolytic enzymes in grains	Meristems of apical buds and roots, young leaves, embryo

22. ENVIRONMENTAL STRESSES

The occurrence of unfavorable environmental factors such as moisture deficit / excess, high radiation, low and high temperature, salinity of water and soil, nutrient deficiency or toxicity and pollution of atmosphere, soil and water are likely to affect the crop growth in terms of morphology (plant size, architecture, malformation of plant organs, growth (height, volume, weight), physiological and metabolic processes and yield of crop plants.

Stress and strain

Any environmental factor potentially unfavorable to plant is termed as **stress**. The effect of stress on plant condition is called **strain**. According to Newton's law of motion, a force is always accompanied by a counterforce, for an action there is always equal and opposite reaction. Stress is the action and whereas strain is the reaction.



I. DROUGHT (Water stress)

Drought is defined as the deficiency of water severe enough to check the plant growth. Drought has been classified into two broad categories viz., soil drought and atmospheric drought. Soil drought leads to atmospheric drought. Atmospheric drought occurs due to low atmospheric humidity, high wind velocity and high temperature which cause a plant to lose most of its water.

Physiological changes occur due to drought

1. Functioning of stomata

In general, stomata lose their function and may die, because wilting after certain limit denatures the starch in the guard cells and also in the mesophyll cells.

2. Carbohydrates metabolism in green leaves

The very first effect of drought on carbohydrates metabolism is that starch disappears from the wilted leaves and sugar accumulates simultaneously.

3. Photosynthetic activity

CO_2 diffusion into the leaf is prevented due to decrease in stomatal opening and there by reduces photosynthetic activity in green cells.

4. Osmotic pressure

The reduced amount of water during drought causes an increase in the osmotic pressure of plant cell. This increase in osmotic pressure permits the plant to utilize better soil moisture.

5. Permeability

The permeability to water and urea increases during drought.

6. Biochemical effects

Water shortage alters the chemical composition. For example, starch is converted to sugar, besides this, there is a considerable increase in nitrate nitrogen and protein synthesis is adversely affected.

Adaptation to drought

Drought resistance

Drought resistance is defined as the capacity of plants to survive during the period of drought with little or no injury. There are three important categories of plants growing in the areas facing drought. They are ephemerals, succulents and non-succulent perennials

1. Ephemerals

These are short lived plants and they complete their life cycle within a short favourable period during rainy season. They pass dry periods in the form of seeds. They are called as *drought escaping plants*.

2. Succulent plants

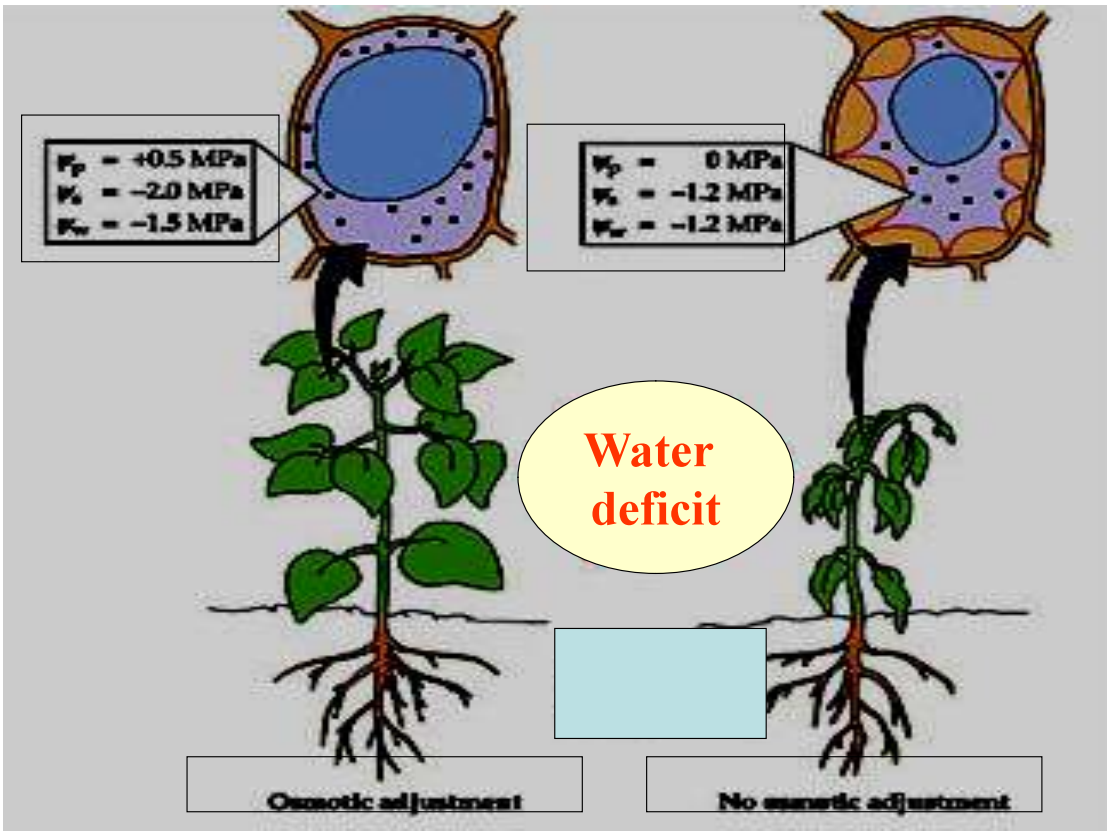
These plants accumulate large quantities of water and use it slowly during dry period. Thus, they pass dry periods or drought without facing it. Such plants develop several morphological adaptations for reducing transpiration such as thick cuticle, reduced leaf area, sunken stomata etc.

3. Non succulent plants

These plants are in fact the real drought enduring (tolerant) plants. They tolerate drought without adapting any mechanism to ensure continuous supply of water. They develop many morphological adaptations which are collectively called *xeromorphy*. They develop, in general, greyish colour, reflecting surfaces, smaller leaves, extensive root system, leaf fall during dry season, sunken stomata and thick cuticle etc. They develop an elaborated conducting system. The stomata remain closed mostly in dry periods.

