BM T36- BIOCHEMISTRY

UNIT-I

2 MARKS:

1. Write the type of carrier protein. (Nov 2016)

- Active Transport
- Facilitated Diffusion. ...
- Sodium-Potassium Pump. ...
- Glucose-Sodium Cotransport. ...

2. a. Differentiate nucleoside from nucleotides (Nov 2016, May 2016, May 2017)

b. What are nucleotides (May 2015)

Nucleotide	Nucleoside
The chemical composition of nucleotide consists of a phosphate group, a sugar and a nitrogenous base.	A nucleoside has a chemical composition that consists of a sugar and a base without the phosphate group.
They are one of the major causes of cancer-causing agents to this very day.	They are used as agents in medicine that are primarily used against viruses and cancer-causing agents.
Some of the major examples of nucleotides are adenosine, guanosine etc.	Some of the key examples of nucleosides are the same as nucleotides only with the addition of phosphate groups.

3. Define redox potential (May 2015)

The oxidation –reduction potential or redox potential, is a electron transfer potential (Eo), oxidation is defined as the loss of electrons and reduction is the gain in electrons, when a substance exist both in reduced state and in oxidised state, the pair is called a redox couple.

4. List the function of any two organelles (Nov 2015)

- Mitochondria – It is involved in the process of oxidative phosphorylation.
- Nucleus – It governs gene expression and facilitates DNA replication.

5. Define active transport (Nov 2015, May 2017)

Active transport is the movement of dissolved molecules into or out of a cell through the cell membrane, from a region of lower concentration to a region of higher concentration. The particles move against the concentration gradient, using energy released during respiration.

6. What are molecular scissors? (May 2016)

- Restriction enzymes are also called 'molecular scissors' as they cleave DNA at or near specific recognition sequences known as restriction sites. These enzymes make one incision on each of the two strands of DNA and are also called restriction endonucleases.
- Example: Eco RI.

7. Which cell organelle is called "power house of the cell"? Explain why? (Nov 2017)

Mitochondria are tiny organelles present inside the cells that are involved in releasing energy from food. It produces energy which is ATP through respiration and regulates cellular metabolism. Since energy gives power to the cell, mitochondria are called the powerhouse of the cell.

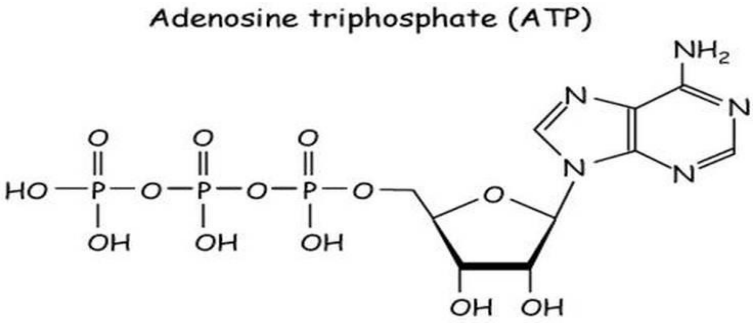
8. What is the significance of oxidative phosphorylation? (Nov 2017, May 2019)

Oxidative phosphorylation is a highly efficient method of producing large amounts of ATP, the basic unit of energy for metabolic processes. During this process electrons are exchanged between molecules, which create a chemical gradient that allows for the production of ATP.

9. What is Svedberg unit? (May 2018)

A Svedberg unit is a non-SI metric unit for sedimentation coefficients. The Svedberg unit offers a measure of a particle's size based on its sedimentation rate under acceleration. The Svedberg is a measure of time, defined as exactly 10^{-13} seconds.

10. Write the structure of ATP. (May 2018)



$C_{10}H_{16}N_5O_{13}P_3$

11. What are chromosomes? In which part of the eukaryotic cell are they present? (Nov 2018)

A chromosome is a long DNA molecule with part or all of the genetic material of an organism. In eukaryotes, all of the cell's chromosomes are stored inside a structure called the nucleus. Each eukaryotic chromosome is composed of DNA coiled and condensed around nuclear proteins called histones.

12. Name the nucleotides of DNA? (Nov 2018)

- Adenine (A)
- Thymine (T)
- Guanine (G) and
- Cytosine (C)

13. What are nucleic acids? (May 2019)

Nucleic acids are naturally occurring chemical compounds that serve as the primary information-carrying molecules in cells. They play an especially important role in directing protein synthesis. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

14. Differentiate between active and passive transport. (Nov 2019)

Active Transport	Passive Transport
Requires cellular energy.	Does not require cellular energy.

It circulates from a region of lower concentration to a region of higher concentration	It circulates from a region of higher concentration to a region of lower concentration
It transports various molecules in the cell.	It is involved in the maintenance of the equilibrium level inside the cell.
Active transport is a dynamic process.	Passive Transport is a physical process.
It is highly selective.	It is partly non-selective
Different types of Active Transport are – Exocytosis, endocytosis, sodium-potassium pump	Different types of Passive Transport are – Osmosis, diffusion, and facilitated diffusion

15. What is differential centrifugation? (Nov 2019)

Differential centrifugation is a common procedure in biochemistry, which is used to separate organelles and other sub-cellular particles based on their sedimentation rate. Differential centrifugation works by a stepwise increase in the centrifugation speed. Lower speeds at the beginning are used to eliminate the heavier food particles from the sample, and the speed is then increased until the targets themselves are pelleted.

11MARKS:

1. **Write the composition of nucleic acid. Explain the structure of DNA. (Nov 2016, May 2017, May 2018, Nov 2018, May 2019, Nov 2019)**

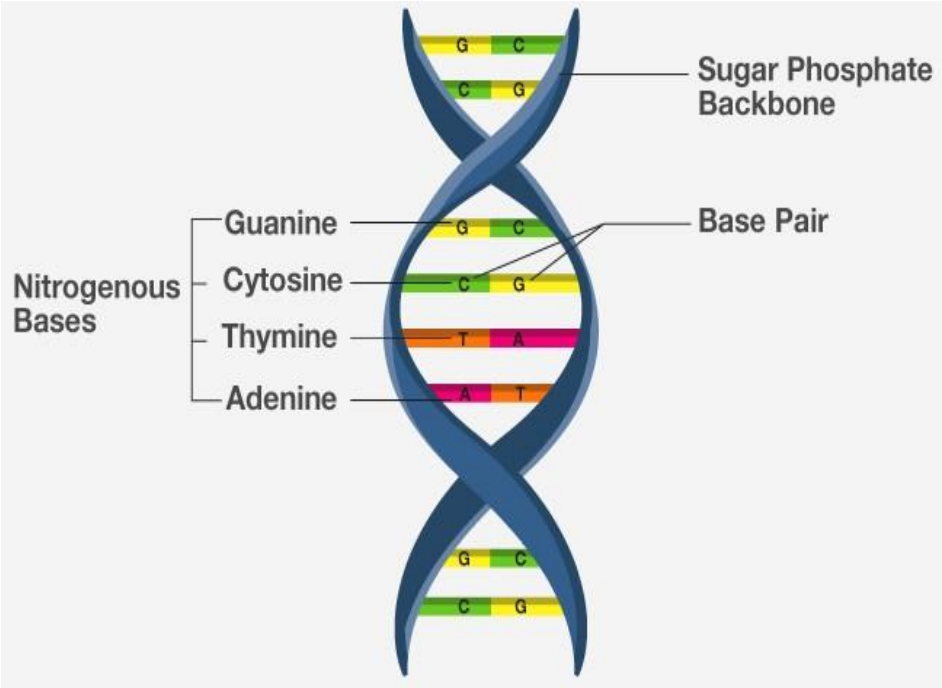
Nucleic acids

They are polynucleotides—that is, long chainlike molecules composed of a series of nearly identical building blocks called nucleotides. Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn attached to a phosphate group. Each nucleic acid contains four of five possible nitrogen-containing bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). A and G are categorized as purines, and C, T, and U are collectively called pyrimidine's. All nucleic

acids contain the bases A, C, and G; T, however, is found only in DNA, while U is found in RNA.

Structure of DNA

Deoxyribonucleic acid, or DNA, is a molecule that contains the instructions an organism needs to develop, live and reproduce. These instructions are found inside every cell, and are passed down from parents to their children.



DNA is made up of molecules called nucleotides. Each nucleotide contains a phosphate group, a sugar group and a nitrogen base. The four types of nitrogen bases are adenine (A), thymine (T), guanine (G) and cytosine (C). The order of these bases is what determines DNA's instructions, or genetic code.

Similar to the way the order of letters in the alphabet can be used to form a word, the order of nitrogen bases in a DNA sequence forms genes, which in the language of the cell, tells cells how to make proteins. Another type of nucleic acid, ribonucleic acid, or RNA, translates genetic information from DNA into proteins.

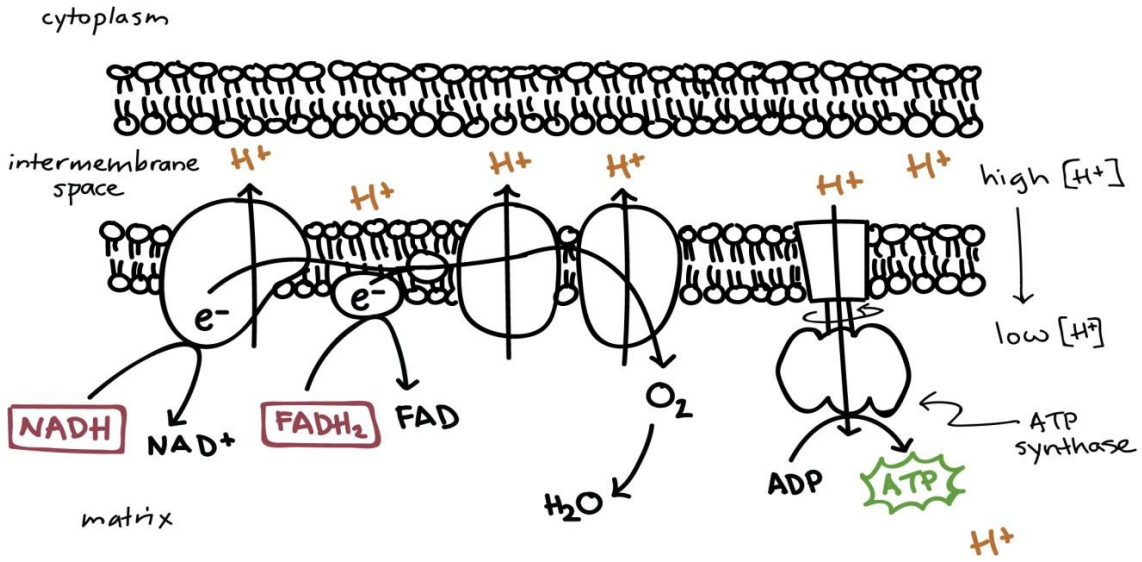
Nucleotides are attached together to form two long strands that spiral to create a structure called a double helix. If you think of the double helix structure as a ladder, the phosphate and sugar molecules would be the sides, while the bases would be the rungs. The

bases on one strand pair with the bases on another strand: adenine pairs with thymine, and guanine pairs with cytosine.

DNA molecules are long — so long, in fact, that they can't fit into cells without the right packaging. To fit inside cells, DNA is coiled tightly to form structures we call chromosomes. Each chromosome contains a single DNA molecule. Humans have 23 pairs of chromosomes, which are found inside the cell's nucleus.

2. Discuss the oxidative phosphorylation. (Nov 2016, May 2017)

"Oxidative phosphorylation is the process of ATP formation, when electrons are transferred by electron carriers from NADH or FADH2 to oxygen"



Oxidative phosphorylation is the final step in cellular respiration. It occurs in the mitochondria. It is linked to a process known as electron transport chain. The electron transport system is located in the inner mitochondrial membrane. The electrons are transferred from one member of the transport chain to another through a series of redox reactions.

Oxidative Phosphorylation Steps:

The major steps of oxidative phosphorylation in mitochondria include:

Delivery of Electrons by NADH and FADH2

Reduced NADH and FADH2 transfer their electrons to molecules near the beginning of the transport chain. After transferring the electrons, they get oxidised to NAD+ and FAD and are utilised in other steps of cellular respiration.

Electron Transport and Proton Pumping

The electrons move from a higher energy level to a lower energy level, thereby releasing energy. Some of the energy is used to move the electrons from the matrix to the intermembrane space. Thus, an electrochemical gradient is established.

Splitting of Oxygen to form Water

The electrons are then transferred to the oxygen molecule which splits into half and uptakes H+ to form water.

ATP Synthesis

The H+ ions pass through an enzyme called ATP synthase while flowing back into the matrix. This controls the flow of protons to synthesize ATP.

Chemiosmosis

Oxidative phosphorylation uses the chemical reactions that release energy to drive a chemical reaction that requires energy. These 2 sets of reactions are coupled and interrelated. The electrons that flow through electron transport chain is an exergonic process and the synthesis of ATP is an endergonic process. These two processes are ingrained within a membrane. As a result, energy will be transmitted from the electron transport chain to ATP synthase by the movement of proteins. This process is termed as chemiosmosis.

Endergonic Process is a chemical reaction in which energy is absorbed. There will be a change in free energy and it is always positive. Exergonic Process is a chemical reaction in which there will be a positive flow of energy from the system to the surrounding environment. Chemical reactions are also considered exergonic when they are spontaneous.

Electron Transport Chain

Most of the biochemical catabolic processes like the citric acid cycle, glycolysis, beta-oxidation, etc. produce the coenzyme NADH. It consists of electrons having high transfer potential.

These reactions release a huge amount of energy on oxidation. These reactions are also known to be the uncontrollable reactions since the energy within the cells is not released at once.

The electrons are separated from the NADH and then passed to the oxygen with a series of enzymes releasing a small amount of energy. All these series of enzymes having complexes is known as electron transport chain.

This chain can be seen in the inner layer or membrane of mitochondria. The salts of succinic acid are also oxidized by this electron chain transport system.

In the case of eukaryotes, the enzymes make use of energy that has been released in the electron transport system from the oxidation of NADH that pumps protons across the inner membrane of the mitochondria. This results in the generation of the electrochemical gradient across the membrane. This can be considered as one of the best examples to understand the concept of oxidative phosphorylation.

3. Describe in detail about the differential centrifugation methods with neat diagram (May 2015, Nov 2017, May 2018)

Sub cellular fractionation using the differential centrifugation method:

- Separation of cellular compartments from one another is an important step for studying a specific intracellular structure or organelle or protein, or to assess possible associations between these macromolecular structures.
- Subcellular fractionation uses one or more of the properties of each compartment, such as buoyant density, surface charge density, size and shape.
- Subcellular fractionation is to provide an enriched source of a protein for further purification, and facilitate the diagnosis of various disease states.

HOMOGENISATION:

- Tissue is typically homogenized in a buffer solution that is isotonic to stop osmotic damage.

- Mechanisms for homogenization include grinding, mincing, chopping, pressure changes, osmotic shock, freeze-thawing, and ultra-sound.
- The samples are then kept cold to prevent enzymatic damage. It is the formation of homogenous mass of cells (cell homogenate or cell suspension). It involves grinding of cells in a suitable medium in the presence of certain enzymes with correct pH, ionic composition, and temperature.
- For example, pectinase which digests middle lamella among plant cells.

FILTRATION:

- Animal tissue however is likely to yield connective tissue which must be removed. Commonly, filtration is achieved either by pouring through gauze or with a suction filter and the relevant grade ceramic filter.

PURIFICATION:

- Purification is achieved by differential centrifugation – the sequential increase in gravitational force results in the sequential separation of organelles according to their density.

CENTRIFUGE:

- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
- The centrifuge works using the sedimentation principle, where the centripetal 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.
- In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

TYPES OF CENTRIFUGE:

1. LOW SPEED CENTRIFUGE:

- Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
- The low-speed centrifuge has a maximum speed of 4000-5000rpm
- These instruments usually operate at room temperatures with no means of temperature control.
- Two types of rotors are used in it,
  1. Fixed angle
  2. Swinging bucket.
- It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

2. HIGH SPEED CENTRIFUGE:

- High-speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber is essential.
- The high-speed centrifuge has a maximum speed of 15,000 – 20,000 RPM
- The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
- Three types of rotors are available for high-speed centrifugation-
  1. Fixed angle
  2. Swinging bucket
  3. Vertical rotors

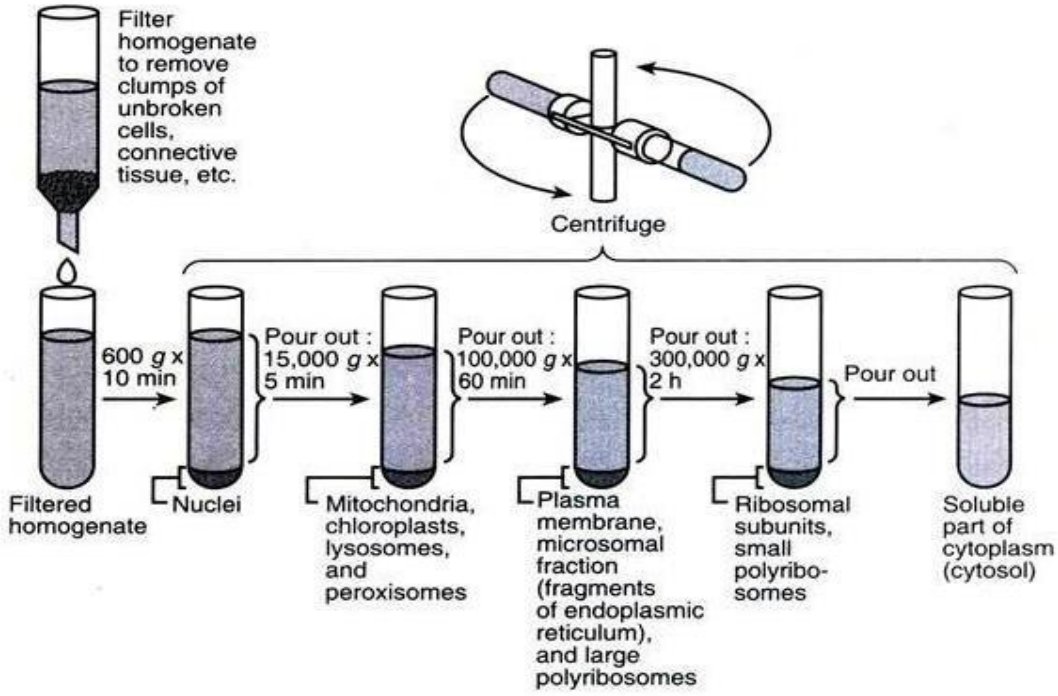
3. ULTRACENTRIFUGE:

- It is the most sophisticated instrument.
- Ultracentrifuge has a maximum speed of 65,000 RPM (100,000's x g).
- Intense heat is generated due to high speed thus the spinning chambers must be refrigerated and kept at a high vacuum.
- It is used for both preparative work and analytical work.
- **Applications of ultra-centrifuge:** The ultra-cetrifuge has found many applications in fields of protein and nucleic acid chemistry. This gives information about
  (a) Determination of molecular weight of biomolecules
  (b) Estimation of purity of macromolecules
  (c) Detection of conformational changes in macromolecules.

DIFFERENTIAL CENTRIFUGATION:

- In differential centrifugation the material to be separated is divided centrifugally into number of fractions by increasing the applied centrifugal field.
- Any type of particle originally present in homogenate may be found in pellet or the supernatant or both fractions, depending upon the time and speed of centrifugation and size and density of particles.
- At the end of each stage the pellet and supernatant are separated and pellet washed several times by re-suspension and re-centrifugation in homogenation medium.
- Initially all particles of homogenate are homogenously distributed throughout the centrifuge tube. During centrifugation particles move down the centrifuge tubes at their respective sedimentation rates and start to form a pellet on the bottom of centrifuge tube.
- Ideally centrifugation is continued enough to pellet all the largest class of particles, the resulting supernatant then being centrifuged at a higher speed to separate medium-sized particles and so on.
- However, since particles of varying sizes and densities were distributed homogenously at the commencement of centrifugation, it is evident that the pellet will not be homogenous but will contain a mixture of all the sedimented components, being enriched with fastest sedimenting particles.
- In the time required for complete sedimentation of heavier particles, some of the lighter and medium sized particles, originally suspended near the bottom of the tube, will also sediment and thus contaminate the fraction.
- Pure preparation of the pellet of the heaviest particle cannot be, therefore, obtained in single centrifugation step. It is only the most slowly sedimenting component of mixture remaining in the supernatant after all the larger particles have been sedimented that can be purified by single centrifugation step.
- The separation achieved by differential centrifugation can be improved by repeated re-suspension and re-centrifugation under similar condition.
- Further centrifugation of the supernatant with gradually increasing centrifugal fields results in sedimentation of intermediate and finally the smallest and least dense particles.

- In spite of its inherent limitations, differential centrifugation is probably the most commonly employed method for isolation of cell organelles from, homogenized tissue.



EXAMPLE - TISSUE LIVER HOMOGENATE:

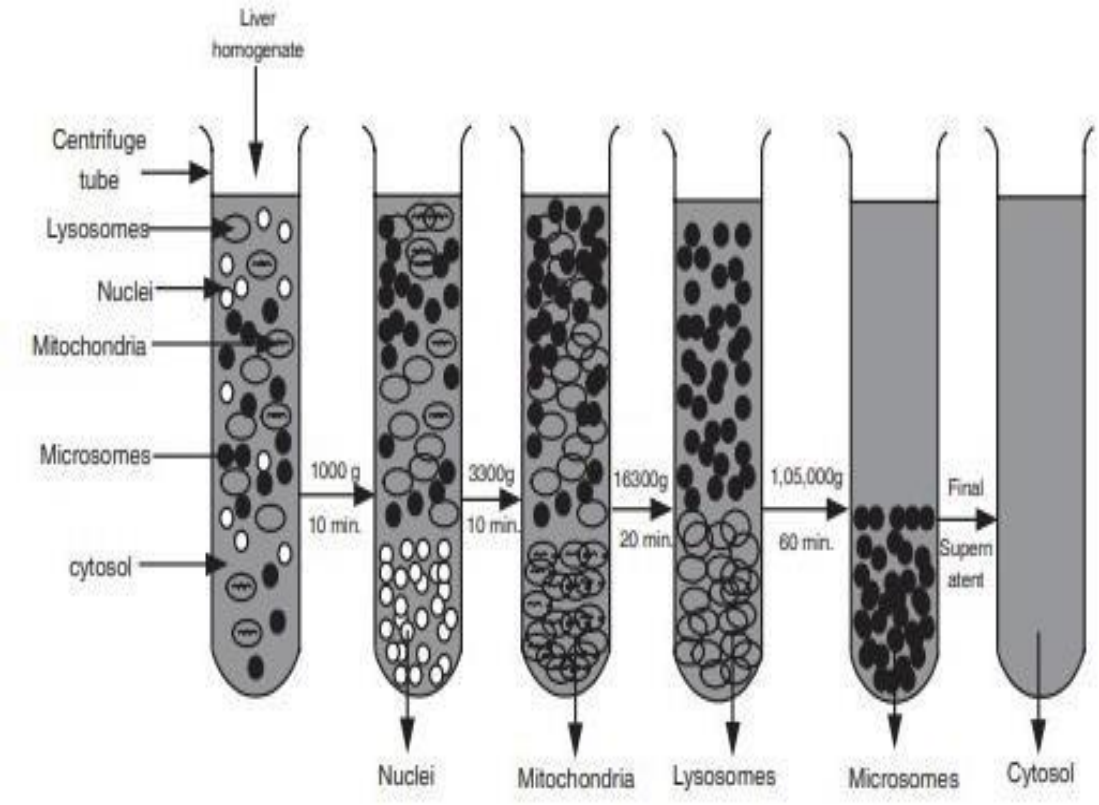
- For example, the sub-cellular organelles (nucleus, mitochondria, lysosomes, microsomes) from a tissue liver homogenate can be isolated by applying these differential centrifugation techniques. The technique has the following steps:
  a) Preparation of liver homogenate – 10% solution in 0.25 molar sucrose.
  b) Centrifugation at 1000 g for 10 minutes.
  c) Isolation of the pellet sedimented which is nucleus.
  d) The supernatant decanted from step (c) is subjected to centrifugation at 3300 g for 10 minutes.
  e) Isolation of the pellet sedimented which contains mitochondria.
  f) The supernatant decanted from step (e) is subjected to centrifugation at 16300 g for 20 minutes.
  g) Isolation of the pellet sedimented which contains lysosomes.

h) The supernatant decanted from step (g) is subjected to centrifugation at 105000 g for 60 minutes.

i) Isolation of the pellet sedimented which contains microsomes.

j) The supernatant obtained in the final step is the cell free cytosol.

- The isolation of sub-cellular organelles is an essential procedure used in many biochemical research laboratories by using these differential centrifugation techniques. A schematic diagram of step-wise isolation sub-cellular organelles from a liver homogenate is given below.



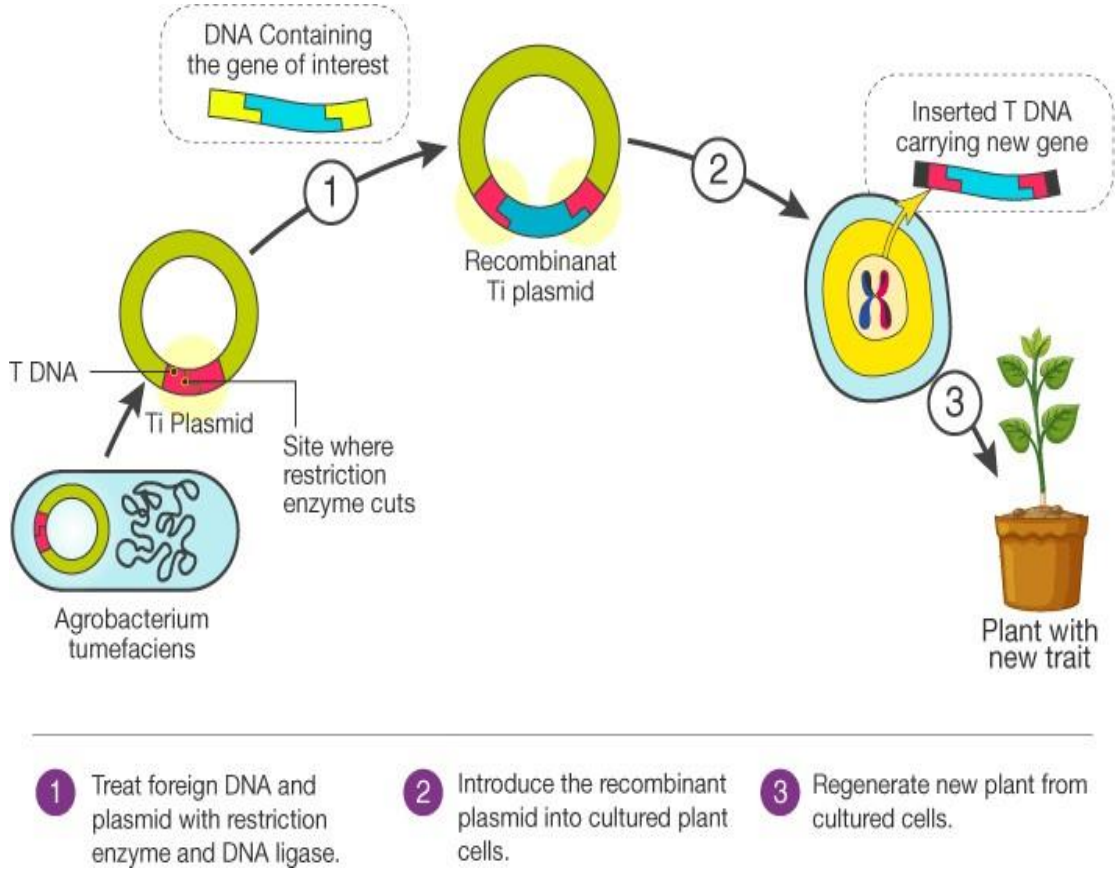
4. Describe the role of r DNA technology and its applications in the current trends. (May 2015, Nov 2015, May 2016, Nov 2017)

Recombinant DNA Technology:

- The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to

as Recombinant DNA Technology. Recombinant DNA technology is popularly known as genetic engineering.

- The recombinant DNA technology emerged with the discovery of restriction enzymes in the year 1968 by Swiss microbiologist Werner Arber.
- Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed.
- Thus the recombinant DNA has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring.



Tools of Recombinant DNA Technology:

- The enzymes which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which

the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases.

- The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points.
- They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.
- The vectors – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism.
- Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have a very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.
- Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.
- There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistic or gene gun, alternate cooling and heating, use of calcium ions, etc.

Process of Recombinant DNA Technology:

- The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step-1: Isolation of Genetic Material.

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

Step-2: Cutting the gene at the recognition sites.

The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestions'.

Step-3: Amplifying the gene copies through Polymerase chain reaction (PCR).

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step-4: Ligation of DNA Molecules.

In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.

Step-5: Insertion of Recombinant DNA Into Host.

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once after the insertion of the recombinant DNA into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

As mentioned in Tools of recombinant DNA technology, there are various ways in which this can be achieved. The effectively transformed cells/organisms carry forward the recombinant gene to the offspring.

Application of Recombinant DNA Technology:

- DNA technology is also used to detect the presence of HIV in a person.
- Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
- Clinical diagnosis – ELISA is an example where the application of recombinant
- Recombinant DNA technology is widely used in Agriculture to produce genetically-modified organisms such as FlavrSavr tomatoes, golden rice rich in proteins, Bt-cotton to protect the plant against ball worms and lot more.

5. Discuss active and passive transport across the biological membrane and explain its functions. (Nov 2015, May 2016, Nov 2018, May 2019, Nov 2019)

Transport of substances across biological membrane

- Membrane transport refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them.
- Cellular membranes possess two key qualities:

- They are **semi-permeable** (only certain materials may freely cross – large and charged substances are typically blocked)
- They are **selective** (membrane proteins may regulate the passage of material that cannot freely cross)

TYPES OF MEMBRANE TRANSPORT

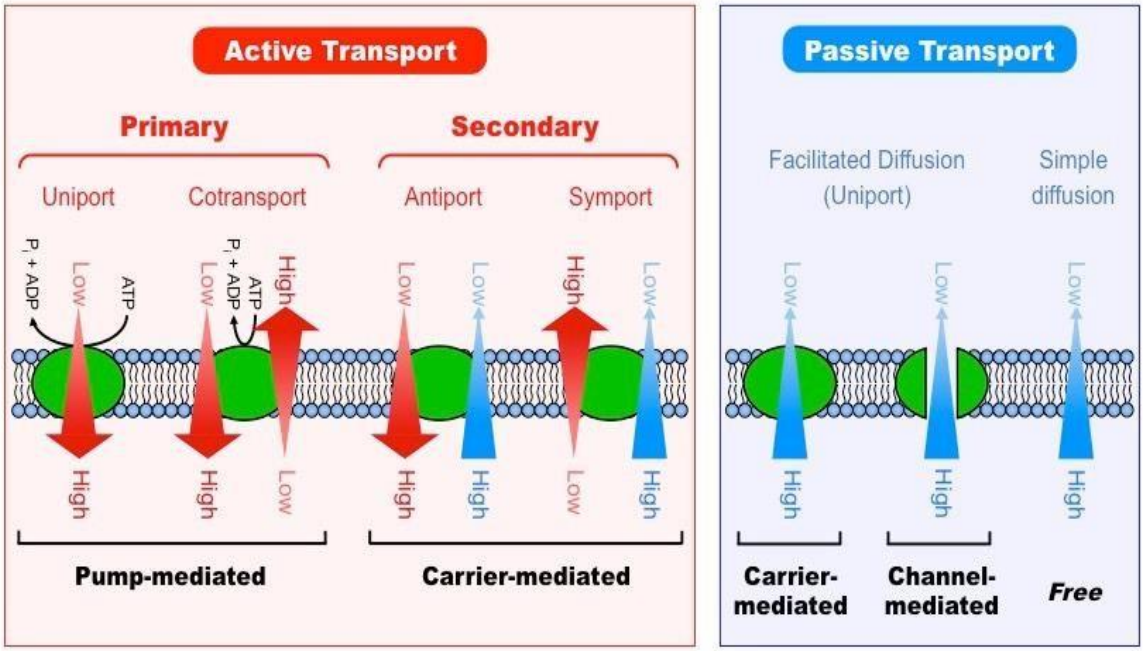
- Movement of materials across a biological membrane may occur either actively or passively.

