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B.Sc. BOTANY

THIRD YEAR

PAPER - VII : PLANT PHYSIOLOGY

Prepared By :
Dr. A. PADMANABAN
Hig-1, TNHB Quarters
Vennampatti Road
Dharmapuri – 636 705

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PLANT PHYSIOLOGY

UNIT I

Movement of materials: Imbibition, diffusion, osmosis. A brief account on absorption of water and minerals. Transpiration: types, mechanism of stomatal movement. Guttation. Role of macro (N,P,K) and micro (B,Mn,Mg,Zn) elements.

UNIT II

Photosynthesis: Photosynthetic pigments, Emerson enhancement effect, Absorption spectrum and Action spectrum. Cyclic and noncyclic elctron transport. Dark reaction. Calvin cycle. Hatch and Slack cycle.

Respiration: aerobic and anaerobic respiration. Glycolysis, Kreb's cycle. Electron transport system. Respiration quotient.

UNIT III

Nitrogen metabolism: Sources of nitrogen, nitrogen fixation, nitrogen cycle, reductive amination and transamination. Protein synthesis.

Enzymes; Nomenclature, Classification (old system) Mode of action, factors affecting enzyme activity.

UNIT IV

Carbohydrates: Classification- monosaccharides (Glucose, Fructose). Disaccharides(Maltose, sucrose). Polysaccharides (starch, cellulose)

Proteins: structure, types of proteins (globular, conjugated).

Lipids: composition of lipids-saturated and unsaturated fatty acid; Break down of fatty acids (alpha oxidation).

UNIT V

Growth: plant growth regulators- auxins, gibberellins, cytokinins, ethylene, abscissic acid- their physiological effects and their role.

Plant movements- geotropism, phototropism, Thigmotropism

UNIT I

Movement of materials: Imbibition, diffusion, osmosis. A brief account on absorption of water and minerals. Transpiration: types, mechanism of stomatal movement. Guttation. Role of macro (N,P,K) and micro (B,Mn,Mg,Zn) elements.

Movement of materials

Imbibition, diffusion and osmosis are essentially physical process which play an important role in the plant physiological process like water relations in plants.

Imbibition:

The absorptionof a liquid (water) by the solid particles of a substances without forming a solution is called imbibition. The substances which can imbibe the liquid are described as imbibant. The plant imbibants are represented by hydrophillic substances such as starch, cellulose, pectic compounds, agar-agar, dry wood, gum etc. Imbibition is accompanied by a swelling in the imbibant, called imbibition pressure.

Imbibition is influenced by a number of factors like temperature, pH, texture of the imbibant, electrolyte etc.

Significance of imbibition:

1. Imbibition initiates seed germination.
2. It causes increase in the volume of seeds and ultimately bursting of testa or seed coat.
3. It helps in initiationof water absorption by roots.
4. It helps in the ripening of ovules into the seeds.
5. During imbibition heat energy is released which further increases the activities of cells of the living seeds.
6. Certain amount of conduction of water in the plant body in known to be through imbibition.

Diffusion:

The movement of molecules of a substances from a region of its higher concentration to a region of its lower concentration is called diffusion. Diffusion can take place in solids, liquids and gasses. The rate of diffusion is faster in gases than in other two substances. The process of diffusion comes to a halt once the molecules of the diffusing substance have spread uniformly in both the regions.

Significance of diffusion in plants:

1. The exchange of oxygen and carbondioxide gases in the atmosphere through stomata of the leaves takes place by the process of diffusion. Oxygen gas participates in respiration whereas carbondioxide in photosynthesis.
2. During stomatal transpiration, the water vapours from intercellular spaces diffuse in the atmosphere through stomata by diffusion.
3. The diffusion of ions of mineral salts during passive absorption also takes place by this process.
4. The absorption of water through roots is also performed by diffusion.

Osmosis

It is a special type of diffusion, which involves the movement of solvent molecules from a region of higher concentration to a region of lower concentration, through a semipermeable membrane. Since, in the biological system, water is a universal solvent, osmosis is defined as the movement of water along the concentration gradient, through a semipermeable membrane. The pressure exerted by water molecules on the semipermeable membrane is known as **osmotic pressure**. It is the **diffusion pressure** of water.

The addition of any solute of pure water decreases its diffusion pressure. Such a decrease is known as **diffusion pressure deficit** (DPD). Hence osmosis is also defined as movement of water from a region of lower DPD to a region of higher DPD.

When a plant cell comes in contact with a medium (**hypotonic**) in which the concentration of dissolved substance is less than that of cell sap, there is an entry of water into the cell. This type of osmosis in known as **endosmosis**.

When a plant cell comes in contact with a medium (**hypertonic**) in which the concentratin of dissolved substance is more than that of cell sap, there is an movement of water from the sap into the medium. This type of osmosis is known as **exosmosis**.

During exosmosis the cytoplasm starts shrinking and finally it gets separated from the cell wall. This physiological process is known as **plasmolysis**. The reverse process is known as **deplasmolysis**.

Absorption of water

Importance of water to the plants

1. The amount of water present in the soil changes the morphology and anatomy of the plants eg. Mesophytes, hydrophytes and xerophytes.

2. Water is a good solvent. It also acts as a reactant in various chemical reactions in plant cells.
3. Water helps in the formation of protoplasm.
4. The absorption of mineral salts and other substance take place through water.
5. During photosynthesis, water is oxidised and oxygen is produced.
6. Water effects transpiration, seed germination, respiration, dispersal of fruits and seeds, activation of enzymes, hydrolysis of ATP, growth and all other metabolic process.
7. Water maintains the temperature in plant tissue.
8. Water maintains the turgidity of plant body.

Soil water:

Soil water is the most important constituent of soil. It provides medium for the absorption of minerals and organic matters by the roots. The vegetation of an area depends upon the quantity of soil water. The water present in the soil may be of following types

1. **Grativational water**: the water which reaches deeply into the soil after rains due to gravitation is called gravitational water. The plants cannot abosrb this water through roots.
2. **Capillary water**: the water which remains present in the inter cellular spaces of soil particles is called capillary water. It is the only available water which is absorbed by plant roots. It is held tightly by hydrogen bonds to soil particles against gravitational force.
3. **Hygroscopic water**: the water that is present around the soil particles in the form of thin vapour layer is called hygroscopic water. It is not utilized by plants. This is a part of capillary water which is held very tightly by soil particle and make it non available to roots.
4. **Crystalline or chemically bound or combined water**: the water which remains chemically bound to the soil is crystalline water eg. $CuSO_4.5H_2O$, $FeSO_4.7H_2O$. It is not available to plants.
5. **Running water**: the non available water which after rain flows down through the slopes is called running water.

Root system is directly related to the absorption of water and its growth under field conditions is very much influenced by soil. In dryland agriculture, particularly root structure has apparently greater significance. The rate of water absorption into roots of different plants differs in their stage of growth. Highest rates of water entry are associated with root hair and unsuberised root and lowest with suberised woody root.

Mechanism of water absorption

Radial flow of water:

Plants absorb water from the soil by roots, mainly near the root hair zone, in the region of maturation. Absorption of water from soil by root hairs and its movement to the xylem is known as radial movement of water.

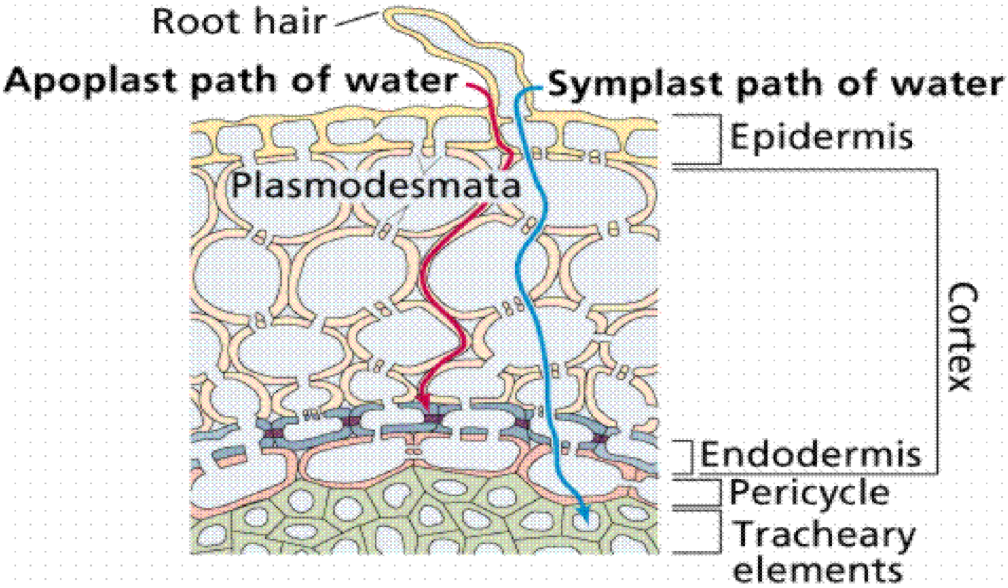
According to Renner (1912, 1915) the mechanism of water absorption is of following two types

1. **Active absorption**- absorption is mainly by roots. It is found in those plants where transpiration is less and water is present in sufficient amounts. It requires ATP.
    a. **Osmotic absorption**: the root acts like a osmometer and water is absorbed according to the osmotic gradient.
    b. **Non-osmotic absorption**. The water absorption is against osmotic gradient and the plant requires more ATP.
2. **Passive absorption**. In this type, the water absorption forces develop primarily in leaves and stem and then reach to root thorugh xylem. It takes place mainly due to transpiration and ATP is not needed.

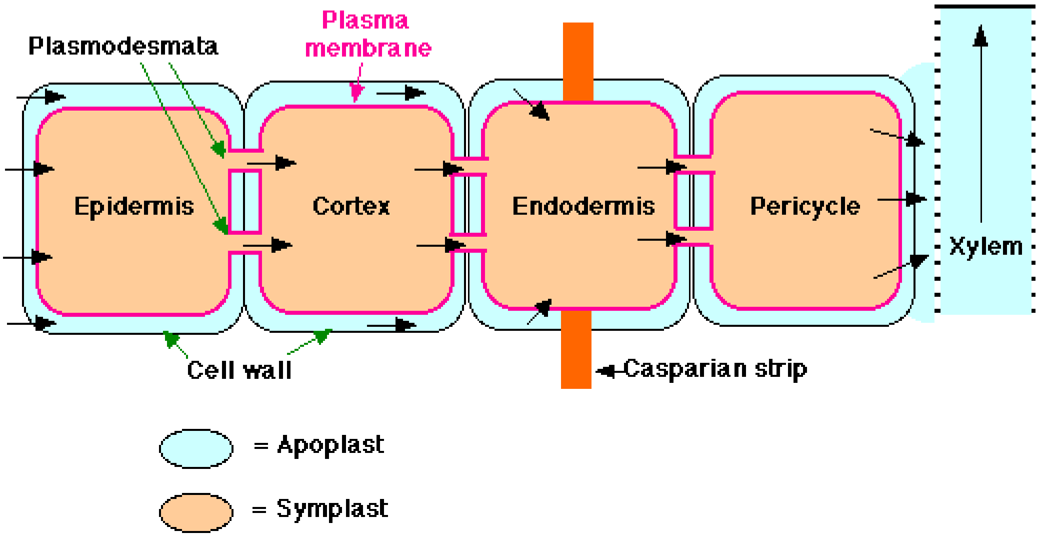
Omsotic active absorption:

Root hairs remain spread in soil water. Root hair cell contain vacuole filled with mienral salts and organic acids. The cell wall of root hair is permeable to water and plasma membrane is semi permeable and it allows only the diffusion of water and important dissolved salts into the cytoplasm. The cell wall of root hair being hydrophilic in nature first absorbs soil water through imbibition. The cytoplasm of root hair is usually concentrated (OP 3-8 atm.) than capillary water of soil (OP= 1 atm.). Thus the DPD, SP become more in root hairs resulting in the osmotic diffusion or endosmosis of water and dissolved substances into the root hairs. Thus the root hair cell becomes turgid, thin and it's OP, DPD and SP is comparitively less than its adjacent first contex cell -resulting in osmosis diffusion. This leads to its turgidity and less OP, DPD, SP compared to second cortex cell reulting in diffusion of water from first to second corex cell. This process continues till the water reaches pericycle through all cortical and endodermal cells. The endodermis possesses passage cell and casparian striped cells. The passage cell are thin walled and found against protoxylems. Through these passage cells, the water diffuses into cells of pericycle and ultimately into xylem vessels. As the water diffuses from root hair to the first cortex cell, the cytoplasm of root hair cell again becomes concentrated and its OP, DPD and SP are also increased resulting again in osmotic diffusion of capillary water of soil into the root hair. This phenomenon continues for long time and thus osmotic active water absorption takes place.

Pathway of water in root: It is by both apoplastic and symplastic pathway. In apoplasitc pathway, the water travels through the cell wall, whereas in symplastic pathway the water passes through the cell protoplasm.



Root hair → epidermal cell → various successive cortex cells → endodermal cell (passage cell) → cells of pericycle → xylem cells → xylem duct → upward movement of water.



Ascent of sap:

The upward movement of water from the underground root to the aerial shoot through xylem is called ascent of sap. It can be demonstrated by the following experiments:

1. Rose (white flowered) plant experiment: the flowering twig is cut under water and placed in a beaker containing a solution of eosin in water. After some time, the minute veins of petals become red. The T.S. of twig also shows red coulor xylem.
2. Balsam plant is cut under water and placed in eosin solution. Minute vertical red lining is seen in the stem. The transverse section shows red coloured tracheids and vessels.
3. Ringing exeriment: in a potted plant, the outer stem is removed in a circular fashion (epidermis, cortex, endodermis, pericycle and phloem) except vessels and trachied of xylem and pits. After a few days the leaves are still green proving the ascending of water and mineral salts thorugh xylem.

Theories of Ascent of sap:

The mechanism of ascent of sap is explained through various theories. They are

1. root pressure theory
2. vital theory
3. physical theories

Root pressure theory:

Root pressure is defined as the positive hydrostatic pressure developed in the root due to the accumulation of absorbed water. Stephen Hales (1927) coined the term root pressure.

The maximum root pressure recorded in plant is 0.2 to 0.3 M Pa (2/3 bars) which can raise the water to a height of about 20 meters. Root pressure may be a force of ascent of sap in herbs and shrubs during rainy season when rate of transpiration is low.

Experiment demonstrating root pressure:

A well potted plant is taken. Its stem is cut off just above the ground level. The stump (cut stem) is connected to a glass tube containing water through a rubber tube. After sometime, the level of water in the glass tube raises. This proves the root pressure.

Objection:

1. Root pressure is not seen in tall gymnosperms.
2. In actively transpiring plant, root pressure is not observed.
3. Water raises upward even in the absence of root.
4. Few plants develop root pressures greater than 30 lb/in^2, and some develop no root pressure at all.
5. The volume of fluid transported by root pressure is not enough to account for the measured movement of water in the xylem of most trees and vines.
6. Those plants with a reasonably good flow of sap are apt to have the lowest root pressures and vice versa.
7. The highest root pressures occur in the spring when the sap is strongly hypertonic to soil water, but the rate of transpiration is low. In summer, when transpiration is high and water is moving rapidly through the xylem, often no root pressure can be detected.

So although root pressure may play a significant role in water transport in certain species (e.g., the coconut palm) or at certain times, it is not satisfactory explanation in most plants.

Vital theories:

Godlewski theory: Godlewski (1884) proposed that the water ascends in the plant due to pumping action to the cells of xylem parenchyma and medullary rays.

Vital force theory: vital force theory states that living cells of stem play an important role in the ascent of sap. J.C. Bose (1923) stated that cells of inner layer of cortex possess pulsating action like heart. This expansion and contraction of cortical cells due to the continuous turgidity and placidity drives water into xylem and it in turn helps the ascent of sap.

Objections:

Strasburger (1983) carried out an experiment with oak tree. He cut the tree and placed it in boiled picric acid for three hours. Later, he placed it in coloured solution. The coloured solution moved upward through the cells killed with picric acid. This experiment shows that the ascent of sap takes place through dead cell and therefore vital theories were discarded.

Physical theories:

1. **Imbibition theory:** Unger (1868) Sachs (1878) supported this theory. According to this theory, the water ascends due to imbibitinal forces

through the walls of xyem trachieds and vessels and their lumen or cavities have no relation with ascent of sap.

Objection: the lumen of trachieds and vessels were blocked with wax then the ascent of sap stopped followed by wilting of leaves.

2. **Capillary force theroy**: Boehm (1899) proposed this theory. Xylem vessels are aslo quite thin like capilary tube and so due to capillary force, the water ascends upwards.

Objection: it fits for small distance transport, but not applicable to very tall trees.

Cohesion tension hypothesis or transpiration pull:

This theory was proposed by Dixon and Jolly (1894) and modified by Dixon (1914).

The water molecules remain attracted by a force called cohesive force. Cohesion is the phenomenon of attraction between similar molecules.

Cohesive force maintains the continuity of water column from the base of plant to apex. The magnitude of cohesive force is about 350 bars.

During transpiration water is lost from the mesophyll cell so that suction pressure called tension (20 to 30 bars) develoops in mesophyll cell. This tension spreads from mesophyll cell to the root thorugh xylem. Thus the water molecules move upwards.

Tension may cause a break in water column. But due to cohesive force of water molecule, the continuous water column is not broken.

Evidence: The osmotic postential (OP) of mesophyll cell is between 20 to 30 bars. This can raise the water to a height of 200 to 300 meters.

Objection: The entry of gases along with water into xylem may break the continuity of water column.

But the several rows of water column running parallel to one another helps in maintaining the continuity.

Cohesion theory assumes trachieds to be more efficient than vessels. Dixon believes that partition walls of the trachieds provide stability on the tensilety stressed transpiration stream. This doesnot explains adaptation of vessels in angiosperms.

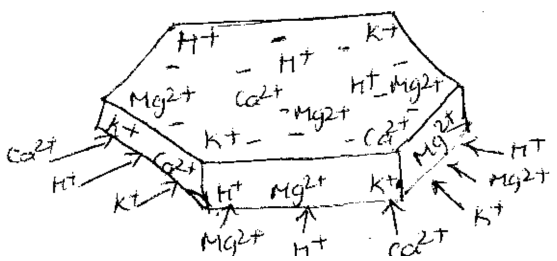
Absorption of minerals:

One might have expected that minerals would enter the root dissolved in water. But, in fact, minerals enter separately:

- Even when no water is being absorbed, minerals enter freely — mostly through the root hairs.

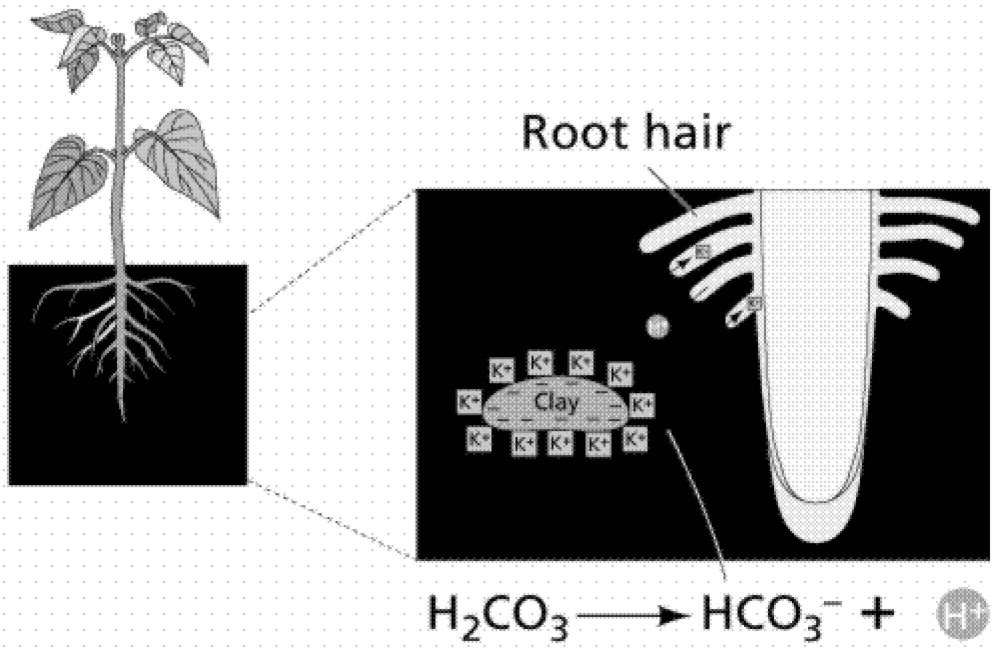
- Plant absorbs salts from soil to build up protoplasm and other vital substances.
- Salts are generally absorbed in the form of ions.
- Absorption of ions may be passive or active absorption.
- The absorption of ions without using metabolic energy is called the passive absorption.
- Passive absorption includes;
  - ion exchange
  - Donnan equilibrium etc.
- In ion exchange, ions of soils may be exchanged with ions absorbed to the surface of the root. This exchange may be through contact exchange or through carbonic acid exchange.
- According to Donnan equilibrium, the fixed nondiffusable ions present in the cells disturb the electrical equilibrium of the cells. To regain neutrality, the cells accumulate ions of the other charge.
- Minerals can enter against their concentration gradient; The accumulation of the mineral ions by utilizing metabolic energy (ATP) is called the active absorption. For example, plants can take up K^+ from the soil against a ten-thousand-fold concentration gradient; e.g., from as little as 10 µM in the soil to 100 mM in the cell.
- Anything that interferes with the metabolism of root hairs interferes with mineral absorption.
- The root hairs are also the point of entry of mycorrhizal fungi. These transport minerals — especially phosphorus — to the root hair in exchange for carbohydrates from the plant.
- In legumes, the root hairs are the point of entry of rhizobia that will establish the mutualistic partnership enabling the plant to convert atmospheric nitrogen into protein.
- The active absorption can be through carrier concept proposed by vander Honert. According to him ions are carried across membrane by certain carrier molecules. Carrier molecules are activated, pick up ions on the outer surface and form carrier-ion-complex. The carrier ion complexes move across the membrane and reach the inner surface.
- The ions absorbed by the root may move across the cortex through apoplastic or symplastic pathway.
- The upward translocation of ions is carried through xylem. The downward translocation/ movement of ion take place through phloem.

Except for carbon, oxygen and hydrogen which are provided by carbondioxide and water, all the oter elements essential for plants are provided by the soil. Hoagland, Stout and Arnon (1923) showed that minerals were taken up from the soil primariloy in ionic form. The rate of uptake of different ions by roots varied and one ion influenced the uptake of other ions. As soil is the medium for the storage and exchange of mineral ions, its properties, ion iexchange capacityl pH and the presence of different cations and anions affect the availability of ions to the plant. In other word, the presence of a certain mineral ions is abundance in the soil cannot ensure its availability to the plants, because ions may adhere to clay or precipitte out of the solution as insoluble salts. The soil with high water holding capacity generally has high mineral holding capacity as well. The fine particles of clay and humus possess a relatively large surface to volume ratio and are negatively charged. Hence they have higher ion-bonding capacity than the soil composed of coarse particles.



The figure shows the colloidal clay crystal (micelles) with innumerable negative surface charges. The cations are loosely bound to negative chage by ionic bond and are capable of exchanging rapidly are reversibly with those in the soil solution. H^+ has greater affinity for charged soil particles than Ca^{2+}, Mg^{2+} or K^+ ions. Therefore these cations are released in soil water by H+ ions andmade available for uptake by roots. The acidity of the soil also incrases due to respiration because carbondioide released react with soil water to form carbonic acid

- Micelle –Mg^{2+}+ + $2H^+$ → micelle H^+ + Mg $^{2+}$ in soil solution.
- From respiration $CO_2 + H_2O$,$\leftrightarrow$ $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$



The ion exchange capacity of mineral ions is affectred by pH of the soil, which in turn affects the availability of different ions to the plants.

The characteristics of nutrient uptake depends on

1. Selectivity: certain mineral elements are taken up preferentially while others are dercriminated against or nearly excluded.
2. Accumulation: the concentration of the mineral element can be much higher in the plant sap than in the external solutin. This means the uptake is against concentration gradient.
3. Genotype: plant species, even cultivar, differ genetically in nutrient uptake characteristics.

Chemically Na $^+$ resembles K^+ very closely but the rate of absorption of K^+ is not influenced by the similar concentrationof Na^{2+} ions in the medium. The process of K^+ ion absorption is therefore selective and uninfluenced by a related ion. Similarly several other monovalent and less related bivalent ions also have no effect on K^+ uptake.

Likewise absorption of Cl^- is unaffected by related halides, fluoride and iodide, as well as other anions like NO_3^-, $H_2PO_4^-$ or SO_4^{2-}.

Interestingly, Ca^{2+}+ is an absolute requirement for this selectivity, for example in the absence of Ca^{2+}, K^+ absorption is inhibited by Na^{2+}.

Eventhough the ion uptake is specific, yet, it can often by “fooled” by similar ions for example

K^+ can be competitively inhibited by Rb^+

Cl^- can be competitively inhibited by Br^-

Ca^{2+}, Sr^{2+} can be competitively inhibited by Mg^{2+}

Sulphate can competitively inhibited by selenate

The selectivity and the rate of utake of the nutrients and metabolites are influenced by temperature, oxygen, poisons, carbohydrate content of the tissues and light. Such effects are similar to enzyme mediated reactions and indicate that proteins are involved in solute uptake.

Mechanisms of uptake of ions

The process of nutrient uptake by plants refers to the transfer of the nutrient ions across the soil-root interfaces into the plant cell. The ions are absorbed from the soil mainly through meristamatic and elongation zones of the root. Partly mineral absorption is also carried by zone of root hair.

The absorption of ions is of two types

1. passive absorption
2. active absorption

Passive absorption:

The absorption of ions without utilising metabolic energy is called the passive absorption. To explain passive absorption many theories were proposed. Some of them are

1.1 ion exchange theory

1.2 Donnan equilibrium etc

Ion exchange theory:

This hypothesis was proposed by Jenny and Overstreet (1938). It can be explained in the two theories as follows:

Contact exchange theory:

The exchange of ions of similar charge between roots and soil, when they are in contact exchange. Several H^+ and OH- ions are absorbed to the surface of root. Cations are absorbed to soil particle. Anions are present freely in soil solution. The absorbed ions are not held tightly but will oscillate within certain volume of space. If the oscillation volume of an ion overlaps the oscillation volume of another ion, exchange of ion takes place.

Many fungi grow in soil in close association or even into the roots in symbiosis called mycorrhizae. The fungal hyphae have superior minearl absorptive ability and supply plant with more nitrogen, potassium and phosporous. Plants in turn provide food to the fungi.

Carbonic acid exchange theory:

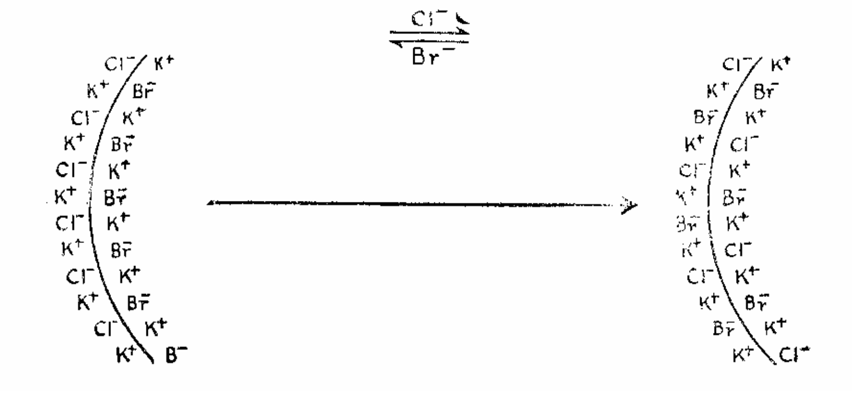
In this theory, soil solutions play an important role in exchange of ions. According to this theory, carbondioxide released by root dissolves in soil solution to form carbonic acid.

Carbonic acid dissociates into H^+ and HCO3- ions. H^+ ions are exchanged with cations absorbed to soil particles. HCO3- ions are exchanged with anions present in soil solution. The ions diffuse to the root surface where they are absorbed.

Donnan equilibrium:

Donnan equlilbrium was proposed by F.G. Donnan (1911). The primary cell wall of the plant cell consists of amorphous matrix of two polysaccharides, hemicelluloses and pectic substances. Hemicelluloses are made up of sugars other than glucose (eg. Xyloglucans) while pectic substances are partly made from polyglacturonic acids. These acids have weak carboxylic acid group (-COOH) the ionize and give rise to negative charges (-COO-) on which hydrogen ions are loosely held. When positively charbed ions such as K^+, $Mg2^+$. $Ca2^+$ pass through the plant cell wall, they displace hydrogen ions of the carboxyl groups and areheld there by the weak inter ionic attractive forces.

The intercellular spaces, the negatively charged regions (Donnan's free space or cation adsorptive sites or cation exchange sites) in the amorphous matrix and the pores in the cellulose microfibrils are readily accessible to water and dissolved ions. The diameter of these pores is in the ragne of 5.0 nm whereas; the dimensions of hydrated ions such as K^+ and $Ca2^+$ are smaller. The fraction of the volume of the plant tissue readily accessible to diffusion of an external solute dissolved in water is termed "free space". The free space in the root is bound by the plasma membrane of the epidermal and cortical cells and the casparian strips of endodermis.



According to Donnan equilibrium theory, a cell accumulates ions because of the presence of certain fixed non-diffusable ions. The fixed ions disturb the ionic eqilibrium of the cell. To regain neutrality (electric equilibrium) the cell accumulates ions of other charges. To explain, if the fixedions are negatively charged, cell accumulated positively charged ions.

Active absorption

There are number of evidences for the existence of active absorbpiton of the substance eg.

1. Rate of absorption is too rapid to the explained by passive absorption.
2. The diffusion substance is at higher concentration within the cell and it at lower conentration outside.
3. The rate and amount of absorption of the substance is directly related to the expenditure of metabolic energy.
4. The cell activity affects the mechanism of absorption.

Mechanism of active absorption:

The carrier concept.

Van den Honert (1937) proposed the role of carrier compound in the transport of ions across the membrane. The concept has derived great support for the following observations:

1. Isotopic exchange.
2. Saturation effects.
3. Specificity in the absorption of ions.

The plasma membranes have carrier proteins that rotate and move across the membrane. The activated carrier proteins combine with ions and form Ion-carrier complexs, move across the membrane, reach inner side and release the ion.

Cytochrome pump hypothesis;

Lundegardh and Burstrom (1933, 1950,195) proposed this theory. The quantative correlation between anion absorption and salt respiration led them to propose that the anions were absorbed via a cytochrome pump in which the cytochromes acted as anion carriers.

Protein lecithin as a carrier protein:

Bennet Clark (1965) proposed a phosphatide- lecithin as a carrier. The carrier is amphoteric and hence both cations and anions combine with it.

The actual absorption of salts by roots is by both passive and active. Themovement of salts inot the cell wall and a part of cytoplasm are passive, while the movement of salt into vacuole is active.

Eventhough lipid bilayer do not permit the entry of polar molecule like ions, sugars, amino acids, nucleotides and cell metabolites, these molecules enters the cell through

1. aqueous protein channels (pores)
2. carrier proteins that rotate and move across the membrane.
3. transmembrane proteins that transport solute be undergoing change in shape or confirmation.

The plant plasma membrane ATPase is a transmembrane protein composed of a single polypeptide chain. The most possible coupling mechanicm is, it exists in two confirmations.

Transpiration

Plants absorb large quantityof water from the soil by the root hairs. Only a very small part (1 to 2 %) of this water is retained in the plant body for the building up proscess, while the most part (98 to 99%) of it is lost in the form of water vapour.

Transpiration is the giving off of water vapour from the internal tissue of living plants through the aerial parts such as leaves, green shoots etc. under the influence of sunlight.

Transpiration is not a simple process of evaporaion. Since it is influenced by the vital activity of the protoplasm and some structural pecularity of the transpiring organs. The total quantity of water that evaporates fron a single plant is considerable for example in a single sunflower plant there is a daily average loss of 187.5 cc water.

Often water loss by transpiration exceeds gain by absorption and results in negative water balance within the plant. Small and moderate deficit that occur due to high temperature during the day are compensated during the night but prolonged deficit causes irreversible damages and threatens theplants survival.

Transpiration is essentially evaporation of water from the aerial protion of plant. However, evaporation of water from open surface meets less resistance while evaporation of watrer from the leaves faces considerable resistance.

Types of transpiration:

Depending on the tissue through which transpirarion occurs, three tyupes are identified

1. cuticular transpiration
2. lenticular transpiration
3. stomatal transpiration

Cuticular transpiration: cuticle is a waxy layer on the epidermis of leaf and stem. Its thickness varies from plant to plant. If the cuticle is thin, cuticualr transpiration takes place. About 5 to 10 % of water is lost through the cuticle.

Lenticular transpiration: in woody plants, lenticels are aperture within the bark which is filled with loosely arranged powdery parenchymatous cells called the complementary cell. Lenticel functions in gas exchange. The water loss through threse cells is called lenticular transpiration. The amount of water lost through lenticls amounts to about 0.1% of the water lost through the crown of the tree and blocking the lenticels reduces bark transpiration by atleast 20 %.

Stomatal transpiration: stomata are minute apertures in the epidermis of leaf and stem. Transpiration carried out through stomata is called the staomatal transpiration. Stomatal transpiration is the rule amounting to 80 to 90 % and in many times 10% excess to cuticular transpiration under ordingary conditions of light, temperature and humidity.

Significance of transpiration

"Transpiratiion is called as a necessary evil". Because stomatal opening is must for the exchange of gases during respiration and photosynthesis. But inevitably water is lost as water varours through the stomatal opening. It has been estimated that more plants perish in their developemnt from a depleted water supply due primarily to its loss by transpiration than from all other factors taken together. Whenever wet cell surfaces are exposed to the atmorphere, evaporation of water is inevitable and transpirational loss must be described as unavoidable though it is a constant threat to the life of the plant.

Transpiration is otherwise regarded as essential for the welfare of the plant for it fulfills these functions:

1. to maintain optimum turgidity
2. to reduce leaf temperature
3. to promote uptake and tanslocatio of mineral ions.
4. an essential requirement for growth.

Mechanism of stomatal movement

A significant fact about somtata is that sometimes these are open and sometimes closed. When open, thy serve as the principal route for the exchange ofgases between the incercellular spaces of the leaf and the surrounding air. When closed, all gaseous exchange between a leaf and its environment are appreciabloy retarded.

Structure of stoma

In most environments, the water concentration outside the leaf is less than that inside the leaf, causing a loss of water through openings in the leaf known as stomata (singular = stoma).

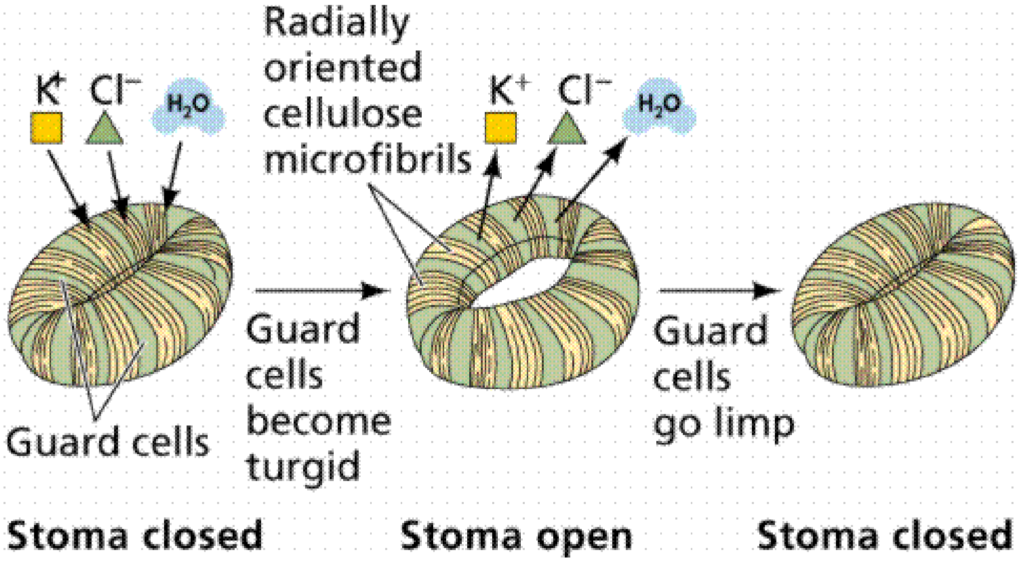
Guard cells are crescent-shaped cells of the epidermis that flank the stoma and regulate the size of the opening. Together, the guard cells and stoma comprise the stomatal apparatus. The inner wall of the guard cell is thicker non elastic than the rest of the wall which are thin and elastic. It has also pecularity, i.e., the cellulose microfibrils of guard cell cell wall are arranged raidally extending from the centre towards the periphery. This arrangement restricts the expansion of cell wall in transverse direction.

In some plants, the epidermal cells bordering on the guard cells are different in configuration from other cells in the same tissues. These are called accessory or subsidary cells. Below the stomata, and directed inwards to the mesophyll, there are large inter cellular spaces known as substomatal chamber. Besides the shape the guard cells differ from other epidermal cells in having rich protoplasm.

Some general features of stomatal movement are

1. Normally, stomata are open in day and closed in night. However, a drop of supply of water leads to the daytime closure of stomata.
2. Stomata open when the internal concentration of CO_2 drops and close when the internal concentration of CO_2 is maximum.
3. Dark CO_2 fixation (CAM plants) occurs in guard cells.’
4. In guard cells there is a change of pH in light (day) and dark (night) which is associated.
5. Opening and closing of stomata are related to the osmotic potential of the guard cells and permeability of membrane.
6. At the time of opening of stomata, there is an inflow of K ions into the guard cells. But when the stomata close, the guard cells loose their acquired potassium to the surrounding cells.
7. Inhibitors of cyclic phosphorylation can also close stomata.
8. Blue light also brings about changes in stomatal movement, serving to open the stomata.
9. Abscissic acid, a plant hormone, at very low concentration can lead to the closure of stomata.

When a guard cell takes up potassium ions, water moves into the cell, causing the cell to become turgid and swell, opening the stoma. When the potassium leaves the guard cell, the water also leaves, causing plasmolysis of the cells, and a closing of the stoma. Stomata occupy 1% of the leaf surface, but account for 90% of the water lost in transpiration.



