18BEBME305 BIOCHEMISTRY 3 0 0 3 100

OBJECTIVES

To study about the biochemistry of living cells, metabolism of biomolecules and the methods of investigation and diagnostic tools.

INTENDED OUTCOMES

1. Understanding the concepts of biochemistry of living cells
2. Understanding the concepts of metabolism of carbohydrates
3. Understanding the concepts of protein biochemistry
4. Understanding the concepts of biochemistry of lipids
5. Understanding the concepts of investigation of metabolism.

UNIT-I BIOCHEMISTRY OF LIVING CELLS (9)

Biochemistry of living cells, sub cellular fractionation using the differential centrifugation method. Functions of each organelles, redox potential, oxidative phosphorylation, Transport of substances across biological membranes.

UNIT II CARBOHYDRATES (9)

Carbohydrates: Definition, classification, biological functions; glycolysis, TCA cycle, glycogenesis, glycogenolysis, Diabetes Mellitus – Blood Sugar analysis and glucose tolerance test.

UNIT III PROTEINS (9)

Proteins: Definition, classification, architecture, biological functions; Classification of amino acids, Oxidative and non oxidative deamination, transamination, decarboxylation, urea cycle, Purification of proteins.

UNIT IV LIPIDS (9)

Lipids: Definition, classification, biological functions; biosynthesis of long chain fatty acids, degradation of fatty acids - oxidation of fatty acids.

UNIT V METHODS OF INVESTIGATION OF METABOLISM (9)

Liver function tests, Real function tests, Gastric function tests. Diagnostic tools: Principles and applications of photometry, spectrophotometry, flurometry, flame photometry, automation in clinical laboratory. Uses of isotopes in biochemistry.

Total : 45

TEXT BOOKS

S.NO.	Author(s) Name	Title of the book	Publisher	Year of publication
1 .	Ambiga Shanmugam	Fundamentals of Biochemistry for Medical Students	Karthick Printers	1997

REFERENCE BOOKS

S.NO.	Author(s) Name	Title of the book	Publisher	Year of publication
1 .	Lehninger.A .L., Nelson D.L., Cox .M.M.,	Principles of Biochemistry	CBS Publications	1993
2	Varley	Clinical Biochemistry	CBS Publications	1988

FACULTY **HEAD OF THE DEPARTMENT** **DEAN**

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOTECHNOLOGY (FoE)

18BEBME305 - BIOCHEMISTRY

LECTURE PLAN

FACULTY NAME: Ms.T R POORANI 21.06.2019

CLASS: II YEAR – III SEMESTER; B.E. – Biomedical Engineering

S.NO	DESCRIPTION OF PORTION TO BE COVERED	HOURS	REFERENCE BOOK & PAGE NOS. USED FOR	TEACHING AIDS
UNIT – I BIOCHEMISTRY OF LIVING CELLS				
1.	Introduction to biochemistry and its role in biomedical engineering	1	[W1]	BB
2.	Biochemistry of living cells	1	[R1] : Pg. No. 3-11	BB
3.	Sub cellular fractionation using the differential centrifugation method	1	[R1] : Pg. No. 8	BB
4.	Functions of each organelles	2	[W2]	BB
5.	Redox potential	1	[W3]	BB
6.	Oxidative phosphorylation	1	[R1] : Pg. No. 691-721	BB
7.	Transport of substances across biological membranes	2	[R1] : Pg. No. 369-389	BB
8.	Tutorial	1	Revision	-
TOTAL HOURS FOR UNIT- I		**Theory hours : 9 Tutorial hour : 1 Total hours : 10**		
UNIT – II CARBOHYDRATES				
9.	Carbohydrates: Definition, classification	1	[R1] : Pg. No. 238-239	BB
10.	Carbohydrates: biological functions	2	[W4]	BB
11.	Glycolysis	2	[R1] : Pg. No. 522-534	BB
12.	TCA cycle	1	[R1] : Pg. No. 602-623	BB

13.	Glycogenesis and Glycogenolysis	1	[R1] : Pg. No. 562	BB
14.	Diabetes Mellitus – Blood Sugar analysis	1	[W5]	PPT
15.	Glucose tolerance test	1	[W5]	PPT
16.	Tutorial	1	Revision	-
TOTAL HOURS FOR UNIT- II		**Theory hours : 9 Tutorial hour : 1 Total hours : 10**		
UNIT – III PROTEINS				
17.	Proteins: Definition, classification	1	[R1] : Pg. No. 85-89	PPT
18.	Architecture	1	[R1] : Pg. No. 116-125	BB
19.	Biological functions	1	[R1] : Pg. No. 158-174	BB
20.	Classification of amino acids	1	[R1] : Pg. No. 75-84	PPT
21.	Oxidative and non oxidative deamination	1	[W12]	BB
22.	Transamination	1	[R1] : Pg. No. 660	BB
23.	Decarboxylation	1	[R1] : Pg. No. 859-860	BB
24.	Urea cycle	1	[R1] : Pg. No. 665-670	BB
25.	Purification of proteins	1	[W6]	
26.	Tutorial	1	Revision	
TOTAL HOURS FOR UNIT- III		**Theory hours : 9 Tutorial hour : 1 Total hours : 10**		
UNIT – IV LIPIDS				
27.	Introduction to Lipids	1	[R1] : Pg. No. 343-347	PPT
28.	Classification of lipids	1	[R1] : Pg. No. 348-355	
29.	biological functions of lipids	2	[W7]	BB and PPT
30.	biosynthesis of long chain fatty acids	2	[R1] : Pg. No. 787-816	BB and PPT
31.	Overview of degradation of fatty acids	2	[R1] : Pg. No. 632-636	BB and PPT
32.	oxidation of fatty acids	1	[R1] : Pg. No. 637-649	BB and PPT

33.	Tutorial	1	Revision	
TOTAL HOURS FOR UNIT- IV		**Theory hours : 9 Tutorial hour : 1 Total hours : 10**		
UNIT – V METHODS OF INVESTIGATION OF METABOLISM				
34.	Liver function tests	1	W8	PPT and BB
35.	Renal function tests	1	W9	PPT and BB
36.	Gastric function tests	1	W10	PPT and BB
37.	Diagnostic tools: Principles and applications of photometry	1	[T2] : Pg. No. 17-18	PPT and BB
38.	Spectrophotometry analysis	1	[T2] : Pg. No. 18-20	PPT and BB
39.	Flurometry analysis	1	[J3]	PPT and BB
40.	Flame photometry analysis	1	[W11]	PPT and BB
41.	Automation in clinical laboratory	1	[J1]	PPT and BB
42.	Uses of isotopes in biochemistry	1	[J2]	PPT and BB
43.	Tutorial	1	Revision	-
TOTAL HOURS FOR UNIT- V		**Theory hours : 9 Tutorial hour : 1 Total hours : 10**		
TOTAL HOURS		**45+ 5 Tutorial hours**		

TEXT BOOKS

[T1]	Ambiga Shanmugam, "Fundamentals of Biochemistry for Medical Students", Karthick Printers, Madras, 1997.
[T2]	Kumar, V., & Gill, K. D., "Basic Concepts in Clinical Biochemistry: A Practical Guide", Springer Singapore, 1st edition, 2018.

REFERENCE BOOKS

[R1]	Lehninger.A .L., Nelson D.L., Cox .M.M., "Principles of Biochemistry", CBS Publications, 1993.
[R2]	Varley, "Clinical Biochemistry", CBS Publications, 1998.

WEBSITE	
[W1]	https://chem.libretexts.org/Courses/University_of_Missouri/MU%3A__1330H_(Keller)/25%3A_Chemistry_of_Life%3A_Organic_and_Biological_Chemistry/25.08%3A_Introduction_to_Biochemistry
[W2]	https://www.toppr.com/guides/biology/the-fundamental-unit-of-life/cell-organelle/
[W3]	http://book.bionumbers.org/what-is-the-redox-potential-of-a-cell/
[W4]	https://courses.lumenlearning.com/wm-biology1/chapter/reading-types-of-carbohydrates/
[W5]	https://www.mayoclinic.org/diseases-conditions/diabetes/diagnosis-treatment/drc-20371451
[W6]	https://www.biologicscorp.com/protein-expression-purification/methods-for-protein-seperation-and-protein-purification.html#.XQtb7B8zaM8
[W7]	https://www.news-medical.net/life-sciences/Lipid-Biological-Functions.aspx
[W8]	https://www.healthline.com/health/liver-function-tests
[W9]	https://www.healthline.com/health/kidney-function-tests#treatment
[W10]	https://lexusorganics.com/blog/gastric-function-tests/
[W11]	https://www.studyandscore.com/studymaterial-detail/flame-photometer-principle-components-working-procedure-applications-advantages-and-disadvantages
[W12]	https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/oxidative-deamination
JOURNAL	
[J1]	Encyclopedia of Analytical Chemistry : Automation in the clinical laboratory
[J2]	Journal of Biomolecular Research & Therapeutics : Radioisotopes and Their Biomedical Applications
[J3]	International Journal of Advances in Pharmaceutical Analysis : Fluorescence spectroscopy and its applications: A Review

FACULTY **HEAD OF THE DEPARTMENT** **DEAN**

18BEBME305 - BIOCHEMISTRY

UNIT – I

BIOCHEMISTRY OF LIVING CELLS

CONTENTS

INTRODUCTION TO BIOCHEMISTRY AND ITS ROLE IN BIOMEDICAL ENGINEERING

The term Biochemistry was introduced by Carl Neubergin 1903. Biochemistry broadly deals with the chemistry of life and living processes. There is no exaggeration in the statement, 'The scope of biochemistry is as vast as life itself!' Every aspect of life-birth, growth, reproduction, aging and death, involves biochemistry. For that matter, every movement of life is packed with hundreds of biochemical reactions. Biochemistry is the most rapidly developing and most innovative subject in medicine. This becomes evident from the fact that over the years, the major share of Nobel Prizes earmarked for Medicine and Physiology has gone to researchers engaged ir: biochemistry. The discipline of biochemistry serves as a torch light to trace the intricate complexicities of biology, besides unravelling the chemical mysteries of life. Biochemical research has amply demonstrated that all living things are closely related at the molecular level. Thus biochemistry is the subject of unity in the diversified living kingdom. Advances in biochemistry have tremendous impact on human welfare, and have largely benefited mankind and their living styles. These include the application of biochernistry in the laboratory for the diagnosis of diseases. The products (insulin, interferon, growth hormone etc.) obtained from genetic engineering, and the possible use of gene therapy in the near future.

Chemistry is fundamental to the biomaterials and medical devices aspects of biomedical engineering. Implantable devices must perform their function in the human body, and coatings can be used to prevent fouling or an immune response and to promote wound healing and biological interactions between the device and the surrounding tissue. For example, vascular stents must have a coating that prevents fouling by proteins and thereby prevent thrombus formation, and more advanced stents have chemical or biochemical coatings that promote endothelialization of the stent. A deep understanding of chemistry is required to design a device coating that is stable over time and biocompatible. Biomaterials and drug delivery systems also require a high level of understanding of polymer chemistry and bioconjugation chemistry (the chemistry of covalently attaching biomolecules to polymers or other synthetic or biological molecules). Biochemistry is important because it provides a very solid fundamental

understanding of proteins and how other biological compounds are created. Biochemistry is a basic fundamental aspect of many areas of biomedical engineering, and its importance is at least equivalent to the importance of fundamental biology and physiology towards biomedical engineering research.

BIOCHEMISTRY OF LIVING CELLS

All Living Matter Contains C, H, O, N, P, and S

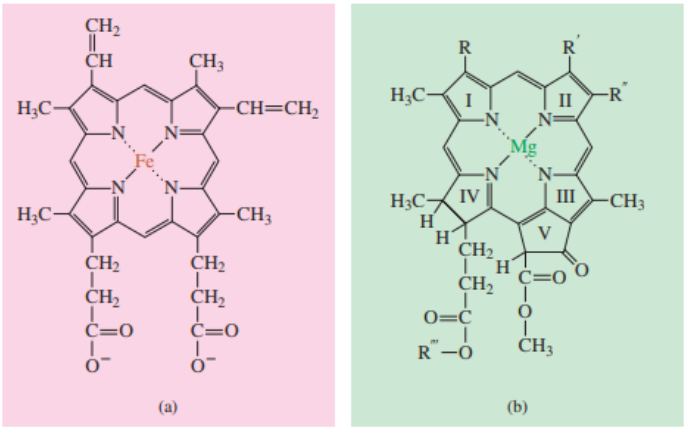
Chemical Elements in Biomolecules Of the 100 plus chemical elements, only about 31 (28%) occur naturally in plants and animals. The elements present in biological material can be divided into three categories:

1. Elements found in bulk form and essential for life: Carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur make up about 92% of the dry weight of living things.
2. Elements in trace quantities in most organisms and very likely essential for life, such as calcium, manganese, iron, and iodine.
3. Trace elements that are present in some organisms and may be essential for life, such as arsenic, bromine, molybdenum, and vanadium. The elements found in biomolecules do not have similar properties and characteristics.

The combination of chemical elements into biomolecules provides for great variety in chemical structure and reactivity. Biomolecules contain functional groups that are common in organic chemistry

Compounds representing all three states of matter (gases, liquids, and solids) are present in living cells. One of the most recent advances in biochemistry is the discovery of an enzyme that catalyzes the synthesis of the gas nitric oxide (NO) in the brain and other organs where it serves to regulate biological processes. Nature's molecules include examples of cations, anions, covalent compounds, ionic compounds, metal ions, coordination complexes, and polymers. Several well-known examples illustrate the diverse array of organic and organometallic chemicals that perform a variety of cellular roles. The amino acids are building blocks for protein structure. The carbohydrates are involved as nutrients in energy metabolism and play roles in cell structure and molecular recognition as well. Lipids are a diverse collection of organic compounds that display low water solubility. The lipids have primary functions as energy

molecules in metabolism, as components for the construction of cell membranes, and as hormones. The vitamins, a broad assortment of organic compounds, ensure proper growth and development by functioning in major biochemical processes. Prominent among the natural organometallic compounds are heme and chlorophyll. Both consist of a substituted porphyrin ring coordinated with a metal ion. Heme, a porphyrin ring with iron, is found in the oxygen transport proteins myoglobin and hemoglobin, in respiratory proteins such as cytochrome c, and in enzymes such as catalase. Chlorophyll, a magnesium– porphyrin complex, is abundant in green plants and algae, where it functions as a receptor of light energy.

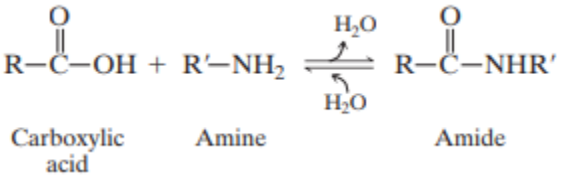


Two natural organometallic compounds: (a) heme, containing a porphyrin ring and iron; and (b) chlorophyll, containing a porphyrin ring and magnesium.

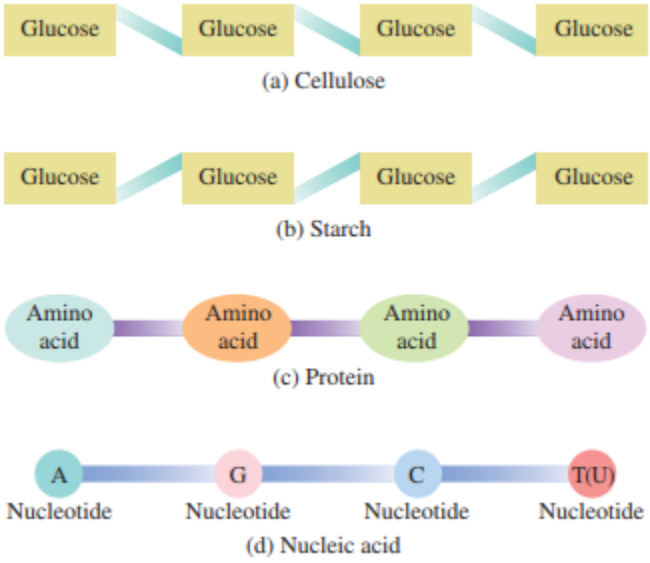
Many molecules present in biological cells are very large by the standards of inorganic and organic chemistry. Three major classes of natural polymeric macromolecules are found in biological cells: the nucleic acids, proteins, and polysaccharides. (Lipids are also considered a major class of biomolecules, but because they are not polymeric macromolecules, they are not described in this section but in the previous paragraph.) The major classes of macromolecules participate in a complex array of biological processes, such as storing and transferring genetic information (nucleic acids), catalyzing biochemical reactions (proteins called enzymes), holding cells and organisms together (structural proteins and polysaccharides), transporting small molecules across cell membranes or from one location in an organism to another location (transport proteins), and protecting an organism against disease agents (protein antibodies). Although their structures and functions are quite different, all natural macromolecules have one

common characteristic; they are polymers constructed by combining together hundreds, thousands, and sometimes millions of smaller, prefabricated molecules called monomers

Cellular Reactions The reaction process that connects monomer units is called condensation, a chemical combining that results in the loss of a small molecule, usually water:



In the example given here, a carboxylic acid is condensed with an amine to form a new compound with an amide bond. This represents the chemistry for combining amino acids to make proteins. During metabolic turnover in the living cell, the reverse process, called cleavage or hydrolysis (if water is used), also becomes important. The product formed by condensation or polymerization of thousands of glucose monomers is either starch or cellulose, depending on the type of chemical linkage.



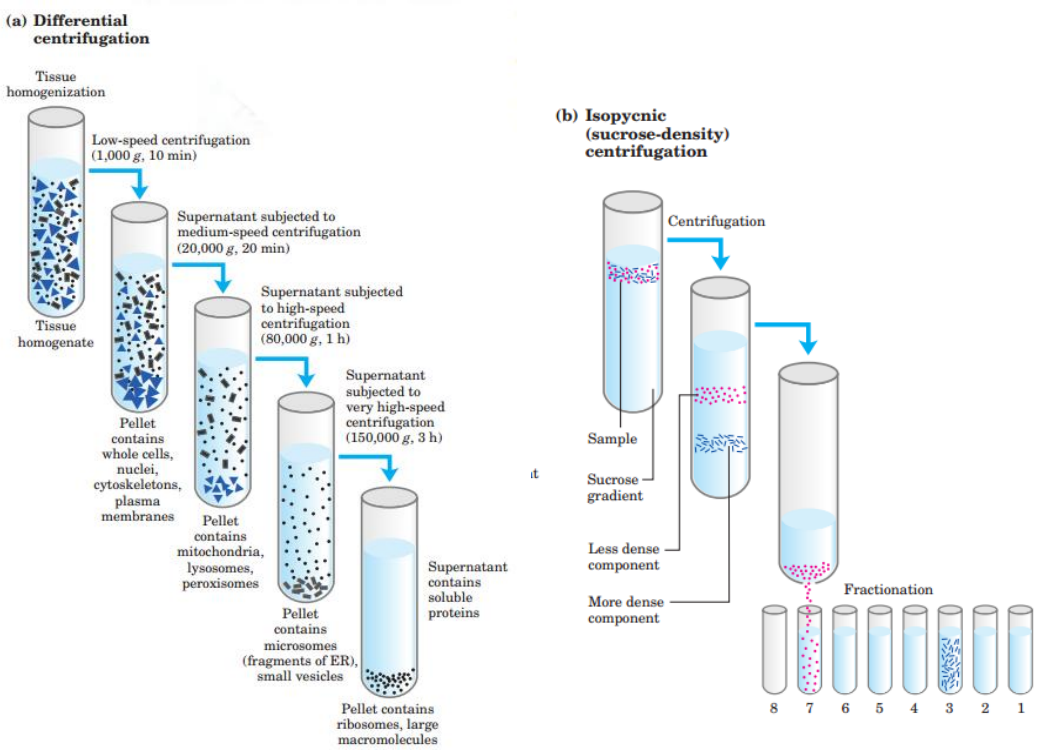
Types of natural polymers. (a) Cellulose, a homopolymer formed by joining many identical glucose units. (b) Starch, a homopolymer formed by joining many identical glucose molecules. Note that different types of bonding are used in starch and in cellulose. (c) Protein, a heteropolymer formed by linking together amino acids. (d) Nucleic acid, a heteropolymer formed by combining different nucleotides, A, G, C, and T or U.

between the glucose units.These macromolecules are called polysaccharides because they are composed of many saccharide (sugar) molecules. Because the monomeric units comprising these polysaccharides are chemically identical, they are, in general, termed homopolymers. Proteins are the products of joining together amino acids by amide bonds. Because 20 different amino acids are available as monomeric building blocks, the resulting proteins are heteropolymers. By combining many different amino acids in proteins, a level of molecular complexity and structural diversity results that is not possible for starch or cellulose. Different proteins are formed by changing the order (sequence) and number of amino acid monomers. This allows the construction of a vast array of different protein molecules, each with its own physical, chemical, and biological characteristics.All molecules of a specific protein (e.g., hemoglobin) within a species of organism, however, normally have an identical sequence of amino acids. The nucleic acids are heteropolymers of monomeric units called nucleotides. Deoxyribonucleic acid (DNA), the chemical storage form of genetic information, is composed of the monomers deoxyadenosine 5-monophosphate (dAMP), deoxyguanosine 5-monophosphate (dGMP), deoxycytidine 5-monophosphate (dCMP), and deoxythymidine 5-monophosphate (dTMP). Each DNA molecule in the human chromosome contains millions of nucleotides. Ribonucleic acid (RNA), which is involved in the transfer of genetic information and in biological catalysis, is a heteropolymer of adenosine 5-monophosphate (AMP), guanosine 5-monophosphate (GMP), cytidine 5-monophosphate (CMP), and uridine 5-monophosphate (UMP).The genetic information present in nucleic acids is coded by the sequence of nucleotides. In the last part of this book, we will focus on the important processes of metabolism.This refers to the thousands of biochemical reactions that define the synthesis (anabolism) and breakdown (catabolism) of all biomolecules including the macromolecules. The metabolic turnover of proteins, nucleic acids, polysaccharides, and lipids requires complex cellular machinery, strict control to assure reproducibility, the presence of reaction catalysts called enzymes (usually proteins), and complicated processes for exchange of chemical energy. Catabolism (exergonic) and anabolism (endergonic) are linked through the transfer of energy in the molecular form of adenosine triphosphate (ATP). All the information necessary to direct cellular tasks resides in the DNA of the organism, but the message in the form of nucleotide sequence must first be decoded and made into functional proteins before biochemical reactions can occur.

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, and is mainly based on differential centrifugation in media of high viscosity at 4°C. Media used for differential centrifugation are mainly sucrose, mannitol, glycerol, Ficoll 400 (a polymer of sucrose), Percoll (a type of colloidal silica) and iodixanol (OptiPrep, e.g., Hela or THP1 cell fractionation for cGAS activity assay. Sucrose is widely used because it is inexpensive. But they all have their advantages and limitations. Mainly these methods will be discussed here, with a preference for the ones that are easily accessible to most labs and are less time-consuming, as speedy recovery is vital. Gel filtration, affinity chromatography, electrophoresis or selective density-shift perturbation can also be used. Variations in the conditions of the available protocols are dependent on the organelle, tissue or cell type and equipment used, and it is highly recommended to read the cited references for full details of each procedure. In the end, the purity and the yield of the fractionation should be assessed by detection of distinct markers in each collected fraction during the entire procedure.

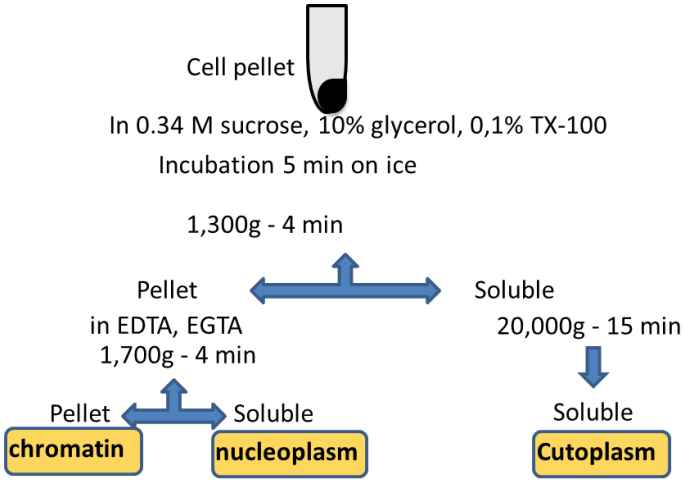
Subcellular fractionation of tissue. A tissue such as liver is first mechanically homogenized to break cells and disperse their contents in an aqueous buffer. The sucrose medium has an osmotic pressure similar to that in organelles, thus preventing diffusion of water into the organelles, which would swell and burst. **(a)** The large and small particles in the suspension can be separated by centrifugation at different speeds, or **(b)** particles of different density can be separated by isopycnic centrifugation. In isopycnic centrifugation, a centrifuge tube is filled with a solution, the density of which increases from top to bottom; a solute such as sucrose is dissolved at different concentrations to produce the density gradient. When a mixture of organelles is layered on top of the density gradient and the tube is centrifuged at high speed, individual organelles sediment until their buoyant density exactly matches that in the gradient. Each layer can be collected separately.



Isolation of cytoplasm, nucleoplasm, and chromatin

Cytoplasmic, nucleoplasmic and chromatin fractions can be easily prepared from a pellet of cultured cells. Cells are resuspended in a buffer containing 0.34 M sucrose, 10% glycerol and low concentration of a mild detergent (0.1% Triton X-100) as well as K^{+} and Mg^{+2} (which protect the nuclei from breaking) and the nuclei are pelleted by low-speed centrifugation while the supernatant is kept as the cytoplasmic fraction. Next, nuclei are lysed in a buffer containing chelating agents EDTA and EGTA and the insoluble chromatin fraction is pelleted by low-speed centrifugation while the supernatant is the nucleoplasmic fraction.

Some researchers use a 'quick and dirty' preparation of chromatin with its associated proteins from the cytoplasm/nucleoplasm. This is simply performed by lysing the cells in a lysis buffer containing 1% Triton X-100. In this buffer, chromatin and some cytoskeletal structures are insoluble and they can be recovered by centrifugation. The pellet can be resuspended in the buffer of choice e.g., Laemmli for SDS PAGE.



Isolation of mitochondria by differential centrifugation

The procedure is as follows

1. Cell pellets, grown as a monolayer or in suspension, are homogenized in a homogenization buffer containing $MgCl_2$ and KCl. Later, sucrose is added up to 0.25 M and the nuclei are pelleted by low-speed centrifugation (1000 g). Second centrifugation of the supernatant at 5000 g wills sediment the mitochondria. The pellet is resuspended in a medium containing sucrose and Mg^{+2} and is subjected to a few gentle strokes in a Dounce homogenizer. The last centrifugation step at 5000 g will enrich mitochondria which can be resuspended in Tris buffer containing 0.25 M sucrose or in the buffer of preference for subsequent analyses (e.g., Laemmli)
2. Yeast cells are treated with zymolase to break the hard outer wall and produce spheroplasts, which are washed in a sorbitol buffer. The pellet is resuspended in homogenization buffer containing 0.6 M mannitol and cells are lysed by a few strokes in a Dounce homogenizer. Nuclei are removed by low-speed centrifugation, while the cytoplasm-containing supernatant is centrifuged in a fixed-angle rotor at 6500 g to pellet mitochondria.

Additionally, there are protocols that utilize density-gradient separations which provide purer mitochondrial fractions, but they are more time-consuming and are avoided. Despite the "contamination" by lysosomes and peroxisomes in fractions obtained by the differential centrifugations, they are the method of choice. Therefore, the desired purity determines which the most suitable method is. For example, if metabolic studies are of interest, differential

centrifugation is preferred; alternatively, if the exact localization of a protein is under investigation or samples of the purest form are a necessity, e.g., in proteomics, the density-gradient preparations are more suitable.

Sub-fractionation of mitochondria

Mitochondria have a complex structure comprising the matrix, inner mitochondrial membrane (IMM), intermembrane space and the outer mitochondrial membrane (OMM) which as attached to the inner membrane at "contact sites". Protocols have been published which enable the enrichment and isolation of the various mitochondrial compartments. Isolation of OMM from yeast and rat liver both involve isolation of mitochondria followed by osmotic swelling/shrinking to release the OMM followed by density gradient centrifugation to yield highly purified OMM. Whilst purity is high, OMM yields are typically 1% of total mitochondrial protein. The detachment of the OMM releases the proteins of the intermembrane space. The 'mitoplasts' devoid of OMM are a source of enriched IMM. Lysis of the mitoplasts releases the proteins of the mitochondrial matrix.

Methods have also been published for the isolation of mitochondrial-associated membranes (MAM) which connect mitochondria and the endoplasmic reticulum and play an important role in phospholipid metabolism.

FUNCTIONS OF EACH ORGANELLES OF LIVING CELLS

CELL THEORY

Cells are the basic unit of life.

The Cell Theory states that:

1) All organisms are made up of one or more cells and the products of those cells.

2) All cells carry out life activities (require energy, grow, have a limited size).

3) New cells arise only from other living cells by the process of cell division.

THE THREE MAIN COMPONENTS OF ANY PLANT OR ANIMAL CELL ARE:

1. PLASMA MEMBRANE/ CELL MEMBRANE

Structure- a bilipid membraneous layer composed of proteins and carbohydrates. It is fluid like.

Function - the cell membrane separates the cell from its external environment, and is selectively permeable (controls what gets in and out). It protects the cell and provides stability.

Proteins are found embedded within the plasma membrane, with some extending all the way through in order to transport materials.

Carbohydrates are attached to proteins and lipids on the outer lipid layer.

2. CYTOPLASM

Structure - The jelly-like substance composed of mainly water and found between the cell membrane and nucleus. The cytoplasm makes up most of the "body" of a cell and is constantly streaming.

Function - Organelles are found here and substances like salts may be dissolved in the cytoplasm.

3. NUCLEUS

Structure - The largest organelle in the cell. It is dark and round, and is surrounded by a double membrane called the **nuclear envelope/membrane**. In spots the nuclear envelope fuses to form pores which are selectively permeable. The nucleus contains genetic information (DNA) on special strands called **chromosomes**.

Function - The nucleus is the "control center" of the cell, for cell metabolism and reproduction.

THE FOLLOWING ORGANELLES ARE FOUND IN BOTH PLANT AND ANIMAL CELLS.

1. "ER" OR ENDOPLASMIC RETICULUM

The Endoplasmic Reticulum is a network of membranous canals filled with fluid. They carry materials throughout the cell. The ER is the "transport system" of the cell.

There are two types of ER: rough ER and smooth ER.

Rough Endoplasmic Reticulum is lined with ribosomes and is rough in appearance and smooth endoplasmic reticulum contains no ribosomes and is smooth in appearance.

2. RIBOSOMES

Ribosomes are small particles which are found individually in the cytoplasm and also line the membranes of the rough endoplasmic reticulum. Ribosomes produce protein. They could be thought of as "factories" in the cell.

3. GOLGI BODY / APPARATUS

Golgi bodies are stacks of flattened membranous stacks (they look like pancakes!). The Golgi Body temporarily stores protein which can then leave the cell via vesiciles pinching off from the Golgi.

4. LYSOSOMES

Lysosomes are small sac-like structures surrounded by a single membrane and containing strong digestive enzymes which when released can break down worn out organelles or food. The lysosome is also known as a suicide sac.

5. MITOCHONDRIA

The mitochondria are round "tube-like" organelles that are surrounded by a double membrane, with the inner membrane being highly folded. the mitochondria are often referred to as the "powerhouse" of the cell. the mitochondria releases food energy from food molecules to be used by the cell. This process is called respiration. Some cells(muscle cells) require more energy than other cells and so would have many more mitochondria.

6. VACUOLES

Vacuoles are fluid filled organelles enclosed by a membrane. They can store materials such as food, water, sugar, minerals and waste products.

ANIMAL CELLS ORGANELLES NOT FOUND IN PLANT CELLS:

CILIA AND FLAGELLA

Both cilia and flagella are hair-like organelles which extend from the surface of many animal cells. the structure is identical in both, except that flagella are longer and whiplike and cilia are shorter. There are usually only a few flagella on a cell, while cilia may cover the entire surface of a cell. The function of cilia and flagella ionclude locomotion for one-celled organisms and to move substances over cell surfaces in multi-celled organisms.

ORGANELLES AND OTHER FEATURES FOUND ONLY IN PLANT CELLS:

1. CELL WALL

The cell wall is a rigid organelle composed of cellulose and lying just outside the cell membrane. The cell wall gives the plant cell it's box-like shape. it also protects the cell. The cell wall contains pores which allow materials to pass to and from the cell membrane.

2. PLASTIDS

Plastids are double membrane bound organelles. It is in plastids that plants make and store food. Plastids are found in the cytoplasm and there are two main types:

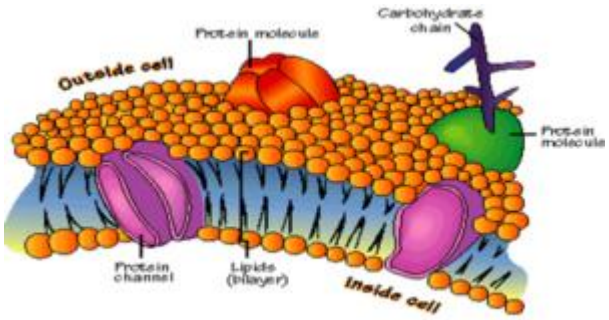
Leucoplasts - colorless organelles which store starch or other plant nutrients. (example - starch stored in a potato)

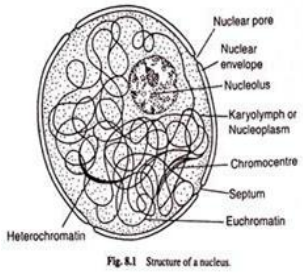
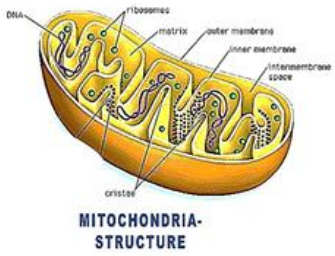
Chromoplasts - contain different colored pigments. The most important type of chromoplast is the **chloroplast**, which contains the green pigment chlorophyll. This is important in the process of photosynthesis.

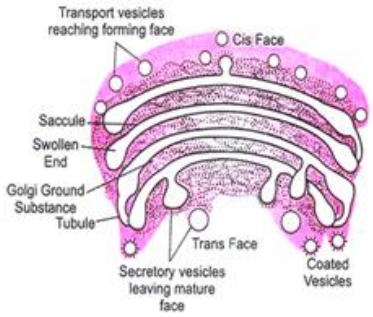
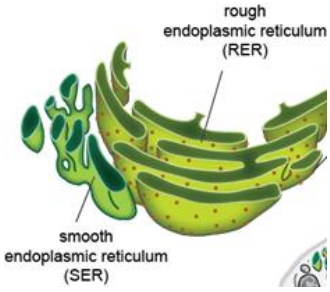
3. CENTRAL VACUOLE

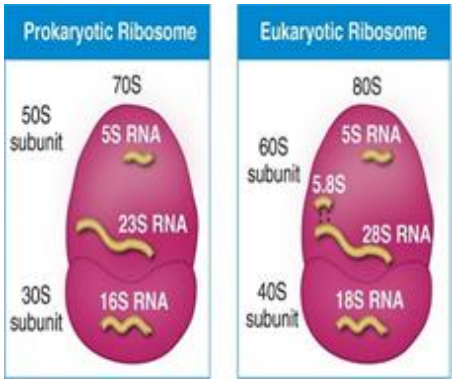
The central vacuole is a large fluid-filled vacuole found in plants.

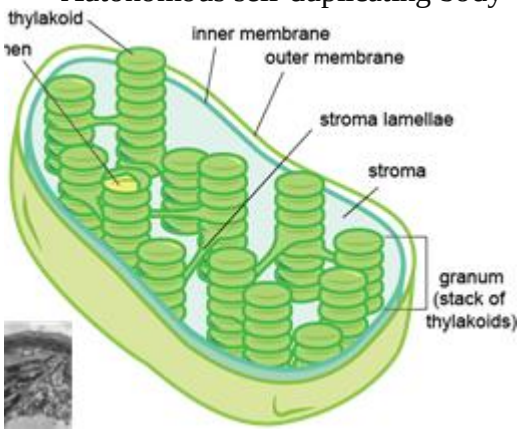
Cell Organelle	Occurrence/ Characteristic & Structure	Function

Cell Membrane/ Plasma Membrane	• Present in both plant cell and animal cell. • Selectively Permeable: Allows the materials in and out of the cell according to the requirement of the cell.  • Made up of bilipid layer and protein (Fluid Mosaic Model)	• Encloses the contents of the cell. • Provides shape: animal cell. • Allows transport: by Diffusion and Osmosis.
Cell Wall	• Present only in a plant cell. • Hard and rigid. • Fully permeable. • Made up of Cellulose in plant and peptidoglycan in bacteria.	• Protection • Gives shape and turgidity.
Cytoplasm	• Contains 80-90% water and many organic and inorganic compounds. • Colloidal, Viscous, Jelly like fluid inside the cell.	• Contains enzymes responsible for all the metabolic activity taking place inside the cell.