ACTIVE TRANSPORT

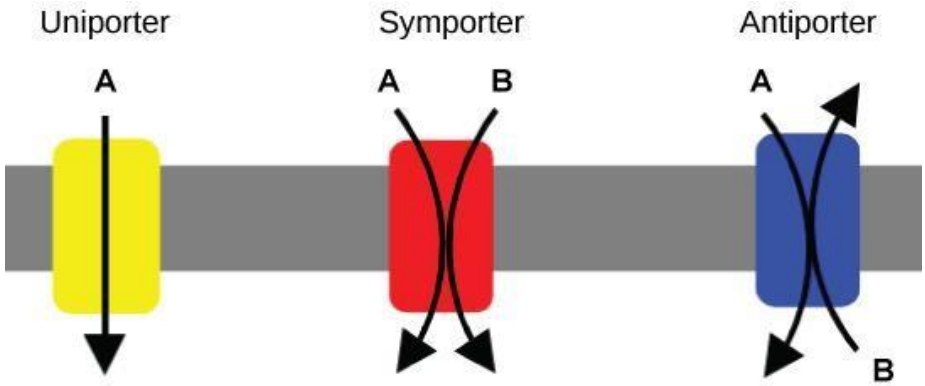
- Active transport involves the movement of materials against a concentration gradient (low concentration ⇒ high concentration)
- Because materials are moving against the gradient, it requires the expenditure of energy (e.g. ATP hydrolysis)
- There are two main types of active transport:
  - Primary (direct) active transport – Involves the direct use of metabolic energy (e.g. ATP hydrolysis) to mediate transport
  - Secondary (indirect) active transport – Involves coupling the molecule with another moving along an electrochemical gradient

Carrier Proteins for Active Transport:

- An important membrane adaption for active transport is the presence of specific carrier proteins or pumps to facilitate movement: there are three types of these proteins or transporters.
  - A uniporter carries one specific ion or molecule.
  - A symporter carries two different ions or molecules, both in the same direction.
  - An antiporter also carries two different ions or molecules, but in different directions.



- All of these transporters can also transport small, uncharged organic molecules like glucose. These three types of carrier proteins are also found in facilitated diffusion, but they do not require ATP to work in that process.



Primary Active Transport:

- In primary active transport, the energy is derived directly from the breakdown of ATP.
- Often times, primary active transport such as that shown below which functions to transport sodium and potassium ions allows secondary active transport to occur.
- The second transport method is still considered active because it depends on the use of energy from the primary transport.

Secondary Active Transport (Co-transport)

- Secondary active transport brings sodium ions, and possibly other compounds, into the cell.
- As sodium ion concentrations build outside of the plasma membrane because of the action of the primary active transport process, an electrochemical gradient is created. If a channel protein exists and is open, the sodium ions will be pulled through the membrane. This movement is used to transport other substances that can attach themselves to the transport protein through the membrane. Many amino acids, as well as glucose, enter a cell this way. This secondary process is also used to store high-energy hydrogen ions in the mitochondria of plant and animal cells for the production of ATP. The potential energy that accumulates in the stored hydrogen ions is translated into kinetic energy as the ions surge through the channel protein ATP synthase, and that energy is used to convert ADP into ATP.

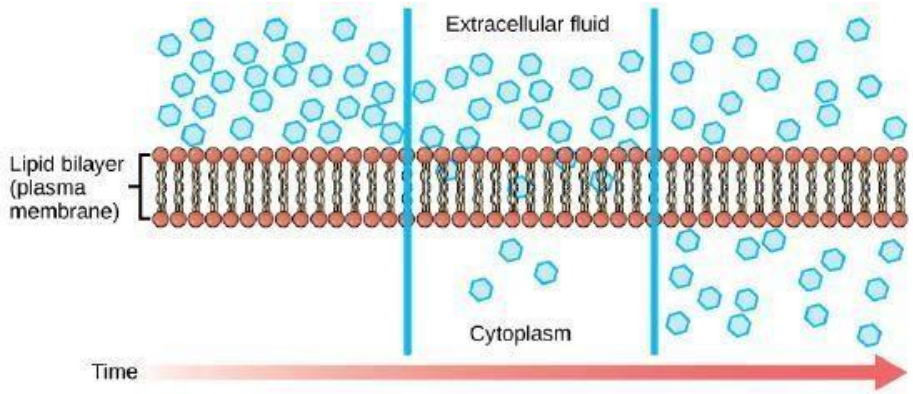
PASSIVE TRANSPORT

- Passive transport involves the movement of material along a concentration gradient (high concentration ⇒ low concentration)
- Because materials are moving down a concentration gradient, it does not require the expenditure of energy (ATP hydrolysis)
- There are three main types of passive transport:
- Simple diffusion – movement of small or lipophilic molecules (e.g. O2, CO2, etc.)
- Osmosis – movement of water molecules (dependent on solute concentrations)
- Facilitated diffusion – movement of large or charged molecules via membrane proteins (e.g. ions, sucrose, etc.)

SIMPLE DIFFUSION

- A single substance tends to move from an area of high concentration to an area of low concentration until the concentration is equal across a space.
- Materials move within the cell's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion.

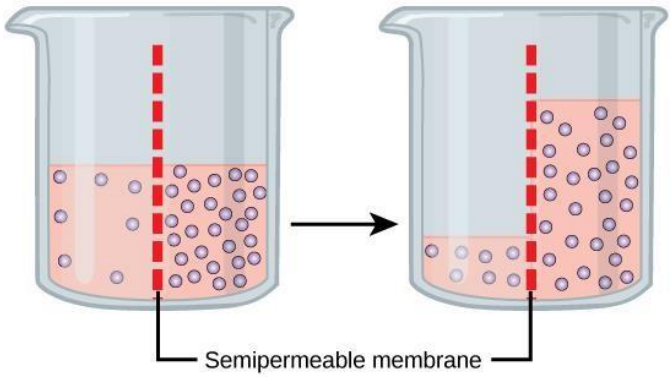
- Diffusion through a permeable membrane moves a substance from an area of high concentration (extracellular fluid, in this case) down its concentration gradient (into the cytoplasm).



- Each separate substance in a medium, such as the extracellular fluid, has its own concentration gradient, independent of the concentration gradients of other materials.
- In addition, each substance will diffuse according to that gradient. Within a system, there will be different rates of diffusion of the different substances in the medium.

OSMOSIS

- Osmosis is the movement of water through a semipermeable membrane according to the concentration gradient of water across the membrane, which is inversely proportional to the concentration of solutes.
- While diffusion transports material across membranes and within cells, osmosis transports only water across a membrane and the membrane limits the diffusion of solutes in the water.
- This diffusion of water through the membrane—osmosis—will continue until the concentration gradient of water goes to zero or until the hydrostatic pressure of the water balances the osmotic pressure.

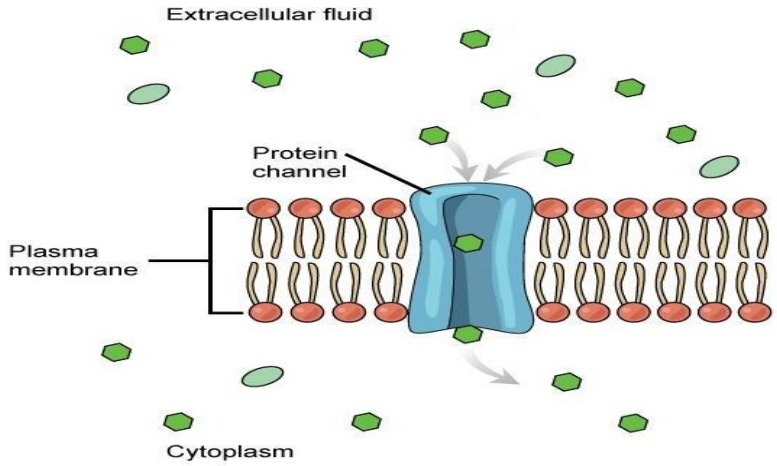


FACILITATED TRANSPORT

- In facilitated transport, also called facilitated diffusion, materials diffuse across the plasma membrane with the help of membrane proteins.
- A concentration gradient exists that allows these materials to diffuse into or out of the cell without expending cellular energy.
- In the case that the materials are ions or polar molecules, compounds that are repelled by the hydrophobic parts of the cell membrane, facilitated transport proteins help shield these materials from the repulsive force of the membrane, allowing them to diffuse into the cell.
- Facilitated transport moves substances down their concentration gradients. They may cross the plasma membrane with the aid of membrane proteins.

MEMBRANE PROTEINS

- Membrane proteins play crucial roles in all organisms, where they serve as, such as membrane receptors, ion channels, GPCR (G protein–coupled receptors) and various kinds of transport proteins.
- Membrane proteins represent about a third of the proteins in living organisms.
- Based on their structure, there are main three types of membrane proteins:
- 1. Integral membrane protein - permanently anchored to the membrane
- 2. Peripheral membrane protein - temporarily anchored to the membrane
- 3. Lipid anchored protein - anchored to lipid bilayer



SODIUM - POTASSIUM PUMP

- One of the most important pumps in animal cells is the sodium-potassium pump (Na+-K+ ATPase), which maintains the electrochemical gradient (and the correct concentrations of Na+ and K+) in living cells. The sodium-potassium pump moves K+ into the cell while moving Na+ out at the same time, at a ratio of three Na+ for every two K+ ions moved in. The Na+-K+ ATPase exists in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either sodium or potassium ions. The process consists of the following six steps.

1. With the enzyme oriented towards the interior of the cell, the carrier has a high affinity for sodium ions. Three ions bind to the protein.
2. ATP is hydrolyzed by the protein carrier and a low-energy phosphate group attaches to it.
3. As a result, the carrier changes shape and re-orients itself towards the exterior of the membrane. The protein's affinity for sodium decreases and the three sodium ions leave the carrier.
4. The shape change increases the carrier's affinity for potassium ions, and two such ions attach to the protein. Subsequently, the low-energy phosphate group detaches from the carrier.
5. With the phosphate group removed and potassium ions attached, the carrier protein repositions itself towards the interior of the cell.

6. The carrier protein, in its new configuration, has a decreased affinity for potassium, and the two ions are released into the cytoplasm. The protein now has a higher affinity for sodium ions, and the process starts again.

- For every three ions of sodium that move out, two ions of potassium move in. This results in the interior being slightly more negative relative to the exterior.
- This difference in charge is important in creating the conditions necessary for the secondary process. The sodium-potassium pump is, therefore, an **electrogenic pump** (a pump that creates a charge imbalance), creating an electrical imbalance across the membrane and contributing to the membrane potential.

BM T36- BIOCHEMISTRY

UNIT-II

2 MARKS:

1. Define apoenzyme. (Nov 2016)

A protein that forms an active enzyme system by combination with a coenzyme and determines the specificity of this system for a substrate. A cofactor required by an Apoenzyme can be a metal ion, e.g. Mg^{2+}, Fe^{3+}, etc. or an organic molecule called coenzyme such as NAD^{+}, $NADP^{+}$, FAD^{2+}, etc.

2. Write the assay of hormone. (Nov 2016)

A hormonal assay test is performed to give an indication of metabolic processes and conditions, or 'hormone imbalance'.

Methods of hormonal assay may be:

1. Bioassay
2. Chemical Assay
3. Microbiological Assay
4. Immuno Assay

3. What are coenzymes? (May 2015, May 2016)

Coenzymes are organic nonprotein molecules that bind with the protein molecule (apoenzyme) to form the active enzyme (holoenzyme). Coenzymes are often broadly called cofactors, but they are chemically different. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme.

4. Name four diagnostic enzymes. (May 2015)

- Acid Phosphatase.
- Alanine Aminotransferase.
- Alkaline Phosphatase.
- Amylase.

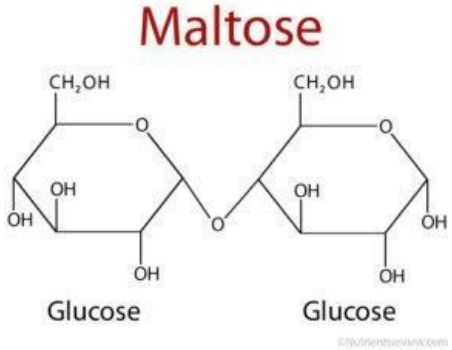
5. Define biocatalyst. (Nov 2015)

Biocatalysts are substances (enzyme or hormone) that activates or speeds up biochemical reactions. e.g.digestive enzymes like pepsin ,trypsin etc.

6. Short note on insulin hormone. (Nov 2015)

Insulin is a hormone that lowers the level of glucose (a type of sugar) in the blood. It's made by the beta cells of the pancreas and released into the blood when the glucose level goes up, such as after eating. Insulin helps glucose enter the body's cells, where it can be used for energy or stored for future use.

7. Draw the structure of maltose. (May 2016)



8. Write a note on optimum pH. (May 2017)

Optimum pH refers to the pH resulting in maximal activity of a particular enzyme. Differing pH levels affect the shape of an enzyme. Each enzyme has an optimal pH at which the enzyme works best. Enzymes in the intestine for instance work best at pH of 7.5 (therefore, the optimum pH).

9. What are diagnostic enzymes? (May 2017)

Diagnostic enzymes refers to the enzymes that are used directly or as components of the assay system for the determination of number of substances.

10. What is an "enzyme active site"? (Dec 2017, Dec 2019)

The active site is region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of amino acid residues that form temporary bonds with the substrate and residues that catalyze a reaction of that substrate.

11. What is the function of a hormone receptor? (Dec 2017)

Hormone receptors are proteins that bind hormones. Once bound, the hormone/receptor complex initiates a cascade of cellular effects resulting in some modification of physiology and/or behavior. Hormones usually require receptor binding to mediate a cellular response.

12. Write the diagnostic importance of CPK. (May 2018)

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme (EC 2.7. 3.2) expressed by various tissues and cell types. CK catalyses the conversion of creatine and uses adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). When a muscle is damaged, CPK leaks into the bloodstream. Finding which specific form of CPK is high helps determine which tissue has been damaged. This test may be used to: Diagnose heart attack.

13. What are hormones? (May 2018, May 2019, Dec 2019)

Hormones are molecules produced by the endocrine system that send messages to various parts of the body. They help regulate your body's processes, like hunger, blood pressure, and sexual desire. While hormones are essential to reproduction, they are fundamental to all the systems of your body.

14. Define specific activity and international unit of enzyme activity. (Dec 2018)

- Specific enzyme activity (usually stated simply as 'specific activity') is the number of enzyme units per ml divided by the concentration of protein in mg/ml. Specific activity values are therefore quoted as units/mg or nmol/min/mg.
- The enzyme unit, or international unit for enzyme (symbol U, sometimes also IU) is a unit of enzyme's catalytic activity. 1 U (μmol/min) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

15. Name any two cardiac market enzymes. (Dec 2018)

Myoglobin, troponin and creatine kinase.

16. Write down the chemical nature of an enzyme. (May 2019)

- All known enzymes are proteins. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds.
- Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000.
- Exception non protein enzyme is ribozyme.

11 MARKS:

1. What is enzyme? Describe the properties of enzyme. (Nov 2016, May 2015, May 2017, Dec 2017)

- **Enzyme** is a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.
- In this type of chemical reaction, the starting molecules are called substrates.
- The enzyme interacts with a substrate, converting it into a new product.
- Most enzymes are named by combining the name of the substrate with the -ase suffix (e.g., protease, urease).
- Nearly all metabolic reactions inside the body rely on enzymes in order to make the reactions proceed quickly enough to be useful.
- Chemicals called activators can enhance enzyme activity, while inhibitors decrease enzyme activity. The study of enzymes is termed enzymology.

PROPERTIES OF ENZYME:

1. Proteinous nature:

Nearly all enzymes are proteins although some catalytically active RNA molecules have been identified.

2. Colloidal nature:

In the protoplasm, enzymes exist as hydrophilic colloids. Due to colloidal nature, they are isolated by dialysis.

3. Substrate specificity:

- A given enzyme only catalyzes one reaction or a similar type of reaction. For example, maltase acts only on maltose while pancreatic lipase acts in a variety of fats.
- Sometimes, different enzymes may act on the same substrate to produce different end products. The substrate specificity of enzyme is based on amino acids sequence in the catalytic site as well as the optical isomeric form of the substrate.

4. Catalytic properties:

(i) Enzyme require in small concentration for any chemical change,

(ii) They don't initiate the catalysis but accelerate the rate of catalysis by lowering the activation energy,

(iii) They remain unchanged at the end of reaction,

(iv) Their presence don't alter the properties of end products,

(v) Enzymes accelerate the forward or reverse reactions to attain the equilibrium but don't shift the equilibrium,

5. Turn over Number (Enzyme efficiency):

- It is the number of substrate molecules changed per unit of time per enzyme.
- Typical turn over number varies form 10^2 to 10^3 $sec^{-1.}$
- For example the turn over number for sucrase is 10^4, that means, one sucrase molecule convert 10,000 sucrose into products.
- Similarly, it is 36 million for carbonic anhydrase (fastest enzyme) and 5 million for catalase (2^{nd} fastest enzymes).
- Enzyme efficiency is very low in Lysozyme.

6. Sensitivity:

- Enzymes are highly sensitive to change in pH, temperature and inhibitors. Enzymes work best at a narrow range of condition called optimum.

(i) Temp:

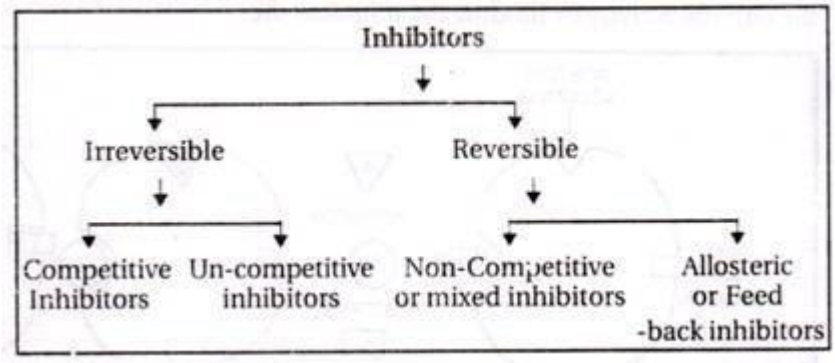
- The optimum temp of enzymes is 20-35°C. They become inactivated at very low temperature and denatured (destroyed) at very high temp i.e. greater than 45°C.
- Low molecular weight enzymes are comparatively more heat stable.
- In archebacterium Pyrococcus furious, the optimum temperature of hydrogenise is greater than 95°C. This heat-stable enzyme enables Pyrococcus to grow at 100°C.

(ii) pH:

- The optimum pH of most endoenzyme is pH 7.0 (neutral pH). However, digestive enzymes can function at different pH.
- For example, salivary amylase act best at pH 6.8, pepsin act best at pH2 etc. Any fluctuation in pH from the optimum causes ionization of R-groups of amino acids which decrease the enzyme activity.
- Sometime a change in pH causes the reverse reaction, e.g. at pH 7.0 phosphorylase break down starch into glucose 1- phosphate while at pH5 the reverse reaction occurs.

(iii) Inhibitors:

- Enzymes are also sensitive to inhibitors. Inhibitors are any molecules like cellular metabolites, drugs or toxins which reduce or stop enzyme activity. Enzyme inhibitors are of 2 types i.e. reversible and irreversible.



2. Explain the various methods adapted for hormonal assay and a note on its significance. (May 2015, Dec 2015, May 2016, Dec 2017, Dec 2019)

ASSAY METHODS:

- Methods of hormonal assay may be:
    1. Bioassay
    2. Chemical Assay
    3. Microbiological Assay
    4. Immuno Assay
- The sensitivity of these methods was low, and large amounts of samples were needed.

BIOASSAY METHOD:

- A bioassay is an analytical method to determine concentration or potency of a substance by its effect on living cells or tissues.
- Bioassays are quantitative biological assays used to estimate the potency of agents by observing their effects on living animals (in vivo) or tissue/cell culture systems (in vitro).
- A bioassay experiment can either be qualitative or quantitative, direct or indirect.
- Bioassay is used to detect biological hazards or give a quality assessment of a mixture.
- Bioassay is often used to monitor water quality and also sewage discharge and its impact on surrounding.
- It is also used to assess the environmental impact and safety of new technologies and facilities.

- Bioassay is a biochemical test to estimate the relative potency of a sample compound to a standard compound.
- Typical bioassay involves a *stimulus* (ex. drugs) applied to a *subject* (ex. animals, tissues, plants) and a *response* (ex. death) of the subject is triggered and measured.
- The intensity of stimulus is varied by doses and depending on this intensity of stimulus, a change/response will be followed by a subject.

BIOASSAY CLASSIFICATION:

1. Direct assay

- - The stimulus/standard sufficiently produces measurable and specific response. The response must be clear, easily recognized, and directly measured.

2. Indirect assay based on quantitative response

- - The relationship between the dose and the response is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately.

3. Indirect assay based on quantal response

- - The assay involves 'all or none' response (ex. life or death). The response is produced by threshold effect.

BIOASSAY EXAMPLES:

1. ELISA (Enzyme-linked immunosorbent assay)

- - quantitative analytical method that measures absorbance of color change from antigen-antibody reaction (ex. Direct, indirect, sandwich, competitive). ELISA is used to measure variety of substances in human body from cortisol levels for stress to glucose level for diabetes.

2. Home pregnancy test

- Home pregnancy test involves ELISA to detect the increase of human chorionic gonadotropin (hCG) during pregnancy.

3. HIV test

- HIV test also uses indirect ELISA to detect HIV antibody caused by infection.

CHEMICAL ASSAY:

- A chemical assay refers to the analysis of a sample material, called analyte, using a set of chemical procedures.
- Qualitative- extraction, distillation, precipitation and other methods that determine physicochemical properties.

- Quantitative- volume or weight of the substance.

PHYSIOCHEMICAL ASSAY TECHNIQUES:

- 1. Photometry
- 2. Colorimetry
- 3. Spectrophotometry
- 4. Fluorimetry
- 5. Flame photometry
- 6. Chromatography
- 7. Column chromatography
- 8. Paper chromatography
- 9. Thin layer chromatography
- 10.Gas chromatography
- 11.High performance liquid chromatography

MICROBIOLOGICAL ASSAY

- Based upon a comparison of the inhibition of growth of micro-organisms by measured concentration of the antibiotics to be examined with that produced by known concentrations of a standard preparation of the antibiotic having a known activity
- 1. The cylinder-plate (or cup-plate) method
- 2. The turbidimetric (or tube assay) method

IMMUNOASSAY METHOD:

- An immunoassay is a test that uses antibody and antigen complexes as a means of generating measurable result
- An immunoassay is an analytical method which uses antibodies as reagents to quantitate or detect specific analytes

PREREQUISITES:

- Antigen
- Antibody
- Analyte
- Label

PRICIPLE AND TYPES:

- Immunoassay uses antibody and antigen complexes as a means of generating measurable result.

- TYPES:

1. Competitive immunoassays.
2. Non-competitive immunoassays.

COMPETITIVE FORMAT:

- In competitive formats, unlabelled analyte (usually antigen) in the test sample is measured by its ability to compete with labeled antigen in the immunoassay.
- The unlabeled antigen blocks the ability of the labeled antigen to bind because that binding site on the antibody is already occupied.

ONE STEP COMPETITIVE FORMAT:

- Both the labeled antigen reagent(Ag*) and the unlabeled specimen (or test sample analyte) compete for a limited amount of antibody.

TWO STEP COMPETITIVE FORMAT:

- The antibody concentration of the reaction solution is present in excess in comparison to the concentration of antigen
- Antibody reagent is first incubated with specimen containing antigens of interest; then in the second step, labeled antigen is added
- Improved assay sensitivity compared to one step assay formats

NON COMPETITIVE FROMAT:

- "Sandwich" assay because analyte is bound (sandwiched) between two highly specific antibody reagents
- Provides highest level of assay sensitivity and specificity
- Applied to the measurement of critical analytes such as cardiac and hepatitis markers.

HOMOGENEOUS AND HETEROGENEOUS IMMUNO ASSAY METHOD

- Immunoassay methods that require separation of bound Ab-Ag* complex are referred to as heterogeneous immunoassays. Those that do not require separation are referred to as homogeneous immunoassays.
- Homogeneous methods have been generally applied to the measurement of small analytes such as abused and therapeutic drugs.

ELISA (Enzyme-Linked Immunosorbent assay):

- Enzyme immunoassay
- Both qualitative and quantitative measurement of Ag-Ab binding
- Direct, Competitive, sandwich ELISA- Ag measurements

- Abs- indirect ELISA
- Advantages (sensitivity, ease of handling multiple samples) without the disadvantages of dealing with radioactivity (like in RIA)

PREREQUISITES:

- Purified antigen (to detect or quantify antibody).
- Purified antibody (detect or quantify antigen).
- Standard solutions (positive and negative controls).
- Sample to be tested.
- Microtiter dishes: plastic trays with small wells in which the assay is done.
- Wash fluid (buffer).
- Enzyme-labeled antibody and enzyme substrate.
- ELISA reader (spectrophotometer) for quantitative measurements.

PERFORMING THE TEST:

- The tubes are filled with the antigen solution (e.g., urine) to be assayed. Any antigen molecules present bind to the immobilized antibody molecules.
- The antibody-enzyme conjugate is added to the reaction mixture. The antibody part of the conjugate binds to any antigen molecules that were bound previously, creating an antibody-antigen-antibody "sandwich".
- After washing away any unbound conjugate, the substrate solution is added.
- After a set interval, the reaction is stopped (e.g., by adding 1 N NaOH) and the concentration of colored product formed is measured in a spectrophotometer. The intensity of color is proportional to the concentration of bound antigen.

ISOTOPIC IMMUNOASSAY:

- Based on competition for antibody between radioactive indicator antigen and unlabelled antigen in test sample.
- Increase in count of unlabeled antigen in test sample decrease the labeled antigen in bound.
- The concentration of the test antigen can be determined by comparison with a standard calibrated curve with known concentration of purified antigen.

NON ISOTOPIC IMMUNOASSAY:

- Differ from isotopic immunoassay in:-

1. Type of label used
2. Means of end point detection

3. Possibility of eliminating a separation test

- Two types of nonisotopic immunoassay are:-

3. What are clinical important enzymes and explain with examples? (Nov 2015, Dec 2019, May 2019)

DIAGNOSTIC ENZYMES

- Enzymes are the preferred markers in various disease states such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc.
- They provide insight into the disease process by diagnosis, prognosis and assessment of response therapy.
- Certain tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed.
- The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage.
- The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance.
- This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood.
- Enzymatic diagnosis is a method to diagnose diseases by measuring the content and changes of certain substances in the body, or through the changes of the original enzyme activity in the body.
- Commonly assayed enzymes are the amino transferases:
- alanine transaminase, ALT (sometimes still referred to as serum glutamate-pyruvate aminotransferase, SGPT)
- aspartate aminotransferase, AST (also referred to as serum glutamate-oxaloacetate aminotransferase, SGOT)
- lactate dehydrogenase, LDH
- creatine kinase, CK (also called creatine phosphokinase, CPK)
- gamma-glutamyl transpeptidase, GGT.

ALKALINE PHOSPHATASE:

- An alkaline phosphatase (ALP) test measures the amount of ALP in your blood.

- ALP is an enzyme found throughout the body, but it is mostly found in the liver, bones, kidneys, and digestive system.
- When the liver is damaged, ALP may leak into the bloodstream.
- High levels of ALP can indicate liver disease or bone disorders.
- Other names: ALP, ALK, PHOS, Alkp, ALK PHOS.
- An alkaline phosphatase test is used to detect diseases of the liver or bones.

CREATININE KINASE:

- Creatine kinase (CK) is an enzyme found in the heart, brain, skeletal muscle, and other tissues.
- Increased amounts of CK are released into the blood when there is muscle damage.
- This test measures the amount of creatine kinase in the blood.
- The small amount of CK that is normally in the blood comes primarily from skeletal muscles.
- Any condition that causes muscle damage and/or interferes with muscle energy production or use can cause an increase in CK.
- For example, strenuous exercise and inflammation of muscles, called myositis, can increase CK as can muscle diseases (myopathies) such as muscular dystrophy.
- Rhabdomyolysis, an extreme breakdown of skeletal muscle tissue, is associated with significantly elevated levels of CK.

ALANINE AMINOTRANSFERASE:

- Alanine aminotransferase (ALT) is an enzyme found primarily in the liver and kidney.
- It was originally referred to as serum glutamic pyruvic transaminase (SGPT).
- Normally, a low level of ALT exists in the serum. ALT is increased with liver damage and is used to screen for and/or monitor liver disease.
- Alanine aminotransferase (ALT) is usually measured concurrently with AST as part of a liver function panel to determine the source of organ damage.
- The reference range for ALT is 20-60 IU/L.

ASPARTATE AMINOTRANSFERASE:

- Aspartate aminotransferase (AST) is an enzyme found in cells throughout the body but mostly in the heart and liver and, to a lesser extent, in the kidneys and muscles.
- In healthy individuals, levels of AST in the blood are low.
- When liver or muscle cells are injured, they release AST into the blood.

- This makes AST a useful test for detecting or monitoring liver damage or related infections and some side effects of medications.
- The test is most useful in detecting liver damage due to hepatitis, drugs toxic to the liver, cirrhosis, or alcoholism.

SORBITOL DEHYDROGENASE:

- Sorbitol dehydrogenase is an enzyme in carbohydrate metabolism converting sorbitol, the sugar alcohol form of glucose, into fructose.
- It has recently been reported that serum levels of the enzyme sorbitol dehydrogenase (SDH) would be a more accurate indicator of liver disease due to the high concentration of this enzyme in the liver.

LACTATE DEHYDROGENASE:

- This test measures the level of lactate dehydrogenase (LDH), also known as lactic acid dehydrogenase, in your blood or sometimes in other body fluids.
- LDH is a type of protein, known as an enzyme.
- LDH plays an important role in making your body's energy. It is found in almost all the body's tissues, including those in the blood, heart, kidneys, brain, and lungs.
- When these tissues are damaged, they release LDH into the bloodstream or other body fluids. If your LDH blood or fluid levels are high, it may mean certain tissues in your body have been damaged by disease or injury.
- Other names: LD test, lactic dehydrogenase, lactic acid dehydrogenase

CHOLIN ESTERASE:

- Cholinesterases are enzymes that are involved in helping the nervous system to function properly.
- There are two separate cholinesterase enzymes in the body:
- (1) acetylcholinesterase, found in red blood cells as well as in the lungs, spleen, nerve endings, and the gray matter of the brain, and
- (2) pseudocholinesterase (butyrylcholinesterase), found in the serum as well as the liver, muscle, pancreas, heart, and white matter of the brain.
- Cholinesterase tests measure the activity of these enzymes.

LIPASE:

- A lipase test measures the level of a protein called lipase in your blood.
- Lipase helps your body absorb fats. It’s released by the pancreas, a long, flat gland between your stomach and spine.

- When your pancreas is inflamed or injured, it releases more lipase than usual.
- A lipase test may also be referred to as a serum lipase or LPS.

AMYLASE:

- Amylase is a digestive enzyme that helps the body break down carbohydrates.
- Both the salivary glands and the pancreas produce amylase. Several different medical conditions can affect amylase levels in the blood.
- An amylase blood test measures the amount of amylase in a person's blood.
- Abnormal levels of amylase may indicate pancreatitis or another problem with the pancreas.