Factors affecting transpiration

Transpiration is affected by several factors which can be grouped into

- environmental factors
- physiological factors

Environmental factors: they are also called as external factors. Some important environmental factors are

1. **Light**: the primary effect of light is to enhance the water permeability of the protoplasm of the evoparating cells and favour sotmatal opening. It also increases the concentration of sugars within guard cells.
2. **Temperature**: it increases the rate of evaporation from surface cell. Stoma closes at low temperature and very high temperature.
3. **Humidity**: as a rule, the rate of water loss is slower when the vapour pressure of the atmospheric air is greater.when the atmosphere is relatiely less humid and the inner atmosphere is saturated , the water vapours diffuses rapidly through the stomata.
4. **Wind velocity**: in the immediate vicinity of transpiring surfce, air becomes more or less staurates with water vapour and the vapour pressure gradient is lowered. On the other hand, if the moving air removes the water vapour concentration around the transpiring cell, the transpiration is enhanced. However, high velocity wind (which probably closes the stomata) is less effective than a gentle breeze.

5. **Atmospheric** pressure: the rate of evaporation is inversly proportional to the pressure of other gases and this takes place with a constant vapour pressure gradient.
6. **Availability** of soil water: transpiration will be high with abundant supply of water in soil, and low with limited supply of water.
7. **Chemical** sprays and dusts: pesticides and fungicides decrease the thickness of cuticle, and increase cuticular transpiration.

Physiological factors

1. **fungus disease**: fungus increase the rate of transpiration due to the increase in the opening and closing of stomata and water lost from the aerial mycelium of the fungus
2. **Plant structure**: different types of plants growing under identical conditions may vary greatly in the rates of transpiration. Such differences in transpiration is due to stomatal behavious, cell-sap concentration, imbibitonalcapacities of protoplasm and cell wall, structural difference in leaves like:leaf area, leaf structure, root-shoot ratio, leaf orientation etc.

Guttation

The loss of excess of waterin theform of liquid through hydathodes or water stomata or water glands is called as guttaiton or exudation. There are specialized organs for theis outlet of liquid- the hydathodes. These are pores in the epidermis situated over intercellular spaces which are lined with a mass of thin walled parenchyma (epithem). Guttation sometimes occurs through lenticels and ordinary stomata. Hydathodes are present on the leaf margin and apexs. Most herbaceous trees and somewoody trees, particularly in the tropics, drops off water accumulated at the apex of margins of leaf in the early morning. As a general rule, guttated water seems to escape by the parts of least resistance. When absorption of water is rapid to excess transpiration; a positive root pressure develops in the xylem cells, followed by guttation. Watering soil with dilute potassium nitrate or sugar solution decreases water potential of soil solution, hinders water intake and stops guttation.

Conditions favouring guttations are

1. abundant supply of water
2. a suitable temperature
3. activity of living cells of root etc.

The volume of guttated liquid is exceeding variable from a few drops to 10 to 100 ml. The composition of guttated liquids appears to vary from almost pure water to dilute solution of inorganic and organic solutes (enzymes, aminoacids, vitamins, sugar) and mineral ions, which roughly constitute 0.005 to 0.5% of the liquid. Osmotic potential varies from -0.51 to -0.91 bars

Significance of guttation is not known. However, some believe that it may paly a more essential role in plants. For eg.

1. Guttation is a means of eliminating surplus clacium from certain species of *Saxifraga*.
2. It may also act as a sort of safety valve wich regulates turgidity.
3. Guttation may speed up transport of minerals to the leaves, if transpiration is extremely slow.
4. Guttation may be important in the movement of materials from roots to shoots in some submerged plants where transpiration cannot occur.

Role of macro and micro elements

Unlike animals (which obtain their food from what they eat) plants obtain their nutrition from the soil and atmosphere. Using sunlight as an energy source, plants are capable of making all the organic macromolecules they need by modifications of the sugars they form by photosynthesis. However, plants must take up various minerals through their root systems for use.

Carbon, Hydrogen, and Oxygen are considered the essential elements. Nitrogen, Potassium, and Phosphorous are obtained from the soil and are the primary macronutrients. Calcium, Magnesium, and Sulfur are the secondary macronutrients needed in lesser quantity. The micronutrients, needed in very small quantities and toxic in large quantities, include Iron, Manganese, Copper, Zinc, Boron, and Chlorine. A complete fertilizer provides all three primary macronutrients and some of the secondary and micronutrients. The label of the fertilizer will list numbers, for example 5-10%, which refer to the percent by weight of the primary macronutrients.

Plants use these minerals in:

1. Structural components in carbohydrates and proteins
2. Organic molecules used in metabolism, such as the Magnesium in chlorophyll and the Phosphorous found in ATP
3. Enzyme activators like potassium, which activates possibly fifty enzymes
4. Maintaining osmotic balance

Nitrogen

Nitrogen is an indispensable elementary constituent of important organic compounds like amino acids, proteins and nucleic acide. Dry plant material contains about 2 to 4 % of nitrogen. In green plants parts, protein nitrogen is by far the largest nitrogen fraction and amounts for 80 to 85% of total nitrogen. In vegetative parts, the proteins are mainly enzyme proteins, whereas in seeds and grains special storage proteins make the major protein fraction. Nitrogen is also an essential constituent of various coenzymes.

Nitrogen (N) deficiency in plants can occur when woody material such as sawdust is added to the soil. Soil organisms will utilise any nitrogen in order to break this down, thus making it temporarily unavailable to growing plants. 'Nitrogen robbery' is more likely on light soils and those low in organic matter content, although all soils are susceptible. Cold weather, especially early in the season, can also cause a temporary shortage.

All vegetables apart from nitrogen fixing legumes are prone to this disorder. Symptoms include poor plant growth, leaves are pale green or yellow in the case of brassicas. Lower leaves show symptoms first. Leaves in this state are said to be etiolated with reduced chlorophyll. Flowering and fruiting may be delayed.

Phosphorus

The inorganic forms of phosphate found in plants are orthophosphate and to a minor extent, pyrophosphate.

The organic forms of phosphates are compounds in which the orthophosphate is esterified with hydroxyl groups or sugars and alcohols or bound through a phosphate bond to another phosphate group. Phosphorylated sugars and alcohols are the intermediiary compounds of metabolism. Phosphate is also present in phospholipids. The nucleotides phsophates: ATP, UTP, GTP and CTP supply energy to many endergonic process including active ion uptake and the synthesis of various organic compounds.

Phosphorus (P) deficiency is a plant disorder that is most common in areas of high rainfall, especially on acid, clay or poor chalk soils. Cold weather can cause a temporary deficiency.

All plants may be affected, although this is an uncommon disorder. Particularly susceptible are carrots, lettuce, spinach, apples, and gooseberries. Symptoms include poor growth, and leaves that turn blue/green but not yellow—oldest leaves are affected first. Fruits are small and acid tasting. Phosphorus deficiency may be confused with nitrogen deficiency. Undersides of tomato plant leaves, and the veins and stems, may turn purple.

Potassium

Potassium is an important cation in plants. The phloem sap is rich in potassium. The concentration in cytoplasm is 5 to 10 times more than in vacoule. Potassium is necessary for meristamatic growth. It is involved in the water status of plants and maintains cell turgour. Potassium plays an important role in opening and clossing of stomata. Potassium also acts as a cofactor.

Potassium deficiency, also known as **potash deficiency**, is a plant disorder that is most common on light, sandy soils, as well as chalky or peaty soils with a low clay content. It is also found on heavy clays with a poor structure.

Plants require potassium ions (K^+) for protein synthesis and for the opening and closing of stomata, which is regulated by proton pumps to make surrounding guard cells either turgid or flaccid. A deficiency of potassium ions can impair a plant's ability to maintain these processes.

The deficiency most commonly affects fruits and vegetables, notably potatoes, tomatoes, apples, currants, and gooseberries, and typical symptoms are brown scorching and curling of leaf tips, and yellowing of leaf veins. Purple spots may also appear on the leaf undersides.

Deficient plants may be more prone to frost damage and disease, and their symptoms can often be confused with wind scorch or drought.

Micronutrient deficiency

A **micronutrient deficiency** (or **trace mineral deficiency**) is a physiological plant disorder which occurs when a micronutrient is deficient in the soil in which a plant grows. Micronutrients are distinguished from macronutrients (such as Nitrogen, Phosphorus, and Potassium) by the relatively low quantities needed by the plant. A number of elements are known to be needed in these small amounts for proper plant growth and development. Nutrient deficiencies in these areas can adversely affect plant growth and development. Some of the best known trace mineral deficiencies include: Boron deficiency, Calcium deficiency, Iron deficiency, Magnesium deficiency, and Manganese deficiency.

Boron

- **Boron** is believed to be involved in carbohydrate transport in plants; it also assists in metabolic regulation. It plays an essential role in elongation of pollen tubes.

Boron (B) deficiency is an uncommon disorder affecting plants growing in deficient soils and is often associated with areas of high rainfall and leached soils. Boron may be present but locked up in soils with a high pH, and the deficiency may be worse in wet seasons.

Extreme case of boron/calcium imbalance induced "watercore" in apple

Symptoms include dying growing tips and bushy stunted growth, extreme cases may prevent fruit set. Crop-specific symptoms include;

- *Apple*- interacting with calcium, may display as "water core", internal areas appearing frozen
- *Beetroot*- rough, cankered patches on roots, internal brown rot.
- *Cabbage*- distorted leaves, hollow areas in stems.
- *Cauliflower*- poor development of curds, and brown patches. Stems, leafstalks and midribs roughened.
- *Celery*- leaf stalks develop cracks on the upper surface, inner tissue is reddish brown.
- *Celeriac*- causes brown heart rot
- *Pears*- new shoots die back in spring, fruits develop hard brown flecks in the skin.
- *Strawberries*- Stunted growth, foliage small, yellow and puckered at tips. Fruits are small and pale.
- *Swede (rutabaga)* and *turnip*- brown or grey concentric rings develop inside the roots.
- *Arecaceae* (*Palm Tree*) - brown spots on fronds & lower productivity.

Manganese

- **Manganese** activates some important enzymes involved in chlorophyll formation. Manganese deficient plants will develop chlorosis between the veins of its leaves. The availability of manganese is partially dependent on soil pH.

Manganese (Mn) deficiency is a plant disorder that is often confused with, and occurs with, iron deficiency. Most common in poorly drained soils, also where organic matter levels are high. Manganese may be unavailable to plants where pH is high.

Affected plants include onion, apple, peas, French beans, cherry and raspberry, and symptoms include yellowing of leaves with smallest leaf veins remaining green to produce a ‘chequered’ effect. The plant may seem to grow away from the problem so that younger leaves may appear to be unaffected. Brown spots may appear on leaf surfaces, and severely affected leaves turn brown and wither.

Magnesium deficiency

Magnesium has a major part in the tetrapyrrolic chlorophyll meolcule formation, it act as an enzyme activator of hexokinase, phosphorylase, carboxylase, dehydrgenase, peptidase, and also it plays as role in photosynthesis and buffer.

Magnesium (Mg) deficiency is a plant disorder with two main causes. Magnesium can be easily washed out of light soils in wet seasons or excessive potassium fertilizer usage can cause also Mg to become unavailable to the growing plant.

This disorder particularly affects potatoes, tomatoes, apples, currants and gooseberries, and chrysanthemums.

Symptoms include, yellowing between leaf veins, which stay green, giving a marbled appearance. This begins with older leaves and spreads to younger growth. Can be confused with virus, or natural aging in the case of tomato plants. Fruits are small and woody.

Zinc

Zinc participates in chlorophyll formation, and also activates many enzymes. It alsotake part in tryptophane synthesis (precurssor for auxin), phosphorylaction enzymes, enzyems in chloroplasts

Symptoms of zinc deficiency include chlorosis and stunted growth.

NOTES

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UNIT II

Photosynthesis: Photosynthetic pigments, Emerson enhancement effect, Absorption spectrum and Action spectrum. Cyclic and noncyclic elctron transport. Dark reaction. Calvin cycle. Hatch and Slack cycle.

Respiration: aerobic and anaerobic respiration. Glycolysis, Kreb's cycle. Electron transport system. Respiration quotient.

Photosynthesis

Photosynthesis The chemical process by which green plants and other phototrophs synthesize organic compounds from carbon dioxide and water in the presence of sunlight.

In plants and most algae it occurs in the chloroplasts and there are two principal types of reactions.

In the **light-dependent** reactions, which require the presence of light, energy from sunlight is absorbed by photosynthetic pigments (chiefly the green pigment chlorophyll) and used to bring about the **photolysis** of water:

$$H_2O \rightarrow 2H^+ + 2e^- + \frac{1}{2}O_2$$

The electrons released by this reaction pass along a series of electron carrier molecules; as they do so they lose their energy, which is used to convert ADP to ATP in the process of **photophosphorylation**. The electrons and protons produced by the photolysis of water are used to reduce NADP:

$$2H^+ + 2e^- + NADP^+ \rightarrow NADPH + H^+$$

The ATP and NADPH produced during the light-dependent reactions provide energy and reducing power, respectively, for the ensuing **light-independent** reactions (formerly called the 'dark reaction'), which nevertheless cannot be sustained without the ATP generated by the light-dependent reactions. During these reactions **carbon dioxide is reduced** to carbohydrate in a metabolic pathway known as the **Calvin** cycle.

Photosynthesis can be summarized by the equation:

$$CO_2 + 2H_2O \rightarrow [CH_2O] + H_2O + O_2$$

Since virtually all other forms of life are directly or indirectly dependent on plants for food, photosynthesis is the basis for all life on earth.

Furthermore virtually all the atmospheric oxygen has originated from oxygen released during photosynthesis.

Photosynthetic Pigments

Pigments are colorful compounds.

Pigments are chemical compounds which reflect only certain wavelengths of visible light. More important than their reflection of light is the ability of pigments to **absorb** certain wavelengths.

Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other **autotrophs** -organisms which make their own food using **photosynthesis**.

In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy.

There are three basic classes of pigments.

All photosynthetic organisms contain one or more organic pigments capable of absorbing visible radiation, which will initiate the photochemical reactions of photosynthesis.

The three major classes of pigments found in plants and algae are the **chlorophylls**, the **carotenoids** and the **phycobilins**. Carotenoids and phycobilins are called **accessory pigments** since the **quanta** (packets of light) absorbed by these pigments can be transferred to chlorophyll.

Chlorophylls are greenish pigments which contain a **porphyrin ring** 'head' and a **phytol** 'tail'. The polar (water-soluble) head is made up of a tetrapyrrole ring and a magnesium ion complexed with the nitrogen atoms of the ring. The phytol tail extends into the lipid layer of the thylakoid membrane.This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll.

- chlorophyll a - present in all higher plants and algae
- chlorophyll b - present in all higher plants and green algae
- chlorophyll c - diatoms and brown algae
- chlorophyll d - red algae
- (chlorophyll a is present in all photosynthetic organisms that evolve O_2.)

The most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups were one of the first clues that they were not as closely related as previously thought.

Carotenoids are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color.

These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. Carotenoids contain a conjugated double bond system of the polyene type (C-C=C-C=C).

Energy absorbed by carotenoids may be transferred to chlorophyll a for photosynthesis. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**.

Types of Carotenoids (carotenes and xanthophylls)

Carotenes: alpha-carotene - higher plants and most algae
beta-carotene - most plants & some algae

xanthophylls: luteol, fucoxanthol and violaxanthol

One very visible accessory pigment is **fucoxanthin** the brown pigment which colours kelps and other brown algae as well as the diatoms.

Phycobilins are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only in Cyanobacteria and Rhodophyta (in blur-green algae and red algae):

These are linear tertapyrroles structurally related to chlorophyll a but lack the phytol side chain and magnesium ion. The red algae have phycoerythrins that enable them to absorb light in the blue-green region of the spectrum which reaches deep-sea depths.

Types of phycobilins:

- phycoerythrin
- phycocyanin
- allophycocyanin

Phycobilins are not only useful to the organisms which use them for soaking up light energy; they have also found use as research tools. Both pycocyanin and phycoerythrin **fluoresce** at a particular wavelength. That is, when they are exposed to strong light, they absorb the light energy, and release it by emitting light of a very narrow range of wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are chemically bonded to antibodies, which are then put into a solution of cells. When the solution is sprayed as a stream of fine droplets past a laser and computer sensor, a machine can identify whether the cells in the droplets have been "tagged" by the antibodies. This has found extensive use in cancer research, for "tagging" tumor cells.

So higher plants and algae have various pigments, which allow them to capture the available solar radiation most efficiently. The relative abundance of the pigments depends on factors like the species, location of the plants and seasons.

Light energy is available in discrete packets called **quanta**. The longer the wavelength of radiation, the less energy that radiation contains. The visible spectrum is a small part of the electromagnetic spectrum, from about 400 nm (blue) to 700 nm (red).

Action & Absorption Spectra

When the pigments involved in photosynthesis are subjected to different wavelengths of light, they absorb some wavelengths more than others. **A graph showing the degree of absorption of light by a pigment is referred to as the absorption spectrum for that pigment**. Chlorophylls absorb strongly in the blue-violet and red regions of the spectrum (and not in the green region, hence leaves containing chlorophyll appear green), while carotenoids absorb in the blue and green regions. The colour of the carotenoids (yellow to orange and red) is usually masked by that of the chlorophylls, which are present in larger quantities.

A graph showing the degree to which different wavelengths affect photosynthesis is called the action spectrum for photosynthesis. The action spectrum for photosynthesis is closely correlated with the action spectra for chlorophylls a and b and the carotenoids. This suggests that these are the main pigments involved in harvesting light in photosynthesis.

Action Spectra

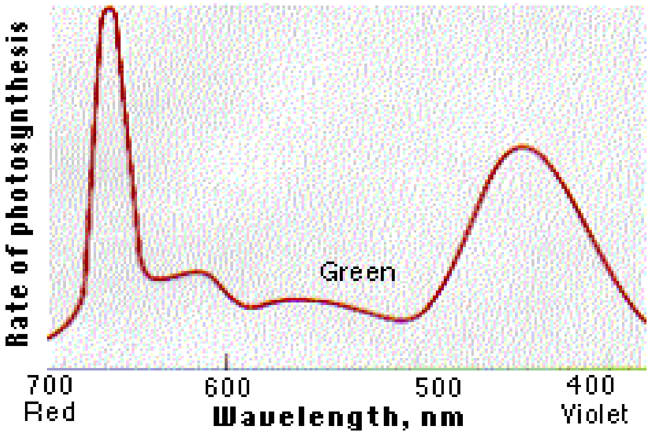
An action spectrum is the rate of a physiological activity plotted against wavelength of light.

In 1881, the German plant physiologist T. W. Engelmann placed a filamentous green alga under the microscope and illuminated it with a tiny spectrum of visible light.

In the medium surrounding the strands were motile, aerobic bacteria. After a few minutes, the bacteria had congregated around the portions of the filament illuminated by red and blue light.

Assuming that the bacteria were congregating in regions where **oxygen** was being evolved in photosynthesis, Engelmann concluded that red and blue light are the most effective colors for photosynthesis.

With modern instruments, a plot of the rate of photosynthesis as a function of wavelength of light produces a graph like this. More precise than Engelmann's but telling the same story.



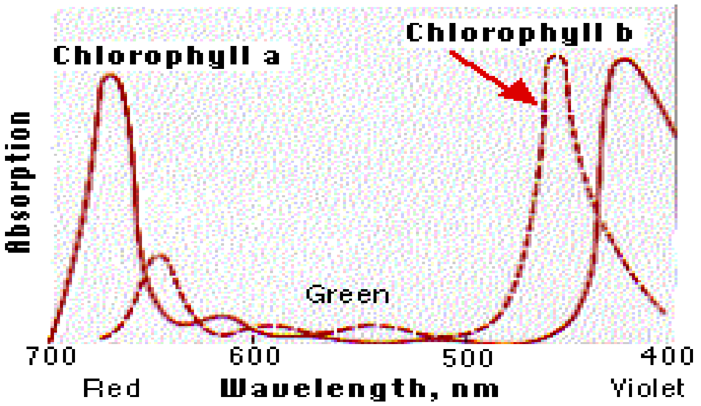
Absorption Spectra

An absorption spectrum is a spectrum of radiant energy whose intensity at each wavelength is a measure of the amount of energy at that wavelength that has passed through a selectively absorbing substance.

The absorption of radiation by a substance can be quantified with an instrument called a **spectrophotometer**. This is a device that

- produces a beam of monochromatic ("single-color") radiation that can be shifted progressively across the spectrum;
- passes the beam through a solution of the substance, and
- measures the radiation that gets through.

The graph shows the absorption spectrum of a mixture of chlorophyll a and chlorophyll b in the range of visible light.



Note that both chlorophylls absorb light most strongly in the red and violet portions of the spectrum. Green light is poorly absorbed so when white light (which contains the entire visible spectrum) shines on leaves, green rays are transmitted and reflected giving leaves their green color.

The similarity of the action spectrum of photosynthesis and the absorption spectrum of chlorophyll tells us that chlorophylls are the most important pigments in the process. The spectra are not identical, though, because carotenoids, which absorb strongly in the blue, play a role as well.

The carotenoids help fill in the absorption gaps of chlorophyll so that a larger part of the sun's spectrum can be used. The energy absorbed by these "antenna pigments"is passed to chlorophyll a where it drives the light reactions of photosynthesis.

Emerson Enhancement effect

This effect is the property of higher plant photosynthesis, discovered by Robert Emerson. Emerson measured the quantum yield of photosynthesis, and found that 8 quanta of light energy would be required for the ruduction of one molecule of carbondioxide to carbohydrate (or for producing 1 molecule of oxygen). The quantum yield is thus 12 percent. Since the transfer of four electrons is involved in the reduction of one CO2 molecule, it wa suggested that it takes two light quanta to move each electron.

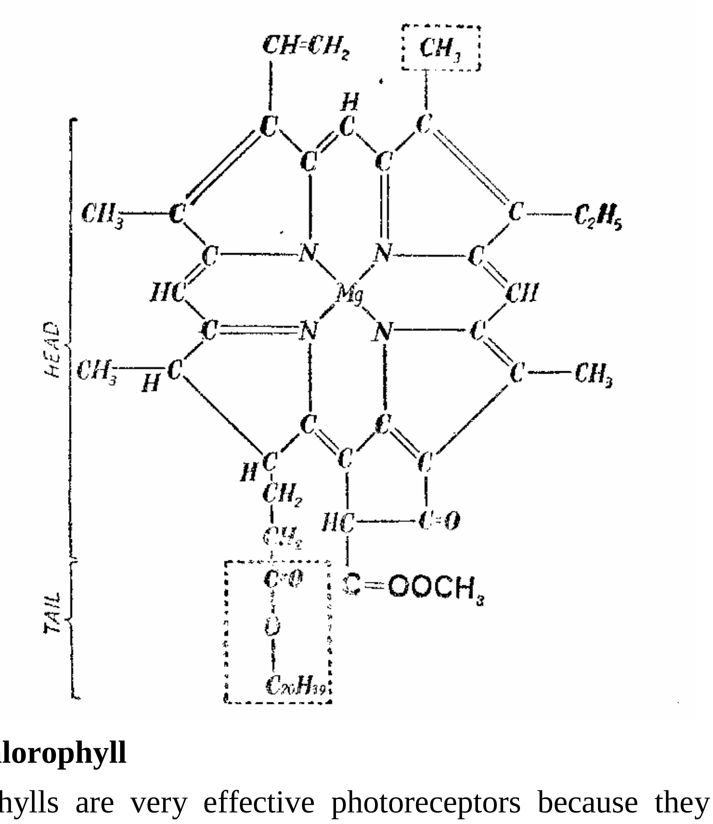
He later determined the quantum yield of photosynbthesis under different wavelenghts and found that there ws a pronounced decrease in quantum yield at wave lengths greater than 680 m□□in the red zone (red drop). It was also found that this decrease could be offset if shorter wavelength was also given to the plant simultaneously. This simultaneous giving of two wavelenthts suprisingly gave a photosynthetic rate higher than the total rate got from the two beams of light used separately. This photosynthetic enhancement is referred to as the Emerson enhancement effect.

The quantum yield of red light (less than 680nm) and far red light (700nm), when shone simultaneously on a plant, is greater than the sum of the yields of the light of the two wavelengths separately. The two photochemical processes are believed to be associated with two different specific groups of pigments called pigment system I and pigment system II. This effect provides evidence for the cooperative interaction of two photosystems in photosynthesis.

Cyclic and non-cyclic electron transport

Light Absorption by Chlorophyll Induces Electron Transfer

The trapping of light energy is the key to photosynthesis. The first event is the absorption of light by a photoreceptor molecule. The principal photoreceptor in the chloroplasts of most green plants is *chlorophyll* a, a substituted tetrapyrrole. The four nitrogen atoms of the pyrroles are coordinated to a magnesium ion. Unlike a porphyrin such as heme, chlorophyll has a reduced pyrrole ring. Another distinctive feature of chlorophyll is the presence of *phytol*, a highly hydrophobic 20-carbon alcohol, esterified to an acid side chain.



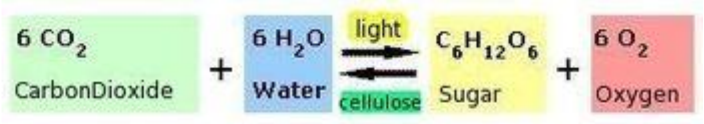
Structure of chlorophyll

Chlorophylls are very effective photoreceptors because they contain networks of alternating single and double bonds. Such compounds are called *polyenes*. They have very strong absorption bands in the visible region of the spectrum, where the solar output reaching Earth also is maximal. The peak molar absorption coefficient of chlorophyll *a* is higher than $10^5\ M^{-1}\ cm^{-1}$, among the highest observed for organic compounds.

What happens when light is absorbed by a molecule such as chlorophyll? The energy from the light excites an electron from its ground energy level to an excited energy level. This high-energy electron can have several fates. For most compounds that absorb light, the electron simply returns to the ground state and the absorbed energy is converted into heat. However, if a suitable electron acceptor is nearby, the excited electron can move from the initial molecule to the acceptor. This process results in the formation of a positive charge on the initial molecule (due to the loss of an electron) and a negative charge on the acceptor and is, hence, referred to as *photoinduced charge separation*. The site where the separational change occurs is called the *reaction center*. We shall see how the photosynthetic apparatus is arranged to make photoinduced charge separation extremely efficient. The electron, extracted from its initial site by absorption of light, can reduce other species to store the light energy in chemical forms.

Photophosphorylation The formation of ATP from ADP and inorganic phosphate using light energy in photosynthesis (compare oxidative phosphorylation). There are two pathways, noncyclic and cyclic photophosphorylation, which occur in the thylakoid membranes of the chloroplasts. In noncyclic photophosphorylation electrons derived from the photolysis of water are raised to higher energy levels in photosystems I and IIand pass along an electron transport chain of carrier molecules (see ferredoxin;plastocyanin; plastoquinone) to NADP reductase. This enzyme transfers electrons to $NADP^+$ to make NADPH, which provides reducing power for the light-independent reactions of photosynthesis. In cyclic photophosphorylationthe electrons from photosystem I that are raised to a higher energy level are recycled through the electron carrier system back to photosystem I. Both pathways of electron flow cause H^+ ions to be pumped by a group ofcytochromes, the cytochrome b_6–f complex, across the thylakoid membrane. This creates a proton gradient that drives the phosphorylation of ADP to ATP by the enzyme ATP synthetase (see chemiosmotic theory).

The Reactions of Photosynthesis



Formula for the type of photosynthesis that occurs in plants.

Photosynthesis[α] is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. Photosynthesis occurs in plants, algae, and many species of Bacteria, but not in Archaea. Photosynthetic organisms are called *photoautotrophs*, since it allows them to create their own food. In plants, algae and cyanobacteria photosynthesis uses carbon dioxide and water, releasing oxygen as a waste product.

Photosynthesis is vital for life on Earth. As well as maintaining the normal level of oxygen in the atmosphere, nearly all life either depends on it directly as a source of energy, or indirectly as the ultimate source of the energy in their food. The amount of energy trapped by photosynthesis is immense, approximately 100 terawatts: which is about six times larger than the power consumption of human civilization. As well as energy, photosynthesis is also the source of the carbon in all the organic compounds within organisms' bodies. In all, photosynthetic organisms convert around 100,000,000,000 tonnes of carbon into biomass per year.

Although photosynthesis can occur in different ways in different species, some features are always the same. For example, the process always begins when energy from light is absorbed by proteins called photosynthetic reaction centers that contain chlorophylls. In plants, these proteins are held inside organelles called chloroplasts, while in bacteria they are embedded in the plasma membrane. Some of the light energy gathered by chlorophylls is stored in the form of adenosine triphosphate (ATP). The rest of the energy is used to remove electrons from a substance such as water. These electrons are then used in the reactions that turn carbon dioxide into organic compounds.

In plants, algae and cyanobacteria this is done by a sequence of reactions called the Calvin cycle, but different sets of reactions are found in some bacteria, such as the reverse Krebs cycle in *Chlorobium*. Many photosynthetic organisms have adaptations that concentrate or store carbon dioxide. This helps reduce a wasteful process called photorespiration that can consume part of the sugar produced during photosynthesis.

Photosynthesis splits water to liberate O_2 and fixes CO_2 into sugar

Photosynthetic organisms are photoautotrophs, which means that they are able to synthesize food directly from carbon dioxide using energy from light. However, not all organisms that use light as a source of energy carry out photosynthesis, since *photoheterotrophs* use organic compounds, rather than carbon dioxide, as a source of carbon.

In plants, algae and cyanobacteria, during photosynthesis releases oxygen. This is called *oxygenic photosynthesis*. Although there are some differences between oxygenic photosynthesis in plants, algae and cyanobacteria, the overall process is quite similar in these organisms. However, there are some types of bacteria that carry out *anoxygenic photosynthesis*, which consumes carbon dioxide but does not release oxygen.

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs to supply both a source of energy to drive this process, and also the electrons needed to convert carbon dioxide into carbohydrate, which is a reduction reaction. In general outline, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy. However, the two processes take place through a different sequence of chemical reactions and in different cellular compartments.

The general equation for photosynthesis is therefore:

$$n\ CO_2 + n\ H_2A + \text{photons} \rightarrow (CH_2O)_n + n\ O_2 + n\ A$$

carbon dioxide + electron donor + light energy → carbohydrate + oxygen + oxidized electron donor

Since water is used as the electron donor in oxygenic photosynthesis, the equation for this process is:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2$$

carbon dioxide + water + light energy → carbohydrate + oxygen

Photosynthesis occurs in two stages. In the first stage, *light-dependent reactions* or *light reactions* capture the energy of light and use it to make the energy-storage molecules ATP and NADPH. During the second stage, the *light-independent reactions* use these products to capture and reduce carbon dioxide.

The proteins that gather light for photosynthesis are embedded within cell membranes. The simplest way these are arranged is in photosynthetic bacteria, where these proteins are held within the plasma membrane. However, this membrane may be tightly-folded into cylindrical sheets called thylakoids, or bunched up into round vesicles called *intracytoplasmic membranes*. These structures can fill most of the interior of a cell, giving the membrane a very large surface area and therefore increasing the amount of light that the bacteria can absorb.

In plants and algae, photosynthesis takes place in organelles called chloroplasts. A chloroplast has both an inner and an outer phospholipid membrane. Between these two layers is the intermembrane space. A typical plant cell contains about 10 to 100 chloroplasts. Within the stroma are stacks of thylakoids, the sub-organelles which are the site of photosynthesis. The thylakoids are arranged in stacks called grana (singular: granum). A thylakoid has a flattened disk shape. Inside it is an empty area called the thylakoid space or lumen. The thylakoid membrane contains many integral and peripheral membrane proteins. The proteins complexes which contain special pigments absorbing light energy are called photosystems.

Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green color. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Algae also use chlorophyll, but various other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthol in brown algae and diatoms resulting in a wide variety of colors.

These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a light-harvesting complex.

Although all cells in the green parts of a plant have chloroplasts, most of the energy is captured in the leaves. The cells in the interior tissues of a leaf, called the mesophyll, can contain between 450,000 and 800,000 chloroplasts for every square millimeter of leaf. The surface of the leaf is uniformly coated with a water-resistant waxy cuticle that protects the leaf from excessive evaporation of water and decreases the absorption of ultraviolet or blue light to reduce heating. The transparent epidermis layer allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

Light reactions

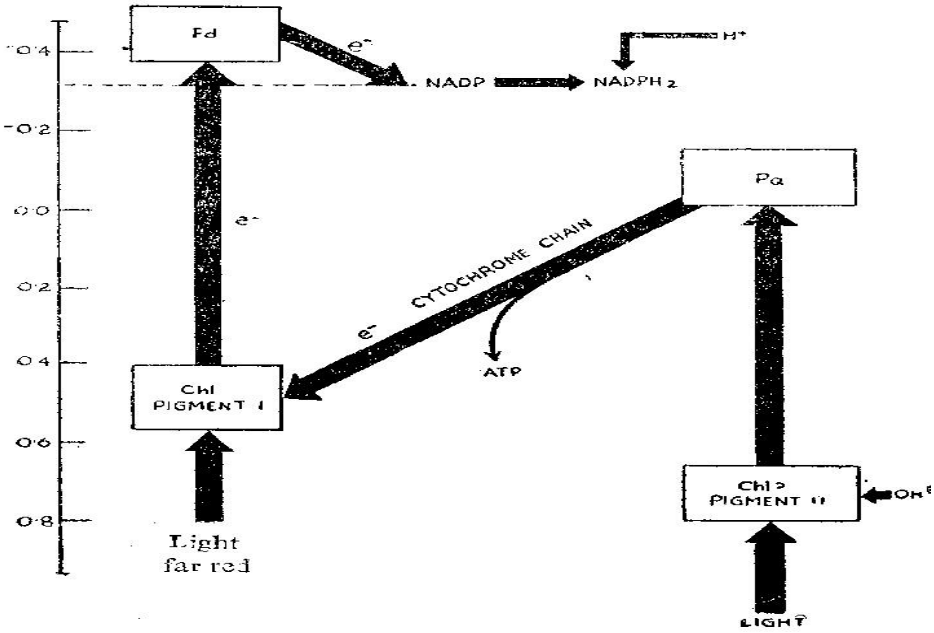
In the light reactions, one molecule of the pigment chlorophyll absorbs one photon and loses one electron. This electron is passed to a modified form of chlorophyll called pheophytin, which passes the electron to a quinone molecule, allowing the start of a flow of electrons down an electron transport chain that leads to the ultimate reduction of NADP to NADPH. In addition, this creates a proton gradient across the chloroplast membrane; its dissipation is used by ATP synthase for the concomitant synthesis of ATP. The chlorophyll molecule regains the lost electron from a water molecule through a process called photolysis, which releases a dioxygen (O_2) molecule. The overall equation for the light-dependent reactions under the conditions of non-cyclic electron flow in green plants is:

$2\ H_2O + 2\ NADP^+ + 2\ ADP + 2\ P_i + light \rightarrow 2\ NADPH + 2\ H^+ + 2\ ATP + O_2$

Not all wavelengths of light can support photosynthesis. The photosynthetic action spectrum depends on the type of accessory pigments present. For example, in green plants, the action spectrum resembles the absorption spectrum for chlorophylls and carotenoids with peaks for violet-blue and red light. In red algae, the action spectrum overlaps with the absorption spectrum of phycobilins for blue-green light, which allows these algae to grow in deeper waters that filter out the longer wavelengths used by green plants. The non-absorbed part of the light spectrum is what gives photosynthetic organisms their color (e.g., green plants, red algae, purple bacteria) and is the least effective for photosynthesis in the respective organisms.

Z scheme

In plants, light-dependent reactions occur in the thylakoid membranes of the chloroplasts and use light energy to synthesize ATP and NADPH. The light-dependent reaction has two forms: cyclic and non-cyclic. In the non-cyclic reaction, the photons are captured in the light-harvesting antenna complexes of photosystem II by chlorophyll and other accessory pigments (see diagram at right). When a chlorophyll molecule at the core of the photosystem II reaction center obtains sufficient excitation energy from the adjacent antenna pigments, an electron is transferred to the primary electron-acceptor molecule, Pheophytin, through a process called photoinduced charge separation. These electrons are shuttled through an electron transport chain, the so called ***Z-scheme*** shown in the diagram, that initially functions to generate a chemiosmotic potential across the membrane.