Nucleus **(Director/ Brain of the Cell)**	• Covered by a double membranous nuclear membrane in a Eukaryotic Cell. • Contains DNA, RNA, Protein, nucleolus, and Chromatin network.  Fig. 8.1 Structure of a nucleus.	• Controls the activity of the cell. • Starts cell division. • It has the chromosomes or DNA which controls the hereditary characters
Mitochondria (The Power House of The Cell / Storage Batteries)	• Double membranous structure. • Autonomous body as contains its own DNA. • Self-duplicates • The main seat of respiration. • Stores energy in the form of ATP molecules.  MITOCHONDRIA-STRUCTURE	

Golgi Bodies **(Shipping Department of Cell)**	• Discovered by Camillo Golgi in 1898. • Originates from RER. • Contains Sac like Cisternae and Vesicles. • Has two faces – cis face or receiving face and trans face or supplying face.  	• Modification, Packaging, and transport of materials • Synthesis of lysosomes, plasma membrane
Endoplasmic Reticulum **(Framework of Cell)**	• A network of membranes. • RER bears ribosomes and appears rough • SER does not have ribosomes  	• Forms the skeletal framework of the cell. • Transport of materials from one cell to other. • Provides a surface for the synthesis of material – Proteins in RER and Lipids in SER. • Formation of lysosomes, Golgi bodies and vacuoles • Membrane Biogenesis • Detoxification of harmful substances in the liver.

Vacuole	• Arise from ER and GB • Surrounded by tonoplast and filled with cell sap	• Store cell sap which may be liquid or solid food, toxic byproduct. • Provide rigidity and turgidity to plant cell
Lysosomes (Suicidal bags of Cell, natural scavenger, cellular housekeeper)	• Membrane-bound organelles • Present in all animal cells and few plant cells • Tiny circular single membrane-bound structures filled with digestive enzymes	• Intracellular digestion of food in unicellular organisms.
Ribosomes (Protein Factories)	• Without a membrane • Consist of two subunits – 60S and 40S in eukaryote both made up of RNA 	• Synthesis of Proteins

Plastids	• Double membrane-bound Types- 1. **Leucoplast** –Colourless plastid; 2. **Chromoplast** –Coloured Plastid – blue, red, yellow 3. **Chloroplast** – Green plastid • Autonomous self-duplicating body 	• Chloroplast – Perform Photosynthesis – Helps in the release of oxygen • Chromoplast – impart colour t o flowers which help in pollination • Leucoplast – Storage • Amyloplast – Store starch • Aleuroplast – Store Protein • Elaioplast – Store fat

REDOX POTENTIAL

Redox potentials are used to characterize the free energy cost and direction of reactions involving electron transfer, one of the most ubiquitous and important of biochemical reactions. Such reduction-oxidation reactions are characterized by a free energy change that shares some conceptual features with that used to describe pKa in acid-base reactions where proton transfer is involved rather than electron transfer. In this vignette, one of the most abstract in the book, we discuss how the redox potential can be used as a measure of the driving force for a given oxidation-reduction reaction of interest. By way of contrast, unlike the pH, there is no sense in which one can assign a single redox potential to an entire cell.

Many enzymatic reactions are oxidation-reduction reactions in which one compound is oxidized and another compound is reduced. The ability of an organism to carry out oxidation-reduction reactions depends on the oxidation-reduction state of the environment, or its reduction

potential (Eh). Strictly aerobic microorganisms are generally active at positive (Eh) values, whereas strict anaerobes are generally active at negative (Eh) values. Redox affects the solubility of nutrients, especially metal ions.

There are organisms that can adjust their metabolism to their environment, such as facultative anaerobes. Facultative anaerobes can be active at positive Eh values, and at negative Eh values in the presence of oxygen bearing inorganic compounds, such as nitrates and sulfates.

Redox potential is a measure of how oxidative (or reductive) an environment is and in a cell it plays an important role in controlling pathway activation *via* modulation of the oxidation state of proteins (through oxidation of thiol, tyrosine or proline side chains). Redox potential changes are associated with cell cycle, differentiation, apoptosis and signalling, and mounting evidence has demonstrated that the dysfunction of redox potential in cells can have a causal role in the initiation or progression of diseases including cardiovascular disorders, neurodegenerative diseases and cancer. Hypoxia is a reductive dysfunction of cells and is considered to be an important factor in driving malignancy and resistance to therapy in tumours. In order to achieve an improved understanding of hypoxia, the factors that control it and its effects, there is a need to develop methods for quantitative, real-time measurements of the redox potentials associated with hypoxia.

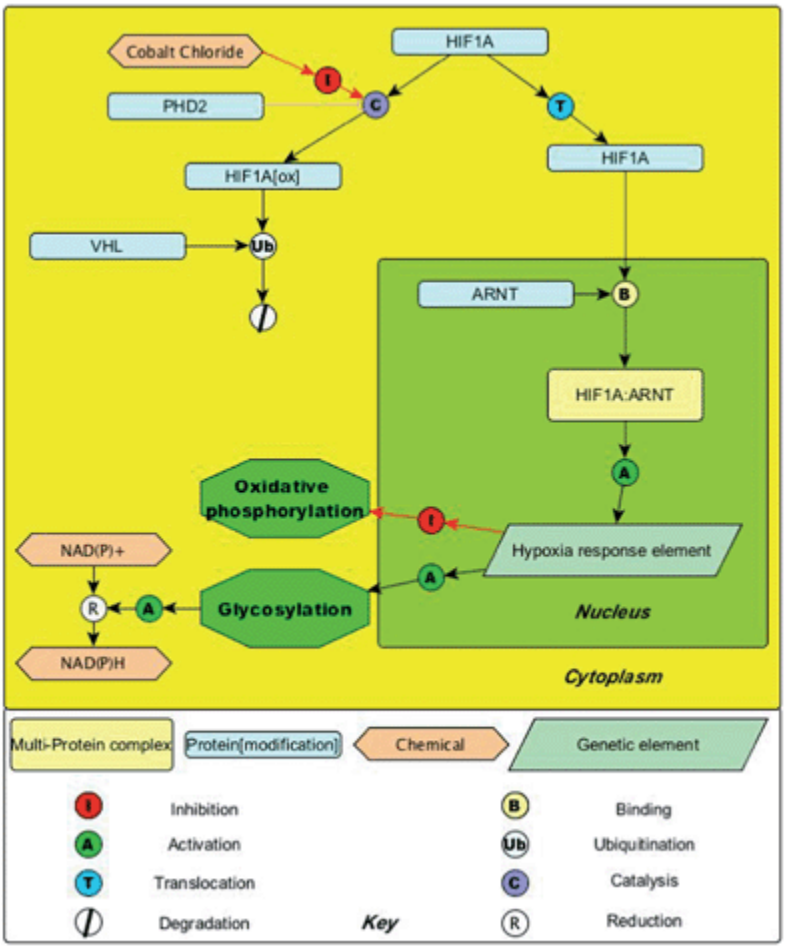
Currently, there are a few quantitative approaches which can measure redox potential in cells; however, they are not able to quantify redox potential in the hypoxic range. Engineered redox-active green fluorescent proteins (roGFPs) can be expressed in cells to report the localized redox potential, but since these measurements are linked with the glutathione redox couple and the roGFPs have standard reduction potentials ranging between −230 to −290 mV *vs.* NHE, they are limited to measurements in a potential-window which is more reflective of normoxia. Since roGFPs function by switching from a dithiol to a disulfide form, protein engineering offers limited opportunities for extension to wider ranges. In addition, various techniques have been used to selectively detect hypoxic cells by utilizing immunostaining, PET imaging, phosphorescence imaging and fluorescence imaging. Among them, fluorescent probes offer obvious advantages including high sensitivity and ease of use. However, none of these hypoxia probes quantify the value of intracellular redox potential – they only report that a cell or tissue was hypoxic. As a result, there is a need for complementary techniques to quantitatively measure redox potential in cells that are in a state of hypoxia. These

quantitative approaches will enable researchers to develop a better understanding of how the dysregulation of cellular redox potential plays a role in hypoxia and hypoxia-associated diseases.

Our group has established a new approach to monitor intracellular redox potential based on the use of Surface-Enhanced Raman Spectroscopy (SERS) nanosensors comprising redox-sensitive reporter molecules assembled on gold nanoshells. Raman spectroscopy measures the vibrational modes of molecules and as a result can differentiate between the oxidation states of the reporter molecules with high specificity. While the Raman effect is typically weak, SERS offers a significant enhancement of the Raman effect of up to 14 orders of magnitude by adsorbing the reporter molecules on metal surfaces (most commonly silver or gold nanoparticles), making it an ideal substitute for fluorescence. Gold nanoshells (NS) are engineered spherical particles composed of a silica core covered by a thin gold shell. NS are ideal substrates for our SERS nanosensors because they possess both tuneable plasmon resonance and biocompatibility. We have demonstrated that NS used for intracellular SERS measurements are non-toxic and do not reduce cell viability, cause redox dysregulation or induce cell death.

In this paper, we describe the development of a novel SERS sensing approach for monitoring intracellular redox potential in the hypoxic range which, to our knowledge, is the first technique reported to quantify the redox potential of cells in hypoxia. We utilize gold nanoshells modified with molecules whose reduction potential is in the hypoxic range and we calculate the intracellular redox potential through the proportions of the oxidized and reduced reporters which can be measured by SERS. By using these nanosensors we have measured the local redox potential in A549 (lung epithelium) cells during hypoxia and correlated its response with that of an established hypoxia assay.

Pathway map illustrating how inhibition of PHD2 results in the production of NAD(P)H and a consequent reductive shift in cellular redox potential is given below.



OXIDATIVE PHOSPHORYLATION

There are a lot of different ways organisms acquire food. Just think about how sharks, bees, plants, and bacteria eat. Almost all aerobic organisms (organisms that require oxygen to live) use oxidative phosphorylation, in one way or another, to produce the basic energy currency of the cell needs to function: ATP (adenosine triphosphate). Oxidative phosphorylation is the fourth step of cellular respiration, and produces the most of the energy in cellular respiration.

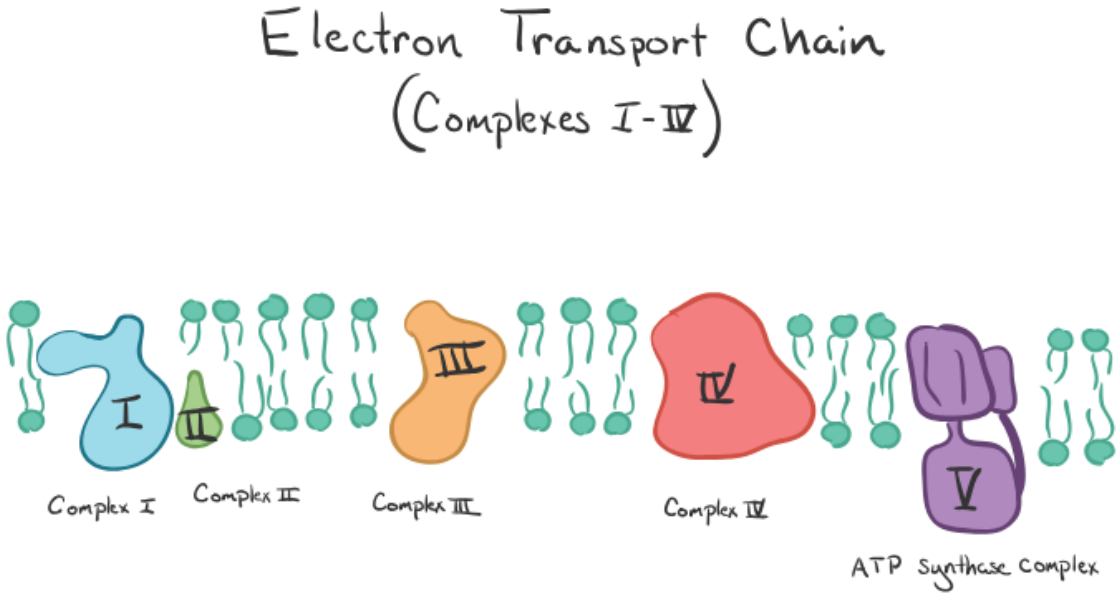
Where does oxidative phosphorylation fit into cellular respiration?

1. Glycolysis, where the simple sugar glucose is broken down, occurs in the cytosol.

2. Pyruvate, the product from glycolysis, is transformed into acetyl CoA in the mitochondria for the next step.
3. The citric acid cycle, where acetyl CoA is modified in the mitochondria to produce energy precursors in preparation for the next step.
4. **Oxidative phosphorylation**, the process where electron transport from the energy precursors from the citric acid cycle (step 3) leads to the phosphorylation of ADP, producing ATP. This also occurs in the mitochondria.

What is oxidative phosphorylation?

Oxidative phosphorylation is the process where energy is harnessed through a series of protein complexes embedded in the inner-membrane of mitochondria (called the electron transport chain and ATP synthase) to create ATP. Oxidative phosphorylation can be broken down into two parts: 1) Oxidation of NADH and $FADH_2$ and 2) Phosphorylation.

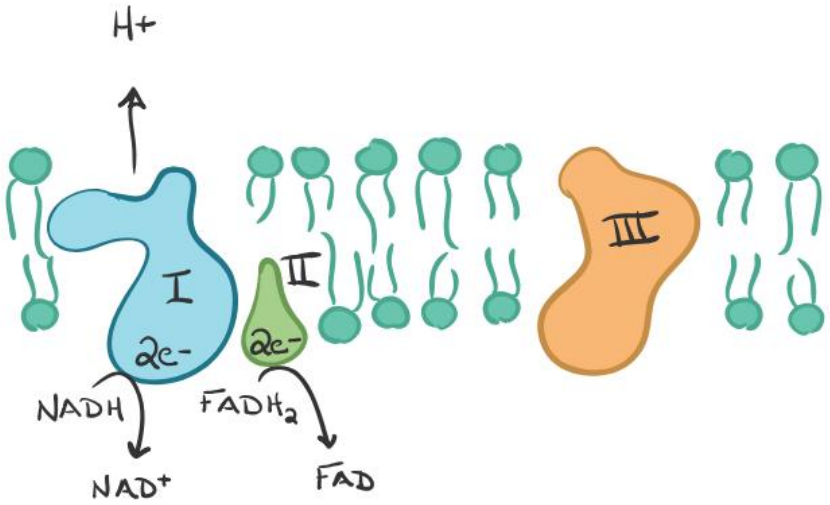


Oxidation of NADH and FADH2 - losing electrons via high energy molecules

Step 1

Oxidative phosphorylation starts with the arrival of 3 NADH and 1 FADH2 from the citric acid cycle, which shuttle high energy molecules to the electron transport chain. NADH transfers its high energy molecules to protein complex 1, while FADH2 transfers its high energy molecules to protein complex 2. Shuttling high energy molecules causes a loss of electrons from NADH and FADH2, called *oxidation* (other molecules are also capable of being oxidized).

The opposite of oxidation is *reduction*, where a molecule *gains electrons* (which is seen in the citric acid cycle).



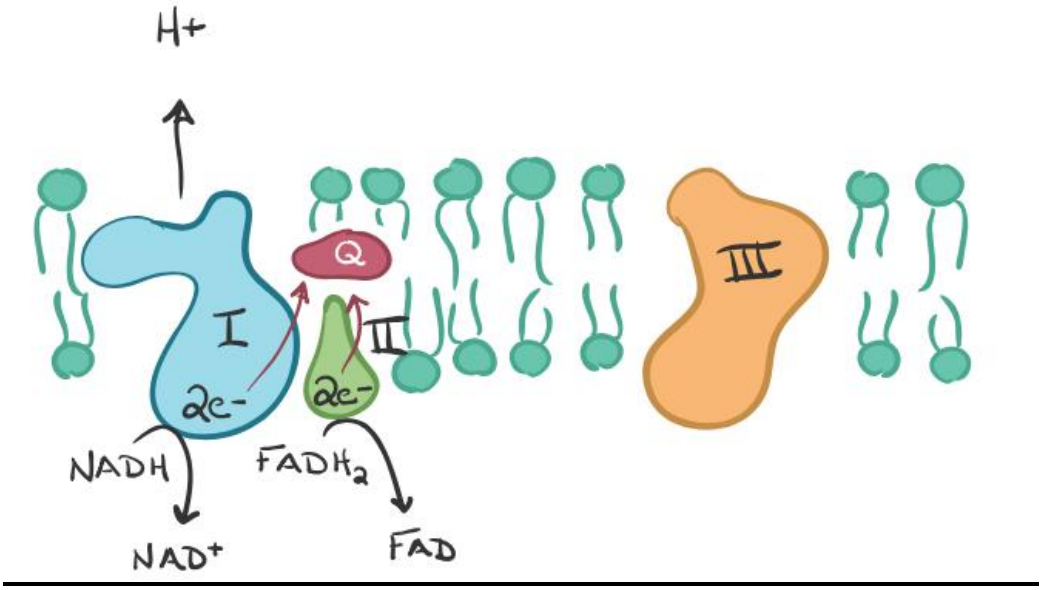
Step 2 - Hitting the gym to pump some serious hydrogens

The process of NADH oxidation leads to the pumping of protons (single positively-charged hydrogen atoms denoted as H+ through protein complex 1 from the matrix to the intermembrane space. The electrons that were received by protein complex 1 are given to another membrane-bound electron carrier called *ubiquinone* or *Q*.

This process of transferring electrons drives the pumping of protons, which is seen as unfavorable. Electron transfer driving proton pumping is repeated in complexes 3 and 4 (which we will discuss in steps 2 - 5). As this action is repeated, protons will accumulate in the

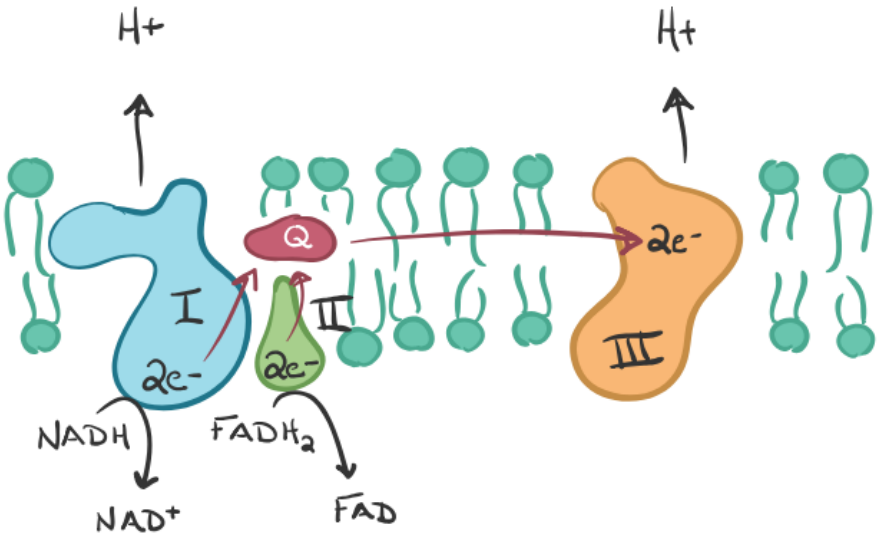
intermembrane space. This accumulation of protons is how the cell temporarily stores transformed energy.

Note – FADH2 has a slightly different route than NADH. After its arrival at protein complex 2, its high energy electrons are directly transferred to Q, to form reduced Q, or *QH2*. There is no hydrogen pumping for the exchange of the FADH2 electrons here.



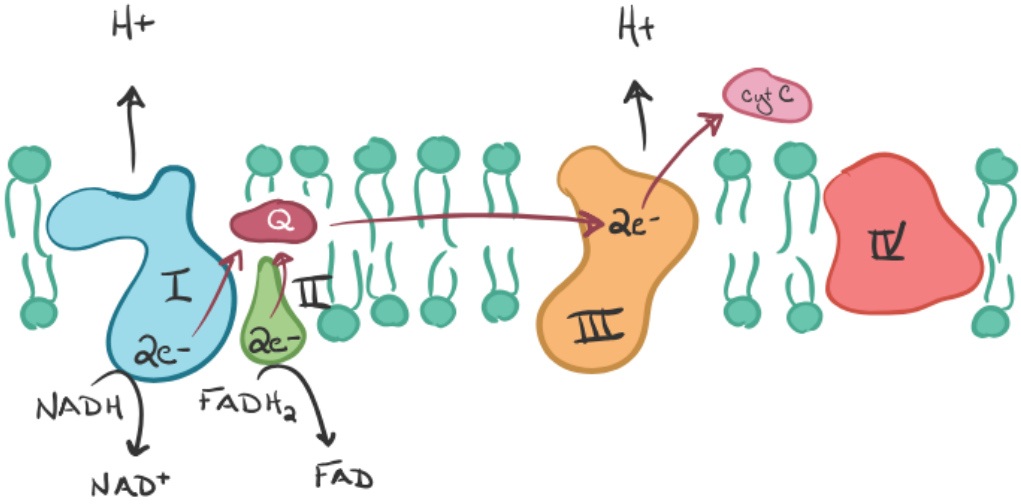
Step 3

The rest of the steps are now the same for the high energy molecules from NADH and FADH2 in earlier steps. Inside the nonpolar region of the phospholipid bilayer, UQH2 (which is also a nonpolar compound) transports the electrons to *protein complex 3*. UQH2 also carries protons. When UQH2 delivers electrons to protein complex 3, it also donates its protons to be pumped.



Step 4

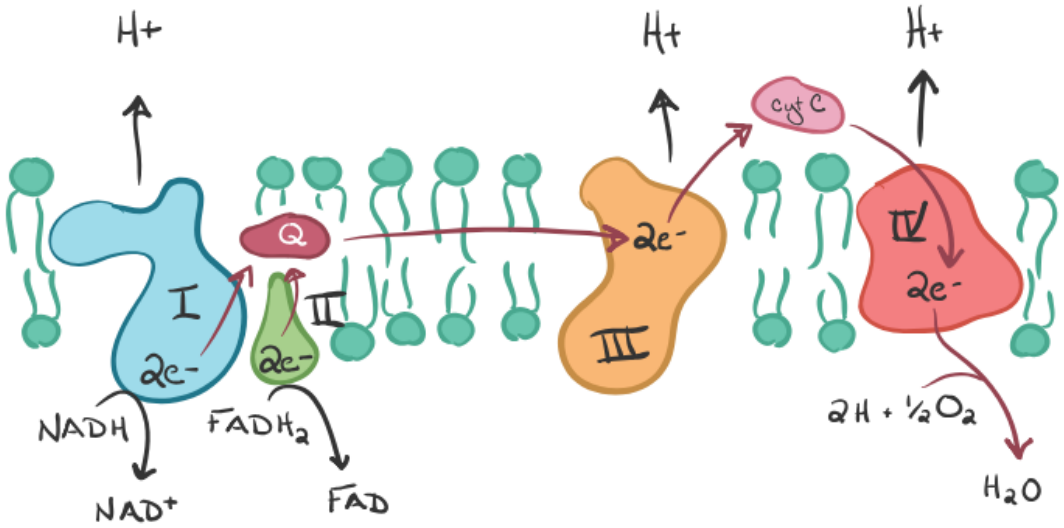
The electrons that arrived at protein complex 3 are picked up by *cytochrome C*(or "*cyt C*"), the last electron carrier. This action also causes protons to be pumped into the intermembrane space.



Step 5

Cytochrome C carries the electrons to the final protein complex, *protein complex 4*. Once again, energy released via electron shuttling allows for another proton to be pumped into the

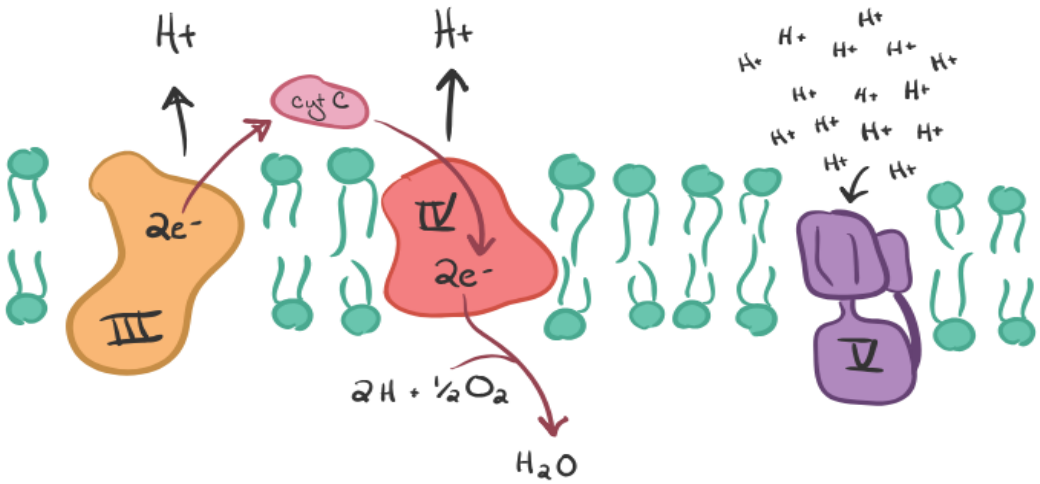
intermembrane space. The electrons are then drawn to oxygen, which is the final *electron acceptor*. Its important to note that oxygen must be present for oxidative phosphorylation to occur. Water is formed as oxygen receives the electrons from protein complex 4, and combines with protons on the inside of the cell.



2. Phosphorylation - the production of ATP

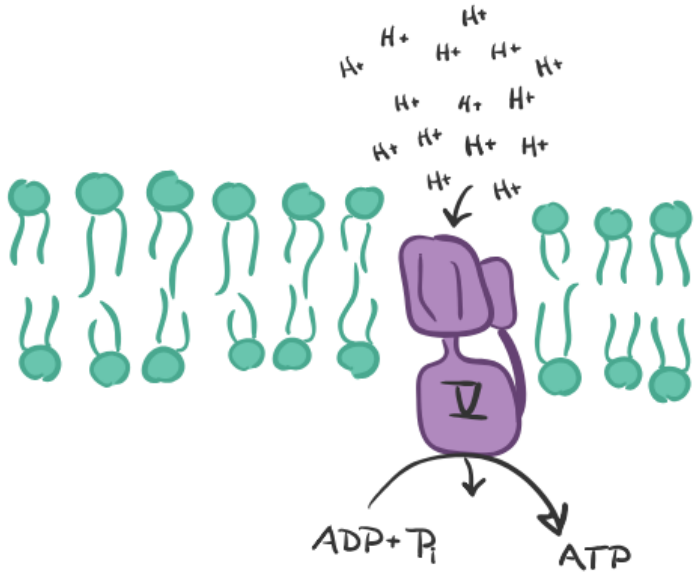
Step 6

As a result of part 1 (Oxidation of NADH and FADH2), an electrochemical gradient is created, meaning there is a difference in electrical charge between the two sides of the inner mitochondrial membrane. The outside, or exterior, of the mitochondrial membrane is positive because of the accumulation of the protons (H+), and the inside is negative due to the loss of the protons. A chemical concentration gradient has also developed on either side of the membrane. The electrochemical gradient is how the cell transfers the stored energy from the reduced NADH and FADH2



Step 7

When there is a high concentration of protons on the outside of the mitochondrial membrane, protons are pushed through *ATP synthase*. This movement of protons causes ATP synthase to spin, and bind ADP and Pi, producing ATP.

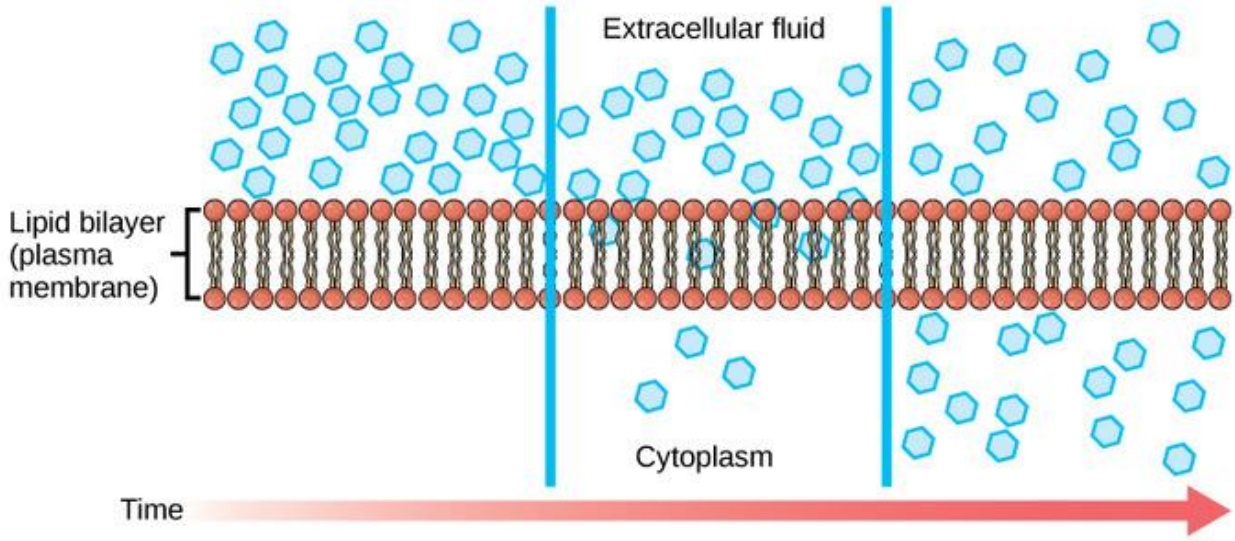


TRANSPORT OF SUBSTANCES ACROSS BIOLOGICAL MEMBRANES

Diffusion

Diffusion is a process of passive transport in which molecules move from an area of higher concentration to one of lower concentration. 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. You are familiar with diffusion of substances through the air. For example, think about someone opening a bottle of ammonia in a room filled with people. The ammonia gas is at its highest concentration in the bottle; its lowest concentration is at the edges of the room. The ammonia vapor will diffuse, or spread away, from the bottle; gradually, more and more people will smell the ammonia as it spreads. Materials move within the cell 's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion. Diffusion expends no energy. On the contrary, concentration gradients are a form of potential energy, dissipated as the gradient is eliminated.

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.



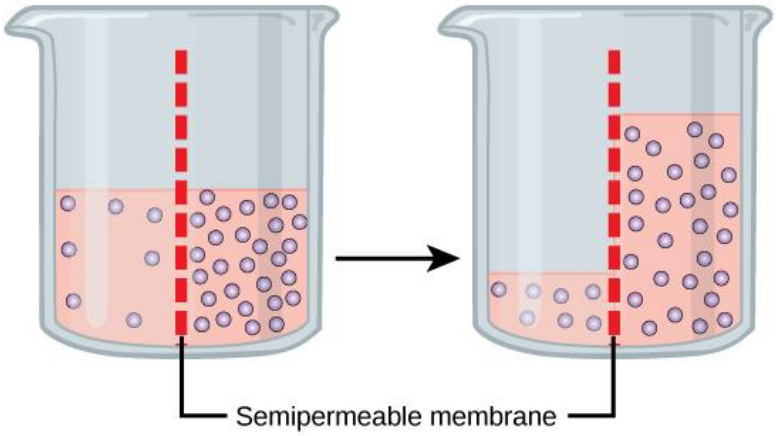
Osmosis

Osmosis is the movement of water across a membrane from an area of low solute concentration to an area of high solute concentration.

Osmosis and Semipermeable Membranes

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. Semipermeable membranes, also termed selectively permeable membranes or partially permeable membranes, allow certain molecules or ions to pass through by diffusion.

While diffusion transports materials across membranes and within cells, osmosis transports only water across a membrane. The semipermeable membrane limits the diffusion of solutes in the water. Not surprisingly, the aquaporin proteins that facilitate water movement play a large role in osmosis, most prominently in red blood cells and the membranes of kidney tubules.



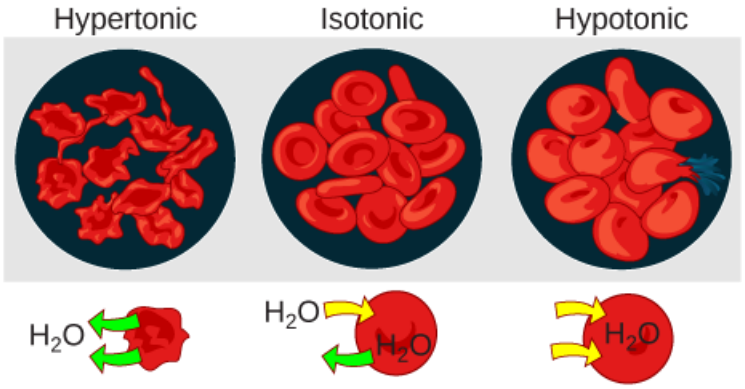
Tonicity

Tonicity, which is directly related to the osmolarity of a solution, affects osmosis by determining the direction of water flow.

Three terms—hypotonic, isotonic, and hypertonic—are used to relate the osmolarity of a cell to the osmolarity of the extracellular fluid that contains the cells.

Hypotonic Solutions

In a hypotonic situation, the extracellular fluid has lower osmolarity than the fluid inside the cell, and water enters the cell. (In living systems, the point of reference is always the cytoplasm, so the prefix hypo- means that the extracellular fluid has a lower concentration of solutes, or a lower osmolarity, than the cell cytoplasm.) It also means that the extracellular fluid has a higher concentration of water in the solution than does the cell. In this situation, water will follow its concentration gradient and enter the cell, causing the cell to expand.



Hypertonic Solutions

As for a hypertonic solution, the prefix hyper- refers to the extracellular fluid having a higher osmolarity than the cell's cytoplasm; therefore, the fluid contains less water than the cell does. Because the cell has a relatively higher concentration of water, water will leave the cell, and the cell will shrink.

Isotonic Solutions

In an isotonic solution, the extracellular fluid has the same osmolarity as the cell. If the osmolarity of the cell matches that of the extracellular fluid, there will be no net movement of water into or out of the cell, although water will still move in and out.

Blood cells and plant cells in hypertonic, isotonic, and hypotonic solutions take on characteristic appearances. Cells in an isotonic solution retain their shape. Cells in a hypotonic solution swell as water enters the cell, and may burst if the concentration gradient is large enough between the inside and outside of the cell. Cells in a hypertonic solution shrink as water exits the cell, becoming shriveled.

Facilitated transport

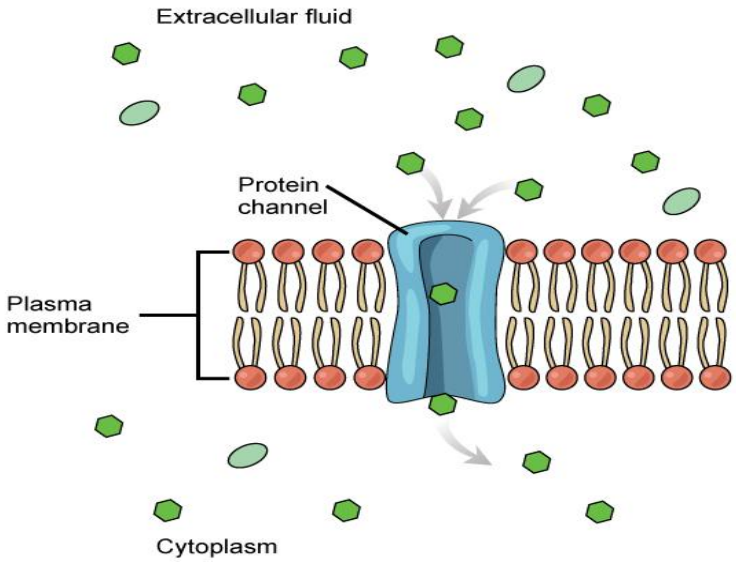
Facilitated diffusion is a process by which molecules are transported across the plasma membrane with the help of membrane proteins.

