

Advance Biochemistry-I

Introduction to Biomolecules and Biotechnology

Introduction to Biochemistry and Biotechnology

Biochemistry is the branch of science concerned with the chemical and physio-chemical processes and substances (bio-molecules and their reactions) that occur within living organisms.

Biotechnology is the exploitation of biological processes for industrial, mechanical, technological, and other purposes, especially the genetic manipulation of microorganisms for the production of antibiotics, hormones, etc.

Biochemistry is the study of metabolic processes within the living system and the disorders which arises due to defect in these processes whereas **Biotechnology** helps to observe and modify the biological mechanisms which helps improving lives and health.

Biochemistry v/s Biotechnology

Biochemistry is a pure science field, while biotechnology is an applied science field. Biochemistry deals with the process and pathways happening inside the living cell, while biotechnology deals with the techniques to study those processes, and applications of those processes.

Historical Perspectives

Carl Alexander Neuberg (29 July 1877 – 30 May 1956) was an early pioneer in biochemistry, and he is often referred to as the "father of modern biochemistry".

The discovery of the first enzyme, diastase in 1833 by Anselme Payen, may have marked the beginning of biochemistry. Although the term "biochemistry" seems to have been first used in 1882, it is generally accepted that the formal coinage of biochemistry occurred in 1903 by Carl Neuberg, a German chemist. Biochemistry is a field of science that broaches the two traditional disciplines of biology and chemistry. If chemistry is the science of matter, then biochemistry is the science of living matter.

Prospects, Scope and Applications in Health, Environment and Agriculture:

Practitioners of biochemistry study the biochemical reactions that occur at the molecular level within living organisms. In **medical biochemistry** (also known as molecular biology), biochemical techniques are applied to human health and disease. The typical scope of medical biochemistry can encompass the following:

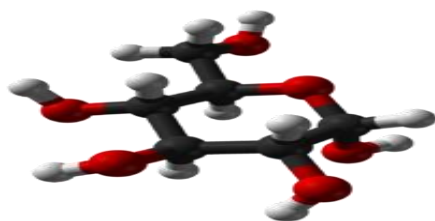
- **The chemical components of the human body**, including carbohydrates and lipids; amino acids and proteins; blood and plasma; biological membranes; nucleic acids (DNA and RNA)
- **The major chemical processes in the human body**, such as cell development; enzyme activity; membrane transport mechanisms; homeostasis; blood coagulation (clotting); oxygen transport; neurotransmitter function; ageing
- **Nutrition and mineral metabolism**, including the role and function of vitamins in the body
- **Molecular genetics**
- **Heredity**
- **Genomics**

Much of biochemical inquiry deals with the structures, functions and interactions of biological macromolecules — large molecules (such as proteins) which provide the structure of cells and perform many of the functions associated with life.

The chemistry of the cell also depends on the reactions of smaller molecules and ions. These molecules can be organic (e.g. the amino acids that are used to synthesize proteins) or inorganic (e.g. water and metal ions).

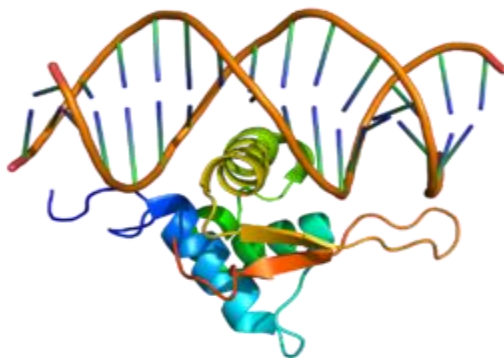
There are four classes of biochemical compounds: carbohydrates, proteins, lipids (fats), and nucleic acids. We get these from our food. Carbohydrates are molecules made up of carbon, oxygen, and hydrogen.

Carbohydrates are molecules made up of carbon, oxygen, and hydrogen. They usually have twice as much hydrogen as oxygen. Examples of carbohydrates include sugars, starches and cellulose.



Example of a carbohydrate (glucose)

Proteins are molecules made up of long chains of amino acids. They're more complex than carbohydrates and contain carbon, oxygen, hydrogen, nitrogen, and sulfur. Examples of proteins include hormones and enzymes.



Example of a protein

Lipids are small, hydrophobic molecules built from fatty acids. They're not soluble in water, but can be dissolved in organic solvents. Like carbohydrates, they usually contain only carbon, oxygen, and hydrogen. Examples of lipids include the fat stores around your body, but also oils and waxes.



Butter contains mostly fats, which are a type of lipid

Nucleic acids are biological polymers made from nucleotides. They're the most complex of the classes of biochemical compounds and are built from many parts, including sugars, which are themselves carbohydrates. They contain the same elements as proteins, except tend to have phosphorus instead of sulfur. But it's the way those elements are bonded together that makes them nucleic acids. Examples of nucleic acids include DNA and RNA.



Example of a nucleic acid: a DNA molecule

Introduction to Biomolecules

The living matter is composed of mainly six elements—Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus and Sulfur. These elements together constitute about 90% of the dry weight of the human body. Several other functionally important elements are also found in the cells. These include Ca, K, Na, Cl, Mg, Fe, Cu, Co, I, Zn, F, Mo and Se.

Carbon—a unique element of life

Carbon is the most predominant and versatile element of life. It possesses a unique property to form infinite number of compounds. This is attributed to the ability of Carbon to form stable covalent bonds and C-C chains of unlimited length. It is estimated that about 90 % compounds found in living system invariably contain Carbon.

Complex Biomolecules

The organic compounds such as amino acids, nucleotides and monosaccharides serve as the **monomeric units** or building blocks of complex biomolecules—proteins, nucleic acids (DNA and RNA) and polysaccharides, respectively.

The important Biomolecules with their respective building blocks and major functions are given in Table (a). The macromolecules (Proteins, Lipids, Nucleic acids and Polysaccharides) form molecules assemblies (e.g membranes) which in turn organize into organelles, cells, tissues, organs and finally the whole organism.

Table (a) Major Complex Biomolecules of cells

| Biomolecules | Building Blocks | Major Functions |
|-----------------------------|------------------------|---|
| Protein | Amino acids | Fundamental basis of structures and function of cell (Static or Dynamic functions) |
| DNA (Deoxyribonucleic acid) | Deoxyribonucleotides | Repository of Hereditary information |
| RNA (Ribonucleic acid) | Ribonucleotides | Essentially required for protein biosynthesis |
| Polysaccharide (glycogen) | Monosaccharides | Storage form of energy to meet short term demands; Structural components of membranes |
| Lipid | Fatty acids, glycerol | Storage form of energy to meet long term demands; Structural components of membranes. |

*As regards Lipids, it may be noted that they are not biopolymers in a strict sense, but majority of them contain fatty acids.

Scope and Applications of Biochemistry

Biochemistry is used in daily life to develop new products and new technologies. ... The use of gene therapy to treat certain medical conditions is another example of **biochemistry used in daily life**. **Biochemistry** is the study of biological processes that occur in cells and organisms.

These are some other possible applications of biochemistry:

- Kidney Function Test. The kidney function is related to the biochemistry study.
- Blood Test. Biochemistry study is also involved in the concept of blood test.
- Liver Function Tests.
- Serum Cholesterol Test.
- Pregnancy Test.
- Breast Cancer Screening.

By using chemical information and procedures, **biochemists** can understand and solve biological problems.

Biochemistry plays an **important** role in **nutrition** and health. Similarly, **biochemistry** in general deals with body substances like enzymes, carbohydrates, amino acids, fats, proteins, hormones, DNA, RNA, pigments, etc.

Biochemistry has become the foundation for understanding all biological processes. It has provided explanations for the causes of many diseases in humans, animals, and plants. It can frequently suggest ways by which such diseases may be treated or cured.

The four main classes of molecules in **biochemistry** are carbohydrates, lipids, proteins, and nucleic acids. Many biological molecules are polymers: in this terminology, monomers are relatively small micromolecules that are linked together to create large macromolecules, which are known as polymers.

Branches of biochemistry

- Animal biochemistry.
- Plant biochemistry.
- Molecular biology.
- Cell biology.
- Metabolism.
- Immunology.
- Genetics.
- Enzymology.

All diseases have a molecular basis, so biochemistry enables us to understand the chemical processes involved in conditions as varied as:

- diabetes
- jaundice
- kidney dysfunction
- hypercholesterolemia
- phenylketonuria
- sickle cell anemia
- dental fluorosis
- rickets
- acidosis and alkalosis

With information gleaned from the chemical nature of pathologies, biochemists working in medicine are able to investigate potential treatments for diseases. The action of a drug almost always involves some change in the biochemical processes taking place in the body. As such, pharmacologists must also be acquainted with the biochemical aspects of the human body. In pharmacy, biochemical testing provides indispensable insights into a drug's:

- mode of action
- half-life
- storage conditions
- metabolism
- potential toxic or adverse effects

Biochemistry makes significant contributions to the fields of :

Cell biology,
Physiology,
Immunology,
Microbiology,
Pharmacology,
And toxicology,

As well as in the fields of inflammation, cell injury, and cancer.

These close relationships emphasize that life, as we know it, depends on biochemical reactions and processes.

Biotechnology including electrophoresis, centrifugation, hybridization, PCR, RFLP, ELISA, sequencing, chromatography, DNA and protein

Types of Biotechnology

- Medical Biotechnology: Medical biotechnology is the use of living cells and other cell materials for the purpose of bettering the health of humans.
- Agricultural Biotechnology.
- Nutrient Supplementation.
- Industrial Biotechnology.
- Biofuels.
- Healthcare.

Some of the subjects in biotechnology are....

- Biochemistry.
- Cell biology.
- Molecular biology.
- Microbiology.
- Industrial microbiology.
- Plant biotechnology.
- Animal biotechnology.

Proteins and Amino Acids

Proteins:

Proteins are the most abundant organic molecules of the living system. They occur in every part of the cell and constitute about 50% of the cellular dry weight. Proteins form the fundamental basis of structure and function of life.

Origin of the Word “Protein”

The term protein is derived from a Greek word *proteios*, meaning holding the first place. Berzelius (Swedish Chemist) suggested the name proteins to group of organic compounds that are utmost important to life. Mulder (Dutch Chemist) in 1838 used the term proteins for the high molecular weight nitrogen-rich and the most abundant substances in animals and plants.

Functions of proteins:

Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as **static** (structural) and **Dynamic**.

Static Functions:

Certain proteins perform brick and mortar roles and are primarily responsible for structures and strengths of body. These include **collagen** and **elastin** found in bone matrix, vascular system and other organs and **α -keratin** present in epidermal tissues.

Dynamic Functions:

The dynamic functions of proteins are moved diversified in nature. These include proteins acting as **enzymes, hormones, blood clotting factors, immunoglobulin**, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc. proteins performing dynamic functions are appropriately regarded as **the working horses** of cell.

Elemental Composition of Proteins:

Proteins are predominantly constituted by five major elements in the following proportion.

| | | |
|----------|---|----------|
| Carbon | : | 50 – 55% |
| Hydrogen | : | 6 – 7.3% |
| Oxygen | : | 19 – 24% |
| Nitrogen | : | 13 – 19% |
| Sulfur | : | 0 – 4% |

Besides the above, proteins may also contain other elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.

Proteins are polymers of Amino acids:

Proteins in complete hydrolysis (with concentrated HCl for several hours) yield L- α -amino acids. This is a common property of all the proteins. Therefore, proteins are the **polymers of L- α -amino acids**.

Structure of Proteins

Proteins are the polymers of L-amino acids. The structure of proteins is rather complex which can be divided into 4 levels of organization.

1. Primary Structure

The linear sequence of amino acids forming the backbone of proteins (Polypeptides).

2. Secondary Structure

The spatial arrangement of protein by twisting of the polypeptide chain.

3. Tertiary structure:

The three dimensional structure of a functional protein.

4. Quaternary Structure:

Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spatial arrangement of these subunits is known as quaternary structure.

Determination of Primary Structure of Protein

Each protein has a unique sequence of amino acids which is determined by the genes contained in DNA. The primary structure of a protein is largely responsible for its function. A vast majority of genetic diseases are due to abnormalities in the amino acids sequence of proteins i.e. changes associated with primary structure of protein.

The primary structure comprises the identification of constituent amino acids with regards to their quality, quantity and sequence in a protein structure. A pure sample of a protein or a polypeptide is essential for the determination of primary structure which involves 3 stages:

1. Determination of amino acids composition.
2. Determination of protein or polypeptide into smaller fragments.
3. Determination of the amino acids sequence.

Determination of Secondary Structure of Protein

The conformation of polypeptide chain by twisting or folding is referred to as secondary structure. The amino acids are located close to each other in their sequence. Two types of secondary structures, α -helix and β -sheet are mainly identified.

α -Helix:

α -Helix is the most common spiral structure of protein. It has a rigid arrangement of polypeptide chain. α -Helix structure was proposed by Pauling and Corey (1951) which is regarded as one of the milestones in the biochemistry research.

β -Pleated sheet:

This is the second type of structure proposed by Pauling and Corey. β -Pleated sheets (or simply β -sheets) are composed of two or more segments of fully extended peptide chains. In the β -sheets, the hydrogen bonds are formed between the neighbouring segments of polypeptide chain.

Determination of Tertiary Structure of Protein

The three-dimensional arrangement of protein structure is referred to as tertiary structure. It is a compact structure with hydrophobic side chains held interior while the hydrophilic groups are on the surface of the protein molecule. This type of arrangement ensures stability of the molecules.

Bonds of Tertiary structure: Besides the hydrogen bonds, disulfide bonds (-S-S), ionic interactions (electrostatic bonds), hydrophobic interactions and van der Waals forces also contribute to the tertiary structure of proteins.

Domains: The term domain is used to represent the basic units of protein structure (tertiary) and function. A polypeptide with 200 amino acids normally consists of two or more domains.

Determination of Quaternary Structure of Protein

A great majority of the proteins are composed of single polypeptide chains. Some of the proteins, however, consist of two or more polypeptides which may be identical or unrelated. Such proteins are termed as oligomers and possess quaternary structure. The individual's polypeptide chains are known as monomers or subunits. A dimer consists of two polypeptides while a tetramer has four.

Bonds in quaternary structure: The monomeric subunits are held together by non-covalent bonds namely hydrogen bonds, hydrophobic interactions and ionic bonds.

Examples of oligomeric proteins: Hemo-globin, aspartate transcarbamylase, lactate dehydrogenase.

Classification of Proteins:

Proteins are classified in several ways. Three major types of classifying proteins based on their function, chemical nature and solubility properties and nutritional importance are discussed here.

A. Functional Classification of proteins

Based on the functions they perform, proteins are classified into the following groups (with examples):

1. **Structural proteins:** Keratin of hair and nails, collagen of bone.
2. **Enzymes or Catalytic proteins:** Hexokinase, Pepsin.
3. **Transport Protein:** Hemoglobin, serum, albumin.
4. **Hormonal proteins:** Insulin, growth hormone.
5. **Contractile proteins:** Actin, Myosin.
6. **Storage proteins:** Ovalbumin, glutelin.
7. **Genetic proteins:** Nucleoproteins.
8. **Defense protein:** Snake venoms, Immunoglobulins.
9. **Receptor proteins:** for hormones and viruses.