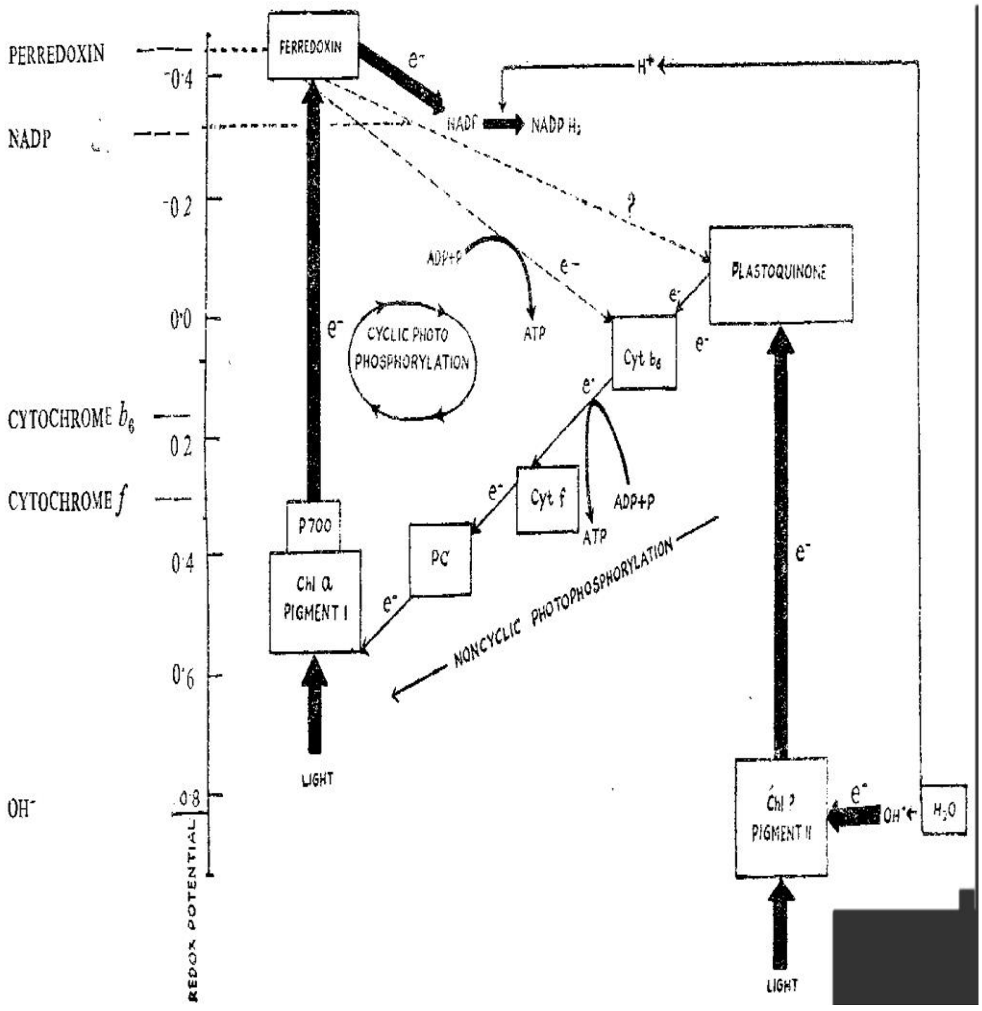


A schematic representation of light-induced electron flow involving pigment system I and II in the chloroplast

An ATP synthase enzyme uses the chemiosmotic potential to make ATP during photophosphorylation, whereas NADPH is a product of the terminal redox reaction in the *Z-scheme*. The electron enters the Photosystem I molecule. The electron is excited due to the light absorbed by the photosystem. A second electron carrier accepts the electron, which again is passed down lowering energies of electron acceptors. The energy created by the electron acceptors is used to move hydrogen ions across the thylakoid membrane into the lumen. The electron is used to reduce the co-enzyme NADP, which has functions in the light-independent reaction. The cyclic reaction is similar to that of the non-cyclic, but differs in the form that it generates only ATP, and no reduced NADP (NADPH) is created. The cyclic reaction takes place only at photosystem I. Once the electron is displaced from the photosystem, the electron is passed down the electron acceptor molecules and returns back to photosystem I, from where it was emitted, hence the name *cyclic reaction*.

Water photolysis

The NADPH is the main reducing agent in chloroplasts, providing a source of energetic electrons to other reactions. Its production leaves chlorophyll with a deficit of electrons (oxidized), which must be obtained from some other reducing agent. The excited electrons lost from chlorophyll in photosystem I are replaced from the electron transport chain by plastocyanin. However, since photosystem II includes the first steps of the *Z-scheme*, an external source of electrons is required to reduce its oxidized **chlorophyll *a*** molecules. The source of electrons in green-plant and cyanobacterial photosynthesis is water. Two water molecules are oxidized by four successive charge-separation reactions by photosystem II to yield a molecule of diatomic oxygen and four hydrogen ions; the electron yielded in each step is transferred to a redox-active tyrosine residue that then reduces the photoxidized paired-chlorophyll *a* species called P680 that serves as the primary (light-driven) electron donor in the photosystem II reaction center.



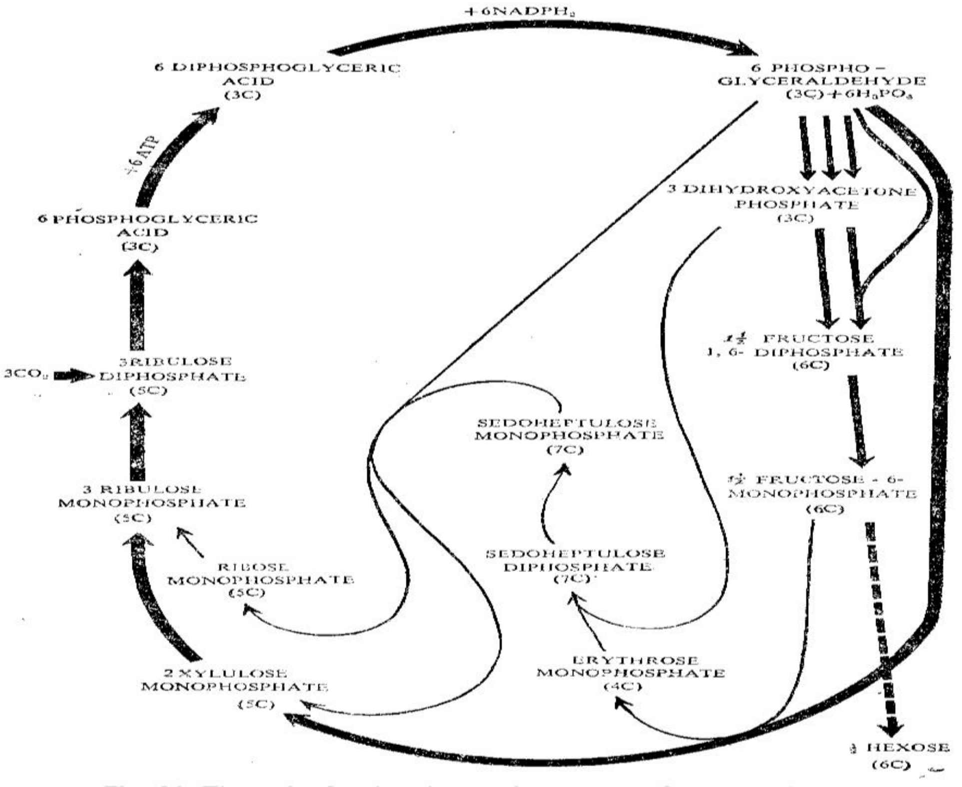
Schematic representation of two photochemical reactions within chloroplast PI=pigment system I, Fd=ferredoxin, Pc=plstocyanin, Cyt f= cytochrome f, Cyt b6= cytochrome b6, PQ= plastoquinone, P II= pigment system II

The oxidation of water is catalyzed in photosystem II by a redox-active structure that contains four manganese ions and a calcium ion; this oxygenevolving complex binds two water molecules and stores the four oxidizing equivalents that are required to drive the water-oxidizing reaction. Photosystem II is the only known biological enzyme that carries out this oxidation of water. The hydrogen ions contribute to the transmembrane chemiosmotic potential that leads to ATP synthesis. Oxygen is a waste product of light-dependent reactions, but the majority of organisms on Earth use oxygen for cellular respiration, including photosynthetic organisms.

The Calvin Cycle

The **Calvin cycle** (or **Calvin-Benson-Bassham cycle**, light-independent reaction, or carbon fixation) is a series of biochemical reactions that take place in the stroma of chloroplasts in photosynthetic organisms. It was discovered by Melvin Calvin, James Bassham and Andrew Benson at the University of California, Berkeley. It is one of the light-independent reactions or dark reactions.

During photosynthesis, light energy is used to generate chemical free energy, stored in glucose. The light-independent Calvin cycle, also (misleadingly) known as the "dark reaction" or "dark stage," uses the energy from short-lived electronically-excited carriers to convert carbon dioxide and water into organic compoundsthat can be used by the organism (and by animals that feed on it). This set of reactions is also called *carbon fixation*. The key enzyme of the cycle is called RuBisCO. In the following equations, the chemical species (phosphates and carboxylic acids) exist in equilibria among their various ionized states as governed by the pH.



The path of carbon in calvin cycle

The enzymes in the Calvin cycle are functionally equivalent to many enzymes used in other metabolic pathways such as gluconeogenesis and the pentose phosphate pathway, but they are to be found in the chloroplast stroma instead of the cell cytoplasm, separating the reactions. They are activated in the light (which is why the name "dark reaction" is misleading), and also by products of the light-dependent reaction. These regulatory functions prevent the Calvin cycle from being respired to carbon dioxide. Energy (in the form of ATP) would be wasted in carrying out these reactions that have no net productivity.

Steps of the Calvin cycle

1. The enzyme RuBisCO catalyses the carboxylation of Ribulose-1,5-bisphosphate, a 5-carbon compound, by carbon dioxide (a total of 6 carbons) in a two-step reaction. The initial product of the reaction is a six-carbon intermediate so unstable that it immediately splits in half, forming two molecules of glycerate 3-phosphate, a 3-carbon compound. (also: 3-phosphoglycerate, 3-phosphoglyceric acid, 3PGA)
2. The enzyme phosphoglycerate kinase catalyses the phosphorylation of 3PGA by ATP (which was produced in the light-dependent stage). 1,3-bisphosphoglycerate (glycerate-1,3-bisphosphate) and ADP are the

products. (However, note that two PGAs are produced for every CO_2 that enters the cycle, so this step utilizes 2ATP per CO_2 fixed.)

3. The enzyme G3P dehydrogenase catalyses the reduction of 1,3BPGA by NADPH (which is another product of the light-dependent stage). Glyceraldehyde 3-phosphate (also G3P, GP, TP, PGAL) is produced, and the NADPH itself was oxidized and becomes $NADP^+$. Again, two NADPH are utilized per CO_2 fixed.

(Simplified versions of the Calvin cycle integrate the remaining steps, except for the last one, into one general step - the regeneration of RuBP - also, one G3P would exit here.)

1. Triose phosphate isomerase converts some G3P reversibly into dihydroxyacetone phosphate (DHAP), also a 3-carbon molecule.
2. Aldolase and fructose-1,6-bisphosphatase convert a G3P and a DHAP into fructose 6-phosphate (6C). A phosphate ion is lost into solution.
3. Then fixation of another CO_2 generates two more G3P.
4. F6P has two carbons removed by transketolase, giving erythrose-4-phosphate. The two carbons on transketolase are added to a G3P, giving the ketose xylulose-5-phosphate (Xu5P).
5. E4P and a DHAP (formed from one of the G3P from the second CO_2 fixation) are converted into sedoheptulose-1,7-bisphosphate (7C) by aldolase enzyme.
6. Sedoheptulose-1,7-bisphosphatase (one of only three enzymes of the Calvin cycle that are unique to plants) cleaves sedoheptulose-1,7-bisphosphate into sedoheptulose-7-phosphate, releasing an inorganic phosphate ion into solution.
7. Fixation of a third CO_2 generates two more G3P. The ketose S7P has two carbons removed by transketolase, giving ribose-5-phosphate (R5P), and the two carbons remaining on transketolase are transferred to one of the G3P, giving another Xu5P. This leaves one G3P as the product of fixation of 3 CO_2, with generation of three pentoses which can be converted to Ru5P.
8. R5P is converted into ribulose-5-phosphate (Ru5P, RuP) by phosphopentose isomerase. Xu5P is converted into RuP by phosphopentose epimerase.
9. Finally, phosphoribulokinase (another plant unique enzyme of the pathway) phosphorylates RuP into RuBP, ribulose-1,5-bisphosphate, completing the Calvin *cycle*. This requires the input of one ATP.

Thus, of 6 G3P produced, three RuBP (5C) are made totalling 15 carbons, with only one available for subsequent conversion to hexose. This required 9 ATPs and 6 NADPH per 3 CO_2.

RuBisCO also reacts competitively with O_2 instead of CO_2 in *photorespiration*. The rate of photorespiration is higher at high temperatures. "photorespiration" turns RuBP into 3PGA and 2-phosphoglycolate, a 2-carbon molecule that can be converted via glycolate and glyoxalate to glycine. Via the glycine cleavage system and tetrahydrofolate, two glycines are converted into serine +CO_2. Serine can be converted back to 3-phosphoglycerate. Thus, only 3 of 4 carbons from two phosphoglycolates can be converted back to 3PGA. It can be seen that photorespiration has very negative consequences for the plant, because, rather than fixing CO_2, this process leads to loss of CO_2. C4 carbon fixation evolved to circumvent photorespiration, but can occur only in certain plants living in very warm or tropical climates.

Products of the Calvin cycle

The immediate product of the Calvin cycle is glyceraldehyde-3-phosphate (G3P) and water. Two G3P molecules (or one F6P molecule) that have exited the cycle are used to make larger carbohydrates. In simplified versions of the Calvin cycle, they may be converted to F6P or F5P after exit, but this conversion is also part of the cycle.

Hexose isomerase converts about half of the F6P molecules into glucose-6-phosphate. These are dephosphorylated and the glucose can be used to form starch, which is stored in, for example, potatoes, or cellulose used to build up cell walls. Glucose, with fructose, forms sucrose, a non-reducing sugar that, unlike glucose, is a stable storage sugar.

In the Light-independent or dark reactions the enzyme RuBisCO captures CO_2 from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson Cycle, releases three-carbon sugars, which are later combined to form sucrose and starch. The overall equation for the light-independent reactions in green plants is.

$$3\ CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ P_i + 6\ NADP^+ + 3\ H_2O$$

Overview of the Calvin cycle and carbon fixation

To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then variously used to form other organic compounds, such as the building material cellulose, as precursors for lipid and amino acid biosynthesis, or as a fuel in cellular respiration. The latter occurs not only in plants but also in animals when the energy from plants gets passed through a food chain.

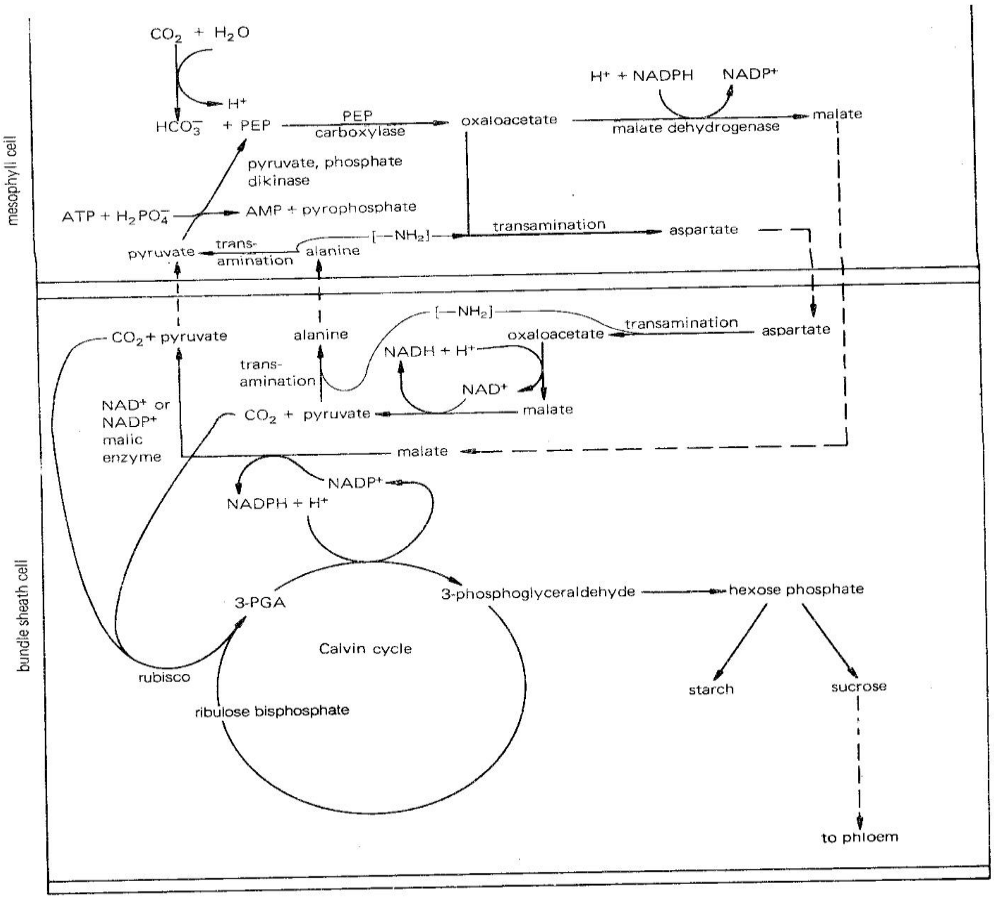
The fixation or reduction of carbon dioxide is a process in which carbon dioxide combines with a five-carbon sugar, ribulose 1,5-bisphosphate (RuBP), to yield two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). GP, in the presence of ATP and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Triose is a 3-carbon sugar (see carbohydrates). Most (5 out of 6 molecules) of the G3P produced is used to regenerate RuBP so the process can continue (see Calvin-Benson cycle). The 1 out of 6 molecules of the triose phosphates not "recycled" often condense to form hexose phosphates, which ultimately yield sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids.

C_4 **and** C_3 **photosynthesis**

In hot and dry conditions, plants will close their stomata to prevent loss of water. Under these conditions, CO_2 will decrease, and dioxygen gas, produced by the light reactions of photosynthesis, will increase in the leaves, causing an increase of photorespiration by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase and decrease in carbon fixation. Some plants have evolved mechanisms to increase the CO_2 concentration in the leaves under these conditions.

Hatch abd Slack cycle (*C_4 plants*)

C_4 plants chemically fix carbon dioxide in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEP carboxylase and which creates the four-carbon organic acid, oxaloacetic acid. Oxaloacetic acid or malate synthesized by this process is then translocated to specialized bundle sheath cells where the enzyme, rubisco, and other Calvin cyle enzymes are located, and where CO_2 released by decarboxylation of the four-carbon acids is then fixed by rubisco activity to the three-carbon sugar 3-Phosphoglyceric acids. The physical separation of rubisco from the oxygen-generating light reactions reduces photorespiration and increases CO_2 fixation and thus photosynthetic capacity of the leaf. C_4 plants can produce more sugar than C_3 plants in conditions of high light and temperature.



Metabolic division of labor in mesophyll and bundle sheath cells of C_4 plants.

Many important crop plants are C_4 plants including maize, sorghum, sugarcane, and millet. Plants lacking PEP-carboxylase are called *C_3 plants* because the primary carboxylation reaction, catalyzed by rubisco, produces the three-carbon sugar 3-phosphoglyceric acids directly in the Calvin-Benson Cycle.

Factors affecting photosynthesis

There are three main factors affecting photosynthesis and several corollary factors. The three main are:

- Light irradiance and wavelength
- Carbon dioxide concentration
- Temperature.

Light intensity (irradiance), wave length and temperature

In the early 1900s Frederick Frost Blackman along with Gabrielle Matthaei investigated the effects of light intensity (irradiance) and temperature on the rate of carbon assimilation.

- At constant temperature, the rate of carbon assimilation varies with irradiance, initially increasing as the irradiance increases. However at higher irradiance this relationship no longer holds and the rate of carbon assimilation reaches a plateau.
- At constant irradiance, the rate of carbon assimilation increases as the temperature is increased over a limited range. This effect is only seen at high irradiance levels. At low irradiance, increasing the temperature has little influence on the rate of carbon assimilation.

These two experiments illustrate vital points: firstly, from research it is known that photochemical reactions are not generally affected by temperature. However, these experiments clearly show that temperature affects the rate of carbon assimilation, so there must be two sets of reactions in the full process of carbon assimilation. These are of course the light-dependent 'photochemical' stage and the light-independent, temperature-dependent stage. Second, Blackman's experiments illustrate the concept of limiting factors. Another limiting factor is the wavelength of light. Cyanobacteria, which reside several meters underwater, cannot receive the correct wavelengths required to cause photoinduced charge separation in conventional photosynthetic pigments. To combat this problem, a series of proteins with different pigments surround the reaction center.This unit is called a phycobilisome.

Carbon dioxide levels and photorespiration

As carbon dioxide concentrations rise, the rate at which sugars are made by the light-independent reactions increases until limited by other factors. RuBisCO, the enzyme that captures carbon dioxide in the light-independent reactions, has a binding affinity for both carbon dioxide and oxygen. When the concentration of carbon dioxide is high, RuBisCO will fix carbon dioxide. However, if the carbon dioxide concentration is low, RuBisCO will bind oxygen instead of carbon dioxide. This process, called photorespiration, uses energy, but does not produce sugars.

RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

1. One product of oxygenase activity is phosphoglycolate (2 carbon) instead of 3-phosphoglycerate (3 carbon). Phosphoglycolate cannot be metabolized by the Calvin-Benson cycle and represents carbon lost from the cycle. A high oxygenase activity, therefore, drains the sugars

that are required to recycle ribulose 5-bisphosphate and for the continuation of the Calvin-Benson cycle.

2. Phosphoglycolate is quickly metabolized to glycolate that is toxic to a plant at a high concentration; it inhibits photosynthesis.
3. Salvaging glycolate is an energetically expensive process that uses the glycolate pathway and only 75% of the carbon is returned to the Calvin-Benson cycle as 3-phosphoglycerate. The reactions also produce ammonia (NH_3) which is able to diffuse out of the plant leading to a loss of nitrogen.

A highly-simplified summary is:

2 glycolate + ATP → 3-phosphoglycerate + carbon dioxide + ADP +NH_3

The salvaging pathway for the products of RuBisCO oxygenase activity is more commonly known as photorespiration, since it is characterized by light-dependent oxygen consumption and the release of carbon dioxide.

Respiration

Cellular respiration is the set of the metabolic reactions and processes that take place in organisms' cells to convert biochemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products. The reactions involved in respiration are catabolic reactions that involve the oxidation of one molecule and the reduction of another.

Nutrients commonly used by animal and plant cells in respiration include glucose, amino acids and fatty acids, and a common oxidizing agent (electron acceptor) is molecular oxygen (O_2). Bacteria and archaea can also be lithotrophs and these organisms may respire using a broad range of inorganic molecules as electron donors and acceptors, such as sulfur, metal ions, methane or hydrogen. Organisms that use oxygen as a final electron acceptor in respiration are described as aerobic, while those that do not are referred to as anaerobic.

The energy released in respiration is used to synthesize ATP to store this energy. The energy stored in ATP can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell membranes. Because of its ubiquity in nature, ATP is also known as the "universal energy currency".

Aerobic respiration

Aerobic respiration is the main means by which both plants and animals utilize energy in the form of organic compounds that was previously created through photosynthesis .

Aerobic respiration requires oxygen in order to generate energy (ATP). It is the preferred method of pyruvate breakdown from glycolysis and requires that pyruvate enter the mitochondrion in order to be fully oxidized by the Krebs cycle. The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation, NADH and $FADH_2$.

The reducing potential of NADH and $FADH_2$ is converted to more ATP through an electron transport chain with oxygen as the "terminal electron acceptor". Most of the ATP produced by aerobic cellular respiration is made by oxidative phosphorylation. This works by the energy released in the consumption of pyruvate being used to create a chemiosmotic potential by pumping protons across a membrane. This potential is then used to drive ATP synthase and produce ATP from ADP. Biology textbooks often state that 38 ATP molecules can be made per oxidised glucose molecule during cellular respiration (2 from glycolysis, 2 from the Krebs cycle, and about 34 from the electron transport system). However, this maximum yield is never quite reached due to losses (leaky membranes) as well as the cost of moving pyruvate and ADP into the mitochondrial matrix and current estimates range around 29 to 30 ATP per glucose.

Aerobic metabolism is 19 times more efficient than anaerobic metabolism (which yields 2 mol ATP per 1 mol glucose). They share the initial pathway of glycolysis but aerobic metabolism continues with the Krebs cycle and oxidative phosphorylation. The post glycolytic reactions take place in the mitochondria in eukaryotic cells, and in the cytoplasm in prokaryotic cells.

Glycolysis or (Embden–Meyerhof pathway)

Glycolysis is a metabolic pathway that is found in the cytoplasm of cells in all living organisms and is anaerobic, or doesn't require oxygen. The process converts one molecule of glucose into two molecules of pyruvate, and makes energy in the form of two net molecules of ATP. Four molecules of ATP per glucose are actually produced; however, two are consumed for the preparatory phase. The initial phosphorylation of glucose is required to destabilize the molecule for cleavage into two triose sugars. During the pay-off phase of glycolysis, four phosphate groups are transferred to ADP by substrate-level phosphorylation to make four ATP, and two NADH are produced when the triose sugars are oxidized. The overall reaction can be expressed this way:

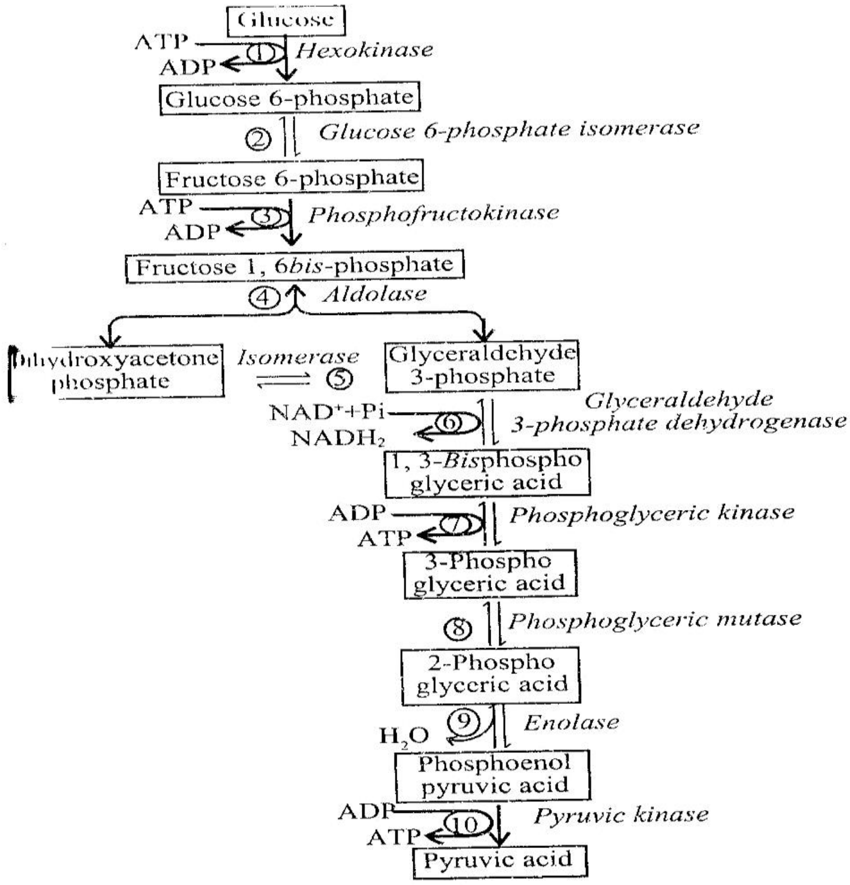
$$\text{Glucose} + 2\ NAD^+ + 2\ P_i + 2\ ADP \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ ATP + 2\ H^+ + 2\ H_2O$$

Glycolysis (from *glycose*, an older term for glucose + *-lysis* degradation) is the metabolic pathway that converts glucose, $C_6H_{12}O_6$, into pyruvate, $C_3H_3O_3^-$. The free energy released in this process is used to form the high energy compounds, ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

Glycolysis is a sequence of ten reactions involving ten intermediate compounds (one of the steps involves two intermediates). The intermediates provide entry points to glycolysis. For example, most monosaccharides, such as fructose, glucose, and galactose, can be converted to one of these intermediates. The intermediates may also be directly useful. For example, the intermediate dihydroxyacetone phosphate is a source of the glycerol that combines with fatty acids to form fat.

Glycolysis is thought to be the archetype of a universal metabolic pathway. It occurs, with variations, in nearly all organisms, both aerobic and anaerobic. The wide occurrence of glycolysis indicates that it is one of the most ancient known metabolic pathways.

The most common type of glycolysis is the *Embden-Meyerhof pathway*, which was first discovered by Gustav Embden and Otto Meyerhof. Glycolysis also refers to other pathways, such as the *Entner-Doudoroff Pathway*. However, the discussion here will be limited to the Embden-Meyerhof pathway.



EMP pathway of glycolysis

The use of symbols in this equation makes it appear unbalanced with respect to oxygen atoms, hydrogen atoms and charges. Atom balance is maintained by the two phosphate (P_i) groups:

- each exists in the form of a hydrogen phosphate anion (HPO_4^{2-}), dissociating to contribute 2 H^+ overall
- each liberates an oxygen atom when it binds to an ADP molecule, contributing 2 O overall

Charges are balanced by the difference between ADP and ATP. In the cellular environment all three hydroxy groups of ADP dissociate into $-O^-$ and H^+, giving ADP^{3-}, and this ion tends to exist in an ionic bond with Mg^{2+}, giving $ADPMg^-$. ATP behaves identically except that it has four hydroxy groups, giving $ATPMg^{2-}$. When these differences along with the true charges on the two phosphate groups are considered together, the net charges of -4 on each side are balanced.

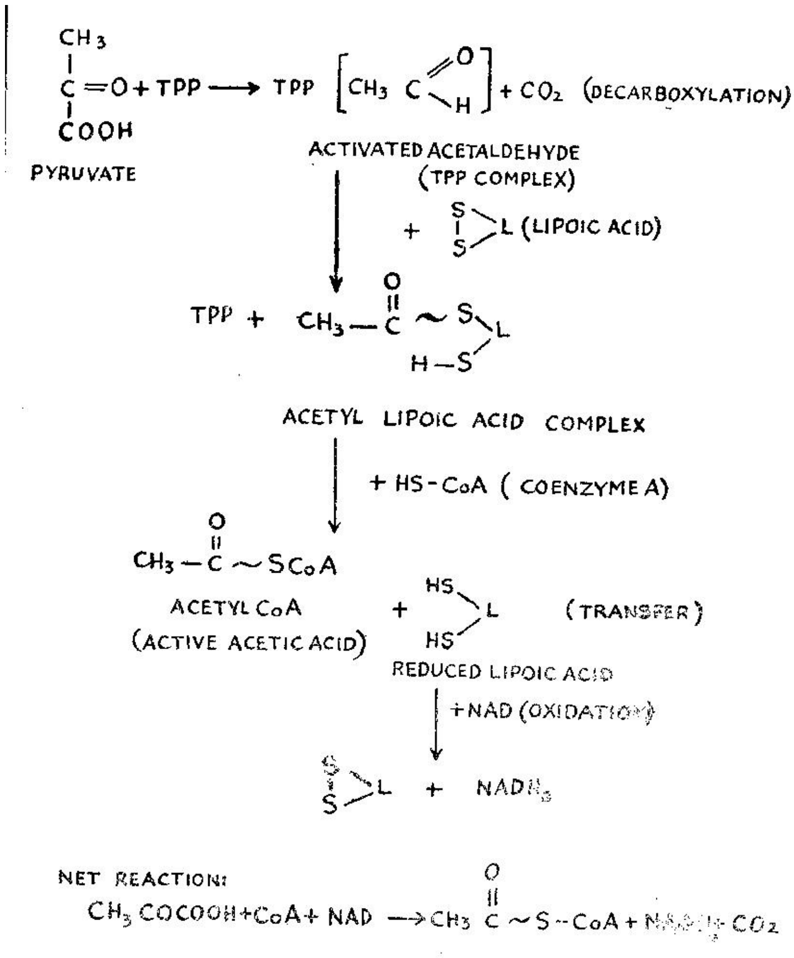
For simple anaerobic fermentations, the metabolism of one molecule of glucose to two molecules of pyruvate has a net yield of two molecules of ATP. Most cells will then carry out further reactions to 'repay' the used NAD^+ and produce a final product of ethanol or lactic acid. Many bacteria use inorganic compounds as hydrogen acceptors to regenerate the NAD^+.

Cells performing aerobic respiration synthesize much more ATP, but not as part of glycolysis. These further aerobic reactions use pyruvate and NADH + H^+ from glycolysis. Eukaryotic aerobic respiration produces approximately 34 additional molecules of ATP for each glucose molecule, however most of these are produced by a vastly different mechanism to the substrate-level phosphorylation in glycolysis.

The lower energy production, per glucose, of anaerobic respiration relative to aerobic respiration, results in greater flux through the pathway under hypoxic (low-oxygen) conditions, unless alternative sources of anaerobically-oxidizable substrates, such as fatty acids, are found.

Oxidative decarboxylation of pyruvate

The pyruvate is oxidized to acetyl-CoA and CO_2 by the Pyruvate dehydrogenase complex, a cluster of enzymes—multiple copies of each of three enzymes—located in the mitochondria of eukaryotic cells and in the cytosol of prokaryotes. In the process one molecule of NADH is formed per pyruvate oxidized, and 3 moles of ATP are formed for each mole of pyruvate.



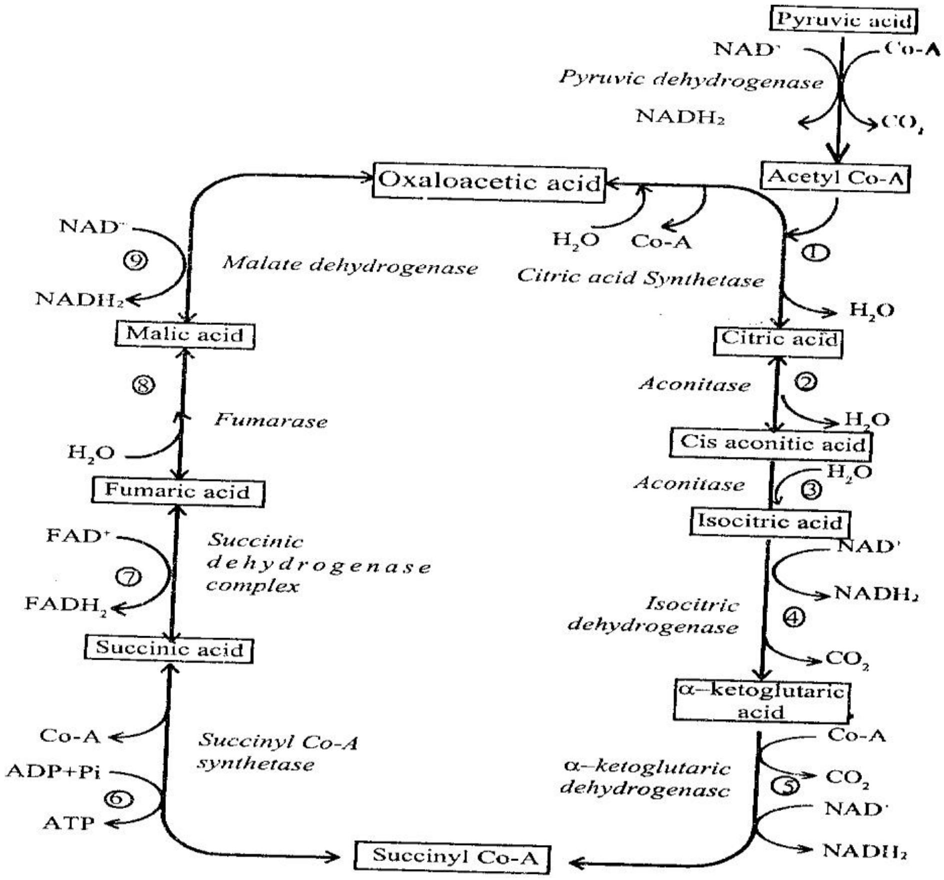
Reactions of oxadative decarboxylation of pyruvic acid

This step is also known as the *link reaction,* as it links glycolysis and the Krebs cycle.

Krebs cycle (citric acid cycle; tricarboxylic acid cycle; TCA cycle)

When oxygen is present, acetyl-CoA is produced from the pyruvate molecules created from glycolysis. Once acetyl-CoA is formed, two processes can occur, aerobic or anaerobic respiration. When oxygen is present, the mitochondria will undergo aerobic respiration which leads to the Krebs cycle. However, if oxygen is not present, fermentation of the pyruvate molecule will occur. In the presence of oxygen, when acetyl-CoA is produced, the molecule then enters the citric acid cycle (Krebs cycle) inside the mitochondrial matrix, and gets oxidized to CO_2 while at the same time reducing NAD to NADH. NADH can be used by the electron transport chain to create further ATP as part of oxidative phosphorylation. To fully oxidize the equivalent of one glucose molecule, two acetyl-CoA must be metabolized by the Krebs cycle. Two waste products, H_2O and CO_2, are created during this cycle.

Kreb cycle is a cyclical series of biochemical reactions that is fundamental to the metabolism of aerobic organisms, i.e. animals, plants, and many microorganisms. The enzymes of the Krebs cycle are located in the mitochondria and are in close association with the components of the electron transport chain. The two-carbon acetyl coenzyme A (acetyl CoA) reacts with the four-carbon oxaloacetate to form the six-carbon citrate. In a series of seven reactions, this is reconverted to oxaloacetate and produces two molecules of carbon dioxide. Most importantly, the cycle generates one molecule of guanosine triphosphate (GTP – equivalent to 1 ATP) and reduces three molecules of the coenzyme NAD to NADH and one molecule of the coenzymeFAD to $FADH_2$. NADH and $FADH_2$ are then oxidized by the electron transport chain to generate three and two molecules of ATP respectively (depending on the values of their respective P/O ratios). This gives a net yield of 12 molecules of ATP per molecule of acetyl CoA.



Krebs cycle

The citric acid cycle is an 8-step process involving 8 different enzymes. Throughout the entire cycle, acetyl-CoA changes into citrate, isocitrate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, malate, and finally, oxaloacetate. The net energy gain from one cycle is 3 NADH, 1 FADH, and 1 ATP. Thus, the total amount of energy yield from one whole glucose molecule (2 pyruvate molecules) is 6 NADH, 2 FADH, and 2 ATP.

Steps

Two carbon atoms are oxidized to CO_2, the energy from these reactions being transferred to other metabolic processes by GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the TCA cycle may later donate its electrons in oxidative phosphorylation to drive ATP synthesis; $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme functioning both in the TCA cycle and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$ thereby facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the Succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

Mitochondria in animals including humans possess two succinyl-CoA synthetases, one that produces GTP from GDP, and another that produces ATP from ADP. Plants have the type that produces ATP (ADP-forming succinyl-CoA synthetase). Several of the enzymes in the cycle may be loosely-associated in a multienzyme protein complex within the mitochondrial matrix.

The GTP that is formed by GDP-forming succinyl-CoA synthetase may be utilized by nucleoside-diphosphate kinase to form ATP (the catalyzed reaction is GTP + ADP → GDP + ATP).

A simplified view of the process

- The citric acid cycle begins with acetyl-CoA transferring its two-carbon acetyl group to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).
- The citrate then goes through a series of chemical transformations, losing two carboxyl groups as CO_2. The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA. The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle. Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric acid cycle in

anabolism, they may not be lost since many TCA cycle intermediates are also used as precursors for the biosynthesis of other molecules.