Facilitated transport is a type of passive transport. Unlike simple diffusion where materials pass through a membrane without the help of proteins, in facilitated transport, also called facilitated diffusion, materials diffuse across the plasma membrane with the help of membrane proteins.

Channels

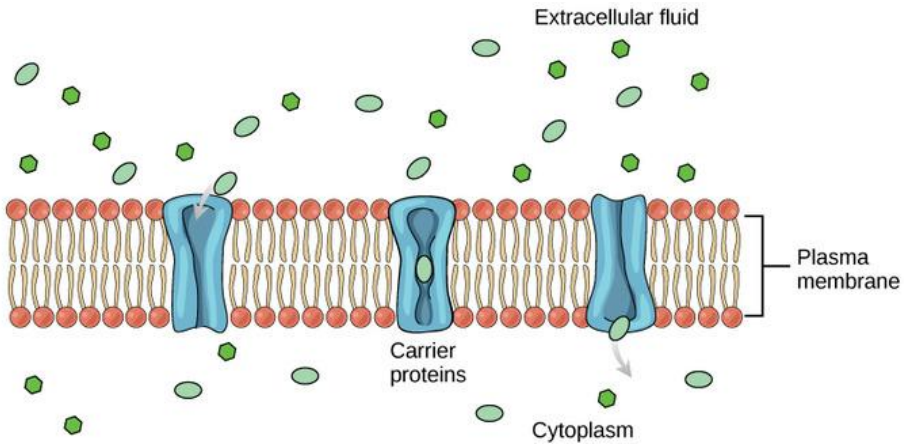
The integral proteins involved in facilitated transport are collectively referred to as transport proteins; they function as either channels for the material or carriers. In both cases, they are transmembrane proteins. Channels are specific for the substance that is being transported. Channel proteins have hydrophilic domains exposed to the intracellular and extracellular fluids; they additionally have a hydrophilic channel through their core that provides a hydrated opening through the membrane layers. Passage through the channel allows polar compounds to avoid the nonpolar central layer of the plasma membrane that would otherwise slow or prevent their entry into the cell.

Channel proteins are either open at all times or they are "gated," which controls the opening of the channel. The attachment of a particular ion to the channel protein may control the opening or other mechanisms or substances may be involved. In some tissues, sodium and chloride ions pass freely through open channels, whereas in other tissues, a gate must be opened to allow passage.



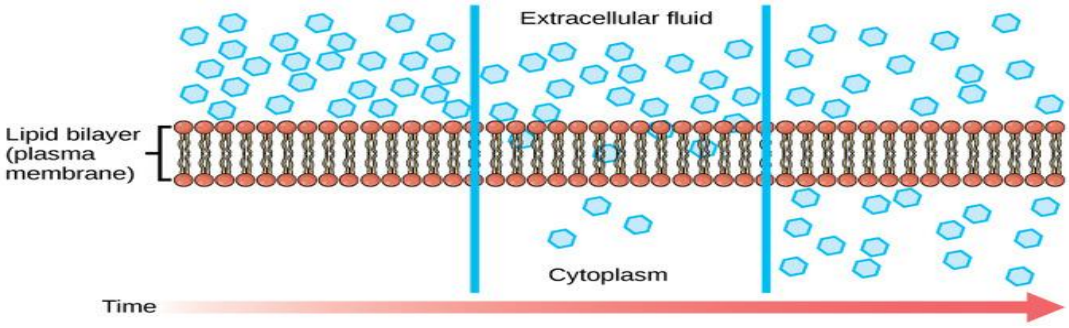
Carrier Proteins

Another type of protein embedded in the plasma membrane is a carrier protein. This protein binds a substance and, in doing so, triggers a change of its own shape, moving the bound molecule from the outside of the cell to its interior; depending on the gradient, the material may move in the opposite direction. Carrier proteins are typically specific for a single substance. This adds to the overall selectivity of the plasma membrane. The exact mechanism for the change of shape is poorly understood. Proteins can change shape when their hydrogen bonds are affected, but this may not fully explain this mechanism. Each carrier protein is specific to one substance, and there are a finite number of these proteins in any membrane. This can cause problems in transporting enough of the material for the cell to function properly.



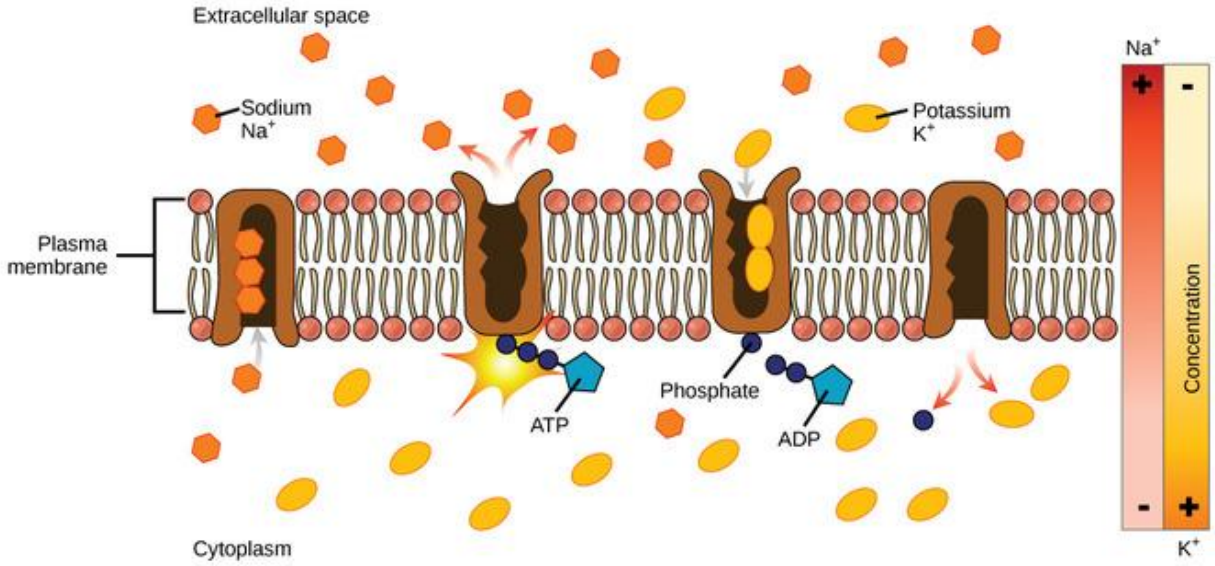
The Role of Passive Transport

Passive transport, such as diffusion and osmosis, moves materials of small molecular weight across membranes. Plasma membranes must allow or prevent certain substances from entering or leaving a cell. In other words, plasma membranes are selectively permeable; they allow some substances to pass through, but not others. This may happen passively, as certain materials move back and forth, or the cell may have special mechanisms that facilitate transport. Some materials are so important to a cell that it spends some of its energy (hydrolyzing adenosine triphosphate (ATP)) to obtain these materials.



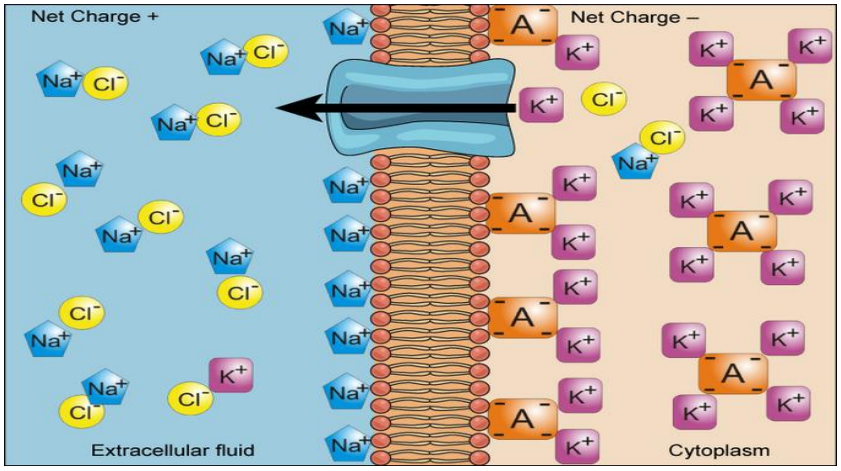
Primary Active Transport

The primary active transport that functions with the active transport of sodium and potassium allows secondary active transport to occur. The secondary transport method is still considered active because it depends on the use of energy as does primary transport.



Electrochemical Gradient

To move substances against the membrane's electrochemical gradient, the cell utilizes active transport, which requires energy from ATP.

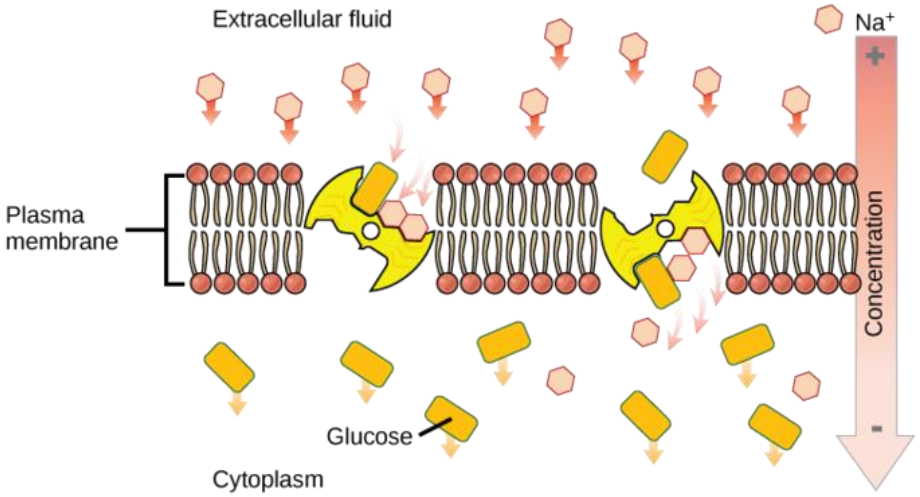


Simple concentration gradients are differential concentrations of a substance across a space or a membrane, but in living systems, gradients are more complex. Because ions move into and out of cells and because cells contain proteins that do not move across the membrane and are mostly negatively charged, there is also an electrical gradient, a difference of charge, across the plasma membrane. The interior of living cells is electrically negative with respect to the extracellular fluid in which they are bathed. At the same time, cells have higher concentrations of potassium (K^+) and lower concentrations of sodium (Na^+) than does the extracellular fluid. The combined gradient of concentration and electrical charge that affects an ion is called its electrochemical gradient.

Secondary Active Transport

In secondary active transport, a molecule is moved down its electrochemical gradient as another is moved up its concentration gradient.

Unlike in primary active transport, in secondary active transport, ATP is not directly coupled to the molecule of interest. Both antiporters and symporters are used in secondary active transport. Co-transporters can be classified as symporters and antiporters depending on whether the substances move in the same or opposite directions across the cell membrane.



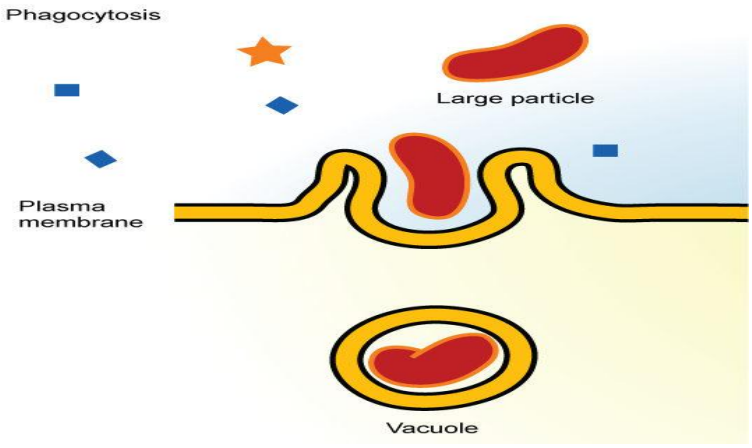
Endocytosis

Endocytosis takes up particles into the cell by invaginating the cell membrane, resulting in the release of the material inside of the cell.

Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell. There are different variations of endocytosis, but all share a common characteristic: the plasma membrane of the cell invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle being contained in a newly-created intracellular vesicle formed from the plasma membrane.

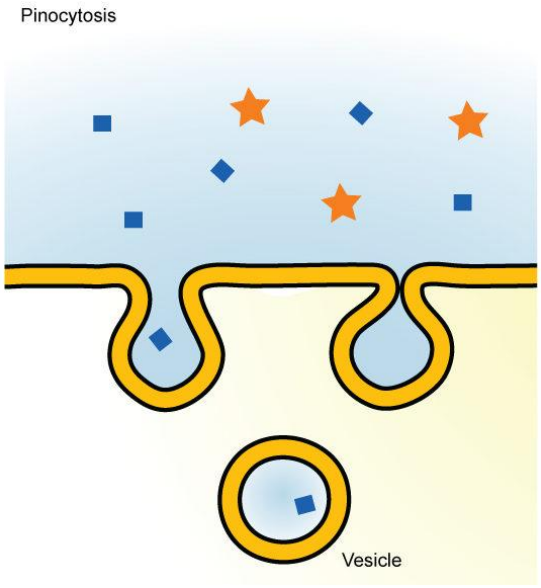
Phagocytosis

Phagocytosis (the condition of "cell eating") is the process by which large particles, such as cells or relatively large particles, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil will remove the invaders through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil.



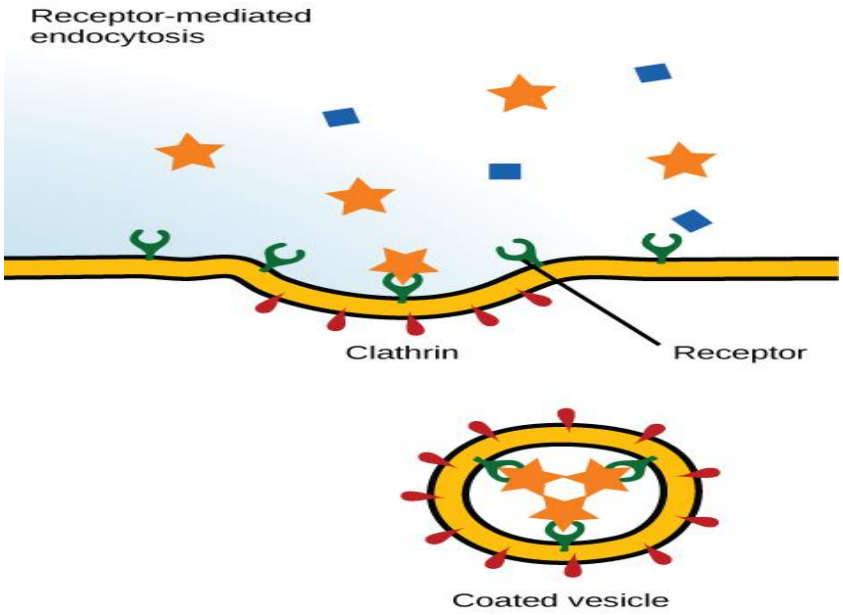
Pinocytosis

A variation of endocytosis is called pinocytosis. This literally means "cell drinking" and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this is a process that takes in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome.



Receptor-Mediated Endocytosis:

In receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds to the receptor on the external surface of the cell membrane.


Receptor-mediated
endocytosis
Clathrin
Receptor
Coated vesicle

18BEBME305 - BIOCHEMISTRY

UNIT – II

CARBOHYDRATES

CONTENTS

1. Carbohydrates: Definition, classification,
2. Biological functions;
3. Glycolysis,
4. TCA cycle,
5. Glycogenesis, glycogenolysis,
6. Diabetes Mellitus – Blood Sugar analysis and glucose tolerance test.

CARBOHYDRATES

General characteristics

- The term carbohydrate is derived from the french: hydrate de carbone
- compounds composed of C, H, and O
- empirical formula: $(CH_2O)_n$
- Most carbohydrates are found naturally in bound form rather than as simple sugars
    - Polysaccharides (starch, cellulose, inulin, gums)
    - Glycoproteins and proteoglycans (hormones, blood group substances, antibodies)
    - Glycolipids (cerebrosides, gangliosides)
    - Glycosides
    - Mucopolysaccharides (hyaluronic acid)
    - Nucleic acids

Functions

- sources of energy
- intermediates in the biosynthesis of other basic biochemical entities (fats and proteins)
- associated with other entities such as glycosides, vitamins and antibiotics)
- form structural tissues in plants and in microorganisms (cellulose, lignin, murein)
- participate in biological transport, cell-cell recognition, activation of growth factors, modulation of the immune system

Classification of carbohydrates

- Monosaccharides- glucose, fructose
- Oligosaccharides
    - Di, tri, tetra, penta, up to 9 or 10
    - Most important are the disaccharides-lactose, sucrose

- Polysaccharides or glycans
    - Homopolysaccharides-starch, glycogen, cellulose
    - Heteropolysaccharides
    - Complex carbohydrates

Monosaccharides

- also known as simple sugars
- Examples-glucose (blood sugar), fructose(sweetest sugar) are principal monosaccharides in food

Oligosaccharides

- Most common are the disaccharides
    - Sucrose, lactose, and maltose
    - Maltose (2 molecules of D-glucose)
    - Lactose (a molecule of glucose and a molecule of galactose)
    - Sucrose (a molecule of glucose and a molecule of fructose)

Polysaccharides(complex carbohydrates)

- homoglycans (starch, cellulose, glycogen, inulin)
- Starch is the most common storage polysaccharide in plants
- Common sources are grains , potatoes, peas, beans, wheat
- heteroglycans (gums, mucopolysaccharides)
- Glycogen is also known as animal starch and is stored in muscle and liver

Fibers

- Found in food derived from plants
- Includes polysaccharides such as cellulose, hemicellulose, pectins, gums and mucilages
- Also includes non-polysaccharides such as lignin, cutins and tannins

- Fibers are not a source of energy because human digestive enzymes cannot break down fibers
- The bacteria in human GI tract can breakdown some fibers.
- **classification of fibers based on their solubilities in water**

(1)Soluble fibers: includes gum,pectin, some hemicellulose and mucilages found in fruits, oats, barley and legumes .

Actions on body:

(i) Delay GI transit(benefits digestive disorders)

(ii) Delay glucose absorption 9benefits diabetes)

(iii) Lowers blood cholesterol(benefits heart disease)

- **classification of fibers based on their solubilities in water**

(2) Insoluble fibers: includes cellulose, many hemicellulose, lignin found in wheat bran , corn bran, whole grain bread, cereals and vegetables (carrot, cabbage)

Actions in body:

(i)Accelerates GI transit and increases fecal weight(promotes bowel movement)

(ii) Slows starch hydrolysis and delays glucose absorption(Benefits diabetes**)**

Functions of Carbohydrate

- **Glucose as a source of energy**
- **Regulation of Fat Metabolism**

The absence of dietary carbohydrate leads to ketone body production due to incomplete breakdown of fats.

- **As a component of Body Compounds**
- **Role in Gastrointestinal Functions**

Lactose for the synthesis of B complex Vitamins and Cellulose to increase bulk in the intestine and prevent constipation

Requirements for carbohydrate

- Carbohydrates are not essential nutrients, because the carbon skeletons of amino acids can be converted into glucose .
- However, the absence of dietary carbohydrate leads to ketone body production ,and degradation of body protein whose constituent amino acids provide carbon skeletons for gluconeogenesis .
- The RDA for carbohydrate is set at 130 g/day for adults and children, based on the amount of glucose used by carbohydrate-dependent tissues, such as the brain and erythrocytes.
- Adults should consume 45–65 percent of their total calories from carbohydrates.
- It is recommended that added sugar represent no more than 25% of total energy because of concerns that sugar may displace nutrient-rich foods from the diet, potentially leading to deficiencies of certain micronutrients.

Simple sugars and disease

- There is no direct evidence that the consumption of simple sugars is harmful. Contrary to folklore, diets high in sucrose do not lead to diabetes or hypoglycemia.
- Carbohydrates are not inherently fattening, and result in fat synthesis only when consumed in excess of the body's energy needs.
- However, there is an association between sucrose consumption and dental caries.

Storing glucose as glycogen

- $1/3^{rd}$ of total glycogen is stored in liver and $2/3^{rd}$ in muscle.
- When blood glucose falls liver cells break down glycogen into single molecules of glucose, which becomes available to supply energy to central nervous system and other organs .
- During exercise the muscle cell themselves use up the glycogen they store.

Making glucose from proteins

- Glycogen stores only last for some hours , if a person does not replenish the depleted glycogen stores, body protein are broken down to make glucose by a process called
- "**gluconeogenesis"**

- Taking adequate amount of carbohydrate prevents the use of protein for energy, this role of carbohydrate is called **protein sparing action.**

Making ketone bodies from fat fragments

- Inadequate supply of carbohydrates causes break down of body fat reserves. This not only supplies energy but also produces ketone bodies.
- Some ketone bodies are used by muscle and other tissues for energy, but when produced in excess they accumulate in blood and cause **ketosis** (disturbance of bodies normal acid-base balance)

Converting glucose to fats

- Excess carbohydrates can be converted to fats when glycogen stores are filled to capacity.
- However storing carbohydrates as fats is an energetically expensive process. So, body fats mainly come from dietary fats.
- Health effects of starch and fibers
- **Weight control:** Food rich in complex carbohydrates provides less energy per bite and also provides satiety.
- **Heart disease**:diets high in soluble fibers and low in animal fats and cholesterol is associated with lower risk of heart disease.
- **Cancer:** high carbohydrate diet protects against some types of cancer (eg. Colon cancer).

Physiological Importance in Body

- Blood clotting,
- Immunity,
- Fertilization

Detoxification of the body by metabolism

- Many drugs and toxic wastes in the body are metabolized for easy excretion in the body. Some of these are water-insoluble and hence they are difficult to be expelled in urine. Body converts them into glucuronosyl conjugates using the glucuronosyl moiety derived from carbohydrates.

- A carbohydrate moiety like glucose combines with uronic acid to form glucuronate. These conjugates of insoluble substances with glucuronosyl are more water-soluble and easily excreted from the body. Thus detoxification of physiological importance is carried out to some extent with carbohydrate derivatives.

As reaction intermediates or accessories

- Carbohydrates participate as reaction intermediates in some vital reactions. This function of carbohydrates is seen extensively in various cellular reaction.

- For example, one of the vitamins, Vitamin B2 also called Riboflavin has a ribose sugar moiety. This ribose is a four carbon type of carbohydrate monomer by its chemical structure. It is involved in vital reactions at the organ and cellular level. Similarly, carbohydrates are also chemical constituents of many hormones, vitamins, enzymes, etc.

Constitute genetic material

- Carbohydrates form a part of DNA and RNA in the form of deoxyribose and ribose sugars. These are five carbon monosaccharides formed form heptulose sugars by the pseudo-heptulose pathway.

They are constituents of all the cellular organelles.

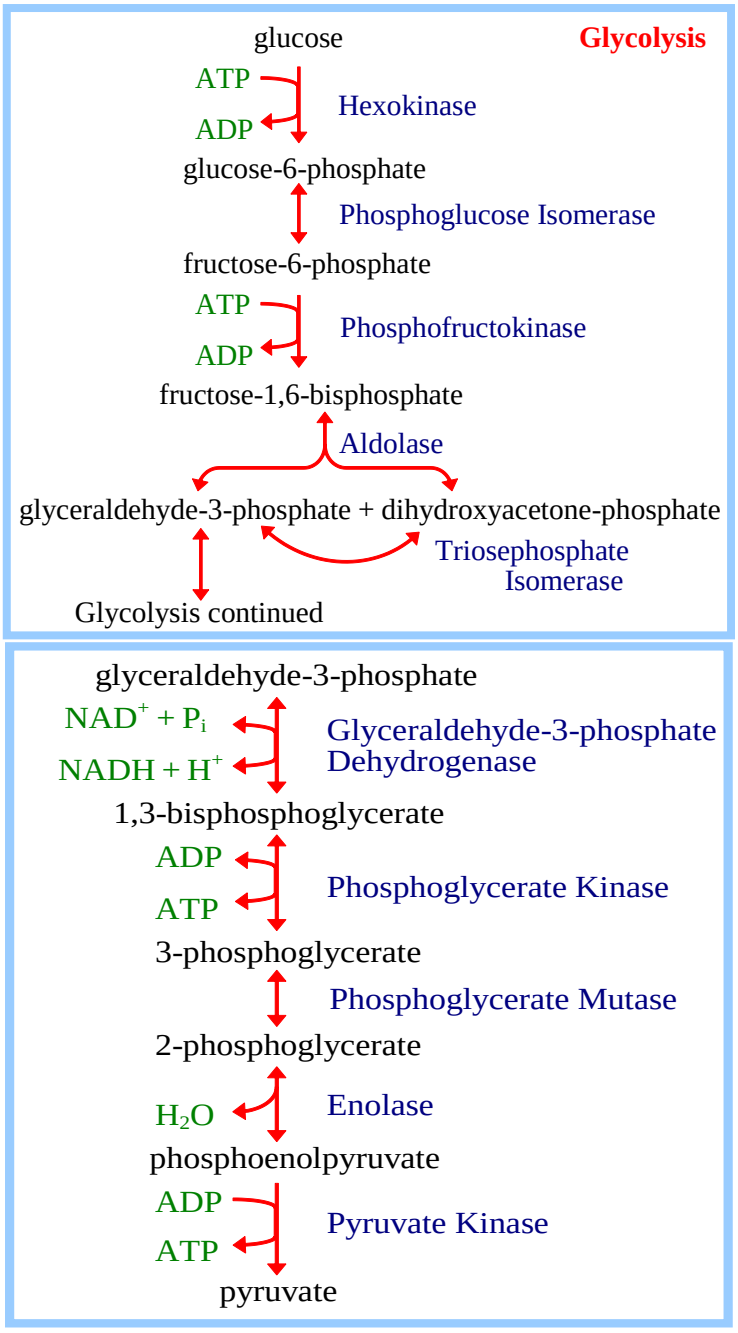
- Carbohydrates are also components of cell organelles like the cell membrane, mitochondria, nucleus, endoplasmic reticulum, etc. They provide structural integrity, mechanical strength in combination with proteins and lipids. They help make up the body mass by being included in all the parts of the cell and tissues. For example, in cell membranes, there are two constituents, i.e., glycolipid layer and glycoprotein layer. Here the term “glyco” is a carbohydrate.

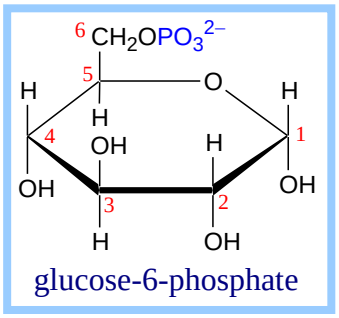
Transport of oxygen

- Glucose is taken by red blood cells. These are the types of blood cells which lack mitochondria and other cell organelles required for producing energy. In such a case, the ATP is produced by a non-oxidative pathway with the end product as lactose and ATP.

- This energy thus produced is necessary for hemoglobin to bind to oxygen molecules. These bound oxygen molecules are transferred from lungs to the different tissues.

Glycolysis

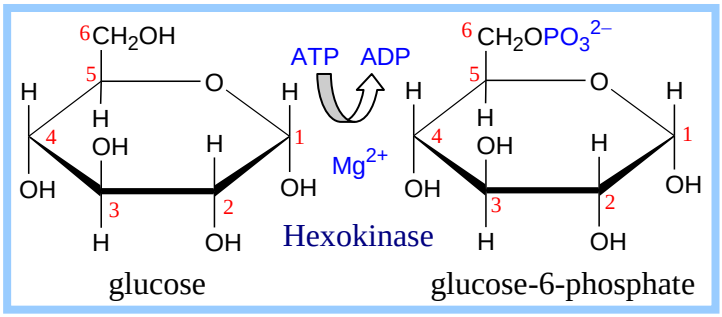

glucose
Glycolysis
ATP
ADP
Hexokinase
glucose-6-phosphate
Phosphoglucose Isomerase
fructose-6-phosphate
ATP
ADP
Phosphofructokinase
fructose-1,6-bisphosphate
Aldolase
glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate
Triosephosphate Isomerase
Glycolysis continued
glyceraldehyde-3-phosphate
NAD+ + Pi
NADH + H+
Glyceraldehyde-3-phosphate Dehydrogenase
1,3-bisphosphoglycerate
ADP
ATP
Phosphoglycerate Kinase
3-phosphoglycerate
Phosphoglycerate Mutase
2-phosphoglycerate
H2O
Enolase
phosphoenolpyruvate
ADP
ATP
Pyruvate Kinase
pyruvate



Glycolysis takes place in the **cytosol** of cells.

Glucose enters the Glycolysis pathway by conversion to **glucose-6-phosphate**.

Initially there is energy input corresponding to cleavage of two ~P bonds of ATP.

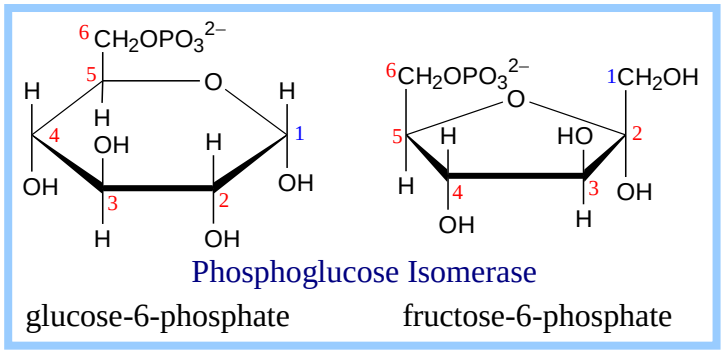


1. Hexokinase catalyzes:

Glucose + ATP → glucose-6-P + ADP

The reaction involves nucleophilic attack of the C6 hydroxyl O of glucose on P of the terminal phosphate of ATP.

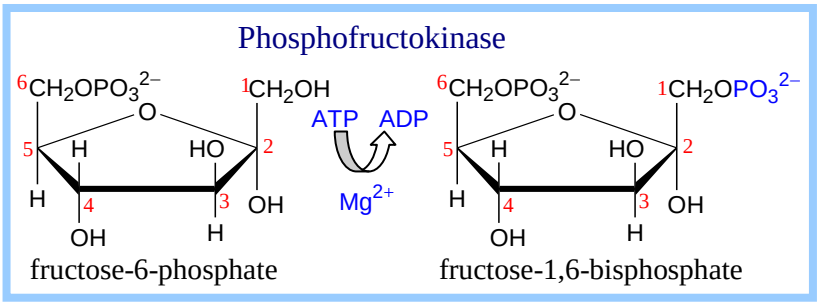
ATP binds to the enzyme as a complex with **Mg^{++}**.



2. Phosphoglucose Isomerase catalyzes:

glucose-6-P (aldose) ←→ **fructose-6-P** (ketose)

The mechanism involves acid/base catalysis, with ring opening, isomerization via an **intermediate**, and then ring closure.



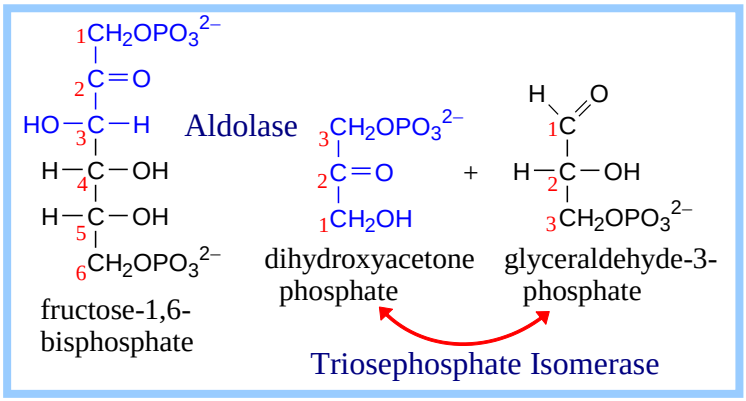
3. Phosphofructokinase catalyzes:

fructose-6-P + ATP → fructose-1,6-bisP + ADP

This highly **spontaneous** reaction has a mechanism similar to that of Hexokinase.

The Phosphofructo kinase reaction is the **rate-limiting step** of Glycolysis.

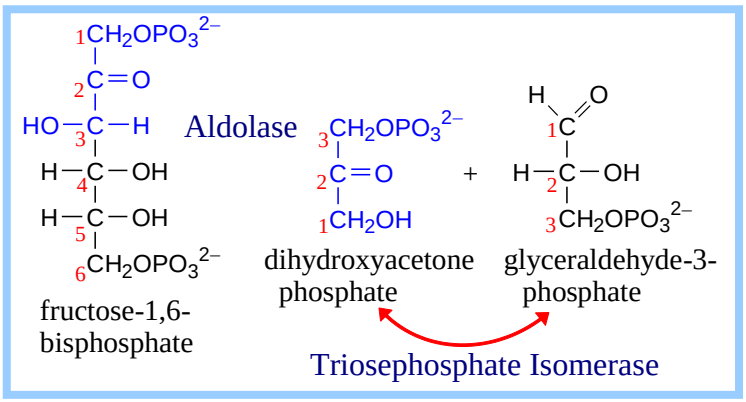
The enzyme is highly **regulated**.



4. Aldolase catalyzes: **fructose-1,6-bisphosphate ←→**

dihydroxy acetone-P + glyceraldehyde-3-P

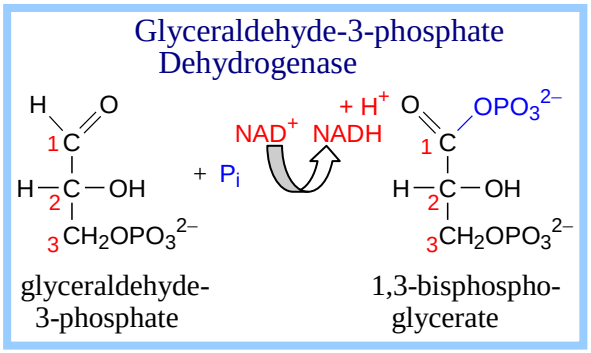
The reaction is an **aldol cleavage**, the reverse of an aldol condensation.



5. Triose Phosphate isomerase catalyzes:

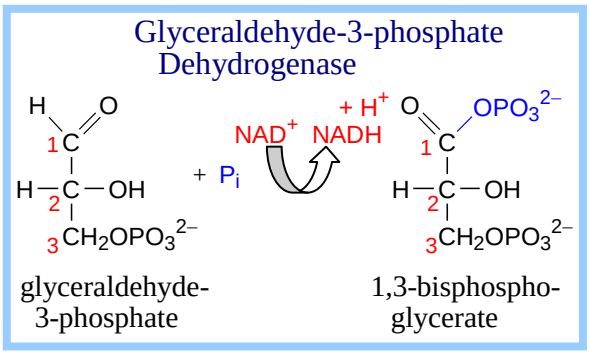
dihydroxy acetone-P ←→ glyceraldehyde-3-P

Glycolysis continues from glyceraldehyde-3-P. K_{eq} favors dihydroxyacetone-P.



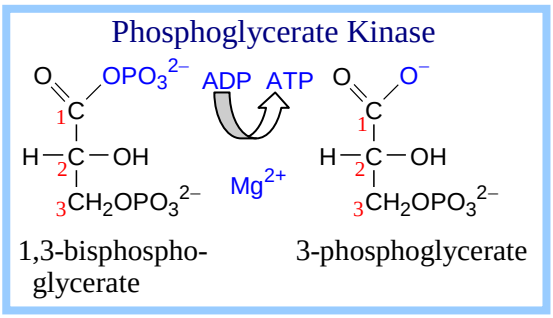
6. Glyceraldehyde-3-phosphate Dehydrogenase catalyzes:

glyceraldehyde-3-P + NAD^+ + P_i ←→ 1,3-bisphosphoglycerate + NADH + H^+



Exergonic oxidation of the aldehyde in glyceraldehyde- 3-phosphate, to a carboxylic acid, drives formation of an **acyl phosphate**, a "high energy" bond (**~P**).

This is the only step in Glycolysis in which **NAD^+** is reduced to NADH.



