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GOVERNMENT OF TAMIL NADU

## HIGHER SECONDARY SECOND YEAR

# BIOCHEMISTRY

A publication under Free Textbook Programme of Government of Tamil Nadu

Department of School Education

**Untouchability is Inhuman and a Crime**

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**Government of Tamil Nadu**

First Edition - 2019

Revised Edition - 2020, 2022

Reprint - 2021, 2023, 2024

(Published under new syllabus)

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**Content Creation**

State Council of Educational
Research and Training

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**Printing & Publishing**

Tamil NaduTextbook and Educational
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# Key features …

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|---|---|---|---|
| | **Scope of BioChemistry** | Awareness about higher education avenues in the field of BioChemistry | |
|  | **Learning objectives** | Describe the specific competency / performance capability acquired by the learner | |
| | **Do you know** | Additional information provided to relate the content to day-to-day life / development in the field |   |
| | **Example Problems** | Model problems worked out for clear-cut comprehension by the learners | |
| | **Evaluate yourself** | To help the students to assess their own conceptual understanding | |
| | **Q.R code** | Quick access to concepts, videos, animations and tutorials |  |
| | **Summary** | A glance on the substance of the unit | |
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# VARIOUS RESEARCH INSTITUTES IN INDIA,
## RESEARCH AREAS AND WEBSITES ARE SUMMARIZED BELOW.

| INSTITUTE | RESEARCH AREAS | WEBSITE |
|---|---|---|
| **Indian institute of Science (IISC)**<br>*Bangalore, Karnataka* | ❖ Peptides and peptidomimetics: Sugar amino acids<br>❖ Delivery of therapeutic oligonucleotides<br>❖ Carbohydrate-protein interactions<br>❖ Functional soft and hybrid materials<br>❖ Studies of dendrimers and liquid crystals<br>❖ Inorganicand organic semiconductors | www.iisc.ac.in |
| **Indian institute of Technology (IITM**)<br>*Chennai, Tamilnadu* | ❖ Statistical Correlations and Information Processing<br>❖ Ionic liquid-templated ordered mesoporous aluminosilicates<br>❖ Energy and fuel research<br>❖ Molecular engineering of materials<br>❖ Environmental science and engineering<br>❖ Catalysis and soft matter<br>❖ System engineering and data science | www.iitm.ac.in |
| **National Institute of Immunology (NII)**<br>*New Dlehi* | ❖ Immunity and Infection,<br>❖ Reproduction, Development and Cell Biology,<br>❖ Genetics, Signalling and Cancer Biology, and<br>❖ Chemical Biology, Biochemistry and Structural Biology. | /www.nii.res.in |
| **National Center for Biological Sciences**<br>*Bengaluru, Karnataka* | ❖ The self-packing polypeptide<br>❖ Computational Approaches to Protein Science<br>❖ Exploring the architecture and function of transmembrane ion channels<br>❖ Computational cancer genomics | www.ncbs.res.in |
| **Council of Scientific Industrial Research (CSIR)**<br>*New Delhi* | ❖ Bio-actives, Cell / Stem cell / Immuno Therapies,<br>❖ Regenerative / Precision / personalized Medicines<br>❖ Reduction of Genetic Disease Burden. | www.csir.res.in |



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| INSTITUTE | RESEARCH AREAS | WEBSITE |
|---|---|---|
| **CSIR - CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE(CFTRI)**<br>*Mysuru, Karnataka* | ❖ Ultra High Pressure System For Food Preservation<br>❖ Non-Aqueous Applications Of Membrane Technology | www.cftri.res.in |
| **Central Salt & Marine Chemicals Research Institute**<br>*Bhavnagar, Gujarat* | ❖ Salt and Marine Chemicals<br>❖ Inorganic Materials and Catalysis<br>❖ Electro Membrane Processes<br>❖ Reverse Osmosis | www.csmcri.org |
| **National Institute of Pharmaceutical Education and Research (NIPER)**<br>*Mohali, Punjab.* | ❖ Development of oral anti diabetic drugs<br>❖ Nano crystalline solid<br>❖ xanthine oxidase inhibitors<br>❖ microbial and marine origin for therapeutic purposes<br>❖ Standardization and quality control of herbal drugs and products<br>❖ Development of chemical process technologies for important natural products involving isolation | www.niper.gov.in |
| **Institute of Nano Science and Technology(INST)**<br>*Mohali, Punjab* | ❖ Bio-inspired soft nanostructures<br>❖ Bio-sensors and online diagnostics<br>❖ Bio-targeting and therapeutics<br>❖ Microfluidics based devices<br>❖ Materials and devices for energy storage and harvesting<br>❖ Nanotechnology in Agriculture and Rural Development<br>❖ Nano toxicology | www.inst.ac.in |
| **Center For Cell And Molecular Biology(Ccmb)**<br>*Hydrabad, Telangana* | ❖ Tumor biology<br>❖ Cataract & other eye diseases<br>❖ Applications of DNA fingerprinting<br>❖ Conservation of endangered species<br>❖ Molecular approaches in animal breeding<br>❖ Aquaculture | www.ccmb.res.in |
| **Tata institute of fundamental research (TIFR)**<br>*Mumbai, Maharashtra* | ❖ Molecular biophysics and imaging<br>❖ Chemical biology and synthetic chemistry<br>❖ Bioinorganic and biomimetic chemistry<br>❖ Nano science and catalysis<br>❖ Chemical physics and dynamics | www.tifr.res.in |



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# LIST OF ENTRANCE EXAMINATIONS AFTER HSC

| NAME OF THE EXAM | MODE OF SELECTION | COURSE | WEBSITE |
|---|---|---|---|
| **NEET** (National Eligibility cum Entrance test | Written test | MBBS., BDS., | www.cbscneet.nic.in |
| **AIIMS** (All India Institute of Medical Sciences) | Written test | MBBS., | www.aiimsexams.org |
| **JIPMER** (Jawaharlal Institute Of Postgraduate Medical Education & Research) | Computer based test | MBBS., | www.jipmer.edu.in |
| **AFMC** (Armed Forces Medical College Entrance Exam | Written test | M.B.B.S., (Should Serve 7 Years in Armed Forces) | www.afmc.nic.in |
| **IIT – JEE** (IIT- Joint Entrance Exam) | Written test | B.E., B.Tech., B.Arch., | www.jeemain.nic.in |
| **CUCET** (Central Universities Common Entrance Test) | Written test | Integrated M.Tech., B.Tech., Integrated B.Sc., B.Ed., Integrated M.Sc., Integrated M.A., B.Des., (craft and design) | www.cucet.co.in |
| **NISER** (National Institute of Science Education and Research | Written test | Integrated M Sc., (Biology, Chemistry, Mathematics and Physics) | www.niser.ac.in |
| **IISC** (Indian Institute of Science Bangalore) | Written test | B.Sc., (4 years) | www.iisc.ernet.in |



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| NAME OF THE EXAM | MODE OF SELECTION | COURSE | WEBSITE |
|---|---|---|---|
| **IISER** (Indian Institutes of Science Education and Research ) | Written test | 5-year BS-MS dual degree (Biology, Chemistry, Mathematics and Physics) **IISER Kolkata offers major in Earth Sciences | www.iiserpune.ac.in |
| **NATA** (National Aptitude Test in Architecture) | Computer Based Test | B.Arch., | www.nata.in |
| **NIFT** (national institute of fashion technology) | Written test | B.Des., BFTech., | www.nift.ac.in |
| **IIFT** (indian institute of fashion technology) | Written test | B.Sc., (in fashion & apparel design) | www.iiftbangalore.com |
| **NID NEED** (Nation al Entrance Exam for Design | Written test | B.Des., (4 years) | www.nid.edu |
| **IIST** (Indian Institute of Space Technology) | Written test | B.Tech., (Avionics/ Aerospace Engineering/ Physical Science) | www.iist.ac.in |
| **CLAT** (Common Law Admission Test) | Written test | Integrated LLB ( 5 years) | www.cbscneet.nic.in |
| **NCHMCT** (National Council for Hotel Management Catering Technology Joint Entrance Exam) | Written test | B.Sc., (Hospitality and Hotel Administration) | www.nchm.nic.in |
| **NDA and NA** (National Defence Academy and Naval Academy) | Written Exam | 3 Years Training for entry into ARMY/ NAVY/AIRFORCE | www.nda.nic.in |
| **AIMNET** (All India Merchant Navy Entrance Test) | Written Exam | B.Tech., Marine Engineering B.Sc., Nautical Science B.Tech.,Navel Architecture and Ship Building | www.aim.net.co.in |



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# VARIOUS SCHOLARSHIP SCHEME OFFERED AFTER HSC

| CENTRAL GOVERNMENT SCHOLARSHIPS OFFERED | OFFERED BY | APPLICATION PERIOD |
|---|---|---|
| **Central Sector Scheme of Scholarship for College and University Students** | Ministry of Human Resource Development, Government of India | July to October |
| **Kishore Vaigyanik Protsahan Yojana (KVPY)** | Department of Science and Technology (DST), Government of India | June to August |
| **Inspire Scholarship** | Department of Science and Technology (DST), Government of India | October to December |
| **ONGC scholarship ( Applicable for SC/ST Students)** | Oil and Natural Gas Corporation Limited | January to March |
| **National Fellowship and Scholarship for Higher Education of ST Students** | Ministry of Tribal Affairs, Government of India | July to October |
| **MOMA scholarship (applicable only for Minority students)** | Ministry of Minority Affairs, Government of India | July to September |
| **Scholarships for Top Class Education for Students with Disabilities** | Ministry of Social Justice and Empowerment, Government of India | July to October |
| **Saksham Scholarship (Applicable for Disable students)** | AICTE | September to November |
| **AICTE Pragati scholarship for Girls** | AICTE | September to November |



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UNIT 1

# CELL MEMBRANES



S. J. Singer and G.L.Nicolson

The Fluid mosaic model was first proposed by S.J Singer (American Cell Biologist) and G L. Nicolson (American Biochemist) in 1972 to describe the structure of cell membrane. They published a paper entitled "The structure of cell membrane" in cell biology journal. In this model cell membrane consists of two phospholipid layers. Each phospholipid macromolecule is itself composed of a hydrophilic head and hydrophobic tail. The hydrophilic heads are attracted to water whereas the hydrophobic tails are repelled by water. This attraction and repulsion gives the stable structure to lipid bilayer.

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## Learning Objectives

After studying this unit the students will be able to

- discuss the structure and composition of a biological cell membrane
- explain the biomemebrane models
- describe the transport of substances across the membrane
- examine the properties of membrane
- explain the various buffer systems and functions



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## INTRODUCTION

Life of a cell is entirely based on planar sandwiches called membranes that are capable of protecting and separating the cells from the world around. These membranes, generally, are impermeable to macromolecules but can facilitate movement of certain molecules across it and contribute to maintain homeostasis at a greater level .

Membranes are comprised of two layers of lipids with embedded proteins. They act as two-dimensional entity, with a polar group, facing water exterior and hydrocarbon tails facing the interior side of cell. While membranes encircling the entire cell act as barriers against external environment, membrane around each organelle creates unique interior space for specialized biochemical reactions of that organelle, finally, contributing to the life of a cell.

The current knowledge on membrane has its origin from the findings of E.coli in the year 1855 which described that a membrane consists of lipid bilayer. In the following century, biochemical experts and X-ray experts had postulated the membrane as a surface coating with proteins diffused in the place of membrane or floating or spanning, with lipid anchors.

The following content of the chapter will provide you a detailed knowledge on structure and composition of a membrane.

## 1.1. CHEMICAL COMPOSITION

### 1.1.1. Lipid

It is understood from the introductory notes that lipids can form the framework of membrane, with the anchored proteins. The lipid molecules are less than 1000 Dalton in size and consist of aliphatic/aromatic hydrocarbons. There are various types of lipids that form the bilayer structure of a membrane.

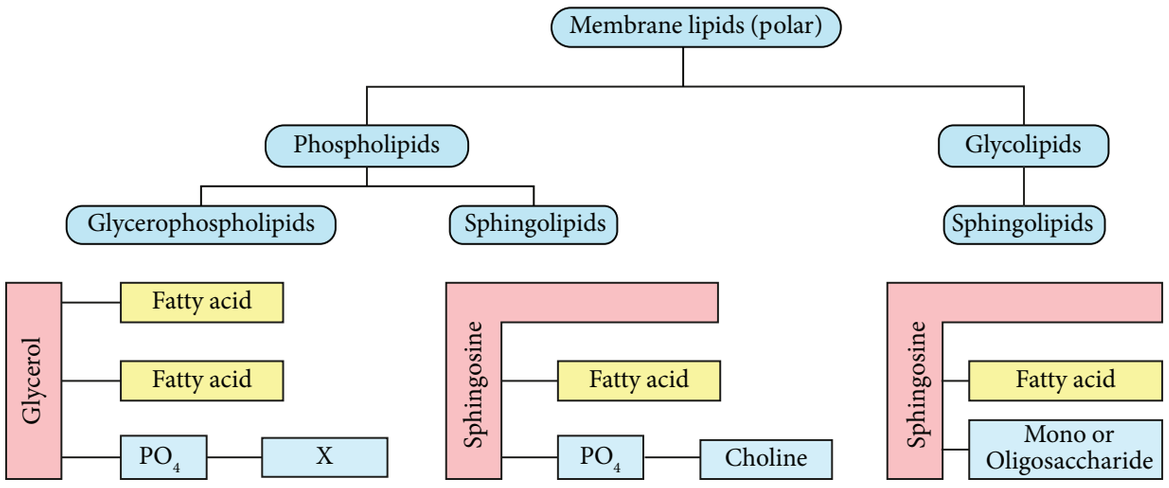



**Figure 1.1 Lipid framework of a bilayered membrane**



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**Phosphoglycerides:**

Phosphoglycerides are also mentioned as glycerolphospholipids because they contain phosphate. They are the major constituents of lipid bilayer of membrane. Phosphoglycerides have three components

(i) tri-carbon (C1, C2, C3) backbone of glycerol,

(ii) 2 long chain fatty acids esterified to C1 and C2 and

(iii) phosphoric acid esterified to C3 of glycerol.

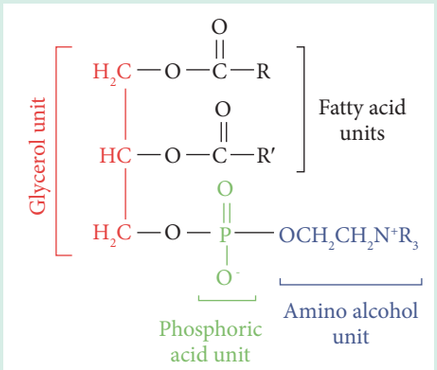



**Figure 1.2 Structure of phosphoglycerides**

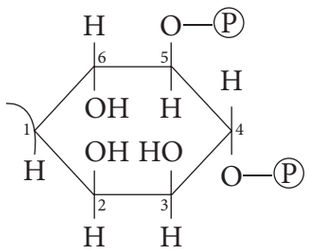
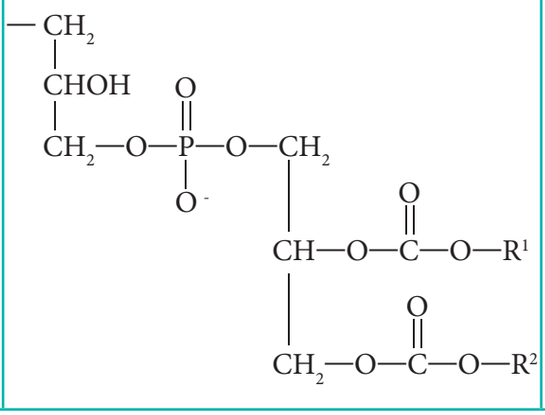
They are amphipathic (as they have both hydrophobic (water fearing) and hydrophilic (water loving) parts. The shape of phosphoglycerides in membrane is roughly rectangular (Figure 1.2). The fatty acids which are aliphatic may have or may not have double bond or can have 2 or more double bonds.

**Table 1: Common head groups found in glycerophospholipids and their basic characteristics. Source: Lafer, Eileen. "Membrane Lipids I and II: Glycerophospholipids and Sphingolipids." Lecture. San Antonio, Texas. December 6, 2011**

| Name of glycerophos-pholipid | Name of X | Formula of X | Net charge (at. pH7) |
|---|---|---|---|
| Phosphatidic acid | - | -H | -1 |
| Phosphatidylethanol-amine | Ethanolamine | $-CH_2-CH_2-\overset{+}{N}H_3$ | 0 |
| Phosphatidylcholine | Choline | $-CH_2-CH_2-\overset{+}{N}(CH_3)_3$ | 0 |
| Phosphatidylserine | Serine | $-CH_2-\underset{\displaystyle COO^-}{\underset{\displaystyle |}{CH}}-\overset{+}{N}H_3$ | -1 |
| Phosphatidylglycerol | Glycerol | $-CH_2-\underset{\displaystyle OH}{\underset{\displaystyle |}{CH}}-CH_2-OH$ | -1 |



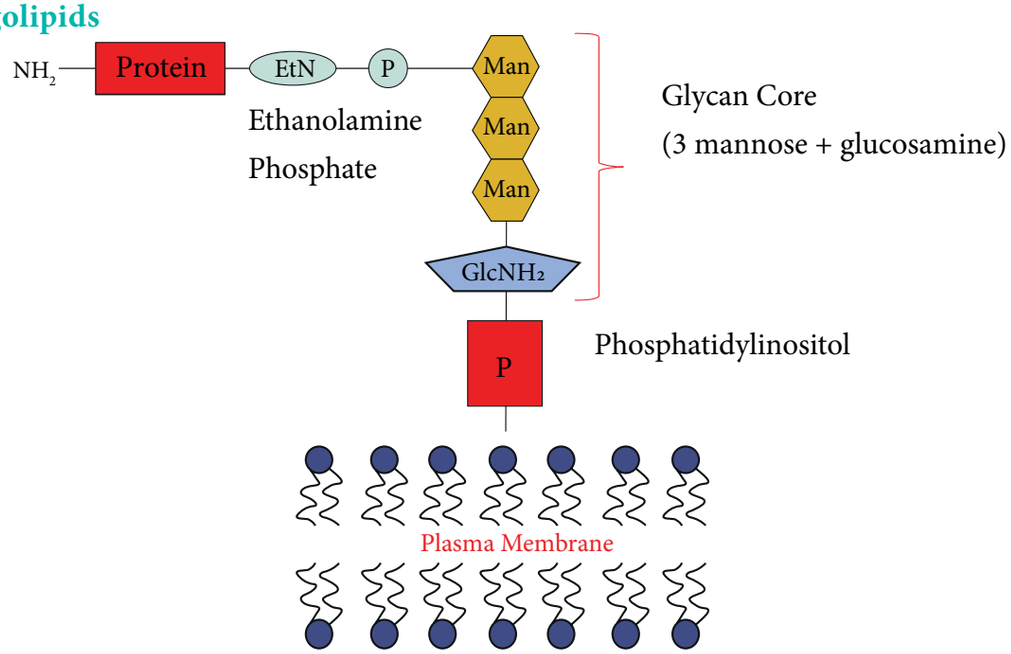
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| | | | |
|---|---|---|---|
| Phosphatidylinositol 4,5-bisphosphate | Myo-Inositol 4,5-bisphosphate |  | -4 |
| Cardiolipin | Phosphatidylglycerol |  | -2 |

## Glycolipids:

Glycolipids as their name indicates are sugar containing lipids. The sugar residues are always present on the external side of the membrane. There are three types of glycolipids (i) sphingolipids (ii) glycerolglycolipids in which sugars are attached to hydroxyl group on C3 of triglycerides (iii) glycosylphosphatidyl inositol (GPI). GPI anchors proteins to the outer leaflet of the plasma membrane. The C-terminal residue of a protein is attached through an amide linkage to the amino group of an ethanolamine which is connected by a sugar backbone (Mannose and Glucosamine) to the phosphaditylinositol.

## Sphingolipids



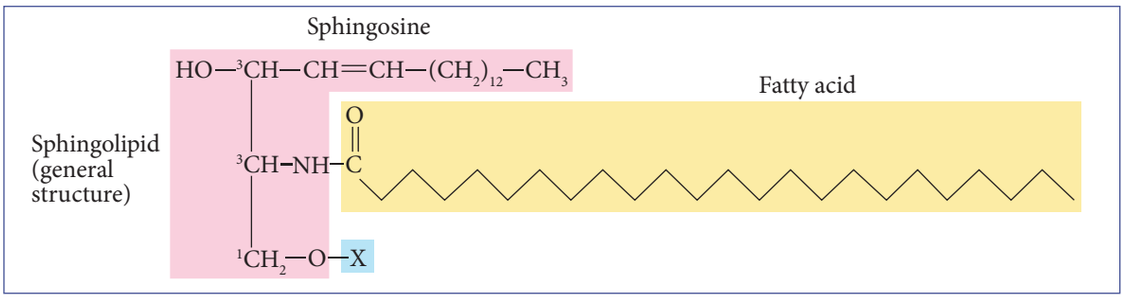



**Figure 1.3. Structure of Sphingolipids.**

In sphingolipids, the hydrophobic region consists of a long chain sphingoid base with generally 18 carbons, which is linked to the acyl group of a fatty acid via an amide bond. The polar head group (X) attached to C1 consists of X=H in the parent compound, ceramide.

In biological membrane, the sphingolipid components that contain a sugar molecule are referred to as glycolipids. These lipids obtain the name from sphingosine with a polar head group attached to C1 (Figure 1.3). Sphingolipids are abundantly present in membrane. There are various sphingolipids depending on (i) the fatty acid attached to C2 by an amide bond, or (ii) nature of polar head group esterified to the hydroxyl group at C1. Sphingolipids with one or more sugars are called glycophospholipids, which may be either neutral or negatively charged. In some case, a phosphate ester can link a base (choline or ethanolamine similar to phosphoglycerides) to C1 of sphingolipids and it is called sphingomyelin. Complex glycolipids like gangliosides contain oligosaccharides as their polar head group and one or more residues of sialic acid as the terminal sugar.

## Sterols

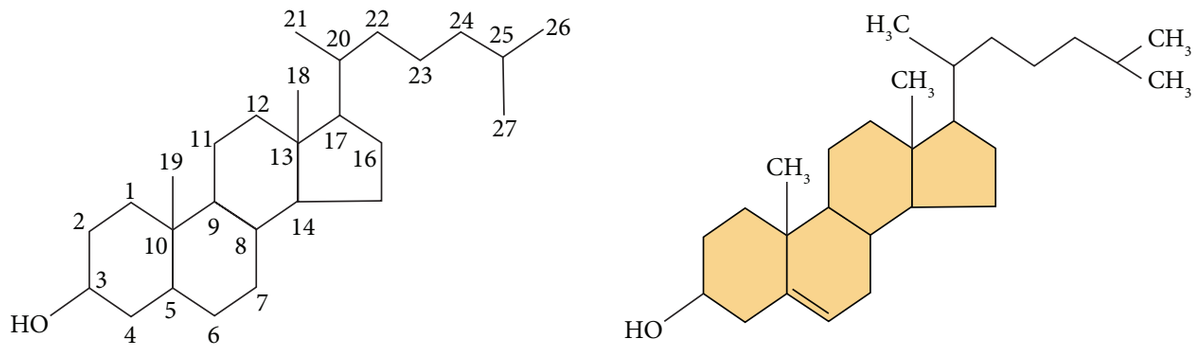



**Figure 1.4: Structure of sterols**

Sterols are third major lipid constituents of the lipid bilayer (Figure 1.4) cholesterol is the major type of sterol found in animal membranes while flat sterols can be found in bacteria, lower eukaryotes and plants. The cyclopentanoperhydrophenanthrene ring of cholesterol is apolar, inserting into the core of the lipid bilayer with the hydroxyl group at C3 situated at the surface. The precursor moieties of cholesterol namely isoprenol, geranyl and farnesylisoprenols act as hydrocarbon anchors for various membrane proteins.



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### Triglycerides

Triglycerides are composed of glycerol with fatty acids esterified to all the 'C's. They do lack polar head group but they are not present in membrane.

Lipoproteins are denoted as proteins that are chemically attached to membrane lipids, and they are present on either sides of a membrane.

Water, the universal solvent is also a component of a membrane. The cell membranes include water molecules, either bound to the polar groups visible on the polar side of the membrane molecules (structured water) or unbound (bulk water) within pores and certain ion channels crossing the membranes .

Ions are associated with membranes by simple adsorption to the two surfaces of the membrane or can just pass through ionic channels (membrane proteins) or ion pumps (membrane proteins with enzymatic character). The ions that contributes to the structure and functions of membrane are: $H^+$, $Na^+$, $K^+$, $Cl^-$, $Ca^{++}$, $HCO_3^-$

## 1.1.2.Proteins

**Membrane proteins**

Most of the functions of a membrane depend on the proteins present in it. You can recall that it was mentioned elsewhere that there are two classes of membrane proteins.

Both integral and peripheral membrane proteins which form the structure of the membrane. Integral proteins have both hydrophobic and hydrophilic interaction with hydrocarbon core and exterior of lipid cage

### 1.1.2.1.Integral Protein

Some of the membrane proteins are tightly embedded in the membrane and they cannot be isolated unless, the membrane is disintegrated. They are called as Integral or Intrinsic membrane proteins. They are again classified into two. (a). Transmembrane proteins, which traverse (pass through) or span the membrane. These proteins will have domains on either side of the membrane. Many cell surface receptors belong to this class. (b). Lipid anchored proteins that are present either on the cytosolic side or on the extracytosolic side. They insert themselves in the membrane by a lipid (acyl chain) attached to the N terminal end. Transmembrane proteins are of two types. Single pass transmembrane proteins that traverse the membrane only once. Multipasstransmembrane proteins that traverse the membrane more than once.

### 1.1.2.2.Peripheral Protein

Those proteins that are present on the surface of the membrane are called as peripheral proteins. They can be easily isolated from the membrane. eg. spectrin present in the RBC membrane.



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## 1.2 MODELS PROPOSED FOR MEMBRANE STRUCTURE

### 1.2.1. Monolayer Model (1917)

If a lipid molecule containing hydrophilic group is dissolved in a highly volatile solvent and several drops of it are carefully applied to the surface of the water, the lipid spreads out to form a thin mono molecular film. Based on this, Langmuir in 1917 proposed the monolayer model of membranes. In the mono molecular structure, it is found that the hydrophilic portion of each molecule projects in to the water surface and the hydrophobic parts are directed away from the water.

### 1.2.2. Lipid Bilayer Model or Bimolecular lipid layer:

The model was proposed by Gorter and Grendal in 1925. Lipids from erythrocytes were extracted from erythrocytes and the amount of surface area that lipid would cover when spread over the surface of water was assessed. It was identified that the membrane contained a bimolecular layer of lipids .They found that the polar groups of each molecular layer were directed towards the outside of the bilayer.

### 1.2.3. Sandwich Model:

Danielli and Davidson proposed the sandwich model in 1935 which was based on the lipid bilayer model. In this model, the plasma membrane is made up of lipid bilayer, sandwiched between the two continuous layers of proteins. The lipid molecules are arranged in an ordered manner and are set at right angles to the surface. The membrane was found to be 75-100 Å thick. In this, the thickness of the protein layer was found to be 20 Å and that of the lipid layer was 35Å .

### Drawbacks

This model cannot be applied to all membranes because it reveals definite proportions of lipids and proteins, which has not been true.

### 1.2.4.Unit membrane Model:

Unit membrane hypothesis of the structure of membrane was proposed in the year 1953 by Robertson. In unit membrane model the protein layers are said to be asymmetrical. The scientist had investigated the structure of a RBC membrane using electron microscope and found that all cellular membranes contains three layers *viz.* two outer layers of the proteins separated by a lighter middle layer of phospholipids. Thus, unit membrane model visualises cell membrane as a trilaminar structure consisting of two dark osmiophilic layers separated by a light osmiophilic layer. The lipid layer was found to be of 35 Å thickness with a dense band of proteins of 20 Å thickness existing on either side of the membrane.Mucoprotein is present on the outer surface and non mucoid protein is present on the inner surface of the RBC membrane.



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**This model was widely accepted because of the following features**

1. The densely packed bimolecular lipid layer shows the presence of 40% lipid by weight in the membrane.
2. The model accounted for the three layered staining pattern of fixed membranes as observed in electron microscopy.
3. Phospholipids spontaneously form a bimolecular system in vitro when added to an aqueous environment and there is no requirement for work input to maintain the minimum energy conformation of the synthetic membrane.
4. The membrane is rich in hydrocarbons, so it is of high electrical resistance.
5. High permeability of natural membranes to non-polar molecules could be explained by their solubility in the non-polar lipid phase and at the same time accounted for relative impermeability to small ions which do not dissolve readily in this medium.

**Drawbacks**

(i) The thickness of the membrane was found to be greater in plasma membrane than in the intracellular membrane of endoplasmic reticulum or golgi complex

(ii) This model is based on the study of the myelin sheath of a nerve fibre, which is a non-typical membrane and hence cannot be used as a reference model for the cell membrane

### 1.2.5.Fluid Mosaic Model:

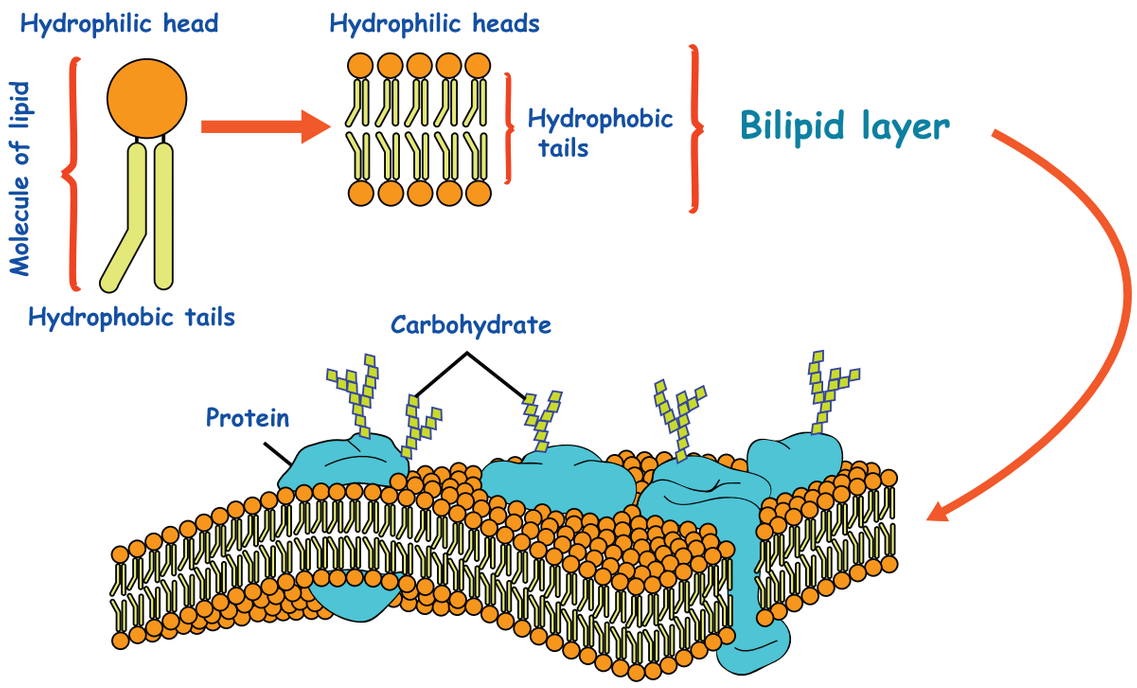



**Figure 1.5: Fluid membrane bilayer**

The fluid mosaic model of cell membrane was proposed in 1972 by S.J. Singer and G.L. Nicolson. The plasma membrane is a unique component of both plant and animal cells. It serves as a barrier between the cell interior and its surrounding and it allows entry



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and exit of certain substances. Hence, it is referred to as a semi-permeable membrane. According to this model, the plasma membrane contains a bimolecular lipid bilayer, both the surfaces are interrupted by protein molecules. The cell membranes have been visualized as mosaics of lipids and proteins.