- Most of the energy made available by the oxidative steps of the cycle is transferred as energy-rich electrons to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced.
- Electrons are also transferred to the electron acceptor Q, forming QH_2.
- At the end of each cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

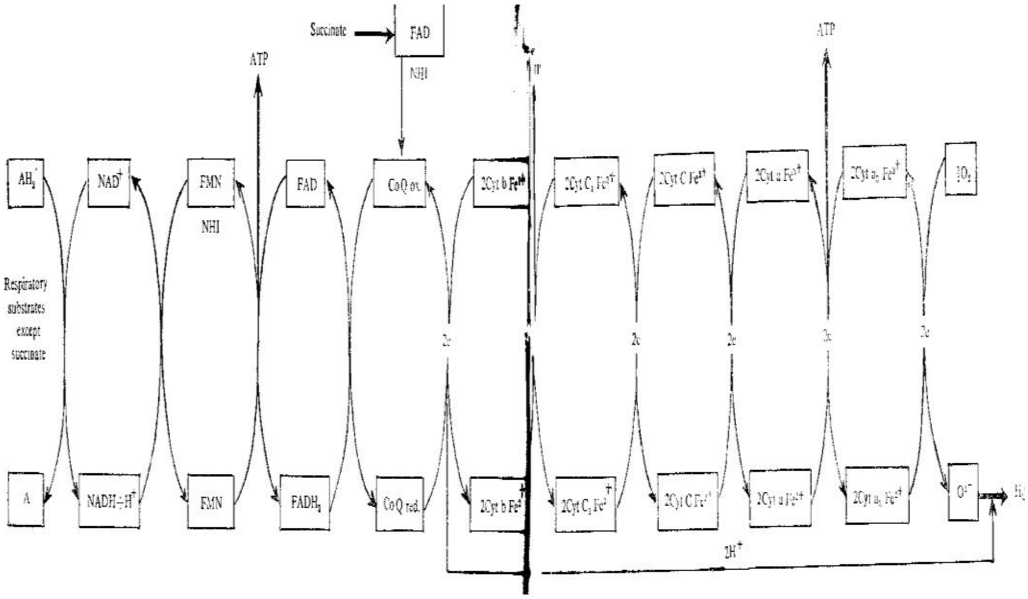
Acetyl CoA can be derived from carbohydrates (via glycolysis), fats, or certain amino acids. (Other amino acids may enter the cycle at different stages.) Thus the Krebs cycle is the central ‘crossroads’ in the complex system of metabolic pathways and is involved not only in degradation and energy production but also in the synthesis of biomolecules.

The total number of ATP obtained after complete oxidation of one glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is estimated to be between 30 and 38. A recent assessment of the total ATP yield with the updated proton-to-ATP ratios provides an estimate of 29.85 ATP per glucose molecule.

Electron and proton transfer molecules

The electron transport chain carries both protons and electrons, passing electrons from donors to acceptors, and transporting protons across a membrane. These processes use both soluble and protein-bound transfer molecules.

Many catabolic biochemical processes, such as glycolysis, the citric acid cycle, and beta oxidation, produce the reduced coenzyme NADH. This coenzyme contains electrons that have a high transfer potential; in other words, they will release a large amount of energy upon oxidation. However, the cell does not release this energy all at once, as this would be an uncontrollable reaction. Instead, the electrons are removed from NADH and passed to oxygen through a series of enzymes that each release a small amount of the energy. This set of enzymes, consisting of complexes I through IV, is called the electron transport chain and is found in the inner membrane of the mitochondrion. Succinate is also oxidized by the electron transport chain, but feeds into the pathway at a different point.



In eukaryotes, the enzymes in this electron transport system use the energy released from the oxidation of NADH to pump protons across the inner membrane of the mitochondrion. This causes protons to build up in the intermembrane space, and generates an electrochemical gradient across the membrane. The energy stored in this potential is then used by ATP synthase to produce ATP. Oxidative phosphorylation in the eukaryotic mitochondrion is the best-understood example of this process. The mitochondrion is present in almost all eukaryotes, with the exception of anaerobic protozoa such as *Trichomonas vaginalis* that instead reduce protons to hydrogen in a remnant mitochondrion called a hydrogenosome.

In mitochondria, electrons are transferred within the intermembrane space by the water-soluble electron transfer protein cytochrome c. This carries only electrons, and these are transferred by the reduction and oxidation of an iron atom that the protein holds within a heme group in its structure.

Within the inner mitochondrial membrane, the lipid-soluble electron carrier coenzyme Q10 (Q) carries both electrons and protons by a redox cycle. This small benzoquinone molecule is very hydrophobic, so it diffuses freely within the membrane. When Q accepts two electrons and two protons, it becomes reduced to the *ubiquinol* form (QH_2); when QH_2 releases two electrons and two protons, it becomes oxidized back to the *ubiquinone* (Q) form. As a result, if two enzymes are arranged so that Q is reduced on one side of the membrane and QH_2 oxidized on the other, ubiquinone will couple these reactions and shuttle protons across the membrane. Some bacterial electron transport chains use different quinones, such as menaquinone, in addition to ubiquinone.

Within proteins, electrons are transferred between flavin cofactors, iron–sulfur clusters, and cytochromes. There are several types of iron–sulfur cluster. The simplest kind found in the electron transfer chain consists of two iron atoms joined by two atoms of inorganic sulfur; these are called [2Fe–2S] clusters. The second kind, called [4Fe–4S], contains a cube of four iron atoms and four sulfur atoms. Each iron atom in these clusters is coordinated by an additional amino acid, usually by the sulfur atom of cysteine. Metal ion cofactors undergo redox reactions without binding or releasing protons, so in the electron transport chain they serve solely to transport electrons through proteins. Electrons move quite long distances through proteins by hopping along chains of these cofactors. This occurs by quantum tunnelling, which is rapid over distances of less than 1.4×10^{-9} m.

Oxidative phosphorylation

Oxidative phosphorylation is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). Although the many forms of life on earth use a range of different nutrients, almost all carry out oxidative phosphorylation to produce ATP, the molecule that supplies energy to metabolism. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.

The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, releasing energy to power the ATP synthase.

In eukaryotes, oxidative phosphorylation occurs in the mitochondrial cristae. It comprises the electron transport chain that establishes a proton gradient (chemiosmotic potential) across the inner membrane by oxidizing the NADH produced from the Krebs cycle. ATP is synthesised by the ATP synthase enzyme when the chemiosmotic gradient is used to drive the phosphorylation of ADP. The electrons are finally transferred to exogenous oxygen, and with the addition of two protons, water is formed.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within mitochondria, whereas, in prokaryotes, these proteins are located in the cells' inner membranes. These linked sets of enzymes are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *chemiosmosis*. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and contributing to disease and, possibly, aging (senescence). The enzymes carrying out this metabolic pathway are also the target of many drugs and poisons that inhibit their activities.

Overview of energy transfer by chemiosmosis

Oxidative phosphorylation works by using energy-releasing chemical reactions to drive energy-requiring reactions: The two sets of reactions are said to be *coupled*. This means one cannot occur without the other. The flow of electrons through the electron transport chain, from electron donors such as NADH to electron acceptors such as oxygen, is an exergonic process – it releases energy, whereas the synthesis of ATP is an endergonic process, which requires an input of energy. Both the electron transport chain and the ATP synthase are embedded in a membrane, and energy is transferred from electron transport chain to the ATP synthase by movements of protons across this membrane, in a process called *chemiosmosis*. In practice, this is like a simple electric circuit, with a current of protons being driven from the negative N-side of the membrane to the positive P-side by the proton-pumping enzymes of the electron transport chain. These enzymes are like a battery, as they perform work to drive current through the circuit. The movement of protons creates an electrochemical gradient across the membrane, which is often called the *proton-motive force*. This gradient has two components: a difference in proton concentration (a pH gradient) and a difference in electric potential, with the N-side having a negative charge. The energy is stored largely as the difference of electric potentials in mitochondria, but also as a pH gradient in chloroplasts.

ATP synthase releases this stored energy by completing the circuit and allowing protons to flow down the electrochemical gradient, back to the N-side of the membrane. This enzyme is like an electric motor as it uses the proton-motive force to drive the rotation of part of its structure and couples this motion to the synthesis of ATP.

The amount of energy released by oxidative phosphorylation is high, compared with the amount produced by anaerobic fermentation. Glycolysis produces only 2 ATP molecules, but somewhere between 30 and 36 ATPs are produced by the oxidative phosphorylation of the 10 NADH and 2 succinate molecules made by converting one molecule of glucose to carbon dioxide and water. This ATP yield is the theoretical maximum value; in practice, some protons leak across the membrane, lowering the yield of ATP.

Anaerobic respiration

Without oxygen, pyruvate is not metabolized by cellular respiration but undergoes a process of fermentation. The pyruvate is not transported into the mitochondrion, but remains in the cytoplasm, where it is converted to waste products that may be removed from the cell. This serves the purpose of oxidizing the hydrogen carriers so that they can perform glycolysis again and removing the excess pyruvate. This waste product varies depending on the organism. In skeletal muscles, the waste product is lactic acid. This type of fermentation is called lactic acid fermentation. In yeast, the waste products are ethanol and carbon dioxide. This type of fermentation is known as alcoholic or ethanol fermentation. The ATP generated in this process is made by *substrate phosphorylation*, which is phosphorylation that does not involve oxygen.

Anaerobic respiration is less efficient at using the energy from glucose since 2 ATP are produced during anaerobic respiration per glucose, compared to the 38 ATP per glucose produced by aerobic respiration. This is because the waste products of anaerobic respiration still contain plenty of energy. Ethanol, for example, can be used in gasoline (petrol) solutions. Glycolytic ATP, however, is created more quickly.

For prokaryotes to continue a rapid growth rate when they are shifted from an aerobic environment to an anaerobic environment, they must increase the rate of the glycolytic reactions. Thus, during short bursts of strenuous activity, muscle cells use anaerobic respiration to supplement the ATP production from the slower aerobic respiration, so anaerobic respiration may be used by a cell even before the oxygen levels are depleted, as is the case in sports that do not require athletes to pace themselves, such as sprinting.

In aerobic organisms, a complex mechanism has evolved to use the oxygen in air as the final electron acceptor of respiration.

- First, pyruvate is converted to acetyl-CoA and CO_2 within the mitochondria in a process called pyruvate decarboxylation.

- Second, the acetyl-CoA enters the citric acid cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD^+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a "hydrogen ion gradient" across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce a large amount of ATP in a process called oxidative phosphorylation.

Respiration quotient

Respiration quotient may be defined as "the ratio between the volume of carbondioxide given out and oxygen consumed during respiraton". This value depends upon the natureof the rspiratory substrate and its rate of oxidation.

$$\text{Respiratory quotient} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ consumed}}$$

(i) *Respiratory quotient of a carbohydrate*

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

$$\begin{array}{l}\text{Respiratory quotient}\\ \text{of glucose}\end{array} = \frac{6 \text{ moles of } CO_2}{6 \text{ moles of } O_2} = 1$$

(ii) *Respiratory quotient of an organic acid*

$$\underset{\text{Malic acid}}{C_4H_6O_5} + 3O_2 \rightarrow 4CO_2 + 3H_2O + \text{Energy}$$

$$\begin{array}{l}\text{Respiratory quotient}\\ \text{of malic acid}\end{array} = \frac{4 \text{ moles of } CO_2}{3 \text{ moles of } O_2} = 1.33 \text{ (more than one)}$$

(iii) *Respiratory quotient of fatty acid*

$$\underset{\text{Palmitic acid}}{C_{16}H_{33}O_2} + 11O_2 \rightarrow \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + 4CO_2 + 5H_2O + \text{Energy}$$

$$\begin{array}{l}\text{Respiratory quotient}\\ \text{of palmitic acid}\end{array} = \frac{4 \text{ moles of } CO_2}{11 \text{ moles of } O_2} = 0.36 \text{ (less than one)}$$

Respiration quotient for anaerobic respiration

In anaerobic respiration, carbondioxide is evolved but oxygen is not consumed. Therefore, the respiratory quotient in such case is infinity.

$$\underset{Glucose}{C_6H_{12}O_6} \xrightarrow{zymase} \underset{Ethanol}{2C_2H_5OH} + 2CO_2 + Energy$$

$$\begin{array}{c}\text{Respiratory quotient of glucose}\\ \text{in anaerobic respiration}\end{array} = \frac{2 \text{ moles of } CO_2}{\text{zero moles of } O_2} = \infty \text{ (infinity)}$$

Factors affecting respiration

There are number of factors, some internal and others external influencing the rate of respiration.

Internal factors

1. **Protoplasmic factors**; the rate of respiration depends upon the quantity of protoplasm. Young meristamatic tissue, rich in protoplasm have higher rates of respiration than older tissues. In addition to the quantity of protoplasm, other conditions like kinds of respiratory enzymes present, water content of protoplasm also control the rate of respiration.
2. **Concentration of respirable material**: the amount of solble substrate present is an important factor controlling respiration. The rate of respiration will increase with increase in concentration of respirable material (if other factors are not limiting). Leaves rich in food material, such as after a period of photosynthesis, show an increase in rate of respiration.

External factors

- **Temperature**: if the temperature range between 0° and 45° C, a rise in temperature causes a marked increase in the rate of respiration. However, at very high temperatures, the rate of respiration declines with time. At very low temperatures, the respiration rate is insignificant.

The vegetables and fruits stored at very low temperature thus increases the quality of food.

- **Light**: most effect of light on respiration is indirect, by enhancing photosynthesis and thus incrase in the amount of respirable material.
- **Oxygen concentration of atmosphere**: oxygen is absolutely essential for the aerobic type of respiration. Its percentage in the atmosphere remains constant. However, a direct relation between the respiration rate and oxygen concentration has been demonstrated by experimentation.
- **Carbon di oxide concentration**: the concentration of carbondioxide in atmosphere is almost constant and therefore, not likely to affect the rate of respiration. In the soil, however, the concentration of carbondioxide is very much variable.
- **Water**: in case of well of well hydrated plant the rate of respiration is not likely to be affected much by slight changes in the content of water. Shortage of water, however, does incrase the rate of respiration. Bery low water content, such as in dry seeds and stored tubers, is responsible for very feeble rates of r espiration.
- **Injury**: in wounded plant tissues, the sugar content issuddenly increased. This increase in the sugar content increases the rate of respiration.
- **Certain chemicals**: certain enzymatic inhibitors like cyanides, azides,carbon monoxide, etc reduce the rate of respiration.

NOTES

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UNIT III

Nitrogen metabolism: Sources of nitrogen, nitrogen fixation, nitrogen cycle, reductive amination and transamination. Protein synthesis.

Enzymes; Nomenclature, Classification (old system) Mode of action, factors affecting enzyme activity.

Sources of nitrogen

Our atmosphere consists of only 0.03% CO_2 which readily diffuses into the green plants, but nitrogen constitutes 78% of atmosphere cannot do so. Unlike the CO_2 the elemental nitrogen diffuses out into the atmosphere before it is converted into a combined form. The higher green plants are thus unable to utilise the gaseous nitrogen and must be supplied with nitrogen in a combined form for synthesizing proteins etc. Nitrogenous compounds absorbed from the soil, therefore, serve as the sole source of nitrogen for all rooted plants. The nitrogen content of the soil is enriched by certain microorganism, which are capable of converting atmospheric nitrogen into combined form.

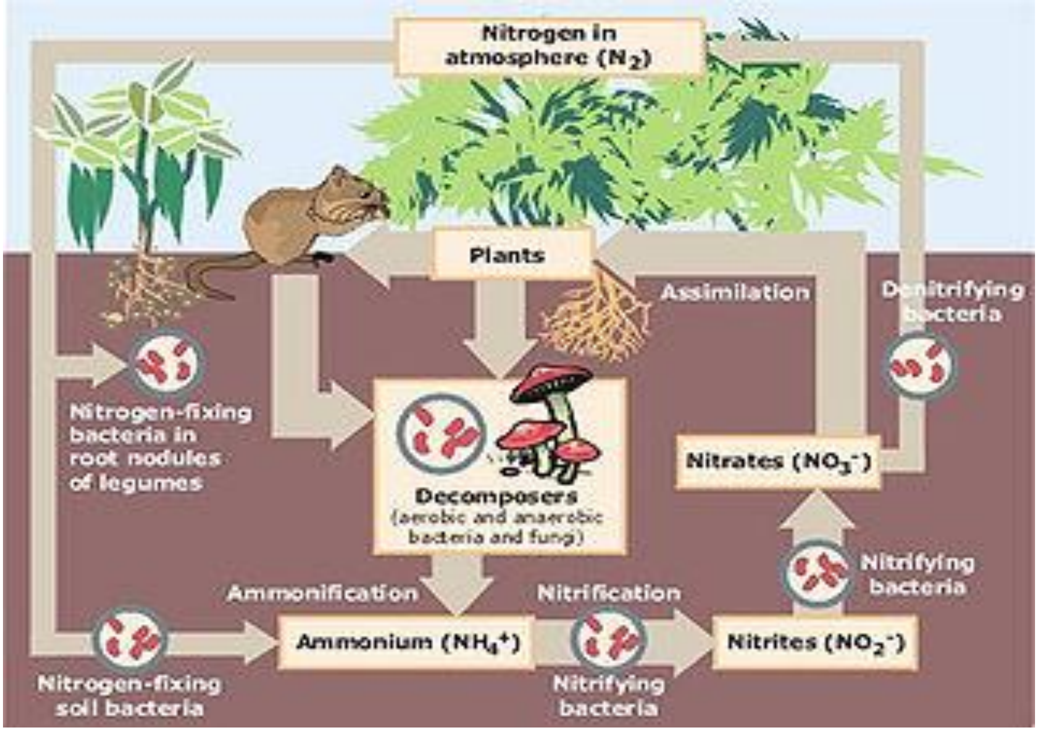
The higher green plants can uptake nitrogen if it is in the form of nitrates, nitrites, ammonium salts or organic nitrogenous compounds. Many plants absorb most of their nitrogen in the form of nitrates. Eventhough they can uptake nitrites, they are rarely important source of nitrogen for plants in nature. Ammonia itself can be absorbed rapidly from the soil and used directly. Ammonia is generally produced in the soil by the action of ammonifying bacteria on rotting nitrogenous compounds (protein etc.). The ammonia itself is, however, oxidized immediately to nitrite and then to nitrate by nitrifying bacteria present in the soil. Certain other nitrogenous compounds such as urea are formed by the decay of remnants of plants and animals in soil. However, these complex substances are generally converted into inorganic forms by bacteria, and then absorbed by plants.

Nitrogen fixation

Nitrogen fixation is the process by which nitrogen is taken from its relatively inert molecular form (N_2) in the atmosphere and converted into nitrogen compounds (such as ammonia, nitrate and nitrogen dioxide). This is an essential process for life because fixed nitrogen is needed to make nucleotides which are needed to make DNA and also to make amino acids which in turn are needed to produce proteins.

Nitrogen fixation is performed naturally by a number of different prokaryotes, including bacteria, actinobacteria, and certain types of anaerobic bacteria. Microorganisms that fix nitrogen are called diazotrophs. Some higher plants, and some animals (termites), have formed associations (symbiosises) with diazotrophs. Nitrogen fixation also occurs as a result of non-biological processes. These include lightning, industrially through the Haber - Bosch process, and combustion. Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck.

Biological nitrogen fixation

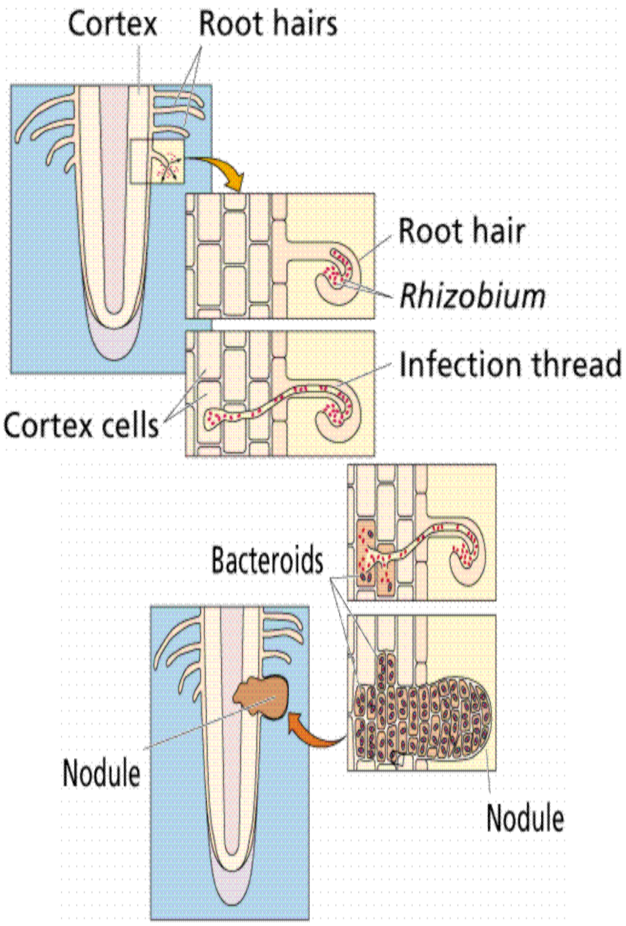


Schematic representation of the flow of Nitrogen through the environment.

Biological Nitrogen Fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by a pair of bacterial enzymes called nitrogenase. The formula for BNF is:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ P_i$$

Although ammonia (NH_3) is the direct product of this reaction, it is quickly protonated into ammonium (NH_4^+). In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway.



In most bacteria, the nitrogenase enzymes are very susceptible to destruction by oxygen (and many bacteria cease production of the enzyme in the presence of oxygen). Low oxygen tension is achieved by different bacteria by: living in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as Leghemoglobin.

The best-known plants which contribute to nitrogen fixation in nature, are in the legume family – Fabaceae – which includes such taxa as clover, beans, alfalfa, lupines and peanuts. They contain symbiotic bacteria called *Rhizobia* within nodules in their root systems, producing nitrogen compounds that help the plant to grow and compete with other plants. When the plant dies, the fixed nitrogen is released; making it available to other plants and this helps to fertilize the soil. The great majority of legumes have this association, but a few genera (e.g., *Styphnolobium*) do not. In many traditional and organic farming practices, fields are rotated through various types of crops, which usually includes one consisting mainly or entirely of clover or buckwheat (family *Polygonaceae*), which were often referred to as "green manure", since the other natural way of adding nitrogen to the soil is via animal waste products. The entire plant is often ploughed back into the field, thus not only adding more nitrogen, but also improving the soil's organic content and volume.

Non-leguminous nitrogen-fixing plants

Although by far the majority of nitrogen-fixing plants are in the legume family *Fabaceae*, there are a few non-leguminous plants that can also fix nitrogen. These plants, referred to as "actinorhizal plants", consist of 22 genera of woody shrubs or trees scattered in 8 plant families. The ability to fix nitrogen is not universally present in these families. For instance, of 122 genera in the *Rosaceae*, only 4 genera are capable of fixing nitrogen.

Rosaceae (rose):

Cercocarpus (mountain mahogany)
Chamaebatia (mountain misery)
Purshia (bitterbrush or cliff-rose)
Dryas

There are also several nitrogen-fixing symbiotic associations that involve cyanobacteria (such as *Nostoc*). These include some lichens such as *Lobaria* and *Peltigera*:

- Mosquito fern (*Azolla* species)
- Cycads
- *Gunnera*

Microorganisms that fix nitrogen

- Diazotrophs
- Cyanobacteria
- *Azotobacteraceae*
- *Rhizobia*
- *Frankia*
- *Acinomycetes*

Nitrogen fixation by cyanobacteria

Cyanobacteria inhabit nearly all illuminated environments on Earth and play key roles in the carbon and nitrogen cycle of the biosphere. Generally, cyanobacteria are able to utilize a variety of inorganic and organic sources of combined nitrogen, like nitrate, nitrite, ammonium, urea or some amino acids. Several cyanobacterial strains are also capable of diazotrophic growth. Genome sequencing has provided a large amount of information on the genetic basis of nitrogen metabolism and its control in different cyanobacteria. Comparative genomics, together with functional studies, has led to a significant advance in this field over the past years. 2-oxoglutarate has turned out to be the central signalling molecule reflecting the carbon/nitrogen balance of cyanobacteria. Central players of nitrogen control are the global transcriptional factor NtcA, which controls the expression of many genes involved in nitrogen metabolism, as well as the P_{II} signalling protein, which fine-tunes cellular activities in response to changing C/N conditions. These two proteins are sensors of the cellular 2-oxoglutarate level and have been conserved in all cyanobacteria. In contrast, the adaptation to nitrogen starvation involves heterogeneous responses in different strains. Nitrogen fixation by cyanobacteria in coral reefs can produce twice the amount of fixed nitrogen than on land – around 1.8kg of nitrogen is fixed per hectare per day.

Chemical nitrogen fixation

Nitrogen can also be artificially fixed for use in fertilizers, explosives, or in other products. The most common method is the Haber process. Artificial fertilizer production is performed on such a scale that it is now the largest source of fixed nitrogen in the Earth's ecosystem.

The Haber process requires high pressures (around 200 atm) and very high temperatures (at least 400 °C). Researchers are trying to develop catalyst systems that convert nitrogen to ammonia at ambient temperatures to reduce the energy use during nitrogen fixation. Many compounds can react with atmospheric nitrogen under ambient conditions (eg. lithium makes lithium nitride if left exposed), but the products of such reactions are not easily converted into biologically accessible nitrogen sources. After the first dinitrogen complex was discovered in 1965 based on ammonia coordinated to ruthenium ($[Ru(NH_3)_5(N_2)]^{2+}$), research in chemical fixation focused on transition metal complexes. Since then a large number of transition metal compounds that contain dinitrogen as a ligand have been discovered. The dinitrogen ligand can be bound either to a single metal or bridge two (or more) metals. The coordination chemistry of dinitrogen is complex and currently under intense investigation. This research may lead to new ways of using dinitrogen in synthesis and on an industrial scale.

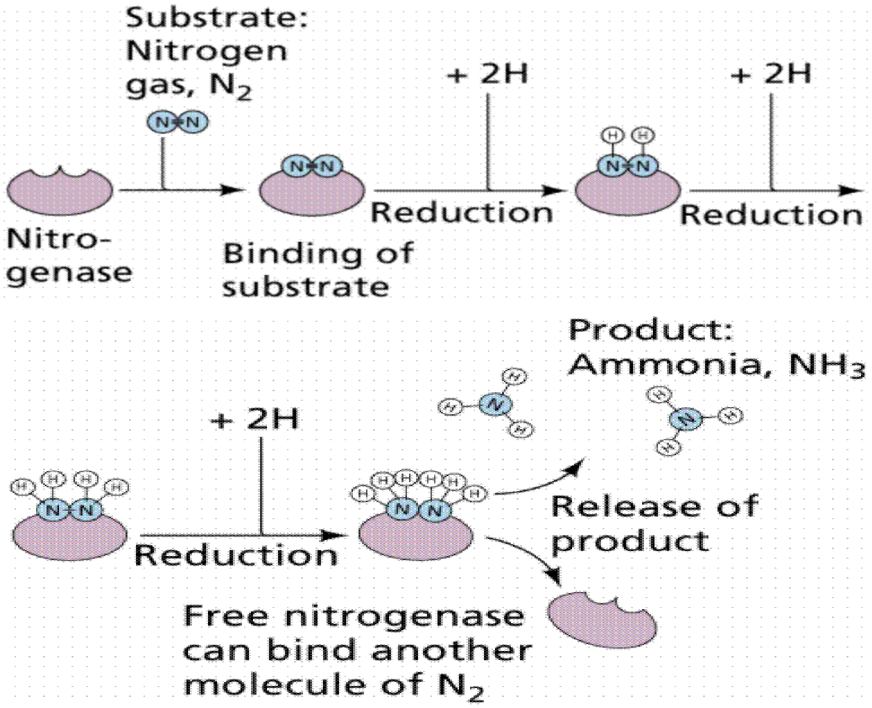
The first example of homolytic cleavage of dinitrogen under mild conditions was published in 1995. Two equivalents of a molybdenum complex reacted with one equivalent of dinitrogen, creating a triple bonded MoN complex. Since this triple bounded complex has been used to make nitriles. The first catalytic system converting nitrogen to ammonia at room temperature and pressure was discovered in 2003 and is based on another molybdenum compound, a proton source and a strong reducing agent. Unfortunately, the catalytic reduction only fixes a few nitrogen molecules before the catalyst no longer works.

Nitrogenase

Nitrogenase (EC 1.18.6.1) is the enzyme used by some organisms to fix atmospheric nitrogen gas (N_2). It is the only known family of enzymes which accomplishes this process. Dinitrogen is relatively inert because each atom of nitrogen has three open orbitals in its outer electron shell to bond with another atom, so that if two nitrogen atoms bond to each other, they do so in all three of these orbitals. To break one nitrogen atom away from another requires breaking all three of these chemical bonds. This is referred to as having a triple bond.

Nitrogenase is a catalyst for the reaction:

$$N_2 + 6H + \text{energy} \rightarrow 2NH_3$$



Whilst the equilibrium formation of ammonia from molecular hydrogen and nitrogen has an overall negative enthalpy of reaction ($\Delta H^0 = -45.2$ kJ mol^{-1} NH_3), the energy barrier to activation is generally insurmountable ($E_A = 420$ kJ mol^{-1}) without the assistance of catalysis.

Nitrogenase thus breaks the triple bond by getting electron donors for each of the three bonds, and then bonds the nitrogen to hydrogen atoms. The process is complex and is not completely understood; each of the dinitrogen bonds is broken individually. Nitrogenase requires both the MoFe protein and ATP, which supplies the energy. Nitrogenase bonds each atom of nitrogen to three atoms of hydrogen to form ammonia or NH_3, and then ammonia is bonded to glutamate and becomes glutamine. Nitrogenase associates with a second protein, and each cycle transfers one electron from an electron donor which is enough to break one of the nitrogen chemical bonds. However, it has not been proven that exactly three cycles are sufficient to fix an atom of nitrogen.

The enzyme therefore requires a great deal of chemical energy, released from the hydrolysis of ATP, and reducing agents, such as dithionite *in vitro* or ferredoxin *in vivo*. The enzyme is composed of the heterotetrameric MoFe protein that is transiently associated with the homodimeric Fe protein. Nitrogenase is supplied reducing power when it associates with the reduced, nucleotide-bound homodimeric Fe protein. The heterocomplex undergoes cycles of association and disassociation to transfer one electron, which is the limiting step in the process. ATP supplies the reducing power.

The exact mechanism of catalysis is unknown due to the difficulty in obtaining crystals of nitrogen bound to nitrogenase. This is because the resting state of MoFe protein does not bind nitrogen and also requires at least three electron transfers to perform catalysis. Nitrogenase is able to bind acetylene and carbon monoxide, which are noncompetitive substrates and inhibitors, respectively. Dinitrogen, however, is a competitive substrate for acetylene. This is because binding of dinitrogen prevents acetylene binding, and acetylene requires only one electron to be reduced, and it does not inhibit.

All nitrogenases have an iron- and sulfur-containing cofactor that includes heterometal atom in the active site (e.g. FeMoCo). In most, this heterometal is molybdenum, though in some species it is replaced by vanadium or iron.

Due to the oxidiative properties of oxygen, most nitrogenases are irreversibly inhibited by dioxygen, which degradatively oxidizes the Fe-S cofactors. This requires mechanisms for nitrogen fixers to avoid oxygen *in vivo*. Despite this problem, many use oxygen as a terminal electron acceptor for respiration. One known exception, a recently-discovered nitrogenase of *Streptomyces thermoautotrophicus*, is unaffected by the presence of oxygen. The Azotobacteraceae are unique in their ability to employ an oxygen-labile nitrogenase under aerobic conditions. This ability has been attributed to a high metabolic rate allowing oxygen reduction at the membrane, but this idea has been shown to be unfounded and impossible at oxygen concentrations above 70 µM (ambient concentration is 230 µM O2), as well as during additional nutrient limitations.

The reaction that this enzyme performs is:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ Pi$$

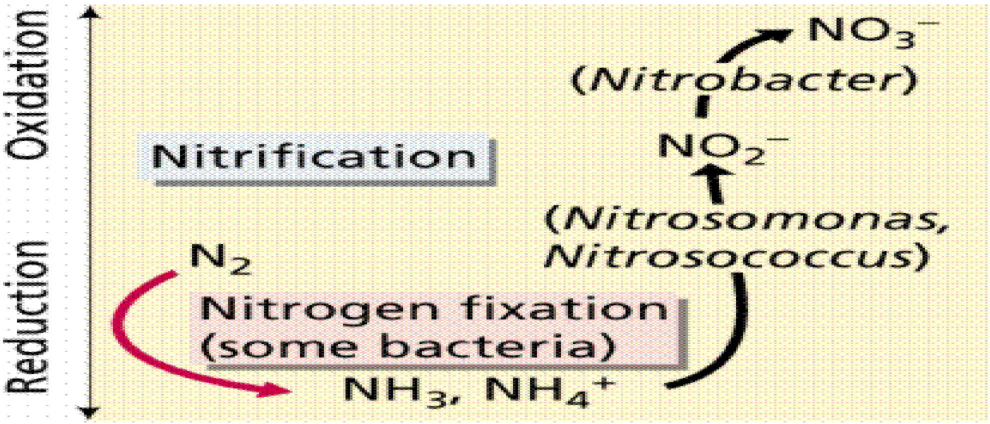
Nitrogen cycle

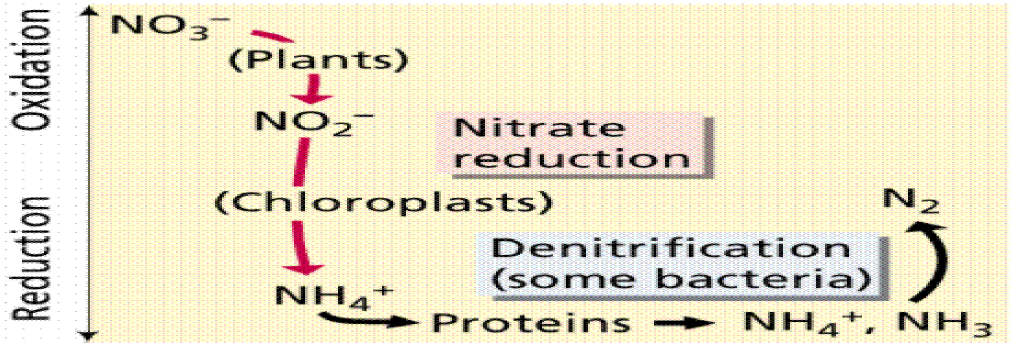
The importance of bacteria in the cycle is immediately recognized as being a key element in the cycle, providing different forms of nitrogen compounds assimilable by higher organisms.

The **nitrogen cycle** is the biogeochemical cycle that describes the transformations of nitrogen and nitrogen-containing compounds in nature. It is a cycle which includes gaseous components.

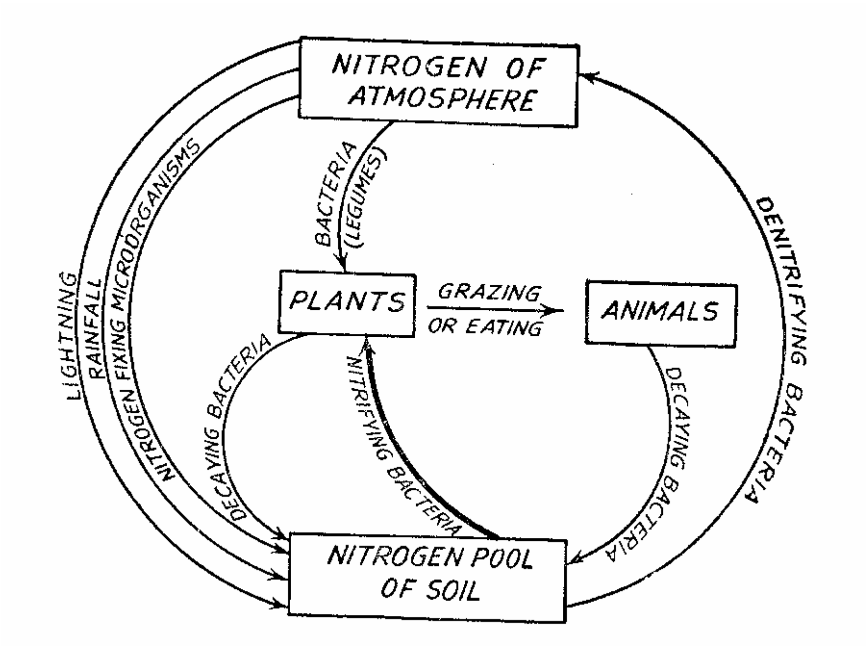
Earth's atmosphere is approximately 78-80% nitrogen, making it the largest pool of nitrogen. Nitrogen is essential for many biological processes; it is crucial for any life here on Earth. It is in all amino acids, is incorporated into proteins, and is present in the bases that make up nucleic acids, such as DNA and RNA. In plants, much of the nitrogen is used in chlorophyll molecules which are essential for photosynthesis and further growth.

Processing, or fixation, is necessary to convert gaseous nitrogen into forms usable by living organisms. Some fixation occurs in lightning strikes, but most fixation is done by free-living or symbiotic bacteria. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by the bacteria to make their own organic compounds. Some nitrogen fixing bacteria, such as *Rhizobium*, live in the root nodules of legumes (such as peas or beans). Here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates. Nutrient-poor soils can be planted with legumes to enrich them with nitrogen. A few other plants can form such symbioses. Nowadays, a very considerable portion of nitrogen is fixated in ammonia chemical plants.





Other plants get nitrogen from the soil, and by absorption of their roots in the form of either nitrate ions or ammonium ions. All nitrogen obtained by animals can be traced back to the eating of plants at some stage of the food chain.



Nitrogen cycle in nature.

Due to their very high solubility, nitrates can enter groundwater. Elevated nitrate in groundwater is a concern for drinking water use because nitrate can interfere with blood-oxygen levels in infants and cause methemoglobinemia or blue-baby syndrome. Where groundwater recharges stream flow, nitrate-enriched groundwater can contribute to eutrophication, a process leading to high algal, especially blue-green algal populations and the death of aquatic life due to excessive demand for oxygen. While not directly toxic to fish life like ammonia, nitrate can have indirect effects on fish if it contributes to this eutrophication. Nitrogen has contributed to severe eutrophication problems in some water bodies. As of 2006, the application of nitrogen fertilizer is being increasingly controlled in Britain and the United States. This is occurring along the same lines as control of phosphorus fertilizer, restriction of which is normally considered essential to the recovery of eutrophied waterbodies.