B. Protein Classification based on chemical nature and solubility

This is a more comprehensive and popular classification of proteins. It is based on the amino acid composition, structure, shape and solubility properties. Proteins are broadly classified into 3 major groups.

1. **Simple Proteins:** They are composed of only amino acid residues.
2. **Conjugated Proteins:** Besides the amino acids, these proteins contain a non-protein moiety known as prosthetic group or conjugating group.
3. **Derived proteins:** These are the denatured or degraded products of simple and conjugated proteins.

C. Nutritional Classification of proteins

The nutritive value of proteins is determined by the composition of essential amino acids. From the nutritional point of view, proteins are classified into 3 categories:

1. **Complete Proteins:** These proteins have all the 10 essential amino acids in the required proportion by the human body to promote good growth e.g. Egg Albumin, Milk Casein.
2. **Partially incomplete proteins:** These proteins partially lack one or more essential amino acids, and can promote moderate growth e.g. Wheat and Rice proteins.
3. **Incomplete proteins:** These proteins completely lack one or more essential amino acids. Hence they do not promote growth at all. e.g. Gelatin, Zein.

Amino Acids

Amino acids are a group of organic compounds containing two functional groups—**amino & Carboxyl**. The amino group (—NH_2) is basic while the carboxyl group (—COOH) is acidic in nature.

General structure of amino acid

The amino acids are termed as α -amino acids, if both the carbonyl group & amino group are attached to the same carbon atom.

The α -carbon atom binds to a side chain represented by R which is different for each the 20 amino acids found in proteins. The amino acids mostly exist in the ionized form in biological system.

Classification of amino acids

There are different ways of classifying amino acids based on the structure and chemical nature, Nutritional requirement, metabolic fate.

A. Amino acids classification based on structure: A comprehensive classification of amino acids is based on their structure & chemical nature. Each amino acid is assigned 3 letters or 1 letter symbol. These symbols are commonly used to represent the amino acids in protein structure e.g. Glycine is represented by Gly or G. These amino acids found in proteins are divided into 7 distinct groups.

1. Amino acids with aliphatic side chains
2. Hydroxyl group containing amino acids
3. Sulfur containing amino acids
4. Acidic amino acids and their amides
5. Basic amino acids
6. Aromatic amino acids
7. Imino acids

B. Amino acids classification based on structure: Amino acids are classified into 4 groups based on their polarity. Polarity is important for protein structure.

1. Non-polar Amino acids
2. Polar Amino acids with no charge on 'R' group
3. Polar amino acids with positive charge 'R' group
4. Polar amino acids with negative charge 'R' group

C. Nutritional classification of amino acids: The amino acids are required for the synthesis of variety proteins, besides other biological functions. However, all these amino acids need not to be taken in the diet. Based on the nutritional requirements, amino acids are grouped into two classes — essential and non-essential.

1. Essential or indispensable amino acid:

The amino acids which cannot be synthesized by the body and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual e.g. Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine.

2. Non-essential or dispensable amino acids:

The body can synthesize about 10 amino acids to meet the biological needs; hence they need not be consumed in the diet. These are glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine and proline.

D. Amino acid classification based on their metabolic fate:

The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose (glycogenic) or fat (ketogenic) or both. From metabolic view point, amino acids are divided into three groups:

1. Glycogenic amino acids

(Serve as precursors for the formation of glucose or glycogen e.g. glycine, alanine etc.)

2. Ketogenic amino acids

(Fat can be synthesized from these amino acids e.g. Leucine, Lysine etc.)

3. Glycogenic and ketogenic amino acids

(These are the precursors for synthesis of glucose as well as fat e.g. isoleucine, tryptophan, tyrosine etc.)

Properties of Amino acids

The amino acids differ in their physico-chemical properties which ultimately determine the characteristics of proteins.

A. Physical Properties

1. Solubility:

Most of the amino acids are usually soluble in water and insoluble in organic solvents.

2. Melting points:

Amino acids generally melt at higher temperatures, often above 200°C.

3. Taste:

Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg).

4. Optical properties:

All the amino acids except glycine possess optical isomers due to the presence of asymmetric carbon atom.

5. Amino acids as ampholytes:

Amino acids contain both acidic and basic groups. They can donate a proton or accept a proton, hence amino acids are regarded as ampholytes.

B. Chemical properties

1. Decarboxylation:

Amino acids undergo decarboxylation to produce corresponding amines. The reactions assume significance in the living cells due to the formation many biologically important amines. These include histamine, tyramine from the histidine and tyrosine respectively.

2. Transamination:

Transfer of an amino group from an amino acid to a keto acid to form a new amino acid is a very important reaction in amino acid metabolism.

3. Oxidative deamination:

The amino acids undergo oxidative deamination to liberate free ammonia.

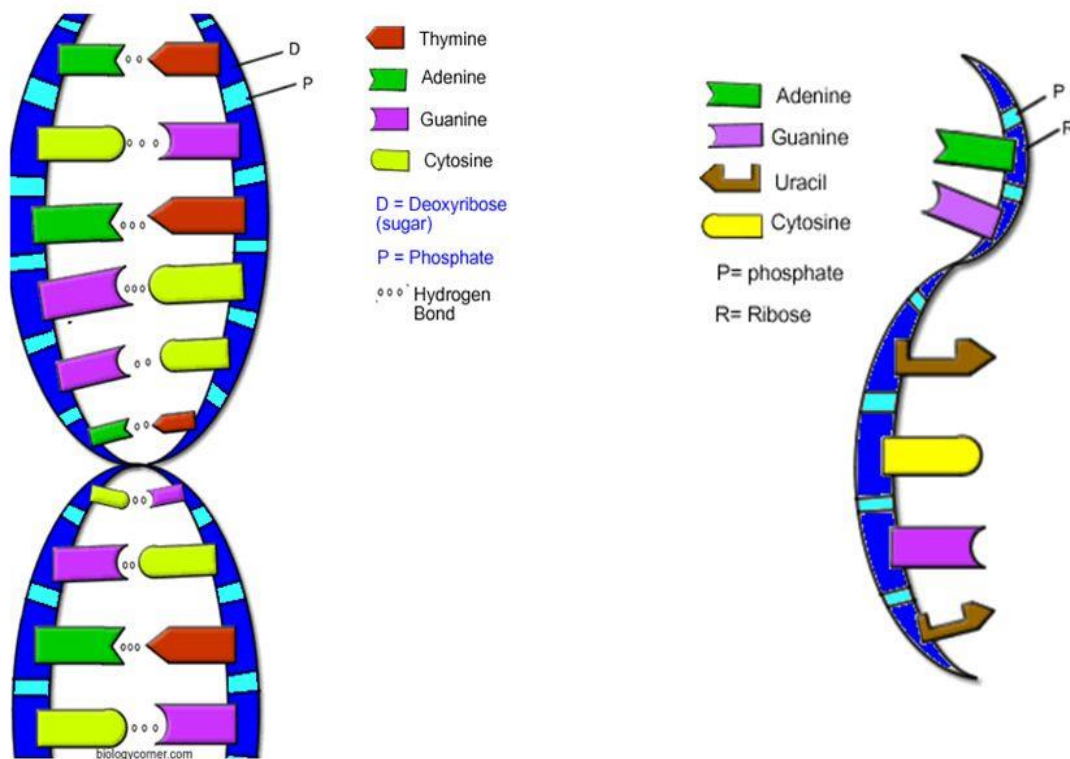
4. Colour reactions of amino acids:

Amino acids can be identified by specific colour reactions.

Introduction to Nucleic acids:

Nucleic acids were isolated in 1869 by F. Miescher from the nuclei of pus cell. Nucleic acids are of 2 types, deoxyribonucleic acid or DNA and ribonucleic acid or RNA.

What are nucleic acids?



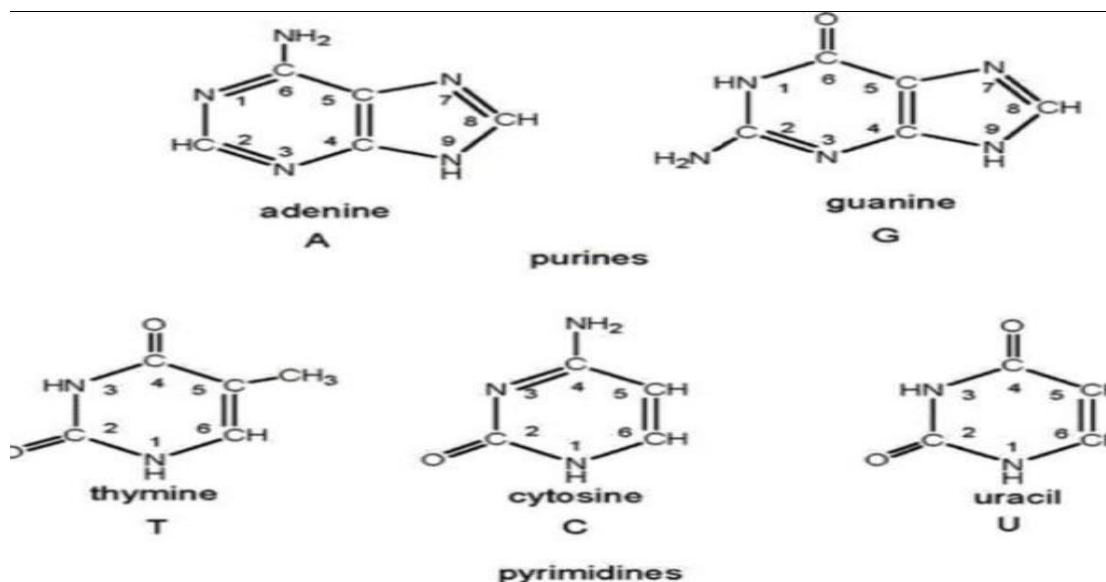
- DNA, occur in chromosomes, in the nuclei of the cells and in much lesser amount in mitochondria and chloroplast. DNA is made up of deoxyribonucleotides. It is a heredity material, made of two polynucleotide chains.
- RNA is present in the nucleolus, in the ribosomes, in the cytosol and in smaller amounts in the other parts of the cell. RNA is made up of ribonucleotides.

NITROGENOUS BASES:

Nitrogen bases are repeating units of RNA and DNA molecules. Levene determined all components of DNA in 1920. There are two main types of nitrogenous bases purine & pyrimidine.

Purines: Adenine and guanine are purines, contain double ring structure.

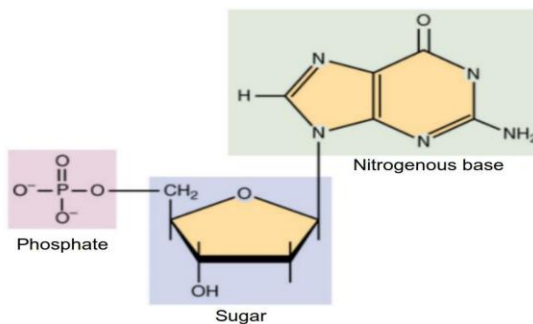
Pyrimidines: Thymine, cytosine and uracil are pyrimidines.



Nucleotides:

The ribose sugar, nitrogenous base and phosphoric acid is collectively known as nucleotide. It forms both DNA and RNA.

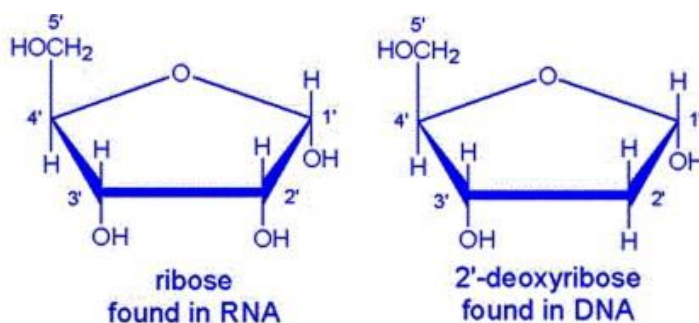
Nucleotide



Nucleosides:

The ribose sugar & nitrogenous base is collectively known as nucleoside.

The ribose sugar in DNA is deoxyribose while in RNA is ribose sugar.



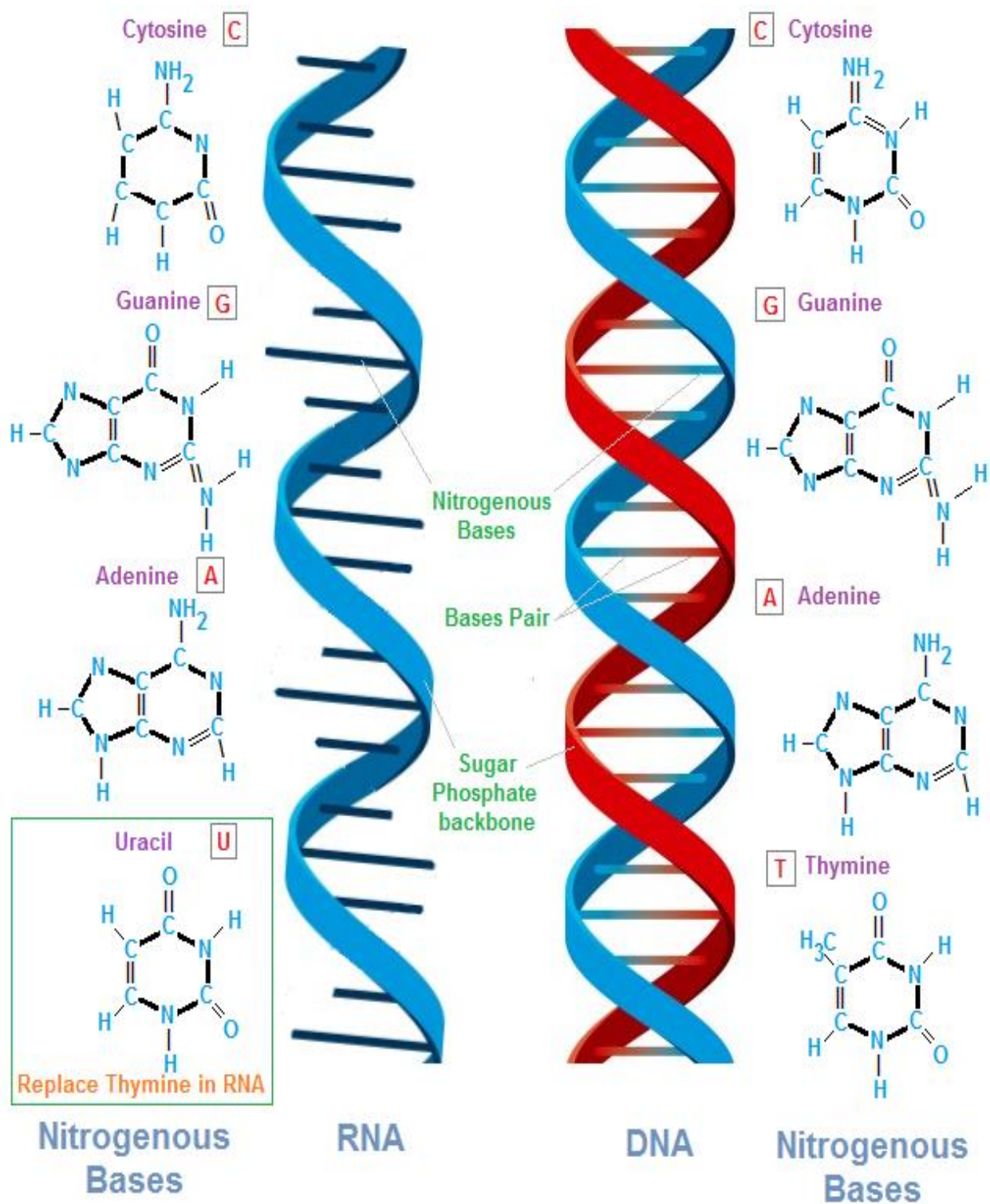
Structure of DNA and RNA:

DNA

- James D. Watson & Francis Crick built the scale model of DNA. The 2 polynucleotides chains or strands are coiled round each other in the form of double helix. Coiling of 2 strands is opposite i.e. they are coiled antiparallel to each other. The 2 chains are held together by weak bonds (hydrogen bonds). Adenine is always opposite to thymine (T), and guanine (G) and cytosine (C) are opposite to each other. There are 2 H-bonds b/w A & T, and 3 in b/w G and C.