As mentioned earlier, the cylindrical shapes and amphipathic ratio of phosphoglycerides and sphingolipids allow the bilayer formation with planar structure. This bilayer formation is associated with increased entropy owing to the hydrophobic chain of the bilayer which in turn assists in membrane assembly.

In this model, the membrane contains various molecules such as embedded protein, carbohydrate, cholesterol, and hence it is described as a mosaic pattern. The lipids are thought to be arranged primarily in a bilayer in which peripheral and integral proteins are embedded to varying degrees. The membrane proteins are not fixed within the lipid layer but are free to move laterally. The globular proteins of the membrane are of two different types: extrinsic (peripheral proteins) and intrinsic (integral proteins).

Some proteins are attached at the polar surface of the lipid, while others either partially penetrate the bilayer or span the membrane entirely to stick out on both sides. The protein usually contains chains of sugars or oligosaccharides. Some lipids present on outer surface are glycolipids. The cell membrane is more like a fluid, rather than being a rigid or solid structure. The membrane is of fluid consistency which enables the lateral movement of lipid and protein molecules within it. The proteins of the membrane are concerned with the enzymatic activities, transport of molecules and with receptor function.

Singer and Nicolson considered the lipoprotein association to be hydrophobic and fluidity of the membrane results due to hydrophobic interaction. It should be noted that phospholipids and many intrinsic proteins are amphipathic molecules, i.e., both hydrophilic and hydrophobic groups occur within the same molecule. The fluidity of lipid is evidenced based on x-ray diffraction, differential thermal analysis and electron spin resonance (ESR) techniques.

**The lipid bilayer has many dynamic properties which are as follows:**

1. The internal rapid motion within each lipid molecule is possible.
2. The lipid molecule might diffuse laterally
3. The transfer of lipid molecule from one side of the bilayer to the other is possible and is referred as flip-flop movement.
4. The lipid molecules might rotate rapidly as a whole about their axis.

The fluid mosaic model of cell membrane is now widely accepted as it can be applied to membranes of all types regardless of their varying characteristics and differences in lipid:protein ratio. In fact this model can account for the molecular organisation and ultrastructure of membranes in-terms of their chemical composition.



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Modern knowledge of the red cell plasma membrane and its membrane skeleton began with Marchesi and Steers's identification of spectrin in 1968. Prior to that year, almost the only thing known about membranes was that they contained a lipid bilayer. Indeed, there was a period in the 1960s when it was believed that red cell membranes contained only a single 22.5 kDa protein called "structural protein".

**Three major factors can influence fluidity of a cell membrane :**

**1. Temperature**

It will affect the mode of movement and closeness of phospholipids. During cold conditions, they are found together but when it is hot they move away farther from each other.

**2. Cholesterol**

These molecules are randomly or arbitrarily distributed across the lipid bilayer, so that the layer can be fluid in various environments. Cholesterol clamps phospholipids thereby controlling the unwarranted movement of molecules across.

**3. Saturated and unsaturated fatty acids**

Fatty acids make the tails of the phospholipid . (i) Saturated fatty acids : they are carbon chains with single bonds between them which makes them straight enabling tight packing. (ii) unsaturated fats are carbon chains with double bonds between them. These double bonds generate bends in the chain, that do not encourage tight packing. This has role in fluidity of the membrane because the twists or bends will increase the space in between the phospholipids. This will eventually leave them not to get frozen at lower temperatures. Further, molecules such as $CO_2$ and $O_2$, do need smaller spaces between the phospholipids through which they can move quicky with ease.The two possible bends that can occur are :

1. Cis-unsaturated fats, both sides of the chain stay on the same side
2. Trans-unsaturated fats, both sides of the chain are opposite from each other

## 1.3.MEMBRANE TRANSPORT

For the maintenance of the integrity of a cell, the internal concentration of various substances should be maintained. The movement of ions, small biomolecules and nutrients should be regulated, while waste products such as carbon dioxide ($CO_2$) should leave the cells. The membranes are equipped with a special feature that aid in regulation of concentration of the substances inside a cell. The lipid bilayer structure so far discussed does the major part of control transport processes. Generally, the movement of substances across the membranes are categorized based on whether or not energy is required.

i. Passive transport - the movement of substances across the membrane without the expenditure of cellular energy.



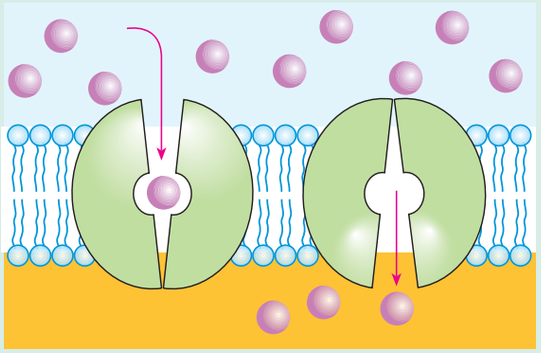
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ii. Active transport - the movement of substances across the membrane using energy from adenosine triphosphate (ATP).

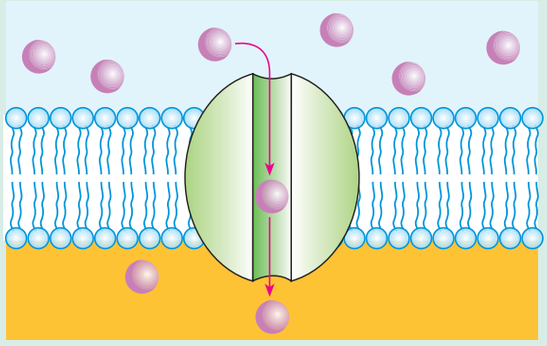
### 1.3.1.Passive transport

To appreciate the movements in the above mentioned passive mode, let us consider the terms viz., gradients and diffusion

A concentration gradient is the difference in concentration of a substance across a space. Molecules (or ions) will spread/diffuse from where they are more concentrated to where they are less concentrated until they are equally distributed in that space. (When molecules move in this way, they are said to move down their concentration gradient.) Three common types of passive transport include simple diffusion, osmosis, and facilitated diffusion.



Transport of matter with facilitated diffusion



Substance passage through carrier protein channel

**Fig 5:. Facilitated Diffusion**

### 1.3.2. Facilitated diffusion

Facilitated diffusion of substances crossing the cell (plasma) membrane takes place with the help of proteins such as channel proteins and carrier proteins. Channel proteins are less selective than carrier proteins, and usually mildly discriminate between their cargo based on size and charge. Carrier proteins are more selective, often only allowing one particular type of molecule only to cross.

### 1.3.3. Active Transport

For the transport methods described above, the cell expends no energy. Membrane proteins that aid in the passive transport of substances do so without the use of ATP. Active transport differs from diffusion in that molecules are transported away from thermodynamic equilibrium (hence, energy is required). The required energy can come from the hydrolysis of ATP, from electron movement, or from light. The energy moves a substance across a membrane, often with the help of protein carriers, and usually against its concentration gradient. One of the most common types of active transport involves proteins that serve as pumps. The word “pump” probably conjures up thoughts of using energy to pump up the tire of a bicycle or a basketball. Similarly, energy from ATP is required for these membrane proteins to transport substances—molecules or ions—across the membrane, usually against their concentration gradients (from an area of low concentration to an area of high concentration).



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The maintenance of electrochemical gradients in biologic systems is so important that it consumes perhaps 30–40% of the total energy expenditure in a cell. In general, cells maintain a low intracellular $Na^+$ concentration and a high intracellular $K^+$ concentration, along with a net negative electrical potential inside. The pump that maintains these gradients is an ATPase that is activated by $Na^+$ and $K^+$ ($Na^+$-$K^+$ ATPase;). The ATPase is an integral membrane protein and requires phospholipids for activity. The ATPase has catalytic centers for both ATP and $Na^+$ on the cytoplasmic side of the membrane, but the $K^+$ binding site is located on the extracellular side of the membrane. These pumps are particularly abundant in nerve cells, which are constantly pumping out sodium ions and pulling in potassium ions to maintain an electrical gradient across their cell membranes. An electrical gradient is a difference in electrical charge across a space. In the case of nerve cells, for example, the electrical gradient exists between the inside and outside of the cell, with the inside being negatively-charged (at around -70 mV) relative to the outside. The negative electrical gradient is maintained because each $Na^+/K^+$ pump moves three $Na^+$ ions out of the cell and two $K^+$ ions into the cell for each ATP molecule that is used . This process is so important for nerve cells that it accounts for the majority of their ATP usage.

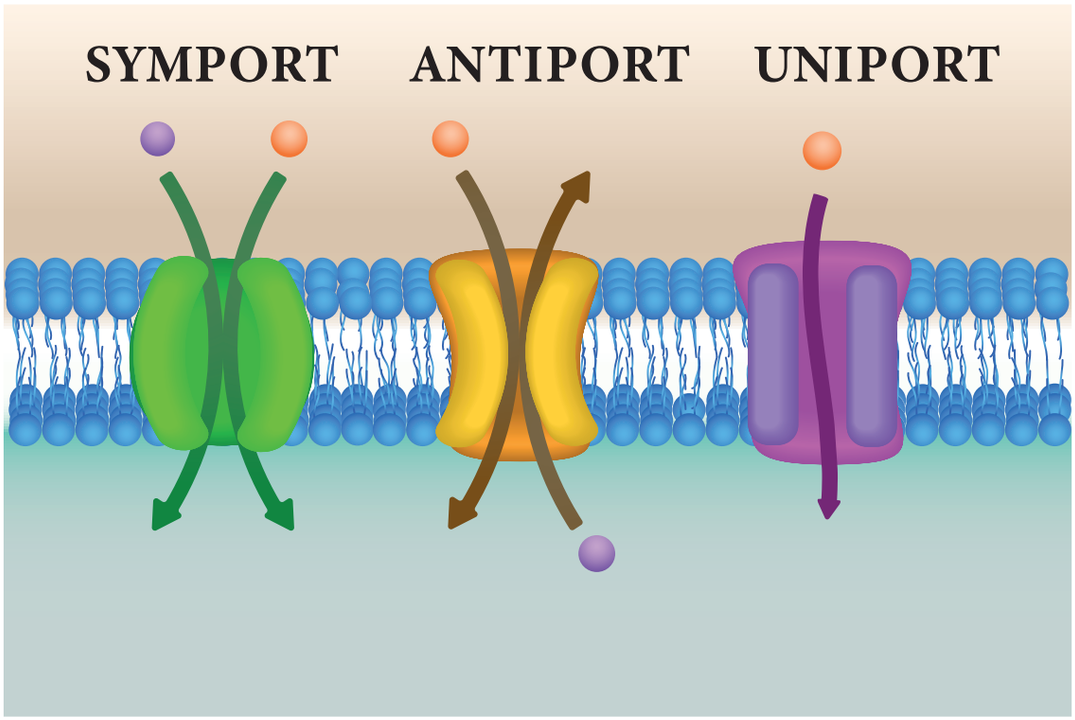



**Figure 6: Active transport across membranes. Primary reaction is mediated by pumps (see text) while the porters (Uniporter, symporter and Antiporter) are referred as secondary reactions**

Like pumps, carriers are found in all membranes, for exchange of molecules for metabolism or expelling out the wastes. Carriers are also known as facilitators or porters and the substrates for carriers include ions and small soluble organic molecules and sometimes, lipid soluble substances.



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Carriers which transport a single substrate through a membrane down its concentration gradient are called uniporters. Many carriers transport substrates against or up concentration gradients also. In such cases, the carrier transport across the membrane is combined with the transport of one more substrate down its electrochemical gradient e.g. Glucose. Its movement from plasma to red blood cells (down the concentration gradient) and to intestines (up its concentration gradient) is along with $Na^+$. It is called as symporter, as both glucose and $Na^+$ are transferred in the same direction.

Another class of carriers are termed as antiporters. These are called anti as they transport substrates in the opposite directions to the ion gradient promoting the reaction. All of the carrier-mediated responses are reversible.

It is well understood that the membrane effectively protects the interior of the cell from the exterior. Though the membrane is generally impermeable, molecules from the outside environment are taken up via a process called endocytosis

### 1.3.4.Endocytosis

In the process of endocytosis, an area or a region of the membrane would surround the substance which needs to be internalised. The section of the membrane that has surrounded the material would bud into the cell forming a vesicle which retains the ingested substance.

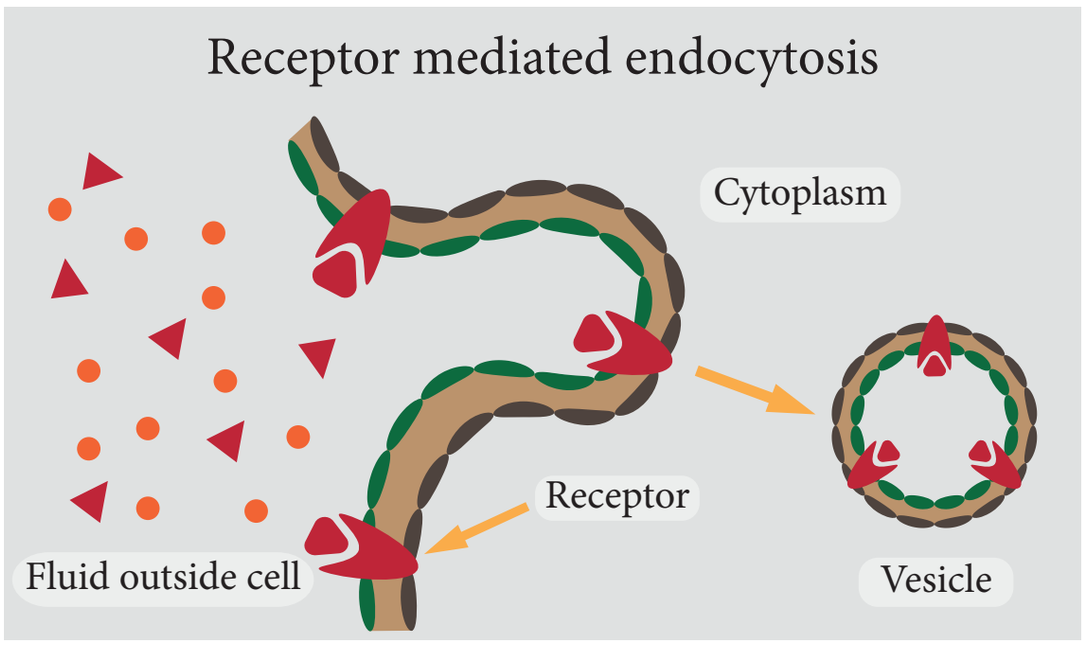



**Figure7 .Receptor-mediated endocytosis. A particular substance binds to a receptor on the outer surface of the membrane and uptaken by endocytosis**



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### Receptor-Mediated Endocytosis

Receptor-mediated endocytosis is a targeted process, unlike endocytosis in general, where receptor molecules are employed on the outer surface of the membrane to bind to a substance that need to be transported into the cell. The receptor exhibits specific binding affinity towards the substance that accomplishes the process. A protein coated vesicle called clathrin coated vesicle is attached to the cytosolic side of the membrane and this would pinch off from the membrane once the substance is transported into the coated vesicle.

Membrane uses a process called pinocytosis, a varied process of endocytosis. Pinocytosis uses smaller vesicles and occur in molecules (including liquid) whenever required by the cell from the extracellular fluid. Another varied process of pinocytosis called Potocytosis, uses a coating protein called caveolin, whose function is same as that of clathrin. In addition to caveolin, membranes contain cavities which form vacuoles and have receptors as well as lipid rafts.




In liver cell, receptor-mediated endocytosis is involved in the uptake of a type of cholesterol, called low-density lipoprotein or LDL bad cholesterol. If the receptor-mediated endocytosis is unsuccessful, bad cholesterol will not be removed and gets accumulated. This may result in familial hypercholesterolemia

## 1.4.VISCOSITY AND SURFACE TENSION

One of the most amazing features of biological membranes is that both the lipid and protein moieties are persistently in motion. This is the property of any molecule of a viscous liquid. The membrane acts as a two-dimensional liquid in which the protein constituents stroll like boats.

The lipid bilayer behaves as a two-dimensional liquid in the absence of covalent bonds between the lipids, whose resistance against shear deformations is characterized by the surface shear viscosity. Any relative motion between the two leaflets of a bilayer is opposed by a frictional force. Living cells actively control these forces by varying the mixture of lipids and sterols present in their membranes




Ouabain, is a plant derived toxic substance and in eastern Africa it was used as an arrow poison warfare and in hunting during ancient times. Ouabain is a cardiac glycoside and at low doses can be useful for treating arrhythmia and hypotension. It can inhibit the ATPase activity.

### 1.4.1.Biological importance of viscosity and surface tension

1. Blood viscosity is useful in streamlining the blood flow. Blood plasma has a normal viscosity of 15 – 20 mpoises. Alterations in the viscosity are an indication of diseased condition. Viscosity increases during macroglobulinemia, retinal hemorrhages and congestive heart failure.



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2. Carbohydrate and protein solutions are highly viscous in nature. The lubricating property of the synovial fluid is achieved mainly by the viscous nature of the mucopolysaccharides present in the synovial fluid.
3. Dipalmitoyl lecithin is a surfactant that is secreted by the lung alveoli, which reduces the surface tension and prevents the collapse of lung alveoli during expiration. Certain premature infants have low levels of this surfactant leading to acute respiratory distress.

## 1.5. OSMOSIS

Osmosis is the net movement of water across a semi-permeable membrane from an area of lower concentration of the solute to an area of higher concentration of the solute.

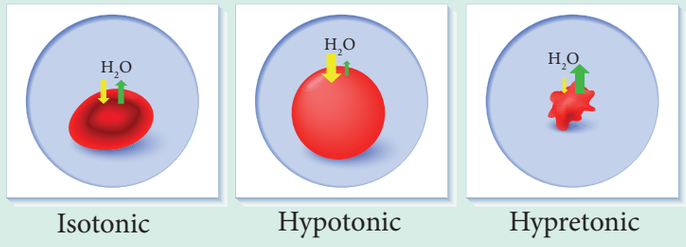



**Figure 8: Osmolarity and the consequent changes in a cell**

Tonicity is responsible for the movement of the water into or out of the cell. A solution's tonicity is related to its osmolarity, which is the total concentration of all solutes in the solution. A solution with low osmolarity has fewer solute particles per liter of solution, while a solution with high osmolarity has more solute particles per liter of solution. When solutions of different osmolarities are separated by a membrane permeable to water, but not to solute, water will move from the side with lower osmolarity to the side with higher osmolarity.

- If the extracellular fluid has lower osmolarity than the fluid inside the cell, it is said to be hypotonic—hypo means less than—to the cell, and the net flow of water will be into the cell.
- In the reverse case, if the extracellular fluid has a higher osmolarity than the cell's cytoplasm, it is said to be hypertonic—hyper means greater than—to the cell, and water will move out of the cell to the region of higher solute concentration.
- In an isotonic solution—iso means the same—the extracellular fluid has the same osmolarity as the cell, and there will be no net movement of water into or out of the cell.

Hypotonic, hypertonic, and isotonic are relative terms. That is, they describe how one solution compares to another in terms of osmolarity. For instance, if the fluid inside a cell has a higher osmolarity, than the surrounding fluid, the cell interior is hypertonic to the surrounding fluid, and the surrounding fluid is hypotonic to the cell interior.



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### 1.5.1. Biological significance

1. Hemolysis and Crenation. The physiological or isotonic saline is 0.9% NaCl. When red blood cells are suspended in 0.3% NaCl (hypotonic solution), water will enter into the cells and the cell will burst releasing all its contents. This kind of lysis is called as hemolysis. The resulting membranes are called as ghosts. On the other hand, when the cells are placed in 1.5% NaCl, water comes out of the cell, leading to shrinkage of cells. The process is called as crenation.

2. The erythrocyte fragility test is based upon the osmotic diffusion property. The ability of the membrane to withstand hypotonic solution depends upon the integrity of the membrane. Certain genetic disorders like sickle cell anemia and deficiency of vitamin E makes the erythrocyte membrane more fragile.

3. Osmotic pressure of blood is largely due to its mineral ions such as sodium, potassium, chloride, calcium and protein. The osmotic pressure exerted by proteins is of considerable biological significance owing to the impermeability of the plasma membrane to the colloidal particles.

4. Absorption of water in the intestine is due to osmosis. Formation of urine in the kidneys may be attributed to osmotic pressure. The net difference in the hydrostatic pressure and osmotic pressure is responsible for the filtration of water at the arterial end of the capillary and the reabsorption of the same at the venous end. At the arterial end, the hydrostatic pressure is 22 mmHg and the osmotic pressure is 15 mm Hg. The pressure to drive out the fluid is 7 mm Hg. At the venous end, the hydrostatic pressure is 15 mm Hg and osmotic pressure is 7 mm Hg. The net absorption pressure to draw water back into the capillaries is 15 – 7 = 8 mm Hg. This is called as Starling's hypothesis.

## 1.6. BUFFERS

A buffer is defined as a solution which resists the change in pH that will occur on addition of small quantities of acid or base to the solution. Buffers are mixtures of weak acid and its salt or weak base and its salt. The pH of the solution is defined as the negative logarithm of hydrogen ion concentration. The pH of buffers are determined by Henderson Haselbach equation

$$pH = pKa + \log \frac{[salt]}{[acid]}$$

### Regulation of blood pH

When there is a deviation of blood pH away from that of normal value, the two systems in the body work togeather for restoring the equilibrium. 1. The respiratory system modifies the rate of respiration that will in turn alter the concentration of carbon dioxide in the blood; 2. The renal system modifies the reabsorption/and or production of bicarbonate/hydrogen ions. This balance is known as “compensation”.



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### 1.6.1 Hemoglobin Buffer system

By the process of metabolism, carbon di oxide is produced in the tissues and enters the blood. This $CO_2$ will be hydrated forming $H_2CO_3$ and this gets ionized yielding $H^+$ and $HCO_3^-$. When the oxygen tension is reduced in the tissues, oxy-hemoglobin will dissociate, thereby producing oxygen and hence reduced hemoglobin is formed.

In lungs, oxy-hemoglobin (strong acid) is formed from reduced hemoglobin releasing hydrogen ions that reacts with bicarbonate and yield carbonic acid. Owing to lower $CO_2$ tension in lung, the shift of equilibrium towards the production of carbon di oxide is enabled which in turn will be continuously eliminated during exhalation

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Reduced hemoglobin will act as anion and accepts the $H^+$ ions to produce acid-reduced hemoglobin (HHb). By the production of weak acid, the arrival of $H^+$ ions are buffered thereby causing little change in pH.

On the return of blood to the lungs, owing to the formation of oxy-hemoglobin (stronger acid ) as mentioned above, these $H^+$ ions are released . Immediately these released $H^+$ ions are neutralized by $HCO_3^-$. This is inevitable for the lungs to release $CO_2$.

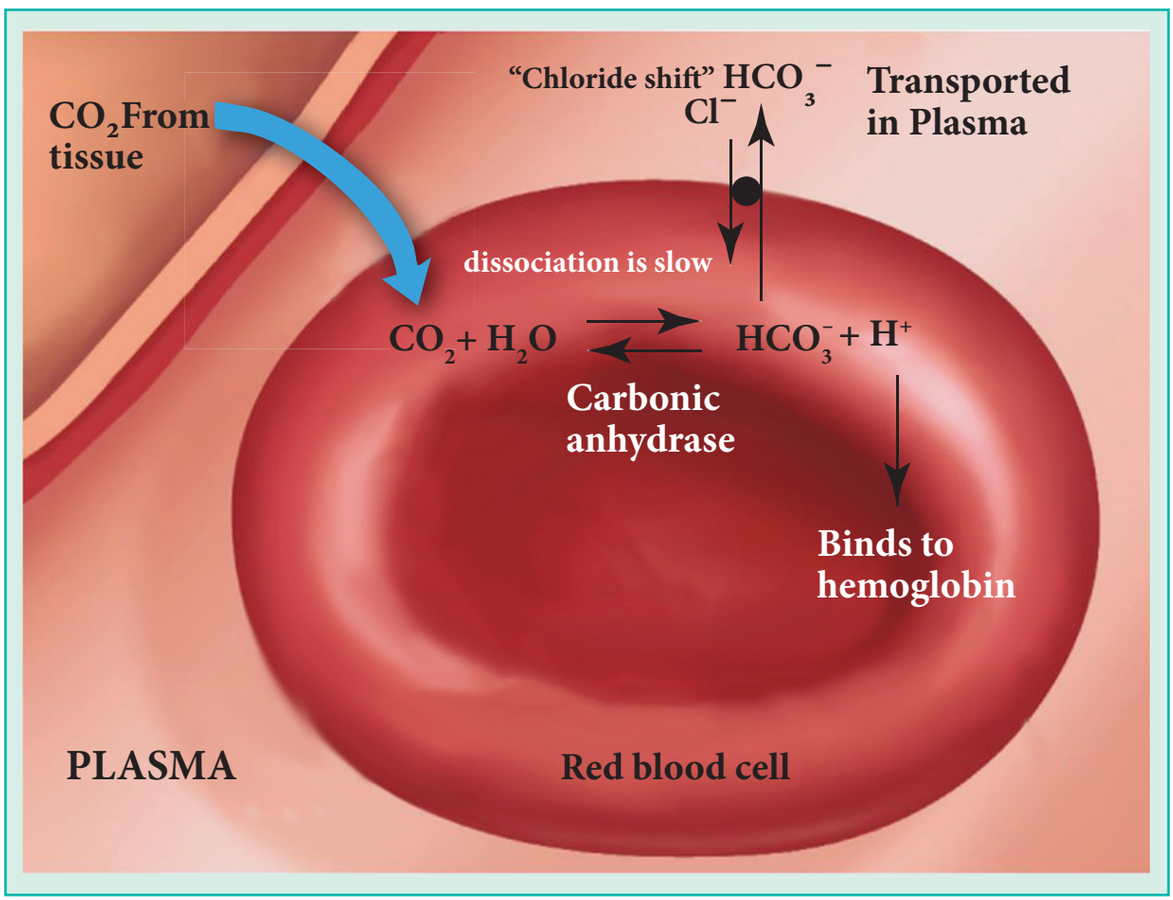



Figure 9



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### Chloride Shift

1. In RBCs $CO_2$ reacts with water forming carbonic acid in a reaction that is catalysed by carbonic anhydrase.
2. The formed carbonic acid is buffered by phosphate buffer as well as haemoglobin buffer.
3. Bicarbonate returns to plasma and gets exchanged with chloride ion that enters into (shifts into) the cell if the tension of $CO_2$ increases in the blood.
4. On the contrary, if the $CO_2$ tension is reduced, chloride will exit the cell and enters the plasma.
5. In general, red blood corpuscles are impermeable to sodium or potassium whereas permeable to hydrogen, bicarbonate and chloride ions. Potassium which is a cation is available to the plasma by anionic (chloride) exchange. This results in added $CO_2$ being carried by plasma as sodium bicarbonate.
6. The cycle continues as $CO_2$ enters and passes to the red blood cells and forms carbonic acid (partial amount of which returns to plasma) by carbonic anhydrase. The remaining carbonic acid then reacts with hemoglobin buffers yielding bicarbonate that travels to the plasma in exchange of chloride and is transported.
7. The reactions mentioned so far are reversible. In lung tissue, chloride shifts back into plasma when blood becomes arterial. This eventually releases intracellular potassium to buffer the oxy-hemoglobin. In plasma, it neutralizes sodium (Figure 9).

### 1.6.2. Regulation by respiratory mechanism

We have seen that the carbonic acid will dissociate into $CO_2$ and $H_2O$. If there is more of $H^+$ within the blood more of $CO_2$ elimination will be carried out by lungs. If there is more of $HCO_3^-$, the lungs will ensure low respiratory rate thus enhancing the retention of $CO_2$ so that it can be useful for forming carbonic acid that can buffer the excess of bicarbonate.

The alveolei and bronchioles of lungs do perform such functions effectively. A phospholipid molecule is secreted by a particular type of cell in the lung lining the alveoli and bronchioles. This surfactant regulates (lowers) the surface tension of the alveolar membranes thereby protecting the alveoli during exhalation and inhalation.

### 1.6.3. Regulation by renal mechanism

The major functions of kidneys are regulation of water and electrolyte balance. This is done by excretion of waste substances in urine. The formation of urine involves three stages (i) filtration, (ii) reabsorption, (iii) secretion. The regulatory role of renal organ is achieved by the buffering capacity of the organ. For this function membrane performs key role. Passive and active transports are involved in addition to osmosis and pinocytosis. (Figure 10).