Ammonia is highly toxic to fish and the water discharge level of ammonia from wastewater treatment plants must often be closely monitored. To prevent loss of fish, nitrification prior to discharge is often desirable. Land application can be an attractive alternative to the mechanical aeration needed for nitrification.

During anaerobic (low oxygen) conditions, *denitrification* by bacteria occurs. This results in nitrates being converted to nitrogen gases (NO, N_2O, N_2) and returned to the atmosphere. Nitrate can also be reduced to nitrite and subsequently combine with ammonium in the anammox process, which also results in the production of dinitrogen gas.

The Processes of the nitrogen cycle

Conversion of N_2

The conversion of nitrogen (N_2) from the atmosphere into a form readily available to plants and hence to animals and humans is an important step in the nitrogen cycle, which distributes the supply of this essential nutrient. There are four ways to convert N_2 (atmospheric nitrogen gas) into more chemically reactive forms:

1. Biological fixation: some symbiotic bacteria (most often associated with leguminous plants) and some free-living bacteria are able to fix nitrogen as organic nitrogen. An example of mutualistic nitrogen fixing bacteria are the *Rhizobium* bacteria, which live in legume root nodules. These species are diazotrophs. An example of the free-living bacteria is *Azotobacter*.
2. Industrial N-fixation : Under great pressure, at a temperature of 600°C, and with the use of a catalyst, atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia (NH_3). In the Haber-Bosch process, N_2 is converted together with hydrogen gas (H_2) into ammonia (NH_3) which is used to make fertilizer and explosives.
3. Combustion of fossil fuels : automobile engines and thermal power plants, which release various nitrogen oxides (NO_x).

4. Other processes : Additionally, the formation of NO from N_2 and O_2 due to photons and especially lightning, are important for atmospheric chemistry, but not for terrestrial or aquatic nitrogen turnover.

Assimilation

Plants can absorb nitrate or ammonium ions from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, intense nucleic acids, and chlorophyll. In plants which have a mutualistic relationship with rhizobia, some nitrogen is assimilated in the form of ammonium ions directly from the nodules. Animals, fungi, and other heterotrophic organisms absorb nitrogen as amino acids, nucleotides and other small organic molecules.

Ammonification

When a plant dies, an animal dies, or an animal expels waste, the initial form of nitrogen is organic. Bacteria, or in some cases, fungi, convert the organic nitrogen within the remains back into ammonium(NH_4), a process called ammonification or mineralization. Enzymes Involved:

- GS: Gln Synthetase (Cytosolic & PLastid)
- GOGAT: Glu 2-oxoglutarate aminotransferase (Ferredoxin & NADH dependent)
- GDH: Glu Dehydrogenase:
  - Minor Role in ammonium assimilation.
  - Important in amino acid catabolism.

Nitrification

The conversion of ammonia to nitrates is performed primarily by soil-living bacteria and other nitrifying bacteria. The primary stage of nitrification, the oxidation of ammonia (NH_3) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO_2^-). Other bacterial species, such as the *Nitrobacter*, are responsible for the oxidation of the nitrites into nitrates (NO_3^-). It is important for the nitrites to be converted to nitrates because accumulated nitrites are toxic to plant life.

Denitrification

Denitrification is the reduction of nitrates back into the largely inert nitrogen gas (N_2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and *Clostridium* in anaerobic conditions. They use the nitrate as an electron acceptor in the place of oxygen during respiration. These facultatively anaerobic bacteria can also live in aerobic conditions.

Anaerobic ammonium oxidation

In this biological process, nitrite and ammonium are converted directly into dinitrogen gas. This process makes up a major proportion of dinitrogen conversion in the oceans.

Human influences on the nitrogen cycle

As a result of extensive cultivation of legumes (particularly soy, alfalfa, and clover), growing use of the Haber-Bosch process in the creation of chemical fertilizers, and pollution emitted by vehicles and industrial plants, human beings have more than doubled the annual transfer of nitrogen into biologically available forms. In addition, humans have significantly contributed to the transfer of nitrogen trace gases from Earth to the atmosphere, and from the land to aquatic systems.

N_2O has risen in the atmosphere as a result of agricultural fertilization, biomass burning, cattle and feedlots, and other industrial sources. N_2O has deleterious effects in the stratosphere, where it breaks down and acts as a catalyst in the destruction of atmospheric ozone. Ammonia (NH_3) in the atmosphere has tripled as the result of human activities. It is a reactant in the atmosphere, where it acts as an aerosol, decreasing air quality and clinging on to water droplets, eventually resulting in acid rain. Fossil fuel combustion has contributed to a 6 or 7 fold increase in NOx flux to the atmosphere. NOx actively alters atmospheric chemistry, and is a precursor of tropospheric (lower atmosphere) ozone production, which contributes to smog, acid rain, damages plants and increases nitrogen inputs to ecosystems. Ecosystem processes can increase with nitrogen fertilization, but anthropogenic input can also result in nitrogen saturation, which weakens productivity and can kill plants. Decreases in biodiversity can also result if higher nitrogen availability increases nitrogen-demanding grasses, causing a degradation of nitrogen-poor, species diverse heathlands.

Waste water

Onsite sewage facilities such as septic tanks and holding tanks release large amounts of nitrogen into the environment by discharging through a drainfield into the ground. Microbial activity consumes the nitrogen and other contaminants in the wastewater. However, in certain areas the soil is unsuitable to handle some or all of the wastewater, and as a result, the wastewater with the contaminants enters the aquifers. These contaminants accumulate and eventually end up in drinking water. One of the contaminants concerned about the most is nitrogen in the form of nitrates. A nitrate concentration of 10 ppm or 10 milligrams per liter is the current EPA limit for drinking water and typical household wastewater can produce a range of 20-85 ppm (milligrams per liter).

The health risk associated with drinking >10 ppm nitrogen water is the development of methemoglobinemia and has been found to cause blue baby syndrome. Several states have now started programs to introduce advanced wastewater treatment systems to the typical onsite sewage facilities. The result of these systems is an overall reduction of nitrogen, as well as other contaminants in the wastewater.

Additional, perhaps greater risks are posed by the increase of fixed nitrogen in aquatic systems leading to eutrophication and hypoxia, altering the chemistry and biology of both freshwater and seawater systems, and spurring the creation and growth of eutrophic lakes and oceanic dead zones. The extent and effects of the human-caused doubling of biologically available nitrogen in the soils, waters, and air of the earth during the past century are still poorly understood.

Ammonia from any source can be combined into organic linkages by three major reactions that appear to occur in all living organisms. These result in the formation of glutamic acid, glutamine and carbamyl phosphate resprectively.

Reductive amination and transamination:

Synthesis of glutamic acid is a reductive amination process. Ammonia reacts with keto-glutaric acid, an intermediate of carbohydrate metabolism, to yield glutamic acid. This reaction is known as **reductive amination** is catalyzed by glutamic dehydrogenase, a cytoplasmic enzyme. The reducing power is supplied by NADH or NADPH in different organisms

$$NH_3 + H^+ + NADH + \alpha\text{-ketoglutaric acid} \rightleftharpoons \text{Glutamic acid} + NAD^+ + H_2O$$

This single reaction of ammonia with alpha keto glutraric acid to form glutamic acid accounts formost of the fixation of ammonia into organic substances, mainly the amino acids. Glutamic acid plays a prime role in the biosynthesis of several amino acids. Most of the non-essential amino acids derive their amino groups from glutamic acids.

Transamination: the amino group of glutamic acid can be transferred to other keto acids by a mechanism called transamination. The process involves the transfer of the amino group from a donor aminoacid to the recipient keto acid to form the analogous amino acid and to produce the ketoacid from the original amino donor. The donor amino acid thus becomes a keto acid. The recipient keto acid becomes an amino acid alanine by the transfer of the amino group from glutamic acid to pyruvic acid, in the course of which ketoglutaic acid is produced.

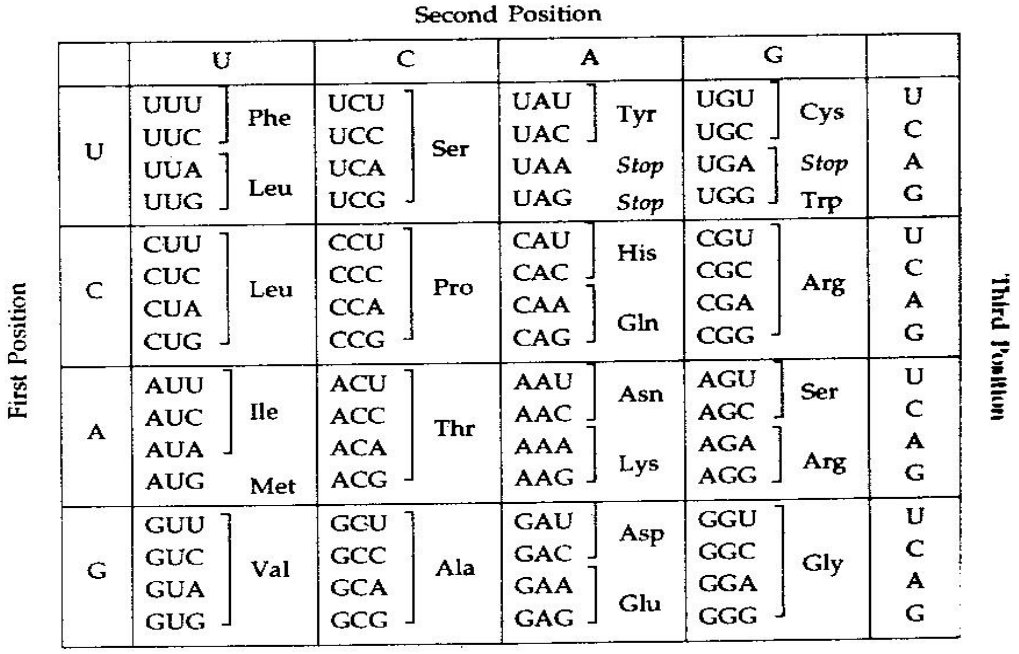
Significance of transamination:

Transamination provides a way for the redustributio of amino nitrogen. For example, if the meal is rich in alanine and poor in aspartic acid, the following pair reaciton woul provide the necessary nitrogen for aspartic acid formation:

1. Alanine + α-ketoglutaric acid $\rightleftharpoons$ Pyruvic acid + Glutamic acid
2. Glutamic acid + Oxaloacetic acid $\rightleftharpoons$ α-ketoglutaric acid + Aspartic acid

Protein synthesis:

The DNA sequence of a gene encodes the amino acid sequence of a protein.Proteins are assembled from amino acids using information encoded in genes. Each protein has its own unique amino acid sequence that is specified by

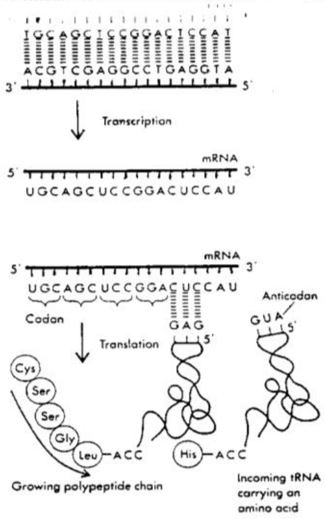


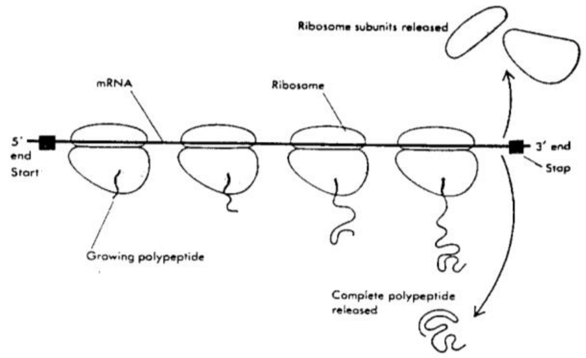
Second Position

First Position	U		C		A		G		Third Position
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	*Stop*	UGA	*Stop*	A
	UUG		UCG		UAG	*Stop*	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

The genetic triplet code

the nucleotide sequence of the gene encoding this protein. The genetic code is a set of three-nucleotide sets called codons and each three-nucleotide combination designates an amino acid, for example AUG (adenine-uracil-guanine) is the code for methionine. Because DNA contains four nucleotides, the total number of possible codons is 64; hence, there is some redundancy in the genetic code, with some amino acids specified by more than one codon. Genes encoded in DNA are first transcribed into pre-messenger RNA (mRNA) by proteins such as RNA polymerase. Most organisms then process the pre-mRNA (also known as a *primary transcript*) using various forms of post-transcriptional modification to form the mature mRNA, which is then used as a template for protein synthesis by the ribosome. In prokaryotes the mRNA may either be used as soon as it is produced, or be bound by a ribosome after having moved away from the nucleoid. In contrast, eukaryotes make mRNA in the cell nucleus and then translocate it across the nuclear membrane into the cytoplasm, where protein synthesis then takes place. The rate of protein synthesis is higher in prokaryotes than eukaryotes and can reach up to 20 amino acids per second.





The mechanism of protein synthesis

Process of synthesizing a protein from an mRNA template is known as translation. The mRNA is loaded onto the ribosome and is read three nucleotides at a time by matching each codon to its base pairing anticodon located on a transfer RNA molecule, which carries the amino acid corresponding to the codon it recognizes. The enzyme aminoacyl tRNA synthetase "charges" the tRNA molecules with the correct amino acids. The growing polypeptide is often termed the *nascent chain*. Proteins are always biosynthesized from N-terminus to C-terminus.

The size of a synthesized protein can be measured by the number of amino acids it contains and by its total molecular mass, which is normally reported in units of *daltons* (synonymous with atomic mass units), or the derivative unit kilodalton (kDa). Yeast proteins are on average 466 amino acids long and 53 kDa in mass. The largest known proteins are the titins, a component of the muscle sarcomere, with a molecular mass of almost 3,000 kDa and a total length of almost 27,000 amino acids.

Enzymes

Within a cell hundreds of different types of chemical reactions occur as part of metabolism. Just about all these reactions would not happen at all at room temperature or else happen very slowly. These reactions are speeded up by the presence of catalysts called enzymes: organic molecules in cells that speed up chemical reactions without getting destroyed in the reaction. Just about all types of chemical reactions in the cell require enzymes. Enzymes are typically proteins, but certain types of RNA can also serve as catalysts. These RNA molecules are called ribozymes

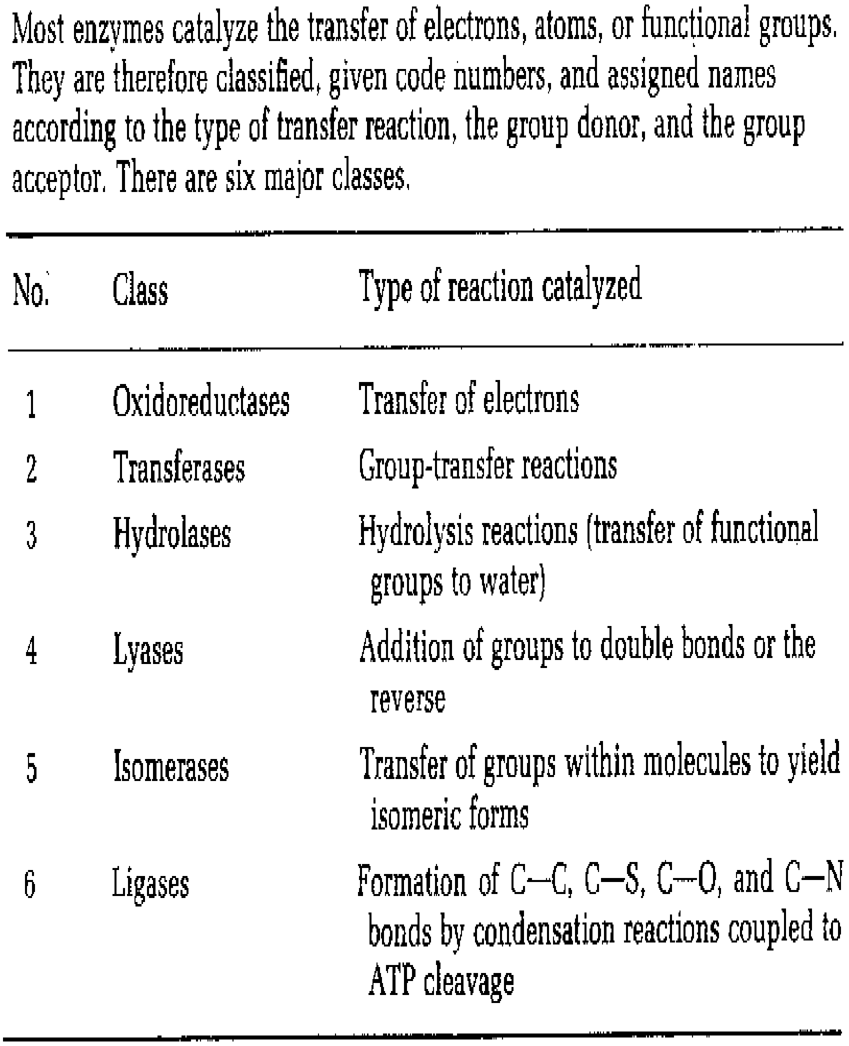
Enzyme: An enzyme is an organic catalyst, usually a protein. A catalyst is a chemical that speeds up a chemical reaction without getting destroyed by the reaction.

Nomenclature and classification

Most of the enzymes are named by adding the suffix *–ase* to the name of the substrate, e.g., sucrase, maltase, lactase, etc. The name of some of the enzymes indicate the nature of the rection in whidh they take part, e.g., oxidases, dehydrogenases etc. Generally the name of the specific substrate is also mentioned along with such names. There are some enzymes which are named in an arbitrary way such as pepsin, trypsin etc.

Classification:

International classification of enzymes are based on the reactions they catalyze. The classification is as shown in the table below:



Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor. There are six major classes.

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons
2	Transferases	Group-transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds or the reverse
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

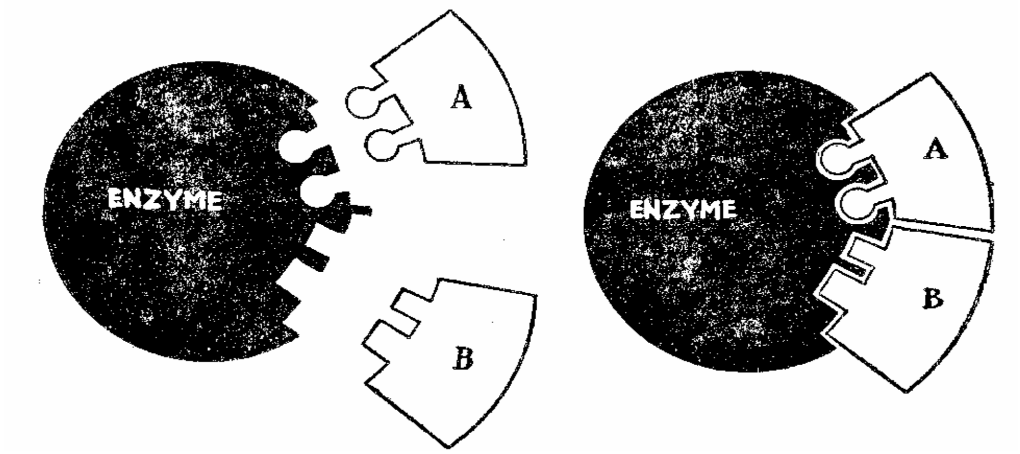
Mode of action

The operation of enzymes depends on the fit between the enzyme and the starting molecule or molecules in the reaction that the enzyme catalyses. This is shown in a simple fashion here.

substrate + enzyme --------> enzyme substrate complex -----------> enzyme + products.

Substrate: The starting molecules for a chemical reaction are called the substrates.

Enzyme substrate complex: The enzyme substrate complex is transitional step when the substrates of a chemical reaction are bound to the enzyme.



Diagramatic representation of enzyme-substrate (A ,B) reaction

Active site: The area on the enzyme where the substrate or substrates attach to is called the active site. Enzymes are usually very large proteins and the active site is just a small region of the enzyme molecule.

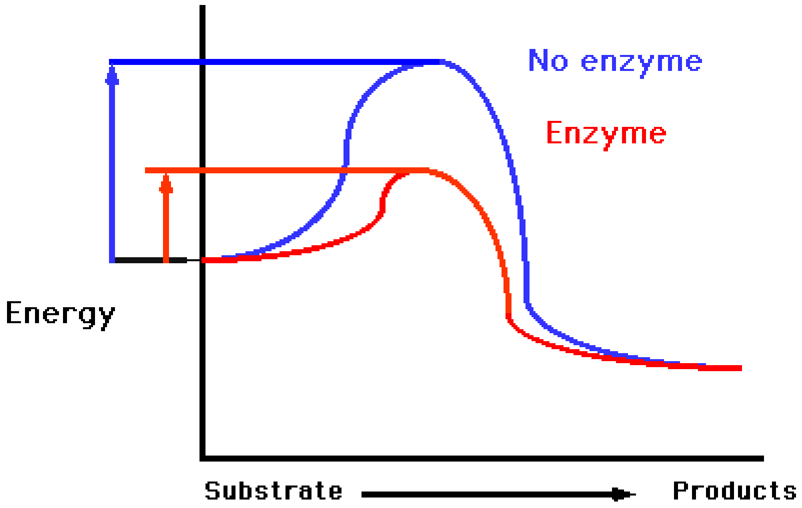
It used to be thought that the substrate and the active site had to have an exact fit for the enzyme to operate. This idea is what used to be called the **'lock and key'** model of enzyme activity. We now know the situation is much more complex.

Induced fit model:

Our current general model of how enzymes operate is called the 'induced fit' model. The idea is that the enzyme's active site does not have the exact shape of substrate, but the substrate brings about or induces a change in the shape of the active site. A good analogy is what happens when you try to catch a softball. You don't hold your hand rigidly in the shape that best fits the ball, but alter the shape of your hand to accommodate the ball. The idea is illustrate in this animation. Here the dark green region represents the active site of an enzyme. The two purplish ovals represent substrates that are going to join together at the active site to form a product(red). Notice how the active site changes shape to conform(more or less) to the substrates.

Enzymes and activation energy

All chemical reactions require some amount of energy to get them started. This energy is called activation energy. The way enzymes operate is by effectively lowering the amount of activation energy required for a chemical reaction to start. Sometimes this happens because enzymes might weaken a covalent bond within a substrate molecule. In other cases this lowering of activation energy seems to happen because the enzyme holds the substrate molecules in a particular position that increases the likely that the molecules are going to react.



Energy hill diagrams are a good way to visualize the effect of enzymes on activation energy. The diagram shows time on the horizontal axis and the amount of energy in the chemicals involved in a chemical reaction on the vertical axis. The point if this diagram again is that without the enzyme, much more activation energy is required to get a chemical reaction to take place.

Factors affecting enzyme activity

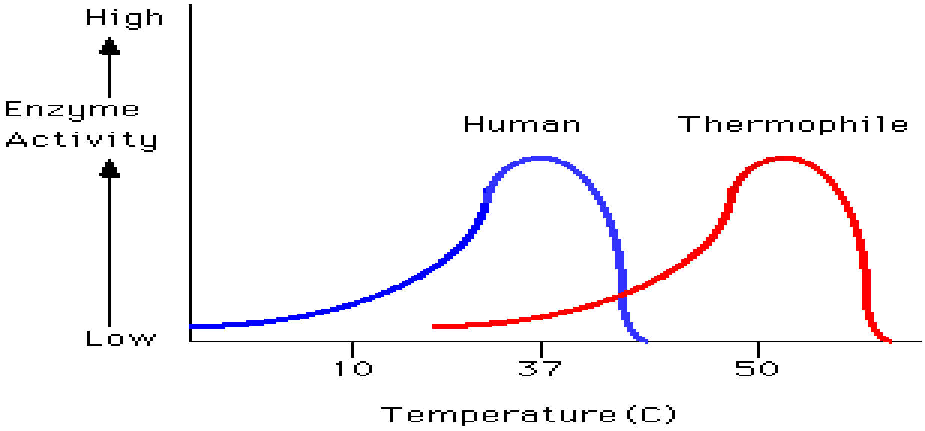
Physical factors

Enzymes typically operate best in a relatively narrow range of environmental conditions.

Many of the enzymes in our bodies work best at body temperature. At significantly lower temperatures the substrate molecules do not have enough kinetic energy for the reaction to take place even in the presence of the enzyme. At body temperatures significantly higher than normal, the enzyme will not work well because the kinetic energy from the molecules in the solution containing the enzyme is so high, that the enzyme's shape is pulled apart to he point that the enzyme is not able to properly function.

Indeed the enzyme's structure may be so disrupted or denatured hat the enzyme molecule cannot return to its original shape. Indeed the danger of high fevers stems in large part from the potential damage to enzymes and other proteins from the high temperature. pH, salinity and concentrations of other ions also affect enzyme shape.

The diagram shows hypothetical relationships between temperature and enzyme activity for a human and for a thermophilic(heat loving) bacteria. Notice the optimum activity level for each enzyme matches the environment that the enzyme has to work in. Human enzymes generally work best at our temperature(37°C) while the thermophilic bacteria's enzyme works best at a higher temperature. Indeed some of these thermophilic organisms live quite nicely at the boiling point of water.



Cofactors and Coenzymes

Many enzymes need companion molecules in order to function. For example, many enzymes such as alcohol dehydrogenase require organic molecules or various metals to function properly. These are called cofactors. If the companion is an organic molecule then the companion is often called a coenzyme. For instance, zinc is a common cofactor for many enzymes including the alcohol dehydrogenase. Many vitamins are coenzymes. For example Vitamin B2(Riboflavin) is an important coenzyme in cellular respiration. Another vitamin, Pantothenic acid is what's called 'Coenzyme A' in cellular respiration. Indeed the group of vitamins called the 'B complex' are all important coenzymes.

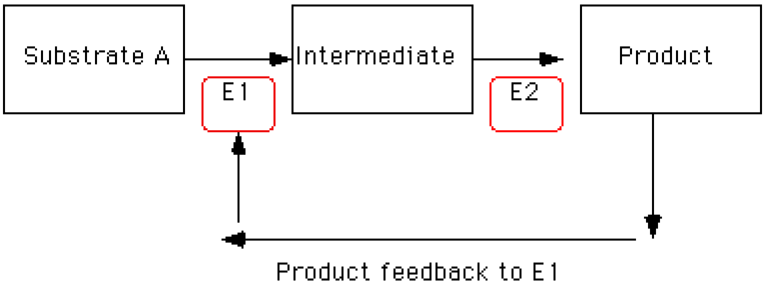
NOTE: the term cofactor refers to either inorganic ions or atoms that work with enzymes or to organic molecules such vitamins that work with enzymes. The word coenzyme refers just to the organic molecules.

Inhibitors

Various substances interfere with the operation of enzymes. These substances are called inhibitors. Organophosphates and certain other pesticides operate by inhibiting key enzymes in the nervous system. Both carbon monoxide and oxygen bind the same active site in the hemoglobin molecule and carbon monoxide binds so strongly to this site that oxygen can't bind properly to the hemoglobin. This type of situation where one substrate out competes another is called competitive inhibition.

Feedback Inhibition

Sometimes the product of a chemical reaction involving an enzyme attaches to a secondary site on the enzyme and inhibits the enzyme's ability to continue the reaction. This type of reversible inhibition is called feedback inhibition and it provides cells with a way to regulate the production of various compounds in the cell. The diagram shows a simple illustration of feedback inhibition for a metabolic pathway involving three chemicals and two enzymes.



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UNIT IV

Carbohydrates: Classification- monosaccharides (Glucose, Fructose). Disaccharides(Maltose, sucrose). Polysaccharides (starch, cellulose)

Proteins: structure, types of proteins (globular, conjugated).

Lipids: composition of lipids-saturated and unsaturated fatty acid; Break down of fatty acids (alpha oxidation).

Carbohydrates

Carbohydrates are one of three macronutrients that provide the body with energy (protein and fats being the other two). The chemical compounds in carbohydrates are found in both simple and complex forms, and in order for the body to use carbohydrates for energy, food must undergo digestion, absorption, and glycolysis. It is recommended that 55 to 60 percent of caloric intake come from carbohydrates.

Definition:

Carbohydrates may be defined as polyhydroxyaldehydes or ketones or compounds which produce them on hydrolysis. The term sugar is applied to carbohydrates soluble in water and sweet to taste. Carbohydrates are also known as saccharides (sakcharon=sugar or sweetness) since many of those of relatively small molecular weight have a sweet taste, although this is not true of those with large molecules.

Chemical Structure

Carbohydrates are a main source of energy for the body and are made of carbon, hydrogen, and oxygen. Most carbohydrates have a ratio of 1:2:1 of carbon, hydrogen, and oxygen, respectively. E.g., sugars, starch, cellulose etc.

Previously, the carbohydrates were regarded as hydrates of carbon and corresponded to general empirical formula $(C.H2O)_n$. But the group name carbohydrate was sometimes found misleading.

Some organic compounds e.g. Formaldehyde (HCHO), acetic acid (CH_3COOH), lactic acid ($C_3H_6O_3$), inositol ($C_6H_{12}O_6$) etc. correspond to the general formula but are not carbohydrates although thy have CH and O and the ratio is also similar to the empirical formula.

Some carbohydrates e.g., rhamnose ($C_6H_{12}O_5$), rhamnohexose ($C_7H_{14}O_6$) digitoxose ($C_6H_{12}O_4$) do not correspond to the above formula.

Besides containing C, H and O some carbohydrates also contain nitrogen e.g. Glucosamine ($C_6H_{13}O_5N$), certain other carbohydrates are now known to possess Phosphorous or sulfur also, so, obviously do not coincide with the above general formula.

Hence carbohydrates are more appropriately referred to as polyhydroxyaldehydes or polyhydroxyketones and their derivatives or the substances which yield these on hydrolysis, but the group name carbohydrates is still retained traditionally.

Sources of carbohydrates:

They are widely distribute molecules in both plants and animal tissues. They are indispensable for living organisms, because-

- They serve as skeletal structures in plants and also in insects and crustaceans.
- They occur as food reserves in storage organs in plants and in liver and muscles in animals
- They serve as important source of energy required for various metabolic activities of living organisms.
- They also serve as lubricants in skeletal joints, to provide adhesion between cells and to confer biological specificity on the surface of animal cells.

Biological significance of carbohydrates:

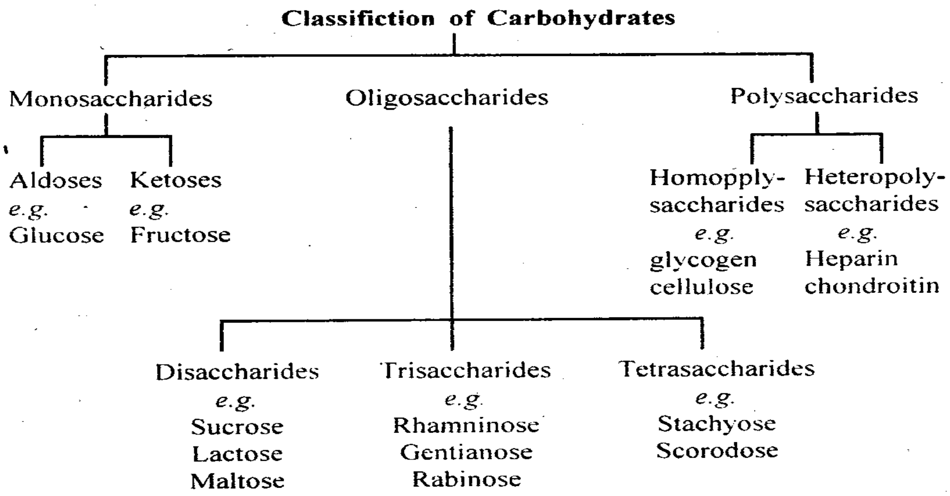
1. Major source of energy: they supply 60-80% of the caloric requirement of the body.
2. As the storage form of energy: Carbohydrates also serve as storage form of energy (glycogen) to the meet the immediate energy demands of the body. About 100 g of glycogen in deposited in the liver and tis breakdown maintains the glucose level in the blood. Muscles contain about 240 g glycogen. This glycogen is however, utilized only by the muscles and is not available for regulating the blood sugar level.
3. As the energy supplier: the muscle glycogen is broken down to lactic acid (glycolysis) to provide energy for muscle contraction.
4. Required for cellular functions: carbohydrates as glycoprotein's and glycolipids participate in the structure of cell membrane and cellular functions such as cell growth, adhesion and fertilization.
5. As structural components: Monosaccharide is important constituents of nucleic acids, coenzymes, flavoprotiens and blood group substances. Vitamin C is related to sugars.
6. Required for central nervous system: the brain and other parts of central nervous system are dependent on glucose for energy. Prolonged hypoglycemia may lead to irreversible brain damage.

7. As precursors for many organic compounds: they serve as precursors for many organic compounds (fats, amino acids).
8. Immunopolysaccharides play a part in resistance to infections.
9. Hyaluronic acid is the viscous substance in the matrix of connective tissue.
10. Heparin prevents the clotting of blood.
11. Glucoronic acid, which occurs in the lever acts as a detoxifying agent.
12. Chondroitin sulphates are found in cornea, cartilage, tendons, skin, heart valves and saliva.
13. Glycosides are components of steroid hormones.
14. Galactolipins are constituents of nervous tissues
15. Regulation of fat metabolism: some carbohydrates are essential for normal oxidation of fats. When carbohydrates are restricted in the diet, there is more rapid metabolization of fats. This results in the accumulation of incompletely oxidized intermediate products leading to ketosis. This is common in uncontrolled diabetes mellitus.
16. Synthesis of Pentoses: Pentoses- ribose and deoxyribose, the constituents of several conpounds in body e.g. nucleic acids (DNA, RNA), co-enzymes (NAD+, FAD) are produced in carbohydrate metabolism.
17. Synthesis of nonessential amino acids: The intermediates of carbohydrate metabolism, mainly the keto acids (e.g., pyruvic acid) serve as precursors for the synthesis of non-essential amino acids.
18. Protein-sparing function: Carbohydrates are preferentially metabolized in the body as a source of energy as long as it is present in the required quantity. This spares protein for building of tissues.
19. Importance of non-digestible carbohydrates: Non digestible carbohydrates are also very important as they improve bowel motility, prevent constipation and lower cholesterol absorption and also improve glucose tolerance.
20. Special function in liver: Liver is the control organ that integrates the body metabolism. Carbohydrates play an active role in this metabolic integration. The liver also utilize certain

products of carbohydrate metabolism e.g. glucoronic acid for detoxification.

21. Gastro intestinal function: lactose promotes the growth of desirable bacteria in the small intestine. These bacteria synthesize certain B-complex vitamins. Lactose also increases calcium absorption.
22. Carbohydrates are utilized as raw materials for several industries e.g., paper, plastic, textiles, alcohols etc.

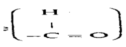
Classification of carbohydrates

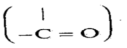


Depending upon their complexity and behaviors on hydrolysis the carbohydrates are classified into 3 categories

I. Monosaccharides (mono=one; sakcharon=sugar). Common monosaccharides (carbohydrates composed of single sugar units) possess a tree **aldehyde** (-CHO) or **ketone** (=CO) group and two or more hydroxyl (-OH) groups. They are in fact the simplest sugars and cannot be hydrolyzed into smaller units. They include glucose, fructose, and galactose.

They have potential aldehyde or keto group and hence, are reducing in nature. The monosaccharides are divided into different categories, based on the **functional group** and the number of carbon atoms.

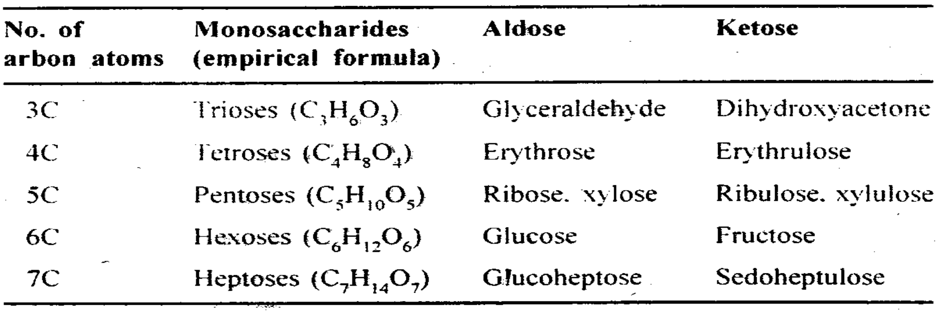
Aldoses: when the functional group in monosaccharide is an aldehyde  $\left(-\overset{\overset{H}{|}}{C}=O\right)$ they are known as aldoses e.g., glyceraldehyde, glucose. Aldehyde trop always lies at carbon number 1 of the monosaccharide molecule.

Ketoses: when the functional group is a keto  $\left(-\overset{|}{C}=O\right)$ group, they are referred as ketoses e.g., dihydroxyacetone, fructose. Keto group always lies at C no.2.

Sometimes a distinction in naming between aldoses and ketoses is maintained by using the suffix –oses to aldose and –uloses for ketoses. E.g., glucose is a hexose and fructose is a hexulose.

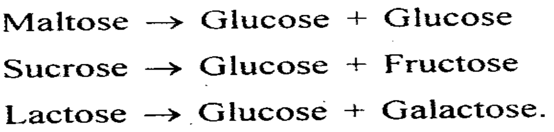
Based on the **number** of carbon atoms, monosaccharides are regarded as **triose, tetroses, pentohexoses** and **heptoses.**

Table: Classification of monosaccharides with selected examples.



No. of arbon atoms	Monosaccharides (empirical formula)	Aldose	Ketose
3C	Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
4C	Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
5C	Pentoses ($C_5H_{10}O_5$)	Ribose, xylose	Ribulose, xylulose
6C	Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
7C	Heptoses ($C_7H_{14}O_7$)	Glucoheptose	Sedoheptulose

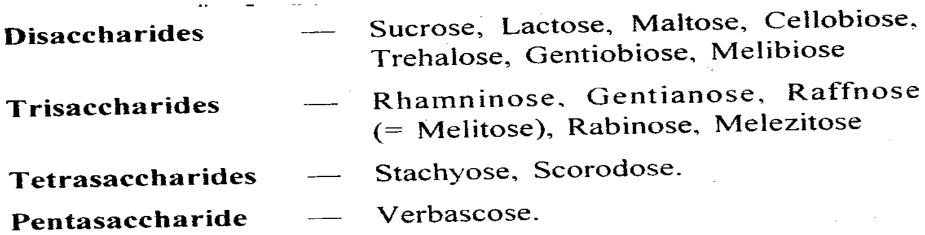
II. Oligosaccharides (oligo= few) contain 2-10 monosaccharides which are liberated on hydrolysis. Based on the number of monosaccharide units present, the oligosaccharides are further subdivided into **disaccharides, trisaccharides, tetrasaccharides, pentasaccharides …**



Maltose → Glucose + Glucose

Sucrose → Glucose + Fructose

Lactose → Glucose + Galactose.