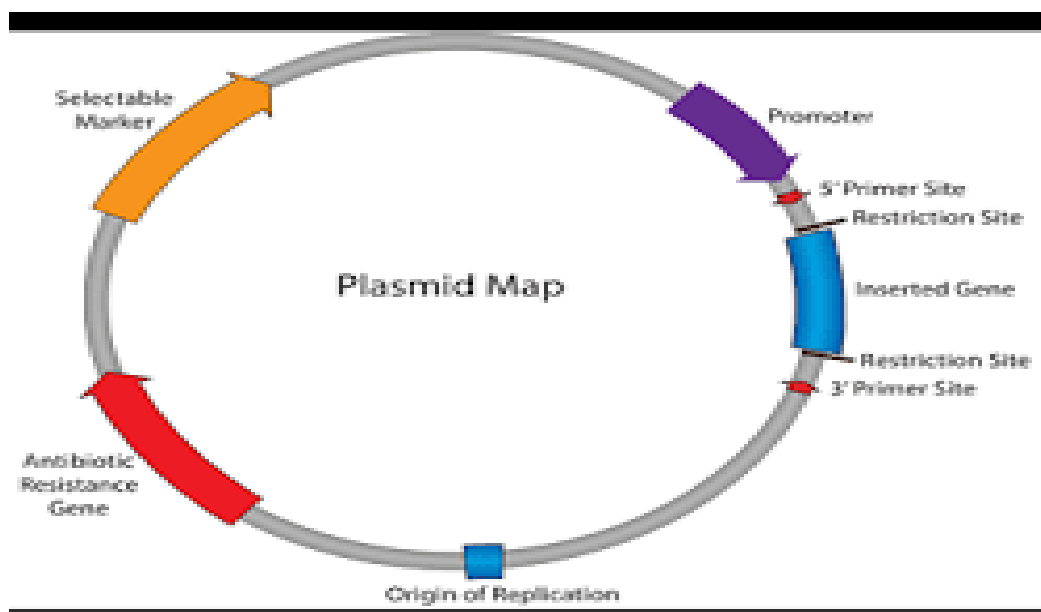
RNA

- RNA is a polymer of ribonucleotides. The RNA molecules occur as single strand, which may be folded back on it, to give double helical characteristics. The nitrogenous bases form the usual complementary pairing viz. cytosine with guanine and uracil with adenine. RNA is synthesized by DNA in a process known as TRANSCRIPTION. It is of 3 main types; rRNA, mRNA and tRNA.



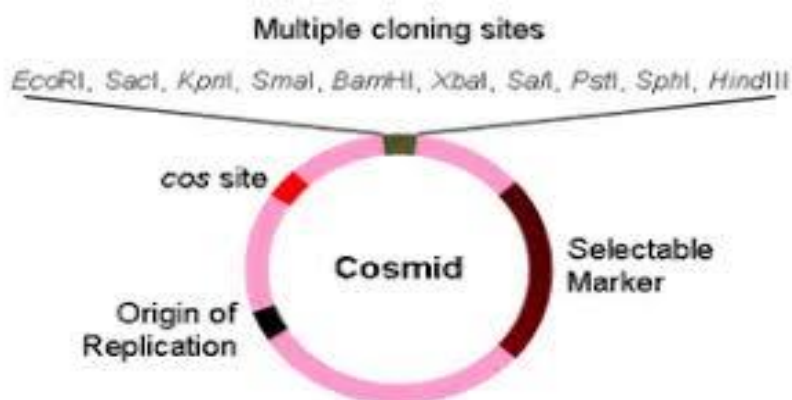
Plasmids:

Many bacteria contain plasmids in addition to chromosomes. These are the circular, double stranded DNA molecules. They are self-replicating and are not essential for bacterial growth and metabolism. They often contain drug resistant, heavy metals, disease and insect resistant genes on them. Plasmids are important vectors, in modern genetic engineering techniques.



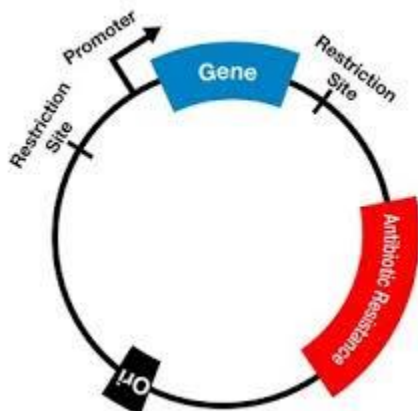
Cosmids:

A cosmid is a type of hybrid plasmid that contains a lambda phage cos sequence. Cosmids (Cos sites + plasmids = Cosmids) DNA sequences are originally from the lambda phage. They are often used as a cloning vector in genetic engineering. Cosmids can be used to build genomic libraries. They were first described by Collins and Hohn in 1978.



Vectors:

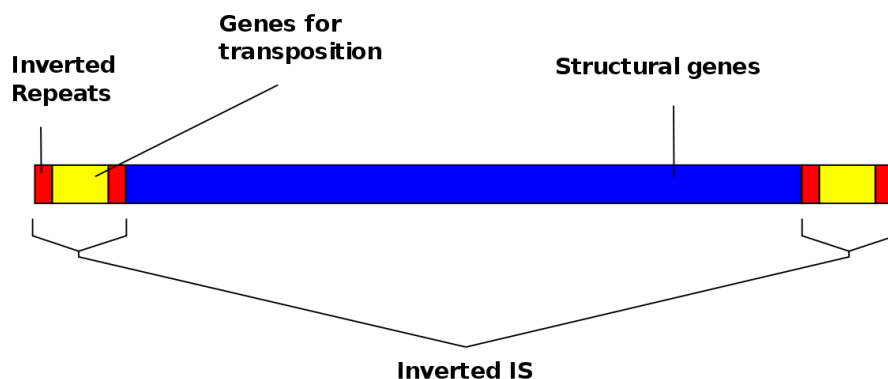
In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed (e.g. Plasmids, Cosmids, and Lambda phages). A vector containing foreign DNA called recombinant DNA. Plasmids are cloning vectors that are maintained in cells for the replication of double stranded DNA molecules. They are good cloning vectors because they are self-replicating, generally small so easy to work with and transform into their hosts



Transposons:

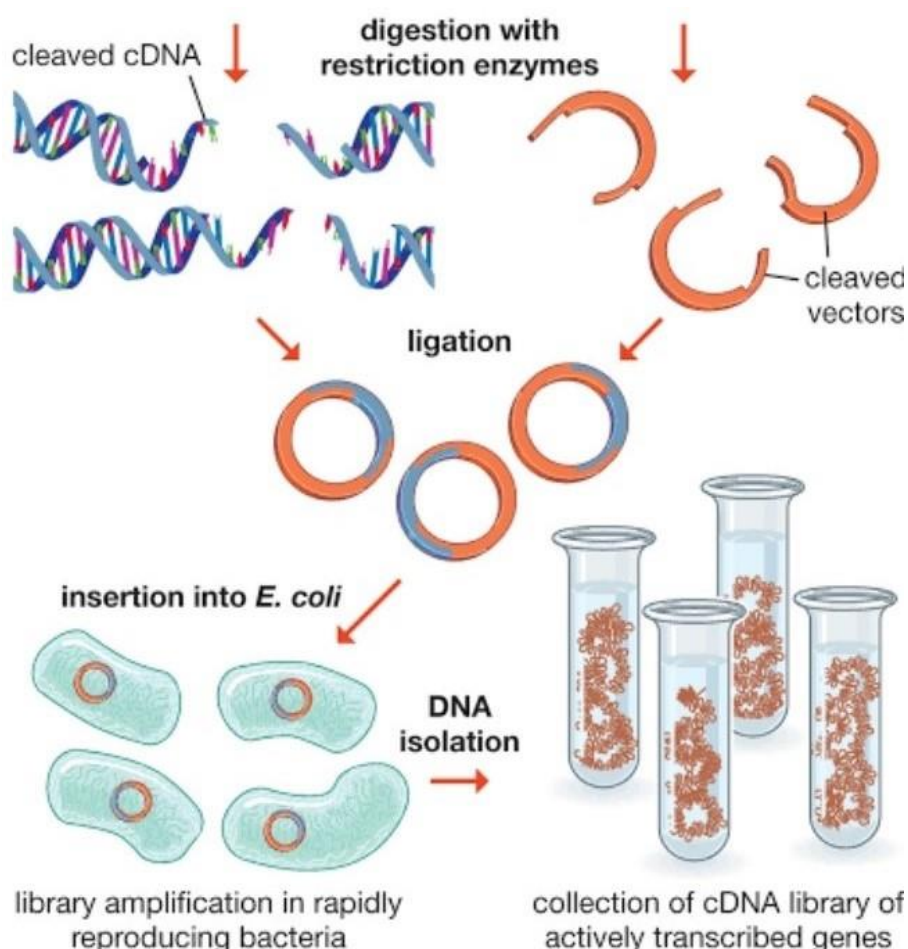
Transposons are called transposable elements because they have ability to jump from one position to another along the DNA or chromosome. The ability of transposons is to increase genetic diversity, together with the ability of the genome results in a balance that makes transposable elements as an important part of evolution and gene regulation in all organisms to carry these sequences. DNA transposons are used to introduce a piece of foreign DNA into a genome.

Bacterial composite transposon



Restriction Endonuclease:

In 1970, Hamilton O. Smith isolated the 1st restriction endonuclease enzyme in bacteria. This enzyme present in bacteria helps in cutting of viral DNA but does not harm to bacterial DNA. Bacteria produce a variety of such enzymes, which cut the DNA at specific sites characterized by specific sequence of nucleotides arranged symmetrically. They are used in DNA recombinant Technology. This restriction enzyme or restriction endonuclease is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.



Storage of the genetic information:

Genetic information is stored in the sequences of bases along a nucleic acid chain. Although, RNA probably functioned as the genetic material very early in evolutionary history, the genes of all modern cells and many viruses are made up of DNA. The genetic information is stored in the chemical structure of DNA molecule.

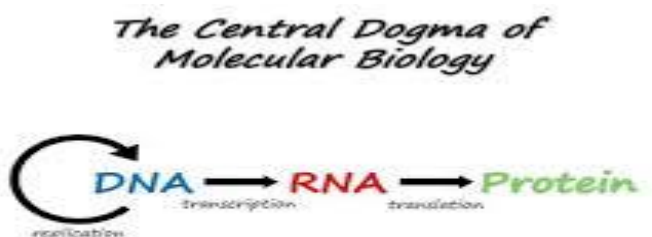
Flow of the genetic information:

The central dogma of molecular biology describes the flow of genetic information in cells from DNA to messenger RNA (mRNA) to protein. It states that genes specify the sequence of mRNA molecules, which in turn specify the sequence of proteins.

Central Dogma:

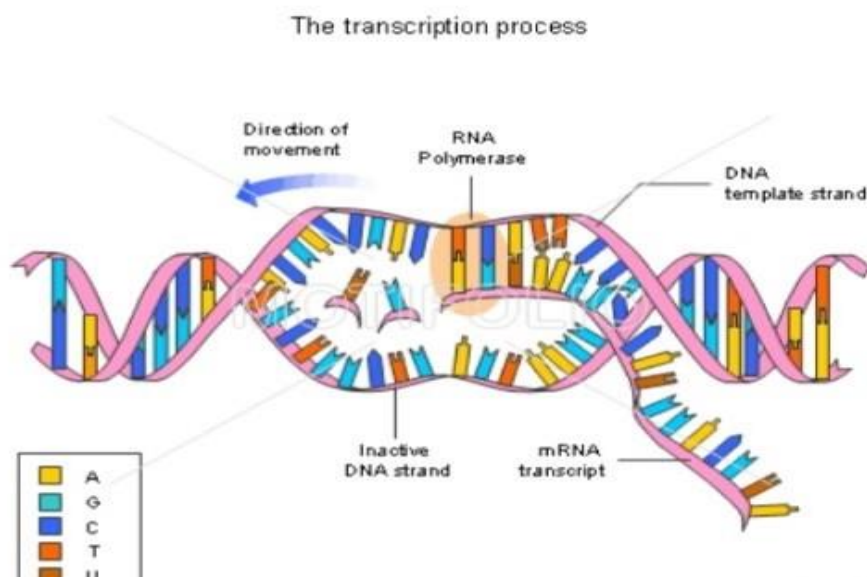
All organisms use the same basic mechanism of reading & expressing genes, which is often referred to as central dogma. There are 2 main steps of central dogma;

- **Transcription** (transfer of genetic information from DNA to RNA).
- **Translation** (transfer of information from RNA to Proteins).



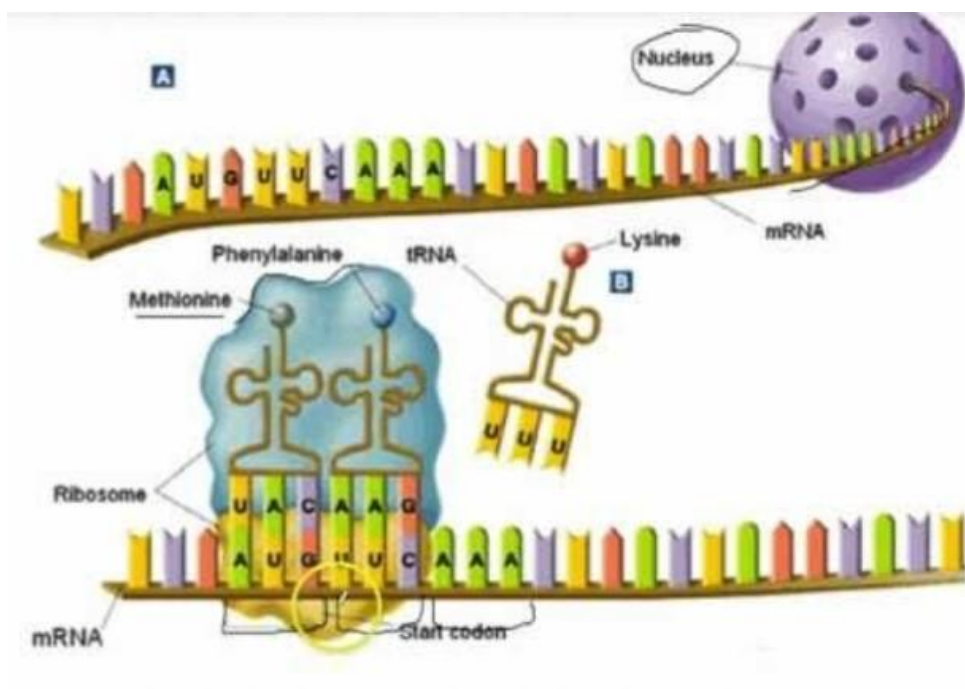
Transcription:

Transcription is the synthesis of RNA from a DNA template where the code in the DNA is converted into a complementary RNA code.