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**Passive transport**
- Simple diffusion
- Facilitated diffusion (glucose in basolateral membrane)

**Active transport**

**Active reabsorption**
- Primary active transport (Sodium-potassium ATPase pump)
- Secondary active transport
- Secondary active reabsorption (glucose by sodium in PT)

**Active Secretion**
- Primary active Secretion
- Secondary active Secretion ($H^+$ by sodium in PT)

**Osmosis**

**Pinocytosis**

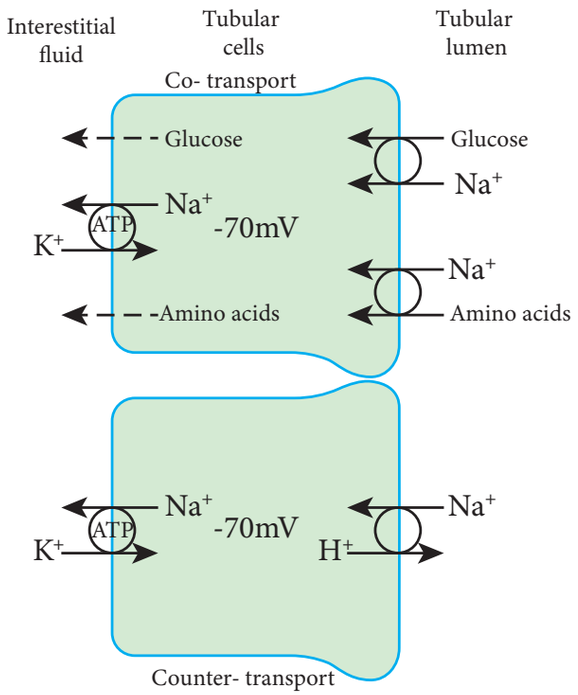



**Figure 10: Movement of substances across the renal tubular membrane that aid in urine formation (by filtration, reabsorption,secretion) by kidneys.**

## Activity

1. Perform the experiment to know the diffusion using coloured liquid or jelly like substance and water that can help you to understand how water, oxygen and small substances are transported across the membrane (Use paper towels as membrane). Do the experiment in the presence and absence of heat i.e hot water and cold water. (Higher temperatures speed up diffusion because molecules have more kinetic energy at higher temperatures).
2. Use potato and sugar solution to understand osmosis. Calculate the difference in height of the solution inside the cut potato (well like pit engraved in potato) which you have kept in a petri dish filled with coloured water.

## Summary

1. The cell membrane is an essential feature for the survival and homeostasis of a cell.
2. The membrane regulates the interaction of a cell with its external environment and it allows substances to enter or leave the cell.
3. The membrane is chiefly made up of lipids and proteins; carbohydrates make up a small percentage.



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4. Lipids and proteins (integral and peripheral) maintain the structure and function of the membrane
5. Many models have been proposed for the structure of a membrane.
6. The fluid-mosaic model is the widely accepted model that best describes the structures of most of cell membranes
7. Cholesterol and the fatty acids of phospholipids control the fluidity of the membrane.
8. The proteins in the lipid bilayer perform several functions of the membrane. They act as receptors, enzymes, etc.
9. Membrane uses passive and active modes of transport.
10. The endocytotic process and variations of endocytosis allow substances to enter the cell, while exocytosis aids the exit of the substances away from the cell (these process maintain the size of membrane)
11. Membrane is bestowed with properties such as viscosity, surface tension and osmosis thus making them indispensable tool in maintenance of integrity of a cell.
12. Buffers maintain the normal physiological pH. Haemoglobin buffers are playing important role in maintaining pH of blood. The regulatory role of respiratory and renal functions are profoundly dependent on the membranes of lung and kidneys respectively

## EVALUATION

### I Multiple Choice Questions

1. In a membrane, carbohydrate moieties glycoproteins or glycolipids are

   A. oriented towards outside
   B. placed towards inside
   C. facing towards outside and inside
   D. randomly dispersed (Ans-A)

2. The lipid bilayer _____ in nature

   A. hydrophilic
   B. hydrophobic
   C. both
   D. the nature depends on the immediate environment (Ans-C)

3. _____membrane contains the largest amount of proteins

   A. red blood corpuscular membrane
   B. myelin sheath
   C. lysosomal membrane
   D. outer mitochondrial membrane (Ans-A)



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4. The distribution of inherent proteins in the membrane is

   A. symmetrical  B. asymmetrical

   C. random  D. uniform (Ans-B)

5. _____statement describes the role of a cell membrane?

   A. free entry and exit of substances into or out of a cell.

   B. controlled movement of substances across the cell.

   C. Block the entry of substances into the cell.

   D. Dismisses the exit of substances from the cell. (Ans-B)

6. ----------can pass through - bilayer without support.

   A. Fat-soluble molecules

   B. Ions

   C. Both A and B

   D. None of the above

7. Channel protein adopts ---------- to transport ions

   A. Facilitated diffusion

   B. Active transport

   C. Both A and B

   D. None of the above

8. Which of the following is/are the major functions of cell membrane.

   A. compartmentalization

   B. protection from from extracellular components

   C. temperature maintenance

   D. All of the above

9. Materials are allowed to permitted through cell membrane thus maintaining a constant

   A. gradient

   B. concentration

   C. nutrients

   D. buffering

10. Fluid Mosaic Model helps to understand that the proteins are embedded in ----------fashion.

    A. eplic  B. criss-cross

    C. zigzag  D. mosaic



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11. This sentence is true about the surface tension of a plasma membrane

    A. Surface tension of membrane is greater than pure lipid assemblies.

    B. Surface tension of membrane is lesser than pure lipid molecules.

    C. Cell membrane do not possess surface tension.

    D. Surface tension of membrane is same as that of the pure lipid.

12. One of the following is not the function of a membrane

    A. chromosomal segregation

    B. transport

    C. extracellular interaction

    D. energy transduction

13. In this process, a vesicle formed at the plasma membrane brings the substances into the cell

    A. Endocytosis

    B. Exocytosis

    C. Plasmolysis

    D. Crenation

14. Sodium and potassium are transported by a -------- protein

    A. carrier

    B. channel

    C. receptor

    D. enzyme

15. Clathrin coated pits assist in

    A. receptor-mediated endocytosis

    B. exocytosis

    C. phagocytosis

    D. diffusion

## II Answer the following

1. What is a membrane?
2. What is the difference between diffusion and osmosis?
3. Give your idea on tonicity.
4. Discuss the different models of membrane structure.



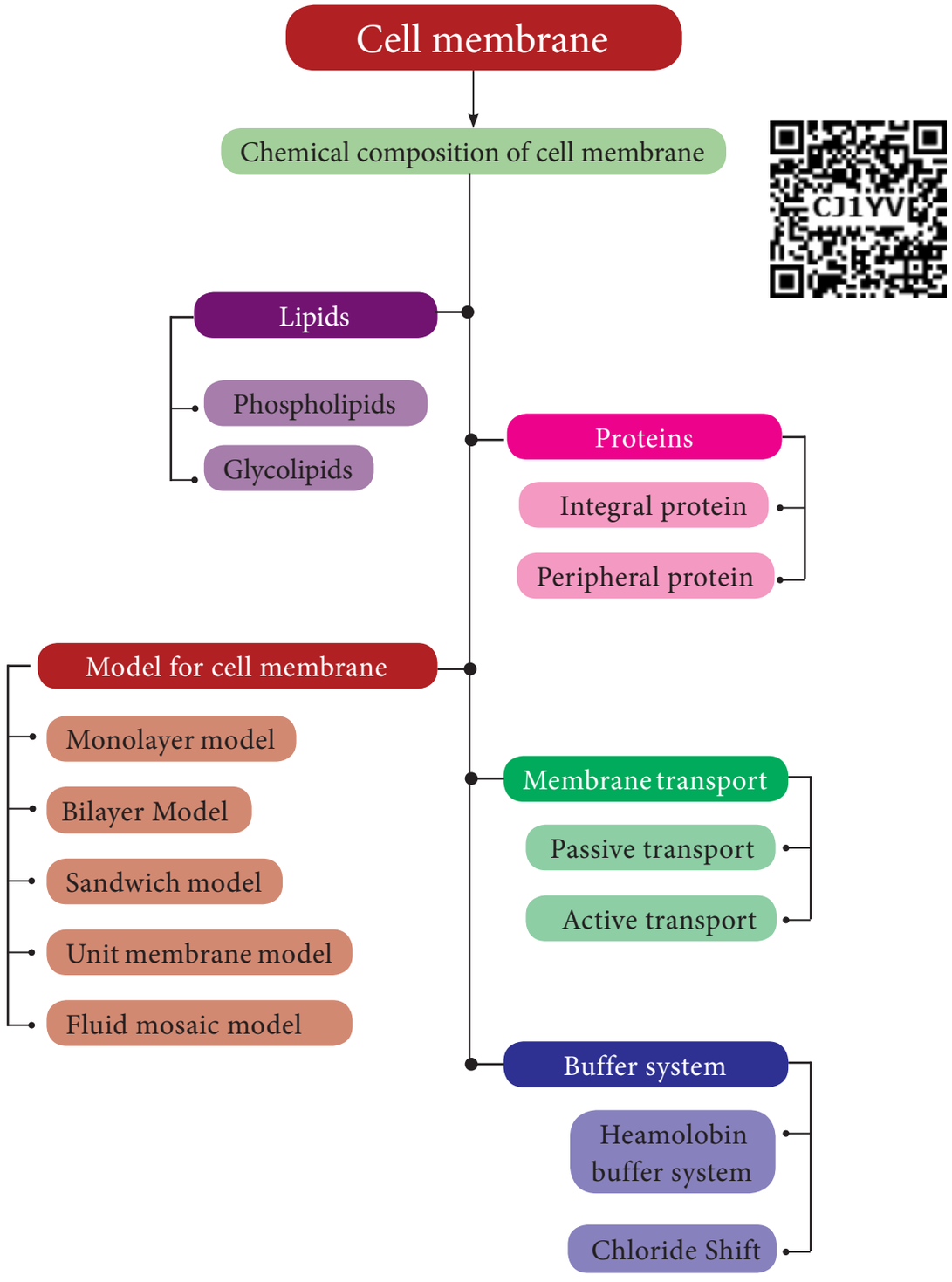
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5. Write about the protein composition of a membrane.
6. Write about the lipid composition of a membrane.
7. Write about the haemoglobin buffer system.
8. How kidneys are efficient in regulation of pH?
9. Give detailed note on membrane transport.
10. What are pinocytosis and potocytosis?
11. What are the three types of glycolipids in the membrane?
12. Give the mosaic model of cell membrane. List the dynamic properties and write the model is accepted.
13. What are uniporters, antiporters and symporters?
14. What do you mean by receptor-mediated endocytosis?
15. Give the biological significance of osmosis.
16. Give the biological significance of viscosity.
17. Give a note on membrane sterols
18. Give a note on sphingolipids present in the membrane.
19. What are the drawbacks of sandwich model?
20. Give a short note on active transport by membrane.



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# CONCEPT MAP






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UNIT 2

# DIGESTION



Dr. Ivan Pavlov

The Nobel Prize in Physiology or Medicine (1904) was awarded to Ivan Petrovich Pavlov in recognition of his work on the physiology of digestion. He was a pioneer who has carried out research in understanding the various processes of digestion, by surgical methods in dogs. He studied the conditional reflexes and the involvement of nervous system in the intestinal movements as well as secretion of gastric juice and other secretions. He also explored the significance of psychic factors in activating secretion of gastric juice.

BP2DR

## Learning Objectives

After studying this unit the students will be able to

- recognise the anatomy of digestive system
- understand the digestive glands and their secretions
- Deliberate the mechanical and chemical aspects of digestion
- explain the role of digestive enzymes in digestion of macromolecules
- describe different modes of absorption and assimilation of nutrients
- recognise the role of gastro-intestinal hormones in digestion



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## INTRODUCTION

Food is vital for sustenance of life for all organisms. However, the billions and trillions of cells in our body cannot utilize the complex constituents present in the food we eat like lip smacking pizzas or burgers and idlies or dosas, directly. Hence, these complex molecules should be broken down into simpler forms for the cells to assimilate and use them. The process by which the complex food is broken down mechanically (i.e. physically, in mouth and stomach), as well as chemically to smaller macromolecules that can be utilized by the cells of our body is called as digestion. Digestion is unique to heterotrophs, as autotrophs like plants are capable of synthesizing their own food. The process of conversion of food to fuel that could be used by cells is aided by the digestive system and it is a unique anatomical feature of the animal kingdom.

## 2.1 DIGESTIVE SYSTEM:

The digestive system is made up of a tubular gastro -intestinal tract (GI tract), extending from mouth till anus, and secretory organs (Figure 2.1). The organs of the GI tract include the oral cavity, pharynx, stomach, small intestine, large intestine, rectum and anus. The accessory digestive organs include the salivary glands, oxyntic and parietal glands of the stomach, duodenal glands, liver, gall bladder and pancreas.

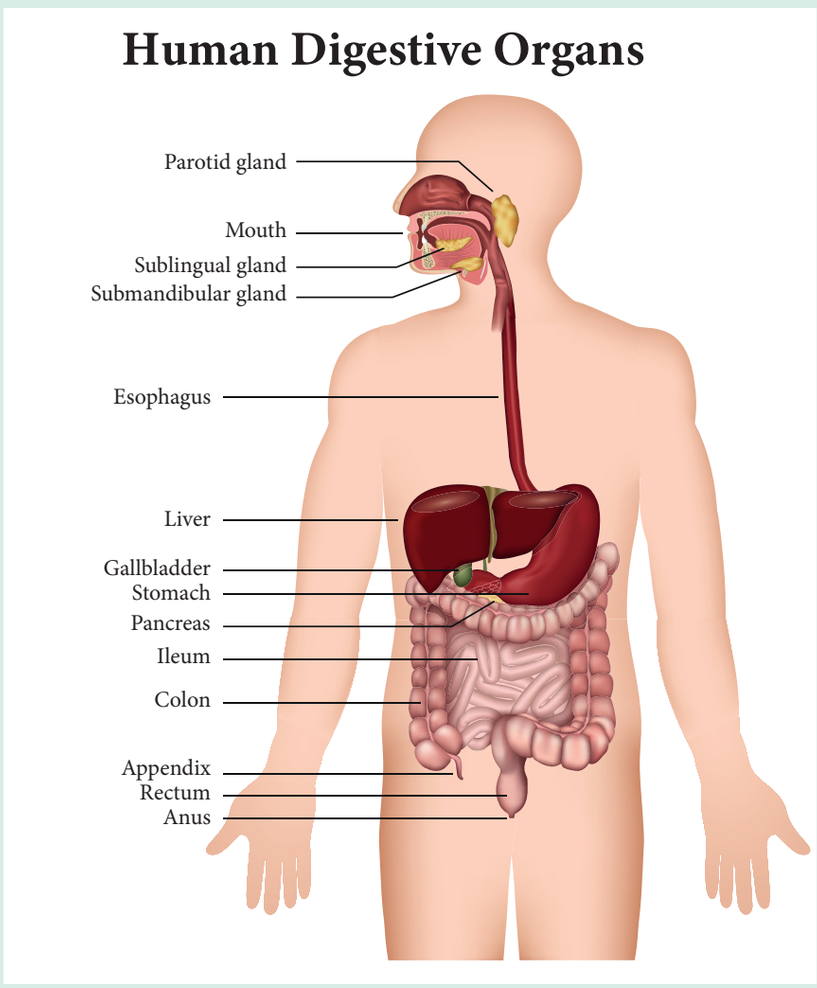



Figure 2.1 Digestive system of human

### 2.1.1 Gastrointestinal Tract:



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### 2.1.1.1 Oral Cavity

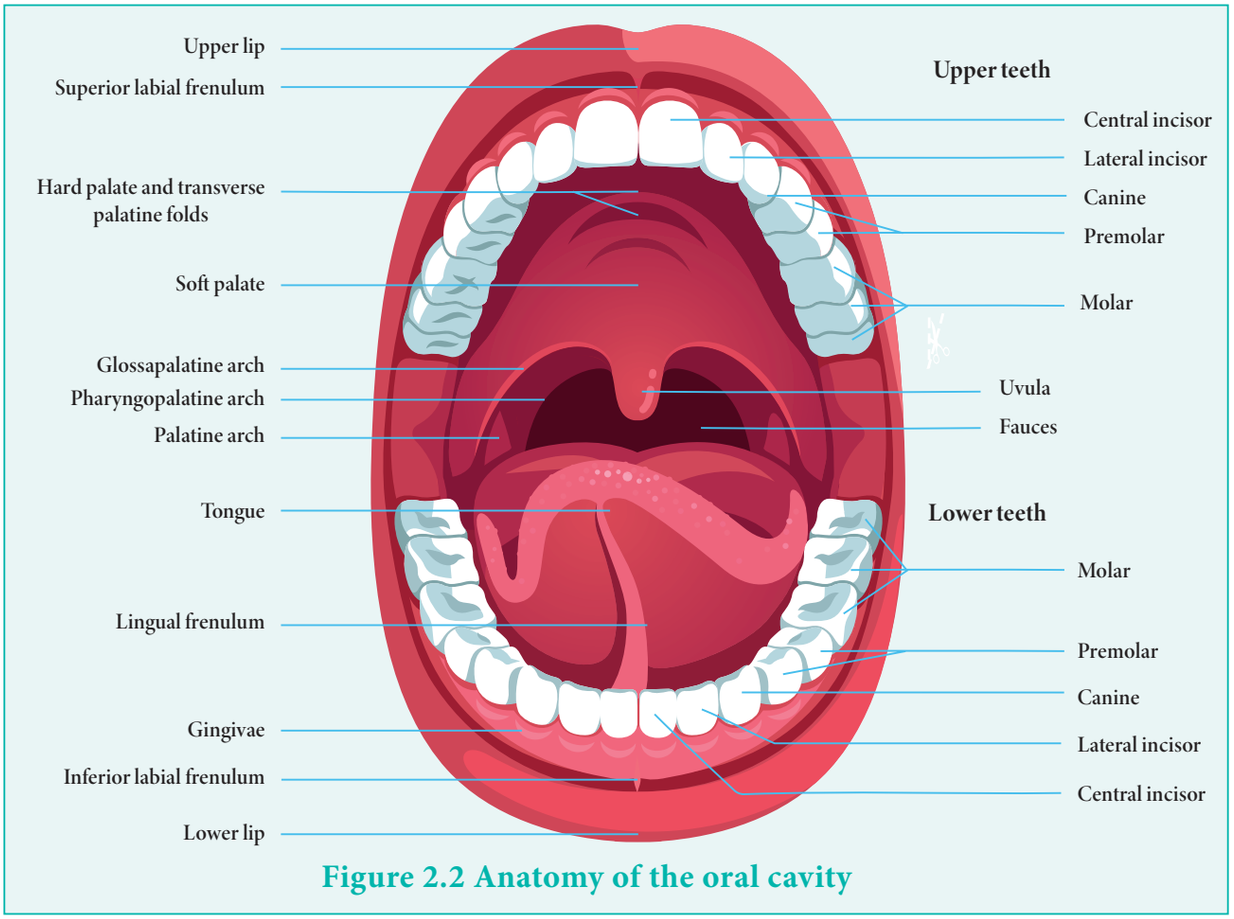



**Figure 2.2 Anatomy of the oral cavity**

The mouth or the oral or buccal cavity is made up of cheeks, hard and soft palates and the tongue along with the teeth embedded in the upper and lower jaws (Figure 2.2). The cheeks form the lateral walls of the oral cavity. The palate is the septum or the wall which separates the oral from the nasal cavity. The hard palate in the front is bony in structure, while the soft palate at the back is muscular in nature and separates the oropharynx from the nasopharynx. The soft palate accommodates a hanging muscular organ called uvula, meaning little grape. The tongue is a muscular organ attached by the frenulum to the floor of the buccal cavity and free in the front. It is involved in mastication of food, swallowing and also bears the taste buds. The tongue and uvula are responsible for the swallowing action. There are four types of teeth – incisors for cutting, canine for holding and tearing, premolars and molars for grinding the food. Human teeth are thecodont (embedded in socket of jaw bones), diphyodont (temporary and permanent sets of teeth) and heterodont in nature. The dental formula for each half of the upper and lower jaw of an adult is 2123/2123.

### 2.1.1.2 Pharynx

The pharynx is a funnel shaped structure that connects the oral and nasal cavity to oesophagus and trachea. The function of pharynx is to help in swallowing the bolus (masticated food). The whole of the pharyngeal stage of swallowing usually occurs within



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6 seconds. During this period, the swallowing center inhibits the respiratory center of the medulla, halting respiration, thereby allowing swallowing to proceed.

### 2.1.1.3 Oesophagus

The bolus passes from the pharynx into the oesophagus. Epiglottis (a cartilageneous flap) prevents the entry of food into the glottis, while the food is being swallowed. Tonsils, the lymphoid organs are present on either side of the pharynx. Oesophagus is the muscular tube located behind the trachea in the thorax, which connects the pharynx to the stomach. The function of oesophagus is to transport the bolus into the stomach. At the end of the esophagus, near the juncture with the stomach, there is a circular muscle called the gastroesophageal sphincter or cardiac sphincter. The gastric secretions are highly acidic and contain many proteolytic enzymes. The oesophageal mucosa, except in the lower one eighth of the oesophagus, is not capable of resisting the action of gastric secretions. The oesophageal sphincter helps to prevent significant reflux of stomach contents into the oesophagus. Gastro esophageal Reflux Disease (GERD) is a condition, which is associated with gastric reflux of acid into the oesophagus resulting in heartburn.

### 2.1.1.4 Stomach

The stomach is a J shaped enlargement of the GI tract that is divided anatomically into cardia, fundus, body, and pyloric part (Figure 2.3).

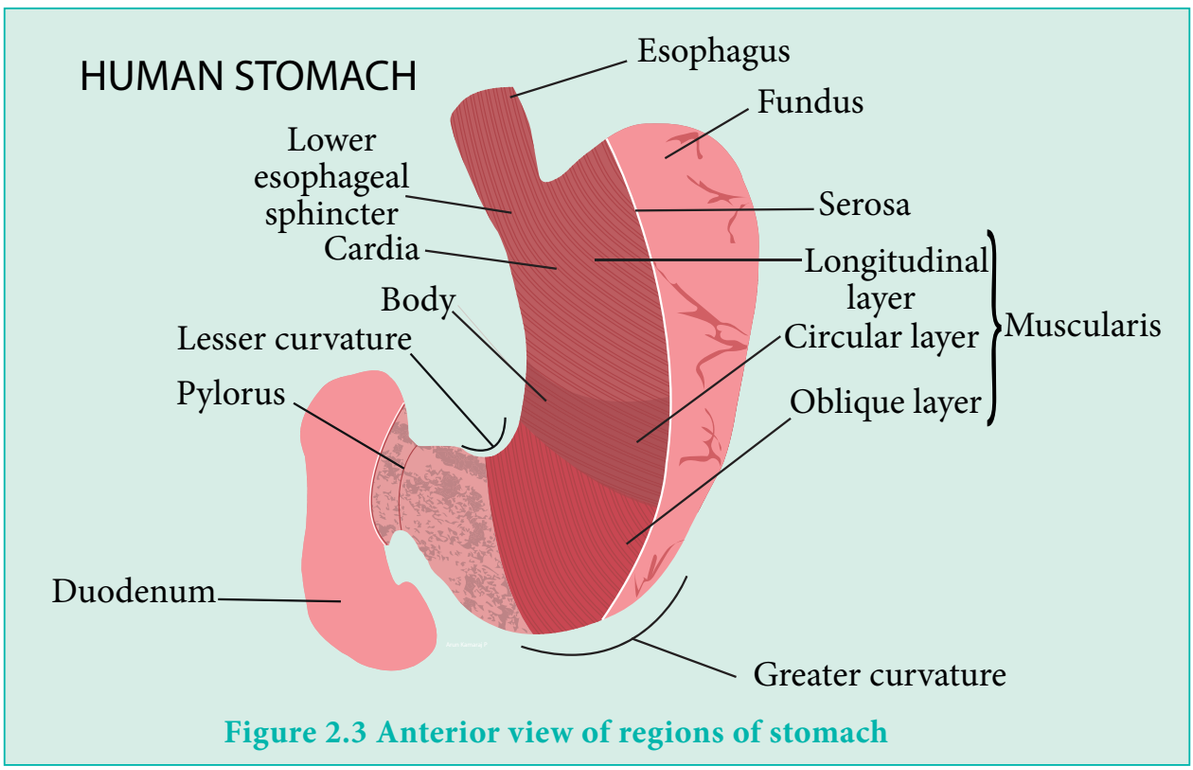



**Figure 2.3 Anterior view of regions of stomach**

The functions of the stomach are:

1. It mixes the semi-solid bolus with the gastric secretions to form a liquid called as chyme.



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2. Digestion cannot occur at the rate at which we eat food. Stomach acts as a storage organ and releases it into the duodenum at regulated intervals.
3. Certain vitamins like B12 are absorbed in the stomach.
4. It secretes gastric juice, which contains HCl that kills bacteria and denatures protein, pepsin that digests proteins and gastric lipase that aids in the digestion of triglycerides.
5. It also secretes gastrin into blood, which aids in gastric motility (movement).

When empty, the inner mucosal layers of the stomach fold into invaginations called rugae that unfold on intake of large meals. The pylorus of the stomach leads to duodenum of the small intestine via a smooth muscle sphincter called the pyloric sphincter.

### 2.1.1.5 The Small Intestine

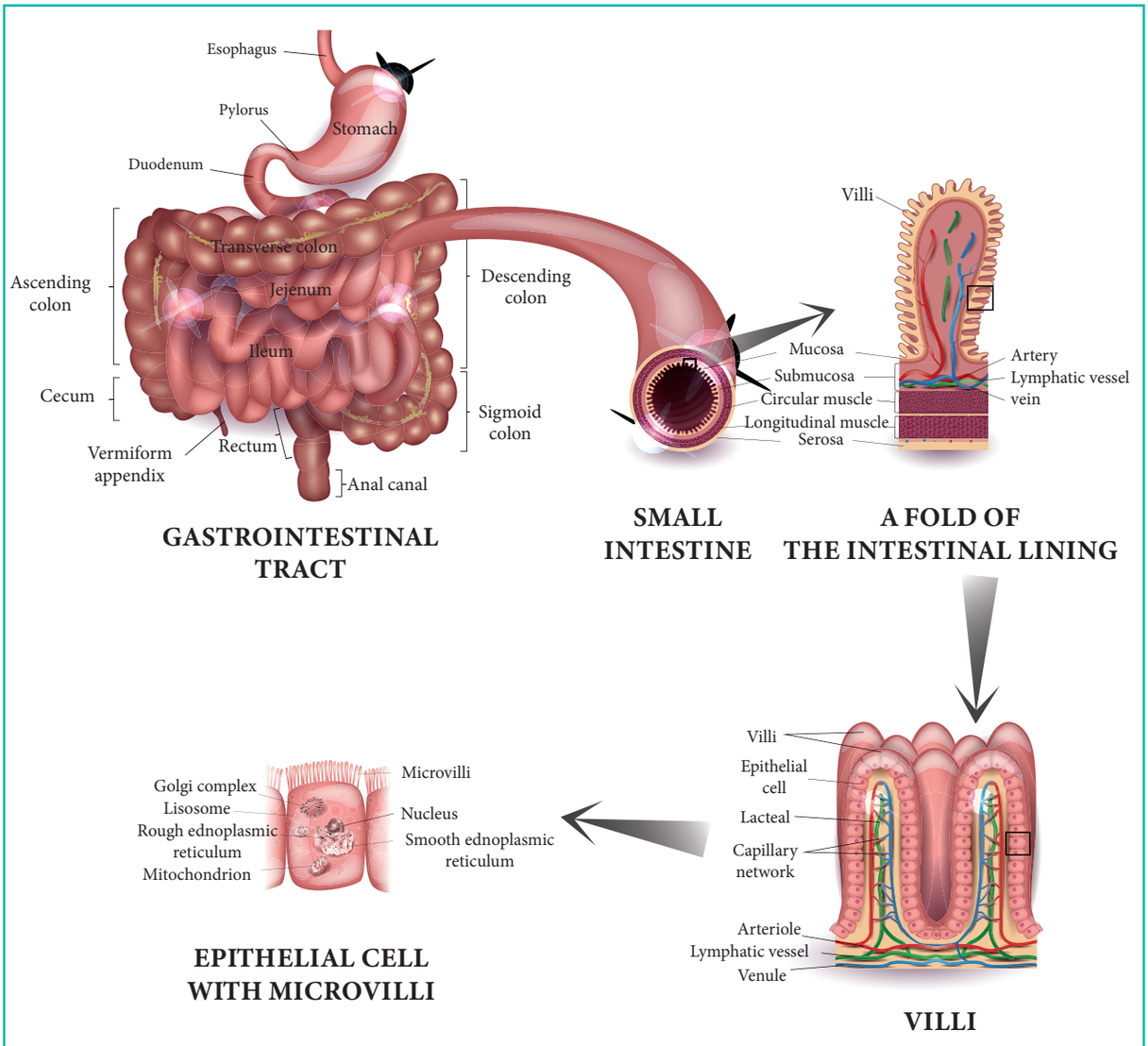



**Figure 2.4 Structure of small intestine and Villi**

The small intestine starts from the pyloric sphincter and opens into the large intestine. It has a diameter of about 2.5 cm and is 10 feet long in a living person. It is the region in the



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gastro intestinal tract where most of the digestion and absorption occurs. The small intestine has a large surface area due to the presence of villi or microvilli to aid absorption of nutrients.

The small intestine is divided into three regions: The duodenum, a C shaped tube, which starts at the pyloric sphincter that extends about 25 cm (10 inches) and merges with the jejunum. The jejunum is about 1 m (3 ft) long and extends to the ileum, the final and longest region of the small intestine, measuring about 2 m (6 ft); ileum joins the large intestine at a smooth muscle sphincter called the ileocecal sphincter (valve) (Figure 2.4).

**Structure of Villi**

An intestinal villus is a small finger like projection that extends into the lumen of the small intestine (Figure 2.5). Each villus has many microvilli projecting from its epithelial surface, collectively forming a brush border. The structure of villi aids in absorption of nutrients by providing large surface area and also have thin wall that reduces the distance for the nutrients to move across the intestine by a process called diffusion. Between the villi there are crypts, called crypts of Lieberkuhn, which are short glands. The Gut associated lymphoid tissue or the Peyer's patches are present in the small intestine and protect the intestine from the foreign pathogens.

Each villus consists of one cell thick thin epithelium surrounding the blood capillary and tiny lymphatic vessels called lacteals.