GLUTAMYL TRANSFERASE:

- A gamma-glutamyl transferase (GGT) test measures the amount of GGT in the blood.
- GGT is an enzyme found throughout the body, but it is mostly found in the liver.
- When the liver is damaged, GGT may leak into the bloodstream.
- High levels of GGT in the blood may be a sign of liver disease or damage to the bile ducts.
- Bile ducts are tubes that carry bile in and out of the liver. Bile is a fluid made by the liver. It is important for digestion.
- A GGT test can't diagnose the specific cause of liver disease. So it is usually done along with or after other liver function tests, most often an alkaline phosphatase (ALP) test.
- ALP is another type of liver enzyme. It's often used to help diagnose bone disorders as well as liver disease.
- Other names: gamma-glutamyl transpeptidase, GGTP, Gamma-GT, GTP

TRYPSIN:

- This test measures levels of trypsin in your blood to see if you have pancreatitis.
- Your pancreas is an organ in your belly. It lies behind your stomach.
- One of its jobs is to make enzymes that go into your small intestine to help you digest foods.
- The pancreas can become inflamed (pancreatitis). This can happen suddenly (acute pancreatitis), or grow worse over a longer time (chronic pancreatitis).
- During acute pancreatitis, enzymes from your pancreas can escape into your blood.
- One of these enzymes is trypsin. It's made from trypsinogen.

- Trypsinogen turns into trypsin in the small intestine, and the names of the two are sometimes used interchangeably.

GLUTATHIONE PEROXIDASE:

- Glutathione, a tripeptide consisting of glutamic acid - cysteine glycine, is the substrate for glutathione peroxidase (GSHPx), which protects cytosolic organelles from the damaging effects of the hydroperoxides formed by normal aerobic metabolism.
- It has been shown that low levels of glutathione peroxidase as measured in the serum may be a contributing factor to vitiligo.
- Lower plasma glutathione peroxide levels were also observed in patients with type 2 diabetes with macroalbuminuria and this was correlated to the stage of diabetic nephropathy.

ACID PHOSPHATASE:

- It is an enzyme primarily found in the body and also in the prostate gland of males.
- It is said that the acid phosphatase enzyme found in the prostate gland of the males is 100 times more than what is found in the body.
- When cancer in the gland rises, the level of the phosphatase enzyme also rises simultaneously. This is used as a basis to diagnose the level of cancer.
- The enzyme present in the prostate gland is referred to as prostatic acid phosphatase.
- It is not only used for diagnosis but also to check for the effectiveness of the treatment.

ALDOLASE:

- Your body converts a form of sugar called glucose into energy. This process requires a number of different steps. One important component in the process is an enzyme known as aldolase.
- Aldolase can be found throughout the body, but concentrations are highest in skeletal muscle and the liver.
- Although there's not a direct correlation, high aldolase levels in the blood can occur if there's damage to your muscle or liver.
- Elevated aldolase is usually a sign of muscle or liver damage. For example, muscle damage from a heart attack releases aldolase in large quantities. Liver damage, such as hepatitis or cirrhosis, raises aldolase levels as well.

GLUTAMATE DEHYDROGENASE:

- Glutamate dehydrogenase (GDH) is a hexameric enzyme that catalyzes the reversible conversion of glutamate to α-ketoglutarate and ammonia while reducing $NAD(P)^+$ to NAD(P)H. It is found in all living organisms serving both catabolic and anabolic reactions.
- GDH is a enzyme if this is identified in your faeces it means you are colonised with the germ (bacteria) called Clostridium difficile (abbreviated 'C.diff' or 'CDT').
- Clostridium difficile are bacteria (germs) which can be present as part of the 'normal' bacteria in the bowel of up to 3% of healthy adults.

HYDROXYBUTRATE DEHYDROGENASE:

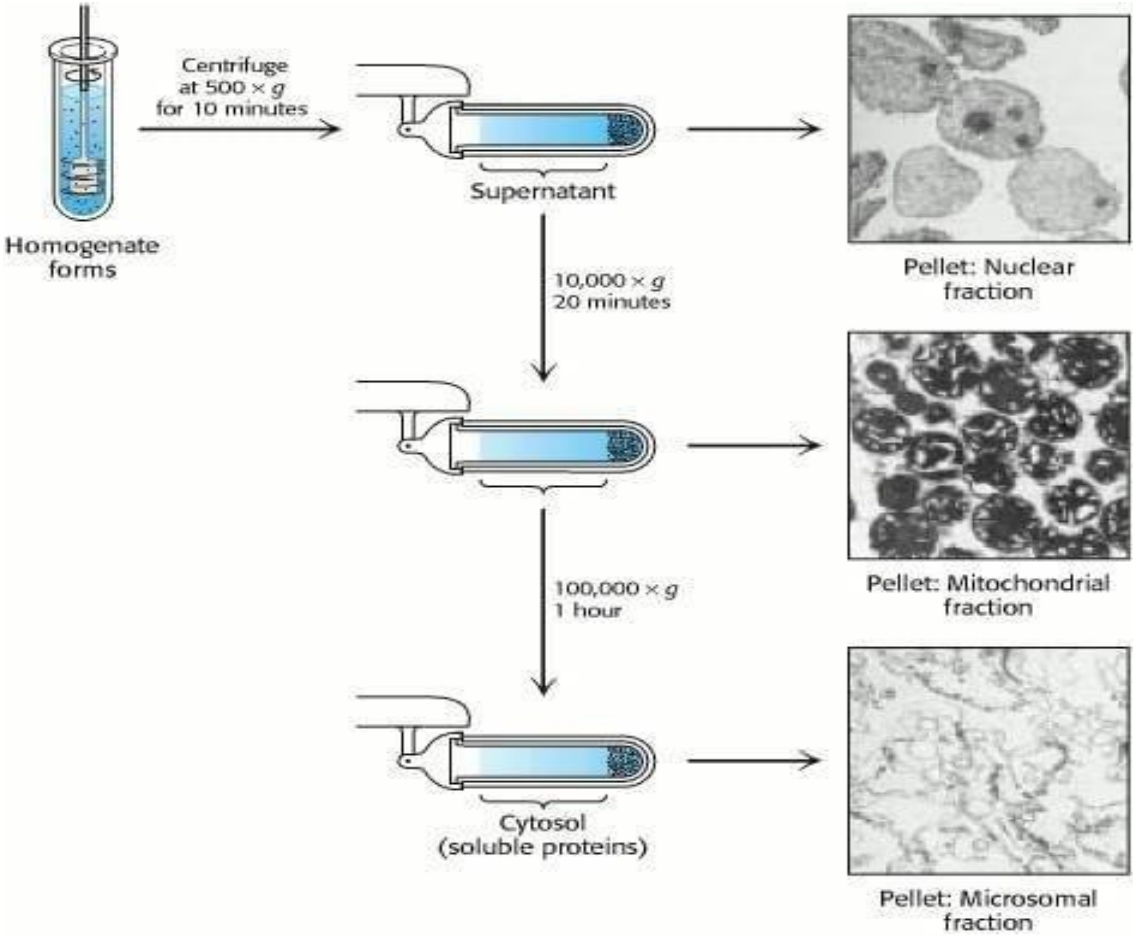
- α-Hydroxybutyrate dehydrogenase (α-HBDH) occurs in higher levels in heart muscle tissue and therefore is more sensitive and specific in diagnosis of myocardial infarction.
- For differentiation between liver and heart diseases the HBDH/LDH ratio can be calculated. A high ratio can be measured in myocardial infarction while a low HBDH/LDH ratio indicates parenchymal liver diseases.
- Normal ratio: HBDH/LDH = 0.63 – 0.81
- Myocardiac lesion: HBDH/LDH > 0.9
  Liver damage: HBDH/LDH < 0.6

4. Discuss about the isolation techniques of enzymes in detail. (May 2016, May 2018)

ISOLATION TECHNIQUES

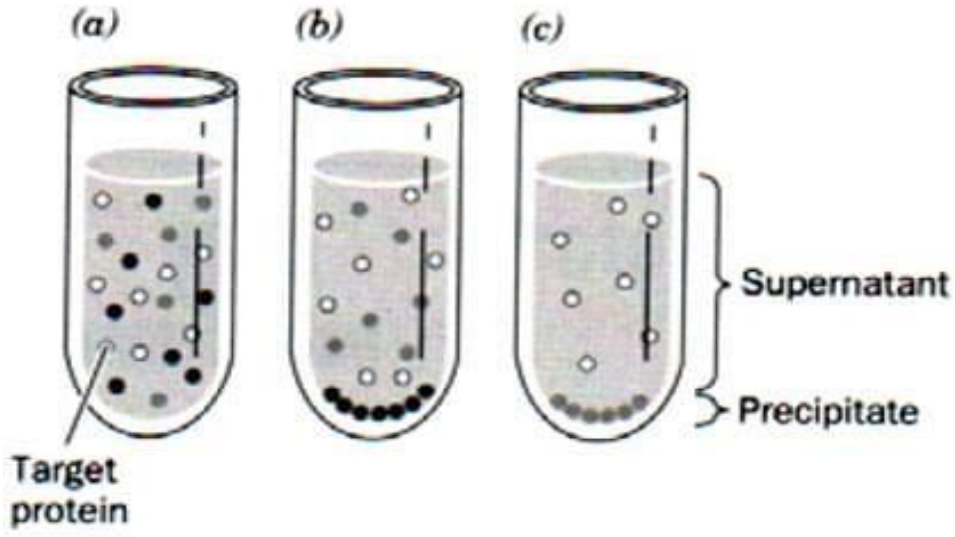
- Initial characterizations of an enzyme in a mixture or sample matrix is practical, such as activity measurement and preliminary quantification, more fundamental knowledge relies on more advanced studies of the enzyme, which can only performed with pure enzyme samples. Pure enzymes also mean easier assays with less interferences.
- Some analysis methods, such as crystallography, are sensitive to sample purity and give desired results only with the highest samples purity.
- In large scale production for industrial applications, enzyme purification is directly related to product quality, in addition to regulatory requirements.

- Therefore, enzyme purification must be thoroughly considered and cautiously operated for both research and production purposes. However, the task is not straightforward.
- Many factors could change the efficiency, the yield, and stability of activity during purification, and the effects of these factors vary largely from one enzyme to another.
- Almost all samples need to be prepared before the actual purification. For the enzymes from cell sources, they need to be fractionated into components before purification.
- The first step usually involves homogenization of cells, which disrupt the cell wall to release the enzyme into the homogenate, along with other components.
- Depending on the cell type, the homogenization could be easy as in the case of mammalian tissue without rigid cell wall, or it may need harsher conditions such as abrasion, freezing, and high pressure due to the rigid cell wall of the plant tissue.
- Sometimes, additional hydrolytic enzymes or detergents are added for better extraction. The mixture is then fractionated by centrifugation, yielding a dense pellet of heavy material at the bottom of the centrifuge tube and a lighter supernatant.
- The supernatant is again centrifuged at a greater force to yield yet another pellet and supernatant. The procedure, called differential centrifugation, yields several fractions of decreasing density, each still containing hundreds of different proteins, which are subsequently assayed for the activity being purified.
- Usually, one fraction will be enriched for such activity, and it then serves as the source of material to which more discriminating purification techniques are applied.
- The choice of temperature, pH, buffering salt, buffer strength, ionic strength, osmolarity, additives (EDTA, SDS, non-ionic detergents etc.), and homogenization technique are important of the success of purification.
- Purification and separation of enzymes are generally based on solubility, size, polarity, and binding affinity. The production scale, timeline, and properties of the enzymes should all be considered when choosing the proper separation method.



1. SOLUBILITY BASED SEPARATION:

- The principle of the type of separation is that enzyme solubility changes drastically when the pH, ionic strength, or dielectric constant changes.
- For example, most proteins are less soluble at high salt concentrations, an effect called salting out. The salt concentration at which a protein precipitates differs from one protein to another.
- Hence, salting out can be used to fractionate proteins. Salting out is also useful for concentrating dilute solutions of proteins, including active fractions obtained from other purification steps.
- Addition of water-miscible organic solvents such as ethanol or acetone will change the dielectric constant of the solvent and therefore precipitate the desired enzyme.
- Neutral water-soluble polymers can also be used for the same purpose instead of organic solvents. However, the risks of losing enzyme activity during precipitation and further separation of the added salt or polymer need to be considered.

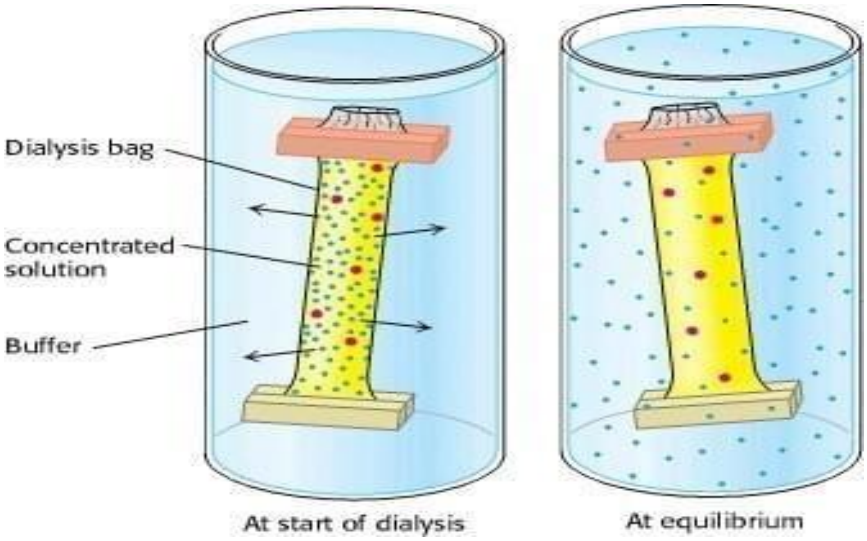


2. SIZE OR MASS BASED METHOD:

- Because enzymes are relatively large molecules, separation based on the size or mass of molecules favors purification of enzymes, especially the ones with high molecular weight.
- Dialysis is a commonly used method, where semipermeable membranes are used to remove salts, small organic molecules, and peptides in the figure below.
- The process usually needs a large volume of dialysate, the fluid outside the dialysis dag, and a period of hours or days to reach the equilibrium.
- Countercurrent dialysis cartages can also be used, in which the solution to be dialyzed flow in one direction, and the dialysate in the opposite direction outside of the membrane.
- Similarly, ultrafiltration membranes, which are made from cellulose acetate or other porous materials, can be used to purify and concentrate an enzyme larger than certain molecular weight.
- The molecular weight is called the molecular weight cutoff and is available in a large range from different membranes.
- The ultrafiltration process is usually carried out in a cartridge loaded with the enzyme to be purified.
- Centrifugal force or vacuum is applied to accelerate the process.
- Both dialysis and ultrafiltration are quick but somewhat vague on distinguishing the molecular weight, whereas size exclusion chromatography gives fine

fractionation from the raw mixture, allowing separation of the desired enzyme from not only small molecules but also other enzymes and proteins.

- Size exclusion chromatography, also known as gel-filtration chromatography, relies on polymer beads with defined pore sizes that let particles smaller than a certain size into the bead, thus retarding their egress from a column.
- In general, the smaller the molecule, the slower it comes out of the column.
- Size exclusion resins are relatively "stiff" and can be used in high pressure columns at higher flow rates, which shortens the separation time. Other factors including the pore size, protein shape, column volumes, and ionic strength of the eluent could also change the result of purification.



3. POLARITY BASED SEPARATION:

- Like other proteins, enzymes can be separated on the basis of polarity, more specifically, their net charge, charge density, and hydrophobic interactions.
- In ion-exchange chromatography, a column of beads containing negatively or positively charged functional groups are used to separate enzymes.
- The cationic enzymes can be separated on anionic columns, and anionic enzymes on cationic column.

ELECTROPHORESIS:

- It is a procedure that uses an electrical field to cause permeation of ions through a solid or semi-solid matrix or surface resulting in separations on constituents on the basis of charge density.

- The most commonly used methods with a SDS-PAGE matrix are quite well standardized and do not differ much between labs.
- The distance a protein migrates in SDS-PAGE is inversely proportional to the log of its molecular radius, which is roughly proportional to molecular weight. Similarly, a matrix with gradient pH can be used in isoelectric focusing separation.
- A protein moves under the influence of an electrical field and stops upon reaching the pH which is the pI for the protein (net charge = 0). The matrix used can be liquid or a gel poured into either a cylindrical shape, or a flat plate.

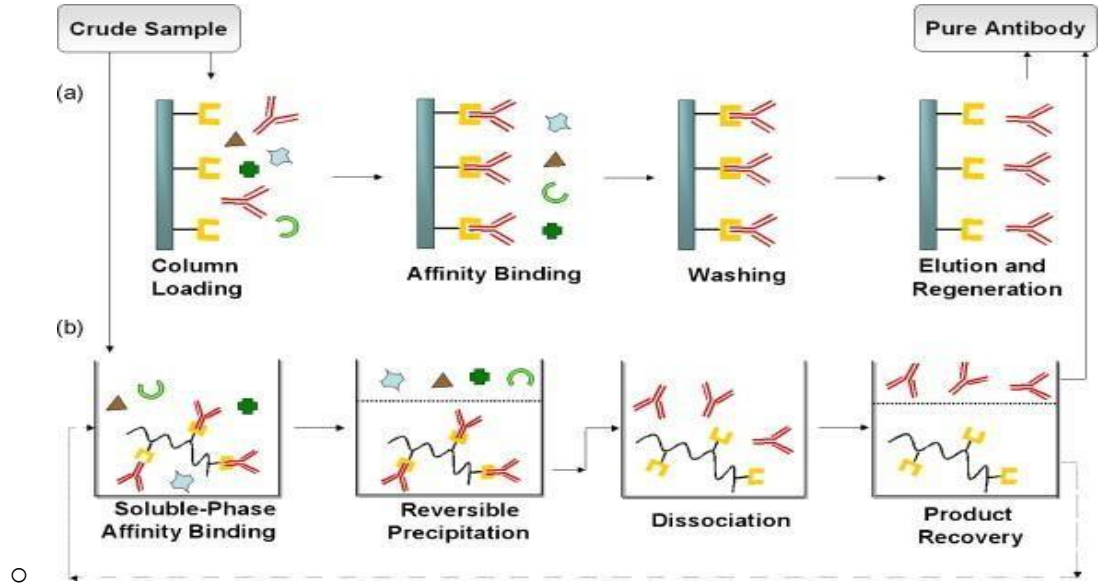
HYDROPHOBIC INTERACTION CHROMATOGRAPHY:

- It employs hydrophobic interactions to distinguish different enzymes, which are adsorbed on matrices such as octyl- or phenyl-Sepharose.
- A gradient of decreasing ionic strength, or possibly increasing non-polar solvent concentration can be used to elute the proteins, giving fractions that usually contain relatively high-pure enzymes.
- High-pressure liquid chromatography (HPLC) uses the same principle of separation of HIC, which is filled with more finely divided and tuned materials and thus allows more choices of eluents and results in better separation.
- Note that HPLC could be based on polarity, affinity, or both.

4. AFFINITY OR LIGAND BASED PURIFICATION:

- Affinity chromatography is another powerful and generally applicable means of purifying enzymes.
- This technique takes advantage of the high affinity of many enzymes for specific chemical groups.
- In general, affinity chromatography can be effectively used to isolate a protein that recognizes a certain group by
    - covalently attaching this group or a derivative of it to a column
    - adding a mixture of proteins to this column, which is then washed with buffer to remove unbound proteins(3) eluting the desired protein by adding a high concentration of a soluble form of the affinity group or altering the conditions to decrease binding affinity.
    - Affinity chromatography is most effective when the interaction of the enzyme and the molecule that is used as the bait is highly specific.

- A special example of ligand-affinity chromatography is the Ni-NTA (nickel – nitrolotriacetic acid-agaraose) affinity chromatography.
- This ligand binds tightly to a 6 amino acid peptide consisting only of histidines (His6).
- The cDNA sequence for His6 can be appended to the cDNA coding for a given recombinant protein, thus yielding a recombinant protein which contains a His-TAG.
- This allows the affinity-purification of such a protein using Ni-NTA without having to design a special ligand-affinity column.
- Other forms of affinity chromatography include dye-ligand chromatography, immunoadsorption chromatography, and covalent chromatography.
- After purification, the enzymes need to be concentrated, and sometimes lyophilized to give the pure, stable form distributed as the product or added into the final formulation.



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IMPORTANCE OF ENZYME ISOLATION:

- It is important to study enzymes in a simple system (only with small ions, buffer molecules, cofactors, etc.) for understanding its structure, kinetics, mechanisms, regulations, and role in a complex system.
- Also isolating pure enzyme is important to use it for medical and industrial purposes.
- Enzyme purification is of great importance in to acquire knowledge about structural and functional properties and to foretell its applications.

5. Explain the properties of steroid hormones. (May 2018)

1. Hormones have low molecular weight so hormones can easily pass out through the capillaries.
2. Hormones are soluble in water, so hormones can be easily transported with blood stream. Hormones are poured into venous blood.
3. Whenever their function is over, hormones are readily destroyed or inactivated or excreted.
4. Hormones are non antigenic.
5. Hormones are organic catalysts and act as coenzymes of the other enzymes in the body.
6. Hormones always act in very low concentration.
7. Many hormones are produced in an inactive form called prohormone.
8. A hormone in its primary action affects one or a limited number of reaction and does not influence directly all other metabolic activities of the cell.
9. Secretion of the hormone is provoked in response to a given situation and its action is therefore, to fulfill a given task.
10. Hormonal activity is not related to heredity.

6. Discuss in detail about spectrophotometric measurement of enzymes (May 2019)

SPECTROPHOTOMETRIC MEASUREMENT OF ENZYMES

SPECTROPHOTOMETRY:

- Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution.
- The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance.
- Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications.
- A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations)

can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

- **UV-visible spectrophotometer**: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- **IR spectrophotometer**: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.
- The basic structure of spectrophotometers. It consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display
- A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer.
- A spectrometer is a device that produces, typically disperses and measures light.
- A photometer indicates the photoelectric detector that measures the intensity of light. or a meter.
- **Spectrometer**: It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromator (prism) to split it into several component wavelengths (spectrum). Then a wavelength selector (slit) transmits only the desired wavelengths.
- **Photometer**: After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photons that is absorbed and then sends a signal to a galvanometer or a digital display.

TRANSMITTANCE:

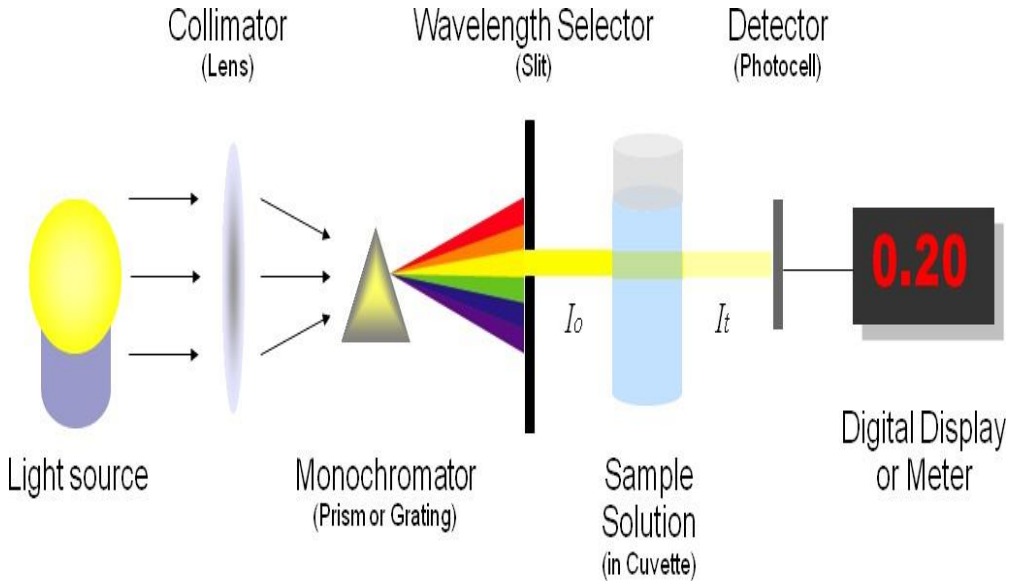
- Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation:

$$\text{Transmittance}(T) = I_t / I_o$$

- Where I_t is the light intensity after the beam of light passes through the cuvette and I_o is the light intensity before the beam of light passes through the cuvette.

ABSORBANCE:

- $\text{Absorbance}(A) = -\log(T) = -\log(I_t / I_o)$
- Absorbance stands for the amount of photons that is absorbed.
- With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample by using Beer-Lambert Law.



BEER – LAMBERT'S LAW:

- Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of a sample. For this reason, Beer's Law can *only* be applied when there is a linear relationship. Beer's Law is written as:

$$A=\epsilon lc$$

- A is the measure of absorbance (no units),
- ϵ is the molar extinction coefficient or molar absorptivity (or absorption coefficient),
- l is the path length, and c is the concentration.

SPECTROPHOTOMETRIC ASSAY:

- In spectrophotometric assays, you follow the course of the reaction by measuring a change in how much light the assay solution absorbs.
- If this light is in the visible region you can actually see a change in the color of the assay, and these are called colorimetric assays.
- The MTT assay, a redox assay using a tetrazolium dye as substrate is an example of a colorimetric assay.
- UV light is often used, since the common coenzymes NADH and NADPH absorb UV light in their reduced forms, but do not in their oxidized forms.

- An oxidoreductase using NADH as a substrate could therefore be assayed by following the decrease in UV absorbance at a wavelength of 340 nm as it consumes the coenzyme.
- Even when the enzyme reaction does not result in a change in the absorbance of light, it can still be possible to use a spectrophotometric assay for the enzyme by using a coupled assay.
- Here, the product of one reaction is used as the substrate of another, easily detectable reaction.
- For example, figure 1 shows the coupled assay for the enzyme hexokinase, which can be assayed by coupling its production of glucose-6-phosphate to NADPH production, using glucose-6-phosphate dehydrogenase.

7. Describe in detail the structure, functions and disorder of adrenal hormones. (Dec 2016)

- The adrenal glands are small glands that sit above the kidneys in the upper abdomen. They produce and release several hormones in the body.
- A range of medical conditions can affect the adrenal glands. These include Addison's disease, Cushing's syndrome, and adrenal cancer, as well as high blood pressure due to the overproduction of aldosterone.

Adrenal gland hormones

- The adrenal glands have two parts: the cortex and the medulla.
- The cortex is the outer part of the gland. It produces the hormones cortisol and aldosterone. The medulla, meanwhile, is the inner part of the gland. It produces the hormones adrenaline and noradrenaline.
- These four hormones are essential to normal functioning in the body. They control many important functions, including:

  - metabolism
  - blood sugar levels
  - blood pressure
  - salt and water balance
  - pregnancy

- sexual development before and during puberty
- stress response
- the balance of sex hormones, including estrogen and testosterone

Disorders affecting the adrenal glands

Sometimes, the adrenal glands produce too much or not enough of their hormones. When this happens, it is known as an adrenal gland disorder. The following sections discuss the most common adrenal gland disorders.

Adrenal insufficiency and Addison's disease

When the adrenal glands do not make enough cortisol, it is known as adrenal insufficiency. There are three types of adrenal insufficiency:

- **Primary adrenal insufficiency, or Addison's disease**. This condition develops when the adrenal gland itself does not function well and cannot make enough cortisol.
- **Secondary adrenal insufficiency**. This occurs when the pituitary gland does not make enough of a hormone called adrenocorticotropin (ACTH). Without ACTH, the adrenals do not receive a signal to make cortisol.
- **Tertiary adrenal insufficiency.** This occurs when the brain cannot produce enough corticotropin-releasing hormone (CRH). Without CRH, the pituitary gland cannot make ACTH. This means that the adrenals cannot make enough cortisol.

There are many potential causes of adrenal insufficiency, including:

- autoimmune disease, which is the most common cause of Addison's disease
- being born with damaged adrenal glands
- tumors on the adrenal glands or those that communicate with the adrenal glands
- infections, such as tuberculosis
- taking corticosteroids for a long time and then suddenly stopping
- having a condition that weakens the immune system, such as HIV or AIDS
- cancer in the adrenal glands
- traumatic brain injury

People with Addison's disease will often have a deficiency in aldosterone as well as cortisol. The symptoms of adrenal insufficiency can be difficult to spot. They may come on slowly, so a person may think that something else is causing them.

The most common signs and symptoms include:

- always feeling tired or weak
- unexplained weight loss
- loss of appetite
- nausea or vomiting
- diarrhea
- dizziness or fainting when standing up
- low blood pressure
- low blood glucose
- irregular or absent menstrual periods before menopause
- cravings for salt or salty foods
- joint pain
- darkening of the skin, especially on scars, lips, skin folds, and joints
- depression

Adrenal insufficiency requires treatment. Without enough cortisol, a person may experience an adrenal crisis. Signs and symptoms of an adrenal crisis include:

- severe vomiting and diarrhea
- fainting
- low blood pressure
- a sharp pain in the abdomen, lower back, or legs

If a person shows any signs of an adrenal crisis, they should seek immediate medical help. Without treatment, an adrenal crisis can be fatal.

Adrenal tumors

Adrenal cancer is rare, affecting as few as 200 people in the United States each year, according to the American Cancer Society (ACS). Benign, or noncancerous, tumors are much more common.

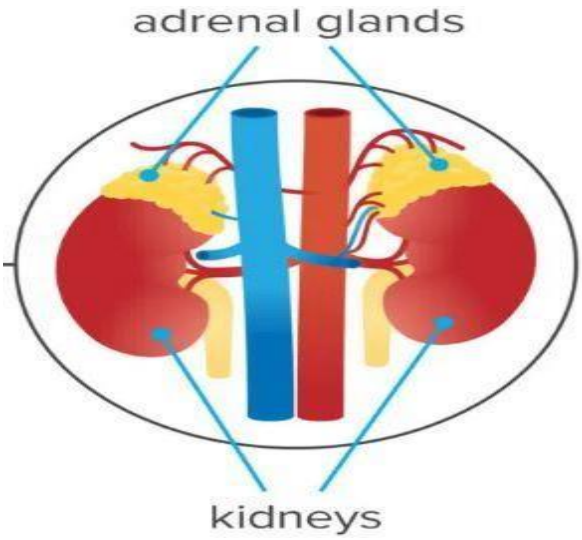
There are several types of adrenal tumor, including:

- **Adenomas**. Most tumors that affect the adrenal cortex are adenomas. These are benign tumors of the adrenal cortex. However, a doctor may need to remove them if they interfere with adrenal gland function or reach a certain size.
- **Adrenocortical carcinoma**. Although rare, this cancer can affect the cortex, which is the outer part of the adrenal gland.
- **Neuroblastoma**. This cancer occurs in childhood and may begin in the medulla, which is the inner part of the adrenal gland.
- **Pheochromocytoma**. This is a neuroendocrine tumor that affects the medulla. It results in high levels of adrenaline.

The ACS say that most tumors in the adrenals do not start there. Instead, they often arise because other cancers, such as breast cancer or lung cancer, spread to the adrenals.

Some signs and symptoms of adrenal cancer include:

- unexplained weight gain or loss
- high blood pressure
- high blood sugar or diabetes
- low blood potassium
- anxiety, nervousness, or panic attacks
- heart palpitations
- excessive sweating
- headache
- abdominal pain
- weakness
- unusual hair growth



- an increase in acne
- changes to genitals or sex drive

Cushing's syndrome

People with Cushing's syndrome have adrenals that produce too much cortisol. The most common cause of this is using medications called glucocorticoids for a long time and at high dosages.

Glucocorticoids help treat many conditions, including asthma, rheumatoid arthritis, and lupus. They act like cortisol in the body.