Translation:

Translation is the synthesis of a protein from an mRNA template where the code in the mRNA is converted into an amino acid sequence in a protein. It involves the conversion of genetic information of mRNA transcript into proteins.



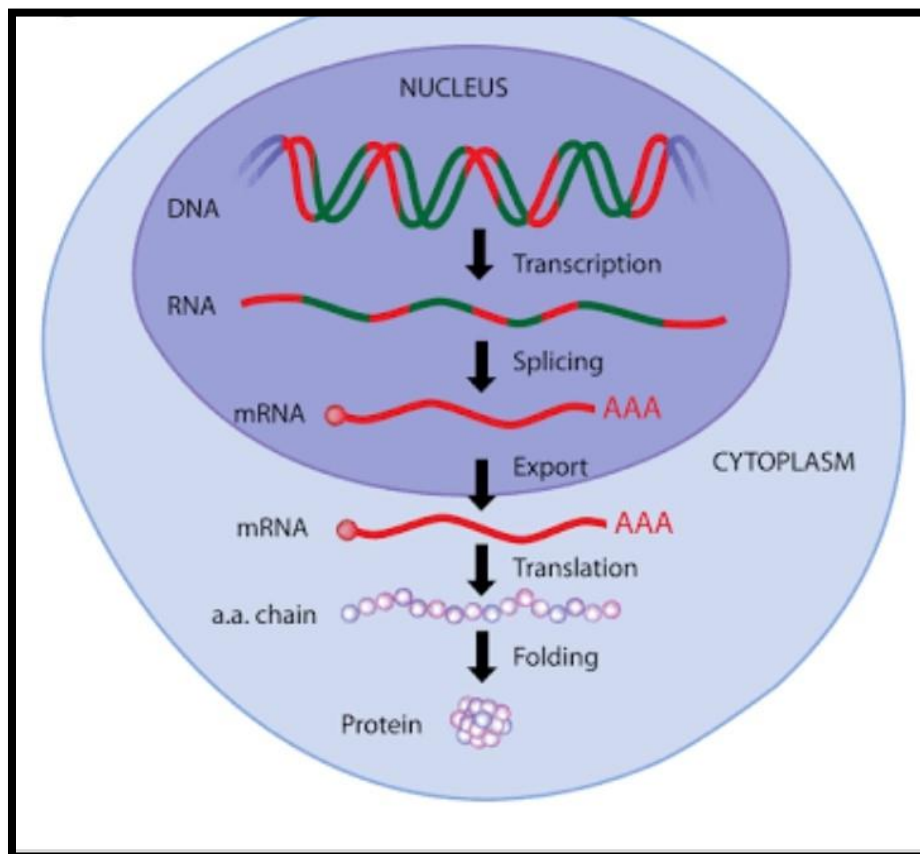
Replication process:

The replication process is the process of duplicating the one's own genetic materials into two more identical copies, so that the similar information may get further transfer to new daughter cells.



Gene Expression:

Gene expression is the process by which the instructions in our DNA are converted into a functional product, such as protein. Gene expression is a tightly regulated process that allows a cell to respond to its changing environment.



Introduction to Microbiology

A microbe, or microorganism, is a microscopic organism that comprises either a single cell (unicellular); cell clusters; or multicellular, relatively complex organisms. Microbes serve many functions in almost any ecosystem on Earth, including decomposition and nitrogen fixation. Many microbes are either pathogens or parasitic organisms, both of which can harm humans. The study of microorganisms is called **microbiology**, a subject that began with **Anton van Leeuwenhoek**'s discovery of microorganisms, using a microscope of his own design.

Examples:

Microorganisms are very diverse; they include bacteria, fungi, algae and protozoa; microscopic plants (green algae); and animals such as rotifers and planarians.

A fundamental understanding of how a cell works has come through the study of microorganisms. But microbiology also is an applied science, helping agriculture, health and medicine and maintenance of the environment as well as the biotechnology industry.

Applications of Microbiology

Microbiology is a scientific discipline that deals with the application of microorganisms and the knowledge about them. Applications include biotechnology, agriculture, medicine, food microbiology and bioremediation.

Classification of Microbiology

Microorganisms are divided into seven types: bacteria, archaea, protozoa, algae, fungi, viruses and multicellular animal parasites. Each type has a characteristic cellular composition, morphology, mean of locomotion and reproduction,

Branches of Microbiology

1. Bacteriology: the study of bacteria.
2. Mycology: the study of fungi.
3. Protozoology: the study of protozoa.
4. Parasitology: the study of parasites.
5. Phycology/Algology: the study of algae.
6. Immunology: the study of immune system.
7. Virology: the study of viruses.
8. Nematology: the study of nematodes.

Cell structure and Function

The Cell:

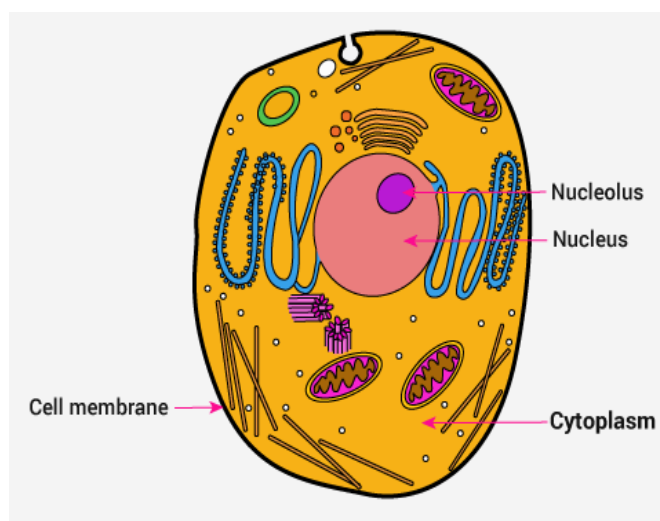
The cell can be defined as the structural and functional unit of life. It is the smallest unit that can carry out all activities of life. Cells are building blocks of complex multicellular organisms. Study of cell began with the discovery of cell by Robert Hooke (1665), who reported his work in famous publication **Micrographia**.

Structure of a Generalized Cell:

Structure of a cell can be studied under light microscope as well as electron microscope. The microscope technology enables us to isolate various components of cells including its organelles by a process of cell fractionation. Some cellular components require very high speeds for separation from other parts of the cells. This is achieved through ultracentrifugation.

A cell consists of the following basic components:

1. Plasma membrane (Cell membrane), also a cell wall in plant cell.
2. Cytoplasm, containing cell organelles.
3. Nucleus, with nuclear or chromatin material.



Function of a Cell

Cell provides six main functions:

1. Structure and Support.
2. Facilitate Growth through mitosis.
3. Allow passive and active transport.
4. Produce Energy.
5. Create metabolic reactions.
6. Aid in reproduction.

Cell components

Plasma membrane (Cell membrane):

Composition:

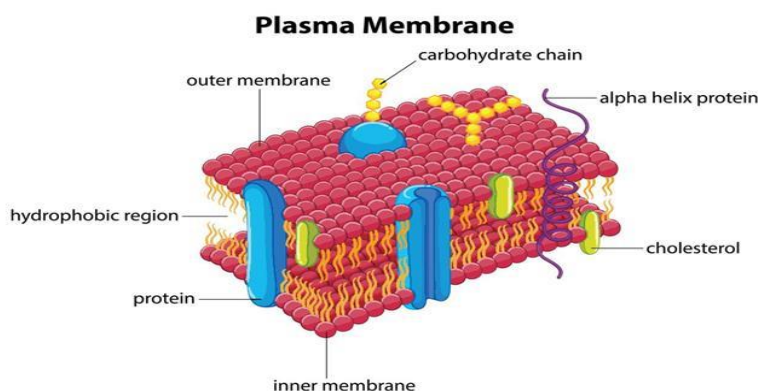
Cell membrane is chemically composed of lipids and proteins; 60-80% are proteins, while 20-40% are lipids. In addition there is a small quantity of carbohydrates.

Occurrence:

Cell membrane is the outermost boundary of the animal cells while in plant cells it is additionally covered by cell wall.

Function:

- Selectively permeable membrane
- Endocytosis (Phagocytosis : engulf solid material, Pinocytosis: to take in liquid material)
- Exocytosis



Cytoplasm:

Composition:

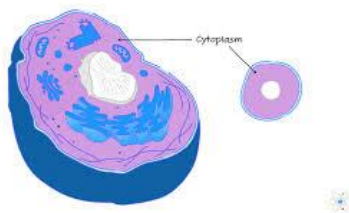
Cytoplasm consists of an aqueous ground substance containing a variety of cell organelles and other inclusions such as insoluble waste and storage products. The soluble part of the cytoplasm is called **cytosol**. Chemically it is about 90% water. Cytosol forms colloidal solutions that may be a **sol** (non-viscous) or a **gel** (viscous).

Occurrence:

It is present in both animal and plants cells.

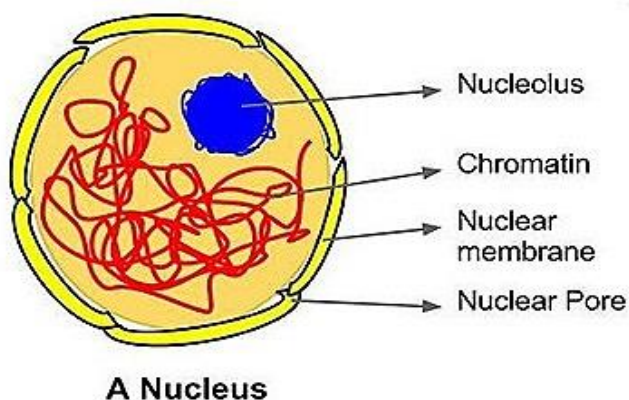
Function:

- Store house of vital chemicals.
- Site for metabolic processes.



Nucleus:

- Nucleus is surrounded by a **nuclear membrane** which separates the nuclear material from the cytoplasm.
- **Nucleolus** is a darkly stained body within the nucleus, and is without any membranous boundary. It helps in the synthesis of rRNA.
- The **nuclear pores** allow the exchange of materials between the nucleus and cytoplasm e.g. undifferentiated cells (such as eggs) have numerous pores (about 30 thousands per nucleus), whereas differentiated cells such as RBCs have only three or four pores per nucleus.
- **Chromatin** is exact replica of the chromosomes which are held together at centromere to form chromosomes.

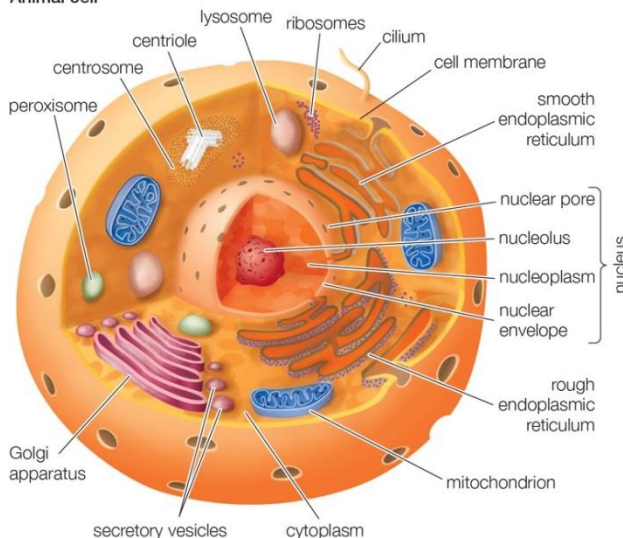


Differentiation between Animal and Plant Cell

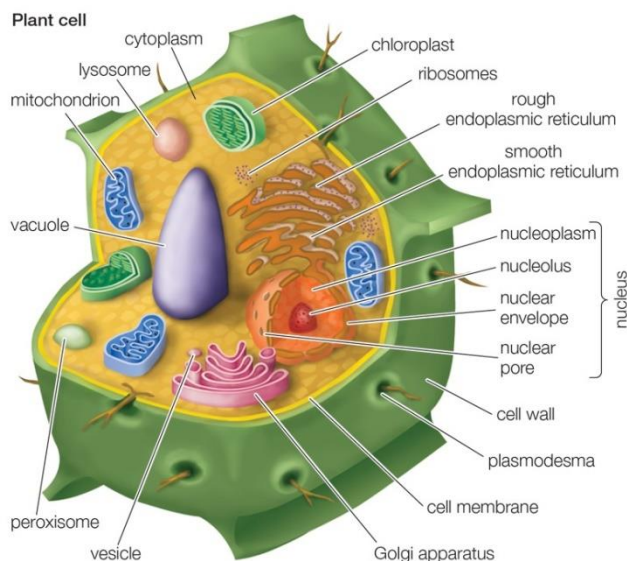
| <u>Organelles</u> | <u>Animal Cell</u> | <u>Plant cell</u> |
|--------------------|----------------------------|---|
| Cell wall | Absent | Present |
| Shape | Round | Rectangular |
| Vacuole | One or more small vacuoles | One, large central vacuole taking up to 90% of cell volume. |
| Centrioles | Present | Only present in lower plants |
| Chloroplast | Absent | Present |
| Ribosomes | Present | Present |
| Plastids | Absent | Present |

Typical animal cell and plant cell

Animal cell



Plant cell



Cell organization

Cellular organization is the basis and evolution of life in which micromolecules form macromolecules, which then form organelles. Organelles form the cells, which become tissue, organ, and system, this then forms a whole organism.

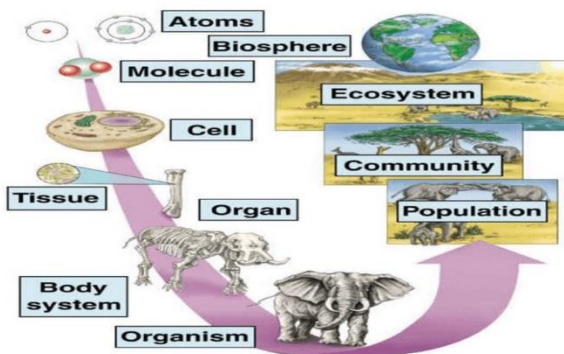
Most organisms have functional parts with five levels; cells, tissues, organs, organ systems and whole organisms. Cells hold genetic material and absorb outside energy. Tissues make the bones, nerves and connective fibers of the body.