### 2.1.1.6 The Large Intestine

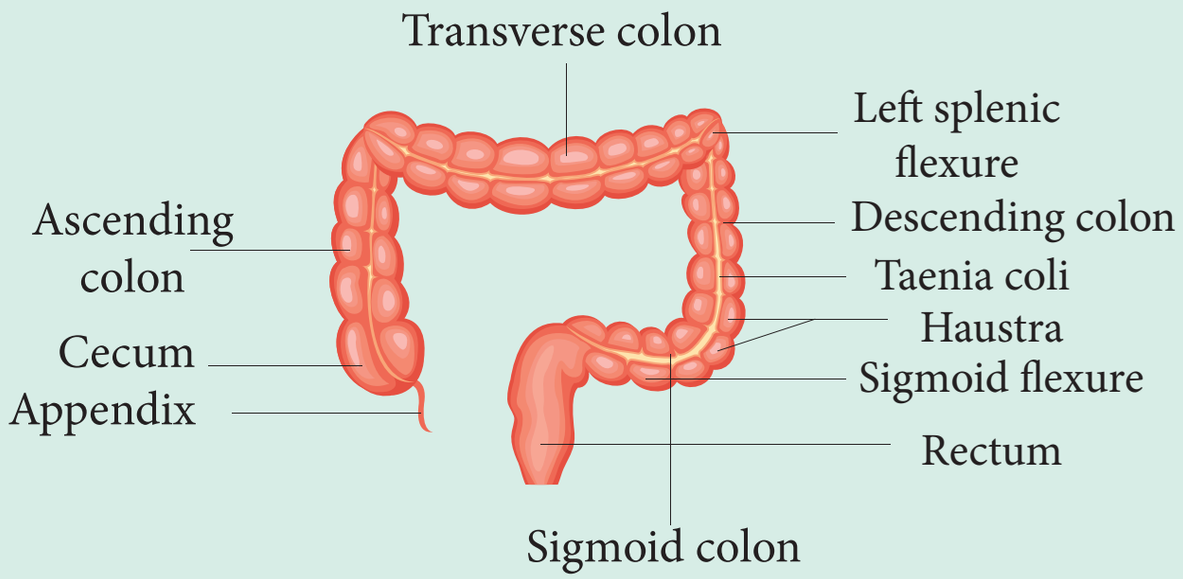



**Figure 2.5 Large Intestine, anterior view**

The large intestine includes the cecum, colon, rectum and the anal canal (Figure 2.5). It is larger in diameter than the small intestine (6.5 cm compared to 2.5 cm), but it is shorter in length. The large intestine absorbs water, salts, and some vitamins. The indigestible material is stored in the large intestine till it is eliminated via the anus. The cecum has a small projection called the vermiform appendix (vermiform -wormlike). The



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colon is divided into the ascending colon, which goes up in the right side of the abdomen to the level of the liver; the transverse colon that crosses the abdominal cavity below the liver and descending colon, that descends in the left side of the body; and the S shaped sigmoid colon, which enters the rectum, where feces is stored. When sufficient amount of feces is collected, the rectum opens at the anus, where defecation occurs. The internal and external anal sphincters help to keep the orifice closed. Getting rid of indigestible remains is the means by which the large intestine helps to maintain homeostasis.

### 2.1.2 Secretory Organs

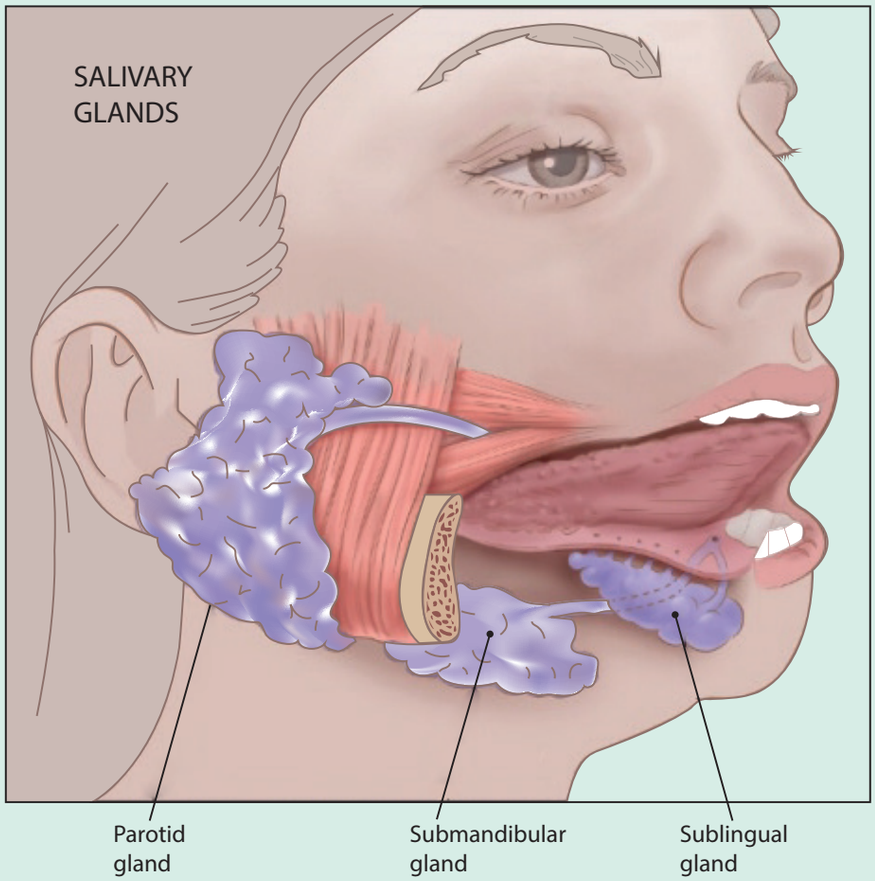



**Figure 2.6 location of salivary gland**

#### 2.1.2.1 Salivary Glands

There are three pairs of major salivary glands namely, the parotid glands present inferior and anterior to the ears, the submandibular glands located in the floor of the mouth and the sublingual glands beneath the tongue and few minor salivary glands that are present in the cheeks, lips and palate (Figure 2.6). The secretion called saliva from the major salivary glands reaches the mouth by Stenson's duct, Wharton's duct and Bartholin's duct. Approximately, an average man secretes about 1 to 1.5 liters of saliva per day. Saliva is 99.5% water and the remaining is mucus, electrolytes, anti-microbial agents and enzymes. The water helps in dissolving the food and thereby stimulates the taste



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receptors (gustatory receptors), while the chloride ions activate amylase to digest starch. The IgA in the saliva acts against the microbes. The saliva helps in rolling the food into a ball so that it can be swallowed.

### 2.1.2.2 Gastric glands

Gastric juice is secreted by the different types of exocrine cells present in the gastric glands lining the stomach. These glandular cells empty their secretions into the gastric pit. There are three types of exocrine cells: Intrinsic factor and HCl secreting parietal cells, chief cells that secrete pepsinogen and gastric lipase, and mucous secreting mucosal cells. The secretions of the mucous, parietal, and chief cells form gastric juice, which amounts to 2 – 3 liters per day. The G cells, which are entero-endocrine (endocrine cells of the GI tract) in nature located mainly in the pyloric antrum secrete gastrin into the bloodstream.

### 2.1.2.3 Liver

The liver is the largest organ in the human body and it lies in the upper right section of the abdomen, just below the diaphragm. The liver has two main lobes, the bigger right lobe and the smaller left lobe, which are further divided into lobules that serve as the structural and functional units of liver (Figure 2.7). A lobule consists of hepatic cells arranged in groups that radiate from a central vein. Hepatic sinusoids separate these groups of cells from each other. Kupffer cells, which are phagocytic in nature are attached to the lining of the hepatic sinusoids (Figure 2.9).

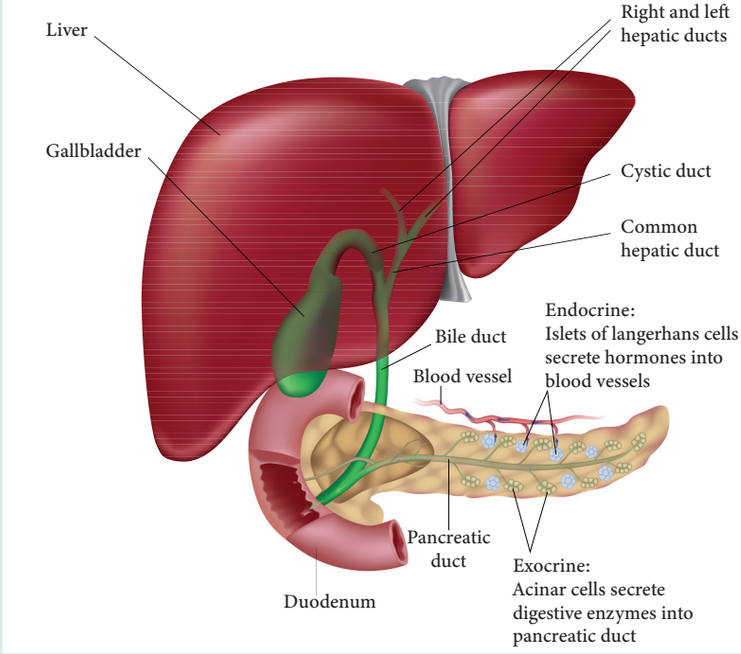



**Figure 2.7 Anterior view of Liver**

Every day, the hepatocytes secrete about 1 liter of bile, a yellow, olive-green or brown liquid with a pH of 7.6 – 8.6. Components of bile include bile salts, cholesterol, lecithin, bile pigments, and several ions.



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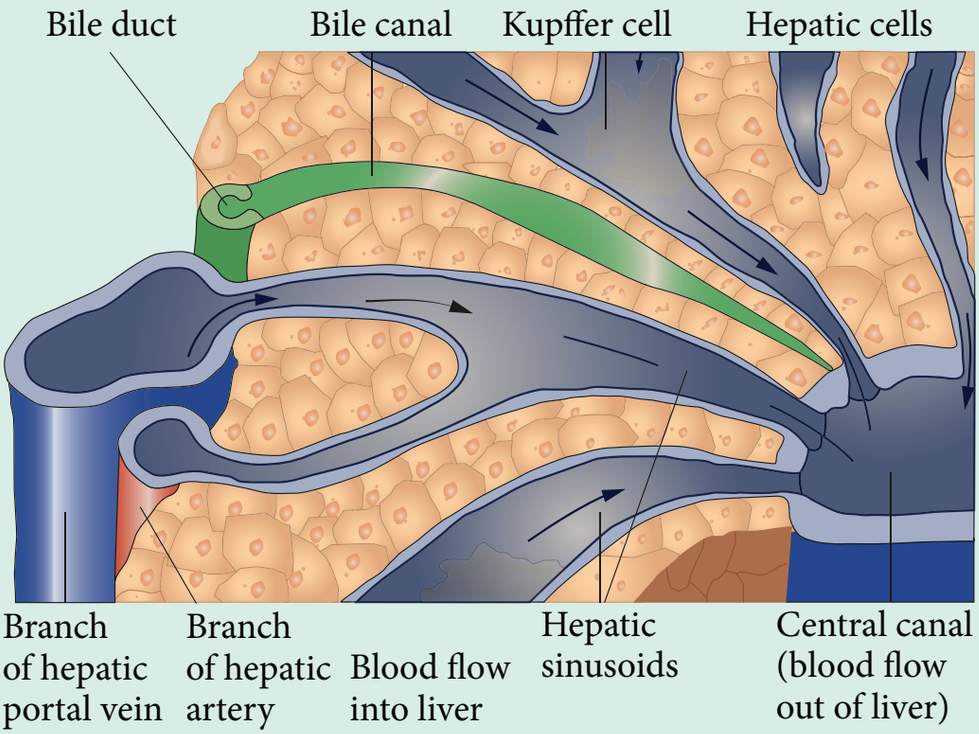



**Figure 2.8 Lobules of liver**

### Functions of liver are:

**Synthetic function**

- Synthesis, storage and release of bile for emulsification of fats
- Synthesis of plasma proteins

**Storage function**

- Stores fat-soluble vitamins and iron
- Stores glucose as glycogen and converts to fats and packages into lipoproteins to be stored in adipose tissue

**Homeostatic function**

Helps in maintenance of blood glucose

- Helps in cholesterol homeostasis by converting some amount of cholesterol to bile salts

**Detoxification function**

- Detoxifies ammonia to urea
- Helps in detoxification by removing poisonous substances
- Phagocytosis of worn out red and white blood cells

### 2.1.2.4 Gall bladder

The gallbladder is a pear-shaped sac, muscular in nature that is located in a depression on the inferior surface of the liver. Excess of bile is stored in the gallbladder. Water is reabsorbed from bile in the gallbladder making bile to become thick, mucus like material. The cystic duct from the gall bladder and the common hepatic duct join to form the



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common bile duct and enter into the duodenum. The cholesterol content of bile can crystallize and grow in size, leading to formation of gallstones. Sometimes, the stones might block the common bile duct and cause obstructive jaundice, in which case, the gallbladder is removed.

### 2.1.2.5 Pancreas

The pancreas lies deep in the abdominal cavity, behind the stomach in the upper left abdomen resting on the posterior abdominal wall. The broad portion of the pancreas is called as the head and it fills the loop formed by the duodenum. The narrow portion is called the tail. The pancreas has both endocrine (as it secretes glucagon and insulin into the blood stream) and exocrine function (by way of secreting pancreatic juice). Pancreatic acinar cells produce pancreatic juice, which is secreted into tiny tubes that merge forming a single pancreatic duct that extends the length of the pancreas. This pancreatic duct joins the common bile duct to form the hepatopancreatic duct Ampulla of Vater that is guarded by the Sphincter of Oddi.

### Glands of Lieberkuhn

These are tubular glands that lie between the villi of the inner surface of the small intestine.

### 2.1.2.6 Brunner's glands

The submucosa of the duodenum above the Oddi of Sphincter contains duodenal glands, also called Brunner's glands, which secrete alkaline mucus into the intestinal lumen. The functions of the mucus are:

- To neutralize the acid in the chyme
- To provide an alkaline pH enabling the intestinal enzymes to act
- To protect the intestinal walls

The secretions of the Brunner's gland and Glands of Lieberkuhn together contribute to intestinal juice. About 1 -2 liters of intestinal juice is secreted every day.

## 2.2. DIGESTION

| GASTROINTESTINAL TRACT | SECRETORY GLANDS |
|---|---|
| Oral cavity | Salivary glands |
| Pharynx | Gastric glands |
| Oesophagus | Liver and Gall bladder |
| Stomach | Pancreas |
| Small Intestine | Duodenal glands (Brunner's glands) |
| Large Intestine | |
| Rectum | |
| Anus | |



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### 2.2.1 MECHANICAL DIGESTION

The mechanical processes involved in digestion are:

1. Ingestion refers to intake of food by the mouth. The amount of food taken by an individual is dependent on hunger and appetite. Hunger is the need for food and appetite is the preferential desire for food.
2. Mastication is the process of chewing the food. It ensures that the food taken is broken down into smaller pieces and ground well. The mouth is equipped with teeth which are well designed for chewing. The incisors provide a strong cutting action and the molars a grinding action. All the jaw muscles when working together can close the teeth with a force as great as 55 pounds on the incisors and 200 pounds on the molars.
3. Deglutition is the act of swallowing which involves the voluntary squeezing or rolling action which pushes the food into the pharynx and the involuntary pharyngeal action that pushes the food into oesophagus.
4. Peristalsis is the wave like motion of the esophagus that propels the food into the stomach (Figure 2.9).
5. Mixing and propulsion - Alternating contractions and relaxations of smooth muscle in the walls of the GI tract mix the food with the secretions of the GI tract and propel them forward. This action of the intestine is called as motility.

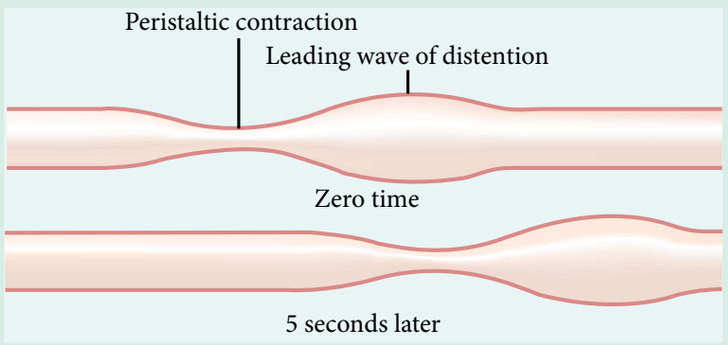



**Figure 2.9 Peristaltic Movements**

6. Defecation- Digested constituents that were not absorbed in the digestive tract and undigested waste leave the body through the anus in a process called defecation. The eliminated material is termed as feces or stool.

### 2.2.2 CHEMICAL DIGESTION

#### 2.2.2.1 Digestion and absorption of carbohydrates

The food we eat contains abundant carbohydrates as they provide a major share of daily caloric requirement. Dietary carbohydrates consist of both digestible compounds such as starch, glycogen, lactose, maltose and sucrose and indigestible fibers such as cellulose and hemi-cellulose (Figure 2.10).

Digestion of carbohydrates takes place briefly in the mouth and largely in the n intestine. The polysaccharides get hydrated during cooking, which is essential for efficient digestion.



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Two types of enzymes are required for digestion of carbohydrates – Amylases and Disaccharidases. Amylases are present in saliva and pancreatic juice, while disaccharidases are present in the brush border membrane of the intestine (Figure 2.11).

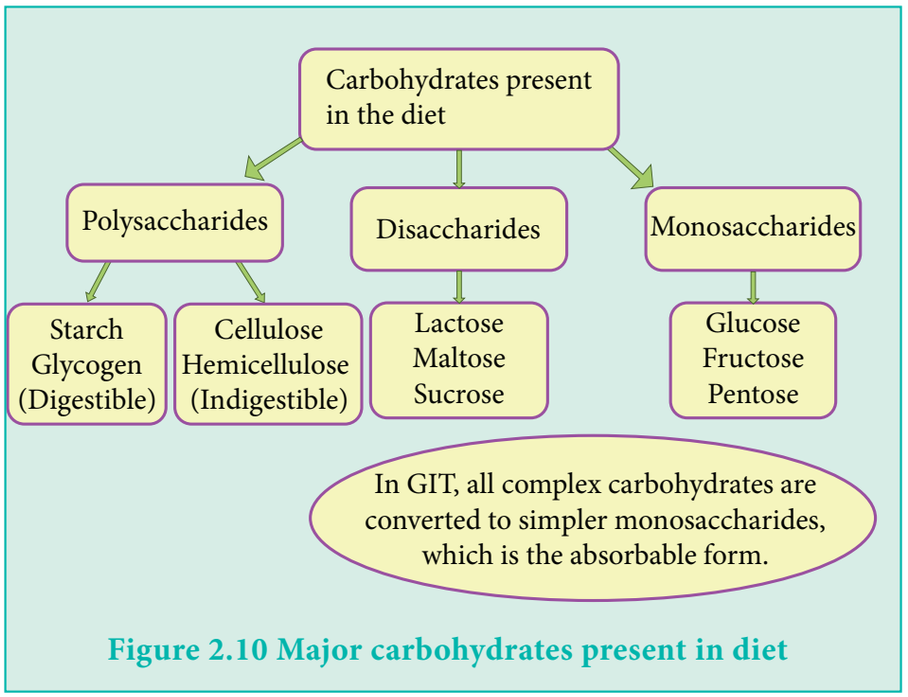



**Figure 2.10 Major carbohydrates present in diet**

## Digestion in mouth

Simpler carbohydrates and carbohydrates present in milk and juices escape digestion in mouth. The digestion of the digestible polysaccharides like starch and glycogen begins in mouth in humans by action of the salivary enzyme, Ptyalin, also called as α-amylase. This enzyme with an optimum pH of 6.8, hydrolyzes the α1→4 glycosidic bonds to release smaller oligosaccharide fragments and a highly branched limit dextrin (as the inner α1→4 linkages are inaccessible to the salivary amylase) (Figure 2.12). However, the digestion of carbohydrates by salivary amylase is limited because of its lower residual time in the mouth and oesophagus. As soon as the bolus reaches the stomach, the salivary amylase is inactivated because of the prevailing low pH.

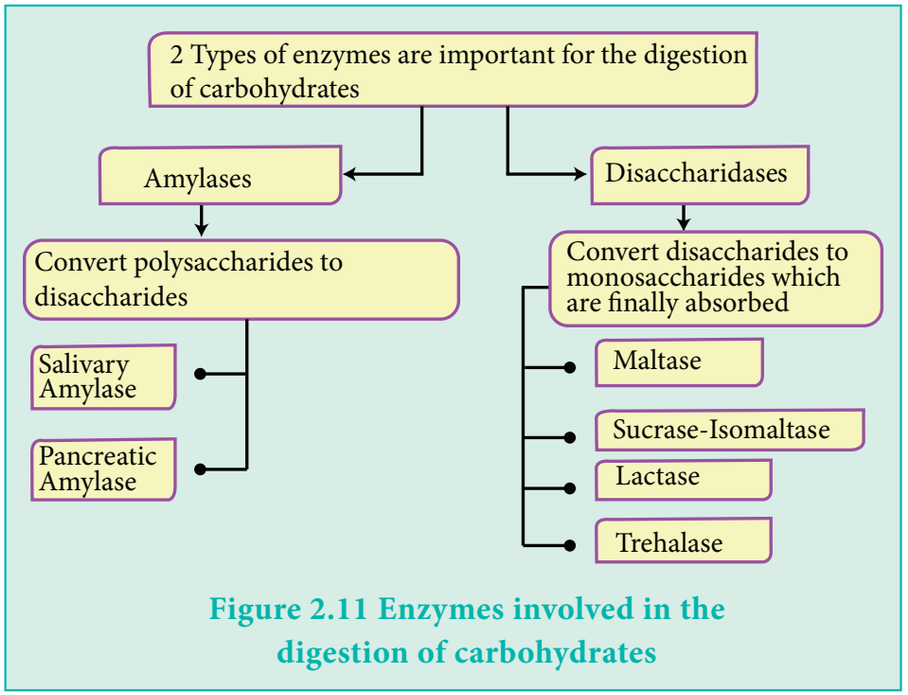



**Figure 2.11 Enzymes involved in the digestion of carbohydrates**



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### Digestion in stomach

There are no enzymes present in the stomach to digest carbohydrates, despite the evidences citing the presence of gastric amylase, which is of lesser significance. Further, the low pH of stomach inactivates salivary amylase. The major digestive action of stomach on carbohydrates is that it helps in the cleavage of the glycosidic bonds present in sucrose. No further digestion of carbohydrates occurs in the stomach and the chyme passes into the duodenum.

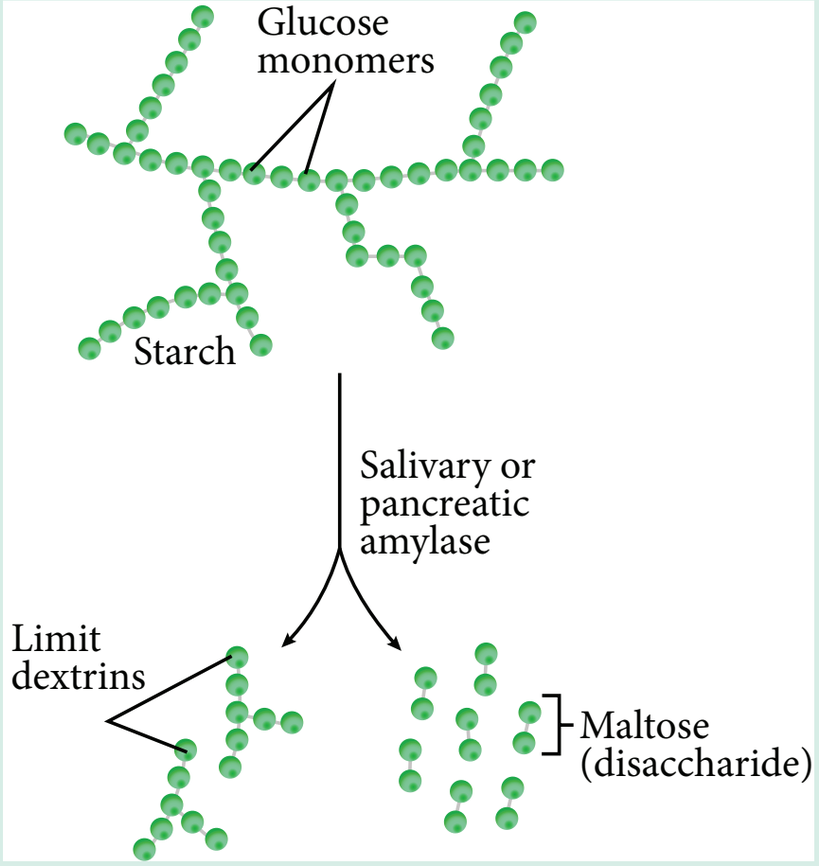



Figure 2.12 Action of amylases

### Digestion in duodenum

The chyme on reaching the duodenum, gets mixed up with the bile juice and pancreatic juice that contains bicarbonate and gets neutralized. The pancreatic amylase, which is similar to the salivary amylase in enzymatic action, with optimum pH 6.9 to 7.1, acts upon the polysaccharides and breaks down the starch and glycogen to disaccharides like maltose and isomaltose (which contains α1→6 linkage)

### Digestion in small intestine

Pancreatic amylase also requires chloride ion for its action and acts on the complex and partially digested carbohydrates. Pancreatic amylase completes the digestion of polysaccharides in the intestine because of

- its ability to break the interior linkages of complex carbohydrates
- the longer residual time of food in the duodenum and intestine

Moreover, pancreas secrete ten times more of pancreatic amylase into the intestinal lumen than that required to digest the carbohydrates in the diet.

### Disaccharidases

There are four important disaccharidases in the human intestinal brush border membrane to hydrolyse the disaccharides (Table 2.1). They are:



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**Sucrase**: Sucrase which acts upon sucrose to cleave it into equimolar quantities of glucose and fructose has an optimum pH of 6.

**Isomaltase:** Isomaltase co-elutes with Sucrase on purification and therefore Sucrase/ isomaltase is a complex protein. Isomaltase acts on the α1→6 linkage and cleaves it into two molecules of glucose.

*Isomall*tose $\xrightarrow{\text{Isomaltase}}$ Glucose+ Glucose

**Lactase:** The β 1→4 glycosidic linkage of lactose is acted upon by Lactase and cleaves it into equimolar quantities of glucose and galactose. The optimum pH for *sucrase* is the optimal pH for *lactase* which is around 6, but it can function in an acidic environment ranging between a pH of 2 to 7.

lactose $\xrightarrow{\text{Lactase}}$ Glucose + Galactose

**Maltase:** Maltose, the end product of polysaccharide digestion by amylases is cleaved into two glucose molecules by cleavage of α1→4 linkage by maltase to liberate glucose into the lumen. It also has an optimum pH around 6 (Figure 2.13).

*Mal*tose $\xrightarrow{\text{Maltase}}$ Glucose+ Glucose

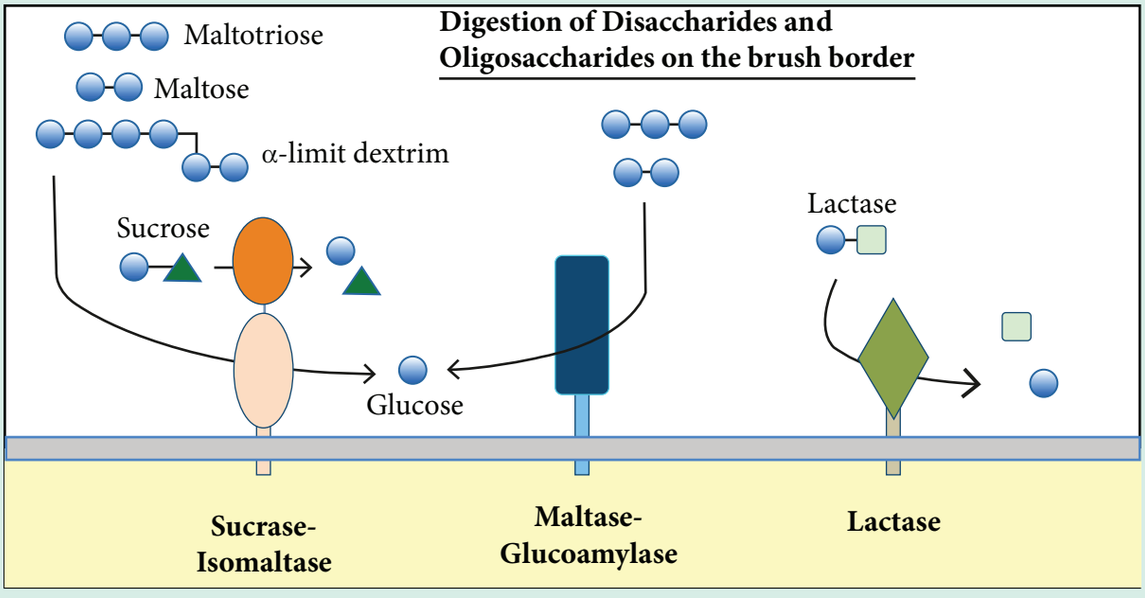



**Figure 2.13 Digestion of disaccharides on the brush border**

**Cerebrosidases :** Cerebrosidases are a group of minor enzymes that hydrolyse the gluco and galactocerebrosides present in glycolipids.



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**Table 2.1 Disaccharidases and oligosaccharidases of the intestinal brush border**

| Enzyme | Cleavage Specificity |
|---|---|
| Maltase | Maltose, maltotriose; also acts as exoglycosidase on $\alpha(1 \rightarrow 4)$ bonds at the non-reducing end of starch and starch – derived oligosaccharides |
| Lactase* | Lactose; also cellobiose# |
| Cerebrosidase* | Gluco – and galactocerebroside |
| Sucrase | Sucrose; also maltose and maltotriose |
| Isomaltase | $\alpha(1 \rightarrow 6)$ bonds in isomaltose and $\alpha$ –limit dextrins |
| Trehalase | • Trehalose |

* The lactase and cerebrosidase activities reside in two different globular domains of the same polypeptide.
# Cellobiose is a disaccharide of two glucose residues in $\beta(1 \rightarrow 4)$ glycosidic linkage.
• Trehalose is a disaccharide with a structure of ∝-D-glucopyranosy l -∝ -D-glucopyranoside (in 1,1 glycosidic linkage); common only in mushrooms.

## Absorption of carbohydrates

- Only monosaccharides can be absorbed by the villi. However, few disaccharides are taken up by pinocytosis and later hydrolysed to monosaccharides.
- The principal monosaccharides that are present in the diet or formed as end products of digestion are glucose (80%), galactose, fructose and pentoses like ribose, xylulose and arabinose.
- The absorption of monosaccharides mainly takes place in the duodenum and the upper part of the small intestine.
- The order of absorption of important monosaccharides are :
  Galactose > Glucose > Fructose > Mannose > Pentoses
- Monosaccharides are absorbed by both passive diffusion as well as active transport. Different monosaccharides are absorbed in different ways. Glucose and Galactose are the prime monosaccharides that are absorbed actively, while fructose and pentoses are absorbed passively by facilitated diffusion.