The plants develop several protoplasmic peculiarities such as cell size, cell structure, increased permeability, increased imbibition power, elasticity, small vacuoles, higher osmotic pressure etc.

Osmotic adjustment



Methods to overcome drought

- Selection of drought tolerant species
- Adjusting the tome of sowing in such a way that the crop completes its lifecycle before the onset of drought
- Seed hardening with KCl, KH_2PO_4, $CaCl_2$ or Thiourea
- Thinning of poorly established plants
- Mulching to minimize the evaporative loss
- Foliar spray of antitranspirants such as Kaolin, PMA, Waxes and Silicone oils
- Foliar spray of KCl
- Foliar spray of growth retardants such as CCC and MC

HIGH MOISTURE STRESS - FLOODING / WATER LOGGING

Water logging refers to a condition when water is present in excess amount than its optimum requirement. It creates an anaerobic situation in the rhizosphere due to which the plant experiences the stress (O_2 deficient stress).

Nature of Water logging Stress

In the water logged soils, water gets filled in the pores of the soil which are previously occupied by O_2. Such soils suffer O_2 deficiency.

This O_2 deficiency depresses growth and survival of plants growing in it.

Flood sensitive plants (eg. Tomato, soybean and sunflower) are killed in the water logged conditions, while the tolerant species (eg. Rice) withstand water logging for a considerable time. However, continuous submergence of rice for more than 10 days is also deleterious resulting in death and decay of the plants.

Plant Water Relations in Flooding Stress

The flooding often induces stomatal closure mostly in C_3 plants. This causes lower water flow in these plants. This also results in leaf dehydration because of reduced root permeability. Ultimately, wilting of leaves occurs due top the restricted water flow from the roots to the shoots.

Occurrence of these changes in leaves, shoots or roots is due to the transfer of toxic substances (acetaldehyde / alcohol) produced under anaerobic conditions in the roots as well as the levels of PGRs transported from the roots to shoots via transpiration stream.

Levels of Endogenous PGRs under Flooding Stress

Endogenous levels of PGRs such as GA and cytokinins (CK) are reduced in the roots. This has enhanced levels of ABA and ethylene in the shoots causing stomatal closure and early onset of senescence respectively.

It is also reported that levels of auxins are reduced and that of Aminocyclopropane -1-Carboxylic Acid (ACC), precursor for the ethylene biosynthesis are increased under flooding stress.

Important roles played by these endogenous PGRs during high moisture (flooding) stress are summarized in the following table.

Effect of flooding stress on the endogenous levels of PGRs and their effect on plants

Sl. No.	Level of PGR in plants	Effects on plants under water logging
01.	Reduced Auxins	Causes "Hypertrophy" (Swelling of stem base by collapse or enlargement of cells in cortex)
02.	Decreased GA	Causes reduction in cell enlargement and stem elongation
03.	Decreased CK	Results in early on-set of senescence and reduced rate of assimilate partitioning to the sinks
04.	Increased ABA	Cause stomatal closure with consequential decrease in the rate of gas exchanges during photosynthesis, respiration and transpiration; results in efflux of K+ from the guard cells; decreases ion transport due to lower rate of transpiration; decrease the starch formation in the guard cells resulting in stomatal closure
05.	Increased Ethylene	Causes "Epinasty" of leaves (uneven growth of leaves due to more cell elongation on upper side than the lower side of the leaf); induces senescence and Hypertrophy in plants.

Thus, the O_2 stress in the roots under flooding produces signals, via transpiration stream, to the leaves affecting stomatal behaviour ultimately.

Mitigation of High Moisture (Water logging) Stress

1. Providing adequate drainage for draining excessive stagnating water around the root system.
2. Spray of growth retardant of 500 ppm cycocel for arresting apical dominance and thereby promoting growth of laterals
3. Foliar spray of 2% DAP + 1% KCl (MOP)
4. Nipping terminal buds for arresting apical dominance and thus promoting growth sympodial branches (as in cotton) for increasing productivity
5. Spray of 40 ppm NAA for controlling excessive pre-mature fall of flowering/buds/young developing fruits and pods
6. Spray of 0.5 ppm brassinolide for increasing photosynthetic activity

7. Foliar spray of 100 ppm salicylic acid for increasing stem reserve utilization under high moisture stress
8. Foliar spray of 0.3 % Boric acid + 0.5 % $ZnSO_4$ + 0.5 % $FeSO_4$ + 1.0 % urea during critical stages of the stress.

SALT STRESS

Salt stress occurs due to excess salt accumulation in the soil. As a result, water potential of soil solution decreases and therefore exosmosis occurs. This leads to physiological drought causing wilting of plants.

Classification of saline soil: 1. Saline soil 2. Alkaline soil

1. Saline soil

In saline soils, the electrical conductivity is greater than 4 dS/m, exchangeable sodium percentage is less than 15% and pH is less than 8.5. These soils are dominated by Cl^- and SO^{2-}_4 ions.

2. Alkaline soil

Alkaline soils are also termed as sodic soils wherein, the electrical conductivity is less than 4 dS/m, exchangeable sodium percentage is greater than 15% and pH of the soil is greater than 8.5. These soils are dominated by CO^-_3 and HCO^-_3 ions.

Classification of plants

Plants are classified into two types based on the tolerance to salt stress. They are halophytes and glycophytes.

1. Halophytes

Halophytes are the plants that grow under high salt concentrations. They are again divided into two types based on extreme of tolerance.

Euhalophytes: can tolerate extreme salt stress

Oligohalophytes: can tolerate moderate salt stress

2. Glycophytes

Glycophytes are the plants that cannot grow under high salt concentration.

Effect of salt stress on plant growth and yield

1. Seed germination

Salt stress delays seed germination due to the reduced activity of the enzyme, α-amylase

2. Seedling growth

The early seedling growth is more sensitive. There is a significant reduction in root emergence, root growth and root length.

3. Vegetative growth

When plants attain vegetative stage, salt injury is more severe only at high temperature and low humidity. Because under these conditions, the transpiration rate will be very high as a result uptake of salt is also high.

4. Reproductive stage

Salinity affects panicle initiation, spikelet formation, fertilization and pollen grain germination.

5. Photosynthesis

Salinity drastically declines photosynthetic process. Thylakoid are damaged by high concentration of salt and chlorophyll *b* content is drastically reduced.