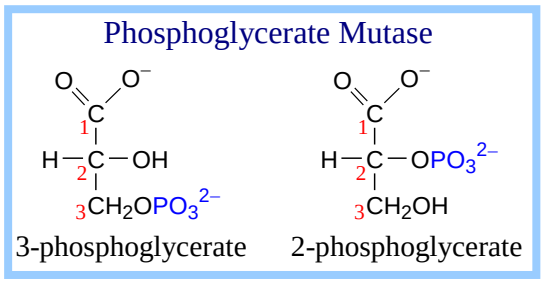
7. Phosphoglycerate Kinase catalyzes:

1,3-bisphosphoglycerate + ADP ←→

3-phosphoglycerate + ATP

This phosphate transfer is reversible (low DG), since one **~P** bond is cleaved & another synthesized.

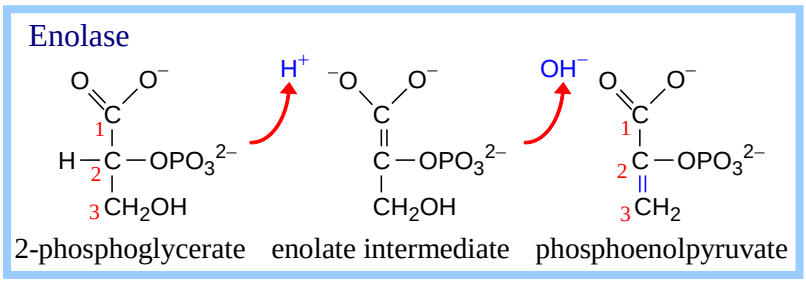
The enzyme undergoes substrate-induced conformational change similar to that of Hexokinase.



8. Phosphoglycerate Mutase catalyzes:

3-phosphoglycerate ←→ 2-phosphoglycerate

Phosphate is shifted from the OH on C3 to the OH on C2.



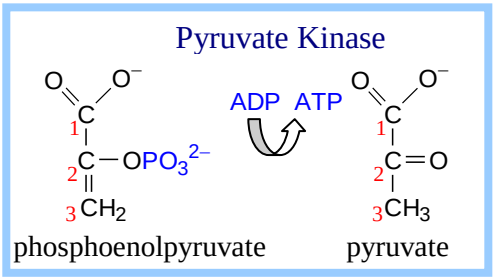
9. Enolase catalyzes:

2-phosphoglycerate ←→ phosphoenolpyruvate + H_2O

This dehydration reaction is **Mg^{++}-dependent**.

2 Mg^{++} ions interact with oxygen atoms of the substrate **carboxyl** group at the active site.

The Mg^{++} ions help to stabilize the enolate anion intermediate that forms when a Lys extracts H^+ from C #2.



10. Pyruvate Kinase catalyzes:

phosphoenolpyruvate + ADP → pyruvate + ATP

Glycolysis - Balance sheet for ~P bonds of ATP:

- How many ATP ~P bonds expended? __2_____
- How many ~P bonds of ATP produced? (Remember there are two 3C fragments from glucose.) ___4_____
- Net production of **~P** bonds of ATP per glucose: _____2__

Balance sheet for **~P** bonds of ATP:

- 2 ATP expended
- 4 ATP produced (2 from each of two 3C fragments from glucose)
- Net production of **2 ~P** bonds of **ATP** per glucose.

Glycolysis - total pathway, omitting H^+:

glucose + 2 NAD^+ + 2 ADP + 2 P_i →

2 pyruvate + 2 NADH + 2 ATP

In **aerobic organisms**:

- **pyruvate** produced in Glycolysis is oxidized to CO_2 via Krebs Cycle

- **NADH** produced in Glycolysis & Krebs Cycle is reoxidized via the respiratory chain, with production of much additional ATP.

TCA Cycle

- Also known as Krebs cycle
- TCA cycle essentially involves the oxidation of acetyl CoA to CO_2 and H_2O.
- TCA cycle –the central metabolic pathway
- The TCA cycle is the final common oxidative pathway for carbohydrates, fats, amino acids.
- TCA cycle supplies energy & also provides many intermediates required for the synthesis of amino acids, glucose, heme etc.
- TCA cycle is the most important central pathway connecting almost all the individual metabolic pathways.

—

— **Definition**

- Citric acid cycle or TCA cycle or tricarboxylic acid cycle essentially involves the oxidation of acetyl CoA to CO_2 & H_2O.
- Location of the TCA cycle
- Reactions of occur in mitochondrial matrix, in close proximity to the ETC.

Reactions of TCA cycle

- Oxidative decarboxylation of pyruvate to acetyl CoA by PDH complex.
- This step is connecting link between glycolysis and TCA cycle.

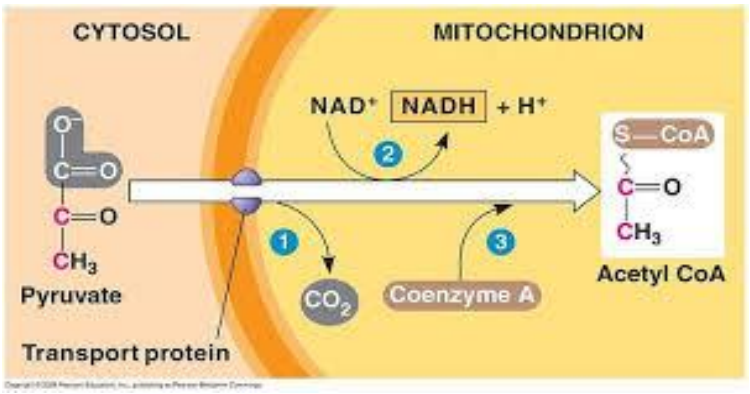
Component enzymes

- Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex

- The pyruvate dehydrogenase complex is a multimolecular aggregate of three enzymes, pyruvate dehydrogenase (E_1, also called a decarboxylase), dihydrolipoyl transacetylase (E_2), and dihydrolipoyl dehydrogenase (E_3).

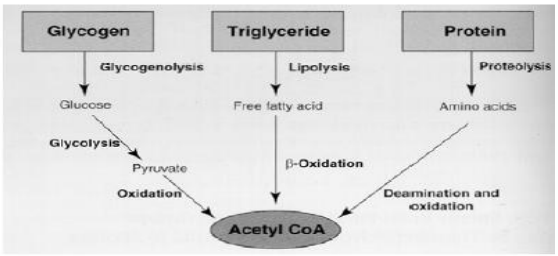
- The complex also contains two tightly bound regulatory enzymes, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase.

Pyruvate oxidation

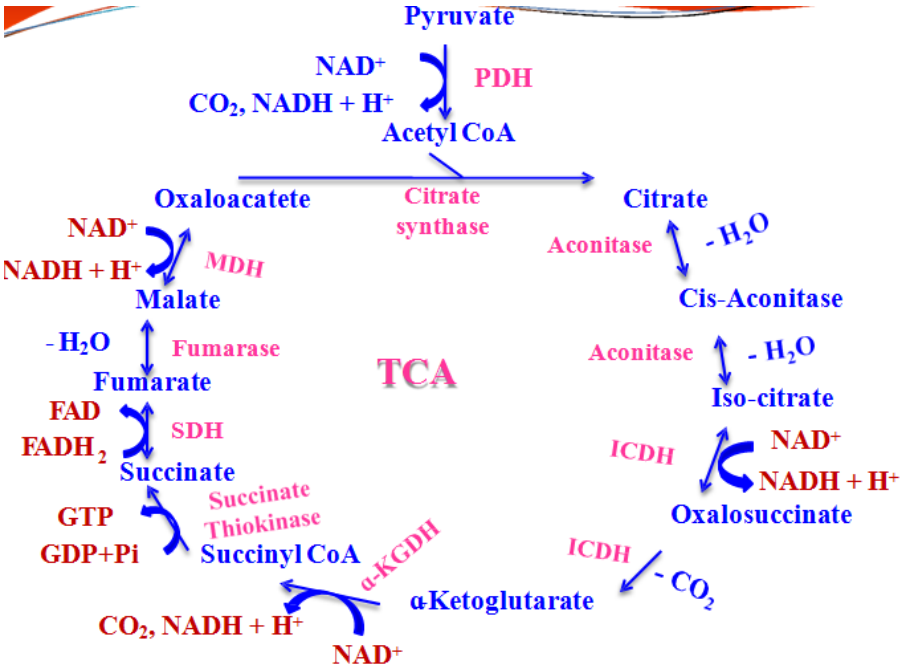


Coenzymes

- The pyruvate dehydrogenase complex contains five coenzymes
- E_1 requires thiamine pyrophosphate, E_2 requires lipoic acid and CoA, and E_3 requires FAD and NAD^+.



Reactions of TCA Cycle



Step:1 Formation of citrate

- Oxaloacetate condenses with acetyl CoA to form Citrate, catalysed by the enzyme citrate synthase
- Inhibited by:
- ATP, NADH, Citrate - competitive inhibitor of oxaloacetate.

Steps 2 & 3 Citrate is isomerized to isocitrate

- Citrate is isomerized to isocitrate by the enzyme aconitase
- This is achieved in a two stage reaction of dehydration followed by hydration through the formation of an intermediate -cis-aconiase

Steps 4 & 5 Formation of a-ketoglutarate

- Isocitrate dehydrogenase (ICDH) catalyses the conversion of (oxidative decarboxylation) of isocitrate to oxalosuccinate & then to a-ketoglutarate.
- The formation of NADH & the liberation of CO_2 occure at this stage.
- Stimulated (cooperative) by isocitrate, NAD^+, Mg^{2+}, ADP, Ca^{2+} (links with contraction).
- Inhibited by NADH & ATP

Step: 6Conversion of a-ketoglutarate to succinyl CoA

- Occurs through oxidative decarboxylation, catalysed by a-ketoglutarate dehydrogenase complex.
- a-ketoglutarate dehydrogenase is an multienzyme complex.
- At this stage of TCA cycle, second NADH is produced & the second CO_2 is liberated.

Step: 7 Formation of succinate

- Succinyl CoA is converted to succinate by succinate thiokinase.
- This reaction is coupled with the phosphorylation of GDP to GTP.
- This is a substrate level phosphorylation.
- GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

Step: 8 Conversion of succinate to fumarate

- Succinate is oxidized by succinate dehydrogenase to fumarate.
- This reaction results in the production of $FADH_2$.

Step: 9 Formation of malate: The enzyme fumarase catalyses the conversion of fumarate to malate with the addition of H_2O.

Step:10 Conversion of malate to oxaloacetate

- Malate is then oxidized to oxaloacetate by malate dehydrogenase.
- The third & final synthesis of NADH occurs at this stage.
- The oxaloacetate is regenerated which can combine with another molecule of acetyl CoA & continue the cycle.

Energy-producing reaction	Number of ATP produced
$3\ NADH \longrightarrow 3\ NAD^+$	9
$FADH_2 \longrightarrow FAD$	2
$GDP + P_i \longrightarrow GTP$	1
	12 ATP/acetyl CoA oxidized

Significance of TCA cycle

- Complete oxidation of acetyl CoA.
- ATP generation.
- Final common oxidative pathway.

- Integration of major metabolic pathways.
- Fat is burned on the wick of carbohydrates.
- Excess carbohydrates are converted as neutral fat
- No net synthesis of carbohydrates from fat.
- Carbon skeleton of amino acids finally enter the TCA cycle.

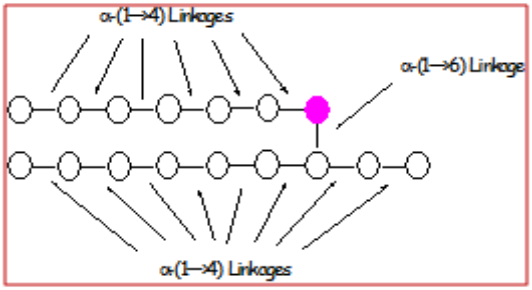
Regulation of TCA Cycle

— Three regulatory enzymes

1. Citrate synthase
2. Isocitrate dehydrogenase
3. α-ketoglutarate dehydrogenase

GLYCOGENESIS

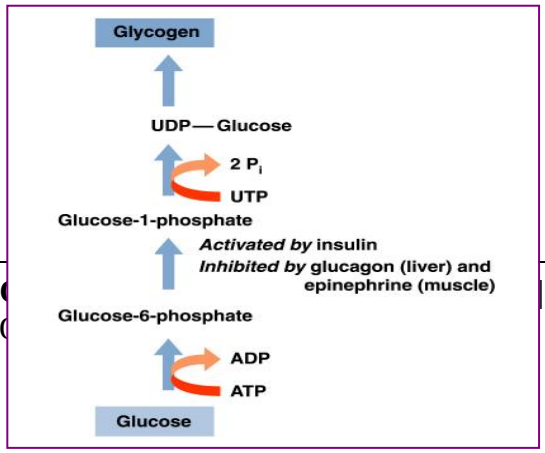
Structure of Glycogen



Glycogenesis - Synthesis of Glycogen

Glycogenesis

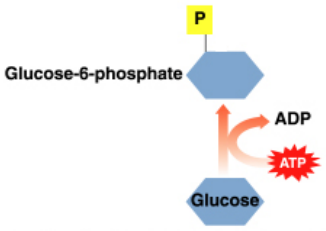
- Stores glucose by converting glucose to glycogen.
- Operates when high levels of glucose ARE VAIALABLE .
- Does not operate when energy stores (glycogen) are full, which means that additional glucose is converted to body fat.



STEP OF GLYCOGENESIS

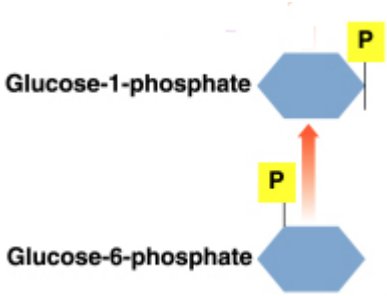
Glucose is converted to glucose-6-phosphate using ATP.

Enzyme: Glucokinase



Glucose-6-phosphate is converted to glucose-1-phosphate.

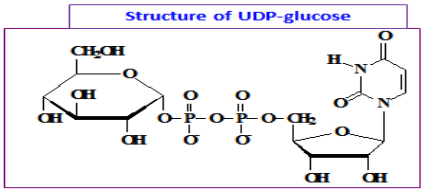
Enzyme: Phospho-glucomutase

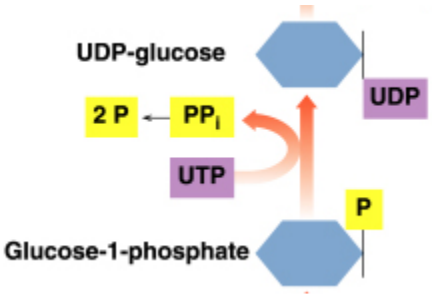


Formation of UDP-Glucose :

UTP activates glucose-1-phosphate to form UDP-glucose and pyrophosphate (PP_i).

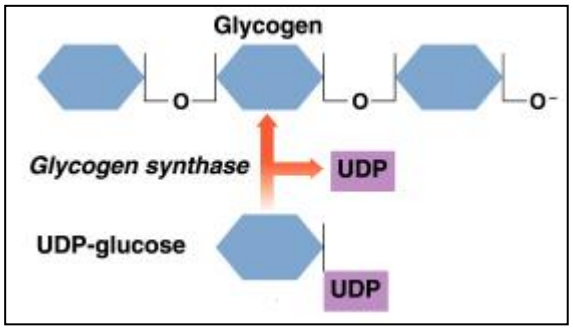
Enzyme: UDP- glucose pyrophosphorylse





The glucose in UDP-glucose adds to glycoge

$$\text{UDP-Glucose} + (\text{glycogen})_n \rightarrow (\text{glycogen})_{n+1} + \text{UDP}$$



A small glycogen molecule or a protein "Glycogenin" is needed to act as Primer for initiation of Glycogen Synthesis.

The UDP reacts with ATP to regenerate UTP.

UDP + ATP → UTP + ADP

Formation of branches in Glycogen is catalyzed by a branching enzyme- Glucosyl 4-6 transferase

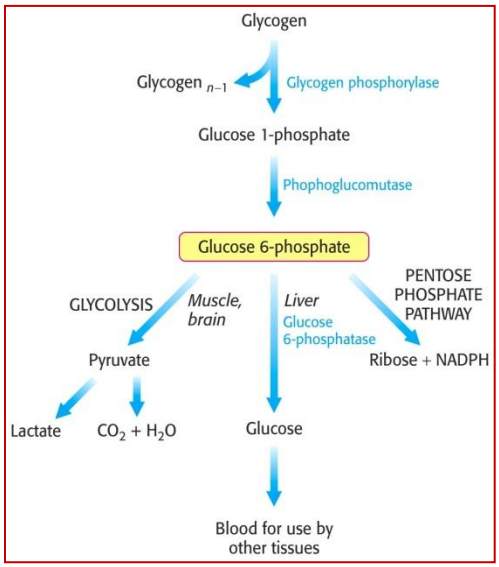
GLYCOGENOLYSIS

Glycogenolysis is a metabolic process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting.

Glycogen is catabolized by removal of a glucose monomer through cleavage with inorganic phosphate to produce glucose-1- phosphate.This derivative of glucose is then converted to glucose-6-phosphate, an intermediate in glycolysis.

It takes place in the muscle and liver tissues, where glycogen is stored, as a hormonal response to epinephrine and/or glucagon.

PATHWAY



- **First step-**

The overall reaction for the 1st step is:

Glycogen (n residues) + Pi ↔ Glycogen (n-1 residues)+ G1P

Here, glycogen phosphorylase cleaves the bond at the C1 position by substitution of a phosphoryl group. It breaks down glucose polymer at [α1-4] linkages until 4 linked glucoses are left on the branch.

Pyridoxal phosphate is an important co- factor in this reaction, as its phosphate group acts as a general acid catalyst, promoting attack by Pi on the glycosidic bond.

- **Second step-**

The second step involves the enzyme oligo α[1→6] to α[1→4] glucantransferase or debranching enzyme, which transfers the three remaining glucose units to another 1,4 terminal of glycogen, which exposes the α[1→6] branching point.

The final action of this enzyme is the hydrolysis of the remaining glucose attached at the α[1→6] branch point, which gives one free glucose molecule.

- **Third step-**

The third and last stage converts G1P (glucose-1-phosphate) to G6P (glucose-6- phosphate) through the enzyme phosphoglucomutase.

G1P ↔ G6P

Here the enzyme donates a phosphoryl group to C-6 of the substrate and accepts a phosphoryl group from C-1.

FATES OF GLUCOSE 6 PHOSPHATE

- This G6P can enter glycolysis and serve as an energy source to support muscle contraction.
- In liver, it releases glucose into blood when blood glucose level drops.

REGULATION OF GLYCOGEN PHOSPHORYLASE

- Glycogen phosphorylase is activated in response to Glucagon or Epinephrine which raise the [cAMP] level and activate Protein KinaseA. PKA phosphorylates and activates Phosphorylase Kinase, which converts Glycogen Phosphorylase B to its active A form.
- Phosphoprotein Phosphatase 1 reverses the phosphorylation of Glycogen Phosphorylase A, inactivating it.
- Glucose binds to the liver isozyme of Glycogen Phosphorylase,favoring its dephosphorylation and inactivation.
- Liver contains glucose 6-phosphatase.
- Muscle and Brain does not have this enzyme. **WHY?**
- The liver releases glucose to the blood to be taken up by brain and active muscle. The liver regulates blood glucose levels.
- The muscle and brain retain glucose 6-phosphate to be use for energy. Phosphorylated glucose is not transported out of muscle cells.

Reulation of Glycogen Metabolism

Three hormones regulate GM

- Epinephrine ,Glucagon and Insulin
  Epinephrine and glucagon stimulate glycogenolysis and inhibit glycogenesis via a cAMP and a **phosphorylation** cascade. ➔ **release glucose**
- **Insulin**
  stimulates Glycogenesis in a pathway ending in the **dephosphorylation and thus activation** of glycogen synthase.

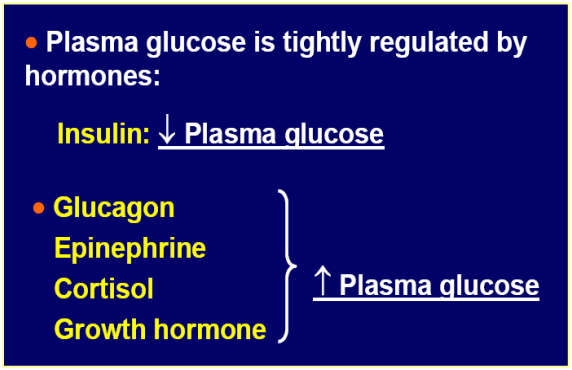
Inhibits Glycogenolysis via **dephosphorylation**.

➔ **takes up glucose**

DIABETES MELLITUS

- Diabetes mellitus (DM) is a chronic condition that is characterised by raised blood glucose levels (Hyperglycemia).

Regulation of Plasma Glucose Level



Classification of DM

1. Type 1 DM

It is due to insulin deficiency and is formerly known as Type I

Insulin Dependent DM (IDDM)

Juvenile onset DM

2. Type 2 DM

It is a combined insulin resistance and relative deficiency in insulin secretion and is frequently known as Type II

Noninsulin Dependent DM (NIDDM)

Adult onset DM

3. Gestational Diabetes Mellitus (GDM):

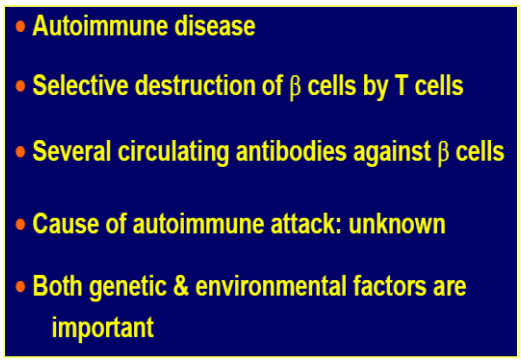
- Gestational Diabetes Mellitus (GDM) developing during some cases of pregnancy but usually disappears after pregnancy.

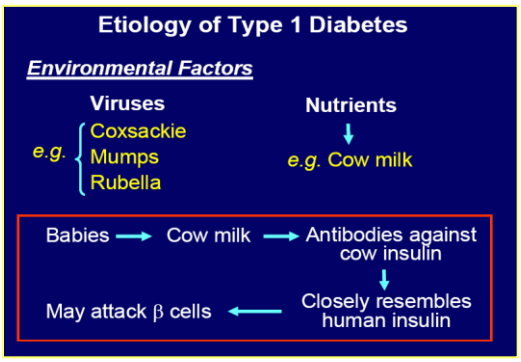
4. Other types:

- Secondary DM

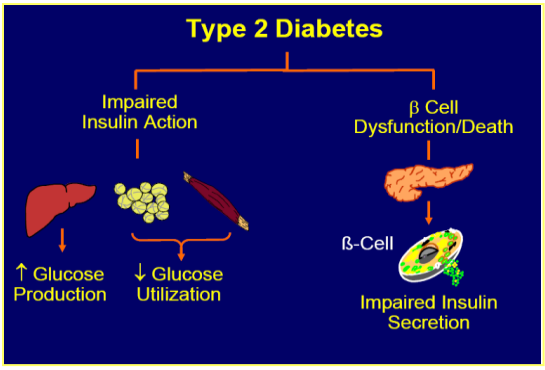
Etiology

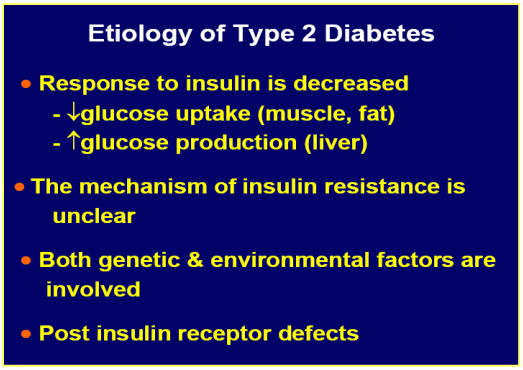
1. Etiology of Type 1 Diabetes





2. Etiology of Type 2 Diabetes





Epidemiology

Type 1 DM

- It is due to pancreatic islet β-cell destruction predominantly by an autoimmune process.
- Usually develops in childhood or early adulthood
- It accounts for upto 10% of all DM cases
- Develops as a result of the exposure of a genetically susceptible individual to an environmental agent

Type 2 DM

- It results from insulin resistance with a defect in compensatory insulin secretion.

- Insulin may be low, normal or high!
- About 30% of the Type 2 DM patients are undiagnosed (they do not know that they have the disease) because symptoms are mild.
- It accounts for up to 90% of all DM cases

LABORATORY TEST FOR DIAGNOSIS

1. **Estimation of blood glucose.**
2. **Oral glucose tolerance test.**

Laboratory Tests

1. Glucosuria

- To detect glucose in urine by a paper strip
- Semi-quantitative
- Normal kidney threshold for glucose is essential

2. Ketonuria

- To detect ketonbodies in urine by a paper strip
- Semi-quantitative

3. Fasting blood glucose

- Glucose blood concentration in samples obtained after at least 8 hours of the last meal

4. Random Blood glucose

- Glucose blood concentration in samples obtained at any time regardless the time of the last meal

5. Glucose tolerance test

- 75 gm of glucose are given to the patient with 300 ml of water after an overnight fast
- Blood samples are drawn 1, 2, and 3 hours after taking the glucose
- This is a more accurate test for glucose utilization if the fasting glucose is borderline

6. Glycosylated hemoglobin (HbA1C)

- HbA1C is formed by condensation of glucose with free amino groups of the globin component of hemoglobin
- Normally it comprises 4-6% of the total hemoglobin.

- Increase in the glucose blood concentration increases the glycated hemoglobin fraction.
- HbA1C reflects the glycemic state during the preceding 8-12 weeks.

7. Serum Fructosamine

- Formed by glycosylation of serum protein (mainly albumin)
- Since serum albumin has shorter half life than hemoglobin, serum fructosamine reflects the glycemic state in the preceding 2 weeks
- Normal is 1.5 - 2.4 mmole/L when serum albumin is 5 gm/dL.
- Self Monitoring Test

Self-monitoring of blood glucose

- Extremely useful for outpatient monitoring specially for patients who need tight control for their glycemic state.
- A portable battery operated device that measures the color intensity produced from adding a drop of blood to a glucose oxidase paper strip.
- e.g. One Touch, Accu-Chek, DEX, Prestige and Precision.

Estimation of blood glucose

1. Measurement of blood glucose is indicative of current state of carbohydrate metabolism.
2. Depending on time of collection:

❖ Fasting blood glucose- after an overnight fast.

❖ Post meal or postprandial blood glucose-2 hrs after the subject has taken a normal meal.

❖ Random blood glucose – Any time of the day.

Folin-Wu and the Somogyi-Nelson

☐ Based on the same principles.

☐ **Principle-**

Cupric ions $\xrightarrow{\text{Blood glucose}}$ cuprous ions $\xrightarrow{\text{phosphomolybdic acid}}$ Blue coloured compound.
(alkaline cupric sulphate)

- Intensity of colour is proportional to glucose present in the blood

Orthotoluidine method

Principle

- Glucose + orthotoluidine $\xrightarrow{\text{hot acidic medium}}$ green coloured complex
- The intensity of the final colour is measured at 620 – 660 nm.
- The measured colour intensity is directly proportional to the concentration of glucose .

Enzymatic method

Glucose Oxidase/Peroxidase method

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Peroxidase}} 2H_2O + 2\,O_2 \xrightarrow[\text{Phenol}]{} \text{pink coloured compound}$$

- Intensity measured at 530 nm.

Hexokinase method

Principle

$$\text{Glucose} + ATP \xrightarrow{\text{HexoKinase}} G6P + ADP$$

$$G6P + NADP \xrightarrow{\text{G6PD}} 6\,PG + NADPH$$

- Glucose concentration proportional to rate of production of NADPH.
- Glucose tolerance means the ability of the body to utilize glucose in blood circulation.

GLUCOSE TOLERANCE TEST

- Glucose tolerance means ability of the body to utilize (tolerate) glucose in blood circulation.
- The effect of ingested carbohydrate can be studied under reasonably standard condition by means of the Glucose Tolerance Test.
- It is indicated by the nature of blood glucose curve following the administration of glucose.
- Temporary rise of blood sugar after food intake for few hours.
- Extent and duration of rise depends on type of food (Glycemic index).

- Glucose level returns to normal within 2-3 hrs.
- If it take >2 hours =Decrease glucose tolerance.

PRINCIPLE

Following the standard oral dose of glucose, plasma and urine glucose levels are monitored at regular intervals, in order to measure tolerance under defined conditions.

Indication of Glucose tolerance test

- In asymptomatic persons with sustained or transient glycosuria.
- In persons with symptoms of diabetes but no glycosuria or hyperglycemia.
- Persons with family history but no symptoms or positive blood findings.
- In persons with or without symptoms of diabetes mellitus showing one abnormal blood findings.
- In patients with neuropathies or retinopathies of unknown origin.

Contraindications of glucose tolerance test

1. There is no indication for doing GTT in a person with confirmed diabetics mellitus.
2. GTT has no role in follow-up of diabetics.
3. The test should not be done in ill patients.

Types of glucose tolerance test

1. Standard Oral glucose tolerance test
2. I/V Glucose tolerance test
3. Mini Glucose tolerance test

Preparation of patient

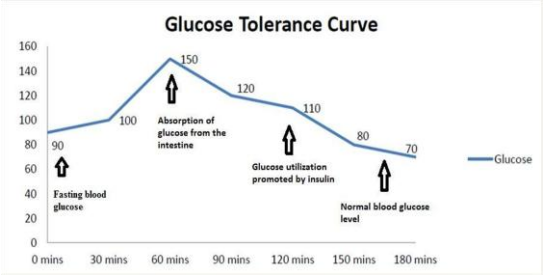
- Patient should on carbohydrate rich unrestricted diet for 3 days.
- Patient should be ambulatory with normal physical activity.
- Medications should be discontinued on the day of testing.
- Exercise, smoking and tea or coffee are not allowed during test period.

- OGTT carried out in the morning after patient has fasted overnight for 8-14 hours.

Test

- A fasting venous blood sample is collected in the morning.
- Patients ingest 75 g of anhydrous glucose in 250-300 ml of water over 5 minutes. (for children, the dose is 1.75 g of glucose per kg).
- In the classical procedures, the blood and urine samples are collected at half hourly interval of the next three hours.
- A curve is plotted with the blood glucose levels on the vertical axis against the time of collection on the horizontal axis.
- The curve so obtained is called glucose tolerance curve.

Normal Glucose tolerance curve



Intravenous Glucose tolerance test

- This test is undertaken for patients with malabsorption (Celiac disease or enteropathies).
- Under these conditions oral glucose load is not well absorbed and the results of oral glucose tolerance test become inconclusive.

I/V Glucose tolerance test- Procedure

- I/V glucose tolerance test is carried out by giving 25 g of glucose dissolved in 100 ml distilled water as intravenous injection within 5 minutes.
- Completion of infusion is taken as time zero.
- Blood samples are taken at 10 minutes interval for the next hour.
- The peak value is reached within a few minutes.

Interpretation

- Normally, blood glucose level returns to normal range within 60 minutes.
- In diabetes mellitus, this decline is slow.

Mini or Modern GTT

- As per current WHO recommendations, in the mini or modern glucose tolerance test, only two samples are collected.
- Fasting (zero hour) and 2 hour post glucose load.
- Urine samples are also collected during the same time.
- The diagnosis is made from the variations observed in these results.

	Zero Hour	After 2 Hours
Normal Person	**< 110 mg/dL**	**< 140 mg/dL**
Increase Glucose Tolerance	**110 – 126 mg/dL**	**140 – 199 mg/dL**

GTT Under special conditions

- Cortisone stress test- used for detecting pre diabetes or Latent diabetes
- Extended GTT- To diagnose the cause of hypoglycemia especially 2-3 hours after meals.

Factors affecting GTT

a) Acute infections- Cortisol is secreted, the curve is elevated and prolonged.

b) Hypothyroidism-A flat curve is obtained in hypothyroidism. Thyroid hormone increases the absorption of glucose from the gut.

c) Starvation- There is rise of counter regulatory hormones, which show increased glucose tolerance.

ADVANTAGES

- It is useful in recognizing of border line cases of diabetes.
- GTT is useful in early diagnosis diabetes malitus.

DISADVANTAGES

- GTT is not necessary in known cases of hyperglycemic patient.
- Oral GTT is also not necessary in known cases of mal -absorption.
- This time I-V glucose tolerance test is required.

18BEBME305 - BIOCHEMISTRY

UNIT – III

PROTEINS

CONTENTS

1. Proteins: Definition, classification,
2. Architecture,
3. Biological functions;
4. Classification of amino acids,
5. Oxidative and non oxidative deamination,
6. Transamination,
7. Decarboxylation,
8. Urea cycle,
9. Purification of proteins.

PROTEINS AND ITS CLASSIFICATION

Introduction

- Protein name is derived form a Greek word PROTOS which means "the first or the supreme.
- Protein are extremely complicated and nitrogenous molecule made up of variable number of amino acid residue joined to each other by a specific covalent bond called peptide bond.
- 20 amino acid which have been found to occur in all proteins, known as standard amino acid.

Importance

- Proteins make up about 15% of the mass of the average person Enzyme act as a biological catalyst
- Storage and transporte – Haemoglobin Defenece -Antibodies
- Hormones – Insulin
- Ligaments and arteries (mainly formes by elastin Protein)
- Muscle – Proteins in the muscle respond to nerve impulses by changing the packing of their molecules (Actin and myosin)
- Hair, nails and skin: Protein keratin as main component

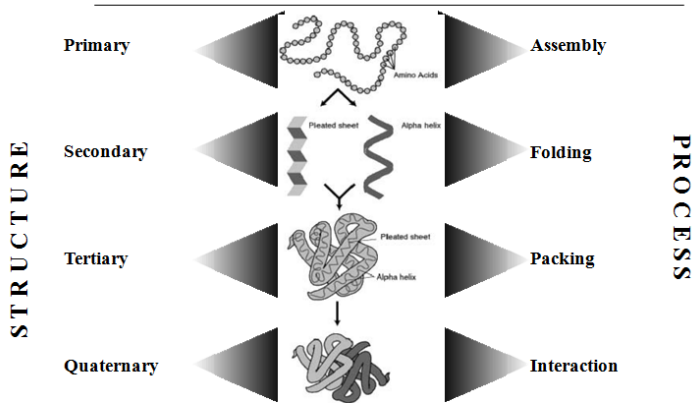
Classification

- ❖ Structure
- ❖ Shape and solubility
- ❖ Biological function
- ❖ Composition
- ❖ Nutritional basis

Levels in Protein structure

1. Majority of protein are compact and highly convoluted molecules.
2. Each polypeptide assumes at least three levels of structural organization termed as primery,secondary and tertiary structure.
3. Proteins which possess more than one polypeptide chain in their molecule also possess a fourth structure called quaternary structure

Chemistry of Protein Structure



Primary structure

- The sequence of amino acid residues along the peptide is called primary structure of the peptide.
- It also include the determination of the number of amino acid residues in a peptide chain.
- Shows whether the peptide chain is open, cyclic or branched. Primary structure is linear, ordered and 1 dimensional.
- Written from amino end to carboxyl end that is N to C.

Secondary Structure

- Primary structure shows that peptide are quite straight and extended.
- X-rays diffraction on protein crystals shows that polypeptide chain tend to twist or coil upon themselves.
- The folding of the polypeptide chain into specific coiled structure held together by H bonds is called secondary structure of protein.
- Secondary structure may take one of the following form.

Alpha(α)- Helix

1. It is a clockwise rodlike spiral shape .
2. Formed by intrachain Hydrogen bonding between
   C=O group of each amino acid and NH**2** group that is present 4 residue ahead.
3. Protein have great strength and elasticity.
   Can easily be stretched due to tight coiling.

β- Pleated Sheath

1. 5 to 10 amino acid in this structure line up side by side just like a sheath of cloth can be folded again and again
2. Hydrogen bond present between the peptide strands that is interstrand.
3. This form is fully expended and can't be further stretched and they are inelastic

Loop or Coil Conformation

1. Present mainly in globular protein.
2. Connect two Alpha helix or Beta sheath.
3. Present in those area where bend is required.

Super secondary Motifs

1. Present in Globular protein.
2. This structure form when two beta pleated sheath are connected to each other by an alpha helix.
3. For example β-α-β supersecondary motif

Tertiary structure

- The tertiary structure mean the overall conformation of a polypeptide.
- Myoglobin chain is when fully extended its length is 20 time than is width.
- X-rays diffraction show that its structure is just like a foot ball i.e. globular.
- The globular structure is due to folding and refolding

Quaternary Structure

- Formed by those protein having more than one peptide chain subunit.
- Each peptide have its own primary, secondary, and tertiary structure.
- The number and arrangement of the over all structure of the peptide subunit is called quaternary structure.
- For example structure of Hemoglobin.