## Facilitated diffusion

Initially, when the glucose concentration in the digested food is very high, facilitated diffusion plays a major role in absorption. Since glucose is a highly polar molecule, glucose is absorbed passively by a transporter, GLUT2 (Glucose Transporter 2). Fructose is absorbed by GLUT5 transporter in a passive manner.



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## Active Transport

Active transport is mediated by a protein called as SGLT1 (Sodium Glucose Transporter 1) in the luminal side and GLUT 2 on the basolateral side (Figure 2.14). The events involved in the transport are:

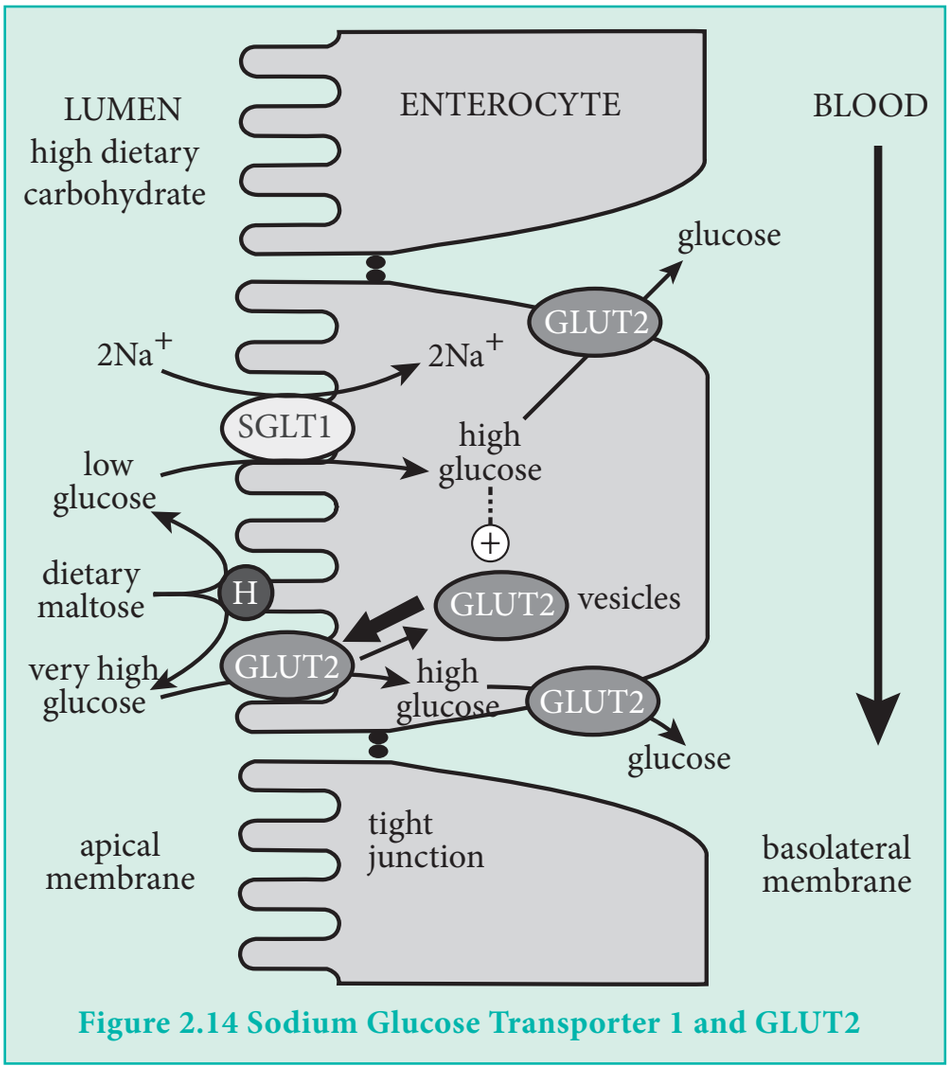



**Figure 2.14 Sodium Glucose Transporter 1 and GLUT2**

1. A high concentration of $K^+$ and low concentration of $Na^+$ is maintained inside the intestinal brush border membrane enterocytes with the help of $Na^+$-$K^+$ ATPases, which utilize ATP for its pumping action.
2. SGLT1 is a symporter, which can bind $Na^+$ and glucose from the gut lumen. With the energy obtained by downhill transport of sodium, glucose is transported in a uphill manner, ie.against the concentration gradient, resulting in high concentrations of glucose inside the enterocyte. The steps involved in this transport are :

- The transporter is initially facing the lumen and oriented such that it can bind sodium and not glucose.
- After the binding of sodium, a conformational change is induced that opens the glucose-binding pocket
- Glucose binding induces a conformational change so that the transporter now faces the cytosol.
- Sodium and glucose dissociate into the cytoplasm
- After delivering the cargo, the transporter reorients back to the luminal side.



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3. GLUT2 on the basolateral membrane, by facilitated diffusion, transports the glucose from the enterocytes into the blood circulation.

- Glucose and galactose are absorbed by the enterocyte by cotransport with sodium using the same transporter.
- Thus, the sodium dependent SGLT1 and sodium independent GLUT2 play major role in absorption of glucose.

Carbohydrates that are not digested and absorbed in the digestive tract so far will reach the large intestine where they are broken down by intestinal bacteria. Indigestible fibers and other carbohydrates are excreted with feces.

### Factors affecting absorption

- Absorption is high with intact mucosa. Any damage to the intestinal mucosa in the form of infections and congenital disorders reduce the rate of absorption.
- Hormones such as thyroid hormones, pituitary hormones and mineralocorticoids increase the absorption, while insulin has no effect on absorption of carbohydrates.
- Vitamin B6, B12 and pantothenic acid deficiencies decrease the absorption of monosaccharides.
- Advancing age decreases the absorption.
- Inhibitors of carbohydrate digestive enzymes and SGLT1 inhibitors like phlorizin decrease the absorption.

## 2.2.2.2 Digestion and absorption of proteins

Proteins have to be broken down to it's constituent amino acids for absorption into the gut. The digestion and absorption of proteins is very effective in a healthy human. The major contribution of the protein is from diets rich in protein like milk, meat, egg, pulses, soybean, nuts, etc. and minor contribution is from the proteins present in the digestive juices and worn out intestinal epithelial cells that are shed into the lumen.

### Digestion in mouth

Proteolytic enzymes are not present in saliva and therefore, no digestion of proteins takes place in the mouth. However, chewing the protein-rich foods especially the harder ones like meat increases the surface area of the food particles and thereby allows digestion to occur more quickly.

### Digestion in stomach

In the stomach the proteins are converted into: Protein → Metaprotein → Proteone → Peptone → Peptide

**HCl:** The partially denatured proteins (denaturation occurring due to cooking process) or the non-denatured proteins present in the raw foods are further denatured by the highly acidic environment present in the stomach, which unveils the sites for action of the peptidases. HCl also initiates the conversion of inactive pepsinogen to active enzyme pepsin.



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**Pepsin:** Pepsin is secreted in the form of inactive pepsinogen, a protein with a molecular weight of 42 kD. Pepsinogen is cleaved into active pepsin of 34 kD, initially by the acidic medium, and then autocatalytically.

### Pepsin

- is an endopeptidase (an enzyme that acts in the interior of the protein)
- has a broad range of specificity
- acts on the peptide linkages contributed by the carboxyl group of aromatic amino acids / acidic amino acids, to a lesser extent.
- breaks down proteins to peptones.
- works optimally at pH 2.0. Hence, any factor that reduces acidity of the stomach like achlorhydria or antacid intake that neutralizes stomach acid or proton pump inhibitors reduces the activity of pepsin.

**Rennin:** Rennin, also called as chymosin is an enzyme that is responsible for curdling of milk and is present only in infants and children. It is responsible for conversion of milk protein into Paracaseinate.

**Gelatinase:** Gelatinase helps in the digestion of gelatin and type IV and V collagen, which are proteoglycans present in meat. Gastricsin is another enzyme present in the gastric juice that digests proteins.

### Digestion in duodenum

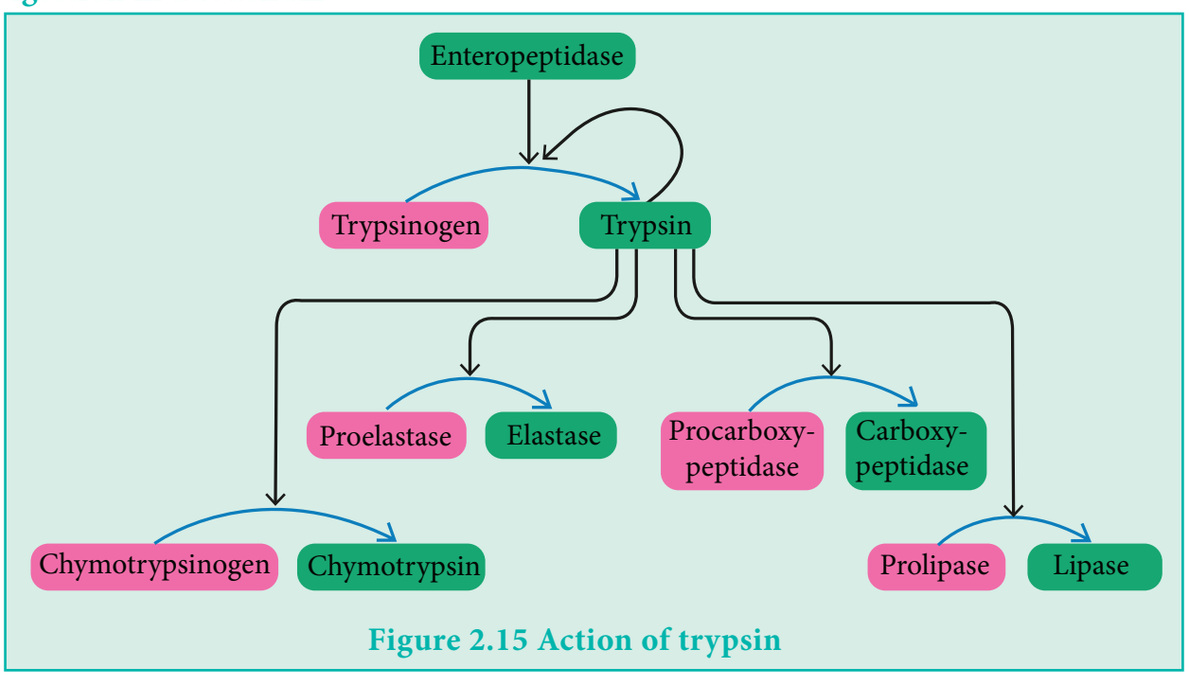



**Figure 2.15 Action of trypsin**

**Enteropeptidase:** Enteropeptidase or Enterokinase is a transmembrane protein expressed by the epithelial cells of the jejunum and the duodenum. It has a substrate specificity of Lys-Lys or Arg-Lys and is involved in activation of trypsin.



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Human pancreatic juice contains zymogens of proteolytic enzymes like trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, pro-elastase along with trypsin inhibitor.

**Trypsin:** Trypsinogen in the pancreatic juice is activated to trypsin by enterokinase and subsequently stabilized by calcium. Trypsin also converts chymotrypsinogen to chymotrypsin, proelastase to elastase and procarboxypeptidases to carboxypeptidases (Figure 2.15). It acts upon the peptide linkages contributed by carboxyl group of Lys and Arg (Figure 2.16). It is ineffective in hydrolyzing the peptide linkages formed by proline. It has an optimum pH of 8-9. Trypsin-inhibitor present in the pancreatic juice prevents the action of trypsin in small intestine and pancreas.

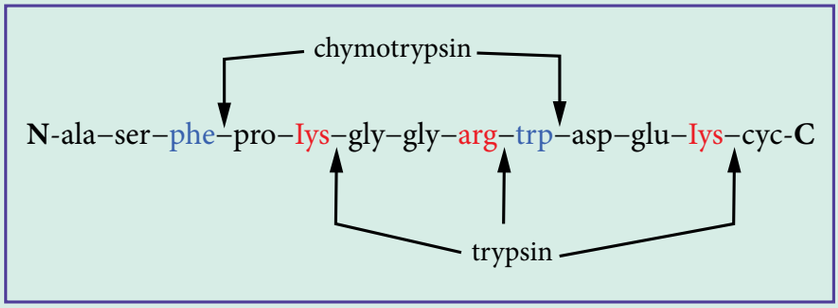



**Figure 2.16 Site of action of chymotrypsin and trypsin**

## Chymotrypsin

Chymotrypsin is also an endopeptidase secreted in the form of its zymogen, chymotrypsinogen, by the pancreas. Its optimum pH is around 8.0. Chymotrypsin catalyzes the hydrolysis of peptide bonds involving the carboxyl group of hydrophobic amino acids like phenylalanine, and tryptophan (Figure 2.17).

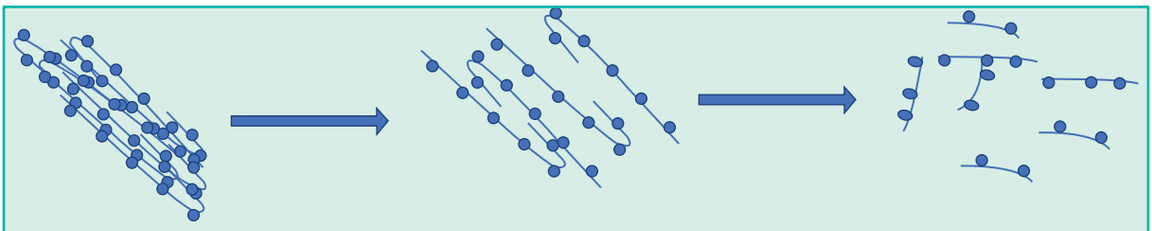


**Figure 2.17 Hydrolysis of peptide bond by chymotrypsin**

## Carboxypeptidases

Carboxypeptidases A and B are zinc containing metallo-enzymes that cleave the peptide bond at the carboxyl or C-terminal end of peptides with a greater preference for aromatic and branched chain amino acids by Carboxypeptidase A and for basic amino acids by Carboxypeptidase B. Elastases and Gelatinases also contribute to the digestion of the proteins. Many dipeptides and tripeptides along with free amino acids are formed in the lumen by the combined action of pancreatic peptidases.



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## Digestion in small intestine

### Digestion at the Brush Border

The di and tripeptides formed by the pancreatic enzymes are finally digested by the amino-peptidases and di and tripeptidases present on the luminal side of the intestinal brush border membrane. Leucine aminopeptidase that releases the N-terminal leucine residue and Proline amino peptidase specific for proline at the end of polypeptides, along with the other brush border membrane enzymes, will bring about the complete digestion of proteins.

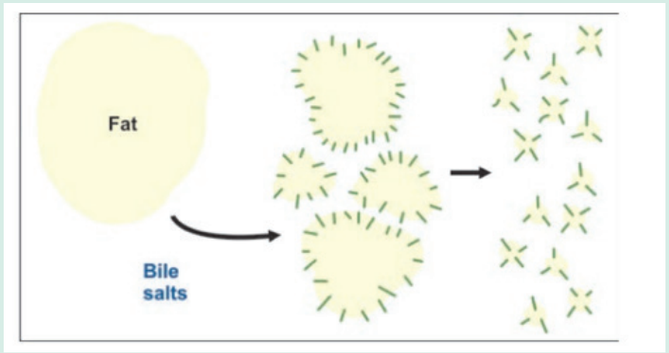



Figure 2.18 Digestion in small intestine

### Absorption of amino acids

1. At physiologic temperature, the amino acids and to some extent, the di and tripeptides are absorbed actively by sodium dependent transporters present in the villi. D amino acids produced by bacteria are absorbed passively, while L amino acids are absorbed actively.

At least five brush border transporters exist - Neutral amino acids (uncharged aliphatic and aromatic), basic amino acids (Lys, Arg, Cys, Cys-Cys), acidic amino acids (Asp, Glu), imino acids (Pro, Hydroxyproline) and di- and tripeptides.

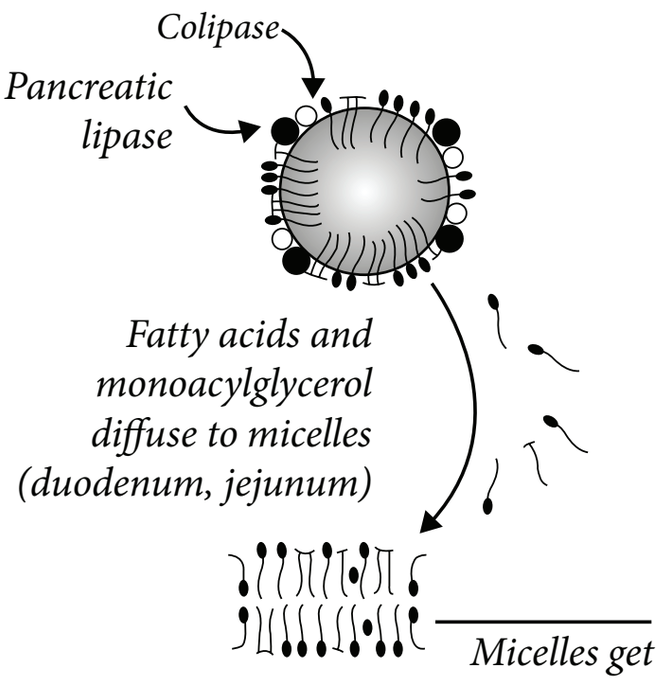



Figure 2.19 Action of pancreatic lipase

### Meister Cycle (Gamma Glutamyl Cycle)

Absorption of neutral amino acids is facilitated by the gamma glutamyl cycle. Glutathione (GSH-gamma glutamyl cysteinyl glycine), a tripeptide is involved in this cycle (Figure 2.18) The steps involved in the reaction are:



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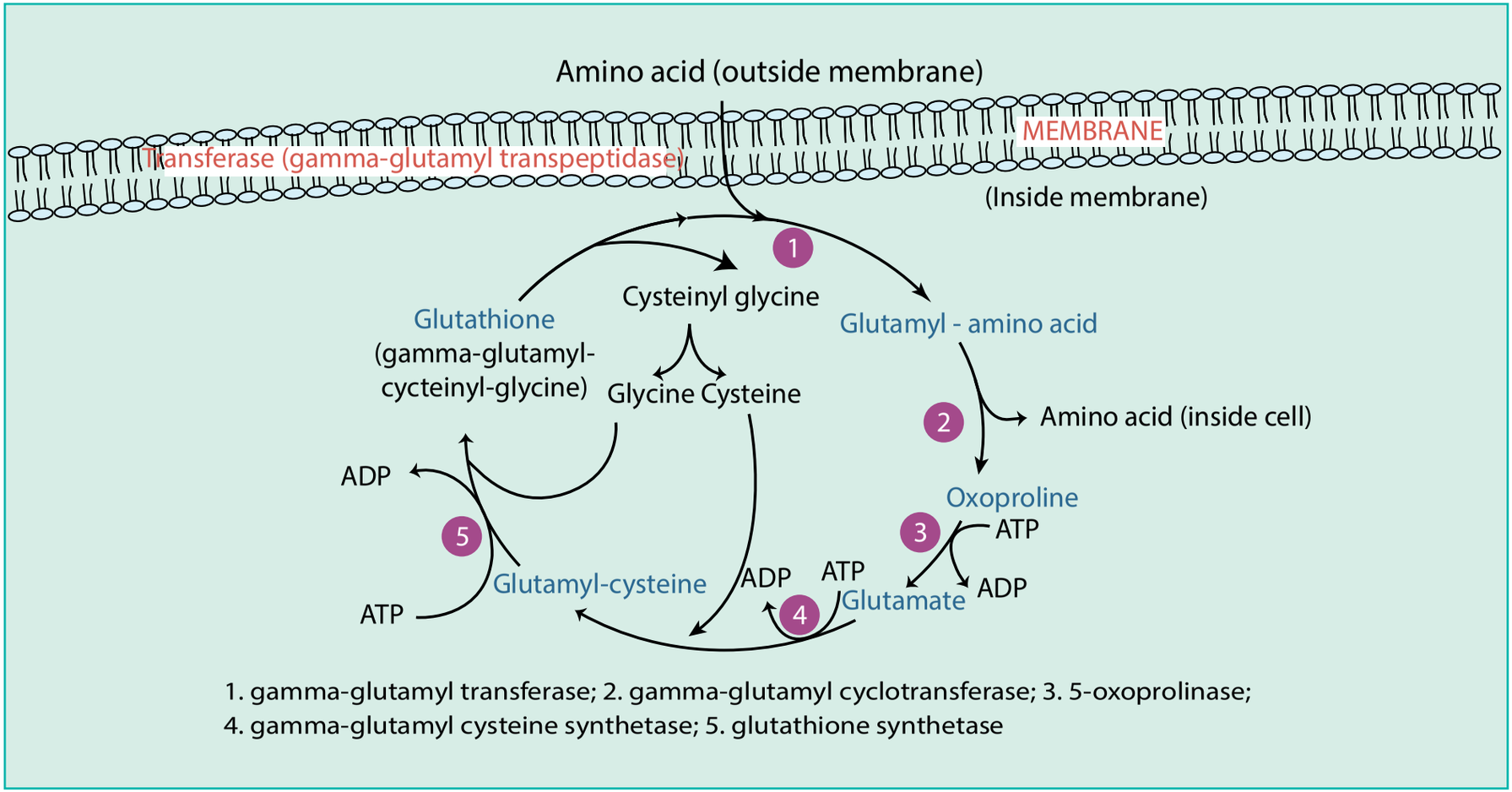



**Figure 2.20 Gamma gutamyl cycle (Meister cycle)**

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1. Gamma glutamyl transferase catalyses the reaction of glutathione (inside the cell) with the neutral amino acid (in the lumen) to form gamma glutamyl amino acid and cysteinyl glycine in the cytoplasm of the brush border membrane cell.
2. The glutamyl amino acid is then cleaved to give the free amino acid and oxo-proline inside the cytosol. The net result is the transfer of an amino acid across the membrane.
3. Oxo-proline is converted to glutamate. Cysteinyl glycine is cleaved into cysteine and glycine.
4. Glutathione is regenerated from the three amino acids.
5. The transport of one molecule of any amino acid along with regeneration of GSH requires 3 molecules of ATP.

After release into the cytosol, the amino acids are released into the blood stream and are transported to the liver by the entero-hepatic circulation.

Some oligopeptides, larger peptides or proteins can cross the brush border membrane by a process called transcytosis. This particularly happens in infants whereby the imunoglobulins in mother's milk can be transferred to the child. The enterocytes located in the Crypts of Lieberkuhn are generally involved in this process. However, this mechanism operates only to a lesser extent in adults.

### Factors affecting absorption

- Glutathione is required for absorption of amino acids and its regeneration should be effective for good absorption.
- Amino acids transported by the same transporter compete with each other for absorption. Therefore, high concentrations of one particular amino acid will have an influence on the other.
- Salicylates decrease the intestinal absorption of amino acids, particularly that of tryptophan
- Dinitrophenol and indomethacin also inhibit the absorption of amino acids.

### 2.2.2.3 Digestion and absorption of lipids

The major difficulty in digestion of fats is that all the enzymes that digest fats are hydrophilic in nature, while fats are insoluble in water. This problem is circumvented by a process called emulsification. During emulsification, the fat is dispersed into smaller droplets, and their surface tension is reduced; and surface area is increased.

**This process is facilitated by :**

- the Detergent action of the Bile salts.
- Mechanical mixing i.e. chewing and peristalsis, where the surface area is increased.
- Presence of Phospholipids for micelle formation.



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### Digestion in mouth

The digestion of fat starts in the mouth by the action of Lingual lipase, which has an optimum pH between 4.5 and 5.4. It acts on short chain triglycerides that are typically present in milk, butter and ghee. The action of lingual lipase is more important for new born infants and children (Table 2.2).

**Table 2.2 Various lipases and the site of action**

| Lipase | Site of action | Preferred substrate | Products (s) |
|---|---|---|---|
| Lingual/acid - stable lipase | Mouth, stomach | TAGs with medium / short chain FAs | FFA+DAG |
| Pancreatic lipase + co-lipase | Small intestine | TAGs with long - chain FAs | 2 FFA+MAG |
| Intestinal lipase with bile acids | Small intestine | TAGs with medium chain FAs | 3 FFA+ glycerol |
| Phospho-lipase $A_2$ + bile acids | Small intestine | PLs with unsat. FA on position 2 | Unsat FFA + lysolecithin |

### Digestion in stomach

Gastric lipase with an optimum pH of 4 -5 is secreted into the stomach by the chief cells. This also acts on breaking down fat. However, the gastric lipase digests the fat to a minimal extent because of the prevailing high acidic pH and absence of emulsification. But, unlike pepsin, it is not dependent on an acid pH, and therefore remains active in the small intestine, and constitutes about 30% of the total lipase secreted over a 3-hour period. Gastric lipase is not essential in fat digestion, but resulting fatty acids and peptides formed by pepsin help to coordinate gastric emptying and pancreatic secretion.

**Satiety:** Fats have a very high satiety value ( fullness of the stomach), by inhibiting the gastric motility ( movement of the chyme from stomach to duodenum). This effect of dietary lipids is mediated by the hormone enterogastrone.

### Digestion in duodenum

The bile (pH 7.7) entering the duodenum serves to neutralize the acid chyme from the stomach and provides a pH favorable for the action of pancreatic enzymes and small intestine (Figure 2.19).

The hydrophobic regions of bile salts intercalate into the lipid aggregates, such that the hydrophilic domains face the surface resulting in the breakdown of larger aggregates into small droplets. This increases the surface area for action of lipase.

The lipolytic enzymes present in the pancreatic secretions are:



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### 1. Pancreatic lipase with Co-lipase

Pancreatic lipase can hydrolyse the ester linkages at the 1st and 3rd carbon atoms of glycerol forming 2-monoacylglycerol. The fatty acid in the second position is shifted to 1st position by an isomerase, the resulting 1 monoacylglycerol is then hydrolysed by the lipase to form glycerol and fatty acid. Co-lipase is a protein that binds to the triacylglycerol molecules at the interface, and this is essential for the action of lipase. The co-lipase is also secreted by the pancreas as zymogen, which is activated by trypsin (Figure 2.20).

Cholesterol esters are hydrolysed to free cholesterol and fatty acid by cholesterol esterase.

The phospholipid phosphatidyl choline is the second most abundant lipid in the intestinal lumen, after triglyceride. Phosphatidyl choline and cholesterol enter the gut via bile and it contributes about three fourths of the gut lipid content. Lingual and gastric lipases are ineffective in hydrolyzing phosphatidyl choline. Pancreatic phospholipase A2 (PLA2) and other lipases secreted act upon the phospholipids. The action of phospholipase A2 produces lysophospholipid and a fatty acid.

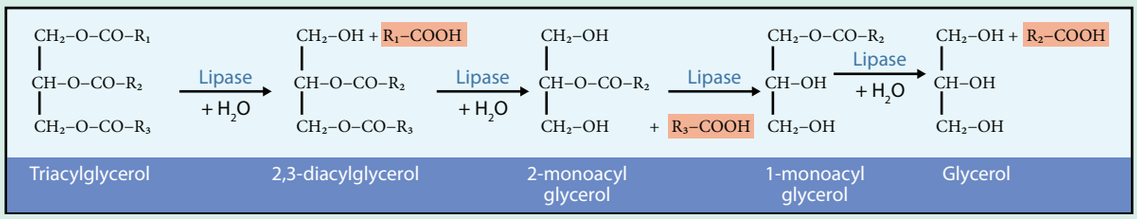



**Figure 2.21 Complete hydrolysis of triglycerides**

## Absorption of lipids

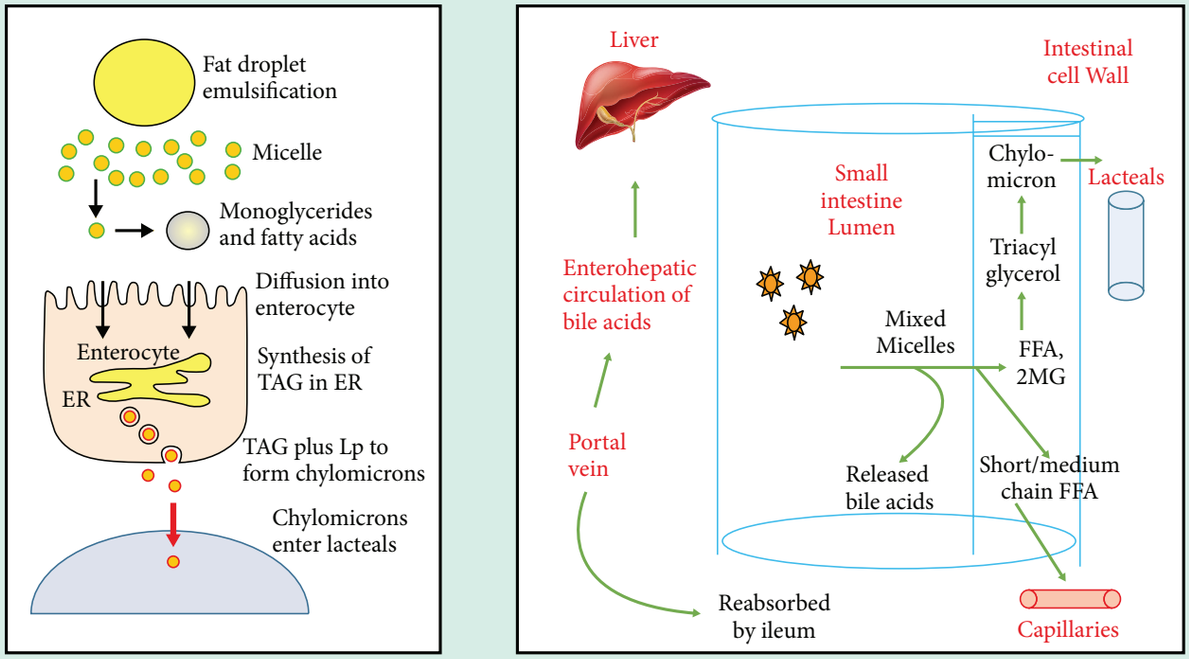



**Figure 2.22 Absorption of fat as a chylomicron**



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Fats are absorbed by passive diffusion mainly in the proximal jejunum (Figure 2.22).

**"Mixed micelle" formation:** The fatty acids, mono and diglycerides and cholesterol, the enzymatic hydrolytic products of lipid digestion along with bile salts, together, form water-soluble molecular aggregates called as mixed "micelles of size 0.1 to 0.5 μm in diameter and are absorbed mainly from duodenum and jejunum. Micelles can also accommodate some fat soluble substances like carotene and vitamins.