Mechanism of salt tolerance

1. Some plants are able to maintain high water potential by reducing the transpiration rate.
2. Salts are accumulated in stem and older leaves in which metabolic processes take place in a slower rate.
3. Na^+ (sodium ion) toxicity is avoided by accumulating high amount of K^+ ions.
4. Accumulation of toxic ions in the vacuole but not in the cytoplasm.
5. Accumulation of proline and abscissic acid which are associated with tolerance of the plants to salt.

Relative salt tolerant crops

Tolerant crops: Cotton, sugar cane, barley

Semi tolerant crops: Rice, maize, wheat, oats, sunflower, soybean

Sensitive crops: Cow pea, beans, groundnut and grams

Mitigation of Salt Stress

1. Seed hardening with NaCl (10 mM concentration)
2. Application of gypsum @ 50% Gypsum Requirement (GR)
3. Incorporation of daincha (6.25 t/ha) in soil before planting
4. Foliar spray of 0.5 ppm brassinolode for increasing photosynthetic activity
5. Foliar spray of 2% DAP + 1% KCl (MOP) during critical stages
6. Spray of 100 ppm salicylic acid
7. Spray of 40 ppm of NAA for arresting pre-mature fall of flowers / buds / fruits
8. Extra dose of nitrogen (25%) in excess of the recommended
9. Split application of N and K fertilizers
10. Seed treatment + soil application + foliar spray of Pink Pigmented Facultative Methnaotrops (PPFM) @ 10^6 as a source of cytokinins.

TEMPERATURE STRESS

Temperature stress includes both high temperature stress and low temperature stress. Low temperature stress causes chilling injury and freezing injury.

Low temperature stress

1. Chilling injury

The tropical origin plants are injured when the temperature drops to some point close to 0°C. The injury which occurs due to low temperature but above zero degree centigrade is called chilling injury.

2. Freezing injury

Freezing injury occurs when the temperature is 0°C or below.

Effect of freezing and chilling injury plants

- The lipid molecules in cell membrane get solidified i.e. changed from liquid state to solid state. Hence, the semi-permeable nature of the membrane is changed and the membrane becomes leaky.
- Inactivation of mitochondria
- Streaming of protoplasm is stopped
- Accumulation of respiratory metabolites which become highly toxic
- Ice formation inside the cell occurs.

Prevention of cold injury

- Some plants change the pattern of growth.
- The growth is completely arrested during this period.
- In cell membrane, unsaturated fatty acid content is increased.
- Intracellular ice formation is reduced.
- The quantity of free enzymes, sugars and proteins increases.

High temperature stress

The effect of high temperature is heat Injury. Heat Injury occurs when plant temperature is higher than that of environment (exceeds 35°C).

General effects of high temperature

High temperature affects

1. Seedling growth and vigour
2. Water and nutrient uptake
3. Solute transport
4. Photosynthesis and respiration
5. General metabolic processes
6. Fertilization and maturation

Cellular Changes during heat stress

When plants are exposed to temperatures higher than 45^0C it experiences heat stress. The cellular changes due to heat stress are

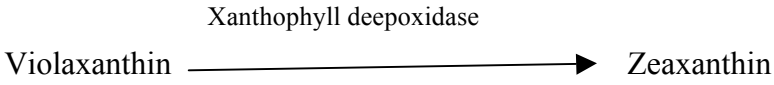
1. Disruption of cytoskeleton and microtubules.
2. Fragmentation of golgi complex
3. Increase in number of lysosomes
4. Swelling of mitochondria thereby resulting in decreased respiration and oxidative phosphorylation
5. Disruption of normal protein synthesis
6. Disappearance of polysomes
7. Disruption of splicing of mRNA precursors
8. Cessation of pre-RNA processing
9. Decline in transcription by RNA polymerase I

10. Inhibition of chromatin assembly
11. Decline in DNA synthesis

Acclimation to high temperature

Morphological Adaptations

- Reflective leaf hair
- Leaf waxes
- Leaf orientation
- Maximize conductive or convective loss of heat



Zeaxanthin decreases membrane fluidity and stabilises the membrane

Heat Shock Proteins (HSPs)

Plants have the capacity to interact with the environment in many different ways and to survive under extreme abiotic and perhaps also biotic stress conditions. The response to heat stress (hs) is highly conserved in organisms but owing to the sessile life style it is of utmost importance to plants. The hs-response is characterised by (i) a transient alteration of gene expression (synthesis of heat shock proteins: HSP) and (ii) by the acquisition of a higher level of stress tolerance (acclimation). The induction of HSP-expression is not restricted to high temperature stress, HSPs are also linked to a number of other abiotic stresses including cold, freezing, drought, dehydration, heavy metal, and oxidative stresses. HSP are molecular chaperones, which either prevent complete denaturation (small HSP: sHSP) or are supporting proper folding (other HSP) of enzymes under or after protein denaturing conditions. Manipulation of the hs-response has the potential to improve common stress tolerance that may lead to a more efficient exploitation of the inherent genetic potential of agriculturally important plants.

HSPs are classified into different families and designated by molecular weight in kDa.

- HSP 100 k Da

- HSP 90
- HSP 70
- HSP 60
- 15 – 30 kDa low molecular mass HSPs or Small HSPs.

Functions

- HSPs 60, 70 and 90 : act as molecular chaperons, involving ATP dependent stabilization and folding of proteins and assembly of oligomeric proteins.
- Some HSPs : assist in polypeptide transport across membranes into cellular compartments.
- Some HSPS : temporarily bind and stabilize an enzyme at a particular stage in cell development, later releasing the enzyme to become active.
- Binding of HSP with particular polypeptide within subcellular compartment avoid denaturation of many proteins at high temperatures.

Mitigation of Low Temperature Stress

1. Seed hardening with 0.01% Ammonium molybdate and foliar spray of 0.1 % ammonium molybdate at critical stages of stress
2. Foliar spray of 2% calcium nitrate spray for membrane integrity
3. Foliar spray of 2% DAP + 1% KCl (MOP)
4. Foliar spray of 500 ppm cycocel for increasing root penetration in search of moisture for alleviation
5. Spray of 100 ppm salicylic acid
6. Brassinolide (0.5 ppm) for enhancing photosynthetic activity of plants
7. Seed treatment + soil application + foliar spray of Pink Pigmented Facultative Methnaotrops (PPFM) @ 10^6 as a source of cytokinins.