Certain tumors can also cause Cushing's syndrome. These include:

- **Pituitary adenomas**. These are benign growths on the pituitary gland. They can make the pituitary gland produce too much ACTH, which results in too much cortisol.
- **Ectopic ACTH-producing tumors.** These produce too much ACTH but are not located on the pituitary gland. Instead, they may be located on the lungs, pancreas, thyroid, or other areas.
- **Adenomas or other adrenal tumors.**

Some signs and symptoms of Cushing's syndrome include:

- fat buildup around the base of the neck
- a fatty hump between the shoulders
- a round face
- unexplained weight gain
- thin arms and legs
- skin that bruises easily
- wide, purple stretch marks on the abdomen, hips, and breasts
- excess hair on the face, neck, chest, abdomen, and thighs (in females)
- irregular or absent menstrual periods (in females)
- low fertility, low libido, or erectile dysfunction (in males)
- obesity and slow growth (in children)

UNIT-III

2 MARKS:

1. Define Electrophoresis. (May 2015)

Electrophoresis is a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge. An electric current is used to move molecules to be separated through a gel. Pores in the gel work like a sieve, allowing smaller molecules to move faster than larger molecules.

2. What are Hetero and homopolysaccharides? (May 2015)

- Polysaccharides consisting of molecules of more than one sugar or sugar derivative are called heteropolysaccharides (heteroglycans).
- Homopolysaccharides are polysaccharides composed of a single type of sugar monomer. They are composed of the same repeating unit.

3. What are hexoses? Give examples. (Nov 2015)

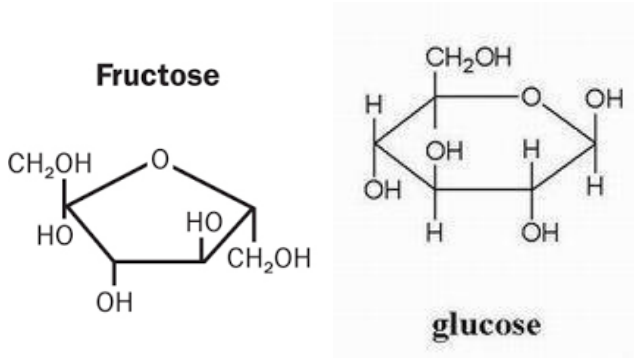
- A hexose is a monosaccharide (simple sugar) with six carbon atoms. Hexoses are extremely important in biochemistry, both as isolated molecules (such as glucose and fructose) and as building blocks of other compounds such as starch, cellulose, and glycosides.
- Three hexoses are particularly abundant: D-glucose, D-galactose, and D-fructose.

4. Differentiate catabolism of anabolism. (Nov 2015)

Catabolism	Anabolism
Breaks down big complex molecules into smaller, easier to absorb molecules.	Builds molecules required for the body's functionality.
The process of catabolism releases energy.	Anabolic processes require energy.
Hormones involved in the processes are adrenaline, cytokine, glucagon, and cortisol.	Hormones involved in the process are estrogen, testosterone, growth hormones and insulin.

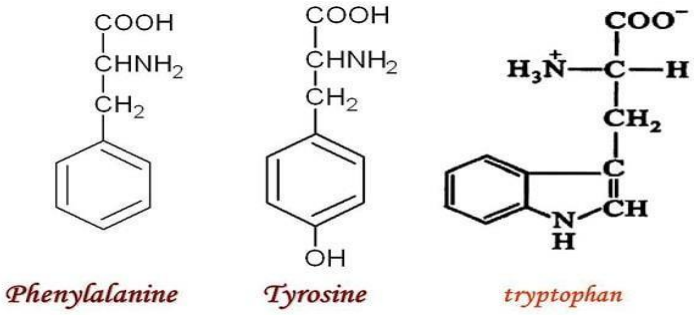
5. What are monosaccharides? Write any two structures of monosaccharides. (Nov 2016, Nov 2019)

- Monosaccharides, also called simple sugars, are the simplest form of sugar and the most basic units of carbohydrates.
- All monosaccharides have the same general formula of $(CH_2O)_n$, which designates a central carbon molecule bonded to two hydrogens and one oxygen.



6. Give an example and structure of Aromatic amino acid. (Nov 2016)

An aromatic amino acid (AAA) is an amino acid that includes an aromatic ring. Among the 20 standard amino acids, the following are aromatic: phenylalanine, tryptophan and tyrosine.



7. What are essential amino acids? Name them. (May 2016)

Essential amino acids cannot be made by the body. As a result, they must come from food. The 9 essential amino acids are:

Histidine,Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryp tophan, and Valine.

8. How will you prescribe fats and oils for those suffering from high level of blood cholesterol and coronary artery diseases? Give examples. (May 2016)

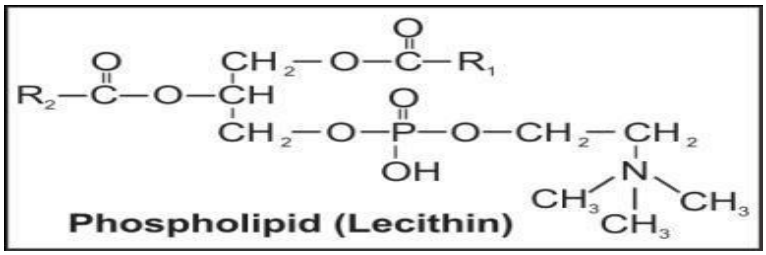
Monounsaturated fatty acids:

Studies show that eating foods rich in monounsaturated fatty acids instead of saturated fats improves blood cholesterol levels, which can decrease your risk of heart disease and may also help decrease the risk of type 2 diabetes.

9. What is called reducing sugars? Examples. (May 2017)

All monosaccharides are reducing sugars because they either have an aldehyde group (if they are aldoses) or can tautomerize in solution to form an aldehyde group (if they are ketoses). This includes common monosaccharides like galactose, glucose, glyceraldehyde, fructose, ribose, and xylose.

10. Give the structure of lecithin. (May 2017)



11. Give two important uses of carbohydrates. (Nov 2017)

- Providing energy and regulation of blood glucose.
- Sparing the use of proteins for energy.
- Breakdown of fatty acids and preventing ketosis.
- Biological recognition processes.

12. Classify the lipids. (Nov 2017)

Lipids have been 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)

13. What are membrane lipids? (May 2018)

Membrane lipids are a group of compounds which form the double-layered surface of all cells. The three major classes of membrane lipids are phospholipids, glycolipids, and cholesterol.

14. What are chromo proteins? (May 2018)

A chromoprotein is a conjugated protein that contains a pigmented prosthetic group. A common example is haemoglobin, which contains a heme cofactor, which is the iron-containing molecule that makes oxygenated blood appear red.

15. How many ATPs are generated during the complete oxidation of glucose to CO2 and H2O? (Nov 2018)

34 molecules of ATP are generated during the complete aerobic metabolism of glucose are synthesized during oxidation of pyruvate to CO_2 and H_2O in mitochondria.

16. Write the principle of Ion exchange chromatography. (Nov 2018)

Ion-exchange chromatography separates molecules based on their respective charged groups. Ion-exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The ion exchange chromatography matrix consists of positively and negatively charged ions.

17. Define Bile acids. (May 2019)

An acid made by the liver that works with bile to break down fats. On a more technical level, bile acids are steroid carboxylic acids derived from cholesterol. The primary bile acids are cholic and chenodeoxycholic acids. They are conjugated with glycine or taurine before they are secreted into the bile.

18. What is amino acid? (May 2019)

Amino acids are organic compounds that contain amino and carboxyl functional groups, along with a side chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, although other elements are found in the side chains of certain amino acids. Amino acids and proteins are the building blocks of life. When proteins are digested or broken down, amino acids are left. The human body uses amino acids to make proteins to help the body: Break down food.

11 MARKS:

1. Discuss the metabolism of beta oxidation and add a note on its energetics. (May 2015, May 2019)

Beta oxidation is a metabolic process involving multiple steps by which fatty acid molecules are broken down to produce energy. More specifically, beta oxidation consists in breaking down long fatty acids that have been converted to acyl-CoA chains into progressively smaller fatty acyl-CoA chains. This reaction releases acetyl-CoA, FADH2 and NADH, the three of which then enter another metabolic process called *citric acid cycle* or *Krebs cycle*, in which ATP is produced to be used as energy. Beta oxidation goes on until two acetyl-CoA molecules are produced and the acyl-CoA chain has been completely broken down. In eukaryotic cells, beta oxidation takes place in the mitochondria, whereas in prokaryotic cells, it happens in the cytosol.

For beta oxidation to take place, fatty acids must first enter the cell through the cell membrane, then bind to coenzyme A (CoA), forming fatty acyl CoA and, in the case of eukaryotic cells, enter the mitochondria, where beta oxidation occurs.

Where Does Beta Oxidation Occur?

Beta oxidation occurs in the mitochondria of eukaryotic cells and in the cytosol of prokaryotic cells. However, before this happens, fatty acids must first enter the cell and, in the case of eukaryotic cells, the mitochondria. In cases where fatty acid chains are too long to enter the mitochondria, beta oxidation can also take place in peroxisomes.

First, fatty acid protein transporters allow fatty acids to cross the cell membrane and enter the cytosol, since the negatively charged fatty acid chains cannot cross it otherwise. Then, the enzyme fatty acyl-CoA synthase (or FACS) adds a CoA group to the fatty acid chain, converting it to acyl-CoA.

Depending on the length, the acyl-CoA chain will enter the mitochondria in one of two ways:

1. If the acyl-CoA chain is short, it can freely diffuse through the mitochondrial membrane.
2. If the acyl-CoA chain is long, it needs to be transported across the membrane by the carnitine shuttle. For this, the enzyme carnitinepalmitoyltransferase 1 (CPT1)—bound to the outer mitochondrial membrane—converts the acyl-CoA

chain to an acylcarnitine chain, which can be transported across the mitochondrial membrane by carnitinetranslocase (CAT). Once inside the mitochondria, CPT2—bound to the inner mitochondrial membrane—converts the acylcarnitine back to acyl-CoA. At this point, acyl-CoA is inside the mitochondria and can now undergo beta oxidation.

As mentioned above, if the acyl-CoA chain is too long to be processed in the mitochondria, it will be broken down by beta oxidation in the peroxisomes. Research suggests that very long acyl-CoA chains are broken down until they are 8 carbons long, after which they are transported and enter the beta oxidation cycle in the mitochondria. Beta oxidation in the peroxisomes yields H_2O_2 instead of FADH2 and NADH, producing heat as a result.

Beta Oxidation Steps:

Beta oxidation takes place in four steps: dehydrogenation, hydration, oxidation and thyolisis. Each step is catalyzed by a distinct enzyme.

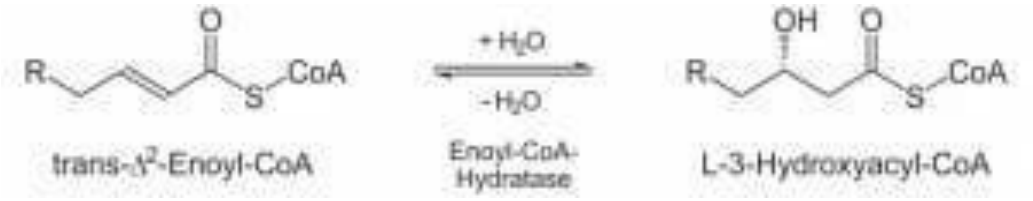
Briefly, each cycle of this process begins with an acyl-CoA chain and ends with one acetyl-CoA, one FADH2, one NADH and water, and the acyl-CoA chain becomes two carbons shorter. The total energy yield per cycle is 17 ATP molecules (see below for details on the breakdown). This cycle is repeated until two acetyl-CoA molecules are formed as opposed to one acyl-CoA and one acetyl-CoA. The four steps of beta oxidation are described below and can be seen in the links to the figures at the end of each explanation.

Dehydrogenation:

In the first step, acyl-CoA is oxidized by the enzyme acyl CoA dehydrogenase. A double bond is formed between the second and third carbons (C2 and C3) of the acyl-CoA chain entering the beta oxidation cycle; the end product of this reaction is trans-Δ^2-enoyl-CoA (trans-delta 2-enoyl CoA). This step uses FAD and produces FADH2, which will enter the citric acid cycle and form ATP to be used as energy. (Notice in the following figure that the carbon count starts on the right side: the rightmost carbon below the oxygen atom is C1, then C2 on the left forming a double bond with C3, and so on.)

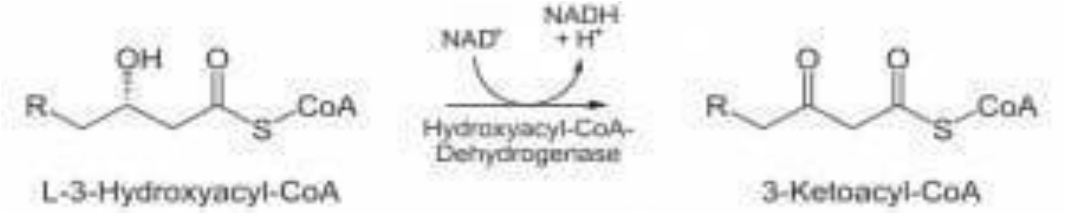
Hydration:

In the second step, the double bond between C2 and C3 of trans-Δ^2-enoyl-CoA is hydrated, forming the end product L-β-hydroxyacyl CoA, which has a hydroxyl group (OH) in C2, in place of the double bond. This reaction is catalyzed by another enzyme: enoyl CoA hydratase. This step requires water.



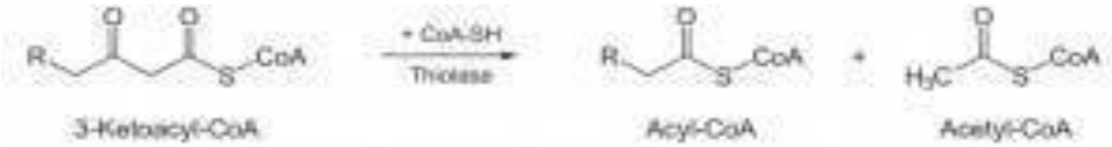
Oxidation:

In the third step, the hydroxyl group in C2 of L-β-hydroxyacyl CoA is oxidized by NAD+ in a reaction that is catalyzed by 3-hydroxyacyl-CoA dehydrogenase. The end products are β-ketoacyl CoA and NADH + H. NADH will enter the citric acid cycle and produce ATP that will be used as energy.



Thiolysis:

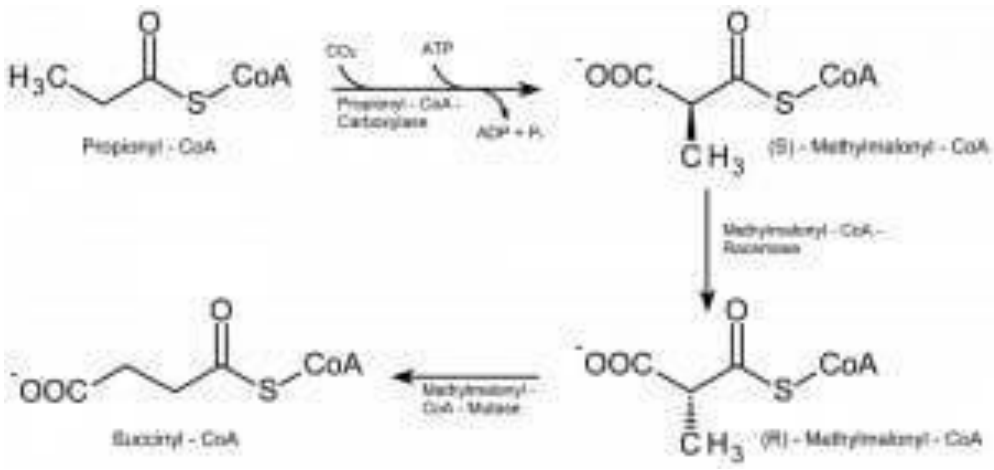
Finally, in the fourth step, β-ketoacyl CoA is cleaved by a thiol group (SH) of another CoA molecule (CoA-SH). The enzyme that catalyzes this reaction is β-ketothiolase. The cleavage takes place between C2 and C3; therefore, the end products are an acetyl-CoA molecule with the original two first carbons (C1 and C2), and an acyl-CoA chain two carbons shorter than the original acyl-CoA chain that entered the beta oxidation cycle.



End of Beta Oxidation:

In the case of even-numbered acyl-CoA chains, beta oxidation ends after a four-carbon acyl-CoA chain is broken down into two acetyl-CoA units, each one containing two carbon atoms. Acetyl-CoA molecules enter the citric acid cycle to yield ATP.

In the case of odd-numbered acyl-CoA chains, beta oxidation ensues in the same way except for the last step: instead of a four-carbon acyl-CoA chain being broken down into two acetyl-CoA units, a five-carbon acyl-CoA chain is broken down into a three-carbon propionyl-CoA and a two-carbon acetyl-CoA. Another chemical reaction then converts propionyl-CoA to succinyl-CoA (see the figure below), which enters the citric acid cycle to produce ATP.



Energy Yield and End Products:

Each beta oxidation cycle yields 1 FADH2, 1 NADH and 1 acetyl-CoA, which in terms of energy is equivalent to 17 ATP molecules:

- 1 FADH2 (x 2 ATP) = 2 ATP
- 1 NADH (x 3 ATP) = 3 ATP
- 1 acetyl-CoA (x 12 ATP) = 12 ATP
- Total = 2 + 3 + 12 = 17 ATP

However, the theoretical ATP yield is higher than the real ATP yield. In reality, the equivalent of about 12 to 16 ATPs is produced in each beta oxidation cycle.

Besides energy yield, the fatty acyl-CoaA chain becomes two carbons shorter with each cycle. In addition, beta oxidation yields great amounts of water; this is beneficial for eukaryotic organisms such as camels given their limited access to drinkable water.

2. Explain in detail about the classification of proteins. (May 2015, May 2016, Nov 2016, Nov 2017, May 2019, Nov 2019)

CLASSIFICATION OF PROTEINS

- Proteins are important macromolecules of the cells, formed by the polymerization of amino acids according to the sequence of genetic code in the mRNA.
- Proteins are the mode of expression of the genetic information.
- They perform a variety of duties in the cells such as they act as the structural components of cells, enzymes, hormones, pigments, storage proteins and some toxins in the cells.
- The proteins are classified into many categories based on different criterions.

Criterions for the classification of proteins:

Proteins are classified based on the following THREE criterions:

I. Classification based on STRUCTURE of Protein
II. Classification based on COMPOSITION of Protein
III. Classification based on FUNCTIONS of Proteins.

I. Classification based on STRUCTURE of Protein

Based on the structure, proteins are classified into 3 groups.

A. Fibrous Proteins
B. Globular Proteins
C. Intermediate Proteins

A. Fibrous Proteins

- They are linear (long fibrous) in shape.
- Secondary structure is the most important functional structure of fibrous proteins.
- Usually, these proteins do not have tertiary structures.
- Physically fibrous proteins are very tough and strong.
- They are insoluble in the water.
- Long parallel polypeptide chains crosslinked at regular intervals.
- Fibrous proteins form long fibres or sheaths.
- Functions of fibrous proteins: perform the structural functions in the cells.
- Examples of fibrous proteins: Collagen, Myosin, Silk and Keratin.

B. GlobularProteins

- Globular proteins are spherical or globular in shape.
- The polypeptide chain is tightly folded into spherical shapes.
- Tertiary structure is the most important functional structure in globular proteins. Physically they are soft than fibrous proteins.
- They are readily soluble in water.
- Most of the proteins in the cells belong to the category of globular proteins. Functions: enzymes, antibodies and some hormones.
- Example: Insulin, Haemoglobin, DNA Polymerase and RNA Polymerase

C. Intermediate Proteins

- Their structure is intermediate to linear and globular structures.
- They are short and more or less linear shaped proteins
- Unlike fibrous proteins, they are soluble in water. Function: blood clotting proteins
- Example: Fibrinogen

II. Classification of Proteins based on Composition:

Two broad categories of proteins according to its composition, they are:

A. Simple Proteins

B. Conjugated Proteins

A. Simple Proteins

- Simple proteins composed of ONLY amino acids.
- Proteins may be fibrous or globular.
- They possess relatively simple structural organization.
- Example: Collagen, Myosin, Insulin, Keratin

B. Conjugated Proteins

- Conjugated proteins are complex proteins.
- They contain one or more non-amino acid components.
- Here the protein part is tightly or loosely bound to one or more non-protein part(s).
- The non-protein parts of these proteins are called prosthetic groups.
- The prosthetic group may be metal ions, carbohydrates, lipids, pliosplioric acids, nucleic acids and FAD.
- The prosthetic group is essential for the biological functions of these proteins.
- Conjugated proteins are usually globular in shape and are soluble in water.
- Most of the enzymes are conjugated proteins.

- Based on the nature of prosthetic groups, the conjugated proteins are further classified as follows:
- Phospho protein: Prosthetic group is phosphoric acid, Example- Casein of milk, itellin of egg yolk.
- Glyco proteins: Prosthetic group is carbohydrates, Example — Most of the membrane proteins, Mticin (component of saliva).
- Nticleo protein: Prosthetic group is nucleic acid, Example — proteins in chromosomes, structural proteins of ribosome.
- Chromo proteins: Prosthetic group is pigment or chrome, Example: Haemoglobin, Phytochrome and Cytochrome.
- Lipoproteins: Prosthetic group is Lipids, Example: Membrane proteins
- Flavo proteins: Prosthetic group is FAD (Flavin Adenine Dinticleotide), Example: Proteins of Electron Transport System (ETS).
- Metallo proteins: Prosthetic group is Metal ions, Example: Nitrate Redtictase.

III. Classification of Protein based on Functions:

(A). Structural Proteins:

- Form the component of the connective tissue, bone, tendons, cartilage, skin, feathers, nail, hairs and horn.
- Most of them are fibrous proteins and are insoluble in water.
- Example: Collagen, Keratin and Elastin.

(B). Enzymes:

- They are the biological catalysts.
- Enzymes reduce the activation energy of reactants and speed-up the metabolic reactions in the cells.
- Most of them are globular conjugated proteins.
- Example: DNA Polymerase, Nitrogenase, Lipase

(C). Hormones:

- They include the proteinaceoiis hormones in the cells.
- Example: Insulin, Glucagon, ACH

(D). Respiratory Pigments

- All of them are conjugated proteins and they contain pigments (chrome) as their prosthetic group.

- They are coloured proteins
- Example: Haemoglobin, Myoglobin

(E).Transport Proteins

- They transport the materials in the cells
- They form channels in the plasma membrane
- They also form one of the components of blood and lymph in animals.
- Example: Serum albumin

(F). Contractile proteins

- They are the force generators of muscles
- They can contract with the expense of energy from ATP molecules. k Example: Actin, Myosin

(G). Storage Proteins

- They act as the store of metal ions and amino acids in the cells.
- Found in seeds, egg and milk, abundantly seen in pulses (legunie seeds).
- Example: Ferritin which stores iron, Casein, Ovalbumin, Gluten of Wheat

(F). Toxins

- They are toxic proteins.
- Example: Snake venom

3. Explain in detail the classification of carbohydrates. (Nov 2015, May 2016, May 2017)

CARBOHYDRATES – CLASSIFICATION

- The carbohydrates are a group of naturally occurring carbonyl compounds (aldehydes or ketones) that also contain several hydroxyl groups.
- It may also include their derivatives which produce such compounds on hydrolysis.
- They are the most abundant organic molecules in nature and also referred to as "saccharides".
- The carbohydrates which are soluble in water and sweet in taste are called as "sugars"

STRUCTURE OF CARBOHYDRATES:

- 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.
- The building blocks of all carbohydrates are simple sugars called monosaccharides.
- A monosaccharide can be a polyhydroxy aldehyde (aldose) or a polyhydroxy ketone (ketose).

The carbohydrates can be structurally represented in any of the three forms:

- Open chain structure.
- Hemi-acetal structure.
- Haworth structure.

PHYSICAL PROPERTIES OF CARBOHYDRATES

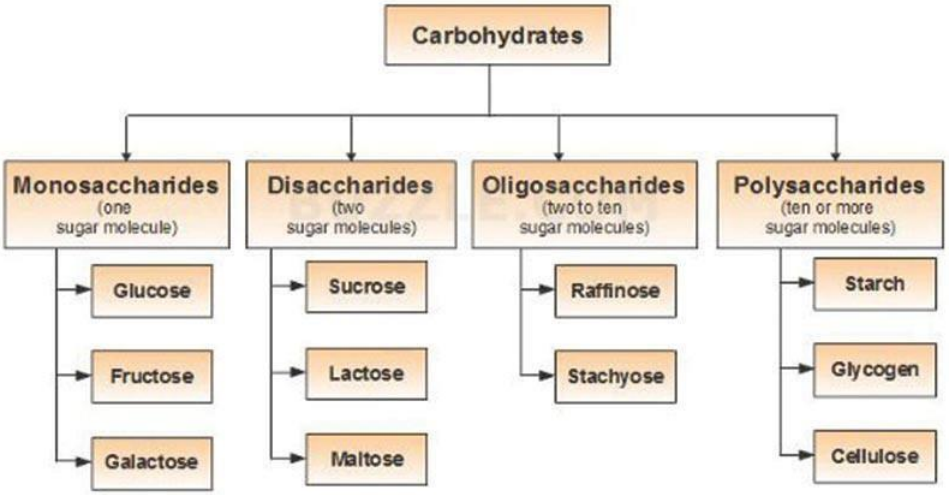
- Stereoisomerism – Compound shaving the same structural formula but they differ in spatial configuration. Example: Glucose has two isomers with respect to the penultimate carbon atom. They are D-glucose and L-glucose.
- Optical Activity – It is the rotation of plane-polarized light forming (+) glucose and (-) glucose.
- Diastereo isomers – It the configurational changes with regard to C2, C3, or C4 in glucose. Example: Mannose, galactose.
- Annomerism – It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

CHEMICAL PROPERTIES OF CARBOHYDRATES:

- Osazone formation: Osazone are carbohydrate derivatives when sugars are reacted with an excess of phenylhydrazine. eg. Glucosazone
- Benedict's test: Reducing sugars when heated in the presence of an alkali gets converted to powerful reducing species known as enediols. When Benedict's reagent solution and reducing sugars are heated together, the solution changes its color to orange-red/ brick red.
- Oxidation: Monosaccharides are reducing sugars if their carbonyl groups oxidize to give carboxylic acids. In Benedict's test, D-glucose is oxidized to D-gluconic acid thus, glucose is considered a reducing sugar.

CLASSIFICATION OF CARBOHYDRATES:

The simple carbohydrates include single sugars (monosaccharides) and polymers, oligosaccharides, and polysaccharides.



Monosaccharides

- Simplest group of carbohydrates and often called simple sugars since they cannot be further hydrolyzed.
- Colorless, crystalline solid which are soluble in water and insoluble in a non-polar solvent.
- These are compound which possesses a free aldehyde or ketone group.
- The general formula is $C_n(H2O)_n$or $C_nH_{2n}O_n$.
- They are classified according to the number of carbon atoms they contain and also on the basis of the functional group present.
- The monosaccharides thus with 3, 4, 5, 6, 7… carbons are called trioses, tetroses, pentoses, hexoses, heptoses, etc., and also as aldoses or ketoses depending upon whether they contain aldehyde or ketone group.
- Examples: Glucose, Fructose, Erythrulose, Ribulose.

Oligosaccharides

- Oligosaccharides are compound sugars that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis.
- The monosaccharide units are joined by glycosidic linkage.
- Based on the number of monosaccharide units, it is further classified as disaccharide, trisaccharide, tetrasaccharide etc.
- Oligosaccharides yielding 2 molecules of monosaccharides on hydrolysis is known as a disaccharide, and the ones yielding 3 or 4 monosaccharides are known as trisaccharides and tetrasaccharides respectively and so on.

- The general formula of disaccharides is $C_n(H2O)_{n-1}$and that of trisaccharides is $C_n(H2O)_{n-2}$ and so on.
- Examples: Disaccharides include sucrose, lactose, maltose, etc.
- Trisaccharides are Raffinose, Rabinose.

Polysaccharides

- They are also called as "glycans".
- Polysaccharides contain more than 10 monosaccharide units and can be hundreds of sugar units in length.
- They yield more than 10 molecules of monosaccharides on hydrolysis.
- Polysaccharides differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bond linking units and in the degree of branching.
- They are primarily concerned with two important functions ie. Structural functions and the storage of energy.
- They re further classified depending on the type of molecules produced as a result of hydrolysis.
- They may be homopolysaccharidese, containing monosaccharides of the same type or heteropolysaccharides i.e., monosaccharides of different types.
- Examples of Homopolysaccharides are starch, glycogen, cellulose, pectin.
- Heteropolysaccharides are Hyaluronic acid, Chondroitin.

FUNCTIONS OF CARBOHYDRATES:

Carbohydrates are widely distributed molecules in plant and animal tissues. In plants and arthropods, carbohydrates from the skeletal structures, they also serve as food reserves in plants and animals. They are important energy source required for various metabolic activities, the energy is derived by oxidation.

Some of their major functions include:

- Living organisms use carbohydrates as accessible energy to fuel cellular reactions. They are the most abundant dietary source of energy (4kcal/gram) for all living beings.
- Carbohydrates along with being the chief energy source, in many animals, are instant sources of energy. Glucose is broken down by glycolysis/ Kreb's cycle to yield ATP.

- Serve as energy stores, fuels, and metabolic intermediates. It is stored as glycogen in animals and starch in plants.

4. Explain in detail of Chromatography techniques. (Nov 2015)

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- The Russian botanist Mikhail Tswett coined the term chromatography in 1906.
- The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures.
- A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties to separate materials.
- It is a powerful separation tool that is used in all branches of science and is often the only means of separating components from complex mixtures.

PRINCIPLE:

- Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.
- The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights.
- Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into the mobile phase, and leave the system faster.

Three components thus form the basis of the chromatography technique.

1. Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface solid support”.
2. Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
3. Separated molecules

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on the separation of molecules from each other.

TYPES:

- Substances can be separated on the basis of a variety of methods and the presence of characteristics such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase.
- This leads to different types of chromatography techniques, each with their own instrumentation and working principle.
- For instance, four separation techniques based on molecular characteristics and interaction type use mechanisms of ion exchange, surface adsorption, partition, and size exclusion.
- Other chromatography techniques are based on the stationary bed, including column, thin layer, and paper chromatography.

TECHNIQUES:

1. Column chromatography
2. Ion-exchange chromatography
3. Gel-permeation (molecular sieve) chromatography
4. Affinity chromatography
5. Paper chromatography
6. Thin-layer chromatography
7. Gas chromatography (GS)
8. Dye-ligand chromatography
9. Hydrophobic interaction chromatography
10. Pseudoaffinity chromatography
11. High-pressure liquid chromatography (HPLC)

APPLICATIONS:

Pharmaceutical sector

- To identify and analyze samples for the presence of trace elements or chemicals.
- Separation of compounds based on their molecular weight and element composition.
- Detects the unknown compounds and purity of mixture.
- In drug development.

Chemical industry

- In testing water samples and also checks air quality.

- HPLC and GC are very much used for detecting various contaminants such as polychlorinated biphenyl (PCBs) in pesticides and oils.
- In various life sciences applications

Food Industry

- In food spoilage and additive detection
- Determining the nutritional quality of food

Forensic Science

- In forensic pathology and crime scene testing like analyzing blood and hair samples of crime place.

Molecular Biology Studies

- Various hyphenated techniques in chromatography such as EC-LC-MS are applied in the study of metabolomics and proteomics along with nucleic acid research.
- HPLC is used in Protein Separation like Insulin Purification, Plasma Fractionation, and Enzyme Purification and also in various departments like Fuel Industry, biotechnology, and biochemical processes.

5. Explain in detail of Electrophoresis. (Nov 2016)

Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field. In the year 1807, Ferdinand Frederic Reuss was the first person to observe electrophoresis. He was from Moscow State University. Anaphoresis is the electrophoresis of negative charge particles or anions whereas cataphoresis is electrophoresis of positive charge ions or cations. Electrophoresis has a wide application in separating and analysing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.

Electrophoresis Principle and its types:

Charged macromolecules are placed in the electric field move towards the negative or positive pole based on their charge. Nucleic acid has a negative charge and therefore it migrates towards the anode.

This technique is divided into two types viz slab electrophoresis and capillary electrophoresis.