- Micellar complexes get destabilized when they come near the brush border of enterocytes and liberates its contents.
- This is followed by passive diffusion of the digested fats across the luminal cell membrane.
- Only few fatty acids like oleic acid and linoleic acid enter the enterocyte by facilitated diffusion.
- In the endoplasmic reticulum of the enterocyte, triacylglycerols and cholesterol esters are re-synthesized
- These resynthesized lipids, together with small quantities of phospholipids and apolipoprotein B-48, form the chylomicrons.
- The chylomicrons cross the other side of the cell membrane and enter into the lymphatic vessels.
- The triacylglycerols of short chain fatty acids and few short chain free fatty acids of chain length (6–10 carbon atoms) can directly enter the portal circulation.

Bile salts of the "micelles" are not absorbed initially, while they are reabsorbed in the lower part of the small intestine and returned to the liver through the portal vein for re-secretion into the bile. Such a process is known as entero-hepatic circulation of bile salts.

### Factors affecting absorption

- Short chain fatty acids enter the circulation at a faster rate than the long chain fatty acids.
- Certain plant sterols like stigmasterol and sitosterol inhibit cholesterol absorption. Drugs like statins and certain phytochemicals present in green tea decrease the absorption of fats.
- Bile salts enhance the digestion and absorption of fats. Presence of unsaturated free fatty acids enhances the absorption of cholesterol.
- Certain conditions like obstructive jaundice/ pancreatic disorders reduce the digestion and absorption of fats.

### 2.2.2.4 Digestion and absorption of nucleic acids

**Digestion in mouth:** No digestion of nucleic acid has been reported in the mouth.

**Digestion in stomach:** Nucleoproteins are denaturated and nucleotides are released by the acidic environment in the stomach. Recent reports suggest that the cleavage of nucleic acids is also facilitated by pepsin in the stomach.



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**Digestion in duodenum and small intestine:** The nucleic acids are hydrolysed to a mixture of nucleotides by ribonucleases and deoxyribonucleases (both endo and exonucleases) present in pancreatic and intestinal secretions. The nucleotides thus formed liberate the phosphate and form nucleosides by the action of nucleotidases. The resulting nucleosides are either absorbed or hydrolyzed by nucleosidases to purine and pyrimidine bases and pentose sugars (Figure 2.23). Some of the unabsorbed purines are metabolized by the intestinal bacteria.

**Absorption of nucleic acids:** The nucleic acid bases are absorbed by active transport in the small intestine and the pentoses are absorbed with the other sugars.

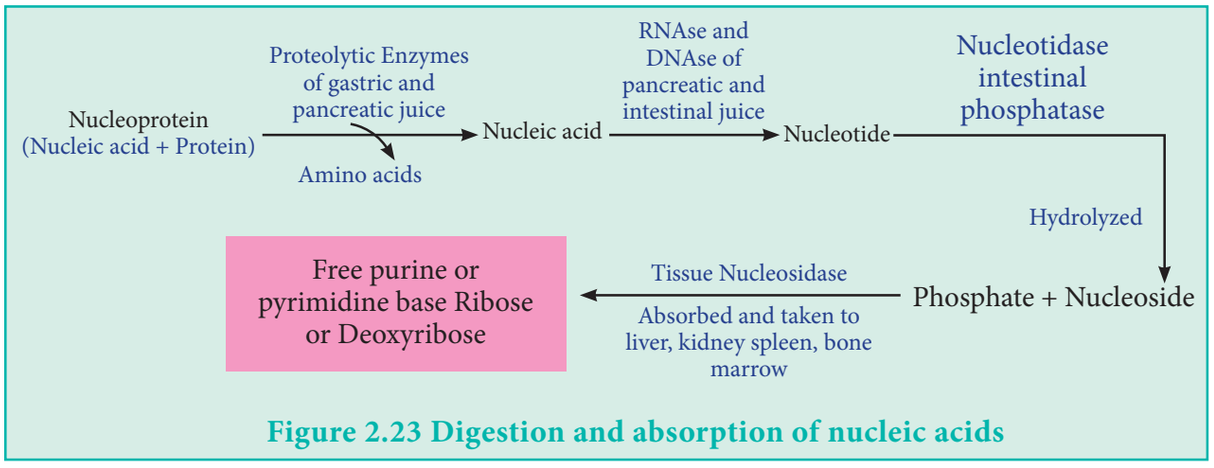



**Figure 2.23 Digestion and absorption of nucleic acids**

## 2.3. Gastrointestinal hormones

**Table 2.3 Gastrointestinal hormone actions, Stimuli for secretion and site of secretion**

| Hormone | Stimuli for Secretion | Site of Secretion | Actions |
|---|---|---|---|
| Gastrin | Protein,<br>Distention<br>(Acid inhibits release) | G cells of the antrum, duodenum, and jejunum | Stimulates<br>Gastric acid secretion<br>Mucosal growth |
| Cholecystokinin | Protein<br>Fat<br>Acid | I cells of the duodenum, jejunum, and ileum | Stimulates<br>Pancreatic enzyme secretion<br>Pancreatic bicarbonate secretion<br>Gallbladder contraction<br>Growth of exocrine pancreas |



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| | | | |
|---|---|---|---|
| Secretin | Acid<br>Fat | S cells of the duodenum, jejunum, and ileum | Stimulates<br>Pepsin secretion<br>Pancreatic Bicarbonate secretion<br>Biliary bicarbonate secretion<br>Growth of exocrine pancreas<br>Inhibits<br>Gastric acid secretion |
| Gastric inhibitory peptide | Protein<br>Fat<br>Carbohydrate | K cells of the duodenum and jejunum | Stimulates<br>Insulin release<br>Inhibits<br>Gastric acid secretion |
| Motilin | Fat<br>Acid<br>Nerve | M cells of the duodenum and jejunum | Stimulates<br>Gastric motility<br>Intestinal motility |

There are five major hormones that help in digestion. They are

1. Gastrin is secreted by the G cells of the gastric pits and it stimulates the gastric glands to secrete pepsinogen and HCl. The stimulation for gastrin secretion is food in the stomach and it is inhibited by low pH. Gastrin also stimulates the growth of the mucosa.
2. The presence of acidic chyme in the duodenum stimulates the secretion of a hormone called Secretin, which in turn stimulates the pancreas to secrete a bicarbonate rich fluid. It also stimulates the secretion of bile in the liver.
3. Cholecystokinin (CCK) is another hormone in the duodenum that stimulates the release of digestive enzymes from the pancreas and also it is involved in the emptying of bile in the gallbladder. This hormone is secreted in response to the abundance of fat in chyme.
4. Enterogastrone and Gastric inhibitory peptide (GIP) released from the duodenum decreases the churning action of stomach and slows the emptying of the stomach. It also induces insulin secretion.
5. Motilin is a duodenal hormone that regulates gastrointestinal motility and stimulates the production of pepsin.
6. Others: Hepatocrinin stimulates bile formation with lesser bile salts. Enterocrinin is involved in the stimulation of the secretion of enzymes by the intestinal mucosa. Chymodenin stimulates the secretion of chymotrypsin from pancreas.

## Appetite-Regulating Hormones

Ghrelin, a hormone which is released by the stomach targets the pituitary gland, signaling to eat. The action of Ghrelin is countered by PYY released by the small intestine signals that you have eaten enough and helps to suppress your appetite.



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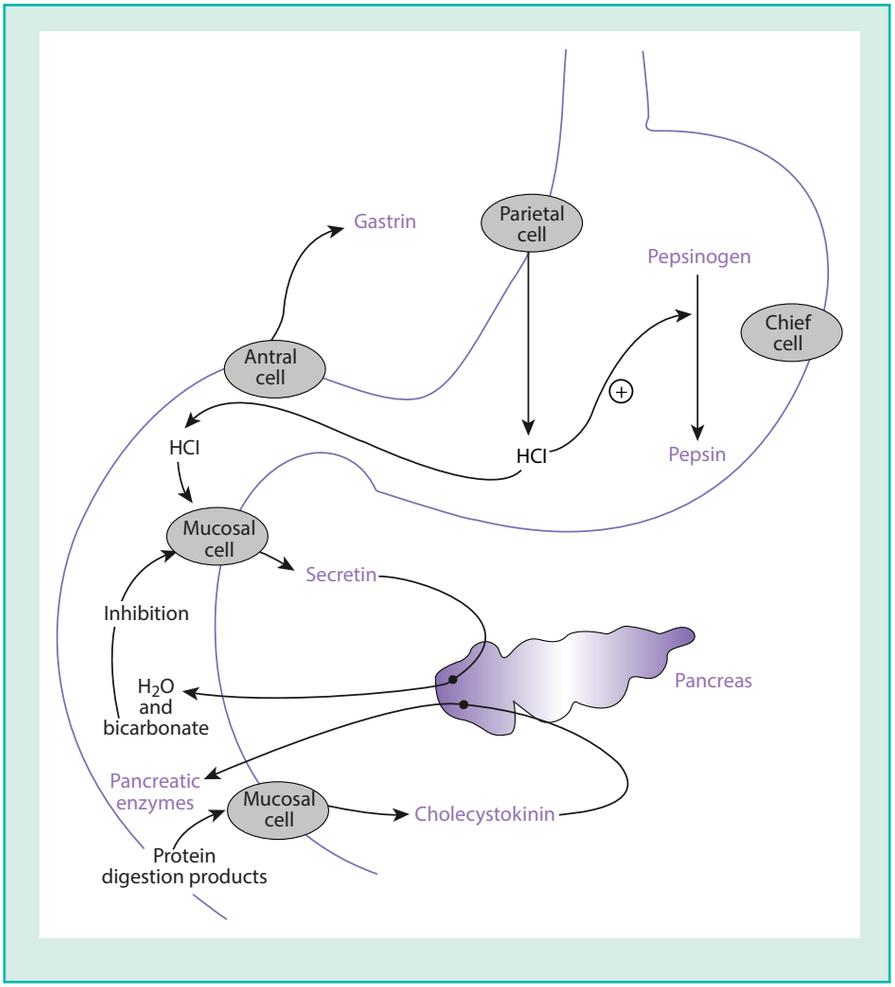



**Figure 2.24. Release and action of intestinal hormones**

## Summary

- The digestive system is made up of a tubular gastro -intestinal tract (GI tract) and secretory organs.
- GI tract includes the oral cavity, pharynx, stomach, small intestine, large intestine, rectum and anus.
- The accessory digestive organs include the salivary glands, glands of the stomach, duodenal glands, liver, gall bladder and pancreas.
- The stomach, a J shaped enlargement is involved in mechanical grinding of food and secretes HCl, gastric lipase and pepsin.
- The small intestine constituted by duodenum, ileum and jejunum has a large surface area due to the presence of villi that aid in absorption of nutrients.
- The large intestine includes the cecum, colon, rectum and the anal canal. The



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indigestible material is stored in the large intestine till it is eliminated via the anus.

- Salivary glands, gastric glands, liver, pancreas, glands of Lieberkuhn and Brunner's glands contribute to the secretions of the GI tract.
- The mechanical processes involved in digestion are ingestion, mastication, deglutition, peristalsis, mixing and propulsion and defecation.

### Digestion and absorption of carbohydrates

- Digestion of carbohydrates takes place briefly in the mouth and largely in the intestine. Amylases present in saliva and pancreatic juice and disaccharidases present in the brush border membrane of the intestine such as sucrase, lactase, maltase and isomaltase are responsible for digestion of carbohydrates.
- Monosaccharides are absorbed by both passive diffusion as well as active transport. Glucose is absorbed passively by a transporter, GLUT2 (Glucose Transporter 2). Active transport is mediated by a protein called as SGLT1 (Sodium Glucose transporter 1) in the luminal side and GLUT2 on the baso-lateral side.

### Digestion and absorption of proteins

- In the stomach, HCl denatures the proteins and exposes the sites for action of the peptidases.
- Pepsin, Rennin, Gelatinase and Gastricsin in the stomach, enteropeptidase, trypsin, chymotrypsin, carboxypeptidases, elastases and gelatinases along with amino peptidases and carboxy peptidases contribute to the digestion of proteins.
- D-amino acids produced by bacteria are absorbed passively, while L amino acids are absorbed actively. Few di and tripeptides are also absorbed.
- Gamma glutamyl cycle is involved in the absorption of neutral amino acids.

### Digestion and absorption of fats

- Fat digestion is facilitated by the detergent action of the Bile salts and micelle formation. Pancreatic lipase, cholesterol esterase and phospholipase A2 are the enzymes that complete lipid digestion. Fat is responsible for the satiety value of foods.
- Fats are absorbed by passive diffusion mainly in the proximal jejunum.

### Digestion and absorption of nucleic acids

- No digestion of nucleic acids has been reported in the stomach.
- The nucleic acids are hydrolyzed to a mixture of nucleotides by ribonucleases and deoxy ribonucleases (both endo and exonucleases) present in pancreatic and intestinal secretions.

### Gastrointestinal hormones

- Gastrin, Cholecystokinin, enterogastrone, secretin and motilin are the five major hormones that help in digestion.



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Mumps is a viral infection accompanied by inflammation and enlargement of the parotid glands presented with moderate fever, malaise (general discomfort), and extreme pain in the throat while swallowing. Swelling occurs on one or both sides of the face, just anterior to the ramus of the mandible. In about 30% of males past puberty, the testes may also become inflamed, however, sterility rarely occurs because of unilateral testicular involvement.

- Hemorrhoids are "cushions" of tissue filled with blood vessels, found at the end of the rectum, just inside the anus. Along with the anal sphincters, they close off the bowel, preventing stool from leaving the body. Enlarged hemorrhoids also known as "piles" can cause unpleasant symptoms.
- -Hartnup Disease is a genetic defect in the neutral amino acid transporter.

  -Symptoms: dermatitis due to tryptophan malabsorption ("niacin" flush)

  -Consequences: Not serious, di- and tripeptide absorption supply minimal amounts of dietary essential neutral amino acids.
- Immediately after birth, the small intestine of infants can absorb intact proteins and polypeptides by a process known as endocytosis or pinocytosis. This is very important for the transfer of maternal immunoglobulins to the child. Colostrum (the first secreted milk after child birth) is rich in immunoglobulins.
- Certain drugs are absorbed by the sublingual area of the oral cavity (i.e. the floor of the mouth). Glyceryl trinitrate, a potent coronary vasodilator used for the rapid symptomatic relief of angina is one of the best known drugs used regularly with great success.

## Activity

1. Testing for salivary amylase

Take two test tubes labeled as A and B. Add 5 ml of 1% starch solution to both the tubes and add 2 ml of saliva to the test tube labeled as B. Incubate it for 15 minutes. Add iodine solution to both the tubes. Observe the color change. Discuss the reason for it in the laboratory.

**Preparation of chart**

- Prepare a chart with the involvement of various enzymes in digestion of carbohydrates, lipids and proteins.

**Preparation of model**

- Prepare a model for the human digestive system and explain.

**Preparation of assignment**

- Try to prepare an assignment with the diseases that are involved with mutations associated with digestion.



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# EVALUATION

## I Multiple choice questions

1. The tongue is a muscular organ attached by the _____ to the floor of the buccal cavity.
   a. Frenulum
   b. Uvula
   c. Oropharynx
   d. nasopharynx
2. _____ teeth are responsible for holding and tearing food.
   a. Incisors canine
   b. Canine
   c. Premolar
   d. Molar
3. Thecodont means _____
   a. Embedded in socket of jaw bones
   b. Temporary set of teeth
   c. Permanent set of teeth
   d. same in structure
4. Dental formula for each half of the upper and lower jaw of an adult is
   a. 2323/2121
   b. 2123/2123
   c. 2321/2321
   d. 2322/2322
5. Which prevents the entry of food into the lung?
   a. Epigottis
   b. Tonsil
   c. Trachea
   d. Pharynx
6. _____ protect the intestine from the foreign microorganism.
   a. Microvilli
   b. The Peyer's patches
   c. Crypts of Lieberkuhn
   d. HCl
7. The length of the small intestine is about
   a. 9 feet
   b. 10 feet
   c. 12 feet
   d. 15 feet
8. _____ has a small projection called the vermiform appendix.
   a. Cecum
   b. Colon
   c. Rectum
   d. Anal canal
9. HCl is secreted by
   a. Chief cells
   b. G cells
   c. Parietal cells
   d. Mucosal cells



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10. Which cells are phagocytic in nature?

a. Kupffer cells
b. Sinusoids
c. Mucosal cells
d. G-cells

11. _____is required for the absorption of aminoacid.

a. Glutathione
b. Lipid
c. Nucleic acid
d. Catecholamines

12. Gall stones are made up of___

a. Uric acid
b.Cholesterol
c. Calcium oxalate
d. Glycine

13. The organ that functions as both exocrine and endocrine

a. Stomach
b. Liver
c. Gall bladder
d. Pancreas

14. The tubular glands that lie between the villi of the inner surface of the small intestine

a. Glands of Lieberkuhn
b. Brunner's glands
c. Adrenal gland
d. Thyroid gland

15. _____ is an example for indigestible fiber present in the dietary carbohydrates

a. amylopectin
b. Glycogen
c. starch
d. Hemi-cellulose

16. _____ is responsible for the conversion of milk protein to Paracaseinate.

a. Gastricsin
b. Renin
c. Gelatinase
d. Promelanin

17. The inner mucosal layer of stomach fold into invagination called_____.

a. Rugae
b. Frenulum
c. Villi
d. Uvula

18. The duodenal gland are also called as_____.

a. Adrenal gland
b. Brunner's gland
c. Thyroid gland
d. Glands of Lieberkuhn



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19. _____ in the saliva acts against the microbes.

a. Salivary amylase

b. Solutes

c. IgA

d. IgG

20. Which is a symporter?

a. GLUT 1

b. GLUT 2

c. SGLT 1

d. GLUT 4

21. Pick the add one out.

a. Pepsin

b. Renin

c. Trypsin

d. Gelatinase

22. Find the exopeptidase.

a. Trypsin

b. Chymotrypsin

c. Carboxy peptidase

d. Pepsin

23. Cholecystokinin is a hormone secreted by the.

a. G cells of gastric pits

b. S cells of duodenum

c. I cells of duodenum

d. C cells of duodenum

24. _____ is a hormone responsible for signaling to eat.

a. Ghrelin

b. PYY

c. Enterogastrone

d. Motilin



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25. Gall bladder is a _____ shaped sac

a. Pear
b. Globular
c. Oval
d. Tubular

## II Give short answer for the following

1) What is digestion? Why it is needed?
2) Mention the different types of salivary glands along with its location.
3) Give the composition and functions of saliva.
4) Discuss about the different secretory cells of the gastric glands.
5) What do you meant by motility?
6) Why is pancreatic amylase superior to salivary amylase in digesting carbohydrates?
7) Give the specificity of pepsin and enteropeptidase.
8) What do you meant by transcytosis?
9) What are chylomicrons?
10) How are mixed micelles formed?
11) Define the term satiety.
12) Write shortly on gastrin.
13) Comment on digestion of fats in mouth.
14) List the enzymes that digest protein in stomach.
15) Brief a note on the specificity and optimum pH of trypsin.

## III Give short answer for the following

1) What is mastication? How is food masticated in the mouth?
2) What are disaccharidases? List the disaccharidases in the small intestine.
3) Brief a note on the anatomy of gall bladder.
4) Write any 3 functions of mucous present in duodenal secretions.
5) What is facilitated diffusion?
6) Enumerate the duodenal enzymes that act upon proteins.
7) Write any 3 factors that affect absorption of amino acids.
8) Bring out the role of bile in digestion of fats.
9) Is there any role for mouth in digestion of protein?
10) Comment on the impact of age on digestion of macromolecules present in milk.



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## IV Answer the following

1) Discuss the anatomy of mouth.

2) Outline the structure and function of oesophagus.

3) List the functions of stomach.

4) Draw and explain the structure of villi.

5) Discuss about the different secretory cells of the gastric glands.

6) Bring out the role of large intestine in digestion.

7) Enumerate the functions of the liver.

8) Explain the mechanical process involved in digestion.

9) Detail on the secretory glands of the digestive system.

10) With a neat diagram, explain the anatomy of the gastro intestinal tract.

11) Discuss about the digestion of carbohydrates in mouth.

12) Outline the digestion of carbohydrates.

13) Illustrate the active transport of glucose across the intestinal brush border membrane.

14) List down the factors that affect the absorption of carbohydrates.

15) Tabulate the enzymes that are involved in digestion of proteins.

16) Write in detail about the absorption of proteins by Gamma Glutamyl Cycle and the factors affecting the absorption.

17) Brief a note on digestion on proteins in stomach.

18) Write the action of gastric lipase on lipids.

## V Analyze the table and match the following:

| Organ | Enzyme | Macromolecules |
|---|---|---|
| Saliva | Pancreatic amylase | Di/Tripeptide |
| Pancreas | Gelatinase | Starch |
| Intestine | Salivary amylase | Type IV Collagen |
| Stomach | Trypsin | Dextrin |
| Duodenum | Leucine amino peptidase | Chymotrypsinogen |



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**Answer Table:**

| Organ | Enzyme | Macromolecules |
|---|---|---|
| Saliva | Salivary amylase | Starch |
| Pancreas | Pancreatic amylase | Dextrin |
| Intestine | Leucine amino peptidase | Di/Tripeptide |
| Stomach | Gelatinase | Type IV Collagen |
| Duodenum | Trypsin | Chymotrypsinogen |

## II. Assertion and Reason:

**Direction:** In each of the following questions a statement of assertion (A) is given and a corresponding statement of reason (R) is given just below it. Mark the correct statement as.

a) If both A and R are true and R is correct explanation of A

b) If both A and R are true but R is not the correct explanation of A

c) If A is true but R is false

d) If both A and R are false.

1. **Assertion** : Phlorizin decreases the absorption of carbohydrates.
   **Reason** : Phlorizin is an inhibitor of GLUT 2.
2. **Assertion** : Liver is responsible for cholesterol homeostasis.
   **Reason** : Liver converts some amount of cholesterol to bile salts.
3. **Assertion** : The secretion of Brunner's gland inactivates the intestinal enzymes.
   **Reason** : The G-cells of Brunner's gland secrete HCl.
4. **Assertion** : Rennin helps in the digestion of milk.
   **Reason** : Rennin is present only in Infants and children.
5. **Assertion** : Digestion of fats starts in the mouth.
   **Reason** : Lingual lipase can act on long chain fatty acid containing Triglycerides.

**Answer:**

1. c) A is true but R is false
2. a) Both A and R are true and R is correct explanation of A
3. d) Both A and R are false
4. b) Both A and R are true but R is the correct explanation of A
5. c) A is true but R is false

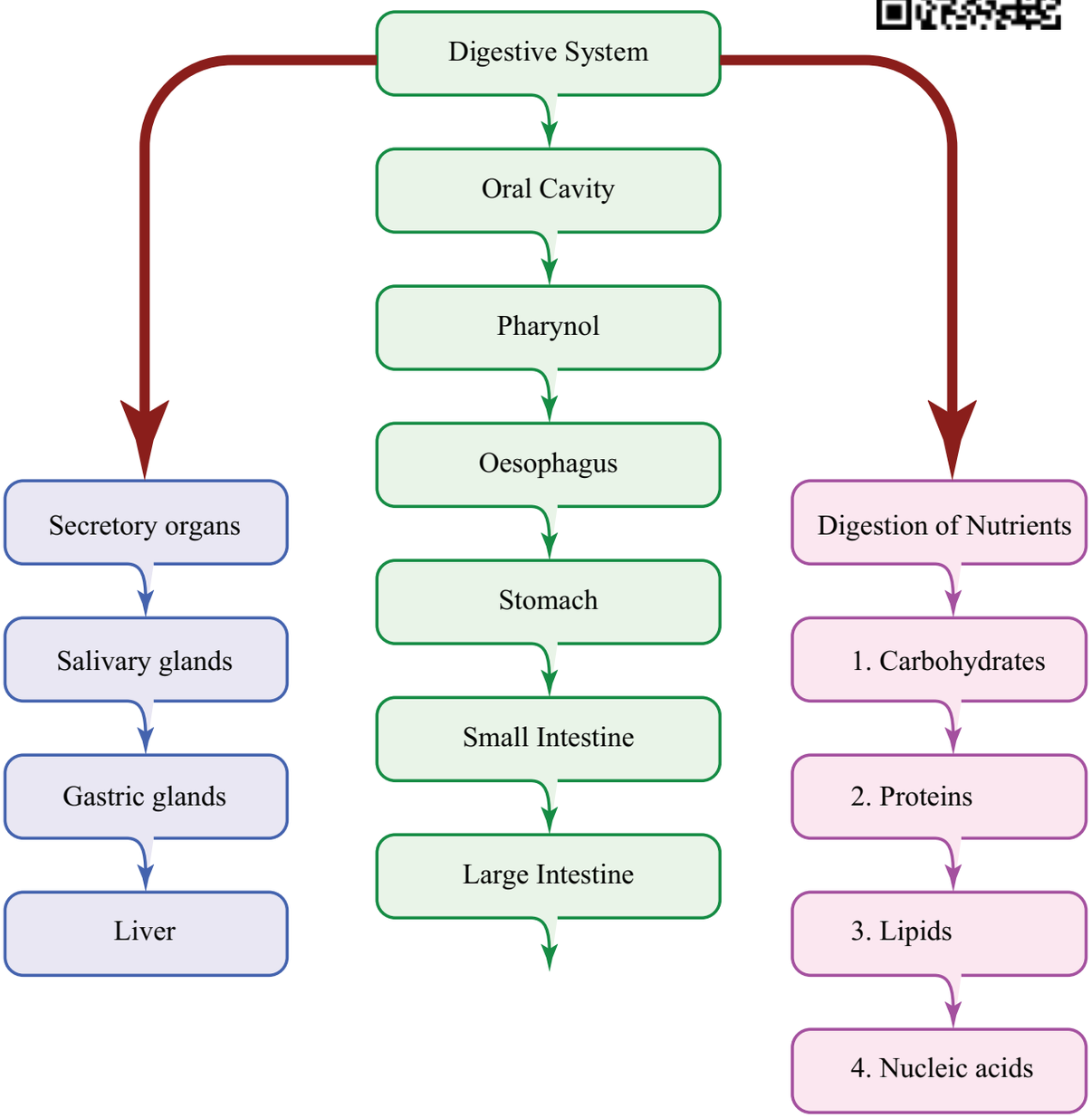


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## CONCEPT MAP

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# Digestion






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UNIT 3

# CARBOHYDRATE METABOLISM



**Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas.**

Gustav Embden (German physiological chemist), Otto Meyerhof (German physician and biochemist), and Jakub Karol Parnas (Jewish-Polish–Soviet biochemist) studied the carbohydrate metabolism and fully explained the reactions of glycolysis. They have combined results of many smaller experiments carried out by other scientists to explain the pathway as a whole. It took almost a century to fully discover the glycolytic pathway. Otto Meyerhof was awarded the Nobel Prize in Physiology and Medicine, with Archibald Vivian Hill, for his work on muscle metabolism, including glycolysis.

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## Learning Objectives

After studying this unit the students will be able to

- Describe the metabolic processes catabolism and alnabolism.
- Describe the reactions of glycolysis and gluconeogenesis.
- Describe the reactions of Hexose mono-phosphate shuttle pathway and TCA Cycle.
- Synthesis of glucose from non – carbohydrate sources
- Discuss the types, symptoms and cases of diabetes.
- Explain the protocol to determine blood glucose level.



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# INTRODUCTION

## 3.1 OVERVIEW OF METABOLISM

We have already learnt in XI standard about the structures and functions of various biomolecules such as proteins, amino acids, carbohydrates etc... We know that biomolecules are important for the normal functioning of any living system. These biomolecules can be obtained either directly from the diet or synthesised in cells. In the next few chapters, we will study the synthesis of various biomolecules and their degradation. These biochemical studies are termed as metabolism. Metabolism consists of series of connected enzymatic reactions that produce specific products. The different species involved in these reactions are referred to as metabolites. Photosynthesis is an example of carbohydrate metabolism in which a series of chemical reactions convert carbon dioxide and water to glucose. In the next few chapters, we will study about the metabolism of various biomolecules such as proteins, lipds etc. Figure 3.1 gives the overview of the various metabolic processes.

We learnt in the previous unit, that digestion of carbohydrates produce three major products namely glucose, galactose and fructose. These monosaccharides are absorbed into the blood stream. After their absorption, monosaccharides are transported to the liver, where fructose and galactose are rapidly converted into glucose.

In this unit we will study the metabolism of carbohydrates. Carbohydrates are simpler when compared to other biomolecules such as nucleic acids, amino acids etc... and their metabolism supplies more than half of the energy requirements of the body. In fact, our brain mainly depends upon carbohydrate metabolism as a source of energy.

### 3.1.1 Catabolism and anabolism

Every cell contains thousands of reactions comprising many metabolic pathways. There are two types of metabolic reactions namely anabolism and catabolism. The process of synthesising the required larger biomolecules from the small molecules is called anabolism. These reactions use up energy for example, in photosynthesis, small molecules $CO_2$ and water are converted into a six carbon carbohydrate. In this reaction sun light provides the energy.

$$6CO_2 + 6H_2O + 2800kJ \xrightarrow[Chlorophyll\&Enzymes]{Sunlight} C_6H_{12}O_6 + 6O_2$$

Catabolism is a reverse process of anabolism, in which bigger biomolecules are broken into simple molecules. Let us consider the oxidation of glucose,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2800kJ$$

The above reaction is the reversal of photosynthesis reaction in which large (6C glucose) molecules are broken into small $CO_2$and $H_2O$ molecules. If the glucose is burnt in the lab we get $CO_2$ & water and large amount of energy is released. Whereas, in living cell, the oxidation of glucose proceeds through a series of enzyme-catalysed intermediate reactions and the part of the energy produced in these reactions is stored in the chemical bonds of ATP (Adenosine triphosphate). When energy is needed, for example during the muscle contraction, ATP is utilised.