Examples of Cell organization:

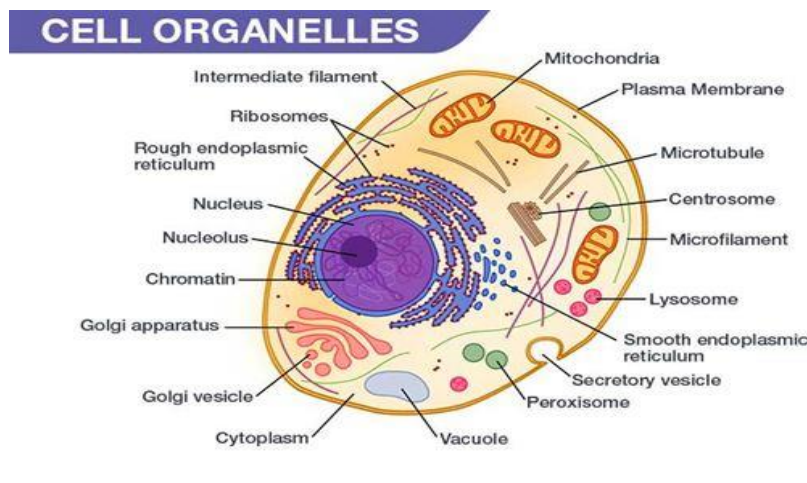
Blood cells, nerve cells, bone cells etc.

- Within an organism, groups of cells with similar functions combine to make up tissues.
- Groups of tissues with similar functions combine to make up organs.
- Group of organs working together combine to make up organ systems.
- All organ systems working together combine to make up the whole organism.

CELL ORGANIZATION



- **Sub cellular Organelles and their functions**



Vacuoles

Occurrence:

Both in animal and plant cells (particularly large and abundant in plant cells)

Appearance:

A single membrane bounded organelle formed by the coalescence of the different smaller vacuoles during growth and development.

Function:

- Serve to expand the cell without diluting its cytoplasm.
- Function as sites for storage of water and cell products.
- Metabolic intermediates.
- Regulates turgor pressure in the plants cells.

Mitochondria

Occurrence:

Present both in animal & plant cell.

Appearance:

A mitochondria is bounded by two membranes, the outer membrane is smooth, while the inner membrane forms infoldings, into the inner chamber called matrix. These infoldings are called Cristae.

Function:

- Mitochondria help in metabolic processes like Krebs cycle, aerobic respiration and fatty acid metabolism.
- Helps in the formation of ATP.

Ribosome

Occurrence:

It is present freely in cytoplasm and also on rough endoplasmic reticulum.

Appearance:

It is tiny granular structure, composed of equal amount of rRNA & protein.

Function:

- It is involved in protein synthesis.

Golgi apparatus/Golgi bodies

Occurrence:

It is also present in both animal & plant cell.

Appearance:

Golgi apparatus consists of stacks of flattened, membrane bound sacs, called Cisternae. The outer convex surface is the forming face, while inner concave face is maturing face.

Function:

- It is concerned with cell secretions.
- Transportation of enzymes & proteins out of the cell.
- Formation of glycoproteins & glycolipid.

Lysosome

Occurrence:

It is also present in both animal & plant cell.

Appearance:

They are bounded by single membrane & are sacks rich in acid phosphatase & several other hydrolytic enzymes.

Function:

- It is involved in phagocytosis of food.
- Also involved in autophagy (self-eating).
- Extracellular digestion.

Smooth Endoplasmic Reticulum

Occurrence:

Present in both animal & plant cell.

Appearance

SER is channel like process with in the cell involve in formation of vesicles.

Function:

- Detoxification of harmful drugs.
- Transmission of impulses.
- Transport of impulses.

Rough Endoplasmic Reticulum

Occurrence:

Present in both plant & animal cell.

Appearance:

RER is cisternae covered by ribosomes.

Function:

- Involved in synthesis of protein.

Fundamental Principles of Bioenergetics

Bioenergetics is a field in biochemistry and cell biology that concerns energy flow through living systems. This is an active area of biological research that includes the study of the transformation of energy in living organisms and the study of thousands of different cellular processes such as cellular respiration and the many other metabolic and enzymatic processes that lead to production and utilization of energy in forms such as adenosine triphosphate (ATP) molecules. That is the goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of **metabolic pathways** is thus essential to bioenergetics.

Principles:

The quantitative study of cellular energy transductions and the chemical reactions underlying these transductions are held in the bioenergetics. A **metabolic pathway** constitutes a series of enzymatic reactions to produce specific products. The term metabolite is applied to a substance or an intermediate or a product in the metabolic reactions.

Metabolism is broadly divided into two categories:

1. Catabolism: The degradative processes concerned with the breakdown of complex molecules to simpler ones, with a concomitant release of energy.
2. Anabolism: The biosynthetic reactions involving the formation of complex molecules from simple precursors.

Carbohydrate Metabolism

Carbohydrates are the major source of energy for the living cells. As such, carbohydrates are the first cellular constituent, synthesized by green plants during photosynthesis from carbon dioxide and water, on absorption of light. Thus, light is the ultimate source of energy for all biological processes. The monosaccharide glucose is the central molecule in carbohydrate metabolism since all the major pathways of carbohydrates metabolism are connected with it.

Major pathways of carbohydrate metabolism

The major pathways of carbohydrate metabolism are listed:

- Glycolysis (Embden-Meyerhof pathway): The oxidation of glucose to pyruvate and lactate.
- Citric Acid cycle (Krebs cycle or tricarboxylic acid cycle): The oxidation of acetyl CoA to CO₂.
- Gluconeogenesis: The synthesis of glucose from non-carbohydrate precursors (e.g. amino acids, glycerol etc.).
- Glycogenesis: The formation of glycogen from glucose.
- Glycogenolysis: The breakdown of glycogen to glucose.

- Hexose monophosphate shunt (pentose phosphate pathway): This pathway is an alternative to glycolysis and TCA cycle for the oxidation of glucose.

Lipid Metabolism

Lipids constitute about 15-20% of the body weight in humans. Triacylglycerol (formerly triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerol (TG; also called neutral fat or deposit fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes.

- Triacylglycerol (TG) is the stored fat in the adipose tissues. The enzyme, namely hormone sensitive triacylglycerol lipase, removes the fatty acid either from carbon 1 or 3 of the triacylglycerol to form diacylglycerol.
- The other two fatty acids of TG are cleaved by additional lipases specific for diacylglycerol and monoacylglycerol.
- The complete degradation of triacylglycerol to glycerol and free acids is called **lipolysis**.

Amino acids Metabolism

Proteins are the most abundant organic compounds and constitute a major part of the body weight. They perform a wide variety of static (structural) and dynamic (enzymes, hormones, clotting factors, receptors etc.) functions. About half of the body protein (predominantly collagen) is present in the supportive tissue (skeleton and connective) while the other half is intracellular.

Proteins are nitrogen-containing macro-molecules consisting of L- α -amino acids as the repeating units. The proteins on degradation (proteolysis) release individual amino acids. Protein metabolism is more appropriately learnt as metabolism of amino acids.

An adult has about 100g of free amino acids which represent the amino acids pool of the body. Glutamate and glutamine together constitute about 50%, and essential amino acids of the body pool (100g). The concentration of intracellular amino acids is always higher than extracellular amino acids. Amino acids enter the cells against a concentration gradient by active transport.

- The amino acids undergo certain common reactions like transamination followed by deamination for the liberation of ammonia.
- The ammonia group of the amino acids is utilized for the formation of urea which is an excretory end product of protein metabolism.

Chemical Equilibrium and principles of thermodynamics

Chemical equilibrium is the thermodynamic equilibrium in a system where direct and reverse chemical reactions are possible. Therefore, the macroscopic parameters of the system do not change and the relationship between concentrations of reacting substance remains constant at a given temperature.

Thermodynamic equilibrium is an axiomatic concept of thermodynamics. Thermodynamics is the science of the relationship between heat, work, temperature and energy. In broad terms,

Thermodynamics deals with the transfer of energy from one place to another and from one form to another.

Basic Principles:

- **Energy is conserved**
 - First law of thermodynamics.
- **All processes must increase entropy**
 - Second Law of thermodynamics.
- **System approaches a constant value as the temperature approaches absolute zero.**
 - Third Law of thermodynamics.

Laws of thermodynamics:

The three laws of thermodynamics define physical quantities (temperature, energy, and entropy) that characterize thermodynamics systems at thermodynamic equilibrium. The laws describe how these quantities behave under various circumstances.

The three laws of thermodynamics are:

❖ **First Law of Thermodynamics:**

The first law, also known as Law of conservation of Energy, states that energy cannot be created or destroyed in an isolated system.

❖ **Second Law of Thermodynamics:**

In a natural thermodynamics process, the sum of the entropies of the interacting thermodynamics systems increases.

❖ **Third Law of Thermodynamics:**

The entropy of a system approaches a constant value as the temperature approaches absolute zero.

Fermentation and its industrial application

Fermentation is the process of converting carbohydrates to alcohol or to organic acids using microorganisms, such as yeasts or bacteria. As the microorganisms divide, lactic acid is formed, which stops the growth of bacteria.

Industrial fermentation is the intentional used of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermented products have applications as food as well as in general industry.

Industrial fermentation is used in many industries, including:

- Microbiology
- Food Processing
- Pharmaceuticals
- Biotechnology
- Chemicals

Applications of Fermentation:

- Yogurt: Yogurt is made from fermented milk
- Alcoholic Beverages: These are created when yeast gives off ethyl alcohol and carbon dioxide as by-products of sugar consumption.
- Pickles: Cucumbers, other fruit and even meat can be preserved through pickling.
- Bread: In processing of bread, fermentation process is mainly used.
- Fuels, drugs, chemicals etc.

Introduction to Lipids:

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. They have the common property of being

- (1) Relatively insoluble in water and
- (2) Soluble in nonpolar solvents such as ether and chloroform.

They are important dietary constituents not only because of their high energy value, but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Combinations of lipid and protein (lipoproteins) serve as the means of transporting lipids in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, e.g., obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

Classification of Lipids:

Lipids are classified as simple or complex

1. **Simple lipids:** Esters of fatty acids with various alcohols.
 - a. **Fats:** Esters of fatty acids with glycerol. Oils are fats in the liquid state.
 - b. **Waxes:** Esters of fatty acids with higher molecular weight monohydric alcohols.
2. **Complex lipids:** Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.
 - a. **Phospholipids:** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, e.g., in glycerophospholipids the alcohol is glycerol.
 - b. **Glycolipids:** Lipids containing a fatty acid and carbohydrate.
 - c. **Other complex lipids:** Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
3. **Precursor and derived lipids:** These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones. Because they are uncharged, acyl glycerol (glycerides), cholesterol, and cholesterol esters are termed neutral lipids.

Chemical and physical Properties of Lipids

Lipids are soluble in organic solvents such as acetone, ethanol and chloroform but insoluble in water. They include fatty acids, **fats**, oils, waxes, phospholipids, glycolipids, steroids and some vitamins. **Lipids** are defined on the basis of a specific **physical property** – namely, solubility.

- Lipids may be either liquids or non-crystalline solids at room temperature.
- Pure fats and oils are colorless, odorless, and tasteless.
- They are energy-rich organic molecules
- Insoluble in water
- Soluble in organic solvents like alcohol, chloroform, acetone, benzene, etc.
- No ionic charges
- Solid triglycerols (Fats) have high proportions of saturated fatty acids.
- Liquid triglycerols (Oils) have high proportions of unsaturated fatty acids.

Chemical properties:

- Lipids are made of the elements Carbon, Hydrogen and Oxygen, but have a much lower proportion of water than other molecules such as [carbohydrates](#).
- Unlike polysaccharides and proteins, lipids are not polymers—they lack a repeating monomeric unit.
- They are made from two molecules: **Glycerol and Fatty Acids**.
- A glycerol molecule is made up of three carbon atoms with a hydroxyl group attached to it and hydrogen atoms occupying the remaining positions.
- Fatty acids consist of an acid group at one end of the molecule and a hydrocarbon chain, which is usually denoted by the letter 'R'.
- They may be **saturated or unsaturated**.
- A fatty acid is saturated if every possible bond is made with a Hydrogen atom, such that there exist no C=C bonds.
- Saturated fatty acids, on the other hand, do contain C=C bonds. Monounsaturated fatty acids have one C=C bond, and polyunsaturated have more than one C=C bond.

Functions of Lipids:

1. **Hydrolysis of triglycerols:** Triglycerols like any other esters react with water to form their carboxylic acid and alcohol— a process known as hydrolysis.
2. **Saponification:** Triacylglycerols may be hydrolyzed by several procedures, the most common of which utilizes alkali or enzymes called lipases. Alkaline hydrolysis is termed saponification because one of the products of the hydrolysis is a soap, generally sodium or potassium salts of fatty acids.
3. **Hydrogenation:** The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids.
4. **Halogenation:** Unsaturated fatty acids, whether they are free or combined as esters in fats and oils, react with halogens by addition at the double bond.
5. **Rancidity:** The term rancid is applied to any fat or oil that develops a disagreeable odor. Hydrolysis and oxidation reactions are responsible for causing rancidity. Oxidative rancidity occurs in triacylglycerol containing unsaturated fatty acids.

Introduction to Carbohydrates:

Carbohydrates are widely distributed in plants and animals; they have important structural and metabolic roles. In plants, glucose is synthesized from carbon dioxide and water by photosynthesis and stored as starch or used to synthesize the cellulose of the plant cell walls. Animals can synthesize carbohydrates from amino acids, but most are derived ultimately from plants. Glucose is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose formed by hydrolysis of dietary starch and disaccharides, and other sugars are converted to glucose in the liver. It is the precursor for synthesis of all the other carbohydrates in the body, including glycogen for storage; ribose and deoxyribose in nucleic acids; galactose in lactose of milk.

Classification of Carbohydrates

Carbohydrates are classified as follows:

- I. Monosaccharaides** are those sugars that cannot be hydrolyzed into simpler carbohydrates. They may be classified as trioses, tetroses, pentoses, hexoses, or heptoses, depending upon the number of carbon atoms, and as aldoses or ketoses, depending upon whether they have an aldehyde or ketone group. In addition to aldehydes and ketones, the polyhydric alcohols (sugar alcohols), in which the aldehyde or ketone group has been reduced to an alcohol group, also occur naturally in foods. They are synthesized by reduction of monosaccharaides for use in the manufacture of foods for weight reduction and for diabetics. They are poorly absorbed, and have about half the energy yield of sugars.
- II. Disaccharides** are condensation products of two monosaccharide units; examples are maltose and sucrose.
- III. Oligosaccharides** are condensation products of three to ten monosaccharaides. Most are not digested by human enzymes.
- IV. Polysaccharides** are condensation products of more than ten monosaccharide units; examples are the starches and dextrans, which may be linear or branched polymers. Polysaccharides are sometimes classified as hexosans or pentosans, depending on the identity of the constituent monosaccharaides (hexoses and pentoses, respectively). In addition to starches and dextrans, foods contain a wide variety of other polysaccharides that are collectively known as non-starch polysaccharides; they are not digested by human enzymes, and are the major component of dietary fiber. Examples are cellulose from plant cell walls (a glucose polymer) and inulin.