Mitigation of High Temperature Stress

1. Seed hardening with 0.5% $CaCl_2$ solution for arresting membrane damage due to high temperature stress
2. Split application of N and K fertilizers

3. Foliar spray of 2% DAP + 1% KCl (MOP) (during the spray, sufficient moisture should be present in the soil for avoiding leaf scorching)
4. Folar spray of 3% Kaoline
5. Foliar spray of 0.5% zinc sulphate + 0.3 % boric acid + 0.5 % Ferrous sulphate + 1% urea
6. Spray of 40 ppm NAA for controlling pre-mature flower / fruit drops due to high temperature stress
7. Foliar spray of 1% Urea + 2 % $MgSO_4$ + 0.5 % $ZnSO_4$ (for arresting chlorophyll degradation due to high temperature stress)
8. Foliar spray of 2% calcium nitrate spray for membrane integrity
9. Foliar spray of 0.5 ppm Brassinolide for increasing photosynthetic activity during stress
10. Spray of 100 ppm salicylic acid for increase stem reserve utilization and increasing Harvest Index of crops under stress
11. Seed treatment + soil application + foliar spray of Pink Pigmented Facultative Methnaotrops (PPFM) @ 10^6 as a source of cytokinins.

Low light and UV radiation stresses

Low Light Stress

In some places (e.g. Thanjavur), the light intensity might be even up to 60000 lux in the first season but it would be low up to 30000 lux in the second season causing very poor productivity. Light quality is also very poor by showing about 400-440nm instead of the normal 600-640nm. The abnormal light intensity and quality causes reduced yield in any crops.

UV-RADIATION STRESS

UV radiation is divided into three categories

1. UV A – wavelength ranges from 320 to 400 nm and this is less lethal to the plants.
2. UV B – wavelength ranges from 280 to 320 nm and this is lethal to the plants.

3. UV C – wavelength is less than 280 nm and it is highly lethal to all biological systems.

The UV radiations cause environmental stress as the cell constituents like proteins and nucleic acids absorb UV radiation in the range of 250-400 nm (UV A and UV B) and cause death of the tissues. In general, on the outer atmosphere of the earth, CO_2, ozone and water vapour form a layer and this layer prevent the entry of UV radiation. However, ozone depletion causes easy entry of UV radiation. In general, 1% reduction in ozone (O_3) causes 2% increase in UV radiation.

UV radiation and plant response

1. UV radiation slows down the growth of plants
2. Damage the process of photosynthesis
3. Prevent maturation and ripening process
4. Accelerate genetic mutation.

Mitigation of Low Light Stress:

1. Foliar spray of 2% DAP + 1% KCl (MOP)
2. Spray of 2% coconut water
3. Spray of 40 ppm of NAA
4. 0.5 ppm Brassinolide spray
5. Spray of 100 ppm Salicylic acid
6. Spray of 500 ppm CC for arresting excessive vegetative growth
7. Split application of N and K fertilizers
8. Foliar spray of 0.3 % Boric acid + 0.5 % Zinc sulphate
9. Seed treatment + soil application + foliar spray of Pink Pigmented Facultative Methanotrophs (PPFM) @ 10^6 as a source of cytokinins.

23. SEED GERMINATION

The process of seed germination starts with the imbibition of water by seed coat and emergence of growing root tip of embryo. The process ends with the development of embryo into a seedling.

Physiological and biochemical changes during seed germination

1. Water uptake

Seed germination starts with the imbibition of water by dry seed coat. Due to imbibition of water, the seed coats become 1) More permeable to O_2 and water and 2) less resistant to outward growth of embryo.

2. Respiration

Rapid increase in respiration rate of embryo occurs. Sucrose is probably the respiratory substrate at this stage which is provided by endosperm.

3. Mobilization of reserve materials

As germination progresses, there is mobilization of reserve materials to provide.

1. building blocks for the development of embryo
2. energy for the biosynthetic process and
3. nucleic acids for control of protein synthesis and embryonic development

Changes in these components are as follows

i) Nucleic acids

In monocots, during imbibition, there is a rapid decrease of DNA and RNA content in the endosperm with a simultaneous increase in the embryonic axis probably due to their transportation as such. High concentration of RNA in the embryonic axis precedes cell division. Due to more cell division, DNA content is increased.

ii) Carbohydrates

Insoluble carbohydrates like starch are the important reserve food of cereals in the endosperm. During germination, starch is hydrolyzed first into maltose in the presence of α-

amylase and β - amylase and then maltose is converted into glucose by maltase. The glucose is further converted into soluble sucrose and transported to growing embryonic axis. During germination, the embryonic axis secretes gibberellic acid into the aleurone layer which causes synthesis of α-amylase.

3. Lipids

Plants like castor bean, peanut etc., store large amount of neutral lipids or fats as reserve food in their seeds. During germination, the fats are hydrolyzed into fatty acids and glycerol by lipase enzyme. Fatty acids are further converted into acetyl – CoA by the process, β - oxidation. The acetyl CoA is further converted into sucrose via glyoxylate cycle and is transported to the growing embryonic axis.

4. Proteins

Some plants store proteins as reserve food in their seeds in the form of aleurone grains. Proteins are hydrolyzed into amino acids by peptidase enzyme. The amino acids may either provide energy by oxidation after deamination (removal of amino group) or may be utilized in the synthesis of new proteins.

5. Inorganic materials

A number of inorganic materials such as phosphate, calcium, magnesium and potassium are also stored in seeds in the form of phytin. These stored materials are liberated during germination due to the activity of various phosphatases including phytase.

Emergence of seedling out of the seed coat

All the changes described above gradually result in splitting of seed coat and emergence of the growing seedling. The radical comes out first and grows downward, and then plumule comes out and grows upward. Due to the continued growth of this seedling, the plumule comes out of the soil, exposed to light and develops its own photosynthetic apparatus.