Classification based on shape

Depend upon the axial ratio the protein are classify into two type of protein.

1. Globular protein
2. Fibrous protein

Fibrous Protein

- Axial ratio more than 10. Long thread like molecule.
- Their helical strands mainly form fibers. These protein are insoluble in water.
- Form structure of the tissue Present where support is required. Example
    1. Collagen
    2. Elastin
    3. Keratin

Globular Protein

- Axial ratio less than 10. Spheroid or ovoid in shape.

- Enzyme are mostly globular in shape. Subdivided into two type of protein,
    1. Albumins: Water soluble.
    2. Globulin: Soluble in dilute salt solution.

Classification based upon Function

Catalytic Protein: These are enzyme which may be simple or conjugated.

1. Alkaline phosphatase
2. Alanine trasaminase

Regulatory or Hormonal protein: Many protein and peptide acts as Hormone.

1. Insulin
2. Growth Hormone

Structural Protein: Contribute to the structure of the tissue.

1. Collagen
2. Elastin

Transport Protein: Serve to carry substances.

1. Transferrin carry Iron
2. Hemoglobin carry Oxygen

Immune Protein: Serve in defense mechanism

1. Immunoglobulin, IgG, IgA, IgM, IgD,IgE

Contractile Protein: Takes part in the muscle contrection.

1. Actin
2. Myosin

Genetic Protein: Protein present in combination with nucleic acid.

1. Histone Protein.

Storage Protein: To store protein for nutritional purposes.

1. Casein in Milk
2. Gliadin in Wheat.

Classification based on composition

ž **Simple proteins** are those which on hydrolysis yield only amino acids and no other major organic or inorganic hydrolysis products. They usually contain about 50% carbon,7% hydrogen, 23% oxygen, 16% nitrogen and 0–3% sulphur.

ž Example:

-Egg (albumin)

-Serum (globulins)

Conjugated proteins are those which on hydrolysis yield not only amino acids but also organic or inorganic components. The non-amino acid part of a conjugated protein is called prosthetic group. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups.

PROTEIN ARCHITECHTURE

INTRODUCTION

- Proteins are an important class of biological macromolecules which are the polymers of amino acids.
- Biochemists have distinguished several levels of structural organization of proteins. They are:
  - Primary structure
  - Secondary structure
  - Tertiary structure
  - Quaternary structure

PRIMARY STRUCTURE

- The primary structure of protein refers to the sequence of amino acids present in the polypeptide chain.
- Amino acids are covalently linked by peptide bonds.
- Each component amino acid in a polypeptide is called a "residue" or "moiety"
- By convention, the 1^0 structure of a protein starts from the amino- terminal (N) end and ends in the carboxyl-terminal (C) end.

IMPORTANCE OF PRIMARY STRUCTURE

- To predict 2^0 and 3^0 structures from sequence homologies with related proteins. (Structure prediction)
- Many genetic diseases result from abnormal amino acid sequences.
- To understand the molecular mechanism of action of proteins.
- To trace evolutionary paths.

METHODS OF AMINO ACID SEQUENCE DETERMINATION

- End group analysis – Edman degradation.
- Gene sequencing method.

SECONDARY STRUCTURE

- Localized arrangement of adjacent amino acids formed as the polypeptide chain folds.
- It consists of
  - α-helix
  - β-pleated sheet
  - β-bends
  - Non repetitive structures
  - Super secondary structures

- Linus Pauling proposed some essential features of peptide units and polypeptide backbone. They are:
  - The amide group is rigid and planar as a result of resonance. So rotation about C-N bond is not feasible.
  - Rotation can take place only about N- C_α and C_α – C bonds.
  - Trans configuration is more stable than cis for R grps at C_α
  - From these conclusions Pauling postulated 2 ordered structures **α** helix and

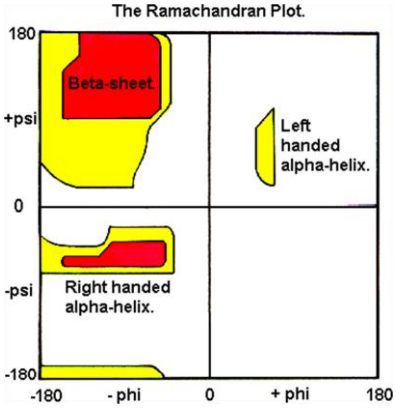
β sheet

POLYPEPTIDE CHAIN CONFORMATIONS

- The only reasonably free movements are rotations around the C α-N bond (measured as ϕ) and the C α-C bond (measured as Ψ).
- The conformation of the backbone can therefore be described by the **torsion angles** (also called **dihedral angles** or **rotation angles**)

RAMACHANDRANPLOT

- A **Ramachandran plot** (also known as a **Ramachandran diagram** or a **[φ,ψ] plot**), originally developed in 1963 by G. N. Ramachandran.
- **White regions :** Sterically disallowed for all amino acids except glycine.
- **Red regions :** allowed regions namely the a-helical and b-sheet conformations.
- **Yellow areas :** outer limit



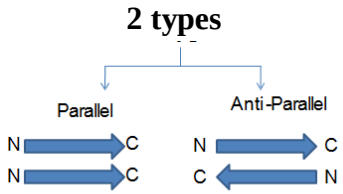
ALPHA HELIX

- Spiral structure
- Tightly packed, coiled polypeptide backbone core.
- Side chain extend outwards
- Stabilized by H bonding b/w carbonyl oxygen and amide hydrogen.
- Amino acids per turn – 3.6
- Pitch is 5.4 A

- Alpha helical segments are found in many globular proteins like myoglobins, troponin-C etc.

BETA PLEATED SHEET

- Formed when 2 or more polypeptides line up side by side.
- Individual polypeptide - β strand
- Each β strand is fully extended.
- They are stabilized by H bond b/w N-H and carbonyl grps of adjacent chains.



BETA BENDS

- Permits the change of direction of the peptide chain to get a folded structure.
- It gives a protein globularity rather than

linearity.

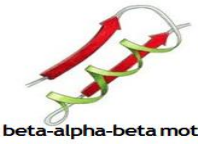
- H bond stabilizes the beta bend structure.
- Proline and Glycine are frequently found in beta turns.
- Beta turns often promote the formation of antiparallel beta sheets.
- Occur at protein surfaces.
- Involve four successive aminoacid residues

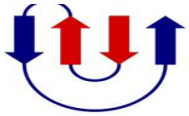
NON REPETITIVE STRUCTURES

- A significant portion of globular protein's structure may be irregular or unique.
- They include coils and loops.
- Segments of polypeptide chains whose successive residues do not have similar ϕ and Ψ values are called **coils**.
- Almost all proteins with more than 60 residues contain one or more loops of 6 to 16 residues, called **Ω loops.**

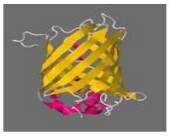
SUPER SECONDARY STRUCTURES (MOTIFS)

Certain groupings of secondary structural elements are called **motifs.**


beta-alpha-beta motif


Greek key motif


β-meander motif


Beta barrel

TERTIARY STRUCTURE

- Tertiary structure is the three- dimensional conformation of a polypeptide.
- The common features of protein tertiary structure reveal much about the biological functions of the proteins and their evolutionary origins.
- The function of a protein depends on its tertiary structure. If this is disrupted, it loses its activity.

DOMAINS

- Polypeptide chains containing more than ,200 residues usually fold into two or more globular clusters known as **domains.**
- Fundamental functional and 3 dimensional structure of proteins.
- Domains often have a specific function such as the binding of a small molecule.
- Many domains are structurally independent units that have the characteristics of small globular proteins.

INTERACTIONS STABILIZING 3^0 STRUCTURE

- This final shape is determined by a variety of bonding interactions between the "side chains" on the amino acids.
- Hydrogen bonds
- Ionic Bonds
- Disulphide Bridges
- Hydrophobic Interactions:

TERTIARY STRUCTURE

DETERMINATION OF TERTIARY STRUCTURE

- The known protein structures have come to light through:
- **X-ray crystallographic studies**
- **Nuclear Magnetic Resonance studies**
- The atomic coordinates of most of these structures are deposited in a database known as the Protein Data Bank (PDB).
- It allows the tertiary structures of a variety of proteins to be analyzed and compared.

QUATERNARY STRUCTURE

- The biological function of some molecules is determined by multiple

polypeptide chains –
multimeric proteins.

- Arrangement of polypeptide sub unit is called quaternary structure.
- Sub units are held together by non covalent interactions.
- Eg: Hemoglobin has the subunit composition a_2b_2

PROTEINS BIOLOGICAL FUNCTION

- Proteins depending upon their physical and chemical structure and location inside the cell, they perform various functions. Proteins are grouped as follows, based on their metabolic function they perform.
- Protein is a chain of amino acids joined by peptide bonds in a specific sequence.
- Protein is an essential nutrient. There is no life without protein. Protein is contained in every part of your body, the skin, muscles, hair, blood, body organs, eyes, even fingernails and bone. Next to water, protein is the most plentiful substance in your body.

FUNCTIONS OF PROTEIN

- Protein has a critical physiological function. Protein is primarily used in the body to build, maintain, and repair body tissues.
- In the event that protein intake is greater than that required by the body for this primary function, excessive protein is converted to energy for immediate use or stored in the body as fat.
- Protein energy will be used only after other energy sources (carbohydrate and fat) are exhausted or unavailable.
- Protein is vital in the maintenance of body tissue, including development and repair.
- Protein is the major source of energy.
- Protein is involved in the creation of some hormones, help control body functions that involve the interaction of several organs and help regulate cell growth.
- Protein produces enzymes that increase the rate of chemical reactions in the body.
- Proteins transport small molecules through the organism. Hemoglobin is the protein that transports oxygen to the cells and it is called as transport protein.
- Proteins called antibodies help rid the body of foreign protein and help prevent infections, illnesses and diseases.

- Protein help store other substance in the organism. For example, iron is stored in the liver in a complex with the protein ferritin.
- Proteins help mediate cell responses, such as the protein rhodopsin, found in the eye and involved in the vision process.
- Proteins make up a large protein of muscle fiber and help in the movement of various parts of our bodies.
- Skin and bone contain collagen, a fibrous protein

(1) Enzymic Proteins

They are the most varied & highly specialized proteins with catalytic activity. Enzymes catalyze a variety of reactions. Example: Urease, Catalase, Cytochrome c.

(2) **Structural Proteins**

These proteins aid in strengthening or protecting biological structures. Example: Elastin, Collagen, Keratin

(3) Transport or Carrier Proteins

These proteins help in transport of ions or molecules in the body. Example: Myoglobin, Hemoglobin

(4) Nutrient and Storage Proteins

These proteins provide nutrition to growing embryos and store ions. Example: Ovalbumin from egg, casein from milk.

(5) Contractile or Motile Proteins

These proteins function in the contractile system. Example: Actin, Myosin

(6) Defense Proteins

These proteins defend against other organisms. Example: Antibodies, Fibrinogen, thrombin.

(7) Regulatory Proteins

They regulate cellular or metabolic activities. Example: Hormones:- Insulin, G proteins ADH

(8) Toxic Proteins

These proteins hydrolyze or degrade enzymes. Example: snake venom, ricin.

AMINO ACIDS CLASSIFICATION

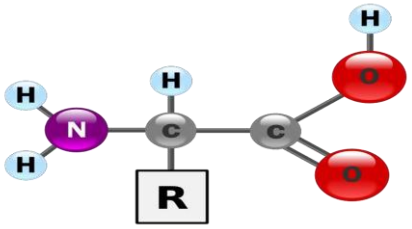
WHAT ARE AMINO ACIDS?

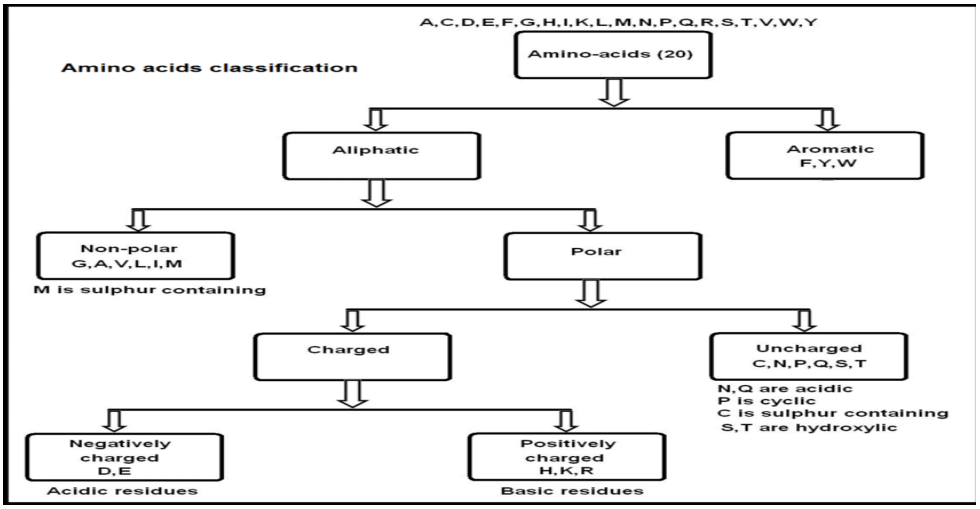
- Amino acids are organic compounds containing
- amine [- NH_2]
- carboxyl [-COOH]
- side chain [R group]

- The major key elements if amino acids are carbon, hydrogen, nitrogen, oxygen.

- About 500 amino acids are known (though only 20 appear in the genetic code) and can be classified in many ways

BASIC STRUCTURE[SKELETON]





NEED FOR CLASSIFICATION

- Classification of amino acids gives the grouping between 20 acids and a basic outline for grouping.
- It makes a clear idea to pick the amino acid type
- This is much useful for biochemists for the easy understanding between each amino acids.

Classification:

Based on,

- R group
- Polarity and R group
- Distribution in protein
- Nutritional requirements
- Number of amino and carboxylic groups

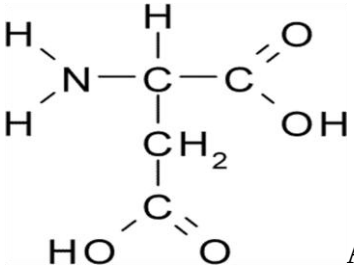
Based on R-Group

- Simple amino acids:
  These have no functional group in their side chain.
  Example: glycine, valine, alanine, leucine, isoleucine
- Hydroxy amino acids:
  These have a hydroxyl group in their side chain
  Eg: serine, threonine
- Sulfur containing amino acids: Have sulfur in their side chain
  Eg: cysteine, methionine
- Aromatic amino acids:
  Have benzene ring in their side chain Eg: phenylalanine, tyrosine
- Heterocyclic amino acids:
  Having a side chain ring which possess at least on atom other than carbon
  Eg: Tryptophan, histidine, proline
- Amine group containing amino acids: Derivatives of amino acids in which one of carboxyl group has been transformed into an amide group
  Eg: Asparagine, glutamine
- Branched chain amino acids:
  A branched-chain amino acid (BCAA) is an amino acid having aliphatic side-chains with a branch
  Eg: leucine, isoleucine, valine
- Acidic amino acids:
  Have carboxyl group in their side chain Eg: Aspartic and Glutamic acid
- Basic amino acids:
  Contain amino group in their side chain Eg: Lysine, Arginine
- Imino acid:
  Amino acids containing a secondary amine group
  Eg: Proline

Polarity and R Group

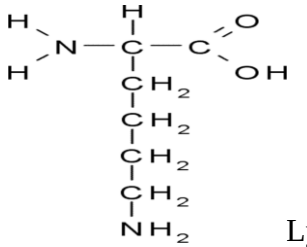
- Amino acids with non polar R group: These are hydrocarbons in nature, hydrophobic, have aliphatic and aromatic groups
  [aliphatic R groups]
  Eg: Alanine, Valine, Leucine, Isoleucine, Proline.
  [Aromatic groups]
  Eg: Phenylalanine, Tryptophan, Methionine(sulfur)
- Amino acids with polar but uncharged R Group:
  These amino acids are polar and possess neutral pH value.
  Eg: Glycine, Serine, Threonine, Cysteine, Tyrosine, Glutamine, Asparagine.

- Negatively charged amino acids:
  Their side chain [R Group] contain extra carboxyl group with a dissociable proton.
  And renders electrochemical behaviour to proteins
  Eg: Aspartic acid and Glutamic acid



Aspartic acid

- Positively charged amino acid:
  Their side chain have extra amino group Rendering basic nature to protein,
  Eg: Lysine, Arginine, Histidine.



Lysine

Distribution in protein:

- Standard protein amino acids:
  The amino acids that are used to form proteins, recognized by ribozyme autoaminoacylation systems
  Eg:
  Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine
- Non standard protein amino acids:
  These amino acids are not required to build proteins.
  Have a vital role as metabolic intermediates.
  Eg. Hydroxyproline, Hydroxylysine, Carboxyglutamate, Diaminopimelate.
- Non standard non protein amino acid:
  These are the derivative of amino acids and have role in metabolism.
  Eg: Alpha amino butyrate, Citruline, Ornithine, beta-alanine.

Based on nutritional requirements:

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

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

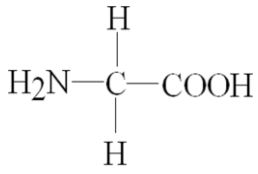
- Non essential amino acids:

An amino acid that can be made by humans and so is essential to the human diet. The nonessential amino acids: Alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

On basis of number of amino and carboxylic groups:

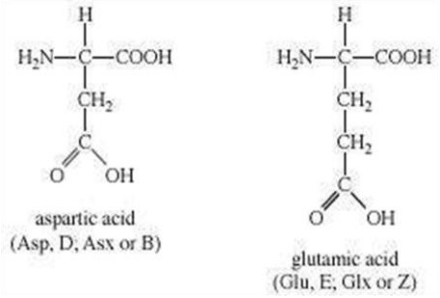
Monoamino- monocarboxylic amino acids

- glycine, alanine
- proline
- phenylalanine
- methionine
- serine, threonine



glycine

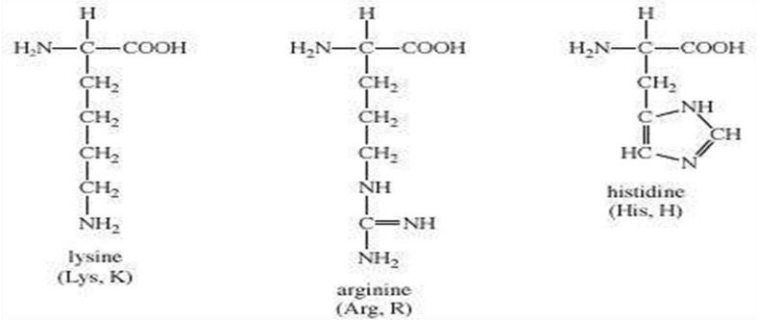
Monoamino-dicarboxyli amino acid: Aspartic and glutamic acid



aspartic acid (Asp, D; Asx or B)

glutamic acid (Glu, E; Glx or Z)

Diamino-monocarboxylic amino acids: Lysine, arginine, histidine.



lysine (Lys, K)

arginine (Arg, R)

histidine (His, H)

Catabolism of amino group occurs in 4 stages

- Transamination
- Oxidative Deamination
- Ammonia Transport
- Urea Cycle

Transamination

- The transfer of an amino (-NH2) group from an amino acid to a ketoacid, with the formation of a new amino acid & a new keto acid.
- Catalysed by a group of enzymes called transaminases (aminotransferases)
- Pyridoxalphosphate (PLP)– Co-factor.
- Liver, Kidney, Heart, Brain - adequate amount of these enzymes.

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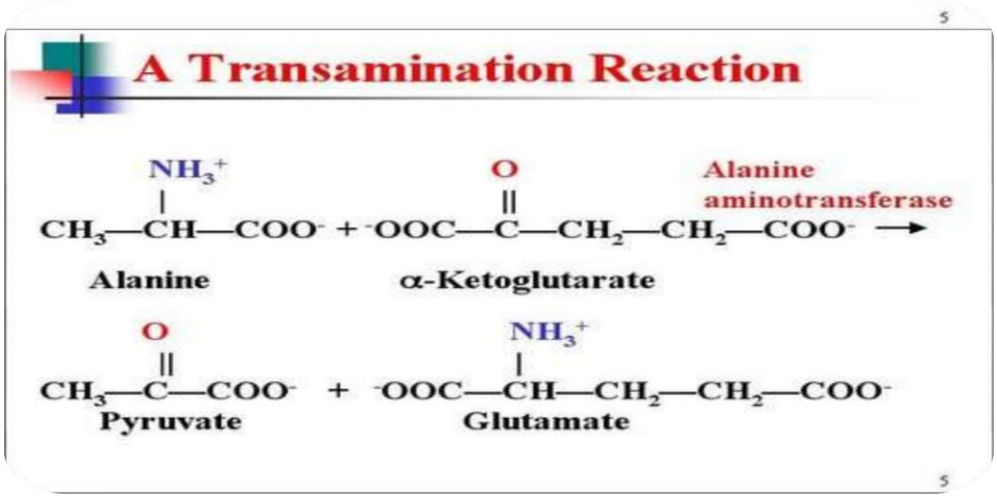

Features of Transamination

- All transaminases require PLP.
- No free NH3 liberated, only the transfer of amino group.
- Transamination is reversible.
- There are multiple transaminase enzymes which vary in substrate specificity.
- AST & ALT make a significant contribution for transamination.
- Transamination is important for redistribution of amino groups & production of non-essential amino acids.
- It diverts excess amino acids towards the energy generation.
- Amino acids undergo transamination to finally concentrate nitrogen in glutamate.
- Glutamate undergoes oxidative deamination to liberate free NH3 for urea synthesis.
- All amino acids except, lysine, threonine, proline & hydroxyproline participate in transamination.
- It involves both anabolism & catabolism, since – reversible.

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$AA_1 + \alpha\text{-KG} \longleftrightarrow ketoacid_1 + Glutamate$

$Alanine + \alpha\text{-KG} \longleftrightarrow Pyruvate + Glutamate$

$Aspartate + \alpha\text{-KG} \longleftrightarrow Oxaloacetetae + Glutamate$

Mechanism of Transamination

- Step:1
- Transfer of amino group from AA_1 to the coenzyme PLP to form pyridoxamine phosphate.
- Amino acid1 is converted to Keto acid2.
- Step:2
- Amino group of pyridoxamine phosphate is then transferred to a keto $acid_1$ to produce a new AA_2 & enzyme with PLP is regenerated.
-
- **Clinical Significance**

- Enzymes, present within cell, released in cellular damage into blood.
- ↑ AST - Myocardial Infarction (MI).
- ↑ AST, ALT – Hepatitis, alcoholic cirrhosis.
- Muscular Dystrophy.

TRANSDEAMINATION

- The amino group of most of the amino acids is released by a coupled reaction, trans-deamination.
- Transamination followed by oxidative deamination.
- Transamination takes place in the cytoplasm.
- The amino group is transported to liver as glutamic acid, which is finally oxidatively deaminated in the mitochondria of hepatocytes.

DEAMINATION

- The removal of amino group from the amino acids as NH3 is deamination.
- Deamination results in the liberation of ammonia for urea synthesis.
- The carbon skeleton of amino acids is converted to keto acids.
- Deamination may be either oxidative or non-oxidative
- Only liver mitochondria contain glutamate dehydrogenase (GDH) which deaminates glutamate to α-ketoglutarate & ammonia.
- It needs NAD^+ as co-enzyme.
- It is an allosteric enzyme.

- It is activated by ADP & inhibited by GTP.

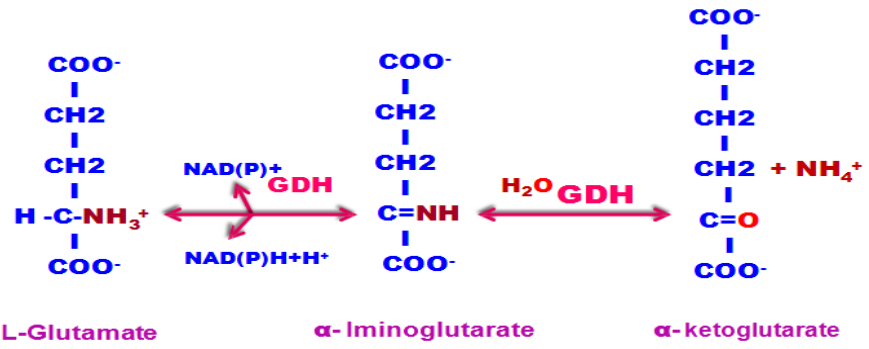
OXIDATIVE DEAMINATION

- Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation.
- Site: Mostly in liver & kidney.
- Oxidative deamination is to provide NH3 for urea synthesis & α-keto acids for a variety of reactions, including energy generation.

Role of Glutamate dehydrogenase

- Glutamate is a 'collection centre' for amino groups.
- Glutamate rapidly undergoes oxidative deamination.
- Catalysed by GDH to liberate ammonia.
- It can utilize either NAD^+ or $NADP^+$.
- This conversion occurs through the formation of an α-iminoglutarate

Oxidation of glutamate by GDH



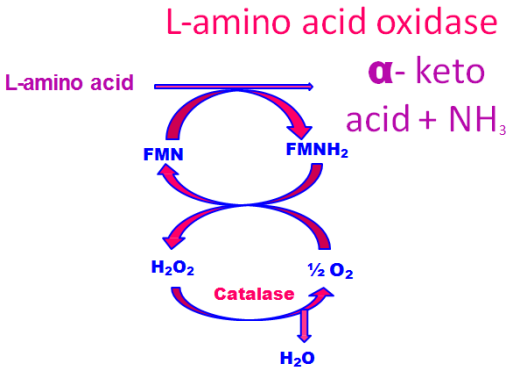
Metabolic Significance

- Reversible Reaction
- Both Anabolic & Catabolic.
- Regulation of GDH activity:
- Zinc containing mitochondrial, allosteric enzyme.
- Consists of 6 identical subunits.
- Molecular weight is 56,000.

Amino acid oxidases

- L-amino acid oxidase & D-Amino acid oxidase.
- Flavoproteins & Cofactors are FMN & FAD.
- Act on corresponding amino acids to produce α-keto acids & NH3

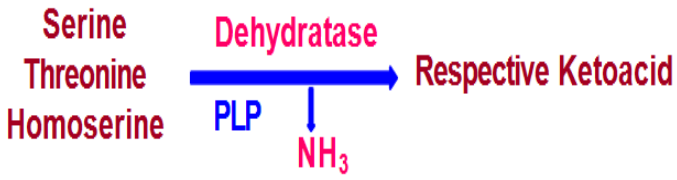
- Site: Liver, kidney, Peroxisomes.
- Activity of L-Amino acid oxidase is low.
- Plays a minor role in Amino acid catabolism.



- L-Amino acid Oxidase acts on all Amino acids, except glycine & dicarboxylic acids.
- Activity of D-Amino oxidase is high than that of L-Amino acid oxidase
- D-Amino oxidase degrades D-Amino acids in bacterial cell wall.

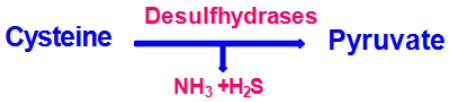
Non-Oxidative deamination

- Direct deamination, without oxidation.
- Amino acid Dehydratases:
- Serine, threonine & homoserine are the hydroxy amino acids.
- They undergo non-oxidative deamination catalyzed by PLP-dependent dehydratases

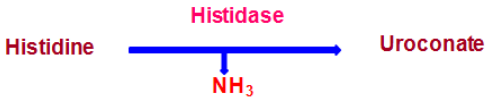


Amino acid desulfhydrases

- Cysteine & homocysteine undergo deamination coupled with desulfhydration to give keto acids.



Deamination of histidine:



DECARBOXYLATION REACTION

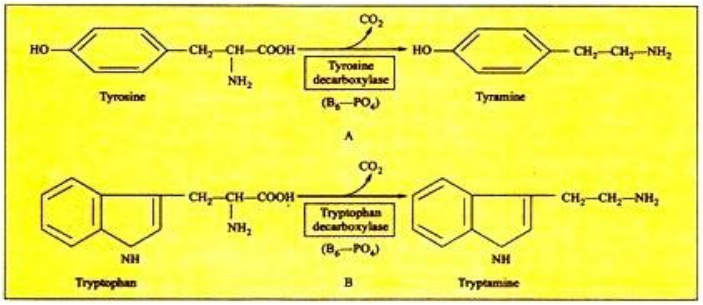
- The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.
- The enzymes, amino acid decarboxylases are pyridoxal phosphate dependent enzymes.
- Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the alpha-carbanion formed by the cleavage of bond between carboxyl and alpha-carbon atom.
- The physiologically active amines epinephrine, nor-epinephrine, dopamine, serotonin, gama-amino butyrate and histamine are formed through decarboxylation of the corresponding precursor amino acids.

Decarboxylation Process # 1. Tyramine:

- Tyramine is formed from tyrosine by tyrosine decarboxylation causing elevation of blood pressure.
- This occurs in the gut by bacterial action.
- The reaction takes place also in the kidney being favoured by oxygen deficiency.
- The tissue deaminates tyrosine in presence of sufficient oxygen.

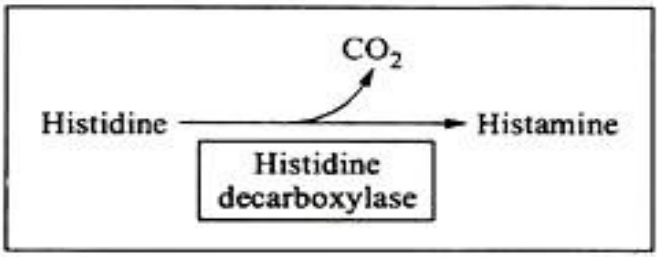
Decarboxylation Process # 2. Tryptamine:

- Tryptamine is formed from the amino acid tryptophan by the enzyme tryptophan decarboxylase present in mammalian kidney, liver and bacteria of gut involving (B_6-PO_4) as a coenzyme.
- Derivative of 5-OH tryptamine is serotonin a tissue hormone.
- It also increases blood pressure.



Decarboxylation Process # 3. Histamine:

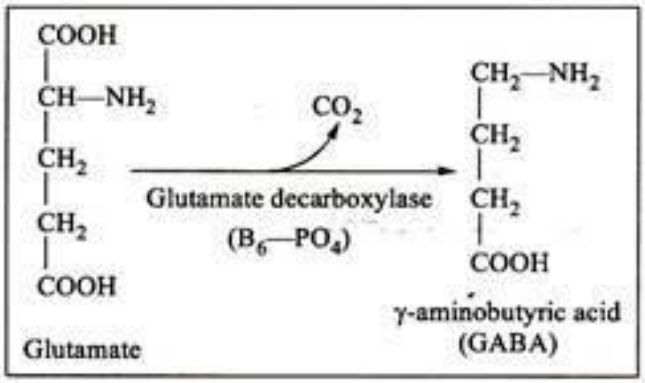
- The enzyme histidine decarboxylase, in presence of the coenzyme (B_6-PO_4), catalyze the conversion of histidine to histamine.
- This is produced by gastric mucosa cells and histaminergic neurons of the central nervous system.
- This is produced in the gut by bacterial decarboxylation of histidine.
- Basophils, another principal source of histamine, in the circulating cells.
- In the hypothalamus, it acts as a neurotransmitter.
- It serves as an anaphylactic and inflammatory agent in response to antigen.
- Effects of released histamine are mediated through H_1 and H_2receptors.
- It is formed in injured tissues. The liberation of histamine excessively is related to traumatic shock.
- Its elevated level depresses blood pressure and large doses cause extreme vascular collapse.
- The increased plasma level of histamine is found in patients with antigen-induced bronchial asthma.
- The anaphylactic reactions of histamine is mixed by promethazine and mepyramine which block H_1 receptors and cimetidine, the blocker of H_2 receptors, reduce the gastric acidity in peptic ulcer patients.
- In the kidneys, the enzyme histaminase oxidatively deaminates histamine to β-imidazole acetaldehyde which is further oxidized in the liver by aldehyde oxidase to form β-imidazole acetic acid which is excreted in urine—being conjugated with ribose.



Decarboxylation Process # 4. GABA (γ-aminobutyric acid):

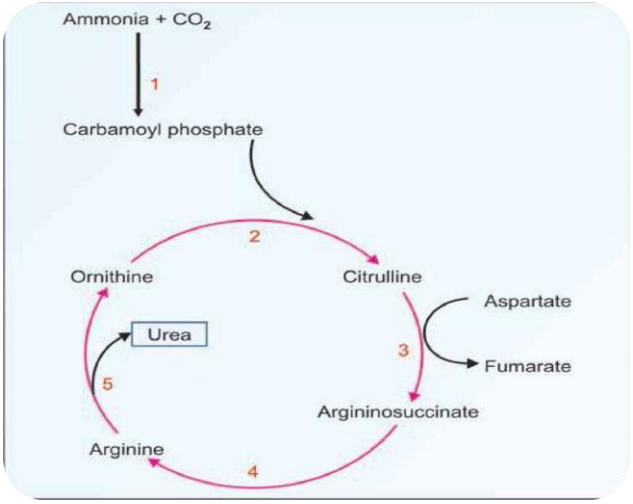
- GABA is formed principally in CN system in grey matter from glutamate by the catalytic activity of the enzyme glutamate decarboxylase with (B_6-PO_4) as coenzyme.
- It acts as a normal regulator of neuronal activity being active as an inhibitor.
- It is released at the axon terminals of neurons in grey matter and acts as inhibitory neurotransmitter by accelerating K^+ permeability of post synaptic membranes.
- The vitamin Pyridoxine (vit. B_6) deficiency forms GABA in less amount leading to neuronal hyper-excitability and convulsions.

- GABA is metabolised to form succinic semi-aldehyde by deamination in presence of (B_6-PO_4) as coenzyme and the ammonia removed is trans-aminated to α-ketoglutarate forming more glutamate.



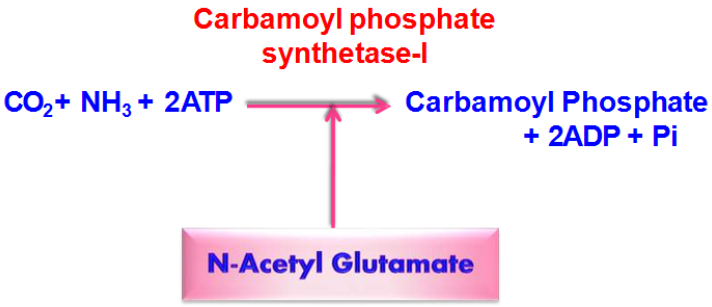
UREA CYCLE

- The urea cycle is the first metabolic pathway to be elucidated.
- The cycle is known as Krebs–Henseleit urea cycle.
- Ornithine is the first member of the reaction, it is also called as Ornithine cycle.
- Urea is synthesized in liver & transported to kidneys for excretion in urine.
- The two nitrogen atoms of urea are derived from two different sources, one from ammonia & the other directly from the a- amino group of aspartic acid.
- Carbon atom is supplied by CO2
- Urea is the end product of protein metabolism (amino acid metabolism).
- Urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- Urea synthesis is a five-step cyclic process, with five distinct enzymes.
- The first two enzymes are present in mitochondria while the rest are localized in cytosol.



STEP 1 : Formation of Carbamoyl formation

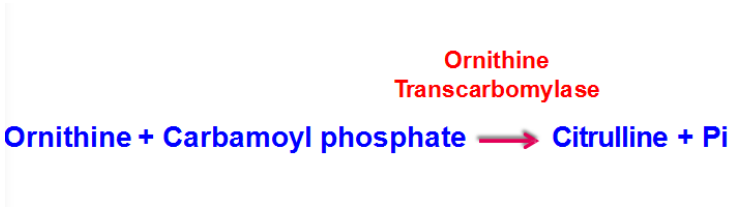
- Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate.
- This step consumes two ATP & is irreversible.
- It is a rate-limiting.
- CPS I requires N-acetylglutamate for its activity.
- Carbamoyl phosphate synthase II (CPS II) - involved in pyrimidine synthesis & it is present in cytosol.
- It accepts amino group from glutamine & does not require N-acetylglutamate for its activity.