Monosaccharaides Structural Aspects-Isomerism

Stereoisomerism is an important character of monosaccharaides. Stereoisomers are the compounds that have the same structural formulae but differ in their spatial configuration. A carbon is called 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.

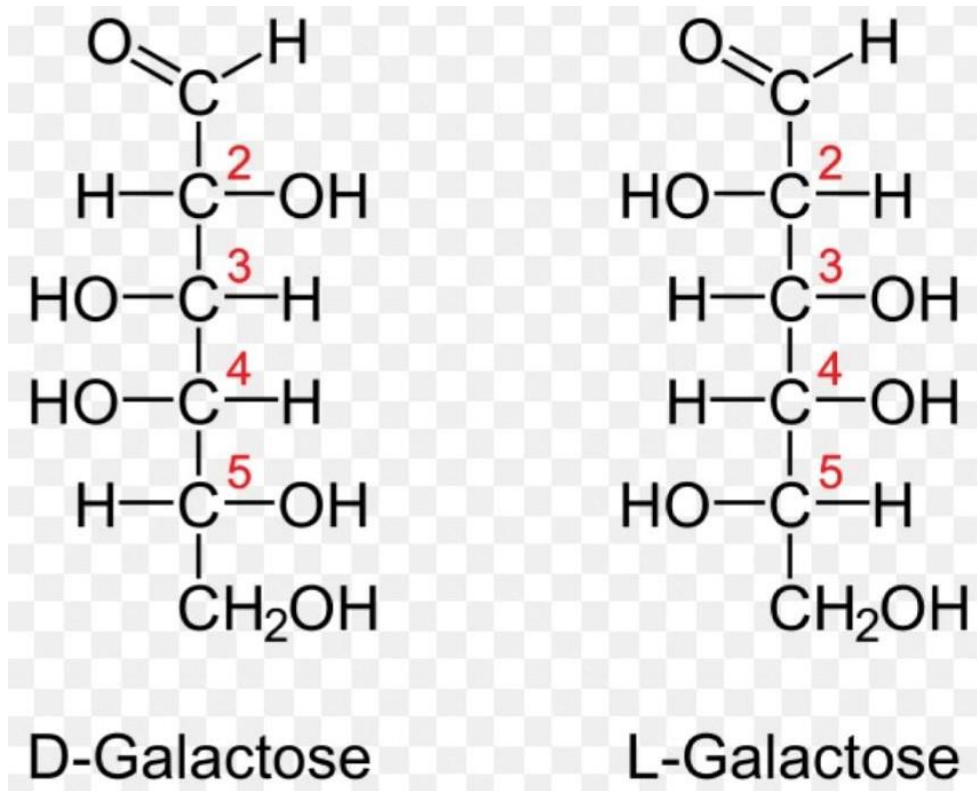
Example:

Glyceraldehyde is the simplest monosaccharide with one asymmetric carbon atom. It exists as two stereoisomers and has been chosen as the reference carbohydrate to represent the structure of all other carbohydrates.

D- and L-isomers

The D- and L-isomers are mirror images of each other. The spatial orientation of –H and –OH groups on the carbon atoms that is adjacent to the terminal primary alcohol carbon determines whether the sugar is D- or L-isomer. If the –OH group is on the right side, the sugar is of D-series, and if on the left side, it belongs to L-series.

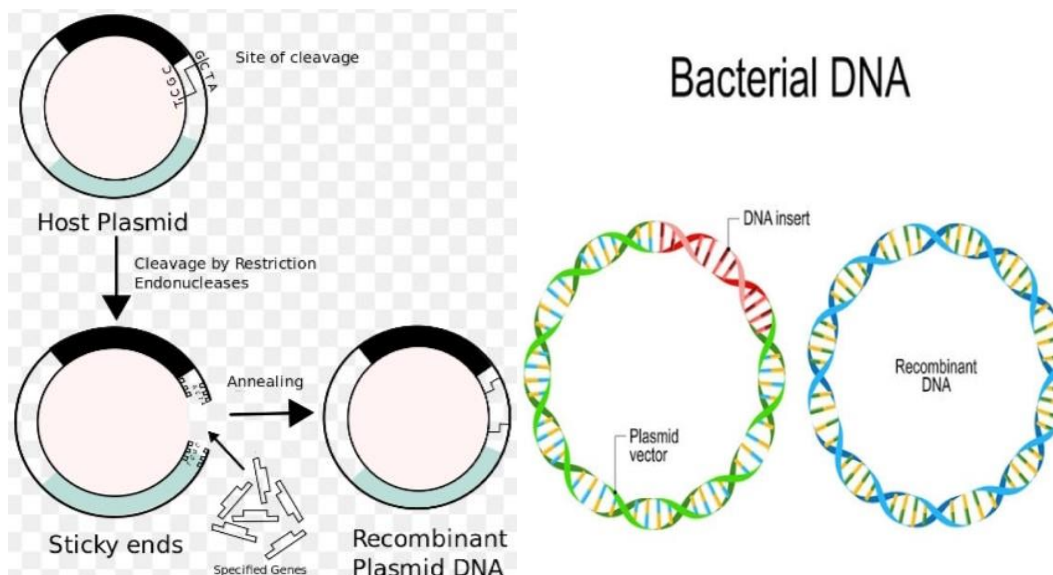
If two monosaccharaides differ from each other in their configuration around a single specific carbon atom, they are referred to as **epimers** to each other. For example, glucose and galactose are epimers.



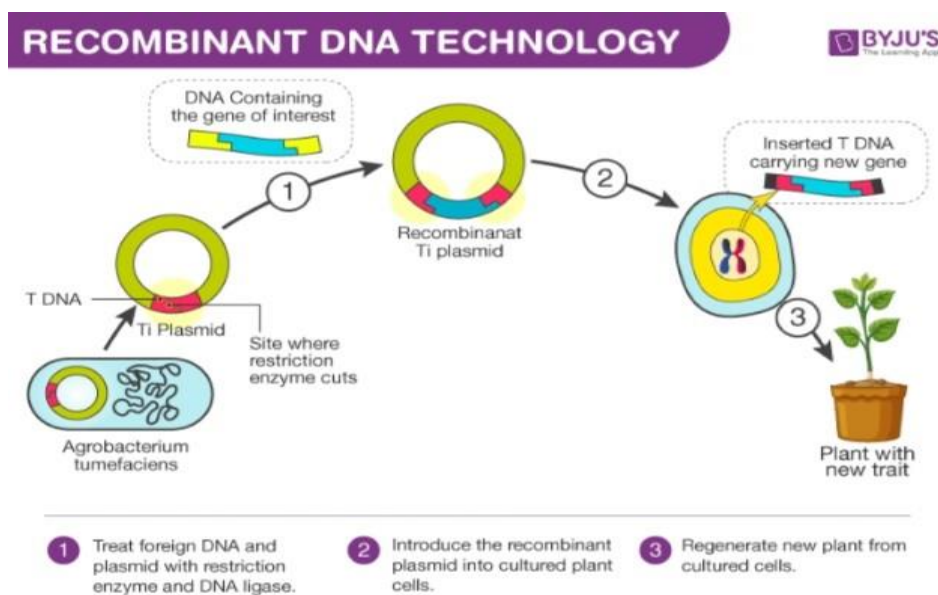
Enantiomers are a special type of stereoisomers that are mirror images of each other. The two members are designated as D- and L-sugars, such as enantiomers of Galactose. **Diastereomers** are used to represent the stereoisomers that are not mirror image of one another.

Recombinant DNA Technology:

Recombinant DNA technology is defined as the joining together of DNA molecules from different organisms and inserting it into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry.



Recombinant DNA molecules are DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. **Examples** of recombinant DNA molecules that are important to humans are pharmaceuticals like human **insulin** and **antibiotics**.

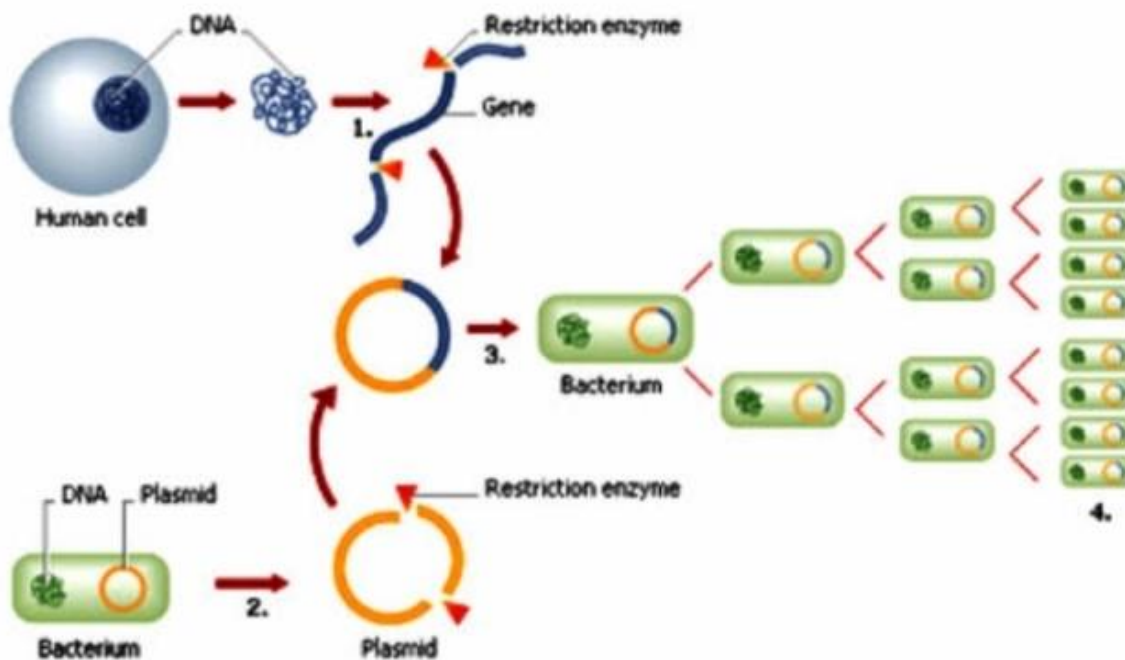


Gene Cloning:

In molecular biology, cloning is a set of experimental methods that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. In the cloning protocols, cloning of any DNA fragment essentially involves four steps:

- Isolation of the DNA of interest.
- Ligation
- Transfection (Transformation)
- Screening (Selection process)

Gene cloning is the process in which gene of interest is located and copied (cloned) out of DNA extracted from an organism. When DNA is extracted from an organism, all of its genes are extracted at one time. Gene cloning is a common practice in molecular biology labs that is used by researchers to create multiple copies of a particular gene for downstream applications such as sequencing, mutagenesis, genotyping or heterologous expression of a protein.



Genetic Engineering:

Genetic engineering, also called Genetic modification or genetic manipulation, is the direct manipulation of an organism's genes.

❖ Stages of Genetic Engineering

There are four stages of genetic engineering:

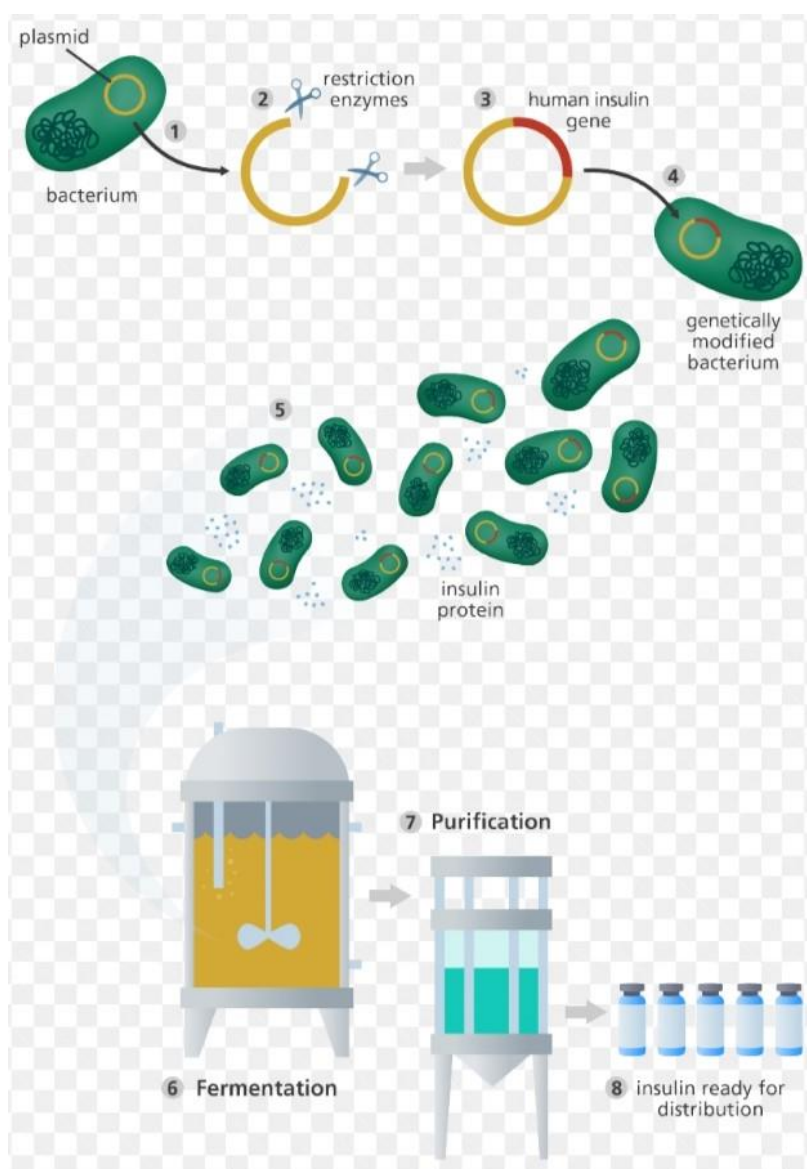
1. DNA cleavage: restriction endonuclease cleaves DNA into fragments.
2. Recombinant DNA production: DNA fragments inserted into two vectors.
3. Cloning: more recombinant DNA created.
4. Screening (Purification): most challenging part of any genetic experiment.

Examples of Genetic Engineering:

Crop plants, farm animals and soil bacteria are some of the more prominent example of organisms that have been subject to genetic engineering.