Splitting of seed coat may take place either by imbibition pressure or by internal pressure created by the growing primary root or by hydrolytic enzymes which act on cell

wall contents of seed coat and digest it (e.g. cellulose and pectinase). Sometimes the seed coat may be extensively rotted by the activity of micro-organisms in the soil.

DORMANCY OF SEEDS

All the viable seeds have capacity to germinate if placed under suitable conditions necessary for germination. But, some seeds fail to germinate sometimes even if placed under the condition favourable for germination. This may be due to some internal factors or due to specific requirement for some environmental factors. During this period, the growth of the seed remains suspended and they are said to be in rest stage or dormant stage and this phenomenon is called as dormancy of seeds.

Factors causing dormancy of seeds

1. Seed coats impermeable to water

The seeds of certain plants especially those belonging to the family's leguminaceae, solanaceae, malvaceae, etc. have very hard seed coats which are impermeable to water. The seeds remain dormant until the impermeable layer decay by the action of soil micro-organisms.

2. Seeds coats impermeable to oxygen

In many plants such as cocklebur and many grasses, the seed dormancy is due to the impermeability of the seed coat to oxygen. However, during the period of dormancy, the seed coat gradually becomes more permeable to oxygen so that they may germinate.

3. Immaturity of the Embryo

In certain orchids, the seed dormancy is due to the immaturity of the embryos which fail to develop fully by the time the seeds are shed. In such cases, the seeds germinate only after a period or rest during which the development of embryo inside the seed is completed.

4. Germination Inhibitors

In certain seeds, the dormancy of the seeds is due to the presence of certain germination inhibitors like coumarin, ferulic acid, abscissic acid, etc. These may be present in endosperm, embryo, testa or juice or pulp of fruit.

5. Chilling or low temperature requirement

In certain plants such as apple, rose, peach etc, the seeds remain dormant after harvest in the autumn as they have a low temperature or chilling requirement for germination. In nature, this requirement is fulfilled by the winter temperatures. In such case the seeds remain dormant throughout the winter season and germinate only in the following spring.

6. Light sensitive seeds

In many species, the germination of the seeds is affected by light resulting in seed dormancy. Such light sensitive seeds are called *photo blastic*. Seeds of lettuce, tomato and tobacco are positively photo blastic and germinate only after they have been exposed to light. On the other hand, the seeds of certain plants are negatively photo blastic and their germination is inhibited by light.

Advantages of dormancy

1. In temperature zones, the dormancy of seeds helps the plants to tide over the severe colds which may be injurious for their vegetative and reproductive growth.
2. In tropical regions, the dormancy of seeds resulting from their impermeable seed coats ensures good chances of survival.
3. Dormancy of seeds in many cereals is of utmost importance to mankind. If these seeds germinate immediately after harvest in the field, they will become useless to man for consumption as food.

24. ABSCISSION AND SENESCENCE

Like human beings, plants a1so grow old and undergo aging and then they die. *Aging is the sum total of changes in the total plant or its organs.* During aging, the plants undergo chemical and structural changes. Aging leads to senescence and later phase of development that u1timately terminates to death.

Senescence

The deteriorative process which naturally terminates the functional life of an organ, organism or other life unit is collectively called senescence. Senescence is a phase of the *aging process.* The major characteristic of senescence is that the metabolic processes are catabolic and eventually become irreversible and terminate to death.

Senescence is not confined only to whole plant. It may be limited to a particular plant organ such as leaf and flowers or cells or cell, organelles. Senescence is closely associated with the phenomenon of aging. Aging leads to senescence. Wheat plant dies after the development of fruit. This is the senescence of an entire plant. *Leaf fall* in a coconut tree is an example of senescence.

Types of senescence

Leopold (1961) has proposed types of senescence patterns in plants which are as follows.

(a) Overall Senescence

This type of senescence occurs in annuals where whole plant is affected. It is also called *whole plant senescence.* The entire plant dies after the development of fruit and seeds. E.g. Paddy, wheat, soybean etc.

(b) Top Senescence

In top senescence, the parts remaining above the ground or (shoot system) may die, but the root system and underground system remain viable. It is also called *shoot senescence.* E.g. Dock, perennial herbs.

(c) Deciduous Senescence

In deciduous woody plants, all the leaves die but the bulk of the stem and root system

remains viable. It is called *deciduous senescence* or *simultaneous* or *synchronous senescence.* E.g. Leaf fall in deciduous trees.

(d) Progressive Senescence

It is a gradual death of old leaves from the base to the top of the plants. It may occur at any time. It is also called *sequential senescence.* E.g. Leaf fall in a coconut tree.

Causes of Senescence

1. Leaf senescence is accompanied by early loss in *chlorophyll,* RNA and enzymes.
2. Cellular constituents are decreased due to slower synthesis or faster break down.
3. Competition between vegetative and reproductive organs for nutrients.
4. A senescence factor (a hormone) is produced in soybean fruits that move to leaves where it causes senescence.
5. Short-day and long-night conditions induce flowering and leaf senescence.
6. Degradation of food reserves and loss of integrity in food storage cells of seeds.
7. Senescence is also hormonally controlled.

Physiology of Senescence

The following physiological changes occur during senescence.

1. *Photosynthesis* stops.
2. *Chlorophyll* degradation: The colour of leaf changes from green to yellow.
3. *Anthocyanin* pigments accumulation in the leaves causing reddening in leaves.
4. The vacuoles function as *lysosomes* and digest the cellular materials.
5. The starch content decreased.
6. RNA and proteins are decreased.
7. DNA molecules are degraded by the enzyme DNase.
8. Growth promoting hormones such as cytokinin decrease.
9. The deteriorative hormones such as *ethylene* and *abscisic acid* (ABA) content are increased.

Senescence Promoters

Senescence is promoted by hormones such as abscisic acid and ethylene. The

senescence accelerating ability of abscisic acid is well documented. The function of *ABA as a promoter of flower tissue senescence including initiaton of colour fading or blueing has been established.* The ABA content of aging leaves increases markedly as senescence is initiated. *Ethylene* plays a very important role in the senescence of certain plant parts, particularly fruit and petals and in the abscission process. It is an inducer in the senescence of flower tissue. Senescence Retardants: The primary plant hormones involved here are auxin, gibberellin and cytokinin.