STEP 2 : Formation of Citrulline

- The second reaction is also mitochondrial.
- Citrulline is synthesized from carbamoyl phosphate & ornithine by ornithine transcarbamoylase.
- Ornithine is regenerated & used in urea cycle.
- Ornithine & citrulline are basic amino acids. (Never found in protein structure due to lack of codons).
- Citrulline is transported to cytosol by a transporter system.

- Citrulline is neither present in tissue proteins nor in blood; but it is present in milk.

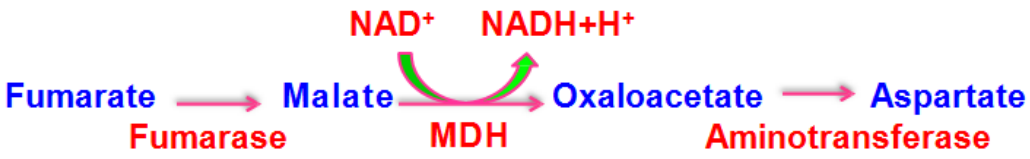


STEP 3 : Formation of Arginosuccinate

- Citrulline condenses with aspartate to form arginosuccinate by the enzyme Arginosuccinate synthetase.
- Second amino group of urea is incorporated.
- It requires ATP, it is cleaved to AMP & PPi
- 2 High energy bonds are required.
- Immediately broken down to inorganic phosphate (Pi).

STEP 4 : Formation of Arginine or cleavage of Arginosuccinate

- The enzyme Argininosuccinase or argininosuccinate lyase cleaves arginosuccinate to arginine & fumarate (an intermediate in TCA cycle)
- Fumarate provides connecting link with TCA cycle or gluconeogenesis.
- The fumarate is converted to oxaloacetate via fumarase & MDH & transaminated to aspartate.
- Aspartate is regenerated in this reaction.



STEP 5 : Formation of Urea

- Arginase is the 5th and final enzyme that cleaves arginine to yield urea & ornithine.
- Ornithine is regenerated, enters mitochondria for its reuse in the urea cycle.
- Arginase is activated by Co^{2+} & Mn^{2+}
- Ornithine & lysine compete with arginine (competitive inhibition).
- Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues.
- Arginine synthesis may occur to varying degrees in many tissues.
- But only the liver can ultimately produce urea.

Significance of Urea Cycle

- Toxic ammonia is converted into non-toxic urea.

- Synthesis of semi-essential amino acid-arginine.
- Ornithine is precursor of Proline, Polyamines.
- Polyamines include putrescine, spermidine, spermine.
- Polyamines have diverse roles in cell growth & proliferation.

Disorders of Urea Cycle

- The main function of Urea cycle is to remove toxic ammonia from blood as urea.
- Defects in the metabolism of conversion of ammonia to urea, i.e., Urea cycle leads to Hyperammonaemia or NH_3 intoxication.

Hyperammonaemia

- Inherited disorders of urea cycle enzymes- familial hyperammonaemia.
- Acquired disorders- Liver Disease, severe Renal disease - Acquired hyperammonaemia.

Amonia Toxicity

- Increased levels of ammonia crosses BBB, formation of glutamate.
- More utilization of α-ketoglutarate.
- Decreased levels of α- Ketoglutarate in Brain.
- α-KG is a key intermediate in TCA cycle.
- Decreased levels impairs TCA cycle.
- Decreased ATP production.

Hepatic Coma

- In diseases of the liver, hepatic failure can finally lead to hepatic coma & death.
- Hyperammonemia is the characteristic feature of liver failure.
- The condition is also known as portal systemic encephalopathy.
- Normally the ammonia & other toxic compounds produced by intestinal bacterial metabolism are transported to liver by portal circulation & detoxified by the liver.
- But when there is portal systemic shunting of blood, the toxins bypass the liver & their concentration in systemic circulation rises.

N- Acetyl Glutamate synthase deficiency

- Autosomal Recessive.
- A severe neonatal disorder with fatal consequences.
- Treatment with structural analog N-carbamoyl-L- glutamate – activates CPS-I.
- Ornithine Transporter Deficiency (ORNT1 gene):
- Ornithine is accumulated in Cytoplasm.
- HHH syndrome – Hyper-ornithinemia, Hyper- ammonemia, Homocitrillinuria.

Blood urea significance

- Normal blood urea concentration is 10-40 mg/dl.
- About 15-30 g of urea (7-15 g nitrogen) is excreted in urine per day.
- Blood urea estimation is a screening test for the evaluation of kidney (renal) function.
- Elevation in blood urea may be broadly classified into three categories.

Pre-Renal

- This is associated with increased protein breakdown, leading to a negative nitrogen balance.
- Observed after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc.
- In leukemia & bleeding disorders also, blood urea is elevated.

Renal

- In renal disorders like acute glomerulonephritis, chronic nephritis, nephrosclerosis, polycystic kidney, blood urea is increased.

Post-renal

- Due to obstruction in the urinary tract (e.g. tumors, stones, enlargement of prostate gland etc.) blood urea is elevated.
- This is due to increased reabsorption of urea from the renal tubules.

PROTEIN PURIFICATION

- Protein purification is a series of processes intended to isolate a single type of protein from a complex mixture.
- Protein purification is vital for the characterization of the function, structure and interactions of the protein of interest.
- The starting material is usually a biological tissue or a microbial culture.
- The various steps in the purification process may free the protein from a matrix that confines it, separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins.
- Separation steps may exploit differences in (for example) protein size, physico-chemical properties, binding affinity and biological activity.

Purpose

- Preparative
- Analytical
- Preparative purifications aim to produce a relatively large quantity of purified proteins for subsequent use. Examples include the preparation of commercial products such as enzymes (e.g. lactase)

- Analytical purification produces a relatively small amount of a protein for a variety of research or analytical purposes, including identification, quantification
- An analytical purification generally utilizes three properties to separate proteins.
- First, proteins may be purified according to their isoelectric points by running them through a pH graded gel or an ion exchange column.
- Second, proteins can be separated according to their size or molecular weight via size exclusion chromatography or by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis
- Thirdly, proteins may be separated by polarity/hydrophobicity via high performance liquid chromatography or reversed-phase chromatography.

Methods of Protein Purification

1. **Extraction**
   - Depending on the source, the protein has to be brought into solution by breaking the tissue or cells containing it.
   - There are several methods to achieve this:
   - Repeated freezing and thawing,
   - sonication,
   - homogenization by high pressure,
   - Filtration, or permeabilization by organic solvents.

ž

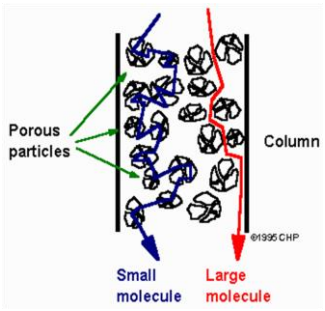
2. **Precipitation and differential solublization**
   - In bulk protein purification, a common first step to isolate proteins is precipitation with ammonium sulfate **(NH4)2SO4.**
   - This is performed by adding increasing amounts of ammonium sulfate and collecting the different fractions of precipitate protein.
   - Ammonium sulphate can be removed by dialysis.
   - The hydrophobic groups on the proteins gets exposed to the atmosphere and it attracts other protein hydrophobic groups and gets aggregated. Protein precipitated will be large enough to be visible.
   - One advantage of this method is that it can be performed inexpensively with very large volumes.

3. **Chromatographic Methods**
   - Usually a protein purification protocol contains one or more chromatographic steps.
   - The basic procedure in chromatography is to flow the solution containing the protein through a column packed with various materials.

- Usually proteins are detected as they are coming off the column by their absorbance at 280 nm.

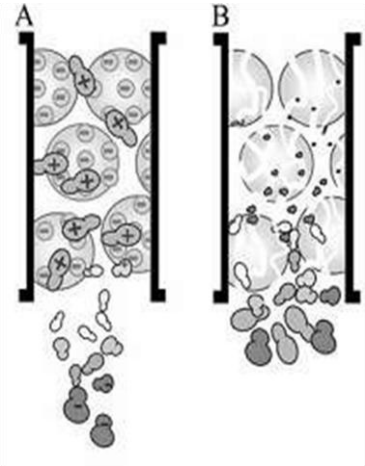
Size exclusion chromatography

- Using porous matrix
- Based on different sizes of proteins



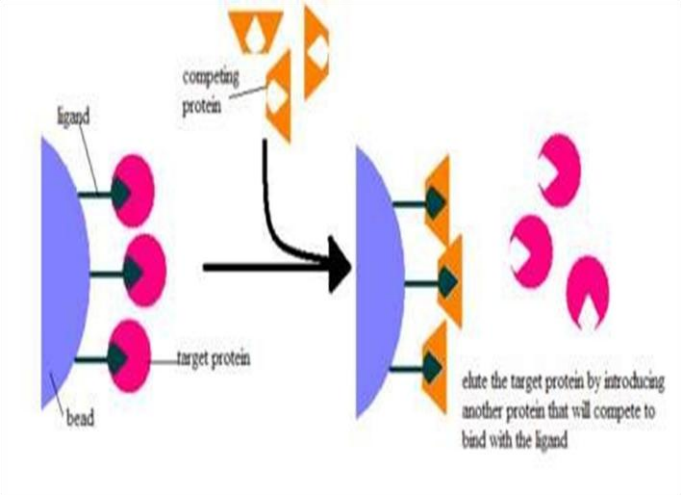
Ion exchange chromatography

- Anion exchange resins (positive charge) separate negatively charged compounds
- cation exchange resins (negative charge) separate positively charged molecules



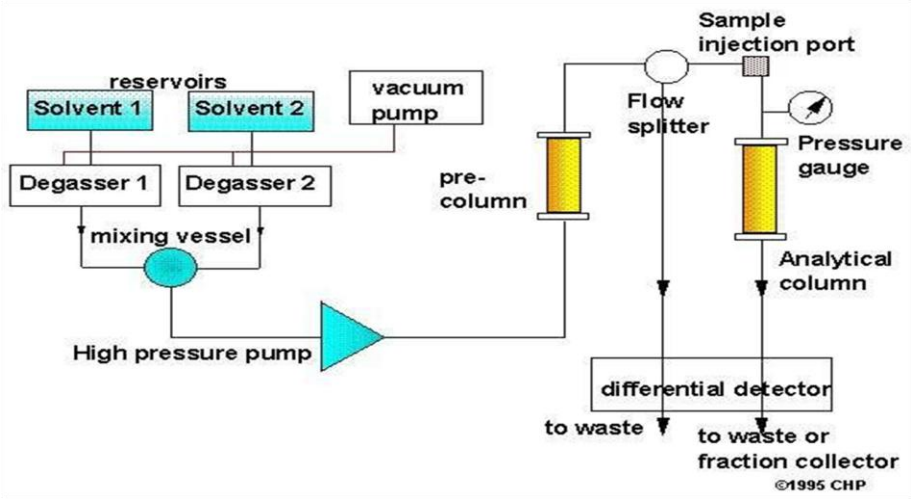
Affinity Chromatography

- Affinity Chromatography is a separation technique based upon molecular conformation
- Resins have ligands attached to their surfaces which are specific for the compounds to be separated.
- Ligands function in a fashion similar to that of antibody-antigen interactions.



ž **HPLC**

- High pressure to drive the solutes through the column faster.
- Diffusion is limited and the resolution is improved.
- The most common form is “reversed phase" hplc, where the column material is hydrophobic.
- The proteins are eluted by a gradient of increasing amounts of an organic solvent, such as acetonitrile.
- The proteins elute according to their hydrophobicity.
- After purification by HPLC the protein is in a solution that only contains volatile

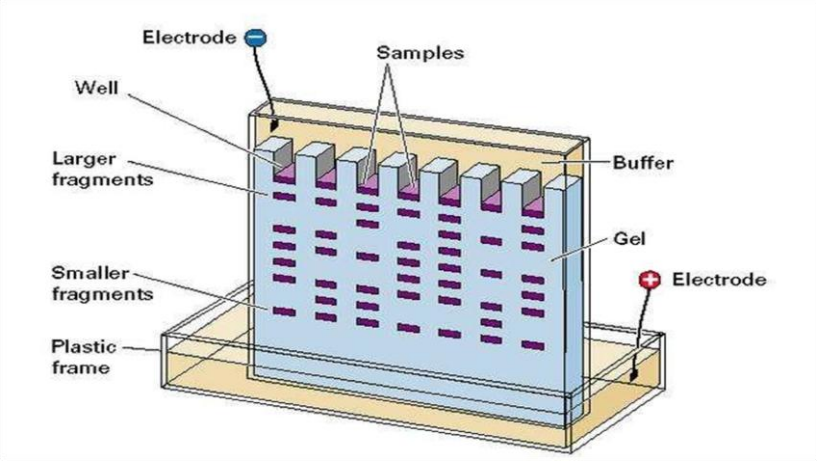


Hydrphobic interaction chromatography

- Resins used in the column are amphiphiles with both hydrophobic and hydrophilic regions.
- The hydrophobic part of the resin attracts hydrophobic region on the proteins.
- The greater the hydrophobic region on the protein the stronger the attraction between the gel and that particular protein.

Gel Electrophoresis

- Gel electrophoresis is a common laboratory technique that can be used both as preparative and analytical method.
- The principle of electrophoresis relies on the movement of a charged ion in an electric field. In these conditions, the proteins are unfolded and coated with negatively charged detergent molecules. The proteins in SDS-PAGE are separated of the sole basis of their size.
- In analytical methods, the protein migrates as bands based on size. Each band can be detected using stains such as Coomassie blue dye or silver stain.
- Preparative methods to purify large amounts of protein require the extraction of the protein from the electrophoretic gel. This extraction may involve excision of the gel containing a band, or eluting the band directly off the gel as it runs off the end of the gel.



18BEBME305 - BIOCHEMISTRY

UNIT – IV

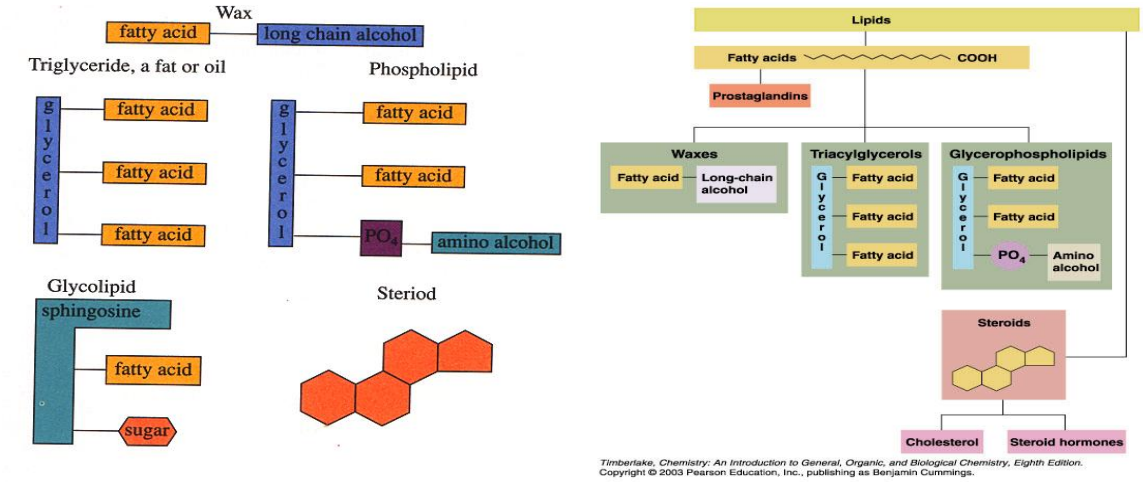
LIPIDS

CONTENTS

1. Lipids: Definition,
2. classification,
3. biological functions;
4. biosynthesis of long chain fatty acids,
5. degradation of fatty acids - oxidation of fatty acids.

LIPIDS

- **Lipids Classification**



- Lipids: A variety of naturally occurring organic compounds classified together on the basis of common solubility properties.
    - insoluble in water.
    - soluble in aprotic organic solvents including diethyl ether, dichloromethane and acetone.
- family of biochemicals that are soluble in organic solvents but not in water
- **Classes**
    - Waxes: fatty acid + a long-chain alcohol
    - Triglycerides (fats & oils): glycerol + 3 fatty acids
    - Phospholipids: glycerol + 2 fatty acids + phosphate + amino alcohol
    - Glycolipids: glycerol or spinogosine + fatty acid + monosaccharide
    - Steroids: 3 cyclohexanes + 1 cyclopentane fused together
- Fatty Acids
    - Fatty acids: long-chain carboxylic acids
    - Form fats and oils (usually have an even number of carbons)
    - Saturated fatty acid: all single bonds

- Unsaturated fatty acid: at least 1 double bond
  - Polyunsaturated: two or more double bonds
  - Most double bonds are *cis*- bonds that cause a bend in the chain

- **Properties**
  - Saturated fatty acids are solid at room temperature and have a high melting point
  - Unsaturated fatty acids are liquid at room temperature and have a low melting point
- Fatty Acids in the Human body
  - Essential fatty acids: polyunsaturated fatty acids that the body can not make
    - Important: linoleic and linolenic acids
    - Lack of in infants cause skin dermatitis
  - Arachidonic acid is converted into prostaglandins which serve as local chemical messengers
- Fats and Oils
  - Fats: 509% or more saturated fatty acids; solid at room temperature
  - Oils: more unsaturated fatty acids; liquid at room temperature
  - Fats and Oils
- Properties:
  - Hydrogenation (adding H_2)
    - Converts a double bond to a single bond
    - Changes liquid oil to a soft semisolid fat
  - Oxidation to carboxylic acid
    - Fat/oil becomes “rancid” with a disagreeable odor
      - Some oils have antioxidants to slow oxidation
      - Refrigeration in an air-tight container slows oxidation

- Microorganisms oxidize body oils during exercise

- Saponification with heat and strong base produces a glycerol and salts of the fatty acids
- Hydrolysis (breakdown)
  - Requires a strong acid catalyst or lipase enzyme
  - Produces 1 glycerol + 3 fatty acids

- Phospholipids

  alcohol + phosphate +fatty acid

  - Glycerophospholid: glycerol + 2 fatty acids + phosphate/amino alcohol
    - Lecithins and cephalins abundant in brain & nerve tissue, egg yolks, wheat germ, and yeast
    - Forms cell membranes
  - Sphingolipid: sphingosin + fatty acid + phosphate/amino alcohol
    - A phospholipid with sphingosin instead of the glycerol
    - Important in the myelin sheath that surrounds most nerve fibers
  - Glycolipid: glycerol + 1 fatty acid + sugar
    - In cell membranes
    - Function in cell adhesion & self-identity markers

- **Lipidoses, lipid diseases**
  - Excess accumulation of sphingolipid or glycolipid
  - Caused by an absent, mismade, or deficient enzyme that breaks down the lipid

- Steroids
  - Cholesterol
    - Synthesized in the liver

- Gives strength to cell membranes
- In myelin sheath, bile salts, vitamin D produced by the skin
- Excess in body leads to gall stones and plaque in the arteries
- Steroids

- Bile salts
  - Synthesized from cholesterol in the liver
  - Sent to the digestive tract to emulsify fats
  - Promotes absorption of cholesterol in the digestive tract
- Fat-soluble vitamins: A, D, E, K
  - A for night vision
  - D promotes absorption of Ca^{+} in intestinal tract and deposition of Ca^{+} in the bones
  - E prevents oxidation of unsaturated fatty acids in cell membranes
  - K functions in blood cloting
- Hormones:
  - Sex hormones control reproduction, sexual characteristics, and general growth
  - Adrenal corticosteroids
    - Cortisone increases blood glucose levels and reduces inflammation
    - Aldosterone acts in the kidneys to maintain Na^{+} and water balance
    - Sex hormones
- Anabolic steroid, derivative of testosterone
  - Prevents breakdown of worn out muscle cells
  - Has dangerous side effects

- Lipids in the Body
- Lipoproteins
  - Water insoluble lipids must be delivered to all parts of the body by the water-based blood
  - Body packages lipids in membrane with proteins to make the lipids water soluble
- Types: VLDL, LDL, HDL, chylomicrons
  - HDL on the way to the liver is "good cholesterol"
  - LDL on way to the cells is prone to deposit in blood vessels
  - Enzyme in blood capillaries releases lipids from the protein carrier

LIPID BIOLOGICAL FUNCTIONS

Chemical messengers

- All multicellular organisms use chemical messengers to send information between organelles and to other cells. Since lipids are small molecules insoluble in water, they are excellent candidates for signalling. The signalling molecules further attach to the receptors on the cell surface and bring about a change that leads to an action.
- The signalling lipids, in their esterified form can infiltrate membranes and are transported to carry signals to other cells. These may bind to certain proteins as well and are inactive until they reach the site of action and encounter the appropriate receptor.

Storage and provision of energy

- Storage lipids are triacylglycerols. These are inert and made up of three fatty acids and a glycerol.
- Fatty acids in non esterified form, i.e. as free (unesterified) fatty acids are released from triacylglycerols during fasting to provide a source of energy and to form the structural components for cells.
- Dietary fatty acids of short and medium chain size are not esterified but are oxidized rapidly in tissues as a source of 'fuel".

- Longer chain fatty acids are esterified first to triacylglycerols or structural lipids.

Maintenance of temperature

- Layers of subcutaneous fat under the skin also help in insulation and protection from cold. Maintenance of body temperature is mainly done by brown fat as opposed to white fat. Babies have a higher concentration of brown fat.

Membrane lipid layer formation

- Linoleic and linolenic acids are essential fatty acids. These form arachidonic, eicosapentaenoic and docosahexaenoic acids. These for membrane lipids.
- Membrane lipids are made of polyunsaturated fatty acids. Polyunsaturated fatty acids are important as constituents of the phospholipids, where they appear to confer several important properties to the membranes. One of the most important properties are fluidity and flexibility of the membrane.

Cholesterol formation

- Much of the cholesterol is located in cell membranes. It also occurs in blood in free form as plasma lipoproteins. Lipoproteins are complex aggregates of lipids and proteins that make travel of lipids in a watery or aqueous solution possible and enable their transport throughout the body.
- The main groups are classified as chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), based on the relative densities
- Cholesterol maintains the fluidity of membranes by interacting with their complex lipid components, specifically the phospholipids such as phosphatidylcholine and sphingomyelin. Cholesterol also is the precursor of bile acids, vitamin D and steroidal hormones.

The "fat-soluble" vitamins

- The "fat-soluble" vitamins (A, D, E and K) are essential nutrients with numerous functions.
- Acyl-carnitines transport and metabolize fatty acids in and out of mitochondria.

- Polyprenols and their phosphorylated derivatives help on transport of molecules across membranes.
- Cardiolipins are a subtype of glycerophospholipids with four acyl chains and three glycerol groups. They activate enzymes involved with oxidative phosphorylation.

FATTY ACID SYNTHESIS

Fatty Acid Synthesis

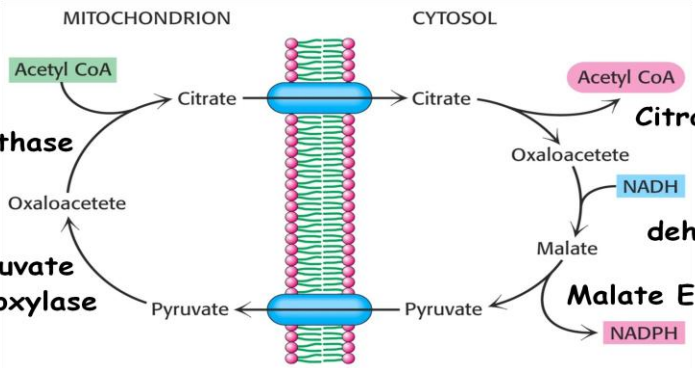
- In mammals fatty acid synthesis occurs primarily in the liver and adipose tissues
- Also occurs in mammary glands during lactation.
- Fatty acid synthesis and degradation go by different routes
- There are four major differences between fatty acid breakdown and biosynthesis

The differences between fatty acid biosynthesis and breakdown

- Intermediates in synthesis are linked to -SH groups of acyl carrier proteins (as compared to -SH groups of CoA)
- Synthesis in cytosol; breakdown in mitochondria
- Enzymes of synthesis are one polypeptide
- Biosynthesis uses NADPH/$NADP^+$; breakdown uses NADH/NAD^+

Fatty Acid Synthesis Occurs in the Cytosol

- Must have source of acetyl-CoA
- Most acetyl-CoA in mitochondria
- Citrate-malate-pyruvate shuttle provides cytosolic acetate units and reducing equivalents for fatty acid synthesis



- Fatty acids are built from 2-C units derived from acetyl-CoA
- Acetate units are activated for transfer to growing FA chain by conversion to malonyl-CoA
- Decarboxylation of malonyl-CoA and reducing power of NADPH drive chain growth
- Chain grows to 16-carbons (eight acetyl-CoAs)
- Other enzymes add double bonds and more Cs

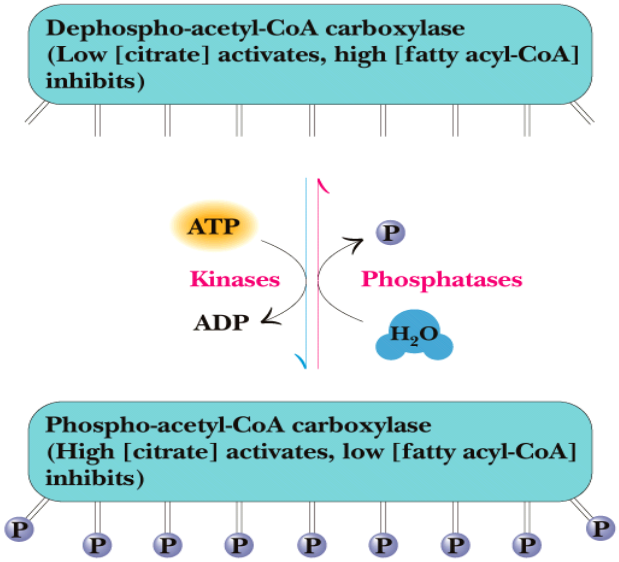
Acetyl-CoA Carboxylase

$$\textbf{Acetyl-CoA + } HCO_3^- \textbf{ + ATP} \rightarrow \textbf{malonyl-CoA + ADP}$$

- The "ACC enzyme" commits acetate to fatty acid synthesis
- Carboxylation of acetyl-CoA to form malonyl-CoA is the irreversible, committed step in fatty acid biosynthesis

Regulation of Acetyl-CoA Carboxylase (ACCase)

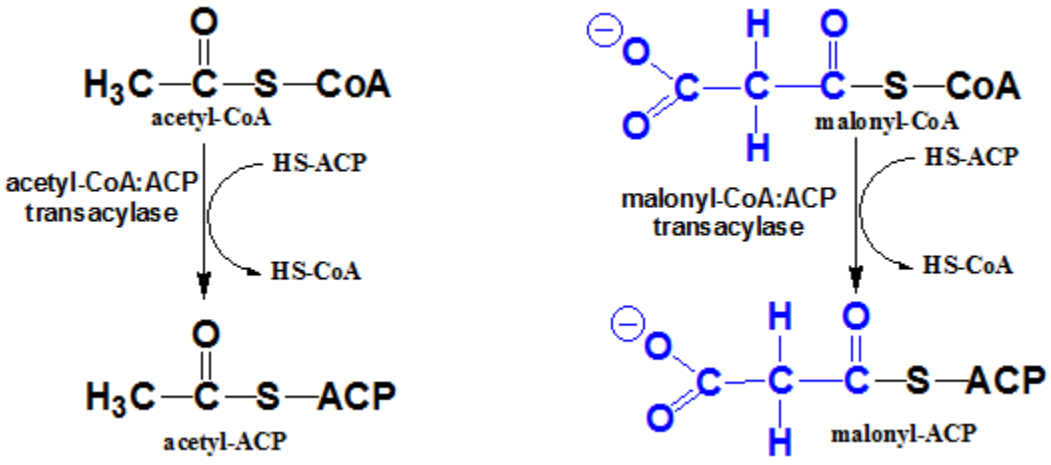
- Unphosphorylated ACCase has low K_m for citrate and is active at low citrate
- Unphosphorylated ACCase has high K_i for palmitoyl-CoA and needs high palmitoyl-CoA to inhibit
- Phosphorylated E has high K_m for citrate and needs high citrate to activate
- Phosphorylated E has low K_i for palmitoyl-CoA and is inhibited at low palmitoyl-CoA



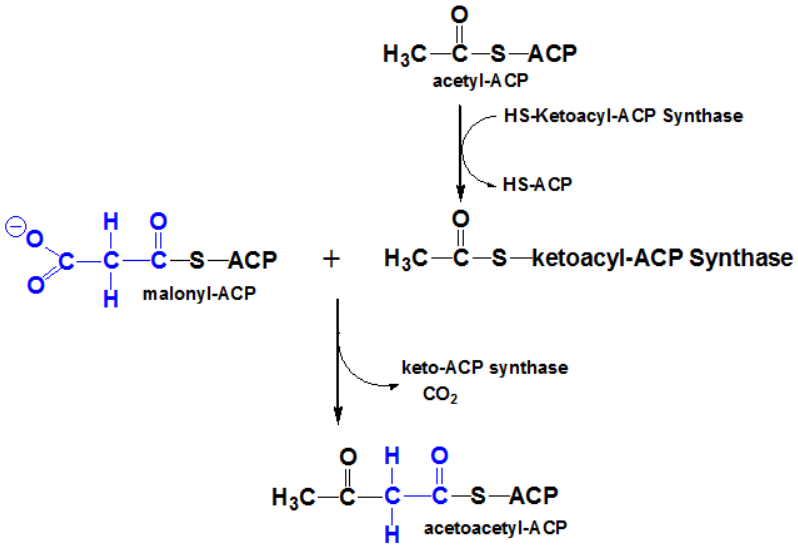
Fatty Acid Synthesis

- Step 1: Loading – transferring acetyl- and malonyl- groups from CoA to ACP
- Step 2: Condensation – transferring 2 carbon unit from malonyl-ACP to acetyl-ACP to form 2 carbon keto-acyl-ACP
- Step 3: Reduction – conversion of keto-acyl-ACP to hydroxyacyl-ACP (uses NADPH)
- Step 4: Dehydration – Elimination of H_2O to form Enoyl-ACP
- Step 5: Reduction – Reduce double bond to form 4 carbon fully saturated acyl-ACP

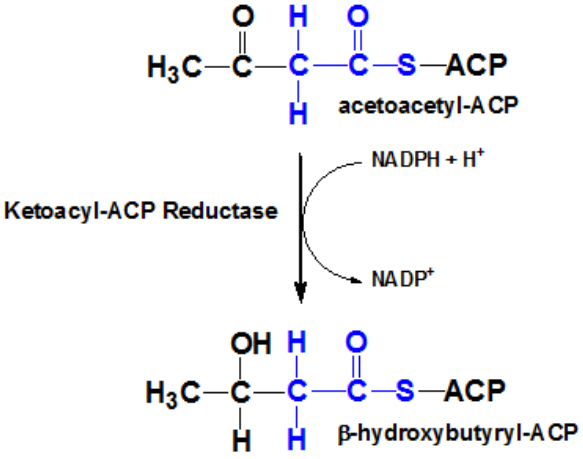
Step 1: Loading Reactions



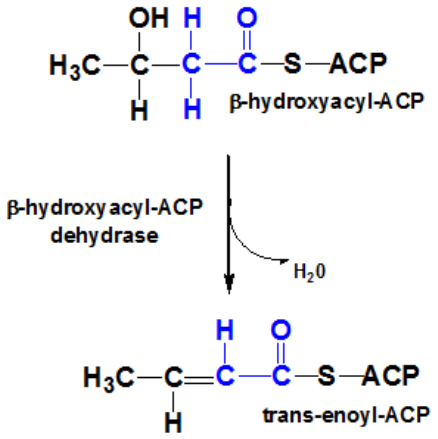
Step 2: Condensation Rxn



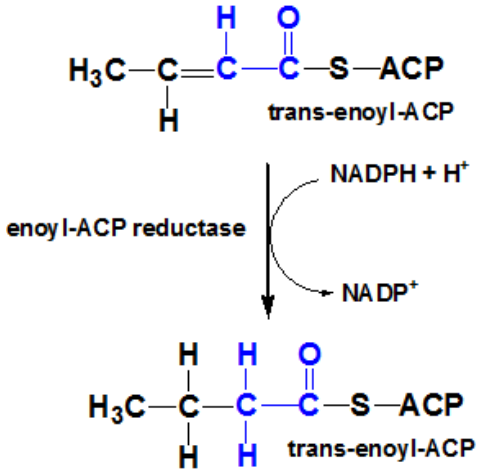
Step 3: Reduction



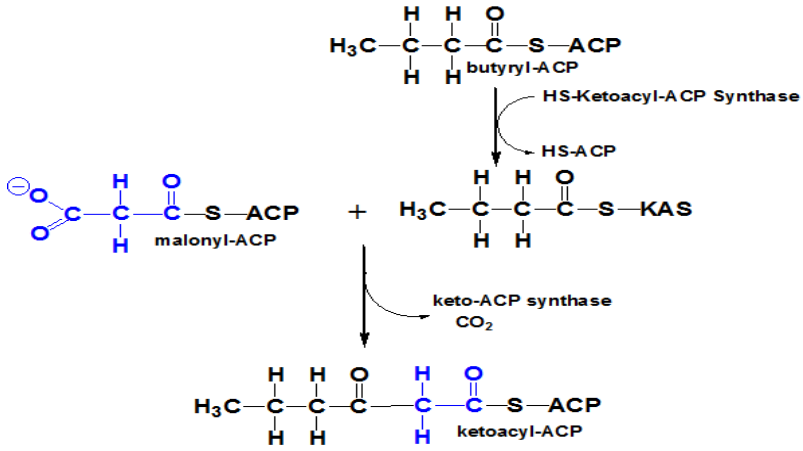
Step 4: Dehydration



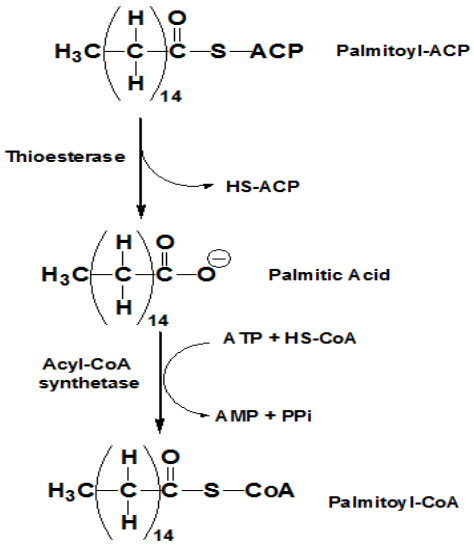
Step 5: Reduction



Step 6: next condensation



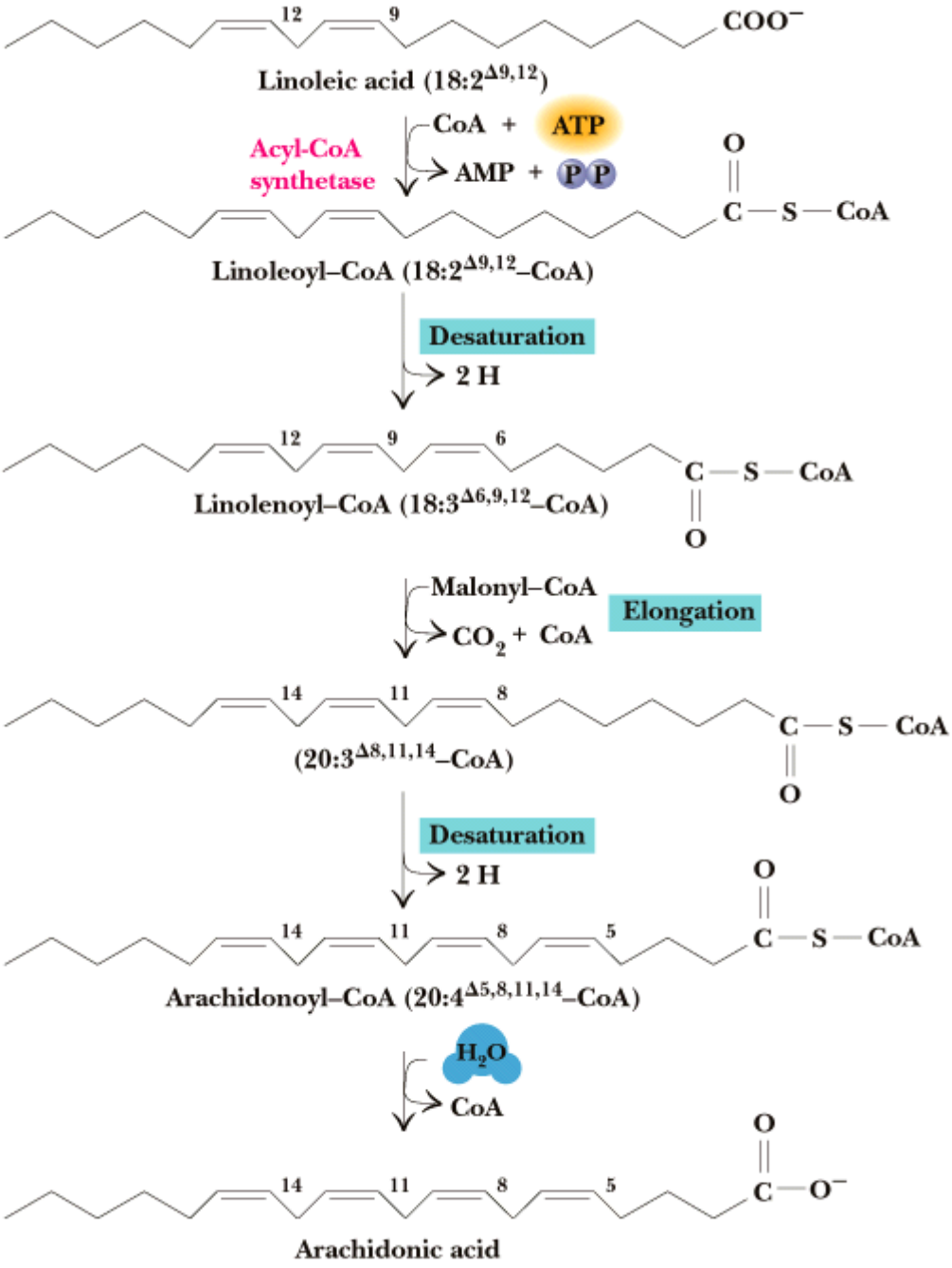
Termination of Fatty Acid Synthesis



Organization of Fatty Acid Synthesis Enzymes

- In bacteria and plants, the fatty acid synthesis reactions are catalyzed individual soluble enzymes.
- In animals, the fatty acid synthesis reactions are all present on multifunctional polypeptide.
- The animal fatty acid synthase is a homodimer of two identical 250 kD polypeptides.