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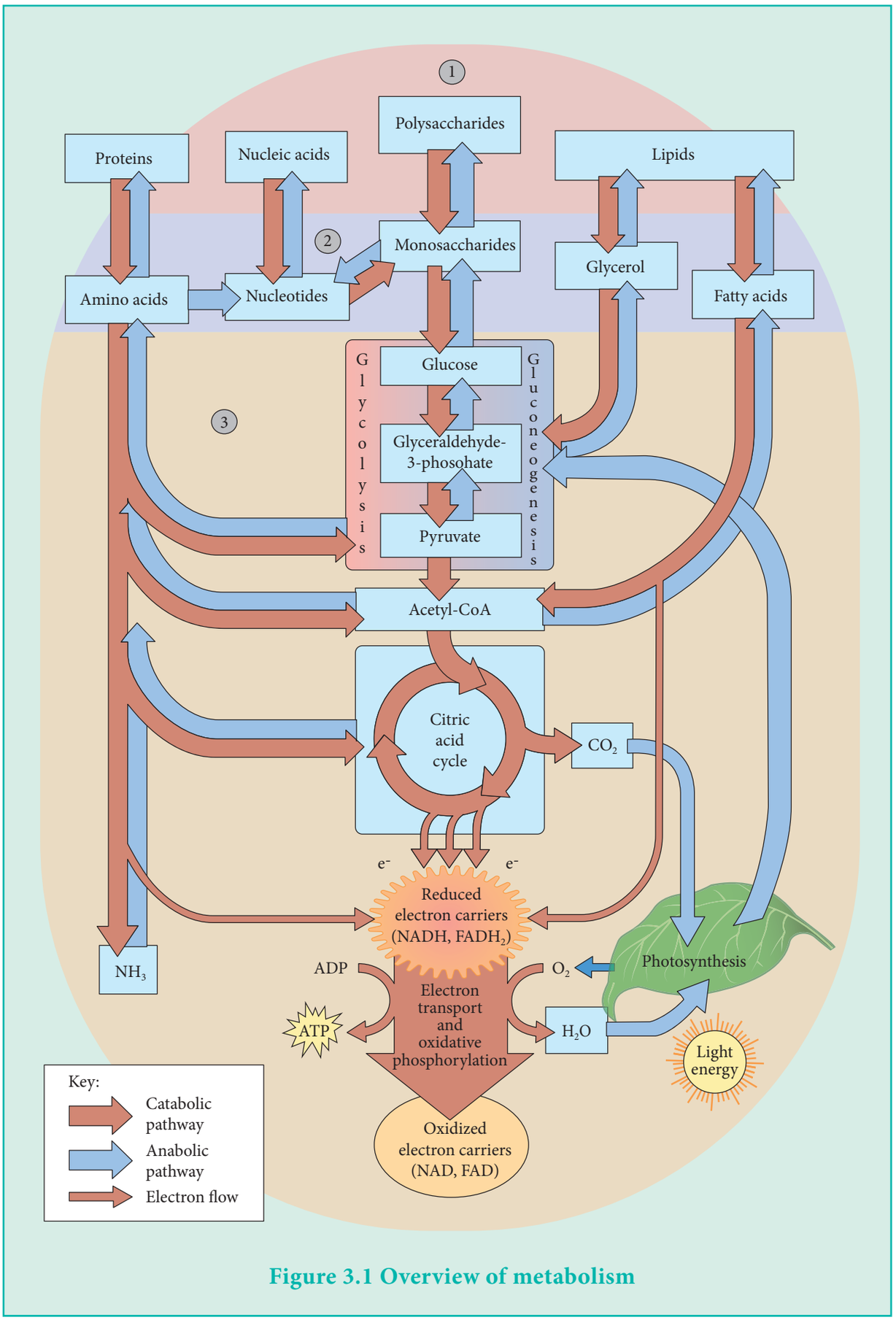


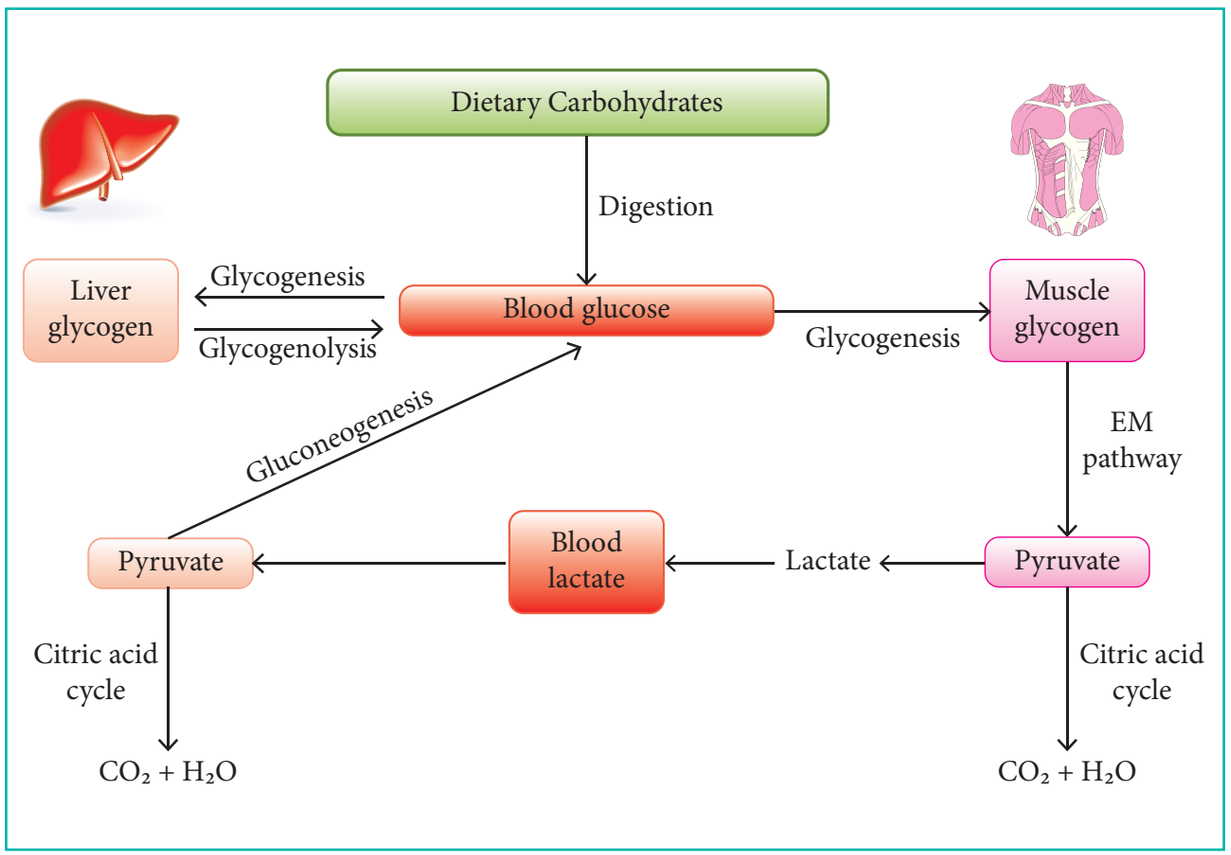

**Figure 3.1 Overview of metabolism**



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## 3.2 CARBOHYDRATE AS A SOURCE OF ENERGY

The major function of carbohydrate is to serve as a fuel. The catabolic enzymatic oxidation of glucose provides energy and the metabolic intermediates are used in various biosynthetic reactions. We require energy to perform any work such as playing. Reading, etc., Carbohydrate metabolism provides us the required energy. Glucose is oxidized via EMP pathway (Embden - Meyerhodf - Parnas) also known as glycolysis. During glycolysis, glucose is converted into pyruvate. Pyruvate is then converted into acetyl CoA which is fully oxidised to carbon dioxide via the citric acid cycle in the presence of oxygen (aerobic conditions). Under oxygen limiting conditions the pyruvate is converted into lactate. Lactate is then transported to liver where it is metabolised further. Glucose can be synthesised from pyruvate by gluconeogenesis pathway which will be discussed later in detail.




The liver maintains the glucose level in the blood. When blood glucose is in excess, it is converted to glycogen in the liver and muscle tissue. The synthesis of glycogen form glucose is called **glycogenesis**. In addition, glycolytic pathway is also accelerated.

When the glucose level is low, the glycogen is hydrolysed to produce glucose. The hydrolysis of glycogen to glucose is called **glycogenolysis**. The glucose level is also increased by activating the gluconeogenesis pathway.



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## 3.3. GLYCOLYSIS

Glycolysis is an oxidative metabolic process in which one mole of glucose is converted into two moles of pyruvate with the generation of chemical energy in the form of ATP in a series of ten enzyme catalysed reactions. In this process the oxidising agent is coenzyme $NAD^+$. No molecular oxygen is utilised in glycolysis and hence it is an anaerobic pathway.

Under aerobic conditions, the pyruvate formed by glycolysis is further oxidized to $CO_2$ in the citric acid cycle. Whereas, in anaerobic conditions, the pyruvate is converted to lactate. This process occurs in the cytoplasm of all the cells.

### 3.3.1. Reactions of glycolysis pathway

There are two stages in the glycolysis namely preparatory stage and pay off stage.

#### Preparatory Stage:

It consists of first five reactions in which one molecule of glucose is converted into two molecules of glyceraldehyde-3-phosphate. This stage is an energy consuming stage in which two ATP molecules are utilized.

#### Pay off Stage:

It consists of the remaining five reactions in which the glyceraldehyde-3-phosphoate is converted into pyruvate. This is an energy producing stage in which two molecules of ATP are produced from ADP, per glyceraldehyde-3-phosphate. i.e. for two glyceraldehyde-3-phosphate molecule, totally four ATP molecules are produced.

In the overall glycolysis process there is a net gain of two ATP molecules for conversion of every glucose molecules into pyruvate. The overall process can be represented by the following equation.

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

#### Reactions of Preparatory stage:

**Step 1:** Glucose is phosphorylated by the transfer of a phosphate group from ATP molecule to form glucose-6-phosphate and ADP. This step is energy consuming step and the required energy is provided by the breakdown of the ATP molecule. Glucose-6-phosphate is more reactive than glucose and also cannot cross the cell membrane. Hence it is trapped inside the cell. This step is catalysed by hexakinase enzyme.

**Step 2:** Glucose-6-phosphate is isomerised to form fructose-6-phosphate. This step is catalysed by the enzyme phosphogluco isomerase

**Step 3:** Like the first step the fructose-6-phosphate is phosphorylated again by ATP to form fructose-1,6-bisphosphate. This step is catalyzed by the enzyme phosphofructokinase.



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**Step 4:** In this step, the six carbon, fructose-1,6-bisphosphate splits to form two three-carbon sugars namely dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate. They are isomers of each other. This step is catalysed by the enzyme aldolase.

**Step 5:** The interconversion of DHAP and glyceraldehyde-3-phosphate is catalysed by triose phosphate isomerase enzyme. The two molecules exist in equilibrium, but the equilibrium shifted towards the formation of glyceraldehyde-3-phosphate as it is consumed in subsequent steps of glycolysis. Eventually as the glycolysis continues, all of the DHAP is converted into glyceraldehyde-3-phosphate(Figure 3.3).

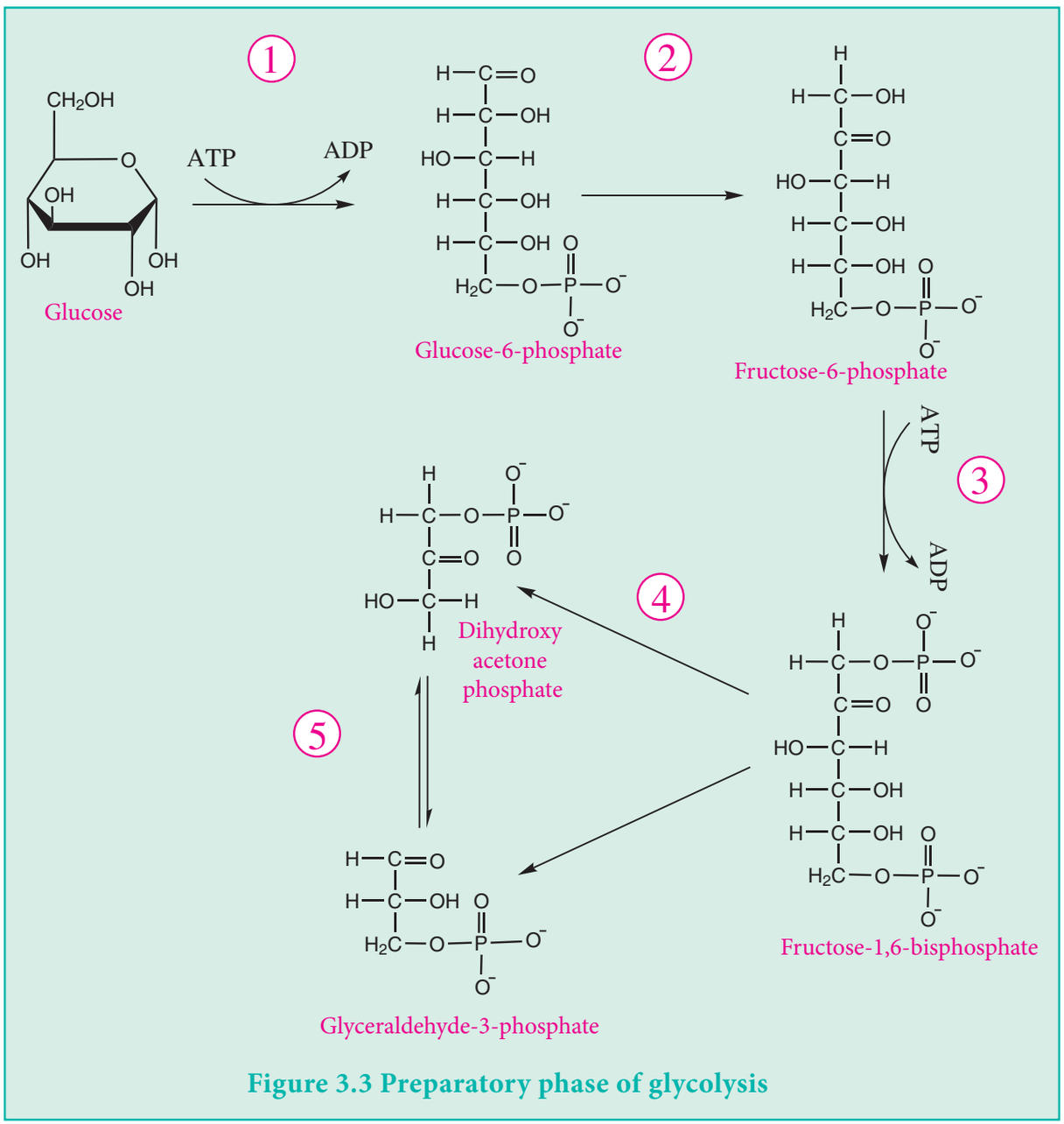



**Figure 3.3 Preparatory phase of glycolysis**



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### Reactions of Payoff phase:

**Step 6**: Glyceraldehyde-3-phosphate is oxidized by $NAD^+$ which gets reduced to NADH and $H^+$. This reaction is exergonic, releasing energy which is utilised to phosphorylate the Glyceraldehyde-3-phosphate to form high energy molecule 1,3-bisphosphoglycerate. Here the inorganic phosphate ($P_i$) is used for phosphorylation. Glyceraldehyde-3-phosphate dehydrogenase catalyses this step.

**Step 7:** The 1,3-bisphosphoglycerate is converted into 3-phosphoglycerate transfering one of its phosphate group to ADP to form ATP. The enzyme involved in this process is phosphoglycero kinase

**Step 8:** 3-Phosphoglycerate is isomerised to 2-phosphoglycerate by the enzyme phosphoglycerate mutase

**Step 9:** 2-Phosphoglycerate loses a molecule of water, becoming phosphoenolpyruvate (PEP). This step is catalysed by enolase enzyme. PEP is an unstable high energy molecule, poised to lose its phosphate group in the final step of glycolysis.

**Step 10:** In the final step, PEP readily loses its phosphate group to ADP and forms pyruvate, the end product of glycolysis. In this reaction one molecule of ATP is formed. This reaction is catalysed by pyruvate kinase (Figure 3.4).

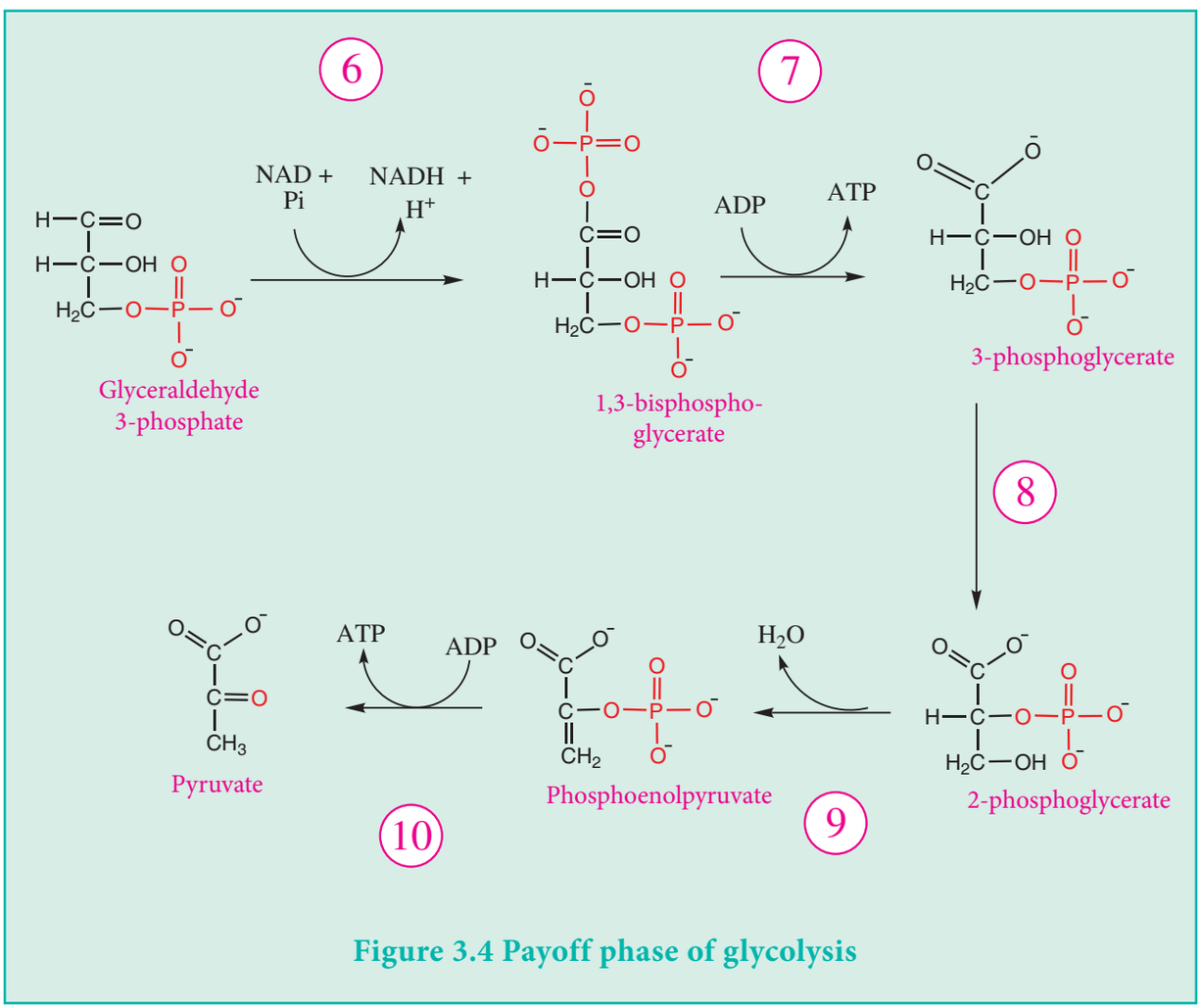
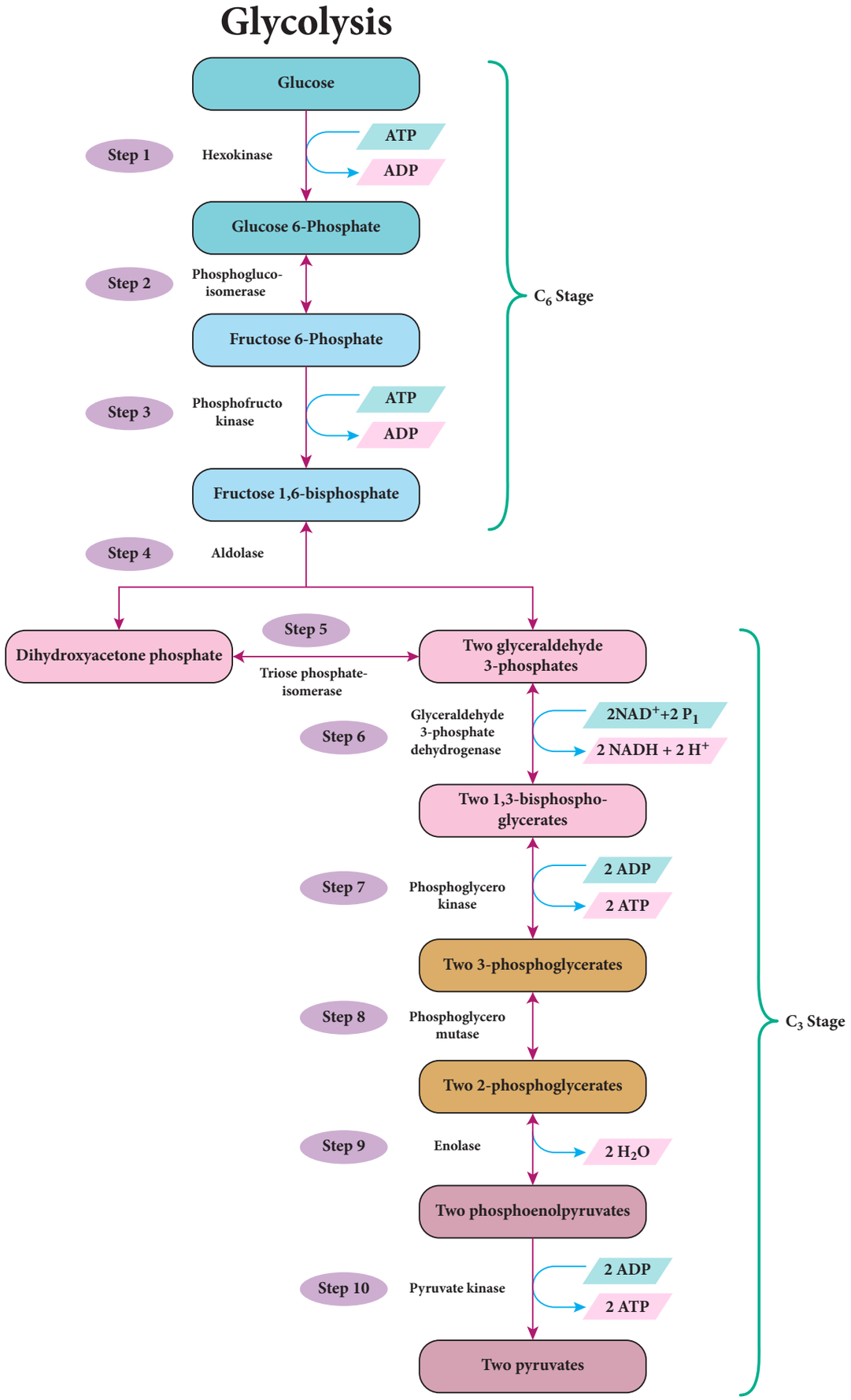



**Figure 3.4 Payoff phase of glycolysis**



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Glycolysis
Glucose
Step 1
Hexokinase
ATP
ADP
Glucose 6-Phosphate
Step 2
Phosphogluco-isomerase
Fructose 6-Phosphate
Step 3
Phosphofructo kinase
ATP
ADP
Fructose 1,6-bisphosphate
C6 Stage
Step 4
Aldolase
Dihydroxyacetone phosphate
Step 5
Triose phosphate-isomerase
Two glyceraldehyde 3-phosphates
Step 6
Glyceraldehyde 3-phosphate dehydrogenase
2NAD+ +2 P1
2 NADH + 2 H+
Two 1,3-bisphospho-glycerates
Step 7
Phosphoglycero kinase
2 ADP
2 ATP
Two 3-phosphoglycerates
Step 8
Phosphoglycero mutase
Two 2-phosphoglycerates
Step 9
Enolase
2 H2O
Two phosphoenolpyruvates
Step 10
Pyruvate kinase
2 ADP
2 ATP
Two pyruvates
C3 Stage

### 3.3.2. Summary of glycolysis

At the end of glycolysis, one glucose molecule is converted into two pyruvate molecules. In addition we also have two ATP, two NADH molecules formed from ADP and $NAD^+$ molecules respectively. To regenerate the coenzyme $NAD^+$, NADH must be re-oxidised. If oxygen is available, the pyruvate is oxidized all the way to carbon dioxide in cellular respiration, regenerates $NAD^+$. During this process more ATP molecules are produced. Under anaerobic conditions it is converted to lactate by lactate dehydrogenase enzyme and regenerates $NAD^+$. Here no further ATP molecules are produced.

### 3.3.3. Energetics of glycolysis

During the glycolysis of one molecule of glucose, two molecules of ATP and two molecules of NADH are produced. The ATP molecules serves as energy source. The NADH molecules are oxidised in mitochondria producing more number of ATP molecules (approximately 3 ATPs per NADH molecule).

| Step No. | Description | Number of ATP/ NADH formed/ consumed |
|---|---|---|
| 6 | Formation of 1,3-bisphospho glycerate from glyceraldehyde 3-phosphate | 2 NADH |
| 7 | Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate | 2 ATP |
| 10 | Formation of pyruvate from phosphoenol pyruvate | 2 ATP |
| 1,3 | Formation of Glucose-6-phosphate & Fructose-1,6-bisphosphate | 2 ATP (consumed) |
| Number of net ATP molecules generated per one molecule of glucose | | 2 ATP |
| Number of net NADH molecules generated one molecule of glucose | | 2 NADH |

### 3.3.4. Glycolysis under anaerobic condition:

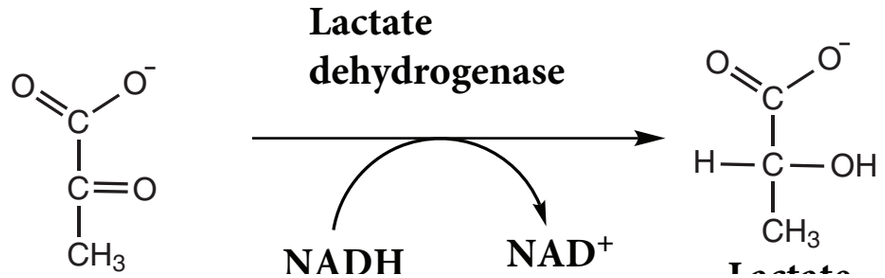
Some organisms are able to continually convert energy without the presence of oxygen. They undergo glycolysis, followed by the anaerobic process to produce ATP.

As discussed earlier anaerobic glycolysis is the transformation of glucose to lactate when limited amounts of oxygen ($O_2$) exist.

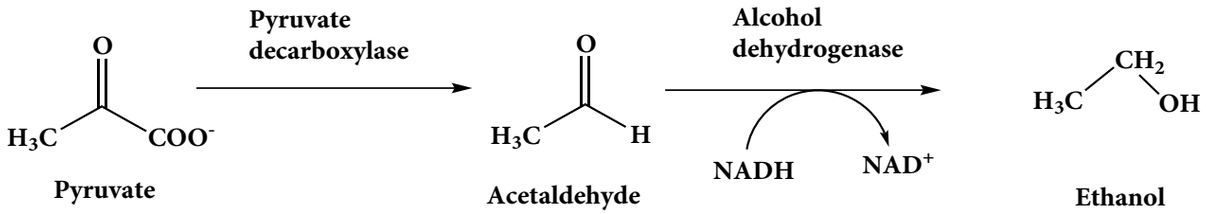
Muscle cells can continue to produce ATP when oxygen runs low using lactic acid fermentation. However, this often results in muscle fatigue and pain due to accumulation of lactate. When sufficient oxygen is not present in the muscle cells, the pyruvate, the terminal electron acceptor is converted to lactate by the enzyme lactate dehydrogenase, regenerating $NAD^+$.



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Many yeasts convert glucose to ethanol and $CO_2$ rather than pyruvate using alcoholic fermentation process. In this process, pyruvate is first converted to acetaldehyde by enzyme pyruvate decarboxylase in the presence of thiamine pyrophosphate and $Mg^{2+}$. Carbon dioxide is released during this reaction. Acetaldehyde is then converted to ethanol by the enzyme alcohol dehydrogenase during which the NADH is oxidized to $NAD^+$.




## 3.4. TRICARBOXYLIC ACID CYCLE:

Citric acid cycle is the common mode of oxidative degradation in eukaryotes and prokaryotes. This process occurs in the mitochondria. This cycle, which is also known as the tricarboxylic acid (TCA) cycle or Krebs cycle, is the central driver of cellular respiration. It accounts for the major part of oxidation of carbohydrate, fatty acid & amino acid and it also generates many biosynthetic precursors. The citric acid cycle is amphibolic, i.e. it operates both catabolically and anabolically.

Unlike glycolysis, the citric acid cycle is a cyclic process. i.e. the last reaction of the pathway regenerates the compound utilised in the first reaction. The citric acid cycle produces only one GTP molecule (ATP equivalent) directly and does not directly consume oxygen.

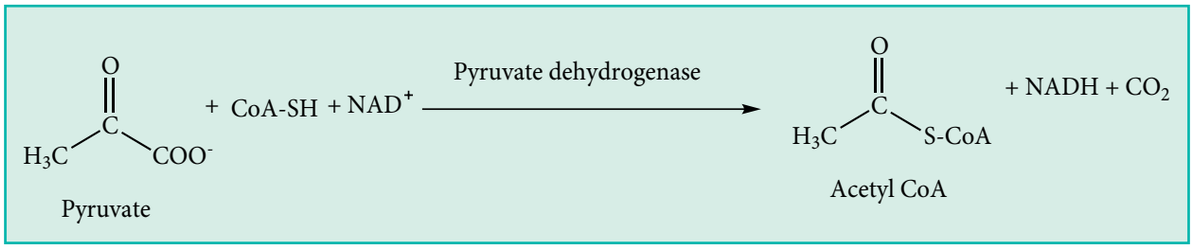
### 3.4.1. Reactions of tricarboxylic acid cycle

TCA cycle takes acetyl CoA produced by the oxidation of pyruvate (derived from glucose and other catabolic processes) as its starting material and in a series of reactions, harvests much of its bond energy in the form NADH, $FADH_2$ and GTP molecules. The reduced electron carriers NADH and $FADH_2$ generated in the TCA cycle, will pass their electrons into the electron transport chain resulting in oxidative phosphorylation, which will generate most of the ATP produced in cellular respiration.

Prior to the start of the first step, a transitional phase occurs during which pyruvate is converted to acetyl CoA. This process occurs in the mitochondrial matrix and provides a link between the glycolysis and the TCA Cycle which consists the following eight steps.