Types of Electrophoresis:

1. Capillary electrophoresis
    1. Gel electrophoresis
    2. Paper electrophoresis
2. Slab electrophoresis
    1. Zone electrophoresis
    2. Immunoelectrophoresis
    3. Isoelectrofocusing

Gel electrophoresis procedure:

Below we have explained the steps conducted during DNA electrophoresis.

Step 1: Prepare sample

Isolate the DNA and prepare the solution by adding blue dye so that it will be easy to observe the movement of the sample taking place in the gel.

Step 2: Prepare an agarose TAE gel solution

TAE buffer solution helps to generate an electric field during the process of electrophoresis. To prepare the solution, for example, if there is a requirement of 1% agarose gel then add 100mL TAE to 1 g of agarose. The higher percentage of agarose will give a denser screen. Dissolve the agarose by heating the agarose TAE solution.

Step 3: Gel casting

Pour the agarose TAE solution in a casting tray. Allow it to cool and solidify. A gel slab along with the wells is ready to use for the experiment.

Step 4: How to set up the electrophoresis chamber

Fill a chamber with TAE buffer. Place the solid gel in the chamber. Place the gel in such a position such that it is near the negative electrode.

Step 5: Gel loading

Load the wells with the DNA sample and DNA ladder (a reference for sizes).

Step 6: Process of electrophoresis

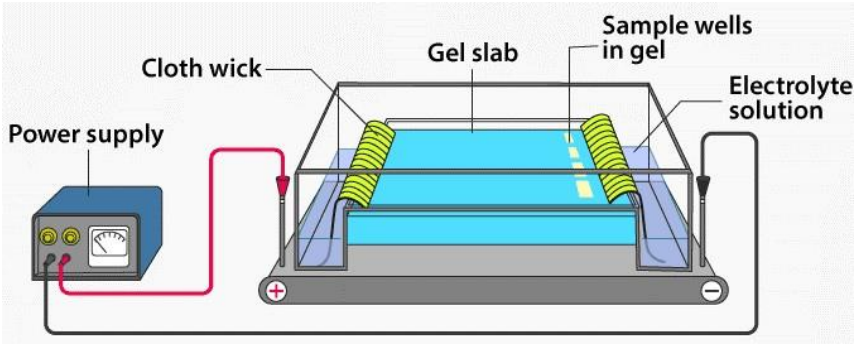
Connect the positive and negative points to the power supply and chamber. Switch on the power and migration in the DNA sample due to the electric field generated. The negatively charged sample will move towards the positive point and away from the negative electrode.

Step 7: Observe the DNA

Once you see the migration of the blue colored DNA samples in the gel switch off the power supply. Remove the gel and place it in the ethidium bromide solution.

Step 8: Expose the ethidium bromide stained gel under UV light and take a picture.

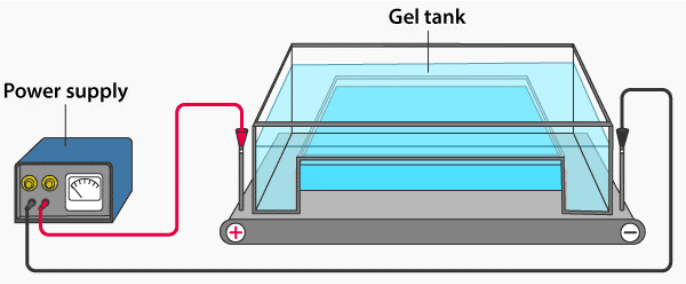
DNA bands appear in the lane of respective well. Also, the DNA ladder is visible. Therefore, the length of DNA bands can be determined. Below is the image of the experiment conducted.



Immuno electrophoresis procedure:

1. Prepare agarose gel on a glass slide in a horizontal position.
2. Use sample template and carefully move the wells to the application zone.
3. Make the sample dilution in the ratio 2:3 with the diluent protein solution.
4. Take a 5 µl pipette and add 5 µl of sample and control across each slit.
5. Place the gel in the chamber for electrophoresis positioning the sample near the cathode side. Carry out the electrophoresis for 20 mins at 100 volts.
6. Take 20 µl of antiserum in a trough and incubate for 8- 20 hours at room temperature on competing the electrophoresis.
7. Soak the agarose gel for 10 minutes in saline solution, dry it and wash it twice.
8. Dry the gel below 70°C and stain it with protein stain solution for 3 minutes. Decolorize the gel in destaining solution for 5 minutes.

9. Determine the results once the gel is dried.



6. Explain Paper chromatography. (May 2017)

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography. It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper. It is a powerful analytical tool that uses very small quantities of material. Paper chromatography was discovered by Synge and Martin in the year 1943.

Paper Chromatography Principle

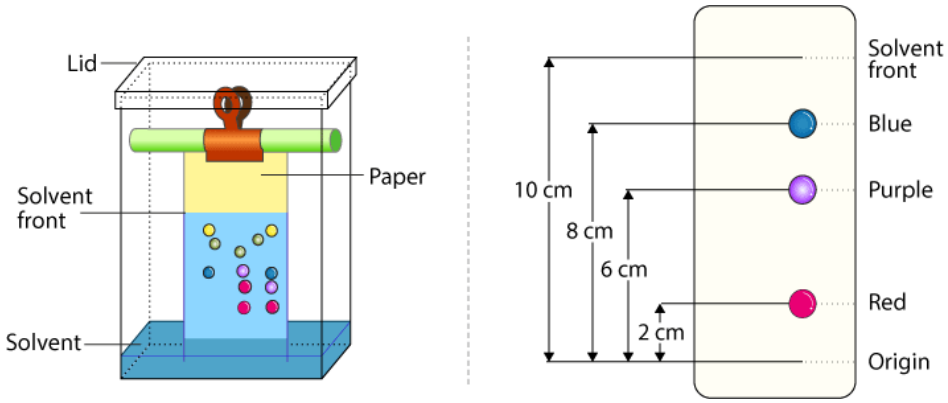
The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place. The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper. Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.

Paper Chromatography Procedure

Below we have explained the procedure to conduct Paper Chromatography Experiment for easy understanding of students.

1. Selecting a suitable type of development: It is decided based on the complexity of the solvent, paper, mixture, etc. Usually ascending type or radial paper

chromatography is used as they are easy to perform. Also, it is easy to handle, the chromatogram obtained is faster and the process is less time-consuming.



2. Selecting a suitable filter paper: Selection of filter paper is done based on the size of the pores and the sample quality.
3. Prepare the sample: Sample preparation includes the dissolution of the sample in a suitable solvent (inert with the sample under analysis) used in making the mobile phase.
4. Spot the sample on the paper: Samples should be spotted at a proper position on the paper by using a capillary tube.
5. Chromatogram development: Chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.
6. Paper drying and compound detection: Once the chromatogram is developed, the paper is dried using an air drier. Also, detecting solution can be sprayed on the chromatogram developed paper and dried to identify the sample chromatogram spots.

Paper Chromatography Applications:

There are various applications of paper chromatography. Some of the uses of Paper Chromatography in different fields are discussed below:

- To study the process of fermentation and ripening.
- To check the purity of pharmaceuticals.
- To inspect cosmetics.

- To detect the adulterants.
- To detect the contaminants in drinks and foods.
- To examine the reaction mixtures in biochemical laboratories.
- To determine dopes and drugs in humans and animals.

Types of paper chromatography:

1. Ascending Paper Chromatography – The techniques goes with its name as the solvent moves in an upward direction.
2. Descending Paper Chromatography – The movement of the flow of solvent due to gravitational pull and capillary action is downwards, hence the name descending paper chromatography.
3. Ascending – Descending Paper Chromatography – In this version of paper chromatography, movement of solvent occurs in two directions after a particular point. Initially, the solvent travels upwards on the paper which is folded over a rod and after crossing the rod it continues with its travel in the downward direction.
4. Radial or Circular Paper Chromatography – The sample is deposited at the centre of the circular filter paper. Once the spot is dried, the filter paper is tied horizontally on a Petri dish which contains the solvent.
5. Two Dimensional Paper Chromatography – Substances which have the same r_f values can be resolved with the help of two-dimensional paper chromatography.

7. Explain the metabolism of glucose to pyruvic acid.(Nov 2015, Nov 2017, May 2018, Nov 2019)

GLYCOLYSIS

- Glycolysis is the central pathway for the glucose catabolism in which glucose (6-carbon compound) is converted into pyruvate (3-carbon compound) through a sequence of 10 steps.
- Glycolysis takes place in both aerobic and anaerobic organisms and is the first step towards the metabolism of glucose.
- The glycolytic sequence of reactions differs from one species to the other in the mechanism of its regulation and the subsequent metabolic fate of the pyruvate formed at the end of the process.

- In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, which together release most of the energy contained in glucose.
- It is also referred to as Embden-Meyerhof-Parnas or EMP pathway, in honor of the pioneer workers in the field.
- A summary of the process of glycolysis cab be written as follows:
- $C_6H_{12}O_6 + 2ADP + 2Pi + 2NAD^+ \rightarrow 2C_3H_4O_3 + 2H_2O + 2ATP + 2NADH + 2H^+$
- In words, the equation is written as:
- Glucose + Adenosine diphosphate + Phosphate + Nicotinamide adenine dinucleotide

  ↓
- Pyruvate + Water + Adenosine triphosphate + Nicotinamide adenine dinucleotide + Hydrogen ions

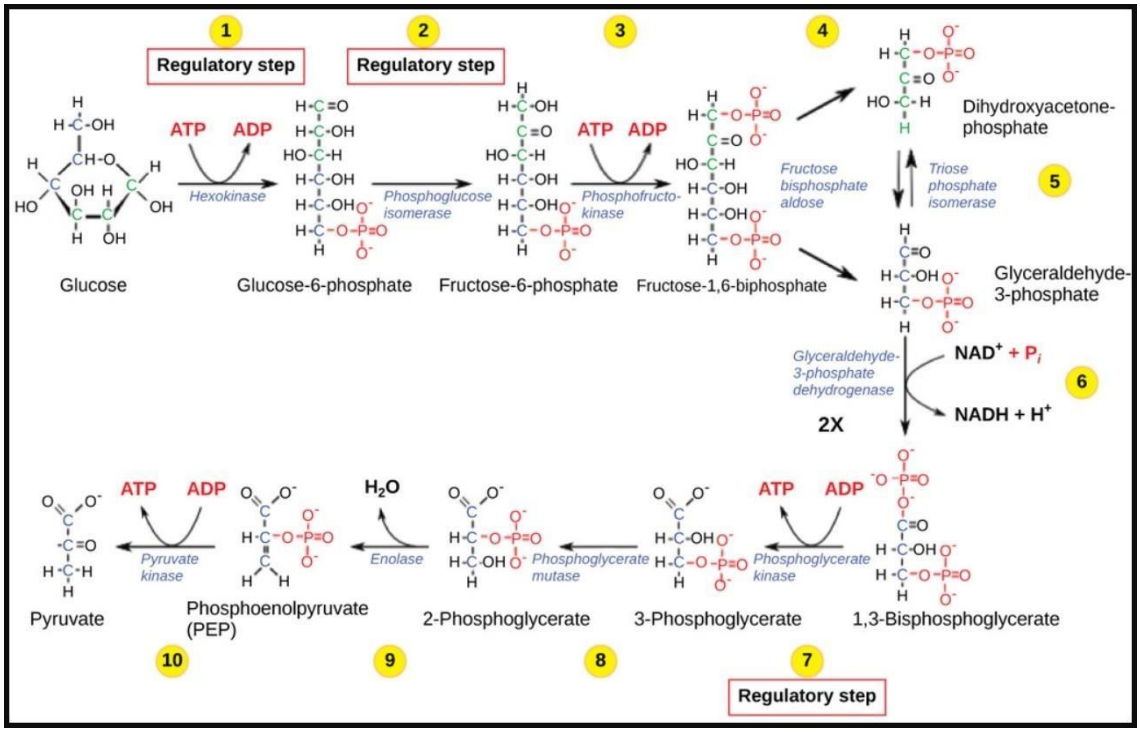
GLYCOLYSIS ENZYMES:

- In most kinds of cells, the enzymes that catalyze glycolytic reactions are present in the extra-mitochondrial fraction of the cell in the cytosol. One common characteristic in all the enzymes involved in glycolysis is that nearly all of them require Mg2+. The following are the enzymes that catalyze different steps throughout the process of glycolysis:
    1. Hexokinase
    2. Phosphoglucoisomerase
    3. Phosphofructokinase
    4. Aldolase
    5. Phosphotrioseisomerase
    6. Glyceraldehyde 3-phosphate dehydrogenase
    7. Phosphoglycerate kinase
    8. Phosphoglyceratemutase
    9. Enolase
    10. Pyruvate kinase

GLYCOLYSIS STEPS:

- During glycolysis, a single mole of 6-carbon glucose is broken down into two moles of 3-carbon pyruvate by a sequence of 10 enzyme-catalyzed sequential reactions. These reactions are grouped under 2 phases, phase I and II.

- Stage I comprises “preparatory” reactions which are not redox reactions and do not release energy but instead lead to the production of a critical intermediate of the pathway.
- Stage I consists of the first five steps of the glycolysis process.
- Similarly, in Stage II, redox reactions occur, energy is conserved in the form of ATP, and two molecules of pyruvate are formed.
- The last five reactions of glycolysis constitute phase II.



STEP 1:

- In the first step of glycolysis, the glucose is initiated or primed for the subsequent steps by phosphorylation at the C6 carbon.
- The process involves the transfer of phosphate from the ATP to glucose forming Glucose-6-phosphate in the presence of the enzyme hexokinase and glucokinase (in animals and microbes).
- This step is also accompanied by considerable loss of energy as heat.

STEP 2:

- Glucose 6-phosphate is reversibly isomerized to fructose 6-phosphate by the enzyme phosphohexoisomerase /phosphoglucoisomerase.

- This reaction involves a shift of the carbonyl oxygen from C1 to C2, thus converting an aldose into a ketose.

STEP 3:

- This step is the second priming step of glycolysis, where fructose-6-phosphate is converted into fructose-1,6-bisphosphate in the presence of the enzyme phosphofructokinase.
- Like in Step 1, the phosphate is transferred from ATP while some amount of energy is lost in the form of heat as well.

STEP 4:

- This step involves the unique cleavage of the C-C bond in the fructose 1, 6-bisphosphate.
- The enzyme fructose diphosphatealdolase catalyzes the cleavage of fructose 1,6-bisphosphate between C3 and C4 resulting in two different triose phosphates: glyceraldehyde 3-phosphate (an aldose) and dihydroxyacetone phosphate (a ketose).
- The remaining steps in glycolysis involve three-carbon units, rather than six carbon units.

STEP 5:

- Glyceraldehyde 3-phosphate can be readily degraded in the subsequent steps of glycolysis, but dihydroxyacetone phosphate cannot be. Thus, it is isomerized into glyceraldehyde 3-phosphate instead.
- In this step, dihydroxyacetone phosphate is isomerized into glyceraldehyde 3-phosphate in the presence of the enzyme triose phosphate isomerase.
- This reaction completes the first phase of glycolysis.

STEP 6:

- Step 6 is one of the three energy-conserving or forming steps of glycolysis.
- The glyceraldehyde 3-phosphate is converted into 1,3-bisphosphoglycerate by the enzyme glyceraldehyde 3-phosphate dehydrogenase (phosphoglyceraldehyde dehydrogenase).
- In this process, NAD+ is reduced to coenzyme NADH by the H– from glyceraldehydes 3-phosphate.
- Since two moles of glyceraldehyde 3-phosphate are formed from one mole of glucose, two NADH are generated in this step.

STEP 7:

- This step is the ATP-generating step of glycolysis.
- It involves the transfer of phosphate group from the 1, 3-bisphosphoglycerate to ADP by the enzyme phosphoglycerate kinase, thus producing ATP and 3-phosphoglycerate.
- Since two moles of 1, 3-bisphosphoglycerate are formed from one mole of glucose, two ATPs are generated in this step.

STEP 8:

- The 3-phosphoglycerate is converted into 2-phosphoglycerate due to the shift of phosphoryl group from C3 to C2, by the enzyme phosphoglyceratemutase.
- This is a reversible isomerization reaction.

STEP 9:

- In this step, the 2-phosphoglycerate is dehydrated by the action of enolase (phosphopyruvatehydratase) to phosphoenolpyruvate.
- This is also an irreversible reaction where two moles of water are lost.

STEP 10:

- This is the second energy-generating step of glycolysis.
- Phosphoenolpyruvate is converted into an enol form of pyruvate by the enzyme pyruvate kinase.
- The enol pyruvate, however, rearranges rapidly and non-enzymatically to yield the keto form of pyruvate (i.e. ketopyruvate). The keto form predominates at pH 7.0.
- The enzyme catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate to ADP, thus forming ATP.

RESULT OF GLYCOLYSIS:

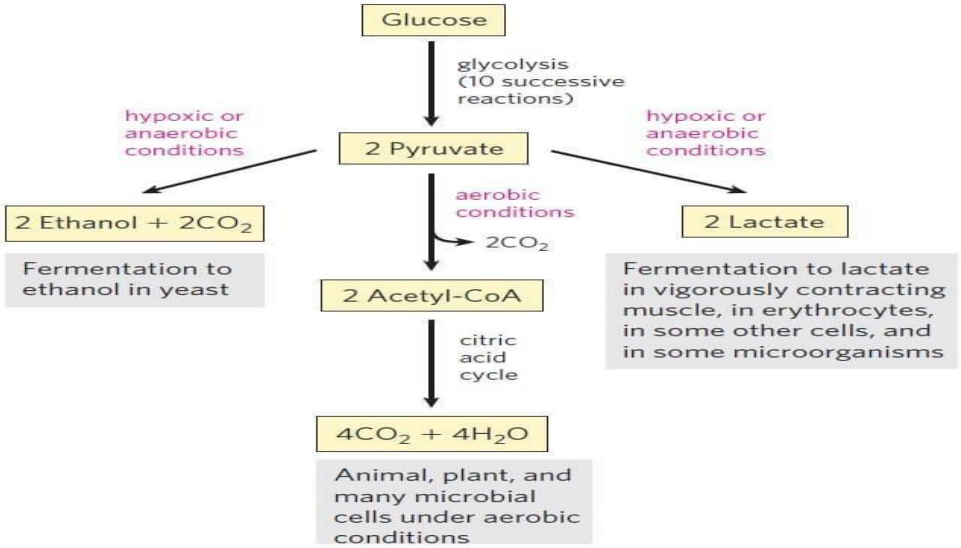
- The overall process of glycolysis results in the following events:
- Glucose is oxidized into pyruvate.
- NAD+ is reduced to NADH.
- ADP is phosphorylated into ATP.

FATES OF PYRUVATE:

- Three possible catabolic fates of the pyruvate formed in glycolysis. Pyruvate also serves as a precursor in many anabolic reactions.

1. OXIDATION OF PYRUVATE:

- In aerobic organisms, the pyruvate is then moved to the mitochondria where it is oxidized into the acetyl group of acetyl-coenzyme A (acetyl Co-A).



- This process involves the release of one mole of CO2.
- Later, the acetyl CoA is completely oxidized into CO2 and H2O by entering the citric acid cycle.
- This pathway follows glycolysis in aerobic organisms and plants.

2. LACTIC ACID FERMENTATION:

- In conditions where the oxygen is insufficient, like in the skeletal muscle cells, the pyruvate cannot be oxidized due to lack of oxygen.
- Under such conditions, the pyruvate is reduced to lactate by the process of anaerobic glycolysis.
- Lactate production from glucose also occurs in other anaerobic organisms by the process of lactic acid fermentation.

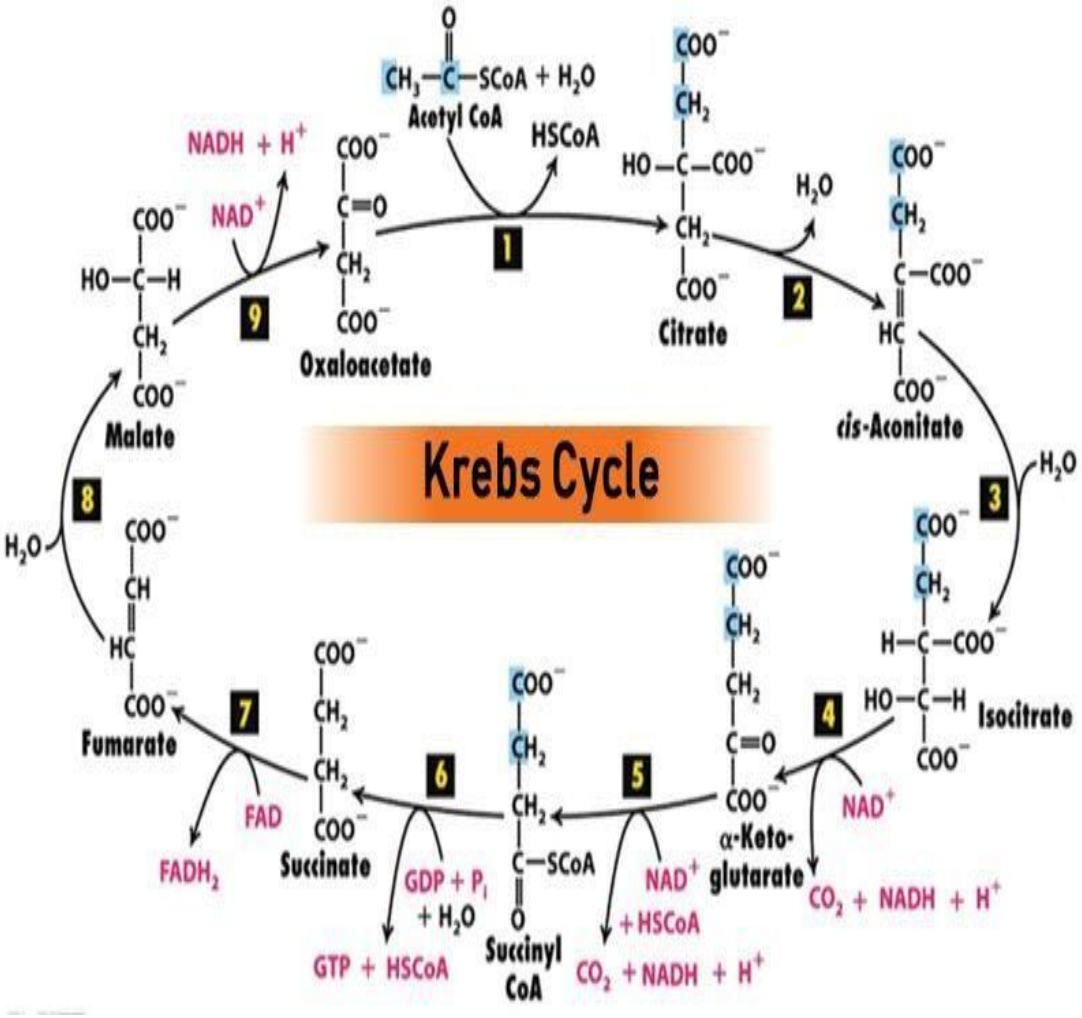
3. ALCOHOLIC FERMENTATION:

- In some microbes like brewer's yeast, the pyruvate formed from glucose is converted anaerobically into ethanol and CO2.
- This is considered the most ancient form of the metabolism of glucose, as observed in conditions where the oxygen concentration is low.

8. Describe the Tricarboxylic acid (TCA) cycle. (Nov 2018)

KREB'S CYCLE / CITRIC ACID CYCLE / TRICARBOXYLIC ACID CYCLE

- The tricarboxylic acid cycle (TCA cycle), also known as the citric acid cycle or the Krebs cycle, is a major energy-producing pathway in living bodies.
- Cells obtain ATP from breakdown of glucose in the absence of oxygen as in glycolysis.
- However, most organisms normally are aerobic and oxidize their organic fuels completely to CO_2 and water.
- Foodstuffs feed into the citric acid cycle as acetyl-CoA and the acetyl-CoA is oxidized to carbon dioxide and water in order to generate energy.
- Thus, under aerobic conditions, the generation of energy from glucose is the oxidative decarboxylation of pyruvate to form acetyl CoA.
- The cycle also serves in the synthesis of fatty acids, amino acids, and glucose.
- The citric acid cycle occurs in the mitochondrial matrix in the eukaryotes.
- All the enzymes of the TCA cycle are in the mitochondrial matrix except succinate dehydrogenase, which is in the inner mitochondrial membrane.
- However, in the prokaryotes, the reaction cycle occurs in plasma membrane.
- The oxidative decaroxylation of pyruvate (end product of glycolysis) to form acetyl CoA (initiator of Kreb's cycle) is the link between Glycolysis and the Citric acid cycle.
- In the conversion of pyruvate to acetyl CoA, each pyruvate molecule loses one carbon atom with the release of carbon dioxide.
- During the breakdown of pyruvate, electrons are transferred to NAD+ to produce NADH, which will be used by the cell to produce ATP.
- In the final step of the breakdown of pyruvate, an acetyl group is transferred to Coenzyme A to produce acetyl CoA.
- The cycle starts with the 4-carbon compound oxaloacetate, adds two carbons from acetyl-CoA, loses two carbons as CO2, and regenerates the 4-carbon compound oxaloacetate. Electrons are transferred by the cycle to NAD+ and FAD. As the electrons are subsequently passed to O2 by the electron transport chain, ATP is generated by the process of oxidative phosphorylation. ATP is also generated from GTP, produced in one reaction of the cycle by substrate-level phosphorylation.
- Oxidation of the carbons of acetyl-CoA to carbon dioxide requires capturing eight electrons from the molecule.



1. Acetyl-CoA and oxaloacetate condense, forming citrate.

- Enzyme: Citrate synthase.
- Cleavage of the high-energy thioester bond in acetyl-CoA provides the energy for this condensation.
- Citrate (the product) is an inhibitor of this reaction.

2. Citrate is isomerized to isocitrate by a rearrangement of the molecule.

- Enzyme: Aconitase.
- Aconitate serves as an enzyme-bound intermediate.
- Under physiological conditions, this is an unfavorable reaction, favoring citrate formation.

3. Isocitrate is oxidized to α-ketoglutarate.

- In a two-step reaction in which there is first an oxidation, and then a decarboxylation. CO2 is produced, and the electrons are passed to NAD+ to form

NADH and H+. This step captures two of the eight electrons present in the carbons of acetyl-CoA.

- Enzyme: Isocitrate dehydrogenase.
- This key regulatory enzyme of the TCA cycle is allosterically activated by ADP and inhibited by NADH.

4. Alpha-Ketoglutarate is converted to succinyl-CoA

- In an oxidative decarboxylation reaction, CO2 is released, and succinyl-CoA, NADH, and H+ are produced. This step captures another two electrons from the carbons of acetyl-CoA.
- Enzyme: Alpha-ketoglutarate dehydrogenase.
- This enzyme requires five cofactors: thiamine pyrophosphate, lipoic acid, CoASH, FAD, and NAD+.

5. Succinyl-CoA is cleaved to succinate.

- Cleavage of the high-energy thioester bond of succinyl- CoA provides energy for the substrate level phosphorylation of GDP to GTP.
- Enzyme: succinate thiokinase (succinyl-CoA synthetase).

6. Succinate is oxidized to fumarate.

- Succinate transfers two hydrogens together with their electrons to FAD, which forms FADH2. After this step, six of the eight electrons from the carbons in acetyl-CoA have been captured.
- Enzyme: Succinate dehydrogenase.

7. Fumarate is converted to malate by the addition of water across the double bond.

- Enzyme: Fumarase.

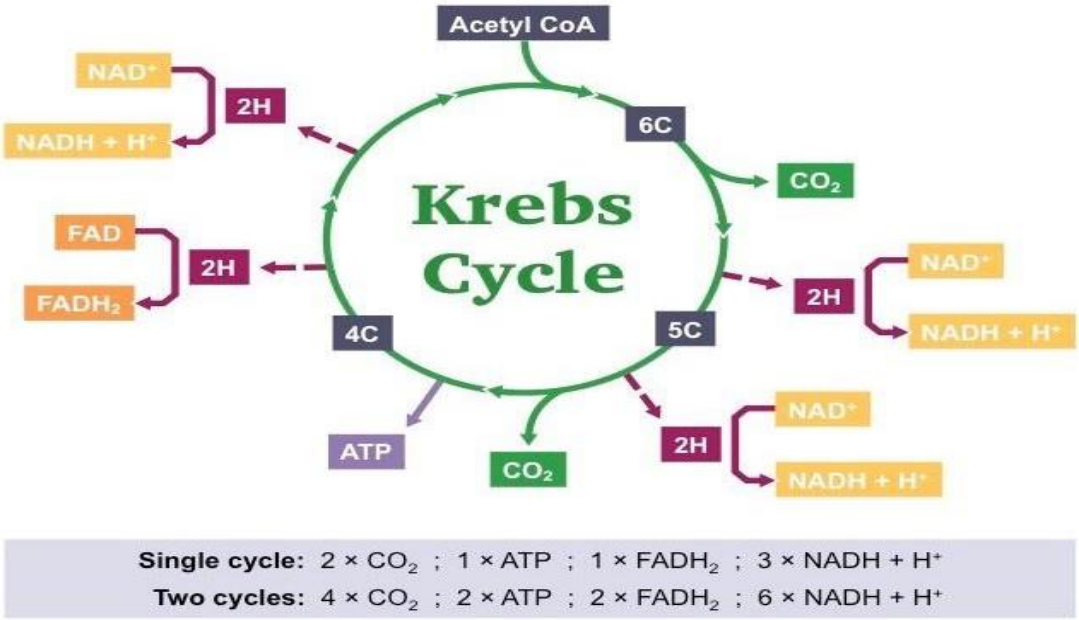
8. Malate is oxidized, regenerating oxaloacetate and thus completing the cycle.

- Two hydrogens along with their electrons are passed to NAD+, producing NADH and H+, and finishing the capture of the eight electrons from the carbons of acetyl-CoA.
- Enzyme: Malate dehydrogenase.

YIELD OF TCA:

- Each molecule of acetyl CoA entering the citric acid cycle yields the following:
- Two CO2
- Three NADH
- One FADH2
- One GTP

- Because each NADH will eventually produce 2.5 ATP and each FADH 2 will produce 1.5 ATP through the electron transport chain, the overall ATP yield from 1 acetyl CoA is 10 ATP (7.5 from NADH, 1.5 from FADH2 , and 1 from GTP).



FUNCTIONS:

- Oxidation of acetyl CoA to CO2.
- Formation of NADH and FADH2 for entrance into the electron transport chain and subsequent ATP generation.
- Synthesis of several important molecules, including succinyl CoA (precursor molecule of heme), oxaloacetate (early intermediate molecule in gluconeogenesis and substrate for amino acid synthesis), α-ketoglutarate (substrate for amino acid synthesis), and citrate (substrate for fatty acid synthesis).
- It is responsible for the major share of energy release and supply during aerobic respiration.
- Due to the many functions of the citric acid cycle is also considered to be the "central hub of metabolism". This is because, as most of the absorbed nutrients, the fuel molecules are oxidized ultimately within the Kreb's Cycle and its intermediates are used for various biosynthetic pathways.