Further Processing of Fatty acids: Desaturation and Elongation



FATTY ACID OXIDATION

FUNCTIONS OF FATTY ACIDS

Fatty acids have four major physiological roles.

1) Fatty acids are **building blocks of phospholipids and glycolipids.**
2) Many proteins are modified by the **covalent attachment of fatty acids, which target them to membrane locations**
3) Fatty acids are **fuel molecules**. They are stored as triacylglycerols. Fatty acids mobilized from triacylglycerols are oxidized to meet the energy needs of a cell or organism.
4) Fatty acid **derivatives serve as hormones and intracellular messengers** e.g. steroids, sex hormones and prostaglandins.

TRANSPORTATION OF FREE FATTY ACIDS

- Free fatty acids—also called unesterified (UFA) or nonesterified (NEFA) fatty acids—are fatty acids that are in the **unesterified state.**
- In plasma, longer-chain FFA are combined with **albumin,** and in the cell they are attached to a **fatty acid-binding protein.**
- **Shorter-chain fatty acids are more water- soluble and exist as the un-ionized acid or as a fatty acid anion.**
- By these means, free fatty acids are made accessible as a fuel in other tissues.

TYPES OF FATTY ACID OXIDATION

Fatty acids can be oxidized by-

1) **Beta oxidation-** Major mechanism occurs in the mitochondria matrix. 2-C units are released as acetyl CoA per cycle.
2) **Alpha oxidation-** Predominantly takes place in brain and liver, one carbon is lost in the form of CO2 per cycle.
3) **Omega oxidation-** Minor mechanism, but becomes important in conditions of impaired beta oxidation
4) **Peroxisomal oxidation-** Mainly for the trimming of very long chain fatty acids.

BETA OXIDATION

Overview of beta oxidation

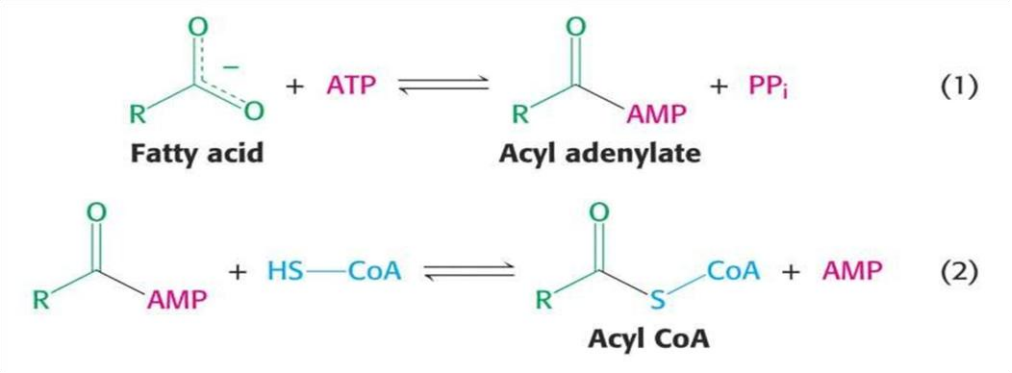
A saturated acyl Co A is degraded by a recurring sequence of four reactions:

1) **Oxidation** by flavin adenine dinucleotide (FAD)
2) **Hydration,**
3) **Oxidation** by NAD^+, and
4) **Thiolysis** by Co ASH

- The fatty acyl chain is shortened by two carbon atoms as a result of these reactions,
- FADH2, NADH, and acetyl Co A are generated.
- Because oxidation is on the β carbon and the chain is broken between the α (2)- and β (3)-carbon atoms—hence the name – β oxidation .

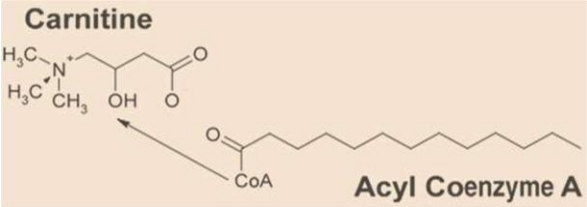
ACTIVATION OF FATTY ACIDS

Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the only step in the complete degradation of a fatty acid that requires energy from ATP. The activation of a fatty acid is accomplished in two steps-



TRANSPORT OF FATTY ACID IN TO MITOCHONDRIAL MATRIX

- Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix.
- Activated long-chain fatty acids are transported across the membrane by conjugating them to *carnitine*, a zwitterionic alcohol.



ROLE OF CARNITINE

1) The acyl group is to the hydroxyl group of carnitine to form *acyl carnitine*. This reaction is catalyzed by ***carnitine acyl transferase I***

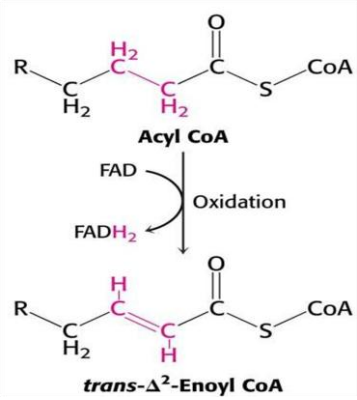
2) Acyl carnitine is then shuttled across the inner mitochondrial membrane by a **translocase.**

3) The acyl group is transferred back to CoA on the matrix side of the membrane. This reaction, which is catalyzed by ***carnitine acyl transferase II.***

Finally, the translocase returns carnitine to the cytosolic side in exchange for an incoming acyl carnitine

STEPS OF BETA OXIDATION

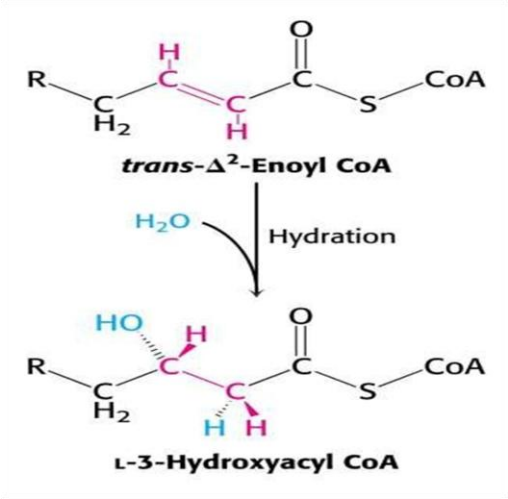
Step-1 Dehydrogenation- The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)- carbon atoms, catalyzed by **acyl- CoA dehydrogenase** and requiring FAD. This results in the formation of Δ^2-*trans*- enoyl-CoA and FADH .



- Electrons from the FADH2 prosthetic group of the reduced acyl CoA dehydrogenase are transferred to *electron- transferring flavoprotein* (ETF).
- ETF donates electrons to *ETF: ubiquinone reductase,* an iron-sulfur protein.
- Ubiquinone is thereby reduced to ubiquinol, which delivers its high-potential electrons to the second proton-pumping site of the respiratory chain

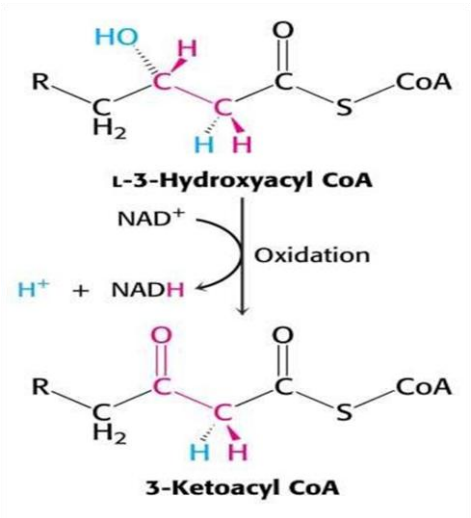
Step-2- Hydration:

Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalyzed by Δ^{2}-enoyl-CoA hydratase.



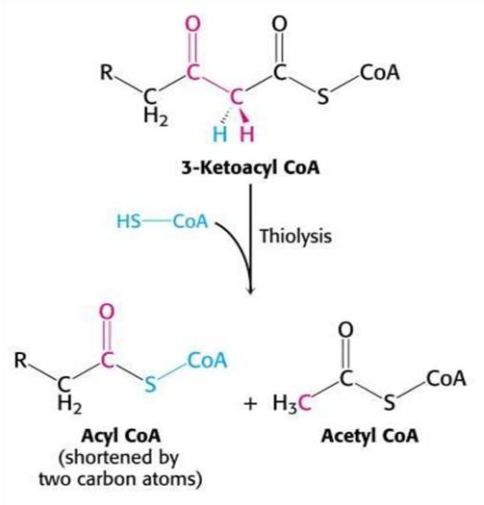
Step-3- dehydrogenation-

The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalyzed by L(+)-3-hydroxyacyl- CoA dehydrogenase to form the corresponding 3-ketoacyl-CoA compound. In this case, NAD^{+} is the coenzyme involved.

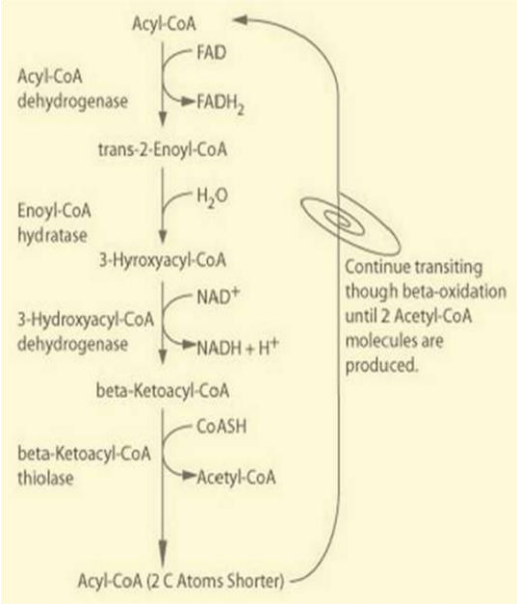


Step-4- Thiolysis-

3-ketoacyl-CoA is split at the 2,3- position by thiolase (3- ketoacyl-CoA- thiolase), forming acetyl-CoA and a new acyl-CoA two carbons shorter than the original acyl-CoA molecule.



- The acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2.
- Since acetyl- CoA can be oxidized to CO_2 and water via the citric acid cycle the complete oxidation of fatty acids is achieved



BETA OXIDATION

The overall reaction can be represented as follows-

$$C_n\text{-acyl CoA} + FAD + NAD^+ + H_2O + CoA \longrightarrow C_{n-2}\text{-acyl CoA} + FADH_2 + NADH + \text{acetyl CoA} + H^+$$

BETA OXIDATION- ENERGY YIELD

Energy yield by the complete oxidation of one mol of Palmitic acid-

The degradation of palmitoyl CoA (C16-acyl Co A) requires seven reaction cycles. In the seventh cycle, the C4-ketoacyl CoA is thiolyzed to two molecules of acetyl CoA.

$$\text{Palmitoyl CoA} + 7\,FAD + 7\,NAD^+ + 7\,CoA + 7\,H_2O \longrightarrow 8\,\text{acetyl CoA} + 7\,FADH_2 + 7\,NADH + 7\,H^+$$

106 (129 As per old concept) ATP are produced by the complete oxidation of one mol of Palmitic acid.

2.5 ATPs per NADH = 17.5
1.5 ATPs per FADH2 = 10.5
10 ATPs per acetyl-CoA = 80
Total = 108 ATPs
2 ATP equivalents (ATP — AMP + PPi
PPi — 2 Pi)
consumed during activation of palmitate to Palmitoyl CoA
Net Energy output- 108-2 = 106 ATP

18BEBME305 - BIOCHEMISTRY

UNIT – V

METHODS OF INVESTIGATION OF METABOLISM

CONTENTS

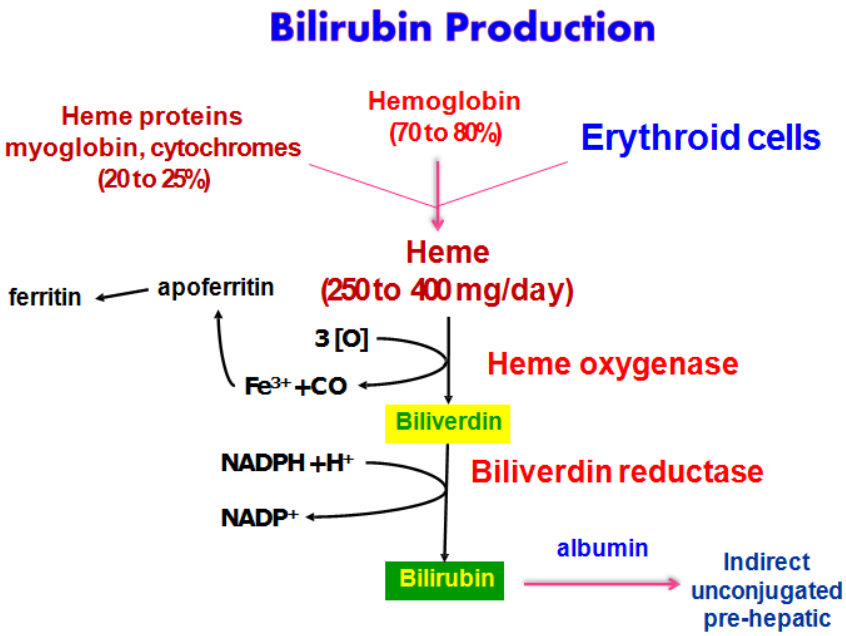
1. Liver function tests,
2. Real function tests,
3. Gastric function tests.
4. Diagnostic tools: Principles and applications of photometry,
5. Diagnostic tools: Principles and applications of spectrophotometry,
6. Diagnostic tools: Principles and applications of flurometry,
7. Diagnostic tools: Principles and applications of flame photometry,
8. Automation in clinical laboratory.
9. Uses of isotopes in biochemistry.

LIVER FUNCTION TESTS

- The liver is the largest organ in the body.
- It is located below the diaphragm in the right upper quadrant of the abdominal cavity.
- The working cells of the liver are known as hepatocytes.

Functions of liver

1. Metabolic function:
    - Liver actively participates in carbohydrate, lipid, protein, mineral & vitamin metabolisms.
2. Excretory function:
    - Bile pigments, bile salts & cholesterol are excreted in bile into intestine.
3. Hematological function:
    - Liver participates in formation of blood (particularly in embryo)
    - Liver is also produces clotting factors like factor V, VII.
    - Fibrinogen involved in blood coagulation is also synthesized in liver.
    - It synthesize plasma proteins & destruction of erythrocytes.
4. Storage function:
    - Glycogen, vitamins A, D & B12 & trace element iron are stored in liver.
    - Protective function & detoxification:
    - Ammonia is detoxified to urea.
    - kupffer cells of liver perform phagocytosis to eliminate foreign compounds.
    - Liver is responsible for the metabolism of xenobiotics.



Plasma bilirubin

- Normal plasma bilirubin: 0.2–0.8 mg/dl.
- Unconjugated bilirubin: 0.2–0.6 mg/dl.
- Conjugated bilirubin: 0–0.2 mg/dl.
- If the plasma bilirubin level exceeds 1mg/dl, the condition is called hyperbilirubinemia.
- Levels between 1 & 2 mg/dl are indicative of latent jaundice.
- When the bilirubin level exceeds 2 mg/dl, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin & mucous membrane resulting in jaundice.

Van den bergh test for bilirubin

- It is a specific test for for identificaion of increased serum bilirubin levels.
- Normal serum gives a negative van den Bergh reaction.

ž **Mechanism of the reaction:**

ž Van den Bergh reagent is a mixture of equal volumes of sulfanilic acid (in dilute HCI)& sodium nitrite.

ž **Principle:**

ž Diazotised sulfanilic acid reacts with bilirubin to form a purple coloured azobilirubin.

ž

ž **Direct and indirect reactions:**

- Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble.
- Van den Bergh reagent reacts with conjugated bilirubin & gives a purple colour immediately (normally within 30 seconds.
- This is direct positive van den Bergh reaction.
- Addition of methanol (or alcohol) dissolves the unconjugated bilirubin & gives the van den Bergh reaction (normally within 30 minutes) positive.
- This is indirect positive.
- lf the serum contains both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensified by the addition of alcohol (indirect positive).
- This type of reaction is known as biphasic.

ž **Major liver function tests may be classified as follows**

1. Tests based on excretory function – Measurement of bile pigments, bile salts, bromosulphthalein.
2. Tests based on serum enzymes derived from liver - Determination of transaminases, alkaline phosphatase, 5'-nucleotidase, γ – glutamyltranspeptidase.
3. Tests based on metabolic capacity – Galactose tolerance, antipyrine clearance.
4. Tests based on synthetic functions – Prothrombin time, serum albumin.
5. Tests based on detoxification - Hippuric acid synthesis.

ž **Tests based on excretory function**

Bromosulphthalein (BSP) test:

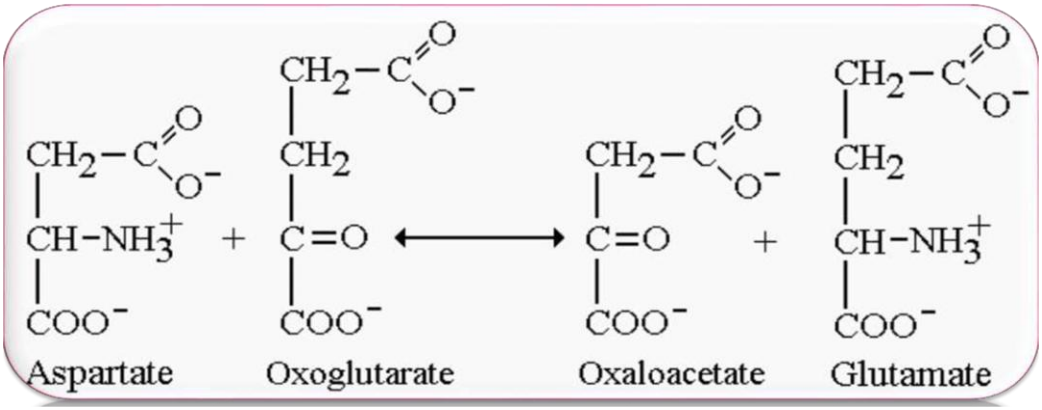
- Bromosulphthalein is a dye used to assess the excretory function of liver.
- It is a non-toxic compound & almost exclusively excreted by the liver (through bile).
- BSP is administered intravenously (5 mg/kg body weight) & its serum concentration is measured at 45 min & at 2 hrs.
- In normal individuals, <5% of the dye is retained at the end of 45 min.
- Any impairment in liver function causes an increased retention of the dye.
- This test is quite sensitive to assess liver abnormality with particular reference to excretory function.

Tests based on serum enzymes derived from liver

Serum enzymes

- A large number of enzyme estimations are available which are used to ascertain liver function.
- They are be divided into two groups:
- Most commonly & routinely done in the laboratory.
- AST & ALT

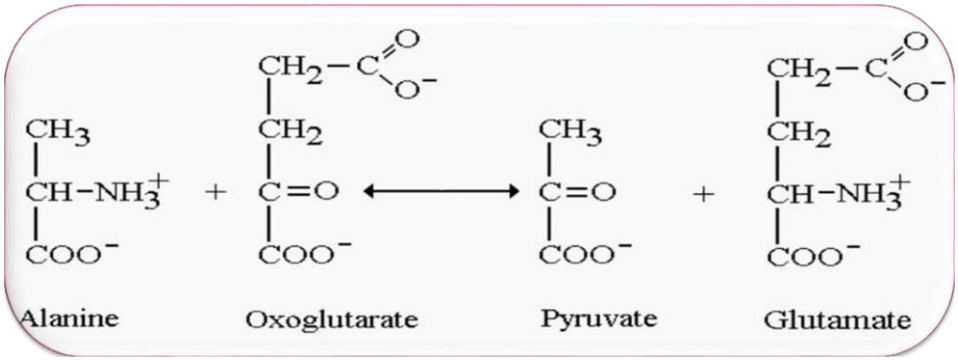
ž **Aspartate transaminase:**



- Normal range: 10-45 U/L.
- AST is found in both cytoplasm & mitochondria
- AST/GOT also reflects damage to the hepatic cells & is less specific for liver disease.
- It can also be released with heart, muscle & brain disorders.
- AST help diagnose various heart, muscle or brain disorders, such as a myocardial infarct (heart attack).

ž **Alanine transaminase**

- ALT is a cytoplasmic enzyme.
- Normal Range: 5-40 U/L.



ž Alanine phosphatase

- ALP occurs in in all tissues, especially liver, bone. Bile duct, kidney & the placenta.
- The ALP used to help diagnose certain liver diseases and bone disorders.
- Normal range: 30 - 95 IU/L (3-13 kings unit)
- ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides & proteins.
- Most effective in an alkaline environment.
- Levels are significantly higher in growing children.
- A rise in serum ALP (normal 3-13 KA units/dl), usually associated with elevated serum bilirubin is an indicator of biliary obstruction (obstructive/posthepatic jaundice).
- ALP is also elevated in cirrhosis of liver & hepatic tumors.

Gama glutamyl transpeptidase

- This is a microsomal enzyme widely distributed in body tissues, including liver.
- Measurement of γ – glutamyl transpeptidase (GGT) activity provides a sensitive index to asses liver abnormality.
- The activity of this enzyme almost parallels that of transaminases in hepatic damage.
- Normal range: 10-15 U/L
- Serum GGT is highly elevated in biliary obstruction & alcoholism.
- Several drugs (e.g. phenytoin) induce (liver synthesis) & increase this enzyme in circulation.

5'Nucleotidase

- Normal range: 2-15 U/L
- The serum activity of 5'-nucleotidase is elevated in hepatobiliary disease & this parallels ALP.
- The 5'-nucleotidase is not altered in bone disease (as is the case with ALP).

ž

ž **Tests based on metabolic capacity**

ž **Galactose tolerance:**

- Galactose is almost exclusively metabolized by the liver.
- The liver function can be assessed by measuring the utilization of galactose.
- The subject is given intravenous administration of galactose (about 300 mg/kg body weight).
- Blood is drawn at 10 minute intervals for the next 2 hours & galactose estimated.
- In the normal individuals, the half-life of galactose is about 10-15 minutes.
- This is markedly elevated in hepatocellular damage (infective hepatitis, cirrhosis).

ž **Tests based on synthetic functions**

ž **Serum albumin:**

- Albumin is solely synthesized by the liver.
- It has a half-life of about 20-25 days.
- It is a good marker to assess chronic (& not acute) liver damage.
- Low serum albumin is commonly observed in patients with severe liver damage.
- Albumin is also decreased in malnutrition.
- Functional impairment of liver is frequently associated with increased synthesis of globulins.
- Cirrhosis of the liver causes a reversal of albumin/globulin ratio (A/G ratio).
- Serum electrophoresis of proteins reveals increased albumin & decreased γ -globulin concentrations.

Prothrombin time

- The liver synthesizes all the factors concerned with blood clotting.
- A decrease in the concentration of plasma clotting factors is found in the impairment of liver function.
- Prothrombin time is prolonged in patients with liver damage, compared to normal.
- It generally falls 10 - 15 seconds.

ž **Tests based on detoxification**

- The liver is the major site for the metabolism of xenobiotics (detoxification).
- Measurement of hippuric acid synthesis is an ideal test for assessing the detoxification function of liver.
- Hippuric acid is produced in the liver when benzoic acid combines with glycine.

- About 6 g of sodium benzoate (dissolved in about 250 ml water) is orally given to the subject, after a light breakfast (usually 2 hrs later) & after emptying the bladder.
- Urine collections are made for the next 4 hours & the amount of hippuric acid excreted is estimated.
- A reduction in hippuric acid excretion (particularly <3 g) indicates hepatic damage.

RENAL FUNCTION TEST

Functions of kidney

- Maintenance of homeostasis
- Excretion of metabolic waste products
- Retention of substances vital to body
- Hormonal functions
- Erythropoietin
- 1,25-Dihydroxycholecalciferol (calcitriol)
- Renin

Formation of urine

- Nephron is the functional unit of kidney.
- Each kidney is composed of approximately one million nephrons.
- Nephron, consists of a Bowman's capsule (with blood capillaries), proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) & collecting tubule.
- The blood supply to kidneys is relatively large.
- About 1200 ml of blood (650 ml plasma) passes through the kidneys, every minute.
- About 120-125 ml is filtered per minute by the kidneys & this is referred to as glomerular filtration rate (GFR).
- With a normal GFR (120-125 ml/min), the glomerular filtrate formed in an adult is about 175-180 litres/day, out of which only 1.5 litres is excreted as urine.
- More than 99% of the glomerular filtrate is reabsorbed by the kidneys.
- Urine formation basically involves two steps- glomerular filtration & tubular reabsorption.

Kidney function tests may be divided into 4 groups.

1. **Glomerular function tests:**

   All the clearance tests (inulin, creatinine, urea) are included in this group.

2. **Tubular function tests:**

   Urine concentration or dilution test, urine acidification test.

3. **Analysis of blood/serum:**

   Estimation of blood urea, serum creatinine, protein & electrolyte are useful to assess renal function.

4. **Urine examination:**

   Routine examination of urine - volume, pH, specific gravity, osmolality & presence of certain abnormal constituents (proteins, blood, ketone bodies, glucose etc).

Clearance test

- Clearance is defined as the volume of plasma that would be completely cleared of a substance per minute.
- In other words, clearance of a substance refers to the milliliters of plasma which contains the amount of that substance excreted by kidney per minute.

$$C = \frac{U \times V}{P}$$

- U = Concentration of the substance in urine, V = Volume of urine in ml excreted per minute, P = Concentration of the substance in plasma.
  - The maximum rate at which the plasma can be cleared of any substance is equal to the GFR.
  - This can be calculated by measuring the clearance of a plasma compound which is freely filtered by the glomerulus & is neither absorbed nor secreted in the tubule.
  - Inulin (a plant carbohydrate, composed of fructose units) and ^{51}Cr-EDTA satisfy this criteria.
  - Inulin is intravenously administered to measure GFR.

Creatinine clearance test

- Creatinine is an excretory product derived from creatine phosphate.
- The excretion of creatinine is rather constant & is not influenced by body metabolism or dietary factors.
- Creatinine is filtered by the glomeruli & only marginally secreted by the tubules.

- Creatinine clearance may be defined as the volume (ml) of plasma that would be completely cleared of creatinine per minute.

ž

ž

ž **Procedure:**

- In the traditional method, creatinine content of a 24 hr urine collection & the plasma concentration in this period are estimated.

The creatinine clearance (C) can be calculated as follows:

$$C = \frac{U \times V}{P}$$

ž U = Urine concentration of creatinine, V = Urine output in ml/min (24 hr urine volume divided by 24 x 60), P = Concentration of creatinine.

ž **Reference values:**

- The normal range of creatinine clearance is around 120-145 ml/min.
- These values are slightly lower in women.
- Serum creatinine normal range:
- Adult male: 0.7-1.4 mg/dl
- Adult female: 0.6-1.3 mg/dl
- Children: 0.5-1.2 mg/dl

Urea clearance test

- Urea is the end product of protein metabolism.
- After filtered by the glomeruli, it is partially reabsorbed by the renal tubules.
- Urea clearance is less than the GFR & it is influenced by the protein content of the diet.
- Urea clearance is not as sensitive as creatinine clearance.
- Urea clearance is defined as the volume (ml) of plasma that would be completely cleared of urea **per minute.**

It is calculated by the formula:

$$Cm = \frac{U \times V}{P}$$

Cm=Maximum urea clearance, U = Urea concentration in urine (mg/dl), V = Urine excreted per minute in ml, P = Urea concentration in plasma.

- If the output of urine is more than 2 ml per minute.
- This is referred to as maximum urea clearance & the normal value is around 75 ml/min.

ž **Standard urea clearance:**

- The urea clearance drastically changes when the volume of urine is less than 2 ml/min.
- This is known as standard urea clearance (C) & the normal value is around 54 ml/min.
    - **Standard urea clearance is calculated by a modified formula**

$$Cs = \frac{U \times \sqrt{V}}{P}$$

Urine concentration test

- This is a test to assess the renal tubular function.
- It is a simple test & involves the accurate measurement of specific gravity which depends on the concentration of solutes in urine.
- A specific gravity of 1.020 in the early morning urine sample is considered to be normal.

Analysis of blood

- Estimation of serum creatinine & blood urea are useful.
- These tests are less sensitive than the clearance tests.
- Serum creatinine is a better indicator than urea.

Urine examination:

- The volume of urine excreted, its pH, specific gravity, osmolality, the concentration of abnormal constituents (such as proteins, ketone bodies, glucose & blood) may help to have some preliminary knowledge of kidney function.

Proteinuria

- Glomerular proteinuria:
- The glomeruli of kidney are not permeable to substances with molecular weight more than 69,000 & plasma proteins are absent in normal urine.

- When glomeruli are damaged or diseased, they become more permeable & plasma proteins may appear in urine.
- The smaller molecules of albumin pass through damaged glomeruli more readily.
- Albuminuria is always pathological.
- Large quantities of albumin are lost in urine in nephrosis.
- Small quantities are seen in urine in acute nephritis, strenuous exercise & pregnancy.

Micro albuminuria

- It is also called minimal albuminuria.
- It is identified, when small quantity of albumin (30-300 mg/day) is seen in urine.
- The test is not indicated in patients with overt proteinuria (+ve dipstick).
- Early morning midstream sample is preferred.
- It is expressed as albumin-creatinine ratio.
- Normal ratio being
- Males < 23 mg/gm of creatinine
- Females < 32 mg/gm of creatinine

Overflow proteinuria

- When small molecular weight proteins are increased in blood, they overflow into urine.
- E.g, hemoglobin having a molecular weight of 67,000 can pass through normal glomeruli & if it exists in free form (as in hemolytic conditions), hemoglobin can appear in urine (hemoglobinuria).

Tubular proteinuria

- This occurs when functional nephrons are reduced, GFR is decreased & remaining nephrons are over-working.
- The tubular reabsorption mechanism is impaired, so low molecular weight proteins appear in urine.
- They are Retinol binding protein (RBP) & α-1 microglobulin.

Nephron loss proteinuria

- In CKD, there is a decrease in the number of functioning nephrons.
- The compensatory rise in glomerular filtration by other nephrons increases the filtered load of proteins.

- Even if there are no glomerular permeability changes, tubular proteinuria is seen.
- This is due to inflammation of lower urinary tract, when proteins are secreted into the tract.
- Accumulation of proteins in tubular lumen can trigger inflammatory reaction.

GASTRIC FUNCTION TEST

- Stomach is a reservoir of ingested foodstuffs.
- It has a great churning ability which promotes digestion.
- Stomach elaborates HCI & proteases (pepsin) which are responsible for the initiation of digestive process.
- The products obtained in the stomach (peptides, amino acids) stimulate the release of pancreatic juice & bile.

Secretion of gastric HCL

- The parietal (oxyntic) cells of gastric glands produce HCL.
- The pH in the gastric lumen is as low as 0.8 (against the blood pH 7.4).
- The protons are transported against concentration gradient by an active process.
- The enzyme K^+ activated ATPase-present in the parietal cells is connected with the mechanism of HCI secretion.

Fractional Test Meal (FTM)

- Fractional test meal involves the collection of stomach contents by Ryle's tube in fasting.
- This is followed by a gastric stimulation, giving a test meal (rice gruel, black coffee etc.).
- The stomach contents are aspirated by Ryle's tube at different time periods (every 15 min for 2 hrs.)
- The samples are analyzed for free & total acidity.
- The results are normally represented by a graph.

Alcohol Test Meal

- In this case, the test meal in the form of 100 ml of 7%alcohol is administered.
- The response to alcohol test meal is more rapid & test time can be reduced to 1½ hour.
- Clear specimens can be collected by this test & the free acidity levels are relatively higher compared to FTM.

Pentagastrin Stimulation Test

- Pentagastrin is a synthetic peptide.
- It stimulates the gastric secretion in a manner similar to the natural gastrin.
- The stomach contents are aspirated by Ryle's tube in a fasting condition.
- This is referred to as residual juice.
- The gastric juice elaborated for the next one hour is collected and pooled which represents the basal secretion.
- Pentagastrin (5mg/kg body weigh) is now given to stimulate gastric secretion.
- The gastric juice is collected at 15 minute intervals for one hour.
- This represents the maximum secretion.
- Each sample of the gastric secretion collected is measured for acidity by titrating the samples with N/10 NaOH to pH 7.4.
- The end point may be detected by an indicator (phenol red) or a pH meter
- Basal acid output (BAO) refers to the acid output (millimol per hour) under the basal conditions i.e. basal secretion.
- Maximal acid output (MAO) represents the acid output (millimol per hour) after the gastric stimulation by pentagastrin i.e. maximum secretion.
- In normal individuals, the BAO is 4-10 mmol/hr while the MAO is 20-50 mmol/hr.

Augmented Histamine Test Meal

- Histamine is a powerful stimulant of gastric secretion.
- The basal gastric secretion is collected for 1 hr.
- Histamine (0.04 mg/kg body weight) is administered subcutaneously & the gastric contents are aspirated for the next one hour (at 15 minute intervals).
- The acid content is measured in all these samples.

Insulin Test Meal

- This is also known as Hollander's test.
- It is mainly done to assess the completeness of vagotomy (vagal resection).

- Insulin (0.1 unit/kg body weight) is administered intravenously, which causes hypoglycemia, usually within 30 minutes, in normal persons.
- lf the vagotomy operation is successful, insulin administration does not cause any increase in the acid output, compared to the basal level.
- This test has to be carefully perfomed, since hypoglycemia is dangerous.

Tubeless Gastric Analysis

- The tubeless gastric analysis involves administration of a cation exchange resin that gets quantitatively exchanged with H^+ ions of the gastric juice.
- The resin is then excreted into urine which can be estimated for an indirect measure of gastric acidity (concentration of H^+ ions).
- Diagnex blue containing azure-A-resin is employed in the tubeless gastric analysis.