Significance of Senescence

1. The whole plant senescence occurs in monocarpic plants coinciding the seed setting and seed dispersal.
2. Due to the formation of abscission layer, the older leaves tend to fall down so that the nutrients will be diverted to the next young leaf.
3. The senescence process helps the mobilization of nutrients and of the vegetative parts of the plant into the fruits.
4. Plants escape the influence of seasonal adversity by undergoing senescence of its organs. Leaf fall in deciduous trees reduces the rate of transpiration to survive under adverse conditions.

Abscission

Shedding of leaves, flowers and fruits is called abscission. Abscission is distinct in deciduous trees and shrubs. In autumn, all the leaves of deciduous plants fall, at about the same time giving the plants a naked appearance. In evergreen plants there is gradual abscission of leaves. The older leaves fall while new leaves are developed continuously throughout the year. In most of the herbaceous species, however the leaves are not shed even after they die. In many cases leaves are retained in withered dry condition even after the whole shoot is dead.

Abscission is a complex physiological process. During abscission, the colour of the leaves, flowers and fruits changes due to degradation of chlorophyll and the synthesis of *anthocyanin* pigment.

Leaf abscission takes place at the base of the petiole. The site of abscission is internally marked by a distinct zone called *abscission zone.* This zone is made up of one or more layers of cells arranged transversely across the petiole base. This is called *abscission layer.* The

abscission zone is pale or brown in colour. The cells of the abscission layer separate from each other due to the dissolution of middle lamellae and the primary cellulose walls under the influence of the activity of enzymes, *pectinase* and *cellulase.*

At this stage, the petiole remains attached to the stem by vascular elements only. But due to its own weight and the wind force, the leaf is detached from the stem. The broken vascular elements are soon plugged with *tyloses* or gums. Wound healing in cells proximal to the breaking point involves formation of a corky layer that protects the plant from pathogen invasion and excess water loss. *Suberin* and *lignin* are synthesized during healing.

Several environmental factors such as drought and N deficiency promote abscission. *Auxin* is synthesized in growing leaf blades and it strongly retards senescence and abscission. Abscission starts when the amount of auxin begins to decrease. Cytokinins and gibberellins arriving from the roots also delay senescence and abscission. Abscission is caused by the formation of cell wall degrading enzymes in the abscission zone, due to ethylene production.

Significance of Abscission

1. It helps in diverting water and nutrients to the young leaves
2. It is a self pruning process through which fruits and injured organs are shed from the parent plant.
3. It helps in disseminating fruits and vegetative propagates.
4. Abscission serves as function in removing plant parts containing waste materials.

25. GLOBAL WARMING - PHYSIOLOGICAL EFFECTS ON CROP PRODUCTIVITY

GLOBAL WARMING (Green house Effect)

In general, delicate plants which require protection from weather are grown in green house (glass house). In green house so many gases are produced like CO_2, water vapour, methane, oxides of nitrogen and chloro fluoro carbon (CFC). These gases are produced from plants and accumulated inside the glass house; as a result glass house gets warming. In natural atmosphere also the same effect occurs i.e. global warming (due to the release of gases from plants).

But in glass house, glass roof is present to prevent the escape of gases from the glass house. In natural atmosphere, the gases such as ozone, water vapour, CO_2 methane etc. form a layer on the lower atmosphere and this layer prevents the heat escaping from the earth. If heat is released or escaped from earth, the temperature of earth would be below freezing point. The accumulation of heat or gases causes the warming of earth surface and leads to global warming.

Global warming leads to the following effects:

1. Rise in temperature
2. Average rise in the level of sea (about 6 cm/decade) due to melting of polar ice.
3. Steady increase (enrichment) in the CO_2.

Atmosphere – (5.1×10^{18}kg)
Lithosphere - (1.5×10^{22} kg) are in a dynamic equilibrium
Hydrosphere – 1.4×10^{22} kg)
Biosphere – 1.2×10^{15} kg (dry wt.)

CO_2 fixation by green plants

Total area of green plants 510×10^6 km^2

CO_2 fixed – 1.39×10^{14} kg $year^{-1}$ (0.27 kg m^{-2} $year^{-1}$)

Light utilization:

A small portion of radiant energy (400 – 700 nm) is reaching the earth's surface

0.1 – 1.0 % utilized under natural vegetation or ordinary agriculture

2.0 – 2.5 % under intensive agriculture

6.0 – 10.0 % in some crop plants

20.0 – 25.0 % under laboratory condition

Biosphere:

O_2 in atmosphere - 1.1×10^{18} kg

O_2 released by photosynthesis - 5.1×10^{14} kg $year^{-1}$

CO_2 content - 5×10^{16} kg (mostly dissolved in sea)

CO_2 consumed by photosynthesis - 7×10^{14} kg $year^{-1}$

Human activities disturbed the global ecosystem (MST- mesospheric, Stratospheric, Troposphere)

Landscape modification

Resource exploitation

Effluent flow

High temperature

Rainfall redistribution

Increased UV-B radiation due to stratospheric O_3 depletion

Increased level of atmospheric CO_2

Other green house gases

Transparent to incoming short wave radiation

Absorb short wave and emit long wave radiation

Net emission of CO_2

Bacterial fermentation in the anaerobic rice fields generate 120 million ton CH_4 every year

Ruminant gut bacteria produce 78 m tons of CH_4 every year and are released by Flatulence

Green house gases

CO_2, CH_4, NO, NO_2, N_2O, (N_xO_x), $CFCl_3$, CF_2Cl_2, CFMs, O_3, H_2O

Fossil fuel reserves are large enough for climatic changes to occur, if these

reserves continue to be exploited at a higher rate in future

CO_2 enrichment and crop productivity

1. CO_2 enrichment leads to increased photosynthesis and productivity
2. CO_2 enrichment also decreases stomatal conductance by closing the stomata, thee by decrease the transpiration / unit area of the leaf.
3. In C_3 plant the efficiency of RuBP carboxylase enzyme is increased
4. Increased CO_2 concentration inhibits photorespiration in plants
5. CO_2 enrichment increased the yield and yield components.

Other green house gases

1. Oxides of nitrogen (NO, NO_2, N_2O molecular N_2) cause phototoxic, bleaching and necrosis (drying of tissues) in plants.
2. Ozone (O_3) causes ozone injury to the plants.