The possible benefits of genetic engineering include:

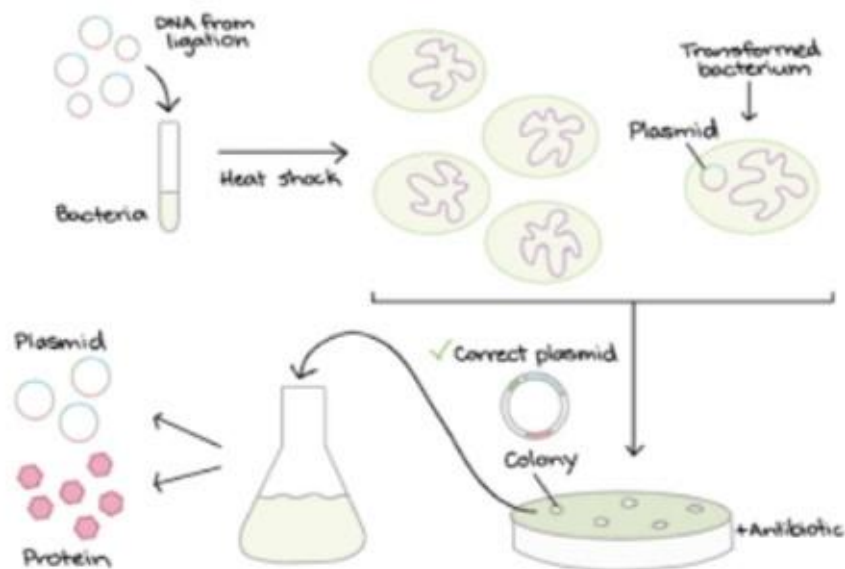
- More nutritious food
- Disease-and drought-resistant plants
- Pharmaceutical applications like insulin and antibiotics
- Less use of pesticides
- Increased supply of food with reduced cost
- Faster growing plants



Transformation:

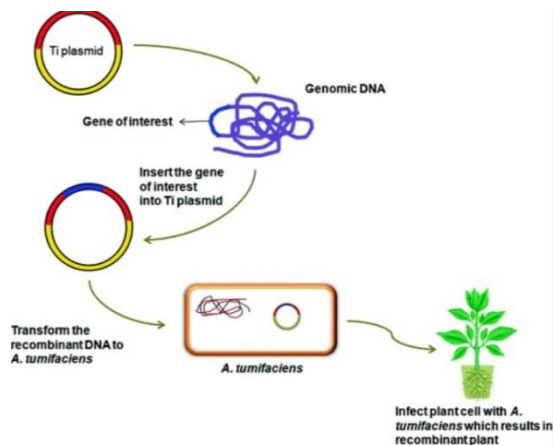
Transformation process is used to make multiple copies of DNA called DNA cloning. To make large amounts of specific human proteins, for example, human insulin or antibiotic, this can be used to treat people.

Genetic transformation is a process by which the genetic material carried by an individual cell is altered by the incorporation of foreign DNA into its genome. In molecular biology and genetics, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane.

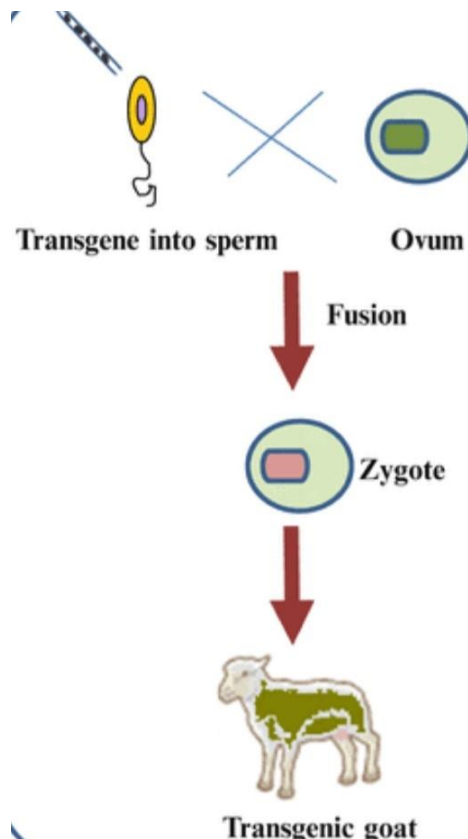


Transgenic plants and animals

Transgenic plants are plants that have been genetically engineered, a breeding approach that uses recombinant DNA techniques to create plants with new novel characteristics. Transgenic crops with insect resistance are corn, cotton, potato and tomato etc.



Transgenic animal is one that carries a foreign gene that has been inserted into its genome. Transgenic animals are those that have been genetically modified such as sheep, goats, cows, rabbits, rats, mice and parasites. Mice, however, are the most popularly tested animals in genetic modification studies.



Introduction to Experimental methodologies

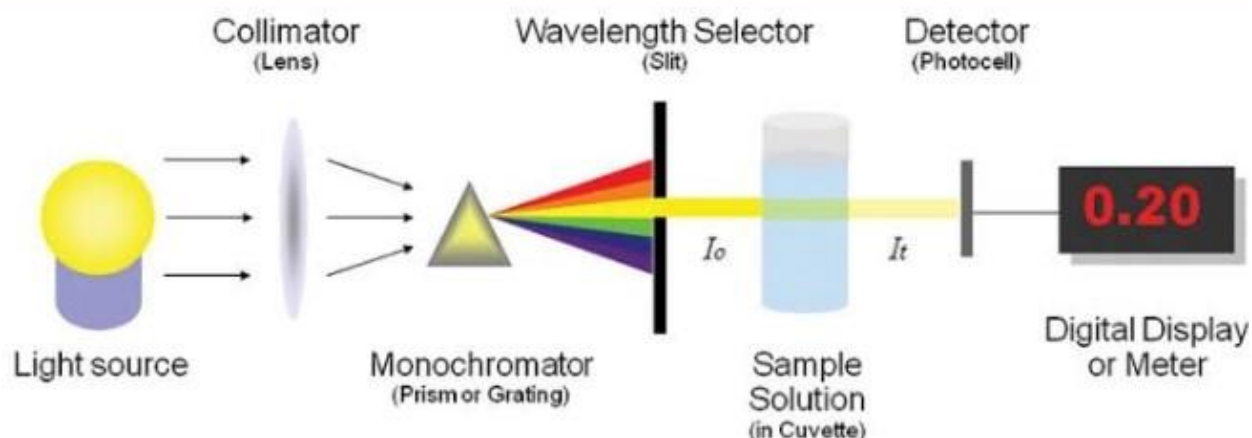
Biochemistry is an experimental rather than a theoretical science. The understanding and development of concepts in biochemistry are a result of continuous experimentation and evidence obtained. Thus, the development of sensitive and sophisticated analytical techniques has tremendously contributed to our understanding of biochemistry.

➤ Spectrophotometry:

Photometry broadly deals with the study of the phenomenon of light absorption by molecules in solution. The specificity of a compound to absorb light at a particular wavelength (monochromatic light) is exploited in the laboratory for quantitative measurements. Colorimeter and spectrophotometer are the laboratory instruments used for this purpose. They both work on the principle discussed below.

Spectrophotometer

Principle, Instrumentation, Applications



Principle of Spectrophotometer:

When a light at a particular wavelength is passed through a solution (incident light), some amount of it is absorbed and, therefore, the light that comes out (transmitted light) is diminished. The nature of light absorption in a solution is governed by **Beer-Lambert Law**.

Beer's law states that the amount of transmitted light decreases exponentially with an increase in the concentration of absorbing material. And according to **Lambert's law**, the transmitted light decreases exponentially with increase in the thickness of the absorbing molecules.

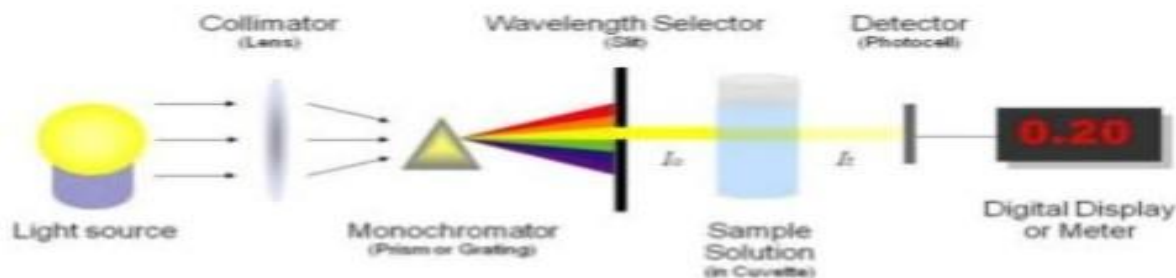
By combining the two laws (Beer-Lambert Law), the following mathematical derivation can be obtained: $I = I_0 e^{-\epsilon ct}$ Where I = Intensity of the transmitted light

I_0 = Intensity of the incident light

ϵ = Molar extinction coefficient

c = Concentration of the absorbing substance

t = Thickness of medium through which light passes



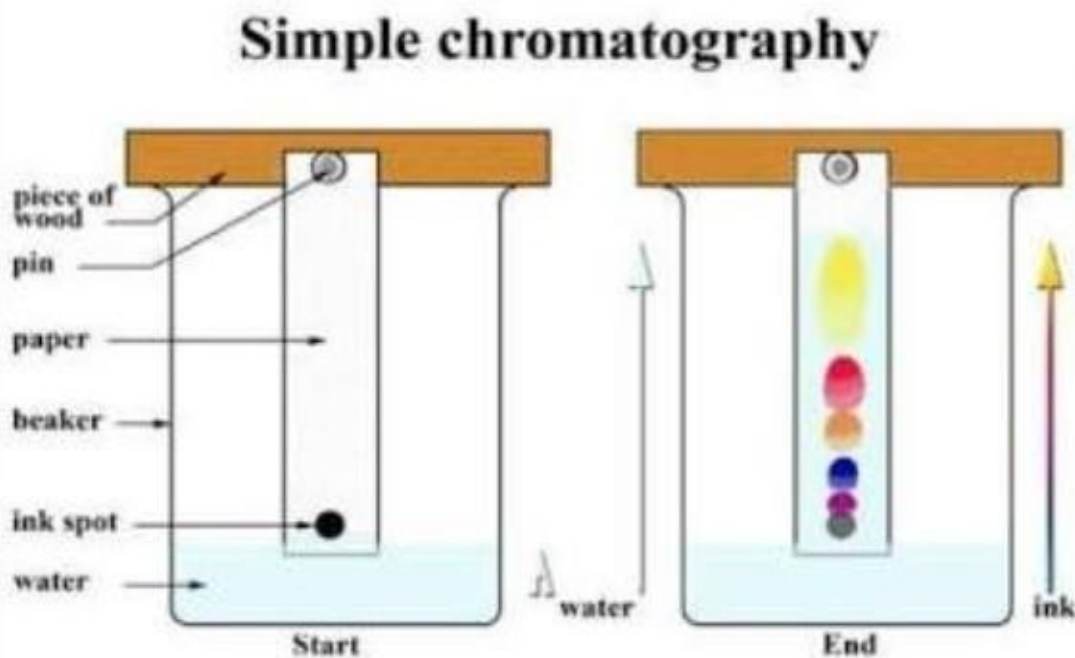
PRINCIPLE (BEER'S LAMBERT'S LAW)

Chromatography:

Chromatography is one of the most useful and popular in tools of biochemistry. It is an analytical technique dealing with the separation of closely related compounds from a mixture. Chromatographic separations partition molecules between two phases, one mobile and the other stationary. The mobile phase refers to the mixture of substances (to be separated), dissolved in a liquid or gas. The stationary phase is a porous solid matrix through which the sample contained in the mobile phase. The interactions between the mobile and stationary phases results in the separation of the compounds from the mixture. For separation of amino acids or sugars, the stationary phase, or matrix, may be separated by a sheet of filter paper or a thin layer of cellulose, silica, or alumina.

Historical perspective:

The credit for the discovery of chromatography goes to the Russian botanist Mikhail Tswett. Tswett described the separation of plant leaf pigments in solution by passing through a column of solid absorbents. He coined the term, chromatography (Greek: chroma_colour; graphein_to write), since technique deals with the separation of colour compounds.



❖ Column Chromatography

Column chromatography of proteins employs as the stationary phase small spherical beads of modified cellulose, acrylamide, or silica whose surface typically has been coated with chemical functional groups. The beads are packed in a cylindrical container, or column, comprised of glass, plastic, or metal. These stationary phase matrices interact with proteins based on their charge, hydrophobicity, and ligand-binding properties.

❖ Partition Chromatography

Column chromatographic separations depend on the relative affinity of different proteins for a given stationary phase and for the mobile phase. In partition chromatography, association between each protein and the matrix is weak and transient. Proteins that interact more strongly with the stationary phase are retained longer. The length of time that a protein is associated with the stationary phase is a function of the composition of both the stationary and mobile phases. Optimal separation of the protein of interest from other proteins thus can be achieved by careful manipulation of the composition of the two phases.

❖ Size Exclusion Chromatography

Size exclusion—or gel filtration—chromatography separates proteins based on their Stokes radius, the radius of the sphere they occupy as they tumble in solution. The Stokes radius is a function of molecular mass and shape. A tumbling elongated protein occupies a larger volume than a spherical protein of the same mass. Size-exclusion chromatography employs porous beads.

❖ Ion Exchange Chromatography

In ion exchange chromatography, proteins interact with the stationary phase by charge-charge interactions. Proteins with a net positive charge at a given pH adhere to beads with negatively charged functional groups such as carboxylates or sulfates (cation exchangers). Similarly, proteins with a net negative charge adhere to beads with positively charged functional groups, typically tertiary or quaternary amines (anion exchangers). Proteins, which are polyanions, compete against monovalent ions for binding to the support—thus the term “ion exchange.”

❖ Peptides Are Purified by Reversed-Phase High-Pressure Chromatography

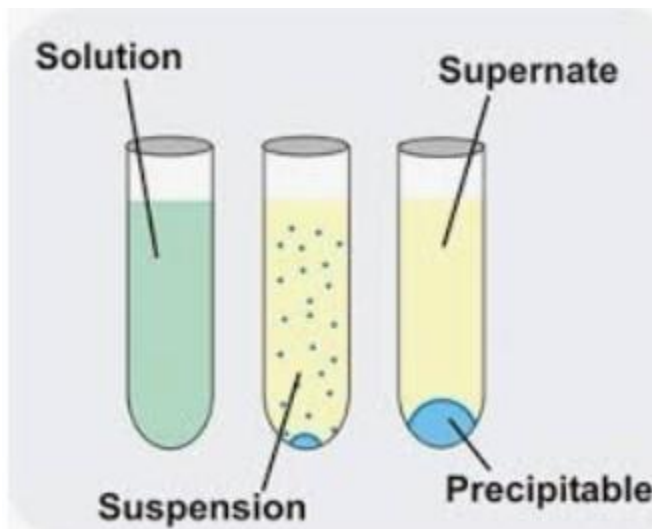
The stationary phase matrices used in classic column chromatography are spongy materials whose compressibility limits flow of the mobile phase. High-pressure liquid chromatography (HPLC) employs incompressible silica or alumina micro beads as the stationary phase and pressures of up to a few thousand. Incompressible matrices permit both high flow rates and enhanced resolution. HPLC can resolve complex mixtures of lipids or peptides whose properties differ only slightly.