REGULATION OF TCA CYCLE:

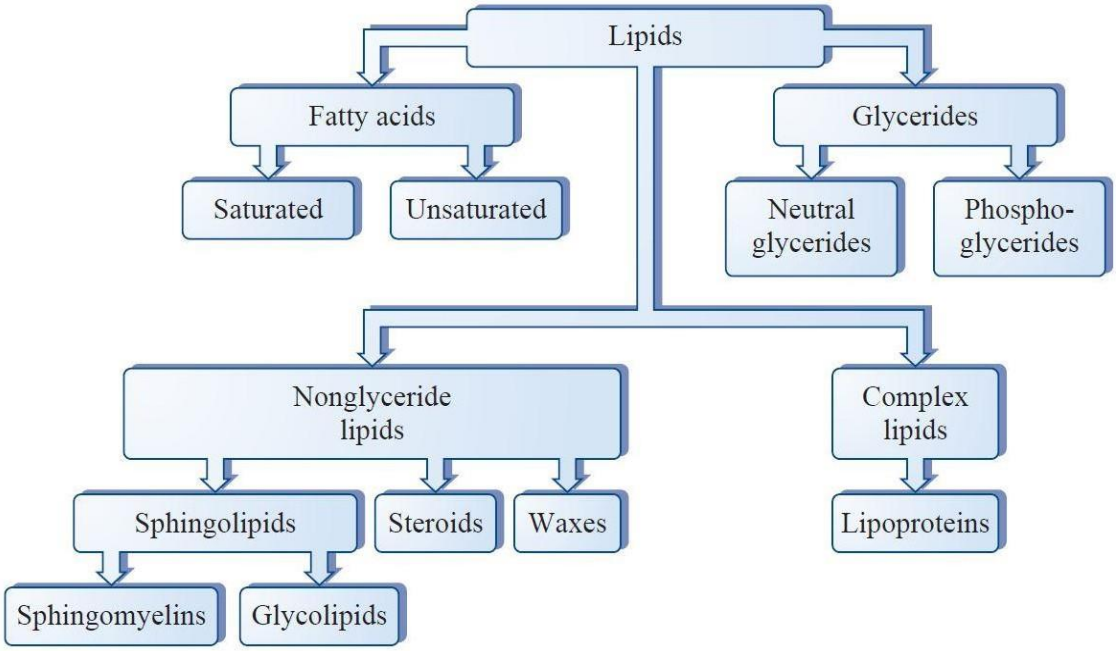
- The TCA Cycle is regulated in a variety of ways including:

- Metabolites: The products of the cycle provide negative feedback on the enzymes that catalyse it. For example, NADH inhibits the majority of the enzymes found in the TCA cycle.
- Citrate: Inhibits phosphofructokinase, a key enzyme in glycolysis. This reduces the rate of production of pyruvate and therefore of acetyl-coA.
- Calcium: Calcium accelerates the TCA cycle by stimulating the link reaction.

9. What are lipids? Give their classification. (Nov 2018)

A lipid is a type of organic molecule found in living things. It is oily or waxy. Fats are made from lipid molecules. ... Lipids are long chains of carbon and hydrogen molecules. Lipids are classified as simple and complex.

LIPID CLASSIFICATION



- Lipids are a heterogeneous group of organic compounds that are insoluble in water and soluble in non-polar organic solvents.
- They naturally occur in most plants, animals, microorganisms and are used as cell membrane components, energy storage molecules, insulation, and hormones.
- Lipids are molecules that contain hydrocarbons and make up the building blocks of the structure and function of living cells.
- Examples of lipids include fats, oils, waxes, certain vitamins (such as A, D, E and K), hormones and most of the cell membrane that is not made up of protein.

SYNTHESIS OF LIPIDS:

- Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown or storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes.
- In animals, these fats are obtained from food or are synthesized by the liver.
- Lipogenesis is the process of synthesizing these fats.
- The majority of lipids found in the human body from ingesting food are triglycerides and cholesterol.
- Other types of lipids found in the body are fatty acids and membrane lipids.
- Lipid metabolism is often considered as the digestion and absorption process of dietary fat; however, there are two sources of fats that organisms can use to obtain energy: from consumed dietary fats and from stored fat.
- Vertebrates (including humans) use both sources of fat to produce energy for organs such as the heart to function.
- Since lipids are hydrophobic molecules, they need to be solubilized before their metabolism can begin. Lipid metabolism often begins with hydrolysis, which occurs with the help of various enzymes in the digestive system.

PROPERTIES OF LIPIDS:

- 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.

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(s). The reaction results in the decolorization of the halogen solution.

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 triacylglycerols containing unsaturated fatty acids.

STRUCTURE OF LIPIDS:

- 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.
- Unsaturated 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.

CLASSIFICATION OF LIPIDS:

- Lipids can be classified into two main classes:
- 1. Nonsaponifiable lipids, and
- 2. Saponifiable lipids.

NON SAPONIFIABLE LIPIDS:

- A nonsaponifiable lipid cannot be disintegrated into smaller molecules through hydrolysis.
- Nonsaponifiable lipids include cholesterol, prostaglandins etc

SAPONIFIABLE LIPIDS:

- A saponifiable lipid comprises one or more ester groups, enabling it to undergo hydrolysis in the presence of a base, acid, or enzymes, including waxes, triglycerides, sphingolipids, and phospholipids.
- 1. Nonpolar lipids, namely triglycerides, are utilized as fuel and to store energy.
- 2. Polar lipids, that could form a barrier with an external water environment, are utilized in membranes. Polar lipids comprise sphingolipids and glycerophospholipids.

TYPES OF LIPIDS:

- Within these two major classes of lipids, there are numerous specific types of lipids important to live, including
- fatty acids,
- triglycerides,
- glycerophospholipids,
- sphingolipids, and
- steroids.
- These are broadly classified as simple lipids and complex lipids.

SIMPLE LIPIDS:

- Esters of fatty acids with various alcohols.
- Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state
- Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols

COMPOUND LIPIDS:

- Esters of fatty acids containing groups in addition to alcohol and a fatty acid.
- Phospholipids: These are lipids containing, in addition to fatty acids and alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
- Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine, and carbohydrate.

- Other complex lipids: Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category

DERIVED LIPIDS:

- These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones.
- Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids.
- These compounds are produced by the hydrolysis of simple and complex lipids.

FATTY ACIDS:

- Fatty acids are carboxylic acids (or organic acid), usually with long aliphatic tails (long chains), either unsaturated or saturated.
- Saturated fatty acids - Lack of carbon-carbon double bonds indicates that the fatty acid is saturated. The saturated fatty acids have higher melting points compared to unsaturated acids of the corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape. 2. Unsaturated fatty acids
- Unsaturated fatty acid is indicated when a fatty acid has more than one double bond. "Often, naturally occurring fatty acids possesses an even number of carbon atoms and are unbranched."
- On the other hand, unsaturated fatty acids contain a cis-double bond(s) which create a structural kink that disables them to group their molecules in straight rod-like shape.

FATS:

- Fats play several major roles in our body. Some of the important roles of fats are mentioned below:
- Fats in the correct amounts are necessary for the proper functioning of our body.
- Many fat-soluble vitamins need to be associated with fats in order to be effectively absorbed by the body.
- They also provide insulation to the body.
- They are an efficient way to store energy for longer periods.

WAXES:

- Waxes are "esters" (an organic compound made by replacing the hydrogen with acid by an alkyl or another organic group) formed from long-alcohols and long-chain carboxylic acids.

- Waxes are found almost everywhere. Fruits and leaves of many plants possess waxy coatings, that can safeguard them from small predators and dehydration.
- Fur of a few animals and the feathers of birds possess same coatings serving as water repellants.
- Carnauba wax is known for its water resistance and toughness (significant for car wax).

PHOSPHOLIPIDS:

- Membranes are primarily composed of phospholipids that are Phosphoacylglycerols.
- Triacylglycerols and phosphoacylglycerols are the same, but, the terminal OH group of the phosphoacylglycerol is esterified with phosphoric acid in place of fatty acid which results in the formation of phosphatidic acid.
- The name phospholipid is derived from the fact that phosphoacylglycerols are lipids containing a phosphate group.

STEROIDS:

- Our bodies possess chemical messengers known as hormones, that are basically organic compounds synthesized in glands and transported by the bloodstream to various tissues in order to trigger or hinder the desired process.
- Steroids are a kind of hormone that is typically recognized by their tetracyclic skeleton, composed of three fused six-membered and one five-membered ring, as seen above. The four rings are assigned as A, B, C & D as observed in the shade blue, while the numbers in red indicate the carbons.

CHOLESTEROL:

- Cholesterol is a wax-like substance, found only in animal source foods. Triglycerides, LDL, HDL, VLDL are different types of cholesterol found in the blood cells.
- Cholesterol is an important lipid found in the cell membrane. It is a sterol, which means that cholesterol is a combination of steroid and alcohol. In the human body, cholesterol is synthesized in the liver.
- These compounds are biosynthesized by all living cells and are essential for the structural component of the cell membrane.
- In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps boost the rigidity of the cell membrane. Without cholesterol, the cell membrane would be too fluid.

- It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D.

LIPOPROTEIN:

- Complex lipids are lipids that are bonded to other types of molecules. The most common and important complex lipids are plasma lipoproteins, which are responsible for the transport of other lipids in the body.
- Lipids are only sparingly soluble in water, and the movement of lipids from one organ to another through the bloodstream requires a transport system that uses plasma lipoproteins. Lipoprotein particles consist of a core of hydrophobic lipids surrounded by amphipathic proteins, phospholipids, and cholesterol.

UNIT-IV

2 MARKS:

1. What is Cystometrogram? (May 2015)

Cystometry, or cystometrogram, with a pressure flow study is part of urodynamic testing (or UDS). These tests measure how well the bladder functions. They help diagnose problems related to urine control. These can be incontinence, difficulty emptying the bladder, overactive bladder, obstructions or frequent infections. Cystometry is used to measure how much urine the bladder can hold. It also measures pressure inside the bladder, and how full it is when you have the urge to go.

2. What are the normal values of Glucose, Cholesterol, urea, uric acid and bilirubin? (May 2015, Nov 2017)

- **Glucose:** A blood sugar level less than 140 mg/dL(7.8 mmol/L) is normal. A reading of more than 200 mg/dL(11.1 mmol/L) after two hours indicates diabetes. A reading between 140 and 199 mg/dL (7.8 mmol/L and 11.0 mmol/L) indicates prediabetes.

- **Cholesterol:** Total cholesterol levels less than 200 milligrams per deciliter (mg/dL) are considered desirable for adults. A reading between 200 and 239 mg/dL is considered borderline high and a reading of 240 mg/dL and above is considered high. LDL cholesterol levels should be less than 100 mg/dL.

- **Urea:** The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl, or 1.8 to 7.1 mmol urea per liter.

- **Uric acid:** The reference ranges for uric acid in the blood are as follows: Adult male: 4.0-8.5 mg/dL or 0.24-0.51 mmol/L. Adult female: 2.7-7.3 mg/dL or 0.16-0.43 mmol/L. Elderly: A slight increase in values may occur.

- **Bilirubin:** Normal results for a total bilirubin test are 1.2 milligrams per deciliter (mg/dL) for adults and usually 1 mg/dL for those under 18.

3. Write the function of liver. (Nov 2015, Nov 2017)

The liver has many functions that are vital to life. Briefly, some of the important functions of the human liver are:

- Detoxification of blood
- Metabolizing (processing) medications and nutrients
- Processing of waste products of hemoglobin and other cells
- Storing of vitamins, fat, cholesterol, and bile.
- Production of important clotting factors, albumin, and many other important proteins.

4. How to perform the stool test? (Nov 2015, Nov 2016)

You need to collect stool samples at home, using latex gloves and plastic wrap. The plastic wrap should be covered in the toilet before using it. The sample should not contain urine or toilet water. After collecting the sample in a container, it should be returned to the lab right away, for getting accurate results.

5. What happens if the abnormal levels of electrolytes are observed in blood? (May 2016)

When these substances become imbalanced, it can lead to either muscle weakness or excessive contraction. The heart, muscle, and nerve cells use electrolytes to carry electrical impulses to other cells.

6. What is the importance of liver function test? (May 2016)

Liver function tests are blood tests used to help diagnose and monitor liver disease or damage. The tests measure the levels of certain enzymes and proteins in your blood.

7. What is the importance of Addis test? (Nov 2016)

The Addis count is a urine test measuring urinary casts over time. It is named for Thomas Addis. A urine test may be done:

- To check for a disease or infection of the urinary tract. Symptoms of a urine infection may include colored or bad-smelling urine, pain when urinating, finding it hard to urinate, flank pain, blood in the urine (hematuria), or fever.

8. Define GTT. (May 2017)

GTT short for Glucose Tolerance Test is a test designed to assess the body response to glucose. In GTT, the patient is given a glucose solution and blood samples are drawn afterword at intervals to measure how well the body cells are able to absorb glucose.

9. What are the abnormal constituents of urine? (May 2017)

Abnormal constituents of urine are sugar, proteins, blood, bile salts, bile pigments and ketone bodies.

10. Define detoxification. (May 2018)

Detoxification or detoxication (detox for short) is the physiological or medicinal removal of toxic substances from a living organism, including the human body, which is mainly carried out by the liver.

11. What is meant by glomerular filtration rate? (May 2018)

Glomerular filtration rate (GFR) is a test used to check how well the kidneys are working. Specifically, it estimates how much blood passes through the glomeruli each minute. Glomeruli are the tiny filters in the kidneys that filter waste from the blood.

12. Name the bile salts and bile pigments. (Nov 2018)

- Bile salts are composed of the salts of four different kinds of free bile acids (cholic, deoxycholic, chenodeoxycholic, and lithocholic acids); each of these acids may in turn combine with glycine or taurine to form more complex acids and salts.
- The two most important bile pigments are bilirubin, which is orange or yellow, and its oxidized form biliverdin, which is green. Mixed with the intestinal contents, they give the brown colour to the faeces (see urobilinogen).

13. What are electrolytes? Write any two functions of electrolytes? Give example. (Nov 2018, May 2019, Nov 2019)

Electrolytes are involved in many essential processes in your body. They play a role in conducting nervous impulses, contracting muscles, keeping you hydrated and regulating your body's pH levels.

Functions:

(i) Proper nerve and muscle function.(ii) maintaining acid-base balance

Examples:

- Sodium, Potassium, Chloride, Calcium, Magnesium, Bicarbonate.

14. List down any two RFT. (May 2019)

- SERUM CREATININE TEST
- GLOMERULAR FILTARTION RATE

15. What is protein denaturation? (Nov 2019)

Denaturation involves the breaking of many of the weak linkages, or bonds (e.g., hydrogen bonds), within a protein molecule that are responsible for the highly ordered structure of the protein in its natural (native) state. Denatured proteins have a looser, more random structure; most are insoluble.

11 MARKS:

1. Discuss in detail about the blood gas analyzer with neat diagram. (May 2015)

BLOOD GAS ANALYSIS

- A blood gas test measures the amount of oxygen and carbon dioxide in the blood. It may also be used to determine the pH of the blood, or how acidic it is. The test is commonly known as a blood gas analysis or arterial blood gas (ABG) test.
- Your red blood cells transport oxygen and carbon dioxide throughout your body. These are known as blood gases.
- As blood passes through your lungs, oxygen flows into the blood while carbon dioxide flows out of the blood into the lungs. The blood gas test can determine how well your lungs are able to move oxygen into the blood and remove carbon dioxide from the blood.
- A blood gas test provides a precise measurement of the oxygen and carbon dioxide levels in your body. This can help your doctor determine how well your lungs and kidneys are working.
- This is a test that is most commonly used in the hospital setting to determine the management of acutely ill patients. It doesn't have a very significant role in the primary care setting, but may be used in a pulmonary function lab or clinic.

- An ABG test requires that a small volume of blood be drawn from the radial artery with a syringe and a thin needle,but sometimes the femoral artery in the groin or another site is used. The blood can also be drawn from an arterial catheter.
- An ABG test measures the blood gas tension values of the arterial partial pressure of oxygen (PaO2), and the arterial partial pressure of carbon dioxide (PaCO2), and the blood's pH. In addition, the arterial oxygen saturation (SaO2) can be determined.
- An ABG test can also measure the level of bicarbonate in the blood. Many blood-gas analyzers will also report concentrations of lactate, hemoglobin, several electrolytes, oxyhaemoglobin, carboxyhemoglobin, and methemoglobin.
- ABG testing is mainly used in pulmonology and critical-care medicine to determine gas exchange across the alveolar-capillary membrane.
- Standard blood tests can also be performed on arterial blood, such as measuring glucose, lactate, hemoglobins,dyshaemoglobin, bilirubin and electrolyte

DIAGNOSIS PF MEDICAL CONDITIONS:

- Imbalances in the oxygen, carbon dioxide, and pH levels of your blood can indicate the presence of certain medical conditions. These may include:
- kidney failure
- heart failure
- uncontrolled diabetes
- hemorrhage
- chemical poisoning
- a drug overdose
- shock

SYMPTOMS:

- Your doctor may order a blood gas test if you’re showing symptoms of an oxygen, carbon dioxide, or pH imbalance. The symptoms can include:
- shortness of breath
- difficulty breathing
- confusion
- nausea

- These symptoms may be signs of certain medical conditions, including asthma and chronic obstructive pulmonary disease (COPD).
- Your doctor may also order a blood gas test if they suspect you're experiencing any of the following conditions:
- lung disease
- kidney disease
- metabolic disease
- head or neck injuries that affect breathing

BLOOD GAS ANALYSIS TEST:

- Arterial blood pH, which indicates the amount of hydrogen ions in blood. A pH of less than 7.0 is called acidic, and a pH greater than 7.0 is called basic, or alkaline. A lower blood pH may indicate that your blood is more acidic and has higher carbon dioxide levels. A higher blood pH may indicate that your blood is more basic and has a higher bicarbonate level.
- Bicarbonate, which is a chemical that helps prevent the pH of blood from becoming too acidic or too basic.
- Partial pressure of oxygen, which is a measure of the pressure of oxygen dissolved in the blood. It determines how well oxygen is able to flow from the lungs into the blood.
- Partial pressure of carbon dioxide, which is a measure of the pressure of carbon dioxide dissolved in the blood. It determines how well carbon dioxide is able to flow out of the body.
- Oxygen saturation, which is a measure of the amount of oxygen being carried by the hemoglobin in the red blood cells.

TEST PROCEDURE

- Blood is most commonly drawn from the radial artery because it is easily accessible, can be compressed to control bleeding, and has less risk for vascular occlusion.
- The selection of which radial artery to draw from is based on the outcome of an Allen's test.
- The brachial artery (or less often, the femoral artery) is also used, especially during emergency situations or with children.

- Blood can also be taken from an arterial catheter already placed in one of these arteries.
- There are plastic and glass syringes used for blood gas samples.
- Most syringes come pre-packaged and contain a small amount of heparin, to prevent coagulation.
- Other syringes may need to be heparinised, by drawing up a small amount of liquid heparin and squirting it out again to remove air bubbles.
- Once the sample is obtained, care is taken to eliminate visible gas bubbles, as these bubbles can dissolve into the sample and cause inaccurate results. The sealed syringe is taken to a blood gas analyzer.
- If a plastic blood gas syringe is used, the sample should be transported and kept at room temperature and analyzed within 30 min.
- If prolonged time delays are expected (i.e., greater than 30 min) prior to analysis, the sample should be drawn in a glass syringe and immediately placed on ice.
- Derived parameters include bicarbonate concentration, SaO2, and base excess.
- Bicarbonate concentration is calculated from the measured pH and PCO2 using the Henderson-Hasselbalch equation.
- SaO2 is derived from the measured PO2 and calculated based on the assumption that all measured hemoglobin is normal (oxy- or deoxy-) hemoglobin.

BLOOD GAS ANALYSER:

- Analyzers used to measure blood gas, pH, electrolytes, and some metabolites in whole blood specimens.
- They can measure pH, partial pressure of carbon dioxide and oxygen, and concentrations of many ions (sodium, potassium, chloride, bicarbonate) and metabolites (calcium, magnesium, glucose, lactate).
- They are also used to determine abnormal metabolite and/or electrolyte levels in blood and the patient's acid-base balance and levels of oxygen/carbon dioxide exchange.
- Handheld device or benchtop device, sometimes placed on a cart, with a display (usually LCD), a keypad to enter information, and a slot to insert a test strip or sample tube.
- Some models may have alarms, memory functions, touchpens, USB ports to transfer data to a computer, and/or a small storage compartment for reagents.

- Blood gas/pH analyzers use electrodes to determine pH, partial pressure of carbon dioxide, and partial pressure of oxygen in the blood.
- Chemistry analyzers use a dry reagent pad system in which a fi lter pad impregnated with all reagents required for a particular reaction is placed on a thin plastic strip.
- Electrolyte analyzers use ion-selective electrode (ISE) methodology in which measurements of the ion activity in the solution are made potentiometrically using an external reference electrode and an ISE containing an internal reference electrode.

INTERPRETATION OF RESULT:

- The results of a blood gas test can help your doctor diagnose various diseases or determine how well treatments are working for certain conditions, including lung diseases. It also shows whether or not your body is compensating for the imbalance.
- Due to the potential for compensation in some values that will cause the correction of other values, it's essential that the person interpreting the result be a trained healthcare provider with experience in blood gas interpretation.

NORMAL VALUES:

- In general, normal values include:
- arterial blood pH: 7.38 to 7.42
- bicarbonate: 22 to 28 milliequivalents per liter
- partial pressure of oxygen: 75 to 100 mm Hg
- partial pressure of carbon dioxide: 38 to 42 mm Hg
- oxygen saturation: 94 to 100 percent

SIDE EFFECTS:

- Possible side effects associated with the blood gas test include:
- bleeding or bruising at the puncture site
- feeling faint
- blood accumulating under the skin
- infection at the puncture site

2. Discuss in detail about the LFT with its significance. (May 2015, Nov 2015, May 2016, Nov 2016, May 2017, Nov 2017, May 2018, May 2019, Nov 2019)

LIVER FUNCTION TEST

- The liver is located in the right upper portion of the abdominal cavity just beneath the rib cage.
- The liver has many functions that are vital to life. Briefly, some of the important functions of the human liver are:
    - Detoxification of blood
    - Production of important clotting factors, albumin, and many other important proteins
    - Metabolizing (processing) medications and nutrients
    - Processing of waste products of hemoglobin and other cells
    - Storing of vitamins, fat, cholesterol, and bile
    - Production of glucose (gluconeogenesis or glucose synthesis/release during starvation)
- Liver function tests are blood tests that measure different enzymes, proteins, and other substances made by the liver.
- These tests check the overall health of your liver.
- The different substances are often tested at the same time on a single blood sample, and may include the following:
    1. Albumin, a protein made in the liver
    2. Total protein. This test measures the total amount of protein in the blood.
    3. ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), and gamma-glutamyltranspeptidase (GGT). These are different enzymes made by the liver.
    4. Bilirubin, a waste product made by the liver.
    5. Lactate dehydrogenase (LD), an enzyme found in most of the body's cells. LD is released into the blood when cells have been damaged by disease or injury.
    6. Prothrombin time (PT), a protein involved in blood clotting.
- If levels of one or more of these substances are outside of the normal range, it may be a sign of liver disease.
- Other names: liver panel, liver function panel, liver profile hepatic function panel, LFT
- Commonly used tests to check liver abnormalities are tests checking:
    1. alanine transaminase (ALT)

2. aspartate aminotransferase (AST)
3. alkaline phosphatase (ALP)
4. albumin
5. bilirubin

- The ALT and AST tests measure enzymes that your liver releases in response to damage or disease. The albumin test measures how well the liver creates albumin, while the bilirubin test measures how well it disposes of bilirubin. ALP can be used to evaluate the bile duct system of the liver.
- Having abnormal results on any of these liver tests typically requires follow up to determine the cause of the abnormalities. Even mildly elevated results can be associated with liver disease. However, these enzymes can also be found in other places besides the liver.
- Liver function tests are used to measure specific enzymes and proteins in your blood.
- Depending on the test, either higher- or lower-than-normal levels of these enzymes or proteins can indicate a problem with your liver.
- Some common liver function tests include:

1. Alanine transaminase (ALT) test:

- Alanine transaminase (ALT) is used by your body to metabolize protein. If the liver is damaged or not functioning properly, ALT can be released into the blood. This causes ALT levels to increase.
- A higher than normal result on this test can be a sign of liver damage.
- According to the American College of Gastroenterology, an ALT above 25 IU/L (international units per liter) in females and 33 IU/L in males typically requires further testing and evaluation.

2. Aspartate aminotransferase (AST) test

- Aspartate aminotransferase (AST) is an enzyme found in several parts of your body, including the heart, liver, and muscles. Since AST levels aren't as specific for liver damage as ALT, it's usually measured together with ALT to check for liver problems.
- When the liver is damaged, AST can be released into the bloodstream. A high result on an AST test might indicate a problem with the liver or muscles.
- The normal range for AST is typically up to 40 IU/L in adults and may be higher in infants and young children.

3. Alkaline phosphatase (ALP) test

- Alkaline phosphatase (ALP) is an enzyme found in your bones, bile ducts, and liver. An ALP test is typically ordered in combination with several other tests.
- High levels of ALP may indicate liver inflammation, blockage of the bile ducts, or a bone disease.
- Children and adolescents may have elevated levels of ALP because their bones are growing. Pregnancy can also raise ALP levels. The normal range for ALP is typically up to 120 U/L in adults.

4. Albumin test

- Albumin is the main protein made by your liver. It performs many important bodily functions. For example, albumin:

- stops fluid from leaking out of your blood vessels
- nourishes your tissues
- transports hormones, vitamins, and other substances throughout your body

- An albumin test measures how well your liver is making this particular protein. A low result on this test can indicate that your liver isn't functioning properly.
- The normal range for albumin is 3.5–5.0 grams per deciliter (g/dL). However, low albumin can also be a result of poor nutrition, kidney disease, infection, and inflammation.

5. Bilirubin test

- Bilirubin is a waste product from the breakdown of red blood cells. It's ordinarily processed by the liver. It passes through the liver before being excreted through your stool.
- A damaged liver can't properly process bilirubin. This leads to an abnormally high level of bilirubin in the blood. A high result on the bilirubin test may indicate that the liver isn't functioning properly.
- The normal range for total bilirubin is typically 0.1–1.2 milligrams per deciliter (mg/dL). There are certain inherited diseases that raise bilirubin levels, but the liver function is normal.

6. Gamma-glutamyltransferase(GGT) test

- High levels of the GGT enzyme could point to liver or bile duct damage.

7. L- lactate dehydrogenase (LD) test

- LD is another enzyme that's high when you have liver damage, but other conditions can also raise its level.

8. Prothrombin time (PT) test

- This test measures how long it takes your blood to clot. If it takes a long time, that could be a sign of liver damage. Medications that thin your blood, such as warfarin (Coumadin), can also lead to a longer PT. You probably won't have this test until you have had other liver tests.

SYMPTOMS OF LIVER DISORDER:

Symptoms of a liver disorder include:

- weakness
- fatigue or loss of energy
- weight loss
- jaundice (yellow skin and eyes)
- fluid collection in the abdomen, known as ascites
- discolored bodily discharge (dark urine or light stools)
- nausea
- vomiting
- diarrhea
- abdominal pain
- abnormal bruising or bleeding

PERFORMANCE OF LFT:

You may have your blood drawn in a hospital or at a specialized testing facility. To administer the test:

1. The healthcare provider will clean your skin before the test to decrease the likelihood that any microorganisms on your skin will cause an infection.
2. They'll likely wrap an elastic strap on your arm. This will help your veins become more visible. They'll use a needle to draw samples of blood from your arm.
3. After the draw, the healthcare provider will place some gauze and a bandage over the puncture site. Then they'll send the blood sample to a laboratory for testing.

RISKS OF LFT:

Blood draws are routine procedures and rarely cause any serious side effects. However, the risks of giving a blood sample can include:

- bleeding under the skin, or hematoma

- excessive bleeding
- fainting
- infection

AFTER A LIVER FUNCTION TEST:

- After the test, you can usually leave and go about your life as usual. However, if you feel faint or lightheaded during the blood draw, you should rest before you leave the testing facility.
- The results of these tests may not tell your doctor exactly which condition you have or the degree of any liver damage, but they might help your doctor determine the next steps. Your doctor will call you with the results or discuss them with you at a follow-up appointment.
- In general, if your results indicate a problem with your liver function, your doctor will review your medications and your past medical history to help determine the cause.
- If you drink alcohol heavily, then you'll need to stop drinking. If your doctor identifies that a medication is causing the elevated liver enzymes, then they'll advise you to stop the medication.
- Your doctor may decide to test you for hepatitis, other infections, or other diseases that can affect the liver. They may also choose to do imaging, like an ultrasound or CT scan. They may recommend a liver biopsy to evaluate the liver for fibrosis, fatty liver disease, or other liver conditions.

NORMAL VALUES:

- ALT. 7 to 55 units per liter (U/L)
- AST. 8 to 48 U/L
- ALP. 40 to 129 U/L
- Albumin. 3.5 to 5.0 grams per deciliter (g/dL)
- Total protein. 6.3 to 7.9 g/dL
- Bilirubin. 0.1 to 1.2 milligrams per deciliter (mg/dL)
- GGT. 8 to 61 U/L
- LD. 122 to 222 U/L
- PT. 9.4 to 12.5 seconds

These results are typical for adult men. Normal results vary from laboratory to laboratory and might be slightly different for women and children.

3. Discuss the various RFT. (Nov 2015, May 2017, Nov 2017, May 2018, Nov 2018, Nov 2019)

RENAL FUNCTION TEST

- A renal panel is a group of tests that may be performed together to evaluate kidney (renal) function.
- The tests measure levels of various substances, including several minerals, electrolytes, proteins, and glucose (sugar), in the blood to determine the current health of your kidneys.
- The kidneys are a pair of bean-shaped organs located at the bottom of the ribcage to the right and left of the spine.
- They are part of the urinary tract and perform a few essential roles and functions within the body.
- Within the kidneys are about a million tiny blood filtering units called nephrons. In each nephron, blood is continually filtered through a cluster of looping blood vessels, called a glomerulus, which allows the passage of water and small molecules but retains blood cells, proteins such as albumin, and larger molecules.
- Attached to each glomerulus are tubes (tubules) that have a number of sections that collect the fluid and molecules that pass through the glomerulus, reabsorb what can be re-used by the body, add other molecules through a process called secretion and, finally, adjust the amount of water that is eventually eliminated along with the waste as urine.
- Besides eliminating wastes and helping to regulate the amount of water in the body, these activities allow the kidneys to maintain normal chemical balance in the body.
- Among the important substances the kidneys help to regulate are sodium, potassium, chloride, bicarbonate, calcium, phosphorus, and magnesium. The right balance of these substances is critical.
- When the kidneys are not working properly, the concentrations of these substances in the blood may be abnormal and waste products and fluid may build up to dangerous levels in the blood, creating a life-threatening situation.
- Kidneys also have a number of other miscellaneous roles in maintaining a healthy body, including the production of a hormone that stimulates red blood cell

production (called erythropoietin), production of a hormone that helps maintain a normal blood pressure (called renin), and turning one form of vitamin D into a more active form, which enhances calcium absorption.

- If the kidneys are not functioning properly, waste products can accumulate in the blood and fluid levels can increase to dangerous volumes, causing damage to the body or a potentially life-threatening situation. Numerous conditions and diseases can result in damage to the kidneys.
- The most common causes of and main risk factors for kidney disease are diabetes and hypertension.
- The individual tests included in a renal panel can vary by laboratory, but the tests typically performed include:

ELECTROLYTES:

- Electrolytes – electrically charged chemicals that are vital to normal body processes, such as nerve and muscle function; among other things, they help regulate the amount of fluid in the body and maintain the acid-base balance. Electrolytes include:
- Sodium
- Potassium
- Chloride
- Bicarbonate (Total CO2)

MINERALS:

- Phosphorus – a mineral that is vital for energy production, muscle and nerve function, and bone growth; it also plays an important role as a buffer, helping to maintain the body's acid-base balance.
- Calcium – one of the most important minerals in the body; it essential for the proper functioning of muscles, nerves, and the heart and is required in blood clotting and in the formation of bones.

PROTEIN:

- Albumin – a protein that makes up about 60% of protein in the blood and has many roles such as keeping fluid from leaking out of blood vessels and transporting hormones, vitamins, drugs, and ions like calcium throughout the body.

WASTE PRODUCTS:

- Urea/Blood Urea Nitrogen (BUN) – urea is a nitrogen-containing waste product that forms from the metabolism of protein; it is released by the liver into the blood and is carried to the kidneys, where it is filtered out of the blood and eliminated in the urine.
- Creatinine – another waste product that is produced by the body's muscles; almost all creatinine is eliminated by the kidneys.

ENERGY SOURCE:

- Glucose – supplies energy for the body; a steady amount must be available for use, and a relatively constant level of glucose must be maintained in the blood.