Disaccharides — Sucrose, Lactose, Maltose, Cellobiose, Trehalose, Gentiobiose, Melibiose

Trisaccharides — Rhamninose, Gentianose, Raffnose (= Melitose), Rabinose, Melezitose

Tetrasaccharides — Stachyose, Scorodose.

Pentasaccharide — Verbascose.

Oligosaccharides are compound sugars.

III. Polysaccharides: (poly= many) are polymers of monosaccharide units with high molecular weight (up to a million). They are usually tasteless (non-sugars) and form colloids with water. Polysaccharides are of two types.

Homopolysaccharides/ homoglycans: they contain monosaccharide units of a single tye and may be represented by a general formula ($C_6H_{12}0_5$) e.g., starch, glycogen, inulin, cellulose.

Heteropolysaccharides/ heteroglycns: They possess 2 or more different types of mono-saccharide units or their derivatives e.g., heoparin, chondroitin sulfate.

MONOSACCHARIDE

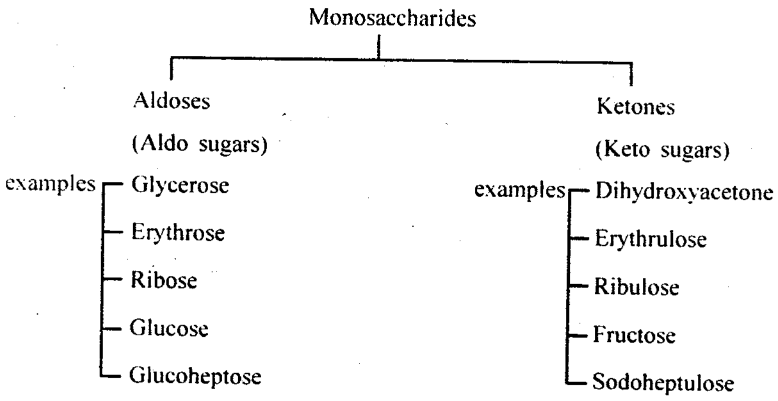
These are simplest of carbohydrate and can not be hydrolyzed. These are known as sugars, sweet-tasting, crystalline and soluble in water. These are the building units of complex carbohydrates.

Properties of monosaccharides:

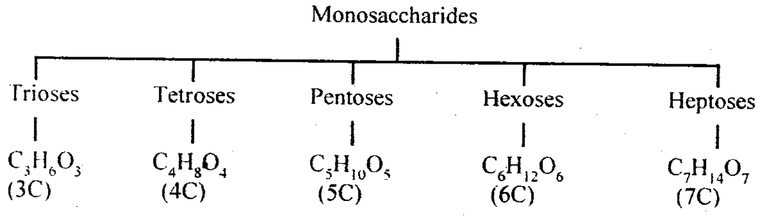
Physical properties: They are colorless crystalline compounds, readily soluble in water and sweet to taste. Their solutions are optically active and exhibit the phenomenon of **mutarotation**.

Classification of monosaccharides:

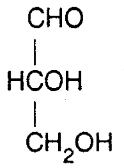
Based on aldehyde and ketone groups monosaccharides are classified as follows:



Monosaccharides may be divided depending upon the number of carbon atoms they possess.

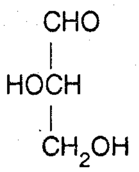


Trioses :



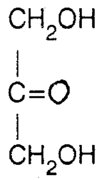
$$\begin{array}{c} CHO \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Glycerose



$$\begin{array}{c} CHO \\ | \\ HOCH \\ | \\ CH_2OH \end{array}$$

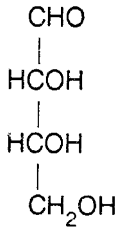
L-Glycerose



$$\begin{array}{c} CH_2OH \\ | \\ C{=}O \\ | \\ CH_2OH \end{array}$$

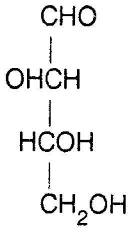
Dihydroxyacetone

Tetroses:



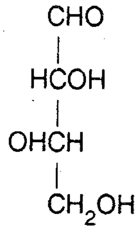
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-erythrose



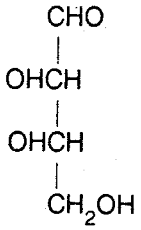
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-threose



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

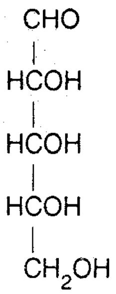
L-threose



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

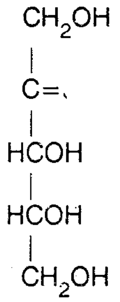
L-erthyrose

Pentoses:



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

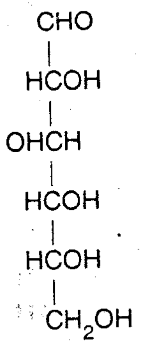
D-ribose



$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

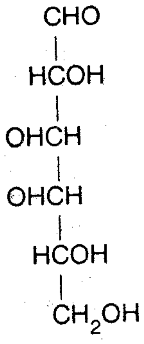
D-ribulose

Hexoses:



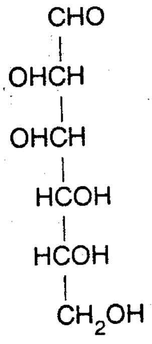
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-glucose



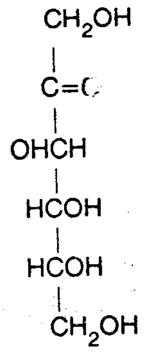
$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-galactose



$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-mannose



$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{C{=}O}\\ |\\ \mathrm{OHCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{CH_2OH}\end{array}$$

D-fructose

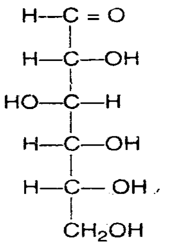
Both these characters (i.e., the number of carbon atoms and the nature of functional group present may also be combined into one. Thus for example, glycerose (= glyceraldehyde) is an aldotriose; ribulose, a ketopentose and glucose an aldohexose. It is noteworthy that except, fructose, ketoses are not as common as aldoses. The most abundant monosaccharide in nature is the 6-carbon sugar D- glucose and also it is the most common type of sugar and the primary form of sugar that is stored in the body for energy. Fructose, the primary sugar found in fruits, also is found in honey and high-fructose corn syrup (in soft drinks) and is a major source of sugar in the diet of Americans. Galactose is less likely than glucose or fructose to be found in nature. Instead, it often combines with glucose to form the disaccharide lactose, often referred to as milk sugar. Both fructose and galactose are metabolized to glucose for use by the body.

Structural aspect

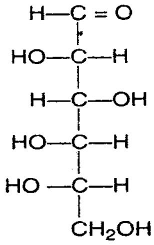
The term **isomer** (iso=equal; meros=part) was originally applied by Jones J Berzelius, in 1827, to different compounds with the same molecular formula, and he phenomenon was called isomerism. The presence of asymmetric carbon atoms in the carbohydrates makes possible the formation of isomers in them.

Stereoisomerism is an important character of monosaccharides. Stereoisomers are the compounds that have the same structural formulae but differ in their **spacial configuration**. A carbon is said to be asymmetric when it is attached to four different atoms or groups. The number of asymmetric carbon atoms (n) determines the possible isomers of a given compound which is equal to 2_n. Glucose contains 4 asymmetric carbons and thus has 16 isomers.

D and L isomers: The D and L isomers are mirror images of each other. The special orientation of -H and –OH groups on the carbon atom (C_5 for glucose) that is adjacent to the terminal primary alcohol carbon determines whether the sugar is D- or L-isomer. If the -OH groups is on the right side, the sugar is of D-series and if on the left side, it belongs to L-series. The structures of D- and L- glucose based on the reference monosaccharide, D- and L-glyceraldehydes (glycerose).



D-Glucose



L-Glucose

It may be noted that the naturally occurring monosaccharides in the mammalian tissues are mostly of D-configuration. The enzyme machinery of cells is specific to metabolise D- series of monosaccharides.

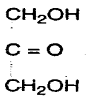
Optical activity of sugars

Optical activity is a characteristic feature of compounds with asymmetric carbon atom. When a beam of polarized light is passed through a solution of an optical isomer, it will be rotated either to the right or left. The term dextrorotatory (+) and levorotatory (-) are used to the compounds that respectively rotate the plane of polarized light to the right or to the left.

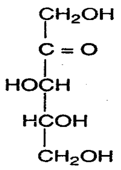
When equal amounts of dextrorotatory and levorotatory isomers are present, the resulting mixture becomes optically inactive because the optical activities of each isomer cancel each other. Such a mixture is called a **racemic** or dl-mixture or (±) conglomerate and this process of converting an optically active compound into the racemic modification is known as **racemisation** (racemus=grape). Compounds produced synthetically are invariably racemic in nature because equal chances exist for the formation of two types of optical isomers or the d and l antipodes.

Configuration of D-aldoses: Starting from D-glyceraldehyde. This is a representation of **Killiani-fischer** synthesis by increasing the chain length of an aldose, by one carbon at time. Thus, starting with an aldotriose (3C), aldotetrose(4C), aldopentoses (5C) and aldohexose(6C) are formed. Of the 8 aldohexose, glucose, mannose and galactose are the most familiar. Among these, D-glucose is the only aldose monosaccharide that predominantly occurs in nature.

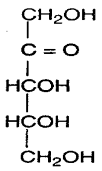
Configuration of D –ketoses L starting from dihydroxyacetone (triose), there are five keto-sugars which are physiologically important.



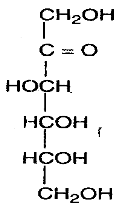
Dihydroxyacetone



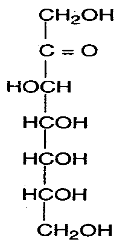
D-Xylulose



D-Ribulose



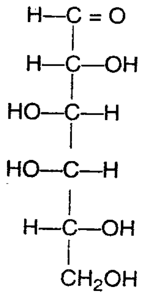
D-Fructose



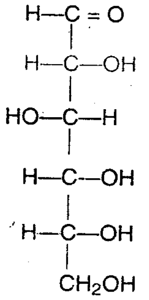
D-Sedoheptulose

Epimers:

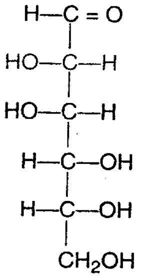
If two monosaccharides differ from each other in their configuration around a single specific carbon (other than anomeric) atom, they are referred to as epimers to each other. For instance, glucose and galactose are epimers with regard to C_4 (C_4- epimer).That is they differ in the arrangement of –OH group at C4. Glucose and mannose are epimers with regard to carbon $_2$.



D-Galactose



D-Glucose



D-Mannose

The interconversion of epimers (e.g., glucose to galactose and vice versa) is known as **epimerization** and a group of enzymes-namely-epimerases catalyse this reaction.

Mutarotation:

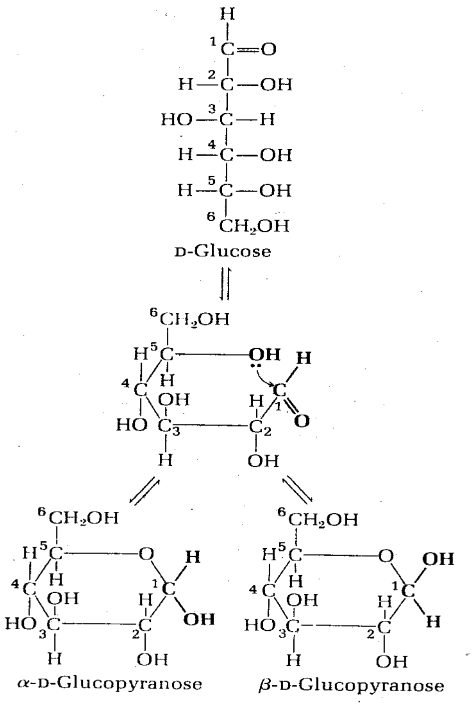
When a monosaccharide is dissolved in water, the optical rotarory power or the solution gradually changes until it reaches a constant value (Dubrunfaut, 1846). A freshly-prepared aqueous solution of □-D-glucose, for instance, has a specific rotation, [□]$^{D}{}_{20}$ a +112.2° , and when it this solution is allowed to stand, the rotation falls to + 52.7° and remains constant at this value. The final stage can be attained more quickly either by heating the solution or by adding some catalyst which may be an acid or an alkali. This gradual change in specific rotation is known as mutarotation or changing rotation. In fact, this term reflects the discovery of the phenomenon by way of changes in the optical rotation of certain carbohydrates. The terms mutatotation and birotation have sometimes also been used for mutarotation. The value of mutarotation of □□□D- glucose is (+112.2)°-(+52.7) ° or +59.5°. A fresh solution of □ -D-glucose, on theother hand, has a rotation value of +18.7 °; on standing, it also changes to the same value, +52.7 °. All reducing sugars (except a few ketoses) undergo mutarotation and the substances interconvert due to a dynamic equilibrium.

Structure of monosaccharides:

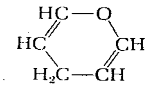
The above presentations of monosaccharides are given as projection formulas, in which the horizontal bonds are assumed to extend forward from the plane of the paper and the vertical bonds to extend to the rear. Although such structures are correct for the trioses and tetroses, monosaccharides with 5 or more carbon atoms in the backbone usually occur in solution as cyclic or ring structures, in which the carbonyl group is not free as written but has formed a covalent bond with one of the hydroxyl groups along the chain. There are two other ways of representing the three dimensional structure of sugar molecules, **Haworth projections and conformational formulas**.

One indication that D-glucose has a ring structure is that it has two crystalline forms of slightly different properties. If D-glucose is crystallized from water, a form called □ -D-glucose results, for which the specific rotation= +112.2°. If D-glucose is crystallized from pyridine, □ -D- glucose results; its rotation is +18.7°. The two forms are identical in chemical composition. From various chemical considerations it has been deduced that the □□□and □□□ isomers of D-glucose are not straight chain structures but two different six-membered ring compounds. Such cyclic forms of sugars are called **pyranoses** because they resemble the six membered ring compound **pyran**. The systematic name for the two ring forms of D-glucose are □–D-glcopyranose and □□D-glucopyranose.

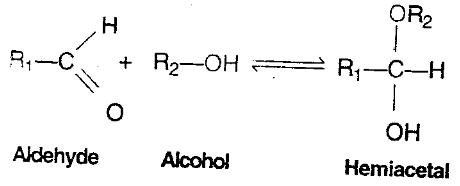
The formation of pyranose rings in D-glucose is the result of a general reaction between aldehydes and alcohols to form derivatives called hemiacetals, which contain an asymmetric carbon atom and thus can exist in two stereoisomeric forms(□ and □).


D-Glucose

α-D-Glucopyranose β-D-Glucopyranose

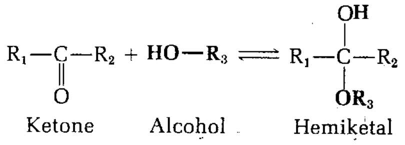

Pyran, the parent compound of pyranoses

Isomeric forms of monosaccharides that differ from each other only in their configuration about the hemiacetal carbon atom are called anomers. The hemiactal or carbonyl carbon atom is called the anomeric carbon.



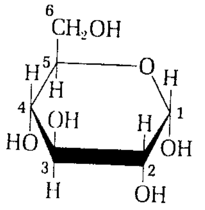
$$R_1-\overset{H}{\underset{O}{C}} + R_2-OH \rightleftharpoons R_1-\overset{OR_2}{\underset{OH}{C}}-H$$

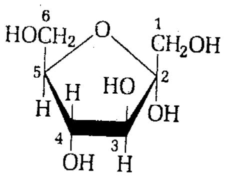
Formation of a hemiketal

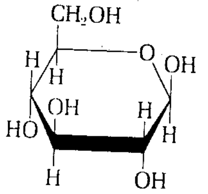


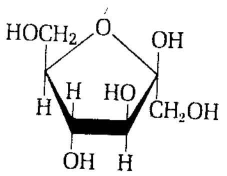
$$R_1-\underset{O}{\overset{}{C}}-R_2 + HO-R_3 \rightleftharpoons R_1-\overset{OH}{\underset{OR_3}{C}}-R_2$$

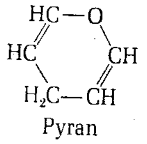
Only aldoses having 5 or more carbon atoms can form stable pyranose rings. Aldohexoses also exist in cyclic forms having five membered rings. Because such rings resemble the five membered ring compound furan, they are called furanoses. However, the six-membered aldopyranose ring is much more stalble than the aldofuranose ring and predominates in aldohexose solution.

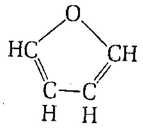

α-D-Glucopyranose


α-D-Fructofuranose

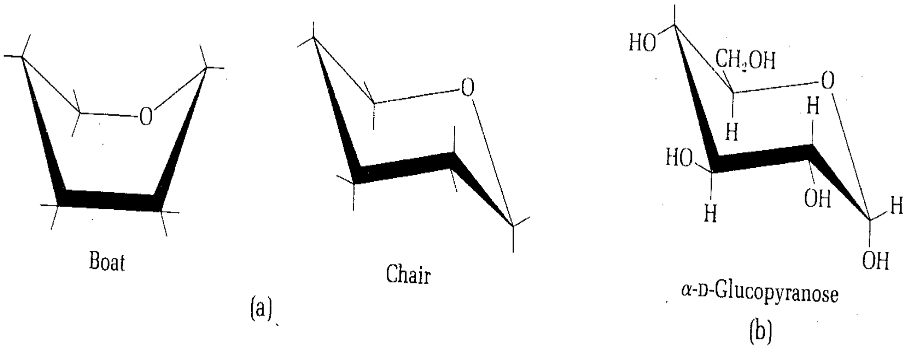

β-D-Glucopyranose


β-D-Fructofuranose


Pyran


Furan

Harworth projection formulas are commonly used to show the ring forms of monosaccharides. Although the edge of the ring nearest the reader is usually represented by bold lines, the six-membered pyranose ring is not planar, as Haworth projections suggest. In most sugars it occurs in the **chair comformation**, but in some is assumes the **boat form**; these forms are represented by conformational formulas. The specific three-dimensional conformations of the simple 6-crbon sugars are important in determining the biological properties and function of some polysaccharides.



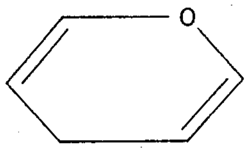
Boat Chair (a)

α-D-Glucopyranose (b)

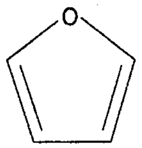
(a) conformational formulas of boat and chair forms of the pyranose ring; (b) chair conformation of □D-glucopyranose

Structure of Glucose:

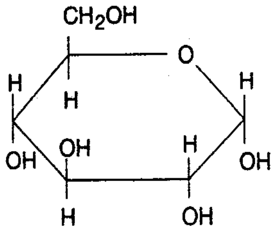
The configuration of glucose is conveniently represented either by Fischer formulae or by Haworth projection formulae. The pyranose and furanose cyclic form of glucose is as shown below.



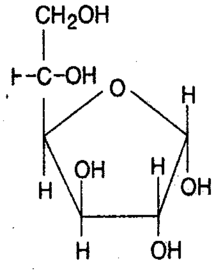
Pyran



Furan

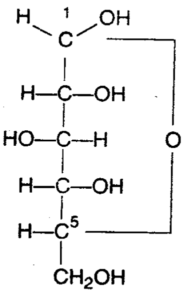


α-D-Glucopyranose

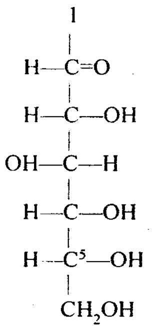


α-D-Glucofuranose

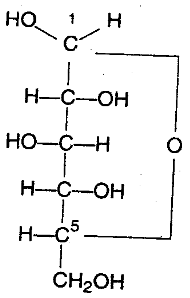
The □ and □□□□cyclic forms of D-glucose are known as **anomers**. The anomers differ in certain physical and chemical properties. Their optical rotation differs. Mutarotation is as depicted below.



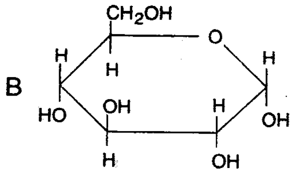
α-D-Glucose
(+ 112.2°)



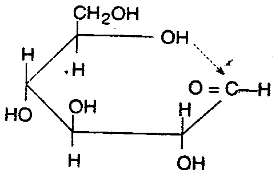
D-Glucose
(aldehyde form)



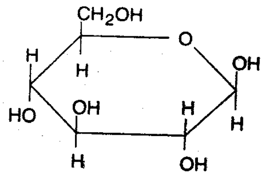
β-D-Glucose
(+ 18.7°)



α-D-Glucopyranose



D-Glucose
(aldehyde form, acyclic)



β-D-Glucopyranose

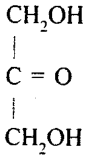
Fructose –a ketohexose:

The major dietary source of fructose is the disaccharide sucrose (cane sugar), containing equimolar quantities of fructose and glucose. It is also found in free form in honey and many fruits. In the body, entry of fructose into the cells is not controlled by the hormone insulin. This is in contrast to glucose which is regulated for its entry into majority of the tissues.

Mutarotation of fructose

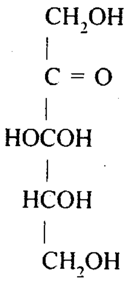
Fructose also exhibits mutarotation.. In case of fructose, the pyranose ring six-membered is converted to furanose (five membered) ring till an equilibrium is attained. And fructose has a specific optical rotation of -92° at equilibrium.

Structure of ketoses of physiological importance



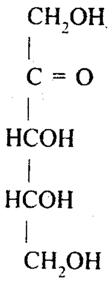
$$\begin{array}{l} CH_2OH \\ | \\ C=O \\ | \\ CH_2OH \end{array}$$

Dihydroxyacetone



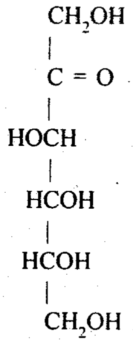
$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HOCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Xylulose



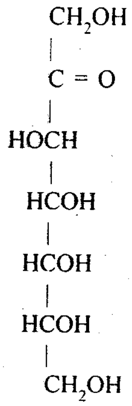
$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Ribulose



$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Fructose



$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

D-Sedoheptulose

Biological importance of monosaccharides

Monosaccharides	Occurrence	Biological Importance
Trioses		
Glyceraldehyde	Found in cells as phosphate	Glyceraldehyde 3-phosphate is an intermediate in glycolysis
Dihydroxyacetone	Found in cells as phosphate	Its 1-phosphate is an intermediate in glycolysis
Tetroses		
D-Erythrose	Widespread	Its 4-phosphate is an intermediate in carbohydrate metabolism
Pentoses		
D-Ribose	Widespread as a constituent of RNA and nucleotides	For the structure of RNA and nucleotide coenzymes (ATP, NAD^+, $NADP^+$)
D-Deoxyribose	As a constituent of DNA	For the structure of DNA
D-Ribulose	Produced during metabolism	It is an important metabolite in hexose monophosphate shunt
D-Xylose	As a constituent of glycoproteins and gums	Involved in the function of glycoproteins
L-Xylulose	As an intermediate in uronic acid pathway	Excreted in urine in essential pentosuria
Hexoses		
D-Glucose	As a constituent of polysaccharides (starch, glycogen, cellulose) and disaccharides (maltose, lactose, sucrose). Also found in fruits	The 'sugar fuel' of life; excreted in urine in diabetes. Structural unit of cellulose in plants
D-Galactose	As a constituent of lactose (milk sugar)	Converted to glucose, failure leads to galactosemia

Disaccharides

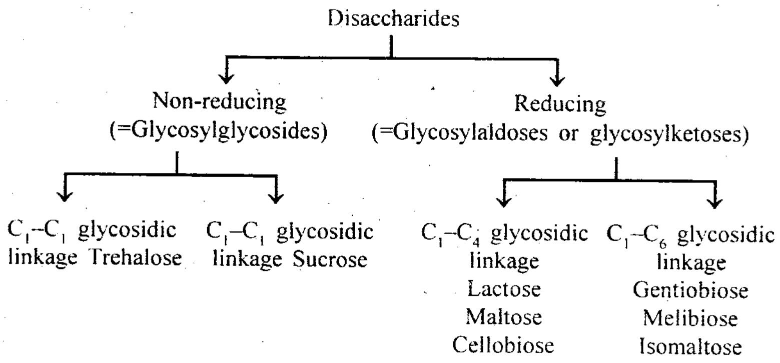
Oligosaccharides are carbohydrates made of two to ten monosaccharides.

- Among the oligosaccharides, disaccharides are the most common.
- These are sweet tasting, crystalline and soluble sugars.
- On hydrolysis they yield the monosaccharide units which may be similar or dissimilar.
- They may or may not have a free –OH group at the reducing centre and accordingly may or may not be reducing.
- These consist of more than one but fewer number of monosaccharide molecules joined together by glycosidic bond as shown below.
- Disaccharide: Those composed of two sugars are specifically referred to as disaccharides, or double sugars. They contain two monosaccharides bound by either an alpha bond or a beta bond. Alpha bonds are

digestible by the human body, whereas beta bonds are more difficult for the body to break down.

- Most of the naturally-occurring representative occurs in plant rather than in animal sources. There are three particularly important disaccharides: sucrose, maltose, and lactose; a few others are cellobiose and trehalose.

Classification of oligosaccharides:

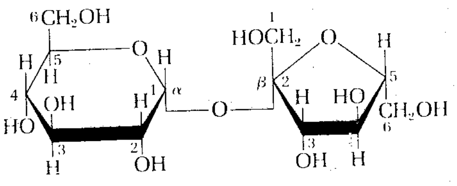


Sucrose is the sugar of commerce as it is mostly produced by sugarcane. It is also known s beat sugar as is produced by sugar beet. It is a non-reducing sugars formed when glucose and fructose are held together by an alpha bond.

Sucrose is a colourless crystalline form soluble in water and with a melting point 160°. It is dextrorotatory and has a specific rotation of +66.5°. It is by far the sweetest of the 3 common disaccharides. It crystallizes in colourless crystals.

ȝSucrose is made up of □ D -glucose and □□□D- fructose; the two monosaccharides are held together by a glycosidic bond (□→□□ between C_1 of □ – glucose and C_2 of □-fructose. The reducing groups of glucose and fructose are involved in glycosidic gond, hence sucrose is a non-reducing sugar and it cannot form osazomes.

Sucrose [O-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside]



It is noteworthy that fructose possesses the furanose ring structre in the sucrose molecule, although the pyranose ring is the dominant and more stable form in the free ketohexose. The linkage between the two disaccharide moieties or sucrose is a glycosidic one between C1 of the glucose and C2 of fructose. Obviously, sucrose does not exhibit mutarotation and is not a reducing sugar.

Inversion of sucrose:

Sucrose as such is dextrorotatory (+ 66.5°). But when hydrolyzed sucrose becomes levorotatory (-28.2°). The process of change in optical rotation from dextrorotatory to levorotatory is referred to as inversion. The hydrolyzed mixture of sucrose, containing glucose and fructose, is known as invert sugar. The process of inversion is as follow:

Upon hydrolysis, sucrose yields equimolar mixture of glucose and fructose which is often called invert sugar. This mixture readily reduces a Fehling's solution and other alike reagents. The name 'invert sugar' is given to this mixture because the levorotatory fructose, thus produced changes (or inverts) the previous dextrorotaroty action of the cusrose. In fact, sucrose (which is dextrorotatory with specific rotation of +66.7°) upon hydrolysisk gives a mixture of equimolar quantities of D(+) glucose (dextrorotaroty of+52.7°) and D(-) fructose (levorotatory of -92.0°). And as the levorotatio nof fructose is greater than the dextrorotation of glucose, the mixture so obtained is levorotatory, contrary to the initial dextrorotatory sucrose.

This reaction of "inversion of sucrose" is catalyzed b the enzyme invertase and also by H+ ions. Invert sugar is more sweeter than the sucrose itself owing to the presence of fructose in invert sugar which is the sweetest of all sugars. This also explains why honey which contains a large proportion of invert sugar is sweeter than sucrose.

Lactose

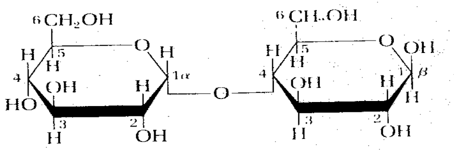
Maltose

- Commonly known as malt sugar.
- it is a reducing sugar.
- It is composed of two glucose units linked by an alpha bond. It is produced from the chemical decomposition of starch.
- Forms osazone (sunflower shaped). It forms osazone with phenyl hydrazine. It is also capable of exhibiting mutaroration.

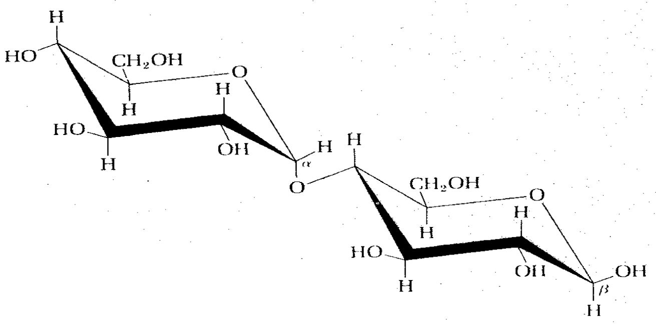
Structure:

Maltose is composed of two □ –D- glucose units held together by a (1→4) glycosidic bond. The free aldehyde group present on C1 of second lactose answers the reducing reactions.

Maltose (β form) [O-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranose]



Haworth projection



Conformational formula

Maltose may be considered as originating by splitting out a molecule of water from the glycosidic OH group of □□D- glucose and the alcoholic OH group on carbon atom 4 of D-glucose. Maltose, like lactose, has one free hemiacetal group. Consequently, it too exists in three forms □□□□ and aldehyde. Maltose isusually found in p-form. Maltose, in solution also exists as an equilibrium mixture of all the three forms.

Hydrolysis

Maltose is easily hydrolyzed into 2 identical units of glucose by the enzyme, maltase, found ill the intestine. Hydrolysis may also be brought about by the enzyme, diastase(=amylase), found in sprouting barley.

Polysaccharides/Complex Carbohydrates

- Complex carbohydrates, or polysaccharides, are composed of simple sugar units in long linear chain or branched chains called polymers.
- They are also called as glycanes or polyholosides or polysides.
- Many are regarded as polymeric anhydrides of simple sugars.
- They are amorphous, tasteless, non-sugars and insoluble in water.
- They are high molecular weight carbohydrates.

Polysaccharides differ form one another not only in the composition but also in molecular weight, in the nature of chain, type of glyucosidic bond and linkage type.

They are concerned with two important functions-structural and storage of energy.

Classification of polysaccharides:

Based on chemical properties polysaccharides are classified into two. They are:

Based on functional aspect, the polysaccharides may be grouped under two heads.

Three polysaccharides are of particular importance in human nutrition: starch, glycogen, and dietary fiber.

Starch

The word "starch" is derived from Middle English *sterchen,* meaning to stiffen. "Amylum" is Latin for starch, from the Greek "amulon" which means "not ground at a mill". The root amyl is used in biochemistry for several compounds related to starch.

Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals including man. It is a digestible forms of complex carbohydrates made of strands of glucose units linked by alpha bonds.

Starch, often contained in seeds, is the form in which plants store energy, and there are two types: amylose and amylopectin.

Humans use an enzyme to break down the bonds linking glucose units, thereby releasing the sugar to be absorbed into the bloodstream. At that point, the body can distribute glucose to areas that need energy, or it can store the glucose in the form of glycogen.

Properties:

Starch is a white soft amorphous powder and lacks sweetness. It is insoluble in water, alcohol and ether at ordinary temperature. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin.

Starch molecules arrange themselves in the plant in semi-crystalline granules. Each plant species has a unique starch granular size: rice starch is relatively small (about 2μm), potato starch have larger granules (up to 100μm). Although in absolute mass only about one quarter of the starch granules in plants consist of amylose, there are about 150 times more amylose molecules than amylopectin molecules. Amylose is a much smaller molecule than amylopectin.

A typical feature of starch is that it becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granule. This process is called starch gelatinization. During cooking the starch becomes a paste and gets its viscosity. During cooling or prolonged storage of the paste, the semi-crystalline structure partially recovers and the starch paste thickens. This is mainly caused by the retrogradation of the amylose. This process is also responsible for the staling, hardening of bread and the water layer on top of a starch gel (syneresis).

Hydrolysis

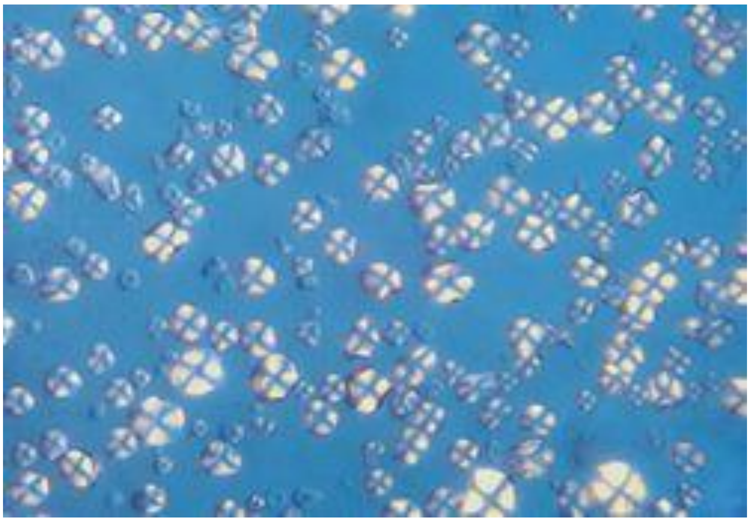
The enzymes that break down or hydrolyze starch into the constituent sugars are known as amylases.

Alpha-amylases are found in plants and in animals. Human saliva is rich in amylase, and the pancreas also secretes the enzyme. Individuals from populations with a high-starch diet tend to have more amylase genes than those with low-starch diets; chimpanzees have very few amylase genes. It is possible that turning to a high-starch diet was a significant event in human evolution. Beta-amylase cuts starch in maltose units. This process is important for the digestion of starch and also used in brewing, where the amylase from the skin of the seed grains is responsible for converting starch to maltose (Malting, Mashing).

The specific rotation of starch, □□□$^{20}_{D}$ is +196°. The microscopic form of the starch grains is characteristic of the source of starch. Starch breaks down into large fragments called dextrins on heating, especially ih the presence of moisture. The resulting dextrins then confer stiffness to clothes that have been starched and ironed. Starch molecule is highly hydrated since it contains many exposed hydroxyl groups. With the result, starch, when extracted from granules with hot water, forms turbid colloidal solutions. In common with other polysaccharides, starch is a non reducing carbohydrate since carbonyl groups of all units (except one of the two terminal ones) participate in the glycosidic linkages.

Structure:

Starch is a homopolymer composed of D-glucose units held by □□glycosidic bonds. It is known as glucosan or glucan. Starch consists of two polysaccharide comonents- water soluble amylase (15-20%) and a water insoluble amylopectin(80-85%).



Starch is one of the main source of human food

Another large non-food starch application is in the construction industry where starch is used in the gypsum wall board manufacturing process. Chemically modified or unmodified starches are added to the stucco containing primarily gypsum.

Adhesives - Starch is used in the manufacture of various glues for book-binding, wallpaper adhesives, paper sack production, tube winding, gummed paper, envelop adhesives, school glues, bottle labeling.

Clothing starch or **laundry starch** is a liquid that is prepared by mixing a vegetable starch in water (earlier preparations also had to be boiled), and is used in the laundering of clothes.

Starch is also used to make some packing peanuts, and some dropped ceiling tiles.

Textile chemicals - To reduce breaking of yarns during weaving, the warp yarns are sized. Starch is one of the main agents used for cotton sizing. Starch is also used as printing thickener.

Printing industry - in the printing industry food grade starch[9] is used in the manufacture of anti-set-off spray powder used to separate printed sheets of paper to avoid wet ink being set off.

Bioplastics - starch is used to produce various bioplastics, synthetic polymers that are biodegradable. An example is polylactic acid.

Body powder - Powdered corn starch is used as a substitute for talcum powder in many health and beauty products.

Oil exploration - starch is used to adjust the viscosity of drilling fluid which is used to lubricate the drill head in (mineral) oil extraction.

Biofuel - Glucose from starch can be further fermented to ethanol.

Hydrogen production - Starch can be used to produce hydrogen, using enzymes.

Tests

Iodine test: Iodine solution is used to test for starch; a darkblue color indicates the presence of starch. The details of this reaction are not yet fully known, but it is thought that the iodine (I_3^- and I_5^- ions) fits inside the coils of amylose, the charge transfers between the iodine and the starch, and the energy level spacings in the resulting complex correspond to the absorption spectrum in the visible light region. The strength of the resulting blue color depends on the amount of amylose present. Waxy starches with little or no amylose present will color red.

Starch indicator solution consisting of water, starch and iodine is often used in redox titrations: in the presence of an oxidizing agent the solution turns blue, in the presence of reducing agent the blue color disappears because triiodide (I_3^-) ions break up into three iodide ions, disassembling the starch-iodine complex. A 0.3% w/w solution is the standard concentration for a starch indicator. It is made by adding 4 grams of soluble starch to 1 litre of heated water; the solution is cooled before use (starch-iodine complex becomes unstable at temperatures above 35 °C).