Remedial measures for green house effect

- Reduction in the use of fossil fuel
- Use of alternative sources of energy (renewable energy)
- Afforestation and community forestry
- Avoiding the use of CFCs and nuclear explosions
- Environmental awareness

Direct effect of CO_2 increase in the absence of climatic change

- Doubling of CO_2 from 340 to 680 ppm increases 0 – 10 % increase in yield of C_4 plants(maize, sorghum, sugarcane) and 10 – 50 % increase in yield in C3 plants (wheat, soybean, rice) depending upon specific crop and growing conditions
- Greater yield benefit accrue to the regions where the C_3 rather than C_4 crops dominate
- Higher CO_2 conc. reduces stomatal aperture, thereby reducing transpiration and WUE
- Doubling of CO_2 will cause about 40 % decrease in stomatal conductance in short term

Law of limiting factor:

- When other environmental factors such as water, light, minerals & temperature limit yield, then higher conc. of CO_2 will have little or no effect.
- This generalizing concept has been challenged
- In certain stressful environments, the relative photosynthesis increased with increased in CO_2 conc.

C_3 – 95 % of world's biomass is of the C_3 category

- In C_3, O_2 compete with CO_2 for the site of Rubisco – In C_4, O_2 is not compete with CO_2 for the site of PEPCase
- At 340 ppm CO_2, in the absence of O_2 Rubisco operating only at ½ to ¾ of its substrate – saturated capacity
- PEPCase has high affinity for CO_2 than Rubisco – PEPCase is close to CO_2 saturation at the present atmospheric CO_2 conc., no significant enhance of C_4 crop growth from increased CO_2 so far as PEP Case is concerned

Carbon Sequestration

Climate change is one of the most important global environmental challenges, with its implications on food production, water supply, healthy energy etc… are detrimental. Historically the responsibility of green house gas emission increase lies largely with the industrialized world and the developing countries are contributing very less amount only. (Jayant & Santhaye, 2006)

The increase in atmospheric concentration of CO_2 by 31% since 1950 from fossil fuel consumption and land use change indicates the threats of global warming since industrial revolution increases the global emission of carbon, estimated at 270 ±30 billion ton emission due to land use change include those by deforestation, due to agriculture and land use changes contributes 78 ± 12 billion ton of carbon to the atmosphere. Well planned management practices enhances bio mass production, purifies surface and ground waters and reduces the rate of enrichment of atmospheric CO_2 due to fossil fuel (Lal, 2004)

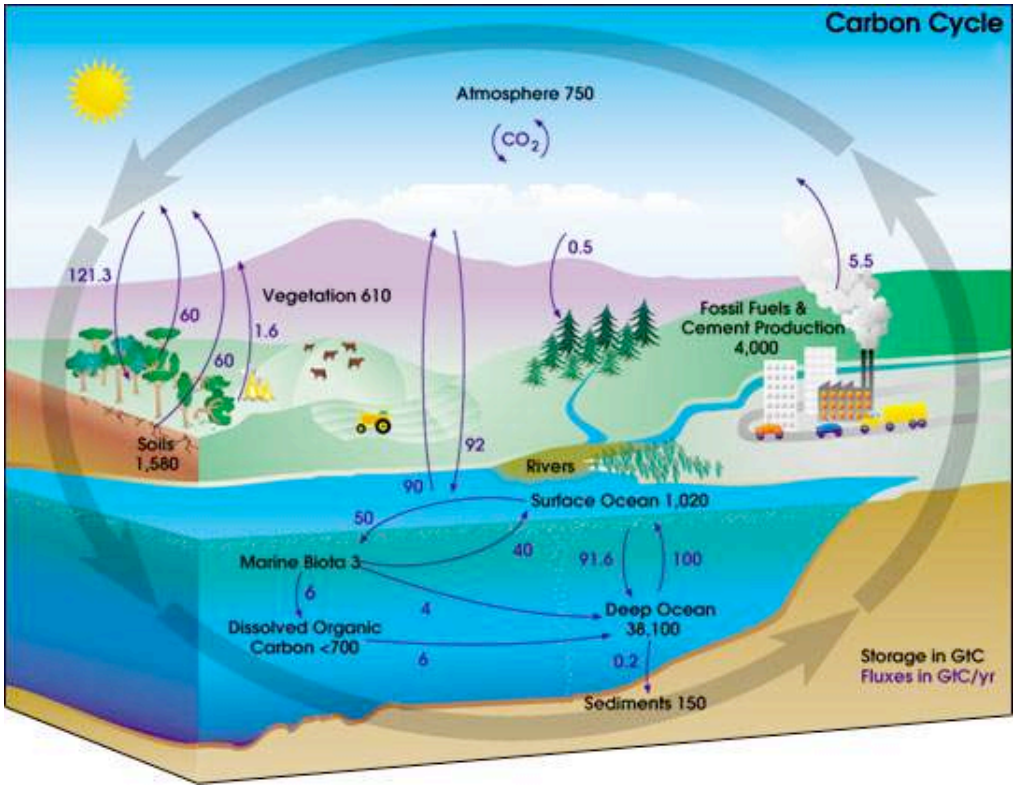
The Kyoto protocol is a positive first step to prevent climatic change at global level and the responsibility going to each and every human being (Ravindranath, 2006).

The annual soil organic carbon (SOC) enrichment of atmospheric CO_2 has to be reduced. The annual SOC sequestration potential is only 0.9 ±0.3 Pg C/year. The CO_2 level

in atmosphere increase at the rate of 2.0-2.6 Pg C / year. So soil carbon sequestration methodologies plays significant role in reducing atmospheric CO_2 control (Lal, 2004)

Besides the artificial CO_2 capture and storage methods also play a significant role to cut down the increasing atmospheric CO_2 concentration. (IPCC, 2001)

Even though all the industrial and other carbon emissions cannot be controlled by soil carbon sequestration, it may be a viable method until a permanent solution found out.