SERUM CREATININE TEST:

- Creatinine is a waste product that comes from the normal wear and tear on muscles of the body.
- Creatinine levels in the blood can vary depending on age, race and body size.
- A creatinine level of greater than 1.2 for women and greater than 1.4 for men may be an early sign that the kidneys are not working properly.
- As kidney disease progresses, the level of creatinine in the blood rises.

GLOMERULAR FILTARTION RATE:

- This test is a measure of how well the kidneys are removing wastes and excess fluid from the blood.
- It is calculated from the serum creatinine level using age and gender with adjustment for those of African American descent.
- Normal GFR can vary according to age (as you get older it can decrease).
- The normal value for GFR is 90 or above.
- A GFR below 60 is a sign that the kidneys are not working properly.
- Once the GFR decreases below 15, one is at high risk for needing treatment for kidney failure, such as dialysis or a kidney transplant.

BLOOD UREA NITROGEN:

- Urea nitrogen comes from the breakdown of protein in the foods you eat.
- A normal BUN level is between 7 and 20. As kidney function decreases, the BUN level rises.

ULTRASOUND:

- This test uses sound waves to get a picture of the kidney.

- It may be used to look for abnormalities in size or position of the kidneys or for obstructions such as stones or tumors.

CT SCAN:

- This imaging technique uses X-rays to picture the kidneys.
- It may also be used to look for structural abnormalities and the presence of obstructions.
- This test may require the use of intravenous contrast dye which can be of concern for those with kidney disease.

KIDNEY BIOPSY:

- A biopsy may be done occasionally for one of the following reasons:
- 1. To identify a specific disease process and determine whether it will respond to treatment
- 2. To evaluate the amount of damage that has occurred in the kidney
- 3. To find out why a kidney transplant may not be doing well
- 4. A kidney biopsy is performed by using a thin needle with a sharp cutting edge to slice small pieces of kidney tissue for examination under a microscope.

URINE TESTS:

- Some urine tests require only a couple tablespoons of urine.
- Other tests require collection of all urine produced for a full 24 hours.
- A 24-hour urine test shows how much urine your kidneys produce, can give an more accurate measurement of how well your kidney are working and how much protein leaks from the kidney into the urine in one day.

URINALYSIS:

- Includes microscopic examination of a urine sample as well as a dipstick test.
- The dipstick is a chemically treated strip, which is dipped into a urine sample.
- The strip changes color in the presence of abnormalities such as excess amounts of protein, blood, pus, bacteria and sugar.
- A urinalysis can help to detect a variety of kidney and urinary tract disorders, including chronic kidney disease, diabetes, bladder infections and kidney stones.

URINE PROTEIN:

- This may be done as part of a urinalysis or by a separate dipstick test.
- An excess amount of protein in the urine is called proteinuria (pro-TEEN-yu-ree-uh).

- A positive dipstick test (1+ or greater) should be confirmed using a more specific dipstick test such as an albumin specific dipstick or a quantitative measurement such as an albumin-to-creatinine ratio.

MICROALBUMINURIA:

- This is a more sensitive dipstick test which can detect a tiny amount of protein called albumin in the urine.
- People who have an increased risk of developing kidney disease, such as those with diabetes or high blood pressure, should have this test or an albumin-to-creatinine ratio if their standard dipstick test for proteinuria is negative.

CREATININE CLEARANCE:

- Creatinine is a waste product that comes from the normal wear and tear on muscles of the body.
- Creatinine clearance test compares the creatinine in a 24-hour sample of urine to the creatinine level in your blood to show how much waste products the kidneys are filtering out each minute.

RESULT:

- Positive results on multiple tests are signs that there is an underlying issue in the kidneys.
- The results of each of these tests help doctors get a better picture of a person's overall kidney health. The tests can also identify markers of kidney disease and justify ordering more tests.
- Once they have identified that there is an issue in the kidneys, doctors will work to thoroughly diagnose the problem and develop a treatment plan.
- Many possible underlying conditions can lead to the loss of kidney function. A thorough diagnosis is key to finding the correct treatment in each case.

4. How is blood electrolytes measured? (May 2016, Nov 2018)

MEASUREMENT OF ELECTROLYTES

- Electrolytes are positively and negatively charged molecules called ions, that are found within the body's cells and extracellular fluids, including blood plasma.
- A test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate. These ions are measured to assess renal (kidney), endocrine

(glandular), and acid-base function, and are components of both renal function and comprehensive metabolic biochemistry profiles.

- Other important electrolytes routinely measured in serum or plasma include calcium and phosphorus. These are measured together because they are both affected by bone and parathyroid diseases, and often move in opposing directions.
- Magnesium is another electrolyte that is routinely measured. Like calcium, it will cause tetany (uncontrolled muscle contractions) when levels are too low in the extracellular fluids.
- Electrolytes are electrically charged minerals that help control the amount of fluids and the balance of acids and bases in your body.
- They also help control muscle and nerve activity, heart rhythm, and other important functions.
- Tests that measure the concentration of electrolytes are needed for both the diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions.
- Abnormal levels of any of these electrolytes can be a sign of a serious health problem, including kidney disease, high blood pressure, and a life-threatening irregularity in heart rhythm.
- An electrolyte panel, also known as a serum electrolyte test, is a blood test that measures levels of the body's main electrolytes:
- Sodium, which helps control the amount of fluid in the body. It also helps your nerves and muscles work properly.
- Chloride, which also helps control the amount of fluid in the body. In addition, it helps maintain healthy blood volume and blood pressure.
- Potassium, which helps your heart and muscles work properly.
- Bicarbonate, which helps maintain the body's acid and base balance. It also plays an important role in moving carbon dioxide through the bloodstream.
- An electrolyte panel is often part of a routine blood screening or a comprehensive metabolic panel. The test may also be used to find out if your body has a fluid imbalance or an imbalance in acid and base levels.
- Electrolytes are usually measured together. But sometimes they are tested individually. Separate testing may be done if a provider suspects a problem with a specific electrolyte.

- Other names: serum electrolyte test, lytes, sodium (Na), potassium (K), chloride (Cl), carbon dioxide (CO2)
- Electrolytes can be acids, bases, or salts. They can be measured by different blood tests. Each electrolyte can be measured separately, such as:
- Ionized calcium
- Serum calcium
- Serum chloride
- Serum magnesium
- Serum phosphorus
- Serum potassium
- Serum sodium

ELECTROLYTE PANEL TEST:

- An electrolyte panel is a blood test that measures the levels of electrolytes and carbon dioxide in your blood.
- Electrolytes are minerals, such as sodium and potassium, that are found in the body. They keep your body's fluids in balance and help keep your body working normally, including your heart rhythm, muscle contraction, and brain function.
- Carbon dioxide (CO2) is also measured in this test. CO2 is a waste product made when the body breaks down food for energy (metabolism). It takes the form of bicarbonate in the blood, so this part of the test is sometimes called a bicarbonate test. Bicarbonate helps your blood stay at the right pH.
- An electrolyte panel measures the blood levels of carbon dioxide, chloride, potassium, and sodium.
- Carbon Dioxide (Bicarbonate)
- Chloride
- Potassium
- Sodium

BICARBONATE TEST:

- Carbon dioxide (CO2) is a gaseous waste product from metabolism. The blood carries carbon dioxide to your lungs, where it is exhaled. More than 90% of it in your blood exists in the form of bicarbonate (HCO3). The rest of it is either dissolved carbon dioxide gas (CO2) or carbonic acid (H2CO3). Your kidneys and

lungs balance the levels of carbon dioxide, bicarbonate, and carbonic acid in the blood.

- This test measures the level of bicarbonate in a sample of blood from a vein. Bicarbonate is a chemical that acts as a buffer. It keeps the pH of blood from becoming too acidic or too basic.
- Bicarbonate is not usually tested by itself. The test may be done on a blood sample taken from a vein as part of a panel of tests that looks at other electrolytes. These may include items such as sodium, potassium, and chloride. It can also be done as part of an arterial blood gas (ABG) test. For this blood gas study, the blood sample comes from an artery.

CHLORIDE TEST:

- A chloride test measures the level of chloride in your blood or urine. Chloride is one of the most important electrolytes in the blood. It helps keep the amount of fluid inside and outside of your cells in balance. It also helps maintain proper blood volume, blood pressure, and pH of your body fluids. Tests for sodium, potassium, and bicarbonate are usually done at the same time as a blood test for chloride.
- Most of the chloride in your body comes from the salt (sodium chloride) you eat. Chloride is absorbed by your intestines when you digest food. Extra chloride leaves your body in your urine.
- Sometimes a test for chloride can be done on a sample of all your urine collected over a 24-hour period (called a 24-hour urine sample) to find out how much chloride is leaving your body in your urine.
- Chloride can also be measured in skin sweat to test for cystic fibrosis.

POTASSIUM TEST:

- A potassium test checks how much potassium is in the blood. Potassium is both an electrolyte and a mineral. It helps keep the water (the amount of fluid inside and outside the body's cells) and electrolyte balance of the body. Potassium is also important in how nerves and muscles work.
- Potassium levels often change with sodium levels. When sodium levels go up, potassium levels go down, and when sodium levels go down, potassium levels go up. Potassium levels are also affected by a hormone called aldosterone, which is made by the adrenal glands.

- Potassium levels can be affected by how the kidneys are working, the blood pH, the amount of potassium you eat, the hormone levels in your body, severe vomiting, and taking certain medicines, such as diuretics and potassium supplements. Certain cancer treatments that destroy cancer cells can also make potassium levels high.
- Many foods are rich in potassium, including bananas, orange juice, spinach, and potatoes. A balanced diet has enough potassium for the body's needs. But if your potassium levels get low, it can take some time for your body to start holding on to potassium. In the meantime, potassium is still passed in the urine, so you may end up with very low levels of potassium in your body, which can be dangerous.
- A potassium level that is too high or too low can be serious. Abnormal potassium levels may cause symptoms such as muscle cramps or weakness, nausea, diarrhea, frequent urination, dehydration, low blood pressure, confusion, irritability, paralysis, and changes in heart rhythm.
- Other electrolytes, such as sodium, calcium, chloride, magnesium, and phosphate, may be checked in a blood sample at the same time as a blood test for potassium.

SODIUM TEST:

- A sodium test checks how much sodium is in the blood. Sodium is both an electrolyte and mineral. It helps keep the water (the amount of fluid inside and outside the body's cells) and electrolyte balance of the body. Sodium is also important in how nerves and muscles work.
- Most of the sodium in the body (about 85%) is found in blood and lymph fluid. Sodium levels in the body are partly controlled by a hormone called aldosterone, which is made by the adrenal glands. Aldosterone levels tell the kidneys when to hold sodium in the body instead of passing it in the urine. Small amounts of sodium are also lost through the skin when you sweat.
- Most foods have sodium naturally in them or as an ingredient in cooking. Sodium is found in table salt as sodium chloride or in baking soda as sodium bicarbonate. Many medicines and other products also have sodium in them, including laxatives, aspirin, mouthwash, and toothpaste.
- Low sodium levels have many causes, such as heart failure, malnutrition, or diarrhea.

- Other electrolytes, such as potassium, calcium, chloride, magnesium, and phosphate, may be checked in a blood sample at the same time as a blood test for sodium.

CALCIUM TEST:

- A calcium blood test measures the amount of calcium in your blood.
- Calcium is one of the most important minerals in your body. Calcium is essential for healthy bones and teeth. Calcium is also essential for proper functioning of your nerves, muscles, and heart. About 99% of your body's calcium is stored in your bones. The remaining 1% circulates in the blood.
- If there is too much or too little calcium in the blood, it may be a sign of bone disease, thyroid disease, kidney disease, or other medical conditions.
- Other names: total calcium, ionized calcium
- There are two types of calcium blood tests:
- Total calcium, which measures the calcium attached to specific proteins in your blood.
- Ionized calcium, which measures the calcium that is unattached or "free" from these proteins.
- Total calcium is often part of a routine screening test called a basic metabolic panel. A basic metabolic panel is a test that measures different minerals and other substances in the blood, including calcium.

ANION GAP TEST:

- Anion gap (AG or AGAP) is a value calculated using the results of an electrolyte panel. It is used to help distinguish between anion-gap and non-anion-gap metabolic acidosis.
- Acidosis refers to an excess of acid in the body; this can disturb many cell functions and should be recognized as quickly as possible, when present. The anion gap is frequently used in the hospital and/or emergency room setting to help diagnose and monitor acutely ill patients.
- If anion-gap metabolic acidosis is identified, the AG may be used to help monitor the effectiveness of treatment and the underlying condition.
- Specifically, the anion gap evaluates the difference between measured and unmeasured electrical particles (ions or electrolytes) in the fluid portion of the blood.

- According to the principle of electrical neutrality, the number of positive ions (cations) and negative ions (anions) should be equal. However, not all ions are routinely measured. The calculated AG result represents the unmeasured ions and primarily consists of anions, hence the name "anion gap."
- The most commonly used formula is:
- Anion Gap (AG) = Sodium - (Chloride + Bicarbonate [total CO2])
- The anion gap is non-specific. It is increased when the number of unmeasured anions increases, indicating a state of anion-gap metabolic acidosis, but it does not tell the healthcare practitioner what is causing the imbalance.
- The metabolic acidosis must be treated to restore the acid/base balance, but the underlying condition must also be identified and treated.
- Causes can include uncontrolled diabetes, starvation, kidney damage, and ingestion of potentially toxic substances such as antifreeze, excessive amounts of aspirin (salicylates), or methanol.
- A low anion gap can also occur; this is most commonly seen when albumin (an anion as well as a protein) is low, while immunoglobulins (cations as well as proteins) are increased.

MEASUREMENT OF ELECTROLYTES:

- The electrolyte panel is used to identify an electrolyte, fluid, or pH imbalance (acidosis or alkalosis). It is frequently ordered as part of a routine health exam. It may be ordered by itself or as a component of a basic metabolic panel (BMP) or a comprehensive metabolic panel (CMP).
- These panels can include other tests such as BUN, creatinine, and glucose.
- Electrolyte measurements may be used to help investigate conditions that cause electrolyte imbalances such as dehydration, kidney disease, lung diseases, or heart conditions. A series of electrolyte panels may also be used to monitor treatment of the condition causing the imbalance.
- Since electrolyte and acid-base imbalances can be present with a wide variety of acute and chronic illnesses, the electrolyte panel is frequently used to evaluate patients who seek medical care in the emergency room as well as hospitalized patients.
- The results for an electrolyte panel may also include a calculation for anion gap that can be used to help detect disorders or the presence of toxic substances.

- If you have an imbalance of a single electrolyte, such as sodium or potassium, your healthcare practitioner may order repeat testing of that individual electrolyte, monitoring the imbalance until it resolves.
- If you have an acid-base imbalance, your healthcare practitioner may also order tests for blood gases, which measure the pH and oxygen and carbon dioxide levels in an arterial blood sample, to help evaluate the severity of the imbalance and monitor its response to treatment.
- Electrolytes are measured by a process known as potentiometry.
- This method measures the voltage that develops between the inner and outer surfaces of an ion selective electrode.
- The electrode (membrane) is made of a material that is selectively permeable to the ion being measured.
- This potential is measured by comparing it to the potential of a reference electrode.
- Since the potential of the reference electrode is held constant, the difference in voltage between the two electrodes is attributed to the concentration of ion in the sample.

SIGNS AND SYMPTOMS:

- An electrolyte panel may be ordered as part of a routine health exam and diagnostic aid when you have signs and symptoms, such as:
- Fluid accumulation (edema)
- Nausea or vomiting
- Weakness
- Confusion
- Irregular heart beat (cardiac arrhythmias)
- It is frequently ordered as part of an evaluation when you have an acute or chronic illness and at regular intervals when you have a disease or condition or is taking a medication that can cause an electrolyte imbalance.
- Electrolyte tests are commonly ordered at regular intervals to monitor treatment of certain conditions, including high blood pressure (hypertension), heart failure, lung diseases, liver disease and kidney disease.

PRECAUTIONS:

- Electrolyte tests are performed on whole blood, plasma, or serum, usually collected from a vein or capillary.

- Special procedures are followed when collecting a sweat sample for electrolyte analysis. This procedure, called pilocarpineiontophoresis, uses electric current applied to the arm of the patient (usually an infant) in order to convey the pilocarpine to the sweat glands where it will stimulate sweating.
- Care must be taken to ensure that the collection device (macroduct tubing or gauze) does not become contaminated and that the patient's parent or guardian understands the need for the electrical equipment employed.

PREPARATIONS, PROCEDURE AND RISKS:

1. Preparation
   - Usually no special preparation is necessary by the patient. Samples for calcium and phosphorus and for magnesium should be collected following an eight-hour fast.
2. Aftercare
   - Discomfort or bruising may occur at the puncture site, or the person may feel dizzy or faint. Pressure to the puncture site until the bleeding stops reduces bruising. Applying warm packs to the puncture site relieves discomfort.
3. Risks
   - Minor temporary discomfort may occur with any blood test, but there are no complications specific to electrolyte testing.

UNITS OF ELECTROLYTES:

- Electrolyte concentrations are similar whether measured in serum or plasma.
- Values are expressed as mmol/L for sodium, potassium, chloride, and bicarbonate.
- Magnesium results are often reported as milliequivalents per liter (meq/L) or in mg/dL.
- Total calcium is usually reported in mg/dL and ionized calcium in mmol/L.
- Since severe electrolyte disturbances can be associated with life-threatening consequences such as heart failure, shock, coma, or tetany, alert values are used to warn physicians of impending crisis.

NORMAL VALUES:

- Typical reference ranges and alert values are cited below:
- serum or plasma sodium: 135–145 mmol/l; alert levels: less than 120 mmol/l and greater than 160 mmol/l
- serum potassium: 3.6–5.4 mmol/l (plasma, 3.6–5.0 mmol/l); alert levels: less than 3.0 mmol/l and greater than 6.0 mmol/l

- serum or plasma chloride: 98–108 mmol/l
- sweat chloride: 4–60 mmol/l
- serum or plasma bicarbonate: 18–24 mmol/l (as total carbon dioxide, 22–26 mmol/l); alert levels: less than 10 mmol/l and greater than 40 mmol/l
- serum calcium: 8.5–10.5 mg/dl (2.0–2.5 mmol/l); alert levels: less than 6.0 mg/dl and greater than 13.0 mg/dl
- ionized calcium: 1.0–1.3 mmol/l
- serum inorganic phosphorus: 2.3–4.7 mg/dl (children, 4.0–7.0 mg/dl); alert level: less than 1.0 mg/dl
- serum magnesium: 1.8–3.0 mg/dl (1.2–2.0 meq/l or 0.5–1.0 mmol/l)
- ionized magnesium: 0.53–0.67 mmol/l
- osmolality (calculated) 280–300 mosm/kg

5. Write in detail about the Biochemistry of Urine and Stools testing. (May 2019)

URINALYSIS

- A urine test checks different components of urine, a waste product made by the kidneys.
- A regular urine test may be done to help find the cause of symptoms.
- The test can give information about your health and problems you may have.
- The kidneys take out waste material, minerals, fluids, and other substances from the blood to be passed in the urine.
- Urine has hundreds of different body wastes.
- What you eat and drink, how much you exercise, and how well your kidneys work can affect what is in your urine.
- More than 100 different tests can be done on urine. A regular urinalysis often includes the following tests:

1. **Color:** Many things affect urine color, including fluid balance, diet, medicines, and diseases. How dark or light the color is tells you how much water is in it. Vitamin B supplements can turn urine bright yellow. Some medicines, blackberries, beets, rhubarb, or blood in the urine can turn urine red-brown.
2. **Clarity:** Urine is normally clear. Bacteria, blood, sperm, crystals, or mucus can make urine look cloudy.

3. **Odor:** Urine does not smell very strong, but it has a slightly "nutty" odor. Some diseases cause a change in the odor of urine. For example, an infection with E. coli bacteria can cause a bad odor, while diabetes or starvation can cause a sweet, fruity odor.
4. **Specific gravity:** This checks the amount of substances in the urine. It also shows how well the kidneys balance the amount of water in urine. The higher the specific gravity, the more solid material is in the urine. When you drink a lot of fluid, your kidneys make urine with a high amount of water in it, which has a low specific gravity. When you do not drink fluids, your kidneys make urine with a small amount of water in it, which has a high specific gravity.
5. **pH:** The pH is a measure of how acidic or alkaline (basic) the urine is. A urine pH of 4 is strongly acidic, 7 is neutral (neither acidic nor alkaline), and 9 is strongly alkaline. Sometimes the pH of urine is affected by certain treatments. For example, your doctor may instruct you how to keep your urine either acidic or alkaline to prevent some types of kidney stones from forming.
6. **Protein:** Protein normally isn't found in the urine. Fever, hard exercise, pregnancy, and some diseases, especially kidney disease, may cause protein to be in the urine.
7. **Glucose:** Glucose is the type of sugar found in blood. Normally there is very little or no glucose in urine. When the blood sugar level is very high, as in uncontrolled diabetes, the sugar spills over into the urine. Glucose can also be found in urine when the kidneys are damaged or diseased.
8. **Nitrites:** Bacteria that cause a urinary tract infection (UTI) make an enzyme that changes urinary nitrates to nitrites. Nitrites in urine show that a UTI may be present.
9. **Leukocyte esterase (WBC esterase):** Leukocyte esterase shows leukocytes (white blood cells [WBCs]) in the urine. WBCs in the urine may mean a UTI is present.
10. **Ketones:** When fat is broken down for energy, the body makes substances called ketones (or ketone bodies). These are passed in the urine. Large amounts of ketones in the urine may mean a very serious condition, diabetic ketoacidosis, is present. A diet low in sugars and starches (carbohydrates), starvation, or severe vomiting may also cause ketones to be in the urine.
11. **Microscopic analysis:** In this test, urine is spun in a special machine (centrifuge) so the solid materials (sediment) settle at the bottom. The sediment is spread on a slide and looked at under a microscope. Things that may be seen on the slide include:Red or

white blood cells. Blood cells aren't found in urine normally. Inflammation, disease, or injury to the kidneys, ureters, bladder, or urethra can cause blood in urine. Strenuous exercise, such as running a marathon, can also cause blood in the urine. White blood cells may be a sign of infection or kidney disease.

12. **Casts:** Some types of kidney disease can cause plugs of material (called casts) to form in tiny tubes in the kidneys. The casts then get flushed out in the urine. Casts can be made of red or white blood cells, waxy or fatty substances, or protein. The type of cast in the urine can help show what type of kidney disease may be present.
13. **Crystals:** Healthy people often have only a few crystals in their urine. A large number of crystals, or certain types of crystals, may mean kidney stones are present or there is a problem with how the body is using food (metabolism).
14. **Bacteria, yeast cells, or parasites:** There are no bacteria, yeast cells, or parasites in urine normally. If these are present, it can mean you have an infection.
15. **Squamous cells:** The presence of squamous cells may mean that the sample is not as pure as it needs to be. These cells do not mean there is a medical problem, but your doctor may ask that you give another urine sample.

PURPOSE OF URINALYSIS:

- A urine test may be done:
- To check for a disease or infection of the urinary tract. Symptoms of a urine infection may include colored or bad-smelling urine, pain when urinating, finding it hard to urinate, flank pain, blood in the urine (hematuria), or fever.
- To check the treatment of conditions such as diabetes, kidney stones, a urinary tract infection (UTI), high blood pressure (hypertension), or some kidney or liver diseases.
- As part of a regular physical examination.

PREPARATION FOR TEST:

- Do not eat foods that can color the urine, such as blackberries, beets, and rhubarb, before the test. Do not exercise strenuously before the test.
- Tell your doctor if you are menstruating or close to starting your menstrual period. Your doctor may want to wait to do the test.
- Your doctor may ask you to stop taking certain medicines that color the urine. These include vitamin B, phenazopyridine (Pyridium), rifampin, and phenytoin

(Dilantin). Be sure to tell your doctor if you are taking diuretics, which may affect the test results.

- Talk to your doctor any concerns you have regarding the need for the test, its risks, how it will be done, or what the results will mean.

PROCEDURE:

- A routine urine test can be done in your doctor's office, clinic, or lab. You may also be asked to collect a urine sample at home and bring it with you to the office or lab for testing.
- Collecting a urine sample from a small child or baby is done by using a special plastic bag with tape around its opening. The bag is placed around the child's genitals until he or she urinates. Then you carefully remove the bag. To collect a urine sample from a very sick baby, a doctor may use a urinary catheter through the urethra or a needle through the baby's belly directly into the bladder (suprapubic tap).

1. Clean-catch midstream one-time urine collection

- Wash your hands to make sure they are clean before collecting the urine.
- If the collection cup has a lid, remove it carefully and set it down with the inner surface up. Do not touch the inside of the cup with your fingers.

2. Double-voided urine sample collection

- This method collects the urine your body is making right now.
- Urinate into the toilet or urinal. Do not collect any of this urine.
- Drink a large glass of water, and wait about 30 to 40 minutes.
- Then get a urine sample. Follow the instructions above for collecting a clean-catch urine sample.
- Return the urine sample to the lab. If you are collecting the urine at home and cannot get it to the lab in an hour, refrigerate it.

3. 24-hour urine collection

- Your doctor may ask you to collect your urine for 24 hours.
- The collection period usually starts in the morning. When you first get up, urinate—but don't save this urine. Write down the time that you urinated to mark the beginning of your 24-hour collection period.
- For the next 24 hours, collect all your urine. Your doctor will usually provide you with a large container that holds about 1 gal (4 L) and has a small amount of preservative in

it. Urinate into a smaller, clean container, and then pour the urine into the large container. Avoid touching the inside of the container with your fingers.

- Keep the large container in the refrigerator during the collection period.
- Urinate for the final time at or just before the end of the 24-hour period. Add this urine to the large container, and write down the time.

FACTORS AFFECTING THE TEST:

- Reasons you may not be able to have the test or why the results may not be helpful include:
- If you are having your menstrual period.
- Taking medicines, such as diuretics, erythromycin, trimethoprim (Trimpex), or high doses of vitamin C (ascorbic acid) taken with an antibiotic, such as tetracycline.
- Having an X-ray test with contrast material in the past 3 days.
- Not getting the urine sample to the lab in 1 hour.

OTHER SUBSTANCES THAT ARE TESTED:

- Other substances that may be checked during a urine test include:

1. **Bilirubin.** This is a substance formed by the breakdown of red blood cells. It is passed from the body in stool. Bilirubin is not found in urine. If it is present, it often means that the liver is damaged or that the flow of bile from the gallbladder is blocked.

2. **Urobilinogen.** This is a substance formed by the breakdown of bilirubin. It is also passed from the body in stool. Only small amounts of urobilinogen are found in urine. Urobilinogen in urine can be a sign of liver disease (cirrhosis, hepatitis) or that the flow of bile from the gallbladder is blocked.

3. **Bence Jones protein.** This is an abnormal protein found in the urine of about 50% of people with a rare type of cancer called multiple myeloma. A urine test is often done when multiple myeloma is suspected. The protein test done during a regular urine test does not check for Bence Jones protein.

FUTURE TESTS:

- To lower the chance of contaminating the urine sample with bacteria, a health professional may collect a urine sample by using a urinary catheter. A catheter may be used to collect urine from a person in the hospital who is very ill or who can't give a clean-catch sample. Using a catheter allows a clean sample to be collected.

- If an abnormal result is found during a urine test, more tests may be done, such as a urine culture, X-ray of the kidneys (intravenous pyelogram [IVP]), or cystoscopy.
  - Urine Culture
  - Intravenous Pyelogram (IVP)
  - Cystoscopy

STOOL TESTING

- A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.
- These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer.
- For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory.
- Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus.
- The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells, and sugars called reducing substances.
- The pH of the stool also may be measured.
- A stool culture is done to find out if bacteria may be causing an infection.
- Stool analysis is done to:

1. Help identify diseases of the digestive tract, liver, and pancreas. Certain enzymes (such as trypsin or elastase) may be evaluated in the stool to help determine how well the pancreas is functioning.
2. Help find the cause of symptoms affecting the digestive tract, including prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
3. Screen for colon cancer by checking for hidden (occult) blood.
4. Look for parasites, such as pinworms or Giardia.
5. Look for the cause of an infection, such as bacteria, a fungus, or a virus.
6. Check for poor absorption of nutrients by the digestive tract (malabsorption syndrome). For this test, all stool is collected over a 72-hour period and then checked for fat (and sometimes for meat fibers). This test is called a 72-hour stool collection or quantitative fecal fat test.

PREPARATION FOR STOOL TESTING:

- Many medicines can change the results of this test. You will need to avoid certain medicines depending on which kind of stool analysis you have.
- You may need to stop taking medicines such as antacids, antidiarrheal medicines, antiparasite medicines, antibiotics, laxatives, or nonsteroidal anti-inflammatory drugs (NSAIDs) for 1 to 2 weeks before you have the test. Be sure to tell your doctor if you have:
- Recently had an X-ray test using barium contrast material, such as a barium enema or upper gastrointestinal series (barium swallow). Barium can interfere with test results.
- Traveled in recent weeks or months, especially if you have traveled outside the country. This helps your doctor look for the parasites, fungi, viruses, or bacteria that may be causing a problem.
- If your stool is being tested for blood, you may need to avoid certain foods for 2 to 3 days before the test. This depends on what kind of stool test you use. And do not do the test during your menstrual period or if you have active bleeding from hemorrhoids
- Do not use a stool sample for testing that has been in contact with toilet bowl cleaning products that turn the water blue.

PROCEDURE FOR STOOL ANALYSIS:

- Stool samples can be collected at home, in your doctor's office, at a medical clinic, or at the hospital. If you collect the samples at home, you will be given stool collection kits to use each day. Each kit contains applicator sticks and two sterile containers.You may need to collect more than one sample over 1 to 3 days.
- Collect the samples as follows:

1. Urinate before collecting the stool so that you do not get any urine in the stool sample.

2. Put on gloves before handling your stool. Stool can contain germs that spread infection.

3. Wash your hands after you remove your gloves.

4. Pass stool (but no urine) into a dry container. You may be given a plastic basin that can be placed under the toilet seat to catch the stool.

5. Either solid or liquid stool can be collected.

- If you have diarrhea, a large plastic bag taped to the toilet seat may make the collection process easier; the bag is then placed in a plastic container.
- If you are constipated, you may be given a small enema.
- Do not collect the sample from the toilet bowl.
- Do not mix toilet paper, water, or soap with the sample.
- Place the lid on the container and label it with your name, your doctor's name, and the date the stool was collected. Use one container for each day's collection, and collect a sample only once a day unless your doctor gives you other directions.You may need to deliver your sample to the lab within a certain time.
- If the stool is collected in your doctor's office or the hospital, you will pass the stool in a plastic container that is inserted under the toilet seat or in a bedpan.
- You will need to collect stool for 3 days in a row if the sample is being tested for quantitative fats. The samples are placed in a large container and then refrigerated.
- You may need to collect several stool samples over 7 to 10 days if you have digestive symptoms after traveling outside the country.
- Samples from babies and young children may be collected from diapers (if the stool is not contaminated with urine) or from a small-diameter glass tube inserted into the baby's rectum while the baby is held on an adult's lap.
- Sometimes a stool sample is collected using a rectal swab that contains a preservative. The swab is inserted into the rectum, rotated gently, and then withdrawn. It is placed in a clean, dry container and sent to the lab right away.

PAIN DURING PROCESS:

- There is no pain while collecting a stool sample.
- If you are constipated, straining to pass stool may be painful.
- If your health professional uses a rectal swab to collect the sample, you may feel some pressure or discomfort as the swab is inserted into your rectum.

RISKS:

- Any stool sample may contain germs that can spread disease.
- It is important to carefully wash your hands and use careful handling techniques to avoid spreading infection.

RESULT:

- A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract.

- The normal value ranges vary from lab to lab, and your lab may have a different range for what's normal. Your lab report should contain the range your lab uses.
- Also, your doctor will evaluate your results based on your health and other factors. This means that a value that falls outside the normal values may still be normal for you or your lab.
- Stool analysis test results usually take at least 1 to 3 days.