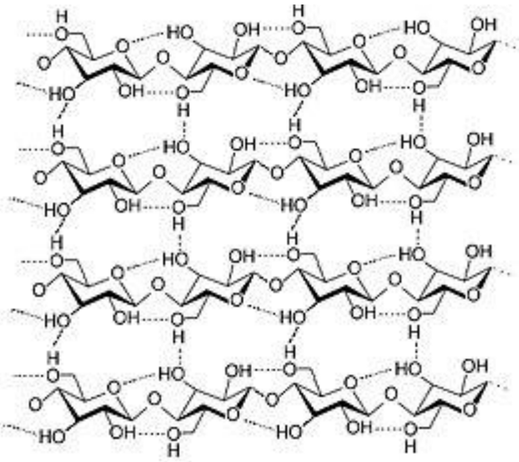
Cellulose

Fiber is derived from plant sources and contains polysaccharides such as cellulose, hemicellulose, pectin, gums, mucilages, and lignins. The indigestible fibers cellulose, hemicellulose, and lignin make up the structural part of plants and are classified as insoluble fiber because they usually do not dissolve in water. Cellulose is a nonstarch carbohydrate polymer made of a straight chain of glucose molecules linked by beta bonds and can be found in whole-wheat flour, bran, and vegetables. Hemicellulose is a nonstarch carbohydrate polymer made of glucose, galactose, xylose, and other monosaccharides; it can be found in bran and whole grains. Lignin, a noncarbohydrate polymer containing alcohols and acids, is a woody fiber found in wheat bran and the seeds of fruits and vegetables.

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on Earth. About 33 percent of all plant matter is cellulose (the cellulose content of cotton is 90 percent and that of wood is 50 percent).

For industrial use, cellulose is mainly obtained from wood pulp and cotton. It is mainly used to produce cardboard and paper; to a smaller extent it is converted into a wide variety of derivative products such as cellophane and rayon. Converting cellulose from energy crops into biofuels such as cellulosic ethanol is under investigation as an alternative fuel source.

Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts. Cellulose is not digestible by humans and is often referred to as 'dietary fiber' or 'roughage', acting as a hydrophilic bulking agent for feces.



A strand of cellulose (conformation I_α), showing the hydrogen bonds (dashed) within and between cellulose molecules.

Structure and properties

Cellulose has no taste, is odourless, is hydrophilic, is insoluble in water and most organic solvents, is chiral and is biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature.

Cellulose is derived from D-glucose units, which condense through β(1→4)-glycosidic bonds. This linkage motif contrasts with that for α(1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. Cellulose is a straight chain polymer: unlike starch, no coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues. The multiple hydroxyl groups on the glucose residues from one chain form hydrogen bonds with oxygen molecules on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming *microfibrils* with high tensile strength. This strength is important in cell walls, where the microfibrils are meshed into a carbohydrate *matrix*, conferring rigidity to plant cells.

Compared to starch, cellulose is also much more crystalline. Whereas starch undergoes a crystalline to amorphous transition when heated beyond 60-70 °C in water (as in cooking), cellulose requires a temperature of 320 °C and pressure of 25 MPa to become amorphous in water.

Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule. Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibers as well as bacterial celluloses have chain lengths ranging from 800 to 10,000 units. Molecules with very small chain length resulting from the breakdown of cellulose are known as cellodextrins; in contrast to long-chain cellulose, cellodextrins are typically soluble in water and organic solvents.

Plant-derived cellulose is usually contaminated with hemicellulose, lignin, pectin and other substances, while microbial cellulose is quite pure, has a much higher water content, and consists of long chains.

Cellulose is soluble in cupriethylenediamine (CED), cadmiumethylenediamine (Cadoxen), *N*-methylmorpholine *N*-oxide and lithium chloride / dimethylformamide]. This is used in the production of regenerated celluloses (as viscose and cellophane) from dissolving pulp.

Commercial products

Cellulose is the major constituent of paper and cardboard and of textiles made from cotton, linen, and other plant fibers.

Cellulose can be converted into cellophane, a thin transparent film, and into rayon, an important fiber that has been used for textiles since the beginning of the 20th century. Both cellophane and rayon are known as "regenerated cellulose fibers"; they are identical to cellulose in chemical structure and are usually made from viscose, a viscous solution made from cellulose. A more recent and environmentally friendly method to produce rayon is the Lyocell process.

Cellulose is the raw material in the manufacture of nitrocellulose (cellulose nitrate) which was historically used in smokeless gunpowder and as the base material for celluloid used for photographic and movie films until the mid 1930s.

Cellulose is used to make water-soluble adhesives and binders such as methyl cellulose and carboxymethyl cellulose which are used in wallpaper paste. Microcrystalline cellulose (E460i) and powdered cellulose (E460ii) are used as inactive fillers in tablets] and as thickeners and stabilizers in processed foods.

Cellulose is used in the laboratory as the stationary phase for thin layer chromatography. Cellulose fibers are also used in liquid filtration, sometimes in combination with diatomaceous earth or other filtration media, to create a filter bed of inert material. Cellulose is further used to make hydrophilic and highly absorbent sponges.

Cellulose insulation made from recycled paper is becoming popular as an environmentally preferable material for building insulation. It can be treated with boric acid as a fire retardant.

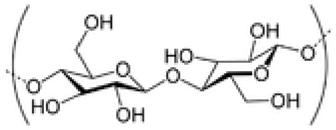
Cellulose source and energy crops

The major combustible component of non-food energy crops is cellulose, with lignin second. Non-food energy crops are more efficient than edible energy crops (which have a large starch component), but still compete with food crops for agricultural land and water resources. Typical non-food energy crops include industrial hemp, switchgrass, *Miscanthus, Salix* (willow), and *Populus* (poplar) species.

Tests for cellulose

Given a cellulose-containing material, the carbohydrate portion that does not dissolve in a 17.5% solution of sodium hydroxide at 20 °C is *α cellulose*, which is true cellulose. Acidification of the extract precipitates *β cellulose*. The portion that dissolves in base but does not precipitate with acid is *γ cellulose*.

Cellulose can be assayed using a method described by Updegraff in 1969, where the fiber is dissolved in acetic and nitric acid to remove lignin, hemicellulose, and xylosans. The resulting cellulose is allowed to react with anthrone in sulfuric acid. The resulting coloured compound is assayed spectrophotometrically at a wavelength of approximately 635 nm.



Proteins

Like carbohydrates, some proteins perform largely structural roles.

Proteins (also known as **polypeptides**) are organic compounds made of amino acids arranged in a linear chain. The amino acids in a polypeptide chain are linked by peptide bonds. In essence, proteins are chains of amino acids. An amino acid consists of a carbon atom bound to four groups. One is an amino group, —NH_2, and one is a carboxylic acid group, —COOH (although these exist as —NH_3^+ and —COO^- under physiologic conditions). The third is a simple hydrogen atom. The fourth is commonly denoted "—R" and is different for each amino acid. There are twenty standard amino acids. Some of these have functions by themselves or in a modified form; for instance, glutamate functions as an important neurotransmitter.

Amino acids can be joined together via a peptide bond. In this dehydration synthesis, a water molecule is removed and the peptide bond connects the nitrogen of one amino acid's amino group to the carbon of the other's carboxylic acid group. The resulting molecule is called a *dipeptide*, and short stretches of amino acids (usually, fewer than around thirty) are called *peptides* or polypeptides. Longer stretches merit the title *proteins*. As an example, the important blood serum protein albumin contains 585 amino acid residues.

The amino acids in a polymer chain are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids, however in certain organisms the genetic code can include selenocysteine — and in certain archaea — pyrrolysine. Shortly after or even during synthesis, the residues in a protein are often chemically modified by post-translational modification, which alter the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins. Proteins can also work together to achieve a particular function, and they often associate to form stable complexes.

Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle. Proteins are also necessary in animals' diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food. Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism.

Proteins are linear polymers built from series of up to 20 different L-α-amino acids. All amino acids possess common structural features, including an α-carbon to which an amino group, a carboxyl group, and a variable side chain are bonded. Only proline differs from this basic structure as it contains an unusual ring to the N-end amine group, which forces the CO–NH amide moiety into a fixed conformation. The side chains of the standard amino acids, detailed in the list of standard amino acids, have a great variety of chemical structures and properties; it is the combined effect of all of the amino acid side chains in a protein that ultimately determines its three-dimensional structure and its chemical reactivity.

Once linked in the protein chain, an individual amino acid is called a *residue,* and the linked series of carbon, nitrogen, and oxygen atoms are known as the *main chain* or *protein backbone.* The peptide bond has two resonance forms that contribute some double-bond character and inhibit rotation around its axis, so that the alpha carbons are roughly coplanar. The other two dihedral angles in the peptide bond determine the local shape assumed by the protein backbone. The end of the protein with a free carboxyl group is known as the C-terminus or carboxy terminus, whereas the end with a free amino group is known as the N-terminus or amino terminus.

The words *protein, polypeptide,* and *peptide* are a little ambiguous and can overlap in meaning. *Protein* is generally used to refer to the complete biological molecule in a stable conformation, whereas *peptide* is generally reserved for a short amino acid oligomers often lacking a stable three-dimensional structure. However, the boundary between the two is not well defined and usually lies near 20–30 residues. *Polypeptide* can refer to any single linear chain of amino acids, usually regardless of length, but often implies an absence of a defined conformation.

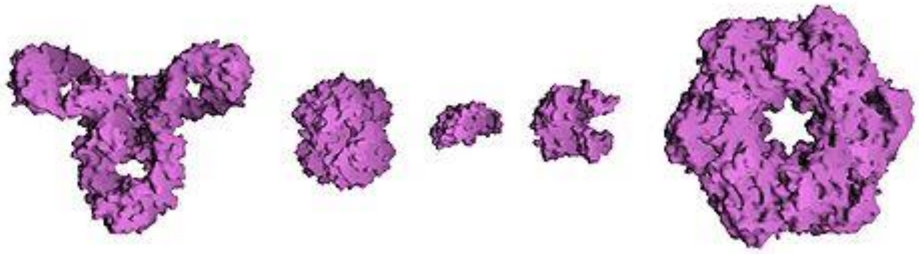
Most proteins fold into unique 3-dimensional structures. The shape into which a protein naturally folds is known as its native conformation. Although many proteins can fold unassisted, simply through the chemical properties of their amino acids, others require the aid of molecular chaperones to fold into their native states. Biochemists often refer to four distinct aspects of a protein's structure:

- *Primary structure*: the amino acid sequence.
- *Secondary structure*: regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha helix and beta sheet. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.
- *Tertiary structure*: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary

structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even post-translational modifications. The term "tertiary structure" is often used as synonymous with the term *fold*. The Tertiary structure is what controls the basic function of the protein.

- *Quaternary structure*: the structure formed by several protein molecules (polypeptide chains), usually called *protein subunits* in this context, which function as a single protein complex.

Proteins are not entirely rigid molecules. In addition to these levels of structure, proteins may shift between several related structures while they perform their functions. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as "conformations", and transitions between them are called *conformational changes*. Such changes are often induced by the binding of a substrate molecule to an enzyme's active site, or the physical region of the protein that participates in chemical catalysis. In solution proteins also undergo variation in structure through thermal vibration and the collision with other molecules.



Molecular surface of several proteins showing their comparative sizes. From left to right are: immunoglobulin G (IgG, an antibody), hemoglobin, insulin (a hormone), adenylate kinase (an enzyme), and glutamine synthetase (an enzyme).

Tertiary proteins are further classified into three types they are

i) simple proteins

ii) globular proteins

iii) conjugated proteins

Simple protein are one of the two main classes of protein tertiary structure (the other being globular proteins).They are also called fibrous proteins.

Characteristic

They form long protein filaments, rod- or wire-like shapes. They are usually inert structural or storage proteins. They are generally water-insoluble and are found as an aggregate due to hydrophobic R groups that stick out of the molecule. The amino acid sequences they are made from often have limited residues with repeats. These can form unusual secondary structures, e.g. collagen triple helix. The structures often contain 'cross-links' between chains, for example cys-cys disulfide bonds between keratin chains.

Globular proteins tend to denature more easily than fibrous proteins.

Functions

They usually play a role which is protective or supportive

They are usually used to construct connective tissues, tendons, bone matrix and muscle fiber.

Attempts at artificial synthesis have been made.Examples of include keratins, collagens and elastins. Another example is fibroin

Globular protein

Globular proteins, are the other type of the two main protein classes, comprising "globe"-like proteins that are more or less soluble in aqueous solutions (where they form colloidal solutions). This main characteristic helps distinguishing them from fibrous proteins (the other class), which are practically insoluble.

The term globin can refer more specifically to proteins including the globin fold.

Globular structure and solubility

The term globular protein is quite old (dating probably from the 19th century) and is now somewhat archaic given the hundreds of thousands of proteins and more elegant and descriptive structural motif vocabulary. The globular nature of these proteins can be determined without the means of modern techniques, but only by using ultracentrifuges or dynamic light scattering techniques.

The spherical structure is induced by the protein's tertiary structure. The molecule's apolar (hydrophobic) amino acids are bounded towards the molecule's interior whereas polar (hydrophilic) amino acids are bound outwards, allowing dipole-dipole interactions with the solvent, which explains the molecule's solubility.

Unlike fibrous proteins which only play a structural function, globular proteins can act as:

- Enzymes, by catalyzing organic reactions taking place in the organism in mild conditions and with a great specificity. Different esterases fulfill this role.
- Messengers, by transmitting messages to regulate biological processes. This function is done by hormones, i.e. insulin etc.
- Transporters of other molecules through membranes
- Stocks of amino acids.
- Regulatory roles are also performed by globular proteins rather than fibrous proteins.

Among the most known globular proteins is Hemoglobin, a member of the globin protein family. Other globular proteins are the immunoglobulins (IgA, IgD, IgE, IgG and IgM), and alpha, beta and gamma globulins. See protein electrophoresis for more information on the different globulins. Nearly all enzymes with major metabolic functions are globular in shape, as well as many signal transduction proteins.

Conjugated protein

A **conjugated protein** is a protein that functions in interaction with other chemical groups attached by covalent bonds or by weak interactions.

Many proteins contain only amino acids and no other chemical groups, and they are called simple proteins. However, other kind of proteins yield, on hydrolysis, some other chemical component in addition to amino acids and they are called conjugated proteins. The non amino part of a conjugated protein is usually called its prosthetic group. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups.

Some examples of conjugated proteins are lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins, metalloproteins, phytochromes, cytochromes and opsins.

Glycoproteins are generally the largest and most abundant group of conjugated proteins. They range from glycoproteins in cell surface membranes that constitute the glycocalyx, to important antibodies produced by leukocytes.

LIPIDS

Lipids are a broad group of naturally-occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment.

Composition of Lipids:

Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

Although the term *lipid* is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

Classification:

Lipids are also classified into

I. Simple lipids- neutral fats and waxes
II. Compound lipids- phospholipids, glycolipids and lipolipids
III. Derived lipids- fatty acids, glycerols and sterols

Fatty acids- they contain the elements C, H, and O. Most of the naturally occurring fatty acids are straight chain derivatives and have an even number of carbon atoms. They may be saturated or unsaturated.

Saturated fatty acids: the can be represented by the general foumula CH3(CH)nCOOH. The simplest is acetic acid and (n=0) Eg. Acetic acid, butyric acid, caproic acid etc.

Unsaturated fatty acids: they conatain one or more double bonds. Some of the important fatty acids are palmitoeic acid, oelic acid, linolenic acid, linolenic acid etc.

Categories of lipids

Fatty acids

Fatty acyls, a generic term for describing fatty acids, their conjugates and derivatives, are a diverse group of molecules synthesized by chain-elongation of an acetyl-CoA primer with malonyl-CoA or methylmalonyl-CoA groups in a process called fatty acid synthesis. They are made of a hydrocarbon chain that terminates with a carboxylic acid group; this arrangement confers the molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water. The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building block of more structurally complex lipids. The carbon chain, typically between four to 24 carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen and sulfur. Where a double bond exists, there is the possibility of either a *cis* or *trans* geometric isomerism, which significantly affects the molecule's molecular configuration. *Cis*-double bounds cause the fatty acid chain to bend, an effect that is more pronounced the more double bonds there are in a chain. This in turn plays an important role in the structure and function of cell membranes. Most naturally occurring fatty acids are of the *cis* configuration, although the *trans* form does exist in some natural and partially hydrogenated fats and oils.

Other major lipid classes in the fatty acid category are the fatty esters and fatty amides. Fatty esters include important biochemical intermediates such as wax esters, fatty acid thioester coenzyme A derivatives, fatty acid thioester ACP derivatives and fatty acid carnitines. The fatty amides include N-acyl ethanolamines, such as the cannabinoid neurotransmitter anandamide.

Glycerolipids

Glycerolipids are composed mainly of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol (triacylglycerols), also known as triglycerides.

Glycerophospholipids

Glycerophospholipids, also referred to as phospholipids, are ubiquitous in nature and are key components of the lipid bilayer of cells, as well as being involved in metabolism and cell signaling. Neural tissue (including the brain) contains relatively high amounts of glycerophospholipids, and alterations in their composition has been implicated in various neurological disorders.

Sphingolipids

Sphingolipids are a complex family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized *de novo* from the amino acid serine and a long-chain fatty acyl CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids and other compounds.

Sterol lipids

Sterol lipids, such as cholesterol and its derivatives, are an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins. The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signaling molecules.

Prenol lipids

Prenol lipids are synthesized from the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid (MVA) pathway.

Saccharolipids

Saccharolipids describe compounds in which fatty acids are linked directly to a sugar backbone, forming structures that are compatible with membrane bilayers. In the saccharolipids, a monosaccharide substitutes for the glycerol backbone present in glycerolipids and glycerophospholipids.

Polyketides

Polyketides are synthesized by polymerization of acetyl and propionyl subunits by classic enzymes as well as iterative and multimodular enzymes that share mechanistic features with the fatty acid synthases. They comprise a large number of secondary metabolites and natural products from animal, plant, bacterial, fungal and marine sources, and have great structural diversity.

Biological functions

Membranes : Eukaryotic cells are compartmentalized into membrane-bound organelles which carry out different biological functions. The glycerophospholipids are the main structural component of biological membranes, such as the cellular plasma membrane and the intracellular membranes of organelles. The glycerophospholipids are amphipathic molecules (containing both hydrophobic and hydrophilic regions) that contain a glycerol core linked to two fatty acid-derived "tails" by ester linkages and to one "head" group by a phosphate ester linkage. While glycerophospholipids are the major component of biological membranes, other non-glyceride lipid components such as sphingomyelin and sterols (mainly cholesterol in animal cell membranes) are also found in biological membranes. In plants and algae, the galactosyldiacylglycerols, and sulfoquinovosyldiacylglycerol, which lack a phosphate group, are important components of membranes of chloroplasts and related organelles and are the most abundant lipids in photosynthetic tissues, including those of higher plants, algae and certain bacteria.

Energy storage: Triacylglycerols, stored in adipose tissue, are a major form of energy storage in animals.

Signaling: In recent years, evidence has emerged showing that lipid signaling is a vital part of the cell signaling. Lipid signaling may occur via activation of G protein-coupled or nuclear receptors, and members of several different lipid categories have been identified as signaling molecules and cellular messengers.

Other functions

The "fat-soluble" vitamins (A, D, E and K) – which are isoprene-based lipids – are essential nutrients stored in the liver and fatty tissues, with a diverse range of functions.

Acyl-carnitines are involved in the transport and metabolism of fatty acids in and out of mitochondria, where they undergo beta oxidation. Polyprenols and their phosphorylated derivatives also play important transport roles, in this case the transport of oligosaccharides across membranes.

Polyprenol phosphate sugars and polyprenol diphosphate sugars function in extra-cytoplasmic glycosylation reactions, in extracellular polysaccharide biosynthesis (for instance, peptidoglycan polymerization in bacteria), and in eukaryotic protein N-glycosylation.

Cardiolipins are a subclass of glycerophospholipids containing four acyl chains and three glycerol groups that are particularly abundant in the inner mitochondrial membrane. They are believed to activate enzymes involved with oxidative phosphorylation.

Breakdown of fatty acids

The neutral fat is hydrolyzed to fatty acids and glycerol in the adipose tissue and liberated into the blood. The former transported an an albuminFAA complex. The glycerol can be converted to glycerophosphate by the enzyme glycerokinase and later converted to dihydroxyacetone phosphate. It can be utilized in the glycolytic pathway.

Oxidation of fatty acids was studied by Knoop by tagging themethylcarbon with phenyl group. His findings are, oxidation of fatty acid occurs at the carbon, resulting in the formation of a molecule of acetate from the terminal two carbons and leaving a reside of a fatty acid containing two carbons less than theoriginal. The process repeats itself until the fatty acid with even number of carbons is completely converted to acetate molecules. The detailes steps are as follows

1. Activation of fatty acid

$$\underset{\text{Fatty acid}}{RCH_2\ CH_2\ COOH} + \underset{\text{Coenzyme A}}{HS\text{-}CoA} \xrightarrow[ATP \qquad AMP+PP]{\text{Thiokinase} \atop Mg^{++}} \underset{\text{Acylcoenzyme A}}{RH_2\ CH_2\ CO\text{-}SCoA}$$

The active fatty acid can now undergo oxidation.

2. Formation of unsaturated acyl-CoA.

$$RCH_2\ CH_2\ CO\text{-}SCoA \xrightarrow[FAD \qquad FAD-H_2]{\text{Acyl CoA dehydrogenase}} \underset{\alpha,\beta\text{ - unsaturated acyl CoA}}{RCH=CHCo\ -\ SCoA}$$

The hydrogenesis taken up by FAD of the enzyme. Different enzymes are ikely to act on fatty acid of varying chain lengths.

3. Formation of β-hydroxy Acyl-CoA.

$$RCH{=}CHCO\text{-}SCoA + H_2O \xrightarrow[\text{(Crotonase)}]{\text{Enoylhydrase}} RCHOH\ CH_2\ CO\text{-}SCoA$$

β-hydroxy acyl CoA

4. Formation of β-keto acyl-CoA

$$RCHOHCH_2\ CO\text{-}SCoA \xrightarrow[NAD^+ \qquad NADH+H^+]{\substack{\text{Hydroxy acylCoA}\\ \text{dehydrogenase}}} RCOCH_2\ CO\text{-}SCoA$$

β-Keto acyl CoA

5. Thiolytic cleavage of acetyl - coenzyme A

$$RCOCH_2\ CO\text{-}SCoA + HSCoA \xrightarrow{\beta-\text{ketothiolase}} RCO\text{-}CoA + CH_3\ CO\text{-}SCoA$$

acylcoenzyme A acetylcoenzyme A

The acyl-CoA formed in this step, is an active fatty acid containing two carbon less than the original one and can undergo a similar set of reactions starting at reaction 2. this can repeat itself till a 4 carbon acyl1-Co A *butryl – CoA) is formed which is finally converted to tow molecules of acetyl coenzyme A. All the enzymes required for oxidation are present in the cell mitochondria. The acetate produced as a result of oxidation can enter the citric acid cycle and be oxidized in that cycle to carbon dioxide and water and yield energy.

NOTES

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UNIT V

Growth: plant growth regulators- auxins, gibberellins, cytokinins, ethylene, abscissic acid- their physiological effects and their role.

Plant movements- geotropism, phototropism, Thigmotropism

GROWTH

Growth is an important characteristic of a living structure. It is a very complex process and cannot be easily defined. However, it can be broadly defined as a permanent and irreversible change in size or volume of a living structure with an accompanied increase in dry weight.

Growth as we know is affected by a host of environmental factors as well as a number of physiological process, *viz.*, absorptin of water and minerals, photosynthesis, respiration etc. to a very large extent.

The growth of plants is affected by following external and internal factors.

External factors:

It includes all those factors which affect various physiological activities of the plants directly or indirectly. Some of those important factors are light, temperature, oxygen, carbondioxide, water and food materials.

Light:

It chiefly affects photosynthesis and transpiration. The favourable length of day for each plant or the light received during day time by a plant is called photoperiod (Garnard and Allard, 1920). According to the light duration the plants may be short day, long day, day neutral. For flowering, the short day plants require less amount of light while long day plants require more amount of light. Day neutral plants usually become weak, thin and yellow. The effect of light on growth is studied under various aspects like

- Light intensity
- light quantity
- duration of light

Temperature:

The plants growing in different regions require different temperature. The termpeature has pronounced effectr on growth. It occurs between 4° C to 45° C. Plants in colder regions grow best between 35°C to 40°C and warmer regions between 38° C to 44° C. Some seeds show germination wshen they are kept at low temperature similarly0some plants require low temperature in additin to sufficient photoperiod for flowering. When they are given low temperature at initial stages, they show earlyflowering later on. This is called as vernalization. Eg. Wheat, rye, rice etc.

Oxygen:

It is necessary for respiration during which the food materials are oxidised to release energy. Growth is direclty proportional to the amount of oxygen.

Carbon di oxide:

It chiefly effects photosynthesis and thus manufacture of food material, which are most important for growth.

Water;

Absorption, translocation of food, photosynthesis, activation of enzymes and protoplasm is affected by water. All these above process are related with growth.

Food material:

The growth rate increases in presence of excess of food materials. They also increase the concentration of cytoplasm and rate of cell division.

Internal factors

‘ Organic substance produced naturally in the higher plants controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts are termed as plant hormones, growth hormones, growth-regulators, phytohormones, growth factors, growth substance etc.

Hormones are characterized by

1. They are usually produced at the tips of root, stem and leaves.
2. The transfer of hormone from the site of production to other parts is through phloem.
3. Their growth promoting action occurs only when they are used in definite quantity and concentration.
4. They are required in traces.
5. They can be isolated from the plant organs by chemical methods.
6. Some growth regulating substances are called vitamins which act like hormone.
7. All hormones are organic in nature.

Natural and synthetic hormones

1. **Auxins**: Indole acetic acid (IAA), 2-4 dichlorophenoxyacetic acid (2,4 D) etc.
2. **Gibberellins**: Gibberellic acid (GA)
3. **Cytokinins**: Kinetin, Zeatin.

4. **Dormins**: Abscissic acid (ABA), xanthoxin etc.
5. **Ethylene**:ethylene
6. **flowering hormone**: florigen, vernalin
7. **Phenolic compounds**: Coumarin.
8. **Other natural substance**: Vitamins, traumatic, phytohormone etc.
9. **Synthetic growth retardants**: Phosphon D, Morphactins.
10. other **synthetic growth substance:** synthetic auxins and cytokinins.

Plant growth regulators

Plant growth regulators (also known as **phytohormones**) are chemicals that regulate plant growth. Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and when moved to other locations, in other locations of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity and even plant death. Hormones are vital to plant growth and lacking them, plants would be mostly a mass of undifferentiated cells.

Characteristics

Plant growth regulators affect gene expression and transcription levels, cellular division and growth. They are naturally produced within plants, though very similar chemicals are produced by fungi and bacteria that can also effect plant growth. A large number of related chemical compounds are synthesized by humans, they are used to regulate the growth of cultivated plants, weeds, and in vitro grown plants and plant cells; these man made compounds are called **Plant Growth Regulators** or **PGRs** for short. Early in the study of plant hormones, "phytohormone" was the commonly-used term, but its use is less widely applied now.

Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. Plants lack glands to produce and store hormones. Plants use more passive means to move chemicals around the plant. Plants utilize simple chemicals as hormones, which move more easily through the plant's tissues. They are often produced and used on a local basis within the plant body.

Hormones are transported within the plant by utilizing four types of movements.

1. For localized movement, cytoplasmic streaming within cells
2. Slow diffusion of ions and molecules between cells.
3. Vascular tissues are used to move hormones from one part of the plant to another; these include sieve tubes that move sugars from the leaves to the roots and flowers
4. Xylem that moves water and mineral solutes from the roots to the foliage.

Not all plant cells respond to hormones, but those cells that do are programmed to respond at specific points in their growth cycle. The greatest effects occur at specific stages during the cell's life, with diminished effects occurring before or after this period. Plants need hormones at very specific times during plant growth and at specific locations. They also need to disengage the effects that hormones have when they are no longer needed. The production of hormones occurs very often at sites of active growth within the meristems, before cells have fully differentiated. After production they are sometimes moved to other parts of the plant where they cause an immediate effect or they can be stored in cells to be released later.

Plants use different pathways to regulate internal hormone quantities and moderate their effects; they can regulate the amount of chemicals used to biosynthesize hormones. They can store them in cells, inactivate them, or cannibalise already-formed hormones by conjugating them with carbohydrates, amino acids or peptides. Plants can also break down hormones chemically, effectively destroying them. Plants also move hormones around the plant diluting their concentrations.

The concentration of hormones required for plant responses are very low (10^{-6} to 10^{-5} mol/L). Because of these low concentrations it has been very difficult to study plant hormones and only since the late 1970s have scientists been able to start piecing together their effects and relationships to plant physiology. Much of the early work on plant hormones involved studying plants that were genetically deficient in one or involved the use of tissue cultured plants grown *in vitro* that were subjected to differing ratios of hormones and the resultant growth compared. The earliest scientific observation and study dates to the 1880s; the determination and observation of plant hormones and their identification was spread-out over the next 70 years.

Classes of plant hormones

It is generally accepted that there are five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next. The chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Other plant hormones and growth regulators are not easily grouped into these classes, they exist naturally or are synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. Each class has positive as well as inhibitory functions, and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

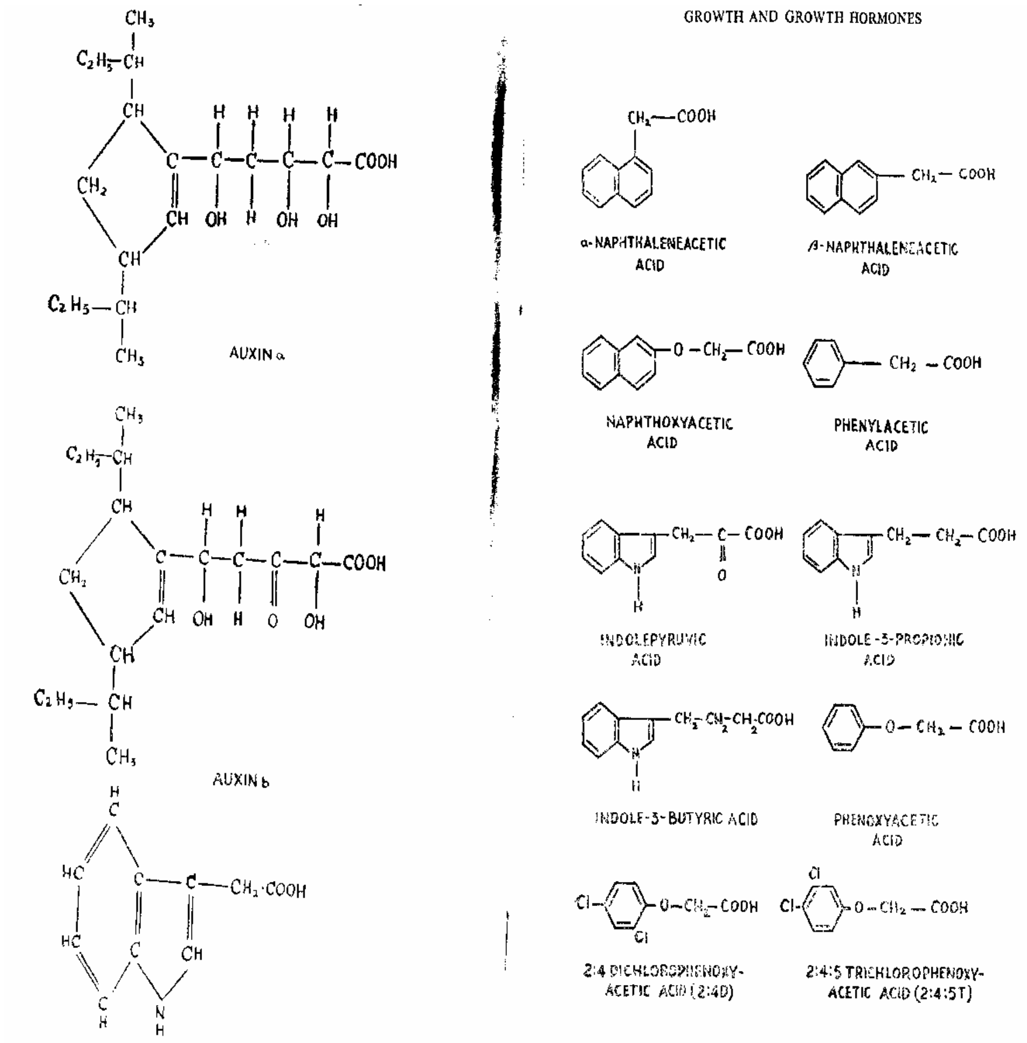
The five major classes are:

Auxins

Auxins are a class of plant growth substance and morphogens (often called phytohormone or plant hormone). Auxins play an essential role in coordination of many growth and behavioral processes in the plant life cycle, they and the behavior they played in plant growth was first revealed by a Dutch scientist named Fritz Went (1903-1990).

Auxins derive their name from the Greek word αυξανω ("auxano" -- "I grow/increase"). They were the first of the major plant hormones to be discovered and are a major coordinating signal in plant development. Their pattern of active transport through the plant is complex. They typically act in concert with (or opposition to) other plant hormones. For example, the ratio of auxin to cytokinin in certain plant tissues determines initiation of root versus shoot buds. Thus a plant can (as a whole) react on external conditions and adjust to them, without requiring a nervous system. On a molecular level, auxins have an aromatic ring and a carboxylic acid group (Taiz and Zeiger, 1998).

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots, fruits and convert stems into flowers. Auxins were the first class of growth regulators discovered. They affect cell elongation by altering cell wall plasticity. Auxins decrease in light and increase where its dark. They stimulate cambium cells to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds lower down the stems (apical dominance), and also to promote lateral and adventitious root development and growth. Leaf abscission is initiated by the growing point of a plant ceasing to produce auxins. Auxins in seeds regulate specific protein synthesis, as they develop within the flower after pollination, causing the flower to develop a fruit to contain the developing seeds. Auxins are toxic to plants in large concentrations; they are most toxic to dicots and less so to monocots. Because of this property, synthetic auxin herbicides including 2,4-D and 2,4,5-T have been developed and used for weed control. Auxins, especially 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA), are also commonly applied to stimulate root growth when taking cuttings of plants. The most common auxin found in plants is indoleacetic acid or IAA.



Chemical structure of natural and sythetic auxins

The most important member of the auxin family is **indole-3-acetic acid** (IAA). It generates the majority of auxin effects in intact plants, and is the most potent native auxin. However, molecules of IAA are chemically labile in aqueous solution, so IAA is not used commercially as a plant growth regulator.

- *Naturally-occurring auxins* include 4-chloro-indoleacetic acid, phenylacetic acid (PAA) and indole-3-butyric acid (IBA).
- *Synthetic auxin analogs* include 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and others.

Auxins are often used to promote initiation of adventitious roots and are the active ingredient of the commercial preparations used in horticulture to root stem cuttings. They can also be used to promote uniform flowering, to promote fruit set, and to prevent premature fruit drop.

Used in high doses, auxin stimulates the production of ethylene. Excess ethylene can inhibit elongation growth, cause leaves to fall (leaf abscission), and even kill the plant. Some synthetic auxins such as 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been used as herbicides. Broad-leaf plants (dicots) such as dandelions are much more susceptible to auxins than narrow-leaf plants (monocots) like grass and cereal crops. These synthetic auxins were the active agents in Agent Orange, a defoliant used extensively by American forces in the Vietnam War.

Hormonal activity

Auxins coordinate development at all levels in plants, from the cellular level to organs and ultimately the whole plant.

The plant cell wall is made up of cellulose, protein, and, in many cases, lignin. It is very firm and prevents any sudden expansion of cell volume, and, without contribution of auxins, any expansion at all.

Molecular mechanisms

Auxins directly stimulate or inhibit the expression of specific genes.[Auxin induces transcription by targeting for degradation members of the Aux/IAA family of transcriptional repressor proteins, The degradation of the Aux/IAAs leads to the derepression of **A**uxin **R**espose **F**actors ARF-mediated transcription. Aux/IAAs are targeted for degradation by ubiquitination, catalysed by an SCF-type ubiquitin-protein ligase.

Another protein called ABP1 (Auxin Binding Protein 1) is a putative receptor, but its role is unclear. Electrophysiological experiments with protoplasts and anti-ABP1 antibodies suggest that ABP1 may have a function at the plasma membrane.

On a cellular level

On the cellular level, auxin is essential for cell growth, affecting both cell division and cellular expansion. Depending on the specific tissue, auxin may promote axial elongation (as in shoots), lateral expansion (as in root swelling), or isodiametric expansion (as in fruit growth). In some cases (coleoptile growth) auxin-promoted cellular expansion occurs in the absence of cell division. In other cases, auxin-promoted cell division and cell expansion may be closely sequenced within the same tissue (root initiation, fruit growth). In a living plant it appears that auxins and other plant hormones nearly always interact to determine patterns of plant development.

According to the acid growth hypothesis for auxin action, auxins may directly stimulate the early phases of cell elongation by causing responsive cells to actively transport hydrogen ions out of the cell, thus lowering the pH around cells. This acidification of the cell wall region activates wall-loosening proteins known as expansins, which allow slippage of cellulose microfibrils in the cell wall, making the cell wall less rigid. When the cell wall is loosened by the action of auxins, this now-less-rigid wall is expanded by cell turgor pressure, which presses against the cell wall.

However, the acid growth hypothesis does not by itself account for the increased synthesis and transport of cell wall precursors and secretory activity in the Golgi system that accompany and sustain auxin-promoted cell expansion

Organ patterns

Growth and division of plant cells together result in growth of tissue, and specific tissue growth contributes to the development of plant organs. Growth of cells contributes to the plant's size, but uneven localized growth produces bending, turning and directionalization of organs- for example, stems turning toward light sources (phototropism), roots growing in response to gravity (gravitropism), and other tropisms.

Organization of the plant

As auxins contribute to organ shaping, they are also fundamentally required for proper development of the plant itself. Without hormonal regulation and organization, plants would be merely proliferating heaps of similar cells. Auxin employment begins in the embryo of the plant, where directional distribution of auxin ushers in subsequent growth and development of primary growth poles, then forms buds of future organs. Throughout the plant's life, auxin helps the plant maintain the polarity of growth and recognize where it has its branches (or any organ) connected.

An important principle of plant organization based upon auxin distribution is **apical dominance**, which means that the auxin produced by the apical bud (or growing tip) diffuses downwards and inhibits the development of ulterior lateral bud growth, which would otherwise compete with the apical tip for light and nutrients. Removing the apical tip and its suppressive hormone allows the lower dormant lateral buds to develop, and the buds between the leaf stalk and stem produce new shoots which compete to become the lead growth. This behavior is used in pruning by horticulturists.