A **carbon dioxide (CO_2) sink** is a carbon dioxide reservoir that is increasing in size, and is the opposite of a carbon dioxide "source". The main natural sinks are (1) the oceans and (2) plants and other organisms that use photosynthesis to remove carbon from the atmosphere by incorporating it into biomass and release oxygen into the atmosphere. The process by which carbon dioxide sinks (natural and artificial) remove CO_2 from the atmosphere is known as carbon sequestration.

Storage in vegetation and soils

Carbon stored in soils oxidizes rapidly; this, in addition to high rainfall levels, is the reason why tropical jungles have very thin organic soils. The forest eco-system may eventually become carbon neutral. Forest fires release absorbed carbon back into the atmosphere, as does deforestation due to rapidly increased oxidation of soil organic matter.

The dead trees, plants, and moss in peat bogs undergo slow anaerobic decomposition below the surface of the bog. This process is slow enough that in many cases the bog grows rapidly and fixes more carbon from the atmosphere than is released. Over time, the peat grows deeper. Peat bogs inter approximately one-quarter of the carbon stored in land plants and soils.

Under some conditions, forests and peat bogs may become sources of CO_2, such as when a forest is flooded by the construction of a hydroelectric dam. Unless the forests and peat are harvested before flooding, the rotting vegetation is a source of CO_2 and methane comparable in magnitude to the amount of carbon released by a fossil-fuel powered plant of equivalent power.

Oceans

Oceans are natural CO_2 sinks, and represent the largest active carbon sink on Earth. This role as a sink for CO_2 is driven by two processes, the solubility pump and the biological pump.[6] The former is primarily a function of differential CO_2 solubility in seawater and the thermohaline circulation, while the latter is the sum of a series of biological processes that transport carbon (in organic and inorganic forms) from the surface euphotic zone to the ocean's interior. A small fraction of the organic carbon transported by the biological pump to the seafloor is buried in anoxic conditions under sediments and ultimately forms fossil fuels such as oil and natural gas.

One way to increase the carbon sequestration efficiency of the oceans is to add micrometre-sized iron particles in the form of either hematite (iron oxide) or melanterite (iron sulfate) to certain regions of the ocean. This has the effect of stimulating growth of plankton. Iron is an important nutrient for phytoplankton, usually made available via upwelling along the continental shelves, inflows from rivers and streams, as well as deposition of dust suspended in the atmosphere. Natural sources of ocean iron have been

declining in recent decades, contributing to an overall decline in ocean productivity (NASA, 2003). Yet in the presence of iron nutrients plankton populations quickly grow, or 'bloom', expanding the base of biomass productivity throughout the region and removing significant quantities of CO_2 from the atmosphere via photosynthesis. A test in 2002 in the Southern Ocean around Antarctica suggests that between 10,000 and 100,000 carbon atoms are sunk for each iron atom added to the water. More recent work in Germany (2005) suggests that any biomass carbon in the oceans, whether exported to depth or recycled in the euphotic zone, represents long-term storage of carbon. This means that application of iron nutrients in select parts of the oceans, at appropriate scales, could have the combined effect of restoring ocean productivity while at the same time mitigating the effects of human caused emissions of carbon dioxide to the atmosphere.

Because the effect of periodic small scale phytoplankton blooms on ocean ecosystems is unclear, more studies would be helpful. Phytoplankton have a complex effect on cloud formation via the release of substances such as dimethyl sulfide (DMS) that are converted to sulfate aerosols in the atmosphere, providing cloud condensation nuclei, or CCN. But the effect of small scale plankton blooms on overall DMS production is unknown.

Other nutrients such as nitrates, phosphates, and silica as well as iron may cause ocean fertilization. There has been some speculation that using pulses of fertilization (around 20 days in length) may be more effective at getting carbon to ocean floor than sustained fertilization.

There is some controversy over seeding the oceans with iron however, due to the potential for increased toxic phytoplankton growth (e.g. "red tide"), declining water quality due to overgrowth, and increasing anoxia in areas harming other sea-life such as zooplankton, fish, coral, etc.

Soils

Methods that significantly enhance carbon sequestration in soil include no-till farming, residue mulching, cover cropping, and crop rotation, all of which are more widely used in organic farming than in conventional farming. Because only 5% of US farmland currently uses no-till and residue mulching, there is a large potential for carbon sequestration.[26]

Conversion to pastureland, particularly with good management of grazing, can sequester even more carbon in the soil.

Terra preta, an anthropogenic, high-carbon soil, is also being investigated as a sequestration mechanism. By pyrolysing biomass, about half of its carbon can be reduced to charcoal, which can persist in the soil for centuries, and makes a useful soil amendment, especially in tropical soils (*biochar* or *agrichar*).

Savanna

Artificial sequestration

For carbon to be sequestered artificially (i.e. not using the natural processes of the carbon cycle) it must first be captured, *or* it must be significantly delayed or prevented from being re-released into the atmosphere (by combustion, decay, etc.) from an existing carbon-rich material, by being incorporated into an enduring usage (such as in construction). Thereafter it can be passively stored *or* remain productively utilized over time in a variety of ways.

For example, upon harvesting, wood (as a carbon-rich material) can be immediately burned or otherwise serve as a fuel, returning its carbon to the atmosphere, *or* it can be incorporated into construction or a range of other durable products, thus sequestering its carbon over years or even centuries. One ton of dry wood is equivalent to 1.8 tons of Carbon dioxide.

Geological sequestration

The method of *geo-sequestration* or *geological storage* involves injecting carbon dioxide directly into underground geological formations. Declining oil fields, saline aquifers, and unminable coal seams have been suggested as storage sites. Caverns and old mines that are commonly used to store natural gas are not considered, because of a lack of storage safety.

Mineral sequestration

Mineral sequestration aims to trap carbon in the form of solid carbonate salts. This process occurs slowly in nature and is responsible for the deposition and accumulation of

limestone (calcium carbonate) over geologic time. Carbonic acid in groundwater slowly reacts with complex silicates to dissolve calcium, magnesium, alkalis and silica and leave a residue of clay minerals. The dissolved calcium and magnesium react with bicarbonate to precipitate calcium and magnesium carbonates, a process that organisms use to make shells. When the organisms die, their shells are deposited as sediment and eventually turn into limestone. Limestones have accumulated over billions of years of geologic time and contain much of Earth's carbon. Ongoing research aims to speed up similar reactions involving alkali carbonates.