Abnormalities of gastric function

- Increased gastric HCI secretion is found in
- Zollinger-Ellison syndrome (a tumor of gastrin secreting cells of the pancreas),
- Chronic duodenal ulcer,
- Gastric cell hyperplasia,
- Excessive histamine production etc.
- A decrease in gastric HCI is observed in
- Gastritis
- Gastric carcinoma
- Pernicious anemia
- Partial gastrectomy
- Chronic iron deficiency anemia.

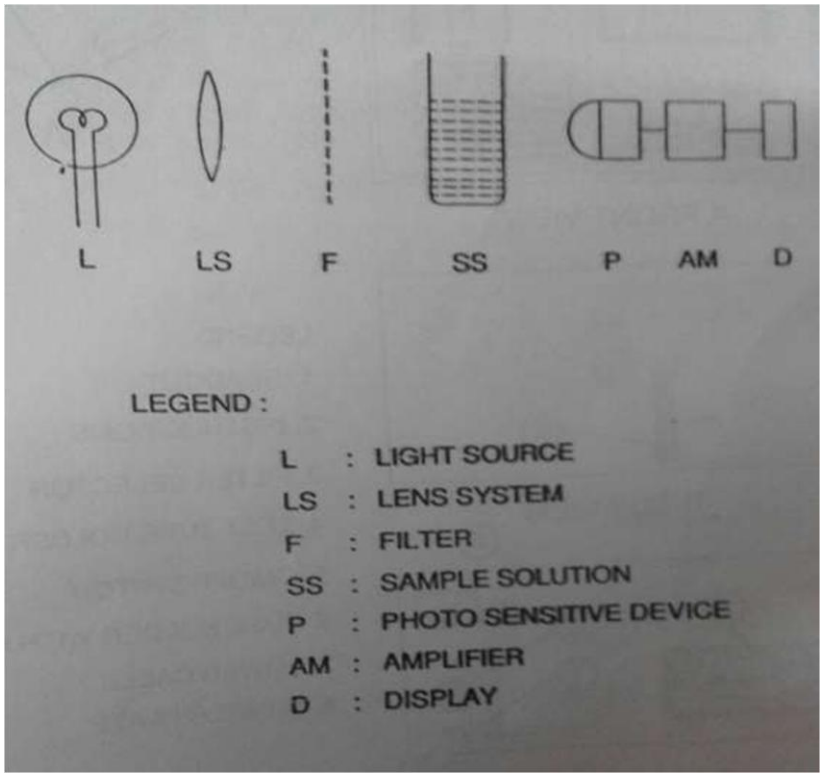
PHOTOMETRY

Principle

- The word is composed of the Greek word photo- "light" and metry – "measure"
- The basis of photometric working is that , the species of alkali metals and alkaline earth metals are dissociated due to the thermal energy provided by the flame source.
- Due to this thermal excitation, some of the atoms are exited to a higher energy level where they are not stable.

- The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques .
- The subsequent loss of energy will result in the movement of exited atoms to the low energy ground state with emission of some radiations, which can be visualized in the visible region of the spectrum.
- The absorbance due to the electron excitation can be measured by using the direct absorption technique while the emititing radiation intensity is measured using the emission techniques .
- The wavelength of emitted light is specific for specific elements .

Principle of operation



Uses of photometry

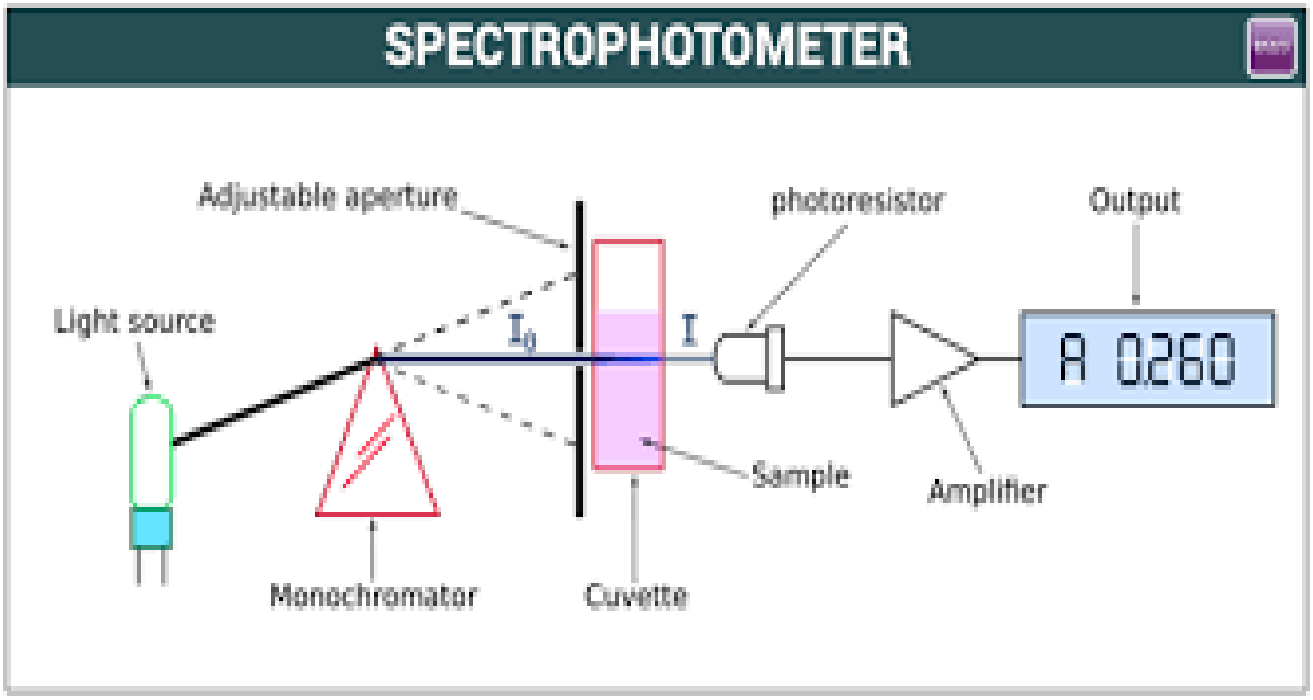
- Photometry is used in various industries like chemicals, soils , agriculture, pharmaceuticals, glass, and ceramics, in plant materials and water , and in biological and microbiological laboratories .
- It is used in determination of potassium, sodium, magnesium and calcium in biological fluids like serum, plasma, urine etc , is routinely carried out by photometer .
- Analysis of industrial water natural water for determining elements responsible for hard water is standard procedure in many laboratories.

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.

Applications

- Qualitative analysis : used to identify classes of compound in both pure state and in biological preparations. This is based on the fact that absorption spectra are specific for a class of compound.
- Denatration of double standard DNA : DNA absorbed at 260 nm. This absorbance increase with rise in temperature , as double standard DNA become single stranded.
- Enzyme assay and kinetic studies: the quantitative assay of enzyme activity is carried out when substrate or product is colored absorbs light in UV range.
- Molecular weight determination: the molecular weight of amines, sugars and many aldehyde and ketone compounds can be determined .
- Control purity : impurity in compound can be detected easily spectrometric studies
- 1, carbon disulfide impurity in carbon tetrachloride can be detected easily by measuring absorbance at 318 nm where only carbon disulfide absorb.
- Protein folding: protein or protein– nucleic acid interaction can be studied.



INSTRUMENTATION

Basic component :

- **Light sources**
- **Monochromatic and filter**
- **Sample holder**
- **Photo detector**

Principle of operation

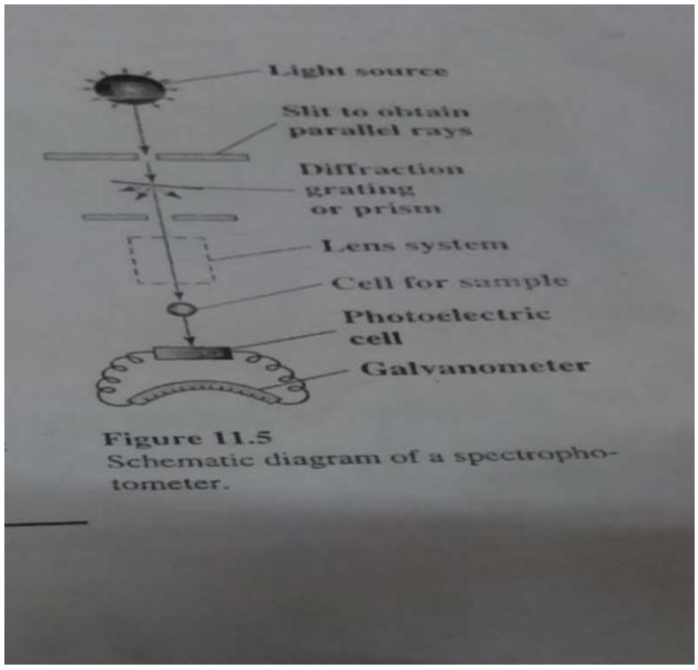


Figure 11.5
Schematic diagram of a spectrophotometer.

Limitation

- Applicable for monochromatic light.
- Applicable lower concentration.
- Applicable only clear solution.

FLUROMETRY

- Absorption of UV/Visible radiation causes transition of electrons from ground state(lowenergy) to excited state (highenergy).
- As excited state is not stable, excess energy is lost by ,
- Collisional deactivation
- Emission of radiation (PhotoLuminescence).

Emission Spectroscopy: Emission of radiation is absorbed. It is called luminescence.

LUMINESCENCE SPECTROSCOPY

The *emission* of radiation from a species after that species has absorbed radiation.

Fluorescence

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

Photo luminiscence:

- Light without heat or coldlight
- Basically of 2 types

Fluorescence: Part of energy is lost due to vibrational transitions and remaining energy is emitted as uv/visible radiation of longer wavelength incident light.

Phosphorescence: Under favorable conditions, excited singlet state undergo transition to triplet state. Emission of radiation when electrons undergo transition from triplet state to ground state.

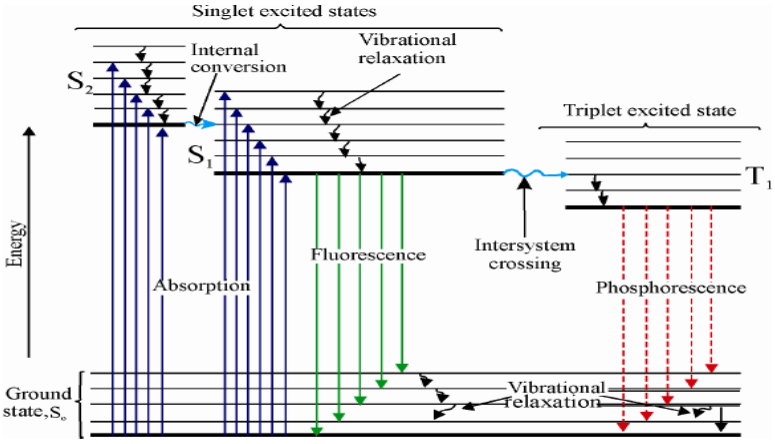
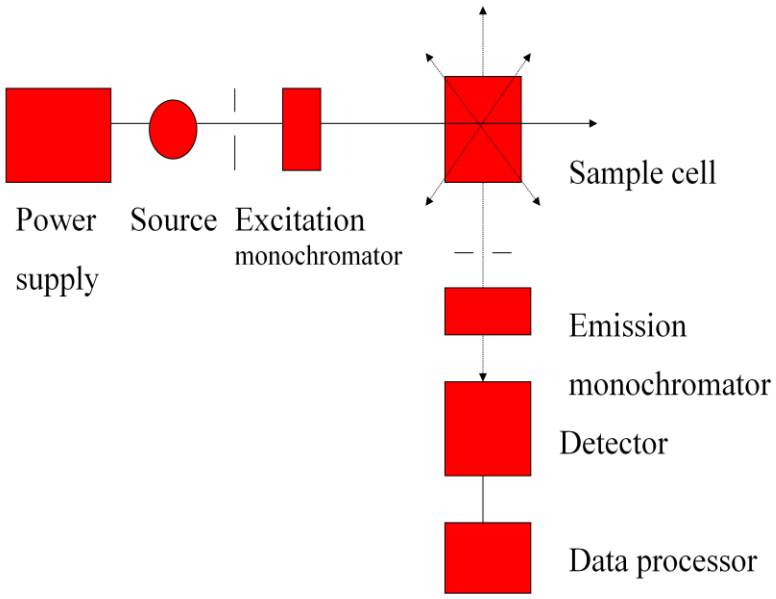


Fig. 5.1: The Jablonski diagram showing the phenomena of fluorescence and phosphorescence

INSTRUMENTATION

- ❖ Source of light
- ❖ Filters and monochromators

- Sample cells
- Detectors



Source of light

- Mercury arc lamp.
- Xenon arc lamp.
- Tungsten lamp.
- Tunable dye lasers.

FILTERS

- Primary filter-absorbs visible light & transmits uv light.
- Secondary filter-absorbs uv radiations & transmits visible light.

MONOCHROMATORS

- Exitation monochromaters-isolates only the radiation which is absorbed by the molecule.
- Emission monochromaters-isolates only the radiation emitted by the molecule.

Sample holder

- The majority of fluorescence assays are carried out in solution.

- Cylindrical or rectangular cells fabricated of silica or glass used.
- Path length is usually 10mm or 1cm.
- All the surfaces of the sample holder are polished in fluorimetry.

Detectors

- Photovoltaic cell
- Photo tube
- Photomultiplier tubes – best and accurate.

Instrument designs

- Single beam fluorimeter
- Double beam fluorimeter
- Spectrofluorimeter(double beam)

Advantages :

- More sensitive when compared to the radiation absorption techniques. Concentrations as low as μg/ml or ng/ml can be determined.
- Precision up to 1% can be achieved easily.
- As both excitation & emission wavelengths are characteristicitis more specific than absorption methods.

Applications

- Determination of Organic substances ,
  - Plant pigments ,steroids ,proteins ,naphthols etc can be determined at low concentrations .
  - Generally used to carry out qualitative as well as quantitative analysis for a grea taromatic compounds present in cigarette smoking ,airpollutant concentrates & automobile exhausts.
- Atomic Fluorescence Spectroscopy (AFS) techniques are useful in other kinds of analysis/measurement of a compound present in air or water, or other media, such

as CVAFS (Cold vapour atomic fluorescence spectroscopy) which is used for heavy metals detection, such as mercury.

- Determination of inorganic substances,
  - Determination of vitaminB1 (thiamine)in food samples like meat,cereals etc.
  - Determination of VitaminB2(riboflavin) .This method is generally used to measure the amount of impurities present in the sample.
- Extensively used in the field of nuclear research for the determination of uranium salts.
- Most important applications are found in the analyses of food products, pharmaceuticals ,clinical samples and natural products
- *Fluorescent indicators:*
  - Intensity and colour of the fluorescence of many substances depend upon the pH of solutions. These are called as forescent indicators and are generally used in acidbase titrations .
  - Eg: Eosin–pH3. 0-4.0–colour less to green

    Fluorescien–pH4.0-6.0– colourless to green

FLAMEPHOTOMETRY

Flame Photometry or Flame Atomic Emission Spectrometry is a branch of spectroscopy in which the species examined in the spectrometer are in the form of atoms.

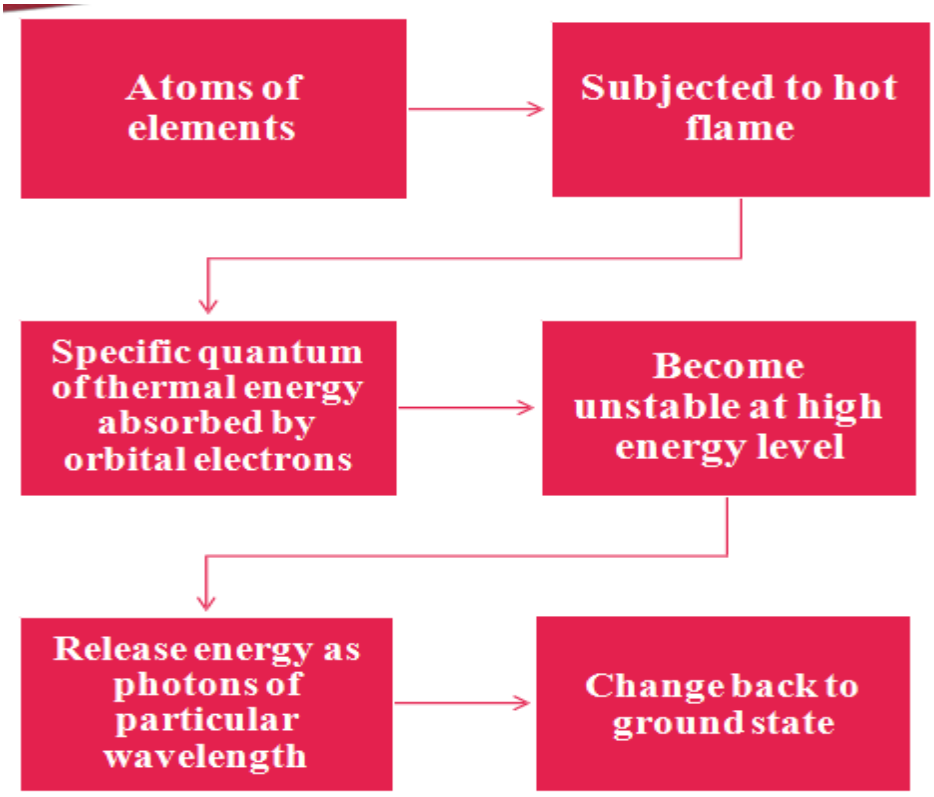
Flame Photometer: "An instrument used in inorganic chemical analysis to determine the concentration of certain metal ions among them sodium, potassium, calcium and lithium.**"**

- Flame Photometry is based on measurement of **intensity of the light** emitted when a metal is introduced into flame.
- The **wavelength of color** tells us what the element is (qualitative).
- The color's intensity tells us how much of the **element present** (quantitative).

Principle

- The **basic principle** upon which Atomic Spectroscopy works is based on the fact that "**Matter absorbs light at the same wavelength at which it emits light."**

- When a metal salt solution is burned, the metal provides a colored flame and each metal ion gives a different colored flame.
- Flame tests, therefore, can be used to test for the absence or presence of a metal ion.



Instrumentation

Parts of a Flame Photometer

- **Source of Flame**

A **burner** that provides flame and can be maintained in a constant form and at a constant temperature.

- **Nebulizer and Mixing Chamber**

Helps to transport the homogeneous solution of the substance into the flame at a steady rate.

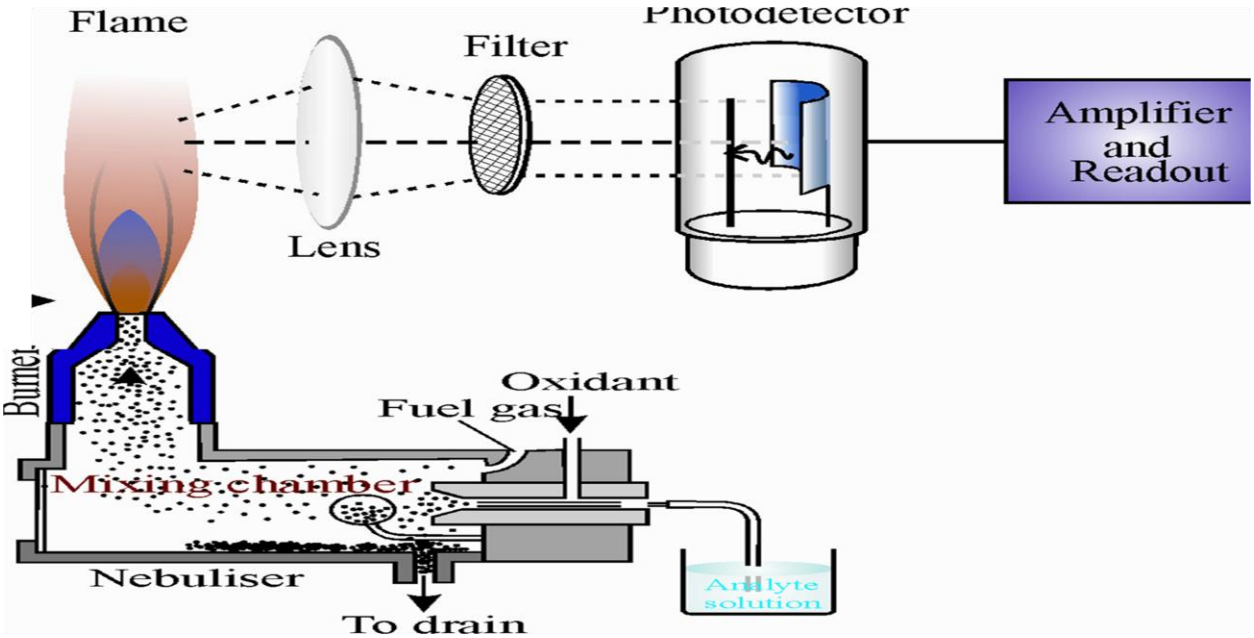
- **Optical System (Optical Filter)**

The optical system comprises three parts: **convex mirror**, **lens** and **filter**. The convex mirror helps to transmit light emitted from the atoms and focus the emissions to the lens. The convex lens help to focus the light on a point called slit. The reflections from the mirror pass through the

slit and reach the filters. This will isolate the wavelength to be measured from that of any other extraneous emissions. Hence it acts as interference type color filters.

- **Photo Detector**

Detect the emitted light and measure the intensity of radiation emitted by the flame. That is, the emitted radiation is converted to an electrical signal with the help of photo detector. The produced electrical signals are directly proportional to the intensity of light.



Working mechanism

Nebulization:

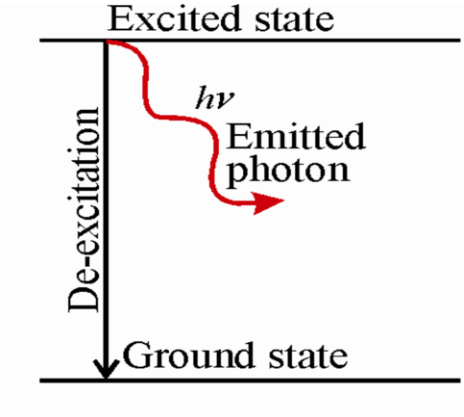
"A dispenser that turns a liquid into a fine mist called nebulizer (such as perfume)."

The solution of the substance to be analyzed is first aspirated into the burner, which is then dispersed into the flame as fine spray particles.

A brief overview of the process

- The solvent is first evaporated leaving fine divided solid particles.
- This solid particles move towards the flame, where the gaseous atoms and ions are produced.
- The ions absorb the energy from the flame and excited to high energy levels.

- When the atoms return to the ground state radiation of the characteristic element is emitted.
- The intensity of emitted light is related to the concentration of the element.



Name of the element	Emitted wavelength range (nm)	Observed color of the flame
Potassium (K)	766	Violet
Lithium (Li)	670	Red
Calcium (Ca)	622	Orange
Sodium (Na)	589	Yellow
Barium (Ba)	554	Lime green

Applications

Flame photometer has both quantitative and qualitative applications.

- Flame photometer with monochromators emits radiations of characteristic wavelengths which help to detect the presence of a particular metal in the sample. This help to determine the availability of alkali and alkaline earth metals which are critical for soil cultivation.
- In agriculture, the fertilizer requirement of the soil is analyzed by flame test analysis of the soil.

- In clinical field, Na+ and K+ ions in body fluids, muscles and heart can be determined by diluting the blood serum and aspiration into the flame.
- Analysis of soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry.

Advantages

- Simple quantitative analytical test based on the flame analysis.
- Inexpensive.
- The determination of elements such as alkali and alkaline earth metals is performed easily with most reliable and convenient methods.
- Quite, convenient, selective and sensitive to even parts per million (ppm) to parts per billion (ppb) range.

Disadvantages

- The concentration of the metal ion in the solution cannot be measured accurately.
- A standard solution with known molarities is required for determining the concentration of the ions which will corresponds to the emission spectra.
- It is difficult to obtain the accurate results of ions with higher concentration.
- The information about the molecular structure of the compound present in the sample solution cannot be determined.
- The elements such as carbon, hydrogen and halides cannot be detected due to its non-radiating nature.

AUTOMATION IN CLINICAL LABORATORY

- Automation, at clinical automation is a automatic equipment which done their process automatically. As this result
- Reduce our work load Nonstop supervision
- Expense should be low because no need much technicians Time save
- The technicians are replaced by auto analyzer because it can work like technicians.

Uses of automation

- Reduce human error
- Safety

- decrease laboratory costs
- improve turnaround time
- increase productivity
- Run more tests
- Test in fewer sites
- Operate with fewer instruments.
- Retain lower operating costs.
- Employ relatively less skilled labor.
- Use more automation in a paperless environment

Analysis steps

1. Identifying the patient
2. Getting the correct sample
3. Identifying and proper labeling of the sample
4. Delivery of sample in proper storage condition and within time
5. Preparation of sample for test
6. Sample loading/aspirating
7. Analysis

Sample collection of automation

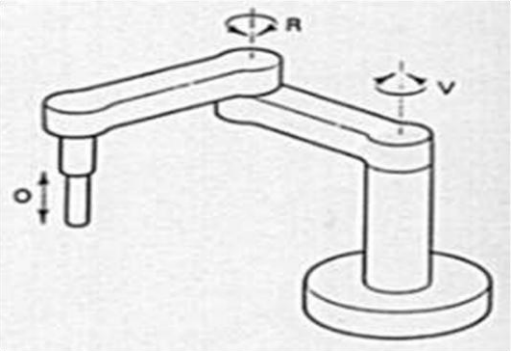
- Measure the blood sugar just prick the finer with less pain
- The phlebotomist need not pull the syringe, blood gets sucked in due to negative pressure filling the vacuum

Sample identification

- By bar coding and labelling
- All details of patient with unique identity
- same details entered into auto analyzer
- To avoid transcriptional error bar coding technique can be used

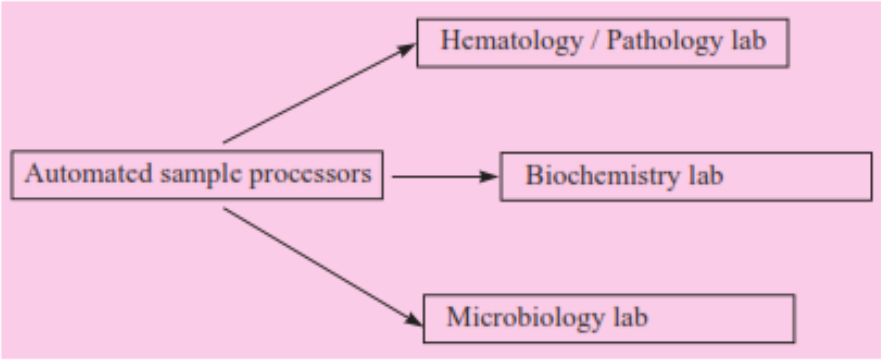
Sample delivery

- The purpose of using for reduce the human error and delay.
- Pneumatic tube system and mobile robots are used.



Sample preparation

- If we are doing sample preparation in automation, the work time and workload should be reduce so we don't want to depend on the techniques peoples.
- The are 2 types of sample preparation
- Fully automatic
- Semi automatic
- This can do the following tasks like, sorting of samples, removing caps, separating samples, bar coding.



Analysis

Continuous Flow Analyzer

- Tubing flow of reagents and patients samples

Flow Injection Analyzer

- Centrifuge force to mix sample and reagents

Dialyzer module

- Separate testing cuvets for each test and sample
- Random and/or irregular access
- Technicon Auto analyzer II (AAII) system Peristaltic Pump Module
- Auto Analyzer is an automated analyzer using a special flow technique named "continuous flow analysis (CFA)" first made by the Technician Corporation. The first applications were for clinical.

Advantage

- More test we can test at same time by using auto analyzer
- Error less
- Time save
- Cost low
- Reduce the workload
- More accurate

Disadvantage

- Reagent waste
- High cost reagent
- Maintance cost high
- job market going to be down

USES OF ISOTOPES IN BIOCHEMISTRY

Radioisotopes

- Radioisotopes are widely used in medicine, industry and scientific research, and new applications for their use are constantly being developed.
- Radioisotopes are radioactive isotopes of an element. Different isotopes of the same element have the same number of protons in their atomic nuclei but differing numbers of neutrons. They can also be defined as atoms that contain an unstable combination of neutrons and protons.

Properties of Radioisotopes

Emits radioactive radiation which

- Have different penetrating ability with materials of different thickness and densities
- Kill cells
- Cause cell mutation
- Ionise molecules
- Have the same chemical properties as non- radioactive isotopes of the same element
- Its activity decreases with time

Examples of formation of radioisotopes

- Beryllium-7 is produced when boron-10 captures a proton

$$^{10}_{5}B + {}^{1}_{1}p \rightarrow {}^{7}_{4}Be + {}^{4}_{2}He$$

- Magnesium-24 is bombarded by a neutron, sodium-24 can be produced

$$^{24}_{12}Mg + {}^{1}_{0}n \rightarrow {}^{24}_{11}Na + {}^{1}_{1}H$$

Occurrence of radioisotopes

- The combination can occur naturally, as in radium- 226, or by artificially altering the atoms. In some cases, a nuclear reactor is used, in others, a cyclotron.
- The best known example is uranium. All but 0.7 % of naturally-occurring uranium is uranium-238; the rest is the less stable, or more radioactive, uranium-235, which has three less neutrons.

Radioisotopes uses:

- Radioisotopes are an essential part of radiopharmaceuticals. In fact, they have been used routinely in medicine.
- Some radioisotopes used in nuclear medicine have very short half-lives, which means they decay quickly; others with longer half-lives take more time to decay, which makes them suitable for therapeutic purposes.
- Radioisotopes are also widely used in scientific research, and are employed in a range of applications, from tracing the flow of contaminants in biological systems, to determining metabolic processes in small Australian animals.

RADIOISOTOPE	USES
1)Calcium-47	Important aid to biomedical researchers studying cellular functions and bone formation in mammals.
2)Cesuim-137	Used to treat cancerous tumors... To measure correct dosages of radioactive pharmaceuticals...
3)Chromium-51	Used in research in red blood cells survival studies.

RADIOISOTOPE	USES
4)Cobalt-57	Used as a tracer to diagnose pernicious anemia.
5)Cobalt-60	Used to sterilize surgical instruments... Used in cancer treatment, food irradiation and radiography.
6)Copper-67	When injected to monoclonal antibodies into a cancer patient, helps the antibodies bind to and destroy the tumor.

questions	opt1	opt2	opt3	opt4
Oxidation of glucose to pyruvate and lactate is called as…..	TCA	Glycolysis	PPP	gluconeogenesis
Pick the glycolysis phase in the following	preparatory	oxidative	non oxidative	All of these
During one Kreb's cycle number of carbon dioxide (CO2), molecules released is	1	2	3	4
____may be defined as polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis.	Carbohydrates	Proteins	Lipids	Amino acids
Which is the total yield of ATP in TCA cycle in eukaryotic cells?	30	32	36	34
Hypoxia refers to	low oxygen	High oxygen	medium oxygen	no oxygen
Glycolysis is otherwise called as	ETR	ETM	EMP	ENP
Which is the product of glycolysis	pyruvate	lactate	acetyl CoA	None of the above
Which of the following is a reduced coenzyme?	FMN	CoA	NADH	FAD
In Kreb's cycle, the hydrogen of malate is accepted by	FMN	CoA	NAD	FAD
Glycolysis occurs in …….	cytoplasm	mitochondria	Endoplasmic recticulum	None of the above
How many molecules of pyruvate produced frrom single molecule of glucose?	2	3	1	4
The oxidation of acetyl CoA to CO2 is called as	TCA	Krebs cycle	Citric acid cycle	All of these
Which of the following is a waste product of the TCA (Krebs)	O2	CO2	H2	NADH
Most enzymes that take part in Kreb's cycle are located in	cytoplasm	inner mitochondrial membrane	plasma membrane	mitochondrial matrix
Last electron acceptor in respiration is	O2	CO2	H2	NADH
In Kreb's cycle , a six carbon compound is formed by the combination of Acetyl CoA and	Citric acid	succinic acid	oxaloacetic acid	malic acid
Net ATP synthesis in glycolysis in anaerobic condition	2	4	6	8
…..is the final common oxidative pathway for carbohydrates, fats or amino acids, through acetyl CoA.	TCA	Glycolysis	PPP	gluconeogenesis
Net ATP synthesis in one turn of TCA cycle	12	24	8	10
How many phases in glycolysis	one	two	three	four
In Kreb's cycle	Energy is liberated from ATP	Energy is stored in the form of ATP	Energy is stored in the form of ADP	Energy is liberated from ADP
FADH2=	2ATP	3ATP	1ATP	None of the above
Which of these enzyme reactions is not irreversible in glycolysis?	Hexokinase	3-phosphoglycerate kinase	Glucokinase	Phosphofructokinase
The formation of glycogen from glucose is called as	Glycogenesis	gluconeogenesis	Glycogenolysis	None of the above
1NADH=	2ATP	3ATP	1ATP	None of the above
Net ATP synthesis in glycolysis in aerobic condition	2	6	8	12
Glucose means	sweet	sugar	both	None of the above
…….is the central molecule in carbohydrate metabolism.	Glucose	pyruvate	lactate	ribose
The synthesis of glucose from non-carbohydrate precursors is called as	Glycogenesis	gluconeogenesis	Glycogenolysis	None of the above
_____are the most abundant dietary source of energ	Proteins	Carbohydrates	Lipids	Amino acids
Carbohydrates are often referred to as	Saccharides	Proteins	Lipids	Amino acids
Carbohydrates are broadly classified into____major groups.	2	3	4	5
_____are the simplest group of carbohydrates and are often referred to as simple sugars.	Monosaccharides	Disaccharides	Oligosaccharides	Polysachharides
When the functional group in monosachharides is an aldehyde they are known as	Aldoses	Ketoses	Amino acids	Both aldoses and ketoses
When the functional group in monosachharides is an ketone they are known as	Aldoses	Ketoses	Amino acids	Both aldoses and ketoses
____ are the compounds that have the same structural formulae but differ in their spatial configuration.	Structural Isomerism	Stereo Isomerism	Functional Isomerism	Positional Isomerism
A carbon is said to be _____when it is attached to 4 different atoms or groups.	Symmetric	Asymmetric	parallel	None of the above
If D and L isomers are present in equal concentration, It is known as	Racemic Mixture	DL Mixture	Both A and B	None of the above
The process of shifting a hydrogen atom from one carbon atom to produce enediols is known as	Isomerism	Tautomerization	racemic mixture	None of the above
Loss of electron is known as	Reduction	Isomerization	Oxidation	All of these
_____is composed of 2 alpha D glucose units held together by glycosidic bond.	Maltose	Sucrose	Lactose	Sucrase

____consist of repeat units of monosaccharides or their derivatives, held together by glycosodic bonds.	Monosaccharides	Oligosaccharides	Polysaccharides	None of the above
____is the carbohydrate reserve in animals	Glycogen	Glucose	Starch	Dextran
Hydrolysis of sucrose by enzyme	Maltase	Lactase	Sucrase	amylose
_____are the breakdown products of starch by the enzyme amylase	Glucose	Starch	Dextrin	Inulin
When the polysaccharides are composed of different types of sugars or their derivatives they are referred to as	Oligosaccharides	Heteroglycans	Homopolysaccharides	Dextrin
When the polysaccharides are composed of same types of sugars or their derivatives they are referred to as	Oligosaccharides	Heteroglycans	Homopolysaccharides	Dextrin
Proteins are covalently bound to carbohydrates are referred to as	Glycoprotein	Lipoprotein	Glucoprotein	Dextrin
The following polysaccharide is composed of β-glycosidic bonds	Starch	Glycogen	Cellulose	Dextrin

answer

Glycolysis

preparatory

2

Carbohydrates

30

low oxygen

EMP

pyruvate

NADH

NAD

cytoplasm

2

All of these

CO2

mitochondrial matrix

H2

oxaloacetic acid

2

TCA

12

two

Energy is stored in the form of ATP

2ATP

3-phosphoglycerate kinase

Glycogenesis

3ATP

8

both

Glucose

gluconeogenesis

Carbohydrates

Saccharides

3

Monosaccharides

Aldoses

Ketoses

Stereo Isomerism

Asymmetric

Both A and B

Tautomerization

Oxidation

Maltose

Polysaccharides

Glycogen
Sucrase
Dextrin

Heteroglycans

Homopolysaccharides

Glycoprotein

Cellulose