Uneven distribution of auxin: To cause growth in the required domains, it is necessary that auxins be active preferentially in them. Auxins are not synthesized everywhere, but each cell retains the potential ability to do so, and only under specific conditions will auxin synthesis be activated. For that purpose, not only do auxins have to be translocated toward those sites where they are needed but there has to be an established mechanism to detect those sites. Translocation is driven throughout the plant body primarily from peaks of shoots to peaks of roots. For long distances, relocation occurs via the stream of fluid in phloem vessels, but, for short-distance transport, a unique system of coordinated polar transport directly from cell to cell is exploited. This process of polar auxin transport is directional and very strictly regulated. It is based in uneven distribution of auxin efflux carriers on the plasma membrane, which send auxins in the proper direction.

Locations

- In shoot (and root) meristematic tissue
- In young leaves
- In mature leaves in very tiny amounts
- In mature root cells in even smaller amounts
- Transported throughout the plant more prominently downward from the shoot apices

Physiological Effects

Crown galls are caused by *Agrobacterium tumefaciens* bacteria; they produce and excrete auxin and cytokinin, which interfere with normal cell division and cause tumors

The plant hormone stimulates cell elongation. It stimulates the Wall Loosening Factors, for example, elastins, to loosen the cell walls. If gibberellins are also present, the effect is stronger. It also stimulates cell division if cytokinins are present. When auxin and cytokinin are applied to callus, rooting can be generated if the auxin concentration is higher than cytokinin concentration while xylem tissues can be generated when the auxin concentration is equal to the cytokinins.

It participates in phototropism, geotropism, hydrotropism and other developmental changes. The uneven distribution of auxin, due to environmental cues (for example, unidirectional light and gravity force), results in uneven plant tissue growth.

It also induces sugar and mineral accumulation at the site of application.

Wounding response

It induces formation and organization of phloem and xylem. When the plant is wounded, the auxin may induce the Cell differentiation and regeneration of the vascular tissues.

Root growth and development

Auxin induces new root formation by breaking root apical dominance induced by cytokinins. In horticulture, auxins, especially NAA and IBA, are commonly applied to stimulate root growth when taking cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation. Removal of the root tip can lead to inhibition of secondary root formation.

Apical dominance

It induces shoot apical dominance; the axillary buds are inhibited by auxin. When the apex of the plant is removed, the inhibitory effect is removed and the growth of lateral buds is enhanced as a high concentration of auxin directly stimulates ethylene synthesis in lateral buds causes inhibition of its growth and potentiation of apical dominance.

Ethylene biosynthesis

In low concentrations, auxin can inhibit ethylene formation and transport of precursor in plants; however, high concentrations of auxin can induce the synthesis of ethylene. Therefore, the high concentration can induce femaleness of flowers in some species.

It inhibits abscission prior to formation of abscission layer and thus inhibits senescence of leaves.

Fruit growth and development

Auxin delays fruit senescence.

It is required for fruit growth and development. When seeds are removed from strawberries, fruit growth is stopped; exogenous auxin stimulates the growth in seed removed fruits. For fruit with unfertilized seeds, exogenous auxin results in parthenocarpy ("virgin-fruit" growth).

Auxin is important for the correct development of fruit. Fruits form abnormal morphologies when auxin transport is disturbed. In Arabidopsis fruits auxin controls the release of seeds from the fruit (pod). The valve margins are a specialised tissue in pods that regulates when pod will open (dehiscence). Auxin must be removed from the valve margin cells to allow the valve margins to form. This process requires modification of the auxin transporters.

Flowering

Auxin plays a minor role in the initiation of flowering. It can delay the senescence of flowers in low concentrations.

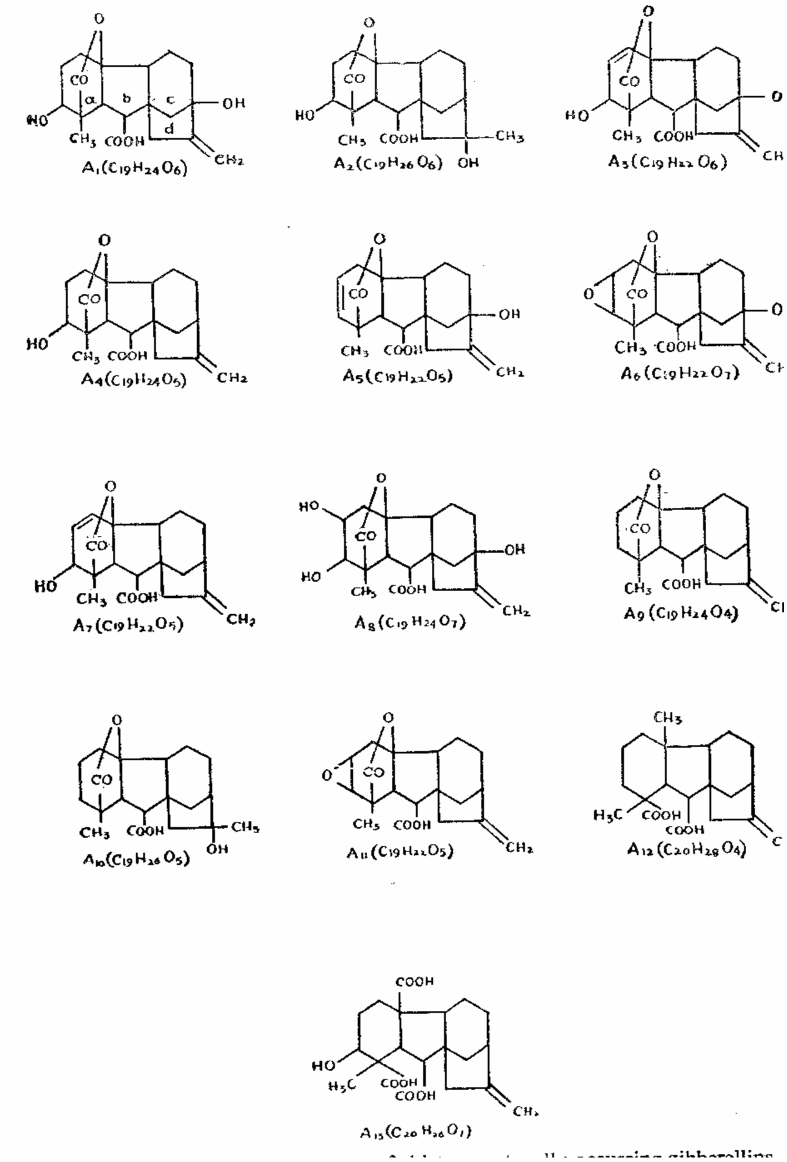
Herbicide manufacture

The defoliant Agent Orange was a mix of 2,4-D and 2,4,5-T. The compound 2,4-D is still in use and is thought to be safe, but 2,4,5-T was more or less banned by the EPA in 1979. The dioxin TCDD is an unavoidable contaminant produced in the manufacture of 2,4,5-T. As a result of the integral dioxin contamination, 2,4,5-T has been implicated in leukaemia, miscarriages, birth defects, liver damage, and other diseases. Agent Orange was sprayed in Vietnam as a defoliant to deny ground cover to the Vietnamese army.

Gibberellins

Gibberellins (GAs) are plant hormones that regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence.

Gibberellin was first recognized in 1926 by a Japanese scientist, Eiichi Kurosawa, studying *bakanae*, the "foolish seedling" disease in rice It was first isolated in 1935 by Teijiro Yabuta, from fungal strains (*Gibberella fujikuroi*) provided by Kurosawa. Yabuta called the isolate gibberellin.



Chemical structure of 13 naturally occuring gibberellins

Interest in gibberellins outside of Japan began after World War II. In the United States, the first research was undertaken by a unit at Camp Dietrick in Maryland, via studying seedlings of the bean *Vicia faba*. In the United Kingdom, work on isolating new types of gibberellin was undertaken at Imperial Chemical Industries. Interest in gibberellins spread around the world as the potential for its use on various commercially important plants became more obvious. For example, research which started at the University of California, Davis in the mid-1950s led to its commercial use on Thompson seedless table grapes throughout California by 1962. A known opponent to gibberellin is Paclobutrazol(PBZ), which in turn is growth inhibiting and inducing early fruitset as well as seedset.

Gibberellins are important in seed germination, affecting enzyme production which mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue. Absoption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling. GAs produce bolting of rosette-forming plants, increasing internodal length. They promote flowering, cellular division, and in seeds growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA.

Chemically all known gibberellins are diterpenoid acids that are synthesized by the terpenoid pathway in plastids and then modified in the endoplasmic reticulum and cytosol until they reach their biologically-active form. All gibberellins are derived from the ent-gibberellane skeleton, but are synthesised via *ent*-kaurene. The gibberellins are named GA1....GAn in order of discovery. Gibberellic acid, which was the first gibberellin to be structurally characterised, is GA3.

Gibberellins are tetracyclic diterpene acids. There are two classes based on the presence of 19-carbons or 20-carbons. The 19-carbon gibberellins, such as gibberellic acid, have lost carbon 20 and in place possess a five-member lactone bridge that links carbons 4 and 10. The 19-carbon forms are generally the biologically active forms of gibberellins. Hydroxylation also has a great effect on the biological activity of the gibberellin. Generally the most biologically active compounds are dihydroxylated gibberellins, which possess hydroxyl groups on both carbon 3 and carbon 13. Gibberellic acid is a dihydroxylated gibberellin.

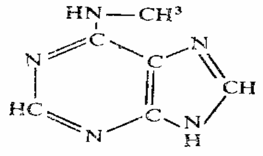
Gibberellins are involved in the natural process of breaking dormancy and various other aspects of germination. Before the photosynthetic apparatus develops sufficiently in the early stages of germination, the stored energy reserves of starch nourish the seedling. Usually in germination, the breakdown of starch to glucose in the endosperm begins shortly after the seed is exposed to water. It is believed that gibberellins in the seed embryo signal starch hydrolysis through inducing the synthesis of the enzyme α-amylase in the aleurone cells. In the model for gibberellin-induced production of α-amylase, it is demonstrated that gibberellins (denoted by GA) produced in the scutellum diffuse to the aleurone cells where they stimulate the secretion α-amylase. α-amylase then hydrolyses starch, which is abundant in many seeds, into glucose that can be utilized in cellular respiration to produce energy for the seed embryo. Studies of this process have indicated that gibberellins cause higher levels of transcription of the gene coding for the α-amylase enzyme, in order to stimulate the synthesis of α-amylase.

Gibberellins are produced in greater mass when it's cold. They stimulate cell elongation, breaking and budding, seedless fruits, and seed germination. They do the last by breaking the seed's dormancy and acting as a chemical messenger. Its hormone binds to a receptor and Ca^{2+} activates a protein, calmodulin, and the complex binds to DNA, producing an enzyme to stimulate growth in the embryo.

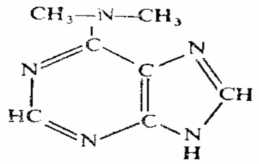
Cytokinins

Cytokinins (CK) are a class of plant growth regulators that promote cell division. They are primarily involved in cell growth, differentiation, and other physiological processes. Their effects were first discovered through the use of coconut milk in the 1940s by Adicott at the University of Wisconsin-Madison named Folke Skoog.

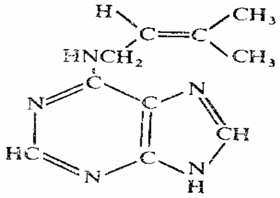
Cytokinins were called kinins in the past when the first cytokinins were isolated from yeast cells. They also help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth. They have a highly-synergistic effect in concert with auxins and the ratios of these two groups of plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins; they in conjunction with ethylene promote abscission of leaves, flower parts and fruits.



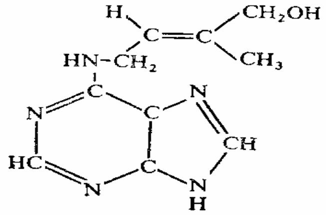
N^6-methylaminopurine



N^6-dimethylaminopurine



N^6-(Δ^2-*iso*pentenylamino)purine



Some naturally occurring cytokinins

There are two types of cytokinins: adenine-type cytokinins represented by kinetin, zeatin and 6-benzylaminopurine, as well as phenylurea-type cytokinins like diphenylurea or thidiazuron (TDZ). The adenine-type cytokinins are synthesised in stems, leaves and roots, which is the major site. Cambium and possibly other actively dividing tissues are also sites of cytokinin biosynthesis. There is no evidence that the phenylurea cytokinins occur naturally in plant tissues. Cytokinins are involved in both local and long distance signalling, the latter of which involves the same *in planta* transport mechanism as used for transport of purines and nucleosides.

The cytokinin zeatin , *Zea*, in which it was first discovered in immature kernels.

The cytokinin zeatin is named after the genus of corn, *Zea*, in which it was discovered.

Mode of Action

Cytokinins are involved in many plant processes, including cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement, auxiliary bud release and senescence. The ratio of auxin to cytokinin is crucial during cell division and the differentiation of plant tissues.

While cytokinin action in vascular plants is described as pleiotropic, this class of plant hormones specifically induces the transition from apical growth to growth via a three-faced apical cell in moss protonema. This bud induction can be pinpointed to differentiation of a specific single cell, and thus is a very specific effect of cytokinin.

Auxin is known to regulate the biosynthesis of cytokinin.

Ethylene

Ethylene (IUPAC name: **ethene**) is the chemical compound with the formula C_2H_4. Ethylene is a gas that forms through the Yang Cycle from the breakdown of methionine, which is in all cells. Ethylene acts physiologically as a hormone in plants. It exists as a gas and acts at trace levels throughout the life of the plant by stimulating or regulating the ripening of fruit, the opening of flowers, and the abscission (or shedding) of leaves. Its biosynthesis starts from methionine with 1-aminocyclopropane-1-carboxylic acid (ACC) as a key intermediate.

Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly-germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stems natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein ETHYLENE INSENSITIVE2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones.

Environmental and biological triggers of ethylene

Environmental cues can induce the biosynthesis of the plant hormone. Flooding, drought, chilling, wounding, and pathogen attack can induce ethylene formation in the plant.

In flooding, root suffers from lack of oxygen, or anoxia, which leads to the synthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC). ACC is transported upwards in the plant and then oxidized in leaves. The product, the ethylene causes epinasty of the leaves.

One speculation recently put forth for epinasty is the downard pointing leaves may act as pump handles in the wind. The ethylene may or may not additionally induce the growth of a valve in the xylem, but the idea would be that the plant would harness the power of the wind to pump out more water from the roots of the plants than would normally happen with transpiration.

Physiological responses of plants

Like the other plant hormones, ethylene is considered to have pleiotropic effects. This essentially means that it is thought that at least some of the effects of the hormone are unrelated. What is actually caused by the gas may depend on the tissue affected as well as environmental conditions. In the evolution of plants, ethylene would simply be a message that was coopted for unrelated uses by plants during different periods of the evolutionary development.

List of plant responses to ethylene

- Seedling triple response, thickening and shortening of hypocotyl with pronounced apical hook. This is thought to be a seedling's reaction to an obstacle in the soil such a stone, allowing it to push past the obstruction.
- In pollination, when the pollen reaches the stigma, the precursor of the ethylene, ACC, is secreted to the petal, the ACC releases ethylene with ACC oxidase.
- Stimulates leaf and flower senescence
- Stimulates senescence of mature xylem cells in preparation for plant use
- Inhibits shoot growth except in some habitually flooded plants like rice
- Induces leaf abscission
- Induces seed germination
- Induces root hair growth – increasing the efficiency of water and mineral absorption
- Induces the growth of adventitious roots during flooding
- Stimulates epinasty – leaf petiole grows out, leaf hangs down and curls into itself
- Stimulates fruit ripening
- Induces a climacteric rise in respiration in some fruit which causes a release of additional ethylene. This can be the one bad apple in a barrel spoiling the rest phenomenon.
- Affects neighboring individuals
- Affects gravistropism
- Stimulates nutational bending
- Disease/wounding resistance
- Inhibits stem growth outside of seedling stage
- Stimulates stem and cell broadening and lateral branch growth also outside of seedling stage
- Synthesis is stimulated by auxin and maybe cytokinin as well
- Ethylene levels are decreased by light
- The flooding of roots stimulates the production of ACC which travels through the xylem to the stem and leaves where it is converted to the gas
- Interference with auxin transport (with high auxin concentrations)

- Inhibits stomatal closing except in some water plants or habitually flooded ones such as some rice varieties, where the opposite occurs (conserving CO_2 and O_2)
- Where ethylene induces stomatal closing, it also induces stem elongation
- Induces flowering in pineapples.

Commercial Issues

- Ethylene shortens the shelf life of many fruits by hastening fruit ripening and floral senescence.
- Tomatoes, bananas and apples will ripen faster in the presence of ethylene. Bananas placed next to other fruits will produce enough ethylene to cause accelerated fruit ripening.
- Ethylene will shorten the shelf life of cut flowers and potted plants by accelerating floral senescence and floral abscission.
- Flowers and plants which are subjected to stress during shipping, handling, or storage produce ethylene causing a significant reduction in floral display.
- Flowers affected by ethylene include carnation, geranium, petunia, rose, and many others.
- Commercial growers of bromeliads, including pineapple plants, use ethylene to induce flowering. Plants can be induced to flower either by treatment with the gas in a chamber, or by placing a banana peel next to the plant in an enclosed area.

Ethylene can cause significant economic losses for florists, markets, suppliers, and growers. Researchers have come up with several ways to inhibit ethylene, including inhibiting ethylene synthesis and inhibiting ethylene perception. Aminoethoxyvinylglycine (AVG), Aminooxyacetic acid (AOA), and silver ions are ethylene inhibitors. Inhibiting ethylene synthesis is less effective for reducing post-harvest losses since ethylene from other sources can still have an effect. By inhibiting ethylene perception, fruits, plants and flowers don't respond to ethylene produced endogenously or from exogenous sources. Inhibitors of ethylene perception include compounds that have a similar shape to ethylene, but do not elicit the ethylene response. One example of an ethylene perception inhibitor is 1-methylcyclopropene (1-MCP).

Abscisic acid

Abscisic acid (ABA), also known as **abscisin II** and **dormin**, is a plant hormone. Once it was determined that the two latter named compounds were the same, it was named abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly-abscissed or freshly-fallen leaves.

It functions in many plant developmental processes, including bud dormancy. This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplasts, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, seed and bud dormancy. It mediates changes within the apical meristem causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly-abscissed leaves, it was thought to play a role in the processes of natural leaf drop but further research has disproven this. In plant species from temperate parts of the world it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy.

In plants under water stress ABA plays a role in closing the stomata. Soon after plants are water stressed and the roots are deficient in water, a signal moves up to the leaves causing the formation of ABA precursors there which then move to the roots. The roots then release ABA which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cells, which then lose turgidity, closing the stomata. ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone, its degradation or more properly catabolism within the plant affects metabolic reactions and cellular growth and production of other hormones. Plants start life as a seed with high ABA levels, just before the seed germinates ABA levels decrease; during germination and early growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves - ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates which mediate another cascade of effects triggering specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.

Function

ABA was originally believed to be involved in abscission - this is now known only to be the case in a small number of plants. ABA-mediated signalling also plays an important part in plant responses to environmental stress and plant pathogens. The plant genes for ABA biosynthesis and sequence of the pathway have been elucidated. ABA is also produced by some plant pathogenic fungi via a biosynthetic route different from ABA biosynthesis in plants.

Abscisic acid owes its names to its role in the abscission of plant leaves. In preparation for winter, ABA is produced in terminal buds. This slows plant growth and directs leaf primordia to develop scales to protect the dormant buds during the cold season. ABA also inhibits the division of cells in the vascular cambium, adjusting to cold conditions in the winter by suspending primary and secondary growth.

Abscisic acid is also produced in the roots in response to decreased soil water potential and other situations in which the plant may be under stress. ABA then translocates to the leaves, where it rapidly alters the osmotic potential of stomatal guard cells, causing them to shrink and stomata to close. The ABA-induced stomatal closure reduces transpiration thus preventing further water loss from the leaves in times of low water availability.

Several ABA mutant *Arabidopsis thaliana* plants have been identified – both those deficient in ABA production and those insensitive to its action. ABA-deficient plants show defects in seed dormancy, germination, stomatal regulation and some mutants show stunted growth and brown/yellow leaves. These mutants reflect the importance of ABA in seed germination and early embryo development.

Location and timing of ABA biosynthesis

- Released during desiccation of the vegetative tissues and when roots encounter soil compaction.
- Synthesized in green fruit and seeds at the beginning of the wintering period
- Mobile within the leaf and can be rapidly translocated from the roots to the leaves by the transpiration stream in the xylem.
- Produced in response to environmental stress, such as heat stress, water stress, salt stress.
- Synthesized in all plant parts, e.g. roots, flowers, leaves and stems

Effects

- Induces stomatal closure, decreasing transpiration to prevent water loss.
- Inhibits fruit ripening
- Responsible for seed dormancy by inhibiting cell growth – inhibits seed germination
- Promotes the synthesis of Kinetin nucleotide

Other known hormones

Other identified plant growth regulators include:

- Brassinolides - plant steroids that are chemically similar to animal steroid hormones. First isolated from pollen of the mustard family and extensively studied in *Arabidopsis*. They promote cell elongation and cell division, differentiation of xylem tissues, and inhibit leaf abscission. Plants that are deficient in brassinolides suffer from dwarfism.
- Salicylic acid - activates genes in some plants that produce chemicals that aid in the defense against pathogenic invaders.
- Jasmonates - are produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are believed to also have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth.
- Plant peptide hormones - encompass all small secreted peptides that are involved in cell-to-cell signaling. These small peptide hormones play crucial roles in plant growth and development, including defense mechanisms, the control of cell division and expansion, and pollen self-incompatibility.
- Polyamines - are strongly basic molecules with low molecular weight that have been found in all organisms studied thus far. They are essential for plant growth and development and affect the process of mitosis and meiosis.
- Nitric oxide (NO) - serves as signal in hormonal and defense responses.
- Strigolactones, implicated in the inhibition of shoot branching.

Hormones and plant propagation

Synthetic plant hormones or PGRs are commonly used in a number of different techniques involving plant propagation from cuttings, grafting, micropropagation, and tissue culture.

The propagation of plants by cuttings of fully-developed leaves, stems, or roots is performed by gardeners utilizing auxin as a rooting compound applied to the cut surface; the auxins are taken into the plant and promote root initiation. In grafting, auxin promotes callus tissue formation, which joins the surfaces of the graft together. In micropropagation, different PGRs are used to promote multiplication and then rooting of new plantlets. In the tissue-culturing of plant cells, PGRs are used to produce callus growth, multiplication, and rooting.

Seed dormancy and seed viability

Plant hormones affect seed germination and dormancy by affecting different parts of the seed.

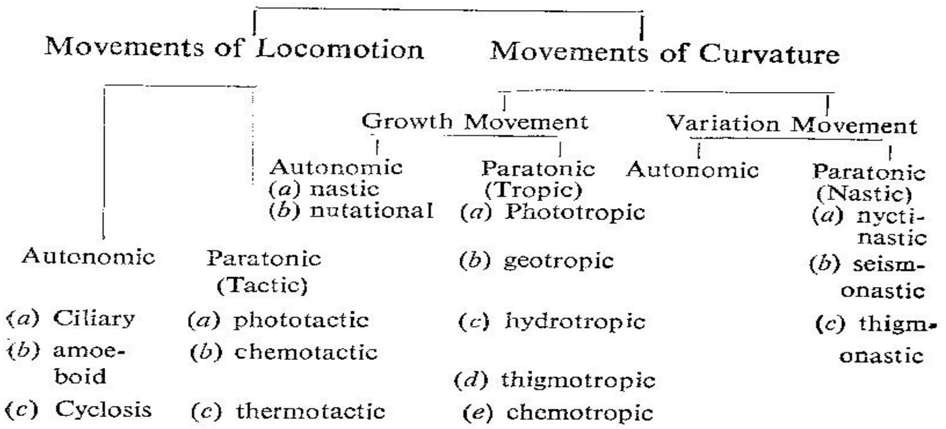
Embryo dormancy is characterized by a high ABA/GA ratio, whereas the seed has a high ABA sensitivity and low GA sensitivity. To release the seed from this type of dormancy and initiate seed germination, an alteration in hormone biosynthesis and degradation towards a low ABA/GA ratio, along with a decrease in ABA sensitivity and an increase in GA sensitivity needs to occur.

ABA controls embryo dormancy, and GA embryo germination. Seed coat dormancy involves the mechanical restriction of the seed coat, this along with a low embryo growth potential, effectively produces seed dormancy. GA releases this dormancy by increasing the embryo growth potential, and/or weakening the seed coat so the radical of the seedling can break through the seed coat. Different types of seed coats can be made up of living or dead cells and both types can be influenced by hormones; those composed of living cells are acted upon after seed formation while the seed coats composed of dead cells can be influenced by hormones during the formation of the seed coat. ABA affects testa or seed coat growth characteristics, including thickness, and effects the GA-mediated embryo growth potential. These conditions and effects occur during the formation of the seed, often in response to environmental conditions. Hormones also mediate endosperm dormancy: Endosperm in most seeds is composed of living tissue that can actively respond to hormones generated by the embryo. The endosperm often acts as a barrier to seed germination, playing a part in seed coat dormancy or in the germination process. Living cells respond to and also affect the ABA/GA ratio, and mediate cellular sensitivity; GA thus increases the embryo growth potential and can promote endosperm weakening. GA also affects both ABA-independent and ABA-inhibiting processes within the endosperm.

Plant movements

Movements in plantws are brought about by definite internal and external stimuli. The movements, which take place spontaneously, without the effect of external stimuli, are termed spontaneous orautonomic. All those movements of plants, which are caused by external stimulii are termed induced or paratonic. The paratonic movements are of three types viz., tactic movements, tropic movements and nastic movements.

Classification of plant movements: The vital plant movements can be classified as under



A **tropism** (*tropos(Gr.)*= turn) is a biological phenomenon, indicating growth or turning movement of a biological organism, usually a plant, in response to an environmental stimulus. In tropisms, this response is dependent on the direction of the stimulus (as opposed to nastic movements which are non-directional responses). The stimuli are however, effective in causing growth movements, only when they are unilateral. Viruses and other pathogens also affect what is called "host tropism" or "cell tropism" in which case tropism refers to the way in which different viruses/pathogens have evolved to preferentially target specific host species, or specific cell types within those species. The word tropism comes from the Greek *trope* ("to turn" or "to change"). Tropisms are usually named for the stimulus involved (for example, a *phototropism* is a reaction to light) and may be either *positive* (towards the stimulus) or *negative* (away from the stimulus).

Tropisms are typically associated with plants (although not necessarily restricted to them). Where an organism is capable of directed physical movement (motility), movement or activity in response to a specific stimulus is more likely to be regarded by behaviorists as a **taxis** (directional response) or a **kinesis** (non-directional response).

Various external factors, often acting together with hormones, are also important in plant growth and development. One important class of responses to external stimuli is that of the tropisms—responses that cause a change in the direction of a plant's growth. Examples are phototropism, the bending of a stem toward light, and geotropism, the response of a stem or root to gravity. Stems are negatively geotropic, growing away from gravity, whereas roots are positively geotropic. Photoperiodism, the response to 24-hour cycles of dark and light, is particularly important in the initiation of flowering. Some plants are short-day, flowering only when periods of light are less than a certain length (see Biological Clocks). Other variables—both internal, such as the age of the plant, and external, such as temperature—are also involved with the complex beginnings of flowering.

Types of tropisms

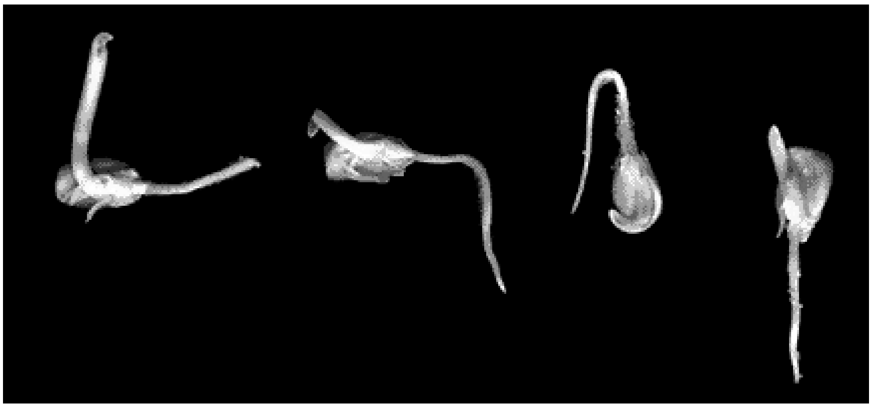
- Chemotropism, movement or growth in response to chemicals
    - Gravitropism (or geotropism), movement or growth in response to gravity
    - Hydrotropism, movement or growth in response to moisture or water
- Heliotropism, movement or growth in response to sunlight
- Phototropism, movement or growth in response to lights or colors of light
- Thermotropism, movement or growth in response to temperature
- Thigmotropism, movement or growth in response to touch or contact
- Host tropism or cell tropism, the host range of pathogens

Geotropism

Geotropism is a turning or growth movement by a plant or fungus in response to gravity. Charles Darwin was one of the first Europeans to document that roots show *positive geotropism* and stems show *negative geotropism*. That is, roots grow in the direction of gravitational pull (i.e., downward) and stems grow in the opposite direction (i.e., upwards). This behaviour can be easily demonstrated with a potted plant. When laid onto its side, the growing parts of the stem begin to display negative gravitropism, bending (biologists say, turning; see tropism) upwards. Herbaceous (non-woody) stems are capable of a small degree of actual bending, but most of the redirected movement occurs as a consequence of root or stem growth in a new direction.

Geotropism in the root

Roots bend in response to gravity due to a regulated movement of the plant hormone auxin known as polar auxin transport. In roots, an increase in the concentration of auxin will inhibit cell expansion, therefore, the redistribution of auxin in the root can initiate differential growth in the elongation zone resulting in root curvature.



Germination of corn seeds occurs regardless of the seed orientation.

A "tropism" is a plant movement triggered by stimuli. The term "geotropic" refers to a plant whose roots grow down into the soil as a response to gravity. Plants commonly exist in a state of "anisotropic growth," where roots grow downward and shoots grow upward. Anisotropic growth will continue even as a plant is turned sideways or upside down. In other words, no matter what you do to a plant within Earth's atmosphere, it will still grow roots down, stem up. The reason for this comes from the nature of a plant, and its general response to gravity.

Upward growth of plant parts, against gravity, is called negative geotropism, and downward growth of roots, positive geotropism.

Geotropism and auxins in the stem

A similar mechanism is known to occur in plant stems except that the shoot cells have a different dose response curve with respect to auxin. In shoots, increasing the local concentration of auxin promotes cell expansion; this is the opposite of root cells.

The differential sensitivity to auxin helps explain Darwin's original observation that stems and roots respond in the opposite way to the gravity vector. In both roots and stems auxin accumulates towards the gravity vector on the lower side. In roots, this results in the inhibition of cell expansion on the lower side and the concomitant curvature of the roots towards gravity (positive geotropism). In stems, the auxin also accumulates on the lower side, however in this tissue it increases cell expansion and results in the shoot curving up (statolithic geotropism).

The negative and the positive responses of the stem and the root tips to gravity are due to increased concentration of auxin on their lower sides.

There are also several cultivated plants that display altered geotropism compared to other varieties within their own species.

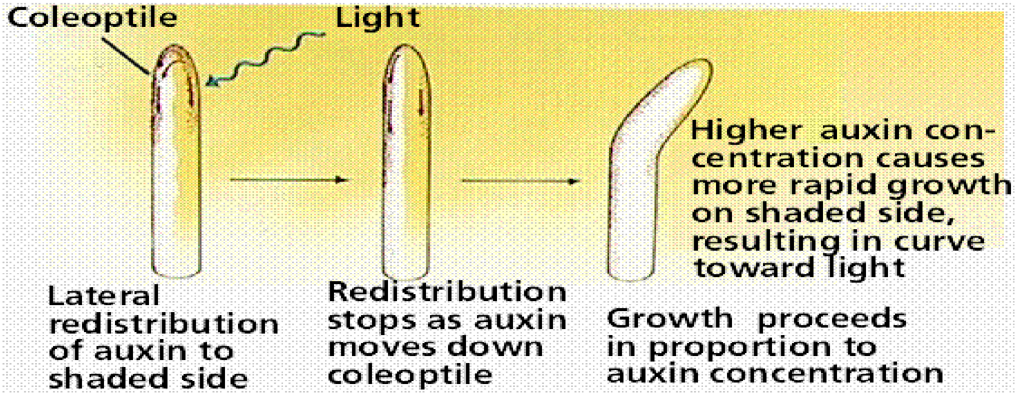
- Some pineapploes can be induced to flower if the stems are kept in a horizontal position.
- In a horizontal stem the buds are normally suppressed on the lower surface.
- In a horizontal stem kept in contact with the soil, adventitious roots are formed.
- Dolk performed an expriment on the pattern of Went's expriment on phototrophism and found a greater concentration of auxin in the lower half of a horizontally placed coleoptile tip.
- Agar blocks containing IAA placed centrally on decapitated roots causes a retardation of growth.
- Application of very low concentration of IAA to decapitated roots results in an acceleration of growth.
- If coleoptile tips are placed on decapitated root tips, the growth is retarded.
- Some are trees that have a weeping or *pendulate* growth habit; the branches still respond to gravity, but with a positive response, rather than the normal negative response. Others are sas the *lazy* varieties of corn *Zea maize*, whose shoots grow along the ground.

Phototropism

Phototropism is directional growth in which the direction of growth is determined by the direction of the light source. In other words, it is the growth and response to a light stimulus. Phototropism is most often observed in plants, but can also occur in other organisms such as fungi. Phototropism is one of the many plant tropisms or movements which respond to external stimuli. Growth towards a light source is a **positive phototropism**, while growth away from light is called **negative phototropism** (or **Skototropism**). Most plant shoots exhibit positive phototropism, while roots usually exhibit negative phototropism, although geotropism may play a larger role in root behavior and growth. Some vine shoot tips exhibit negative phototropism, which allows them to grow towards dark, solid objects and climb them.

Phototropism in plants such as *Arabidopsis thaliana* is regulated by blue light receptors called phototropins. Other photosensitive receptors in plants include **phytochromes** that sense red light and **cryptochromes** that sense blue light. Different organs of the plant may exhibit different phototropic reactions to different wavelengths of light. Stem tips exhibit positive phototropic reactions to blue light, while root tips exhibit negative phototropic reactions to blue light. Both root tips and most stem tips exhibit positive phototropism to red light.

Phototropism and auxins:



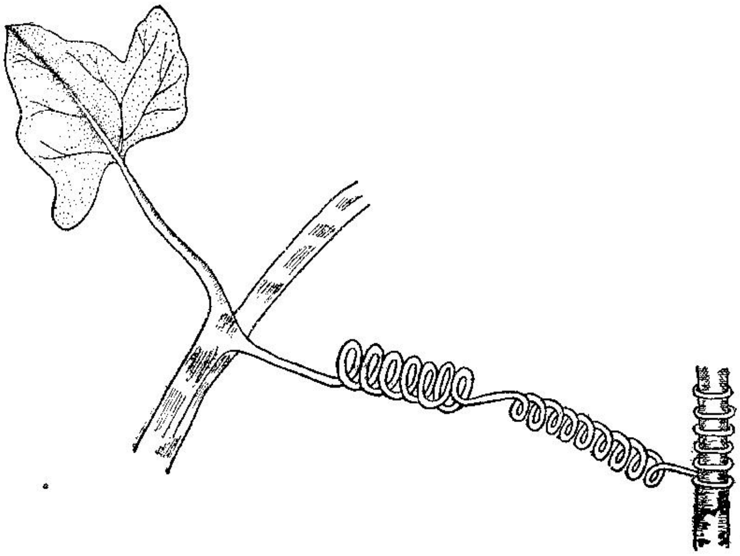
The curvature in stem tip and root tip is due to the unequal amount of growth in the two havles of the stem and root tips, which in turn is due to the light induced unequal distribution of growth hormone, auxin. Thus phototropism is enabled by **auxins**. Auxins are plant hormones that have many functions. In this respect, auxins are responsible for expelling protons (by activating proton pumps) which decreases pH in the cells on the dark side of the plant. This acidification of the cell wall region activates enzymes known as expansins which break bonds in the cell wall structure, making the cell walls less rigid. In addition, the acidic environment causes disruption of hydrogen bonds in the cellulose that makes up the cell wall. The decrease in cell wall strength causes cells to swell, exerting the mechanical pressure that drives phototropic movement.

Other light responses

- Etiolation is the response of a plant when light is nearly (or completely) absent.
- Heliotropism is the diurnal motion of plant parts (flowers or leaves) in response to the direction of the sun. It is not a phototropism since it does not involve growth.
- Photonasty involves the movement of plant parts that does not involve growth but is triggered by light. The plant movement is not determined by the direction of light so it is not a phototropism. Photonasty in prayer plant (*Maranta leuconeura*) involves the downward movement of leaves when they receive light in the morning.
- Phototaxis is movement of an entire organism in which the direction of movement is determined by the direction of light. It occurs in some motile microbes such as Euglena and algae. It is not a phototropism because growth is not required.
- Photo-orientation occurs within a plant cell when chloroplasts change their positions depending upon light intensity. This was discovered in 1987 by Chelsea Polevy and Kelsey Joyce when experimenting in their laboratory. When the light intensity is high, chloroplasts move to the edge of the cell to reduce photobleaching (destruction of chlorophyll). In low light, chloroplasts tend to spread out within the protoplasm to maximize their capture of light energy. Photo-orientation is also not a phototropism.

Thigmotropism

If actively growing tendril-tips come in contact with solid abjects having rough surfaces, they exhibit rapid growth movements. The tendril fails to develop if its tip does not get the touch stimulus.



After it gets the contact stimulus the tendril starts twining around the supports. This is because the growth of the tendril is unequal on the two sides. The side in contct with the rupporting material has less growth, while the other side has more growth. This results in the coiling of the tendril around the support.

The sensitive tip of the tendril transmits the simulus backwards and the whole of the tendril, therefore, gets spirally coiled.

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