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**Step 1:** In the first step of the cycle, the acetyl CoA (two-carbon) reacts with an oxaloacetate molecule (four-carbon) to form citrate (six-carbon) in a condensation reaction. This reaction is catalysed by the enzyme citrate synthase. The CoA is bound to a thiol group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

**Step 2:** In second step, citrate is converted into its isomer, isocitrate, by the enzyme aconitase.

**Step 3:** In the third step, the isocitrate is oxidized, to produce a five-carbon molecule, α-ketoglutarate releasing a molecule of $CO_2$. In this process two electrons are also released which reduce a $NAD^+$ molecule to NADH. This step is catalysed by isocitrate dehydrogenase.

**Step 4:** In the step four, α-ketoglutarate is oxidised to succinyl CoA a four carbon molecule in the prescence of Coenzyme A. In this step also a $NAD^+$ molecule is reduced to NADH. This reaction is catalysed by α-ketoglutarate dehydrogenase enzyme.

**Step 5:** In step five, the succinyl CoA is converted into succinate with the release of large amount of energy. This energy is utilised for the phosphorylation of a guanosine diphosphate (GDP) to guanosine triphosphate (GTP) by the addition of inorganic phosphate. GTP is energetically equivalent to ATP; it is primarily used in protein synthesis, however, its use is more restricted. This reaction is catalysed by the enyme, succinyl-CoA synthetase (succinate thiokinase).

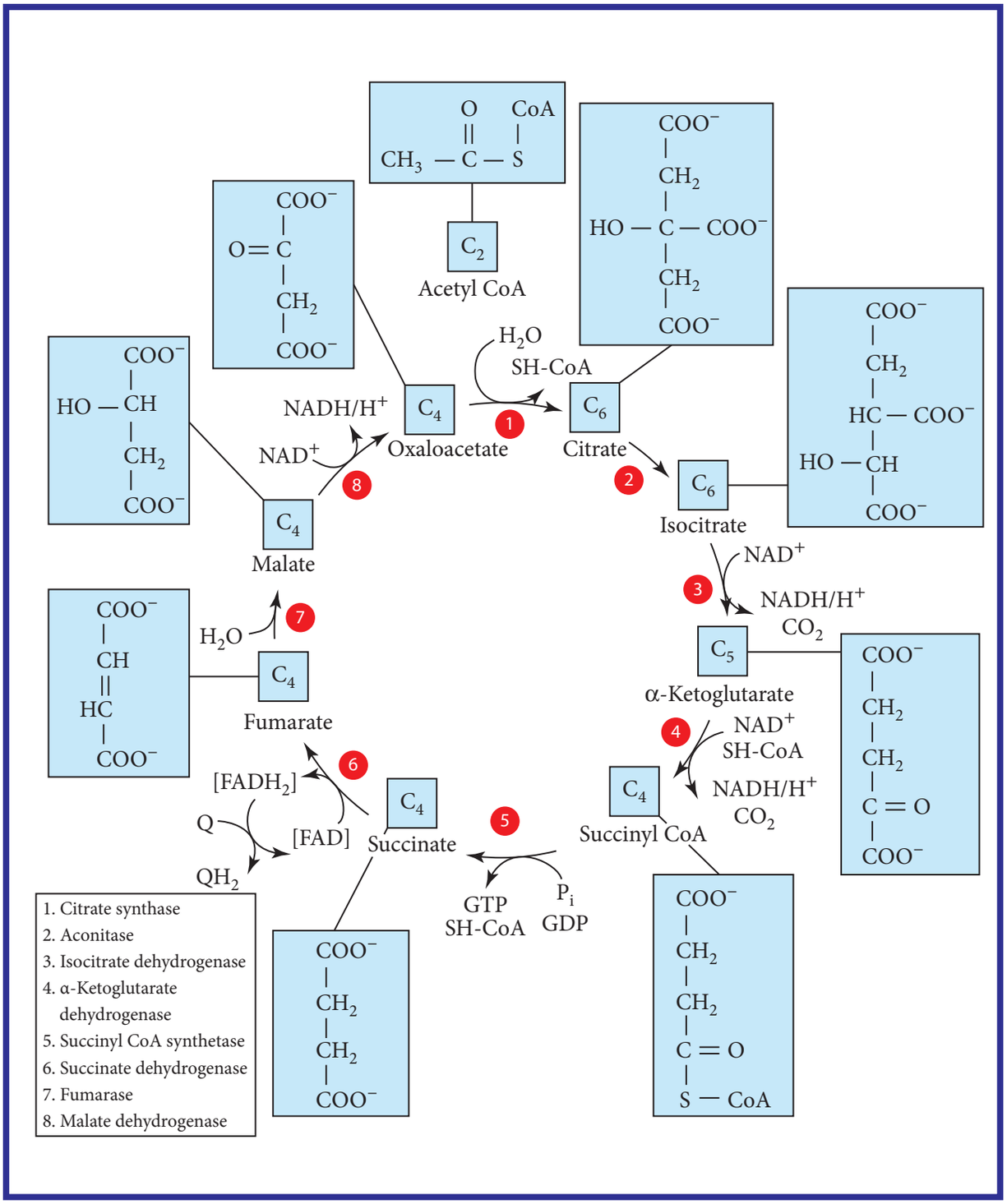
**Step 6:** Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. Unlike NADH, this carrier remains attached to the enzyme succinate dehydrogenase which catalyses this reaction, and transfers the electrons to the electron transport chain directly.

**Step 7:** Water is added to fumarate in step seven, and malate is produced. This reaction is catalysed by fumarase.

**Step 8:** The last step of the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced in this reaction, which is catalysed by malate dehydrogenase.



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### 3.4.2. Energetics of Tricarboxylic acid cycle

In a single turn of the cycle which consumes one acetyl CoA molecule,

- Two molecules of carbon dioxide are released
- Three molecules of NADH and one molecule of $FADH_2$ is generated
- One molecule of GTP is produced

| High energy molecule | Total number formed during TCA | No. of ATP generated per molecule | Total number of ATPs |
|---|---|---|---|
| NADH | 3 | 3 | 9 |
| $FADH_2$ | 1 | 2 | 2 |
| GTP | 1 | 1 | 1 |
| Total no. of ATP per acetyl coA molecule | | | 12 |

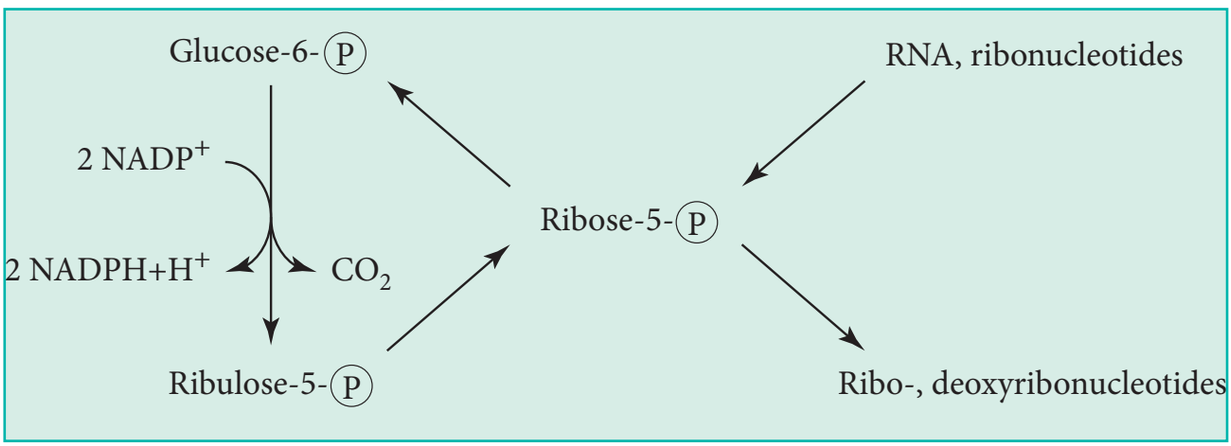
## 3.5. HEXOSE MONOPHOSPHATE SHUNT

So far we have learnt that glucose is oxidised by glycolysis & citric acid cycle, to generate energy and produce the necessary precursor metabolites. However, a number of alternative pathways have also been discovered. Hexose Monophosphate Shunt Pathway (HMP shunt). is one such important pathway. This occurs in the extra mitochondrial soluble portion of the cells.

This pathway, starts with glucose-6-phosphate as a key metabolite. Since, this pathway comprises both pentoses and hexoses, it is also referred to as the pentose phosphate pathway. This pathway consists of two phases namely the oxidative phase and the regenerative phase (non oxidative phase).

The oxidative phase, in which glucose-6-phosphate is oxidized and decarboxylated to give ribulose-5-phosphate. In this phase two NADPH (NADH equivalent) is formed. The fundamental difference between NADPH and NADH is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis of molecules such as fatty acids and cholesterol.

In cells that are not using ribose-5-phosphate for biosynthesis, the nonoxidative phase recycles six molecules of the pentose into five molecules of the hexose, glucose-6-phosphate, allowing continued production of NADPH and converting glucose-6-phosphate (in six cycles) to $CO_2$.



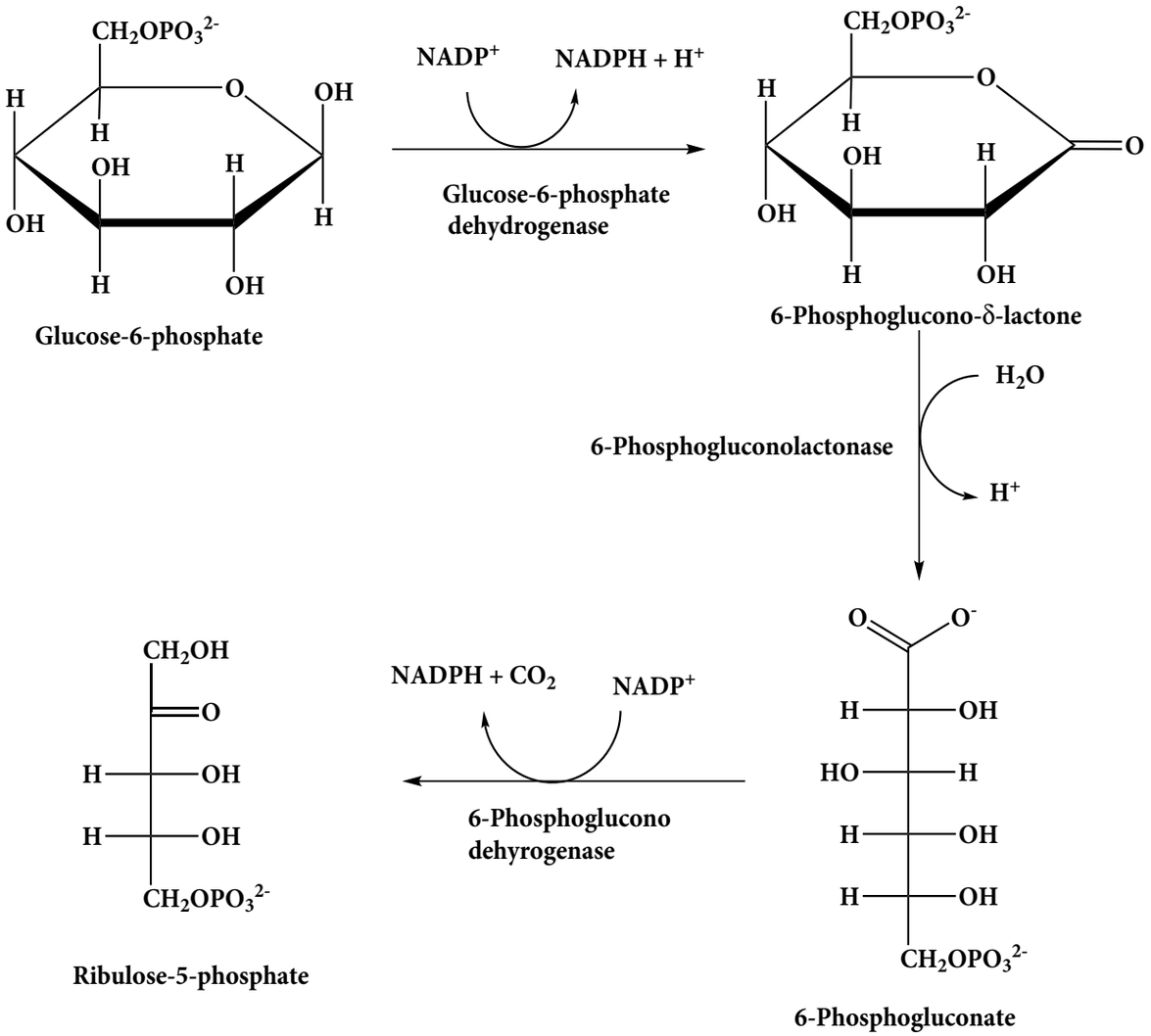
### 3.5.1. Reactions of oxidative phase

**Three enzymes are required in the oxidative phase:**

**Step 1:** In the first step, glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphoglucono-δ-lactone and reduces one molecule of $NADP^+$ to NADPH.

**Step 2:** In the second step, gluconolactonase cleaves the internal ester bond, which gives 6-phosphogluconate.

**Step 3:** In the third step, 6-Phosphogluconate dehydrogenase reduces another molecule of $NADP^+$ and decarboxylates 6-phosphogluconate to the pentose, ribulose-5-phosphate.




### 3.5.2. Non-oxidative phase

The non-oxidative phase involves the enzymes ribulose-5-phosphate epimerase (RE), ribulose-5-phosphate isomerase (RI), transketolase (TK), and transaldolase (TA). They bring about the following reactions:

**Step 1.** Two molecules of ribulose-5-phosphate are converted to xylulose-5-phosphate by ribulose-5-phosphate epimerase, and a third one is converted to ribose-5-phosphate by ribulose-5-phosphate isomerase.

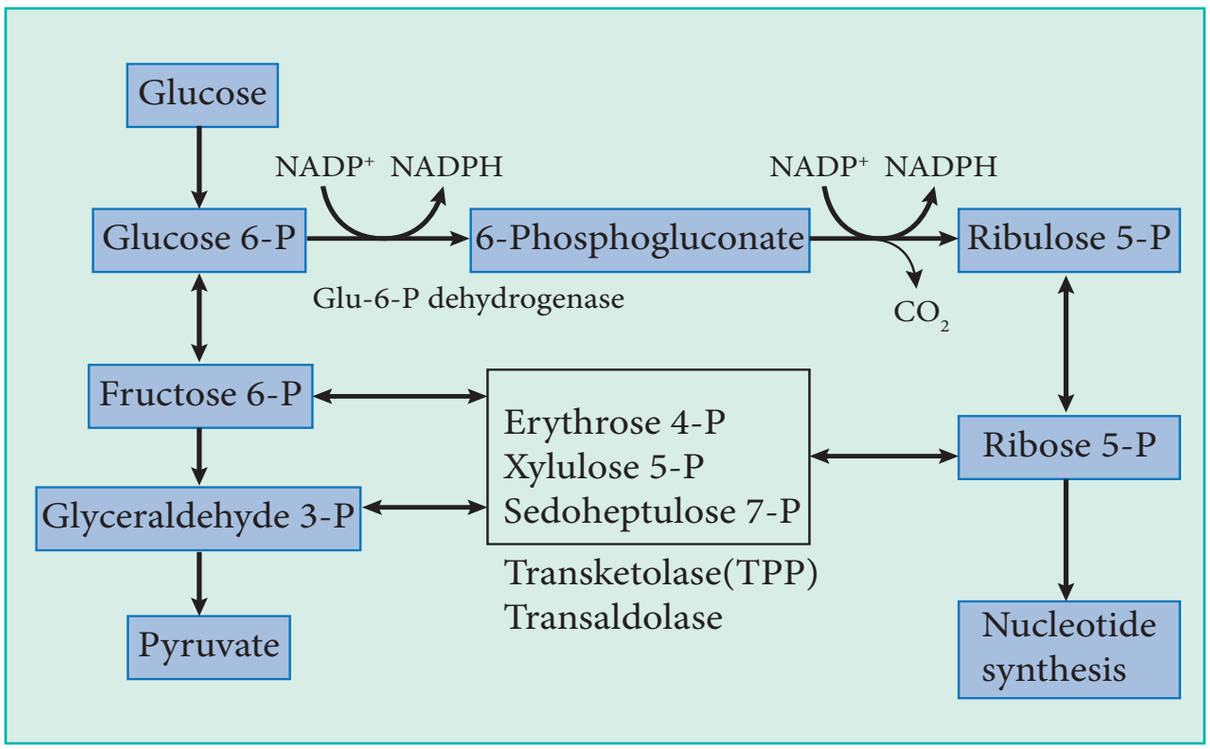


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**Step 2:** Transketolase transfers a C2 unit from one xylulose-5-phosphate to the ribose-5-phosphate, yielding glyceraldehyde-3-phosphate and the C7 sugar sedoheptulose-7-phosphate.

**Step 3:** Transaldolase transfers a C3 unit from sedoheptulose-7-phosphate back to glyceraldehyde-3-phosphate, which yields fructose-6-phosphate and the C4 sugar erythrose-4-phosphate.

**Step 4:** Transketolase transfers a C2 unit from the second molecule of xylulose-5-phosphate to erythrose-4-phosphate. This yields a second molecule of fructose-6-phosphate and again glyceraldehyde-3-phosphate




## 3.6. GLYCOGEN METABOLISM

Glycogen is the major storage form of carbohydrates in animals, present in all cells, but found mostly in liver and muscle, where it occurs as cytoplasmic granules. It is a highly branched form of amylopectin, with branch points occurring every 8 to 14 glucose residues. Glycogen's highly branched structure, which has many non-reducing ends, permits the rapid mobilization of glucose in times of metabolic need.

### 3.6.1. Glycogenesis

The biosynthesis of glycogen from glucose is called Glycogenesis. Glycogenesis takes place when blood glucose levels are sufficiently high to allow excess glucose to be stored.

Glycogenesis is stimulated by the hormone insulin. Insulin facilitates the uptake of glucose into muscle cells, though it is not required for the transport of glucose into liver cells. However,



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insulin has profound effects on glucose metabolism in liver cells, stimulating glycogenesis and inhibiting glycogenolysis, the breakdown of glycogen into glucose.

The pathway of glycogenesis includes a series of steps that result in complex glycogen formation in the cytoplasm of the liver and cells of the muscles. The steps of glycogenesis are as follows:

**Step 1:** Glucose phosphorylation – In the initial phase, glucose is phosphorylated into glucose-6-phosphate, a usual reaction in glycolysis. It is catalyzed by glucokinase (liver) and hexokinase (muscle).

**Step 2:** Conversion of Glucose-6-Phosphate to Glucose-1-Phosphate – An enzyme Phosphoglucomutase will catalyze the conversion of glucose-6-P is to glucose-1-phosphate.

**Step 3:** UTP (uridine triphosphate) attaches to Glucose-1-phosphate in the third step. This step focuses on the reaction of glucose-1-phosphate to UTP thereby forming active nucleotide UDP-Glucose (Uridine diphosphate glucose). The one responsible for such reaction is the enzyme UDP Glucose pyrophosphorylase.

**Step 4:** UDP-Glucose attaches to glycogen primer, a small fragment of already existing glycogen that serves as a primer, in order to stimulate the synthesis of glycogen. The glucose from UDP-Glucose will be accepted by glycogenin (a protein that acts as a primer). The initial glucose unit is attached to the hydroxyl group of tyrosine of glycogenin. The first molecule of glucose is transferred to glycogenin, which will then take up for glucose residues forming a fragment of primer.

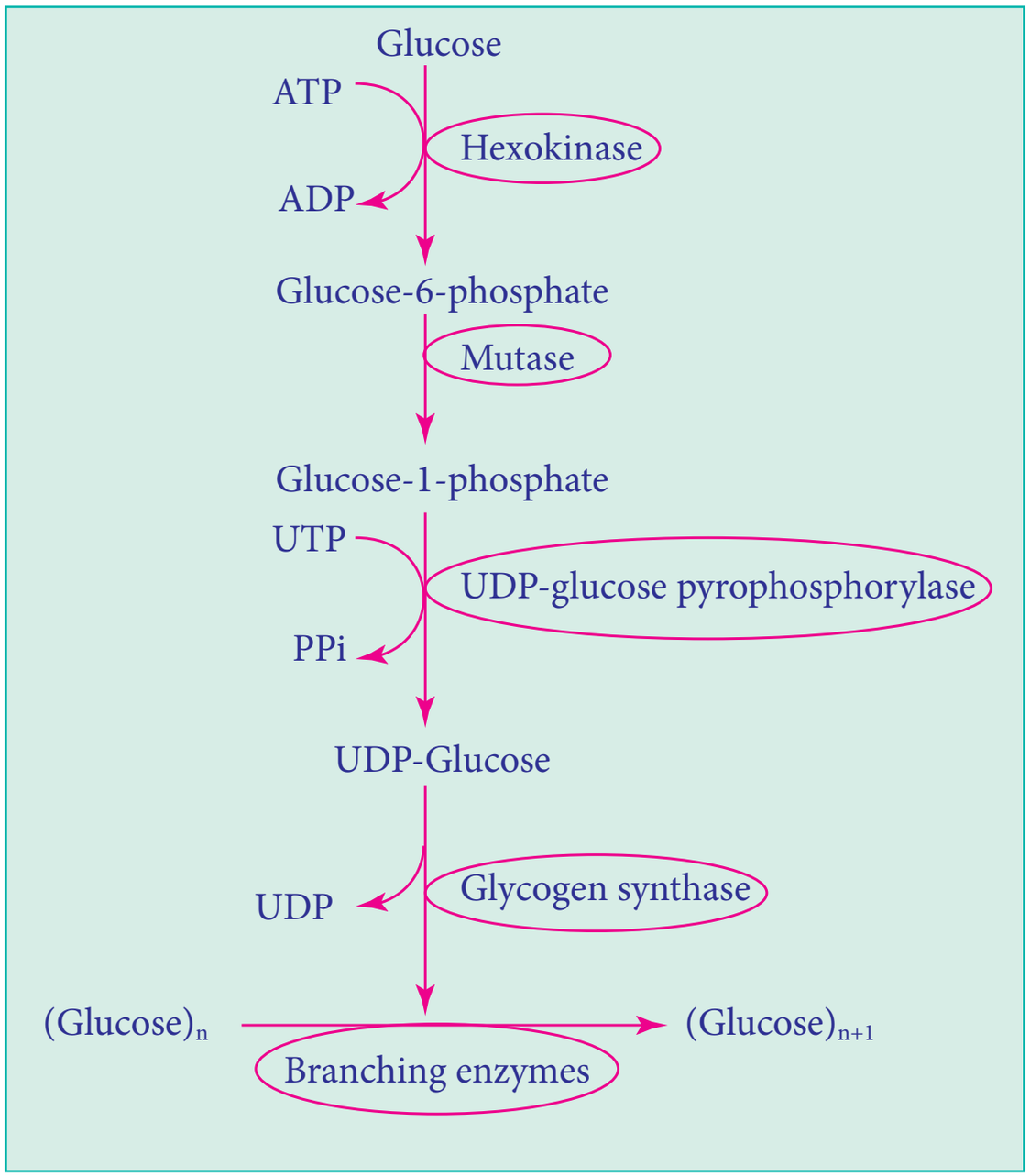
**Step 5:** Glycogen synthase transfers glucose from UDP-Glucose to glycogen (non-reducing end) forming alpha 1,4-linkages. The same enzyme catalyzes the synthesis of the unbranched molecule with alpha-1,4-glycosidic linkages.

**Step 6:** The formation of glycogen branches – The final step is the formation of glycogen branches caused by the effect of branching enzyme, which transfers a small fragment of about five to eight residues of glucose from the non-reducing end of the glycogen chain to another glucose residue linked by alpha-1,6 bond. This action causes the formation of a new non-reducing end. The final result is the elongation and branching out of the glycogen chain.

The process of glycogenesis utilizes two molecules of ATP. One molecule is needed for glucose phosphorylation and another molecule is needed to convert UDP to UTP.



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### 3.6.2. Glycogenolysis

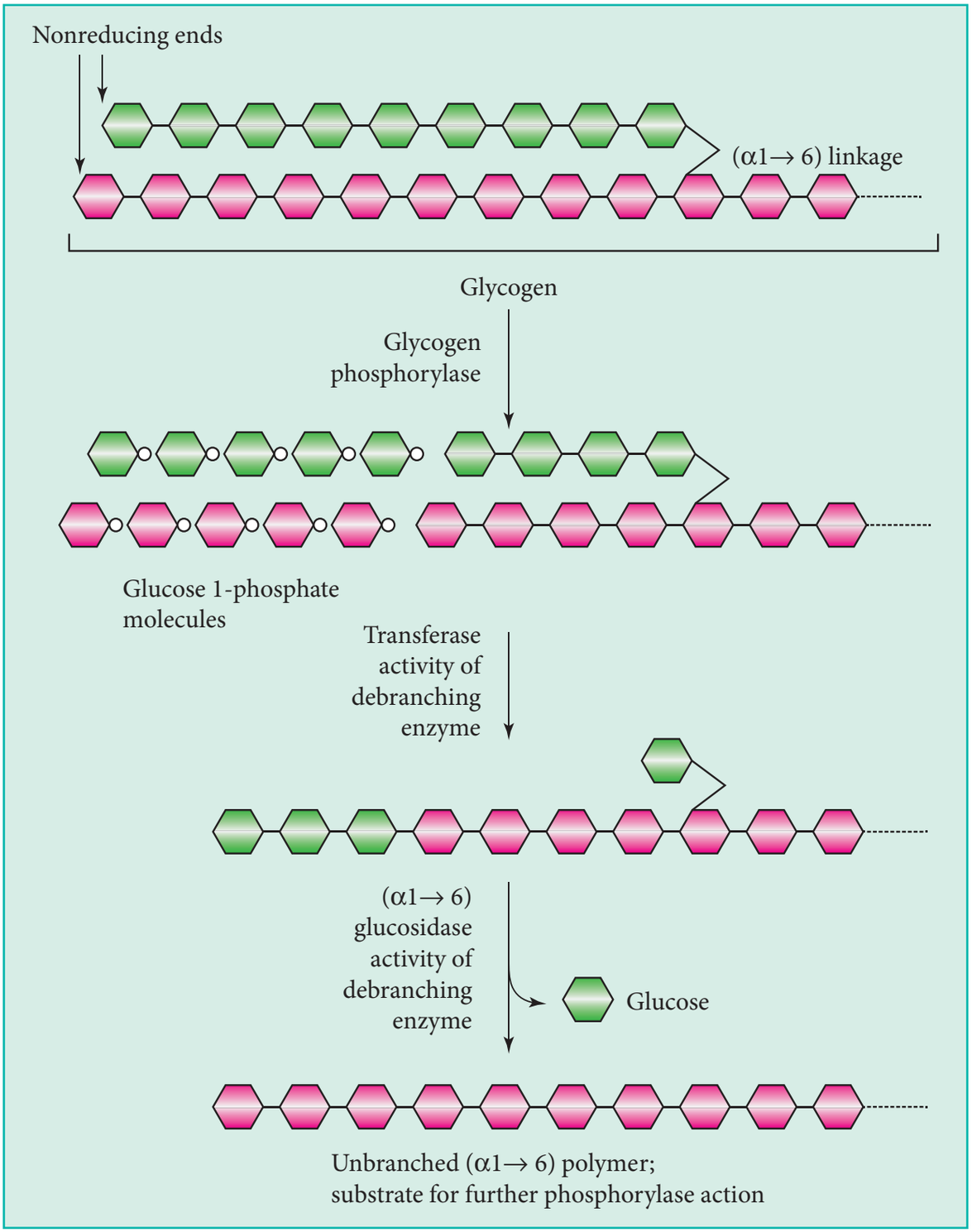
Glycogenolysis is the biochemical breakdown of glycogen to glucose, to provide immediate energy. It takes place in the cells of muscle and liver tissues in response to hormonal and neural signals. In particular, glycogenolysis plays an important role in the adrenaline-induced fight-or-flight response and the regulation of glucose levels in the blood. It is stimulated by the hormones glucagon and epinephrine (adrenaline).

The process of glycogenolysis involves the sequential removal of glucose monomers by phosphorolysis, a reaction catalysed by the phosphorylated 'a' form (active form) of the enzyme glycogen phosphorylase. This enzyme cleaves the glycosidic bond linking a terminal glucose to a glycogen branch by substituting a phosphoryl group for the α [1→4] linkage producing glucose-1-phosphate and glycogen that contains one less glucose molecule.



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A second enzyme, phosphoglucomutase, converts the glucose-1-phosphate to glucose-6-phosphate. Glycogen involves two types of glycosidic linkage: the linear α [1→4] linkage and the branching α [1→6] linkages. During glycogenolysis glucose units are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with an α [1→6] linkage.



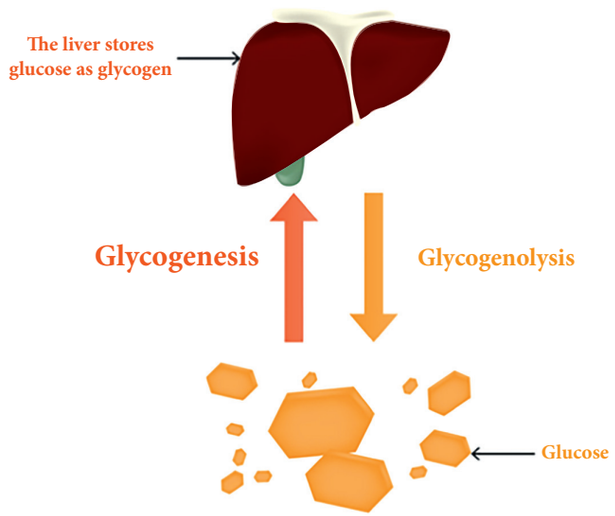



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A third enzyme, glycogen debranching enzyme transfers three of the remaining four glucose units to the end of another glycogen branch, exposing the α [1→6] branching point. This glycosidic bond is hydrolysed by a fourth enzyme α [1-6] glucosidase which eliminates the branch by removing the final glucose as a molecule of glucose, rather than glucose-1-phosphate. In muscle, but not liver cells, the glucose is subsequently phosphorylated to glucose-6-phosphate by a fifth enzyme hexokinase and enters the glycolytic pathway.

In liver cells, the main purpose of the breakdown of glycogen is for the release of glucose into the bloodstream for uptake by other cells. The phosphate group of glucose-6-phosphate is removed by the enzyme glucose-6-phosphatase and the free glucose exits the cell via the membrane localized GLUT2 glucose transporter.

In muscle, glycogenolysis serves to provide an immediate source of glucose-6-phosphate for glycolysis to provide energy for muscle contraction but not for other body tissues. Muscle cells lack the enzyme glucose-6-phosphatase and thus cannot convert glucose-6-phosphate (which cannot be transported across the cell membrane) to glucose.