TYPES OF STOOL TESTING:

- Stool may be checked for hidden (occult) blood.
- A stool culture is done to find the cause of an infection, such as bacteria, a virus, a fungus, or a parasite.
- A bowel transit time test is done to help find the cause of abnormal movement of food through the digestive tract.
- The D-xylose absorption test is done to help diagnose problems that prevent the small intestine from absorbing nutrients in food. This test may be done when symptoms of malabsorption syndrome (such as chronic diarrhea, weight loss, and weakness) are present.
- A stool analysis to measure trypsin or elastase is not as reliable as the sweat test to detect cystic fibrosis.
- High levels of fat in the stool may be caused by diseases such as pancreatitis, sprue (celiac disease), cystic fibrosis, or other disorders that affect the absorption of fats.
- The presence of undigested meat fibers in the stool may be caused by pancreatitis.
- A low pH may be caused by poor absorption of carbohydrate or fat. Stool with a high pH may mean inflammation in the intestine (colitis), cancer, or antibiotic use.
- Blood in the stool may be caused by bleeding in the digestive tract.
- White blood cells in the stool may be caused by inflammation of the intestines, such as ulcerative colitis, or a bacterial infection.
- Rotaviruses are a common cause of diarrhea in young children. If diarrhea is present, testing may be done to look for rotaviruses in the stool.
- High levels of reducing factors in the stool may mean a problem digesting some sugars.
- Low levels of reducing factors may be caused by sprue (celiac disease), cystic fibrosis, or malnutrition. Medicine such as colchicine (for gout) or birth control pills may also cause low levels.

FACTORS THAT AFFECT STOOL ANALYSIS:

- Reasons you may not be able to have the test or why the results may not be helpful include:

  1. Taking medicines such as antibiotics, antidiarrheal medicines, barium, bismuth, iron, ascorbic acid, nonsteroidal anti-inflammatory drugs (NSAIDs), and magnesium.

  2. Contaminating a stool sample with urine, blood from a menstrual period or a bleeding hemorrhoid, or chemicals found in toilet paper and paper towels.

  3. Exposing the stool sample to air or room temperature or failing to send the sample to a laboratory within 1 hour of collection.

UNIT-V

2 MARKS:

1. Define Beer lambert Law. (Nov 2016, May 2017, Dec 2017, May 2018)

Beer's Law

- This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.
- $Log_{10}\ I_0/I_t = a_s c$
  - where,
    - a_s = Absorbency index
    - c = Concentration of Solution

Lambert's Law

- The Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.
- $A = \log_{10} I_0/I_t = a_s b$

  Where,

  A = Absorbance of test

  a_s = Absorbance of standard

  b = length / thickness of the solution.

2. Write the instrumentation of Spectrophotometry. (Nov 2016)

- Energy source
- Monochromator
- Transport vessels
- Photosensitive detector

3. What is Flame Photometry ?(May 2015)

- Flame Photometry is also called as flame emission spectroscopy. Flame Photometry is branch of atomic spectroscopy. It is used to detected certain metal ions like sodium, potassium, magenisum etc.
- The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame.

- The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.It is also known as flame emission spectroscopy.

4. Differentiate calorimeter from colorimeter. (May 2015)

Calorimeter	Colorimeter
➢ An apparatus for measuring the heat generated or absorbed by either a chemical reaction, change of phase or some other physical change.	➢ Any of various instruments designed to determine the color of something, by comparison with standard colors or by spectroscopy. ➢ An analytic instrument that estimates the concentration of a substance in a sample by measuring its color against the solution's complimentary color.

5. Mention the uses of Isotopes in Biochemistry. (Nov 2015)

- Biochemical assays are used to detect the presence and absence of radioisotopes. Therefore radioactive isotopes are used to label biological molecules.
- Such assays estimate the concentration of different constituents of plasma, body fluids, urine, blood etc. This technique is called radioimmuno-assays.
- An example is iodine bioassay which uses gamma emitters'radio nuclides of Iodine 125 and Iodine-131that accrues inside thyroid. Therefore gamma detector can be used toquantify the iodine content (uptake and intake) of the person's thyroid.
- The amount of measured radioiodine in the thyroid is compared with the Annual Limit on Intake (ALI).

6. Outline the Principle of Calorimeter with applications. (May 2016, Dec 2019, Nov 2015)

Principle

- When two bodies of different temperatures (preferably a solid and a liquid) are placed in physical contact with each other, the heat is transferred from the body with higher temperature to the body with lower temperature until thermal equilibrium is attained between them.

- The body at higher temperature releases heat while the body at lower temperature absorbs heat. The principle of calorimetry indicates the law of conservation energy, i.e. the total heat lost by the hot body is equal to the total heat gained by the cold body.

Applications

- In biochemistry/chemistry labs
- In thermodynamics study
- In the study of different materials, such as nanomaterials, zeolites and ceramics
- For assessing the thermal hazard potential of Li batteries
- For examining polymeric materials to determine their thermal transitions
- In the study of liquid crystals

7. Mention the uses of radioisotopes in biochemistry. (May 2016)

- It is possible to predict the fate of individual carbon atoms of (14^oC) acetate through TCA cycle.
- Methods have been developed to isolate intermediates of the cycle & to ascertain the distribution of carbon atoms within each intermediate(this is called as specific labelling pattern) .
- Radioisotopes are widely used in study of the mechanism & rate of absorption , accumulation& translocation of inorganic & organic compounds in the animal .
- Radio labeled drugs are useful in pharmokinetic studies (site of accumulation , rate of accumulation , rate of metabolism & metabolic products) .

8. List and few isotopes used Biochemistry.(May 2017)

- Carbon-11 - Brain scans
- Chromium-51 - Blood Volume determination
- Cobalt-57 - Measuring vitamin B12uptake
- Cobalt-60 - Radiation cancer therapy
- Gadolinium-153 - Determining bone density
- Gallium-67 - Scan for lung tumors
- Iodine-131 - Thyroid therapy
- Iridium-192 - Breast cancer therapy.

9. What is a monochromatic light ?(Dec 2017)

- Light of a single wavelength is known as monochromatic light. The term light signifies the visible and near-visible portions of the electromagnetic radiation.

- These radiations exist over a huge range of wavelengths, ranging from picometers to kilometres.

10. What is the Principle of fluorometry ?(May 2019)

- Fluorescence spectrophotometry is based on fluorescence, which is a photoluminescence event (photo = light; luminescence = the emission of light).
- In simple terms, it is the emission of light because of an exposure to (and resultant absorption of) light. This exposure to and absorption of light is called excitation.
- A common form in which photoluminescence is often observed, is as phosphorescence – what you see in glow-in-the-dark toys.

11. Write any two applications of Flame photometry. (Dec 2019)

- **Beverage industry** Determination of the content of sodium, potassium and calcium in various liquids, such as fruit juices, vegetable juices and soft drinks.
- **Food industry** Monitoring compliance with sodium and potassium limits in foods. In the production of pre-milk, pre-food and milk powder, the quality can also be controlled and monitored with a flame photometer.

11 MARKS

1. Explain in detail about the principle and application of photometry. (Nov 2016)

- When light is passed through a coloured solution, certain wavelengths are selectively absorbed giving a plot of the absorption spectrum of the compound in solution. The wavelength at which maximum absorption is called the absorption maximum (λmax) of that compound. The light that is not absorbed is transmitted through the solution and gives the solution its colour.
- Photometric instruments measure transmittance, which is defined as follows:

  - **Transmittance (T)**= $\frac{\text{Intensity of the emergent (or transmitted) light } Ie}{\text{Intensity of the incident light } Io} = \ldots$

- Transmittance is usually expressed on a range of 0 to 100%.
- If the concentration of the substance in solution is increased linearly, or if the path length that the light beam has to traverse is increased, transmittance falls

exponentially. So a term **absorbance** is defined so that it is directly proportional to the concentration of the substance.

- **Absorbance (A)**= $\log 1/T = \log \frac{Ie}{Io}$

  - Absorbance has no units. Photometric instruments electronically convert the measured transmittance to absorbance values.
  - Much of photometry is therefore based on two laws:
  - When a parallel beam of monochromatic light passes through a solution, the absorbance (A) of the solution is directly proportional to concentration(c)of the compound in the solution. This is **Beer's law**.
  - Each successive layer of the solution absorbs a constant proportion of the light entering the solution, although the absolute amount entering each layer diminishes progressively. Therefore, absorbance is directly proportional to the thickness or length of the light path (l) through the solution. This is **Lambert's law.**
  - A a c (Beer's law) ---------- 1)
  - A a l (Lambert's law) ----------2)
  - By combining 1) and 2) , we get

$$A = \varepsilon_l \, c \, l$$

- ε_l, the proportionality constant is termed the **molar absorption coefficient**.
- It is specific for a given substance at a given wavelength. It is the absorbance of a one molar solution of a substance with a light path of one centimetre (if c is expressed in mol/L).
- But Beer's law applies to only dilute solutions and in practice the concentrations of the solutions that are used in photometry are usually in the mmol/L range. In colorimetry, the absorption coefficient is not usually used. Concentration of an unknown solution can be determined by using equation 1, which is derived as follows:
- Absorbance of test sample (A_t) = ε x concentration of test (C_t) x l
- Absorbance of standard sample (A_s) = ε x concentration of standard (C_s) x l
- Hence,

$$\text{Conc. of Test (Ct)} = \frac{\text{Absorbance of Test sample (At)}}{\text{Absorbance of Standard sample (As)}} \times \text{Conc. of standard } (C_s)$$

- The light path, l, is usually kept constant in photometric measurements at 1 cm. This is the diameter of the tube (called the cuvette) containing the solution.
- A standard (or calibrator) is representative of the substance whose concentration is sought to be determined. The concentration of the compound in the test sample is obtained by comparing its absorbance with that of a known concentration of a standard solution.
- Ideally, a series of standards of known concentration are prepared to obtain a standard (calibration) curve. This helps to determine the range of concentrations over which Beer's law is obeyed.

APPLICATIONS:

- Photon counting
- Photography
- Visible light reflectance photometry
- UV and visible light transmission photometry
- Infrared light transmission photometry
- Atomic absorption photometry

2. Explain in detail about the automation in clinical lab. (Nov 2016, May 2016, Dec 2019)

- **Laboratory automation** is a multi-disciplinary strategy to research, develop, optimize and capitalize on technologies in the laboratory that enable new and improved processes. Laboratory automation professionals are academic, commercial and government researchers, scientists and engineers who conduct research and develop new technologies to increase productivity, elevate experimental data quality, reduce lab process cycle times, or enable experimentation that otherwise would be impossible.
- The most widely known application of laboratory automation technology is laboratory robotics. More generally, the field of laboratory automation

comprises many different automated laboratory instruments, devices (the most common being auto samplers), software algorithms, and methodologies used to enable, expedite and increase the efficiency and effectiveness of scientific research in laboratories.

- The application of technology in today's laboratories is required to achieve timely progress and remain competitive. Laboratories devoted to activities such as high-throughput screening, combinatorial chemistry, automated clinical and analytical testing, diagnostics, large-scale biorepositories, and many others, would not exist without advancements in laboratory automation.
- An autosampler for liquid or gaseous samples based on a microsyringe.

Low cost laboratory automation

- A large obstacle to the implementation of automation in laboratories has been its high cost. Many laboratory instruments are very expensive. This is justifiable in many cases, as such equipment can perform very specific tasks employing cutting-edge technology. However, there are devices employed in the laboratory that are not highly technological but still are very expensive. This is the case of many automated devices, which perform tasks that could easily be done by simple and low-cost devices like simple robotic arms, universal (open-source) electronic modules, or 3D printers.
- So far, using such low-cost devices together with laboratory equipment was considered to be very difficult. However, it has been demonstrated that such low-cost devices can substitute without problems the standard machines used in laboratory. It can be anticipated that more laboratories will take advantage of this new reality as low-cost automation is very attractive for laboratories.
- A technology that enables the integration of any machine regardless of their brand is scripting, more specifically, scripting involving the control of mouse clicks and keyboard entries, like AutoIt. By timing clicks and keyboard inputs, different software interfaces controlling different devices can be perfectly synchronized.

3. **Explain the principle, construction and working of flurometry with neat diagram. (May 2015, Dec 2018)**

- A large number of substances are unknown which can absorb ultraviolet or visible light energy.
- But these substances lose excess energy through heat through collisions with neighboring atoms or molecules.
- However, a number of essential substances are also known which lose only part of this excess energy in the form of heat and release the remnant energy as electromagnetic radiation of a wavelength longer than that absorbed.
- The process of emitting radiation is collectively known as luminescence.
- In luminescence, light is produced at low temperatures.
- Thus, the light emitted by this process is regarded as 'light without heat' or 'cold light'.

Luminescence is of 2 types:

i. Fluorescence:

- When a beam of light is incident on certain materials, they emit visible light or radiations.
- This phenomenon is known as fluorescence and the substance showing this phenomenon is known as fluorescent substances.
- The phenomenon of fluorescence is instantaneous and starts immediately after the absorption of light and stops as soon as the incident light is cut off.

ii. Phosphorescence:

- When light radiation is incident on certain materials, they continue to emit light even after the incident light is cut off.
- This type of delayed fluorescence is called phosphorescence and the substances are called phosphorescent substances.
- A material exhibiting fluorescence generally re-emits excess radiation within 10-6 to 10-4 seconds of absorption.
- On the other hand, materials exhibiting phosphorescence re-emit excess radiation within 10-4 to 20 seconds or longer.

Principle of Fluorometry:

- When molecules are irradiated with light of the appropriate frequency, it will be absorbed in about 10-15 seconds.
- In the process of absorption, the molecules may move from ground to the first excited singlet electronic state.
- Although at room temperature molecules may be present in their ground vibration level.
- After absorption, the excitation molecules can end up in any one of the vibrational levels in the first excited electronic state.
- From the excited singlet state, one of the following three phenomena will probably occur, depending on the molecule involved and the conditions:
- The first possibility is that the excited singlet state is relatively unstable, in such a situation, the excited molecules will return to the ground state by collisional deactivation without emitting any radiation.
- The second possibility is that the molecules in the excited singlet state may emit an ultraviolet or visible light photon. This process is known as fluorescence.
- The third possibility is that the molecule with a relatively stable excited state may undergo transition and sometime thereafter returns to the ground state, usually by the emission of an ultraviolet or visible light photon. This is known as **phosphorescence emission.**
- The instruments used for the measurement of fluorescence are known as fluorometers.
- In these, filters are used to isolate the wavelength of excitation.
- Thus, a fluorometer is a manual instrument and is best used for measurements at one or two wavelengths because a change in filters is to be made each time the wavelength is changed.
- A fluorometer employs a mercury vapor lamp, a condensing lens, a primary filter, a sample container, a secondary filter, and a receiving photocell.

- Generally, the primary filter is used to select ultraviolet but not visible radiation whereas the secondary filter is used to transmit visible fluorescent radiation and to absorb incident ultraviolet radiation.
- The light from the mercury vapor lamp is allowed to pass through the condensing lens followed by its passage through a primary filter.
- The primary filter selects only UV radiation but absorbs visible radiation.
- The UV radiation from the primary filter is passed through a sample container.
- From the sample, UV and fluorescent radiations are obtained which are passed through a secondary filter that absorbs the primary radiant energy but transmits the fluorescent radiation.
- This is received by a photocell placed in a position at right angles to the incident beam.
- The output of the photocell is measured by a sensitive galvanometer or another device.

Applications of Fluorometry:

- Determination of uranium in salts used extensively in the field of nuclear research.
- Estimation of traces of boron in steel by means of the complex formed with benzene.
- Estimation of calcium by fluorometry with a calcium solution.
- Determination of Vitamin B (B1 thiamine and B2 riboflavin) in the food samples like meat, cereals, etc.
- Fluorometry is employed to carry out both qualitative and quantitative analyses for various aromatic compounds present in cigarette smoke, air-pollutant, concentrates, and automobiles exhaust.

4. Explain the principle, construction and working of densitometry with neat diagram. (May 2015)

- A densitometer is a device that measures the degree of darkness (the optical density) of a photographic or semitransparent material or of a reflecting surface. A densitometer does not measure color but measures density. In print work, density is

caused by the light-stopping ability of the pigments in the printing ink that are deposited on the paper by the printing process. Densitometers are widely used in the graphics industry to help control color in each step of the printing process.

WORKING

- Within a densitometer the light passes through the optical system bundled from a stabilized light source on the printed surface. The amount light absorbed depends on the ink density and pigmenting of the ink. The non-absorbed light penetrates the translucent (transparent) ink layer and is weakened. The remainder is re-emitted by the surface of the material, i.e. diffusely reflected or scattered A part of this scattered light passes through the ink layer and is weakened again.
- A lens system captures the light rays coming from the ink layer and sends them to a photodiode. The light striking the photodiode is converted into electric energy. The electronics compares this current with a reference value. The difference between the measured current and the reference value forms the basis for calculating the absorption behavior of the measured ink layer.
- Densitometric values are relative measurements. Despite the same measuring conditions, the values from different instruments are not the same. This can be due to differences in:

- The spectral transparency of the filters (aging etc.)
- The spectral distribution of the light sources
- The photodiode
- The measurement geometry
- The hues/ friction of the print scale inks
- The right handling and calibration can lead satisfactory correspondence within acceptable tolerances.

5. Explain the principle, mechanism, application of spectrophotometry. (Nov 2015, May 2016, Dec 2017, Dec 2018, May 2019, Dec 2019)

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.

- Spectrophotometer techniques are mostly used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer.

Principle:

- The spectrophotometer technique is to measure light intensity as a function of wavelength. It does this by diffracting the light beam into a spectrum of wavelengths, detecting the intensities with a charge-coupled device, and displaying the results as a graph on the detector and then on the display device.

1. In the spectrophotometer, a prism (or) grating is used to split the incident beam into different wavelengths.
2. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm.
3. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

- The essential components of spectrophotometer instrumentation include:
  1. A table and cheap radiant **energy source**
  2. Materials that can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.
  3. A **monochromator,** to break the polychromatic radiation into component wavelength (or) bands of wavelengths.
  4. A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands.

Prisms:

- A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent
- Two types of Prisms are usually employed in commercial instruments. Namely, 600 cornu quartz prism and 300 Littrow Prism.

Grating:

- Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.

Transport vessels (cuvettes), to hold the sample

- Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as "CUVETTES".
- Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz.

A Photosensitive **detector** and an associated **readout system**

- Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it.
- Radiation detectors generate electronic signals which are proportional to the transmitter light.
- These signals need to be translated into a form that is easy to interpret.
- This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.

Applications:

- Some of the major applications of spectrophotometers include the following:

- Detection of concentration of substances
- Detection of impurities
- Structure elucidation of organic compounds
- Monitoring dissolved oxygen content in freshwater and marine ecosystems
- Characterization of proteins
- Detection of functional groups
- Respiratory gas analysis in hospitals
- Molecular weight determination of compounds
- The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

6. Explain the principle and instrumentation of flame photometry. (Nov 2015, May 2017, Dec 2017, May 2018)

- Flame Photometry is also called as flame emission spectroscopy. Flame Photometry is branch of atomic spectroscopy. It is used to detected certain metal ions like sodium, potassium, magenisum etc.
- The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame.

- The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.
- It is also known as flame emission spectroscopy.
- Currently, it has become a necessary tool in the field of analytical chemistry.
- Flame photometer can be used to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium and cesium etc.
- In flame photometer spectra the metal ions are used in the form of atoms.

PRINCIPLE:

- Sample is sprayed into a flame and it converted to droplets.
- Due to the thermal energy of the flame the solvent in the droplets evaporate, leaving behind fine residue, which are converted to neutral atoms.
- This neutral atoms are get energy form thermal energy and go exited state but they are unstable at exited state so they are return to ground state with emission of specific wave length radiation.
- The wavelength of the radiation emitted is characteristic of the elements and is used to identify the elements (Qualitative Analysis).
- The intensity of the radiation emitted depends upon the concentration of the element analysed (Quantitative Analysis).
- The wavelength of the radiation emitted is given by the following equation :-
- $\lambda = hc/ E2-E1$

Where,

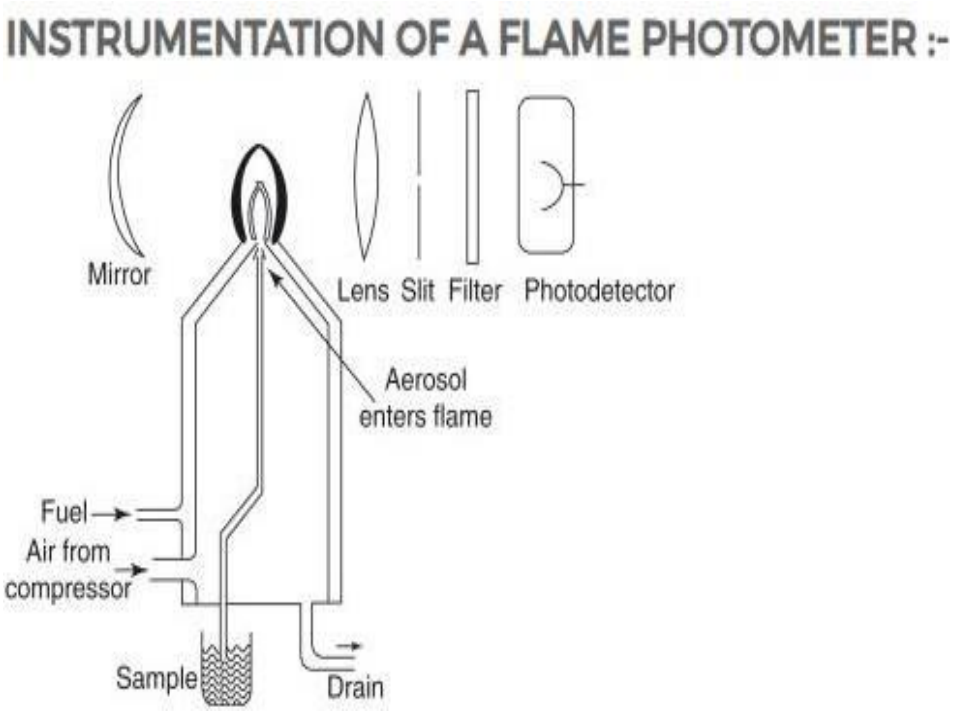
- h = Planks constant
- c= Velocity of light
- E2,E1= energy levels of exited and ground state respectively

BOLTZMAN LAW:

- The fraction of free atom that are thermally exited is governed by a Boltzman Distribution

$N^* / N = Ae^{-\Delta E/kT}$

- N^* =is the number of exited atom
- N = is the number of atom remaining in the ground state
- AE = is the difference in energies levels
- k = The Boltzman constant
- T = the tempeature



COMPONENTS:

- Burner (With fuel and oxidant)
- Filter/Monochromator
- Detector
- Read out device

BURNER :-

- There are different burners available which are used to spray the sample solution into fine droplet mix with fuel and oxidant so that a homogenous flame of stable intensity is obtained. The most common ones are mecker burner, total consumption burner and laminar flow burner.

TOTAL CONSUMPTION BURNER

- Due to the high pressure of fuel and oxidant the sample solution is aspirate through capillary and burnt at the tip of burner.
- Hydrogen and oxygen are generally employed as fuel and oxidant.
- The advantage over other is the entire consumption of sample,
- It's disadvantage is the production of non uniform flame and turbulent.

PREMIX BURNER

- In this burner the sample , fuel oxidant are thoroughly mixed before aspiration and reaching to flame.
- The main advantage of is the uniformity of flame produced.
- The main disadvantage is the heavy loss of mix up to 95%.

FILTER / MONOCHROMATOR :-

- In flame photometry the wavelength as well as intensity of the radiation emitted by the elements has to be monitored. Hence a filter or monochromator is to be used.
- A simple flame photometer contains a filter wheel and when a particular elements has to be analysed the specific filter is selected.

DETECTOR :-

- The radiation emitted by the elements is mostly in the visible region. Hence conventional detectors like photo voltaic cell or photo tubes can be used. In a flame spectrophotometer, photomultiplier tube is used as detector.

READ OUT DEVICE :-

- The signal from the detector is shown as a response in the digital read out device. The readings are displayed in an arbitrary scale (% Flame Intensity).

APPLICATIONS:

- Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to particular metal. Hence with the help of Flame photometer we can detect the presence of any specific element in the given sample.

- The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertiliser.
- The concentrations of Na+ and K+ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.
- Soft drinks, fruit juices and alcoholic beverages can also be analysed by using flame photometry to determine the concentrations of various metals and elements.

7. Explain the principle, application of calorimetry. (May 2017, May 2018)

- It is the most common analytical technique used in biochemical estimation in clinical laboratory.
- It involves the quantitative estimation of color.
- A substance to be estimated colorimetrically, must be colored or it should be capable of forming chromogens (colored complexes) through the addition of reagents.
- Colored substance absorb light in relation to their color intensity.
- The color intensity will be proportional to the conc. of colored substance.
- The instruments used in this method are colorimeter or photometer or absorptiometers.
- When a monochromatic light passes through a coloured solution, some specific wavelengths of light are absorbed which is related to colour intensity.
- The amount of light absorbed or transmitted by a colour solution is in accordance with two law i.e. Beer's & Lambert's Law.
- The measurement of colour intensity of a coloured solution by photometry is governed by two laws.

BEER'S LAW:

- When a monochromatic light passes through a colored solution, amount of light transmitted decreases exponentially with increase in concentration of colored substance.

- The amount of light absorbed by a colored solution is directly proportion to the conc. of substance in the colored solution.

LAMBERT'S LAW:

- The amount of light transmitted decreases exponentially with increase in pathlength (diameter) of the cuvette or thickness of colored solution through which light passes.
- The amount of light absorbed by a colored solution depends on pathlength of cuvette or thickness or dept of the colored solution.

BEER – LAMBERT'S LAW:

- Combined beer's- lambert's law is thus expressed as amount of light transmitted through a colored solution decreases exponentially with increases in conc. of colored solution & increase in conc. of colored solution & increase in the path length of cuvette or thickness of the colored solution.

PARTS OF A CALORIMETER:

Light source :

- Tungsten filament lamp Slit
- It is adjustable which allows only a beam of light to pass through.
- It prevents unwanted or stray light Condensing lenses: light after passing through slit falls on condenser lense which gives a parllel beam of light.

Filter :

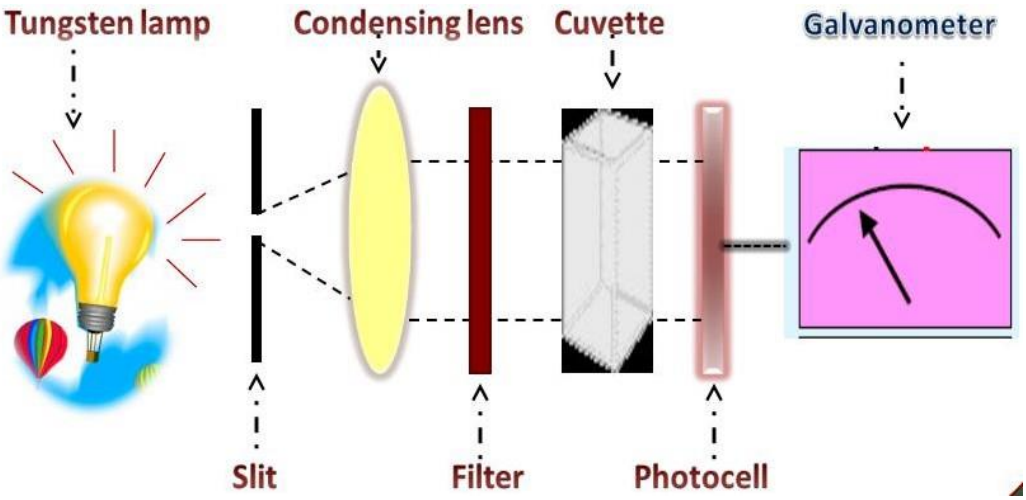
- made of colored glass.
- Filters are used for selecting light of narrow wavelength.
- Filters will absorb light of unwanted wavelength and allow only monochromatic light to pass through.
- For ex: a green filter absorbs all color, except green light which is allowed to pass through.
- Light transmitted through a green filter has a wavelength from 500-560 nm.
- Filter used is always complimentary in color to the color of solution.

Cuvette(sample holder) :

- The monochromatic light from the filter passes through the colored solution placed in a cuvette.
- It is made up of special glass/plastic/quartz material.
- It may be square/rectangular/round shape with fixed diameter (usually 1 cm)& having uniform surface.
- The colored solution in the cuvette absorbs part of light & remaining is allowed to fall on detector.
- For ex : a solution of red color transmits red light & absorbs the complimentary color green.

Detector (photocell):

- Detectors are photosensitive elements which converts light energy into electrical energy.
- The electrical signal generated is directly proportional to intensity of light falling on the detector.
- **Output :** the electrical signal generated in photocell is measured by galvanometer, which displays percent transmission & optical density.



Approx. wavelength	Colour absorbed (Filter)	Colour of solution
<4oonm	Ultra violet (UV-rays)	Not visible
400-420nm	Violet	Green-Yellow
420-500nm	Blue	Yellow
500-570nm	Green	Red
570-600nm	Yellow	Blue
600-630nm	Orange	Green-Blue
630-700nm	Red	Green
>700nm	Infrared (IR-rays)	Not visible

APPLICATIONS:

- It is widely used in hospital & laboratory for estimation of biochemical samples , like plasma, serum, cerebrospinal fluid (CSF) , urine.
- It is also used to quantitative estimation of serum components as well as glucose, proteins and other various biochemical compound.
- They are used by the food industry and by manufacturers of paints and textiles.
- They are used to test for water quality, by screening for chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine.
- They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or hemoglobin in the blood and to identify substandard and counterfeit drugs.

8. Write an essay on the uses of isotopes in biochemistry. (May 2015, Nov 2017, May 2019)

- Isotopes are atoms with the same atomic number but different mass numbers.
- Radioisotopes/radioactive isotopes of an element can be defined as atoms that contain an unstable nucleus and dissipate excess energy by spontaneously emitting radiation in the form of alpha, beta and gamma rays.
- One of the most important applications of radioisotopes in biochemistry is their use in determining metabolic pathways and for measuring the flow of metabolites through pathways.

- Radioisotopic methods have been developed to trace and assay enzymic reactions.
- If the enzyme reaction is simple, either the utilization of radiolabeled substrate or the formation of radiolabeled product may be monitored.
- Radioisotopes are also used for tracing metabolic pathways. Such experiments usually involve pulse-chase techniques where the radioactive substance is presented to the biological system as a pulse and the radiolabel chased through various metabolites or cellular compartments.
- Every chemical element has one or more radioactive isotopes. For example, hydrogen, the lightest element, has three isotopes with mass numbers 1, 2, and 3.
- Only hydrogen-3 (tritium), however, is a radioactive isotope, the other two being stable. More than 1,000 radioactive isotopes of the various elements are known.
- Approximately 50 of these are found in nature; the rest are produced artificially as the direct products of nuclear reactions or indirectly as the radioactive descendants of these products.
- Radioactive isotopes have many useful applications. In medicine, for example, cobalt-60 is extensively employed as a radiation source to arrest the development of cancer.
- Other radioactive isotopes are used as tracers for diagnostic purposes as well as in research on metabolic processes.
- When a radioactive isotope is added in small amounts to comparatively large quantities of the stable element, it behaves exactly the same as the ordinary isotope chemically; it can, however, be traced with a Geiger counter or other detection device. Iodine-131 has proved effective in treating hyperthyroidism.
- Another medically important radioactive isotope is carbon-14, which is used in a breath test to detect the ulcer-causing bacteria Heliobacter pylori.
- In industry, radioactive isotopes of various kinds are used for measuring the thickness of metal or plastic sheets; their precise thickness is indicated by the strength of the radiations that penetrate the material being inspected.

- They also may be employed in place of large X-ray machines to examine manufactured metal parts for structural defects.
- Other significant applications include the use of radioactive isotopes as compact sources of electrical power—e.g., plutonium-238 in spacecraft.
- In such cases, the heat produced in the decay of the radioactive isotope is converted into electricity by means of thermoelectric junction circuits or related devices.