❖ Protein Purity Is Assessed by Polyacrylamide Gel Electrophoresis (PAGE)

The most widely used method for determining the purity of a protein is SDS-PAGE—polyacrylamide gel electrophoresis (PAGE) in the presence of the anionic detergent sodium dodecyl sulfate (SDS). Electrophoresis separates charged biomolecules based on the rates at which they migrate in an applied electrical field. For SDS-PAGE, acrylamide is polymerized and cross-linked to form a porous matrix. SDS denatures and binds to proteins at a ratio of one molecule of SDS per two peptide bonds.

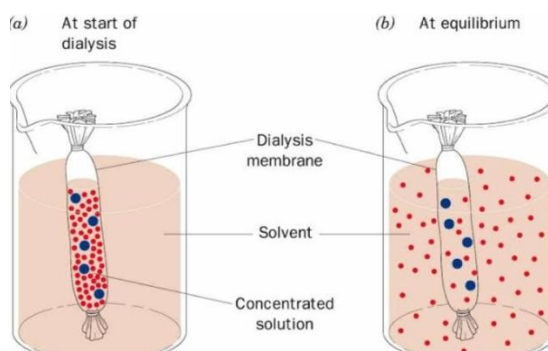
Precipitation Process:

In chemistry, a precipitate is an insoluble solid that emerges from a liquid solution. The emergence of the insoluble solid from solution is called precipitation. Often the precipitate emerges as a suspension. Precipitation is the process of conversion of a solution into solid by converting the substance into insoluble form. This process can be used for making pigments, removing salts from water in water treatment and in classical qualitative inorganic analysis.



Dialysis process:

In chemistry, dialysis is the separation of particles in a liquid on the basis of difference in their ability to pass through a membrane. Dialysis is an operation to separate dissolved molecules based on molecular weight.



In medicine, dialysis is the process of removing excess water, solutes, and toxins from the blood in people whose kidneys can no longer perform these functions naturally. There are two main types of dialysis:

1. **Hemodialysis** uses a machine and a filter to remove waste products and water from the blood.
2. **Peritoneal dialysis** uses a fluid (dialysate) that is placed into the patient's abdominal cavity to remove waste products and fluid from the body.

Physical and chemical properties of Carbohydrates:

Physical Properties:

The simple carbohydrates include single sugars (monosaccharaides) and polymers, oligosaccharides, and polysaccharides. Simplest group of carbohydrates and often called simple sugars since they cannot be further hydrolyzed. They are Colorless, crystalline solids which are soluble in **water** and insoluble in a non-polar solvent.

Chemical Properties:

Carbohydrates consist of carbon, hydrogen, and oxygen. The general empirical structure for **carbohydrates** is $(CH_2O)_n$. They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain.

Functions of Carbohydrates:

The four primary **functions of carbohydrates** in the body are to provide energy, store energy, build macromolecules, and spare protein and fat for other uses. Glucose energy is stored as glycogen, with the majority of it in the muscle and liver.

Qualitative Analysis of Carbohydrates:

Carbohydrates are defined by the following classifications: aldehyde (known as aldose) or ketone (known as ketoses) groups, reducing molecules and water soluble. Additionally, carbohydrates can be grouped in several ways, as monosaccharaides, disaccharides or polysaccharides. Monosaccharaides have one carbohydrate unit. Disaccharides have two carbohydrate units. Polysaccharides have many carbohydrate units. An example for each includes: glucose (**monosaccharaides**), sucrose (**disaccharides**) and starch (**polysaccharides**). The initial purpose of the identification of unknown carbohydrates experiment was to identify unknown carbohydrates based on chemical and physical properties and understand that similarities and differences.

Most of the tests of the **carbohydrates** are based on their reducing properties (due to the presence of reducing aldehyde or ketone groups). Fehling's test, Benedict's test is the example of this. Specific complex formation is sometimes used as specific **test for carbohydrates**.

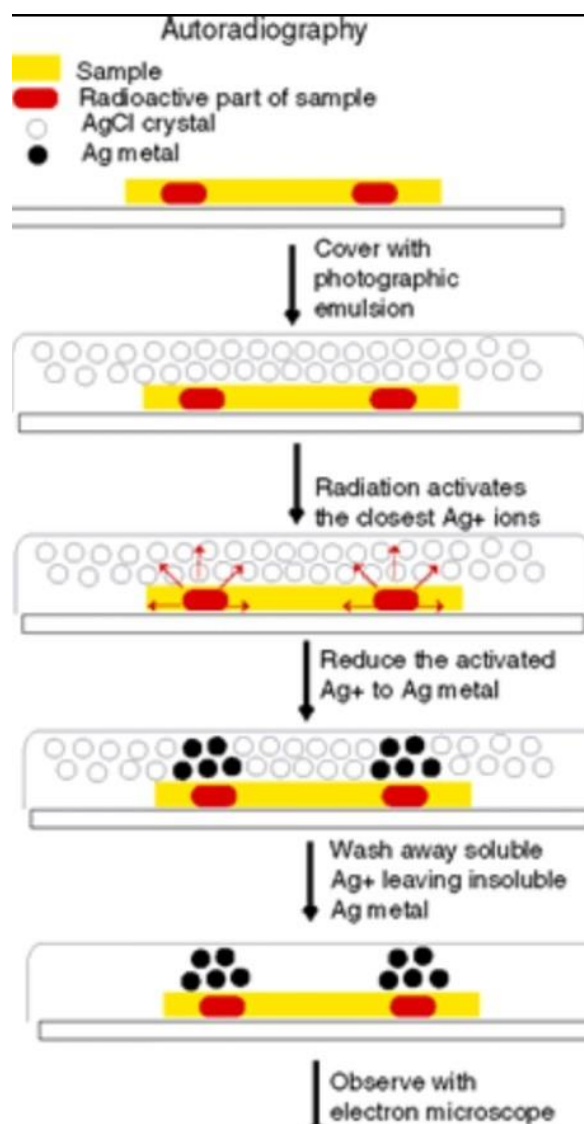
- **Benedict's test** – Given sample food + Benedict's reagent → Red precipitate confirms the presence of carbohydrates.
- **Tollen's test** – Given sample food + Tollen's reagent → Silver mirror confirms the presence of carbohydrates.
- **Benedict's test** would determine if the sample is a reducing sugar, and Bradford's test would determine if it is a monosaccharide or disaccharide.
- **Molisch's test** is a general test for carbohydrates. This test is given by almost all of the carbohydrates to form a purple coloured product.

Autoradiography:

Autoradiography is an imaging technique that uses radioactive sources contained within the exposed sample. In vitro **autoradiography** methods involve the isolation of cellular components such as DNA, RNA, proteins or lipids, followed by labeling with suitable radioisotopes. **Autoradiography** is a technique using X- ray film to visualize molecules or fragments of molecules that have been radioactively labeled. For example, **Autoradiography** can be **used to** analyze the length and number of DNA fragments after they are separated from one another.

Principle of Autoradiography

Autoradiography is based upon the ability of radioactive substance to expose the photographic film by ionizing it. In this technique a radioactive substance is put in direct contact with a thick layer of a photographic emulsion (thickness of 5-50 mm) having gelatin substances and silver halide crystals.



Radioactive and non-radioactive tracers:

A **radioactive tracer**, radiotracer, or **radioactive** label, is a chemical compound in which one or more atoms have been replaced by a radionuclide so by virtue of its **radioactive** decay it can be **used to** explore the mechanism of chemical reactions by tracing the path that the radioisotope follows from reactants to products.

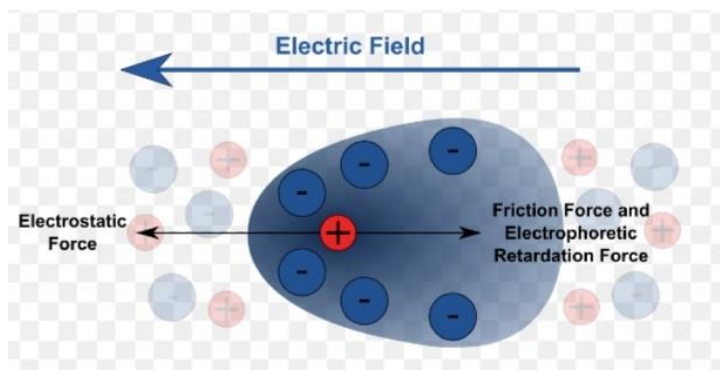
Stable **isotopes** are **non-radioactive** forms of atoms. Although they do not emit radiation, their unique properties enable them to be used in a broad variety of applications, including water and soil management, environmental studies, nutrition assessment studies and forensics.

Electrophoresis:

Electrophoresis is an electro kinetic process which separates charged particles in a fluid using a field of electrical charge. It is most often used in life sciences to separate protein molecules or DNA and can be achieved through several different procedures depending on the type and size of the molecules. Gel **electrophoresis** is **used to** separate macromolecules like DNA, RNA and proteins. DNA fragments are separated according to their size. Proteins can be separated according to their size and their charge (different proteins have different charges).

Principle:

Electrophoresis is a general term that describes the migration and separation of charged particles (ions) under the influence of an electric field. An **electrophoretic** system consists of two electrodes of opposite charge (anode, cathode), connected by a conducting medium called an electrolyte.



TYPES OF ELECTROPHORESIS

1. Zone **Electrophoresis**
 - a) Paper **Electrophoresis**
 - b) Gel **Electrophoresis**
 - c) Thin Layer **Electrophoresis**
 - d) Cellulose acetate **Electrophoresis**
2. Moving Boundary **Electrophoresis**
 - a) Capillary **Electrophoresis**
 - b) Isoelectric Focusing
 - c) Immuno **Electrophoresis**

Microscopy:

Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye). A **microscope** (from the Ancient Greek: mikrós, "small" and skopeîn, "to look" or "see") is an instrument **used** to see objects that are too small to be seen by the naked eye. **Microscopy** is the science of investigating small objects and structures using such an instrument. **Microscopy** is the act of using a **microscope** to view tiny things that cannot be seen with the unaided eye.



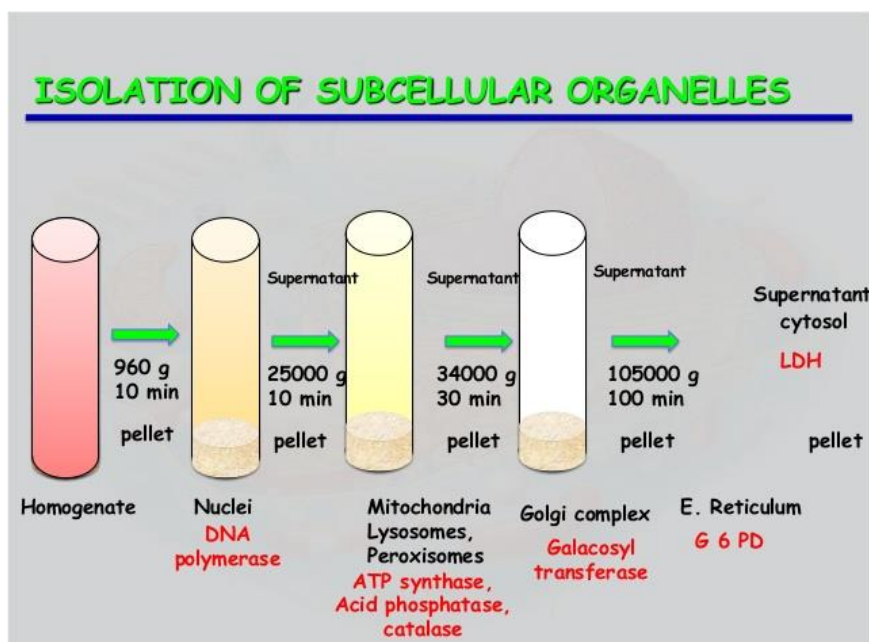
Principle of Microscope:

A general biological **microscope** mainly consists of an objective lens, ocular lens, lens tube, stage, and reflector. An object placed on the stage is magnified through the objective lens. When the target is focused, a magnified image can be observed through the ocular lens. There are three main **types**: optical **microscopy**, scanning probe **microscopy**, and electron **microscopy**.

Isolation of cells and organelles:

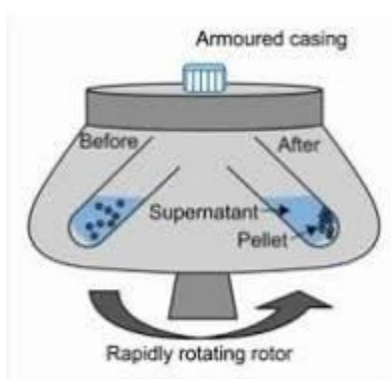
Isolation of **organelles** is accomplished by **cell** membrane lysis and density gradient centrifugation to separate **organelles** from contaminating cellular structures. Intact nuclei and **organelles** have distinctive sizes in mammalian **cells**, enabling them to be separated by this method, called **Cell Fractionation**.

The process is pretty simple; you take some **cells**, throw them in a blender, and then centrifuge them to **separate** the **organelles**. Centrifugal force will move denser material away from the center of the spin, displacing less dense material, and therefore creating a gradation with the least dense closest to the center, and the densest further to the outside. Applying this to separation of **organelles** is called **cell** fractionation.



Centrifugation:

Centrifugation is a technique used for the separation of particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. The particles are suspended in a liquid medium and placed in a **centrifuge** tube. The tube is then placed in a rotor and spun at a define speed. **Centrifugation** is the process where a mixture is separated through spinning. It is **used** to separate skim milk from whole milk, water from your clothes, and blood cells from your blood plasma. **Centrifugation** uses a **centrifuge**, or a device that can rapidly spin, to speed up this process.

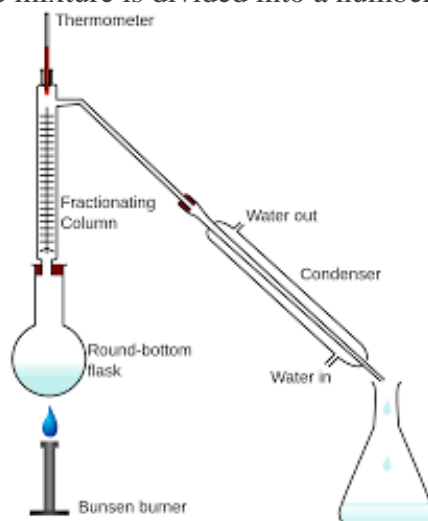


Principle of Centrifuge:

The **centrifuge** works using the sedimentation **principle**, where the centrifugal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the center.

Fractionation:

Fractionation is a separation process in which a certain quantity of a mixture (gas, solid, liquid, enzymes, suspension, or isotope) is divided during a phase transition, into a number of smaller quantities (fractions) in which the composition varies according to a gradient. Fractionation is a separation process in which one mixture is divided into a number of smaller parts.



Lyophilization:

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. **Lyophilization**, also known as **freeze-drying**, is a process **used** for preserving biological material by removing the water from the sample, which involves first freezing the sample and then drying it, under a vacuum, at very low temperatures. **Lyophilized** samples may be stored much longer than untreated samples.

Principle of Lyophilization

Freezing of the product is happened to convert the water in the product to ice form, Sublimation of ice directly into water vapor under vacuum. Drawing off all the water vapors and once the ice has been sublimated; the products are **freeze-dried** and can be removed from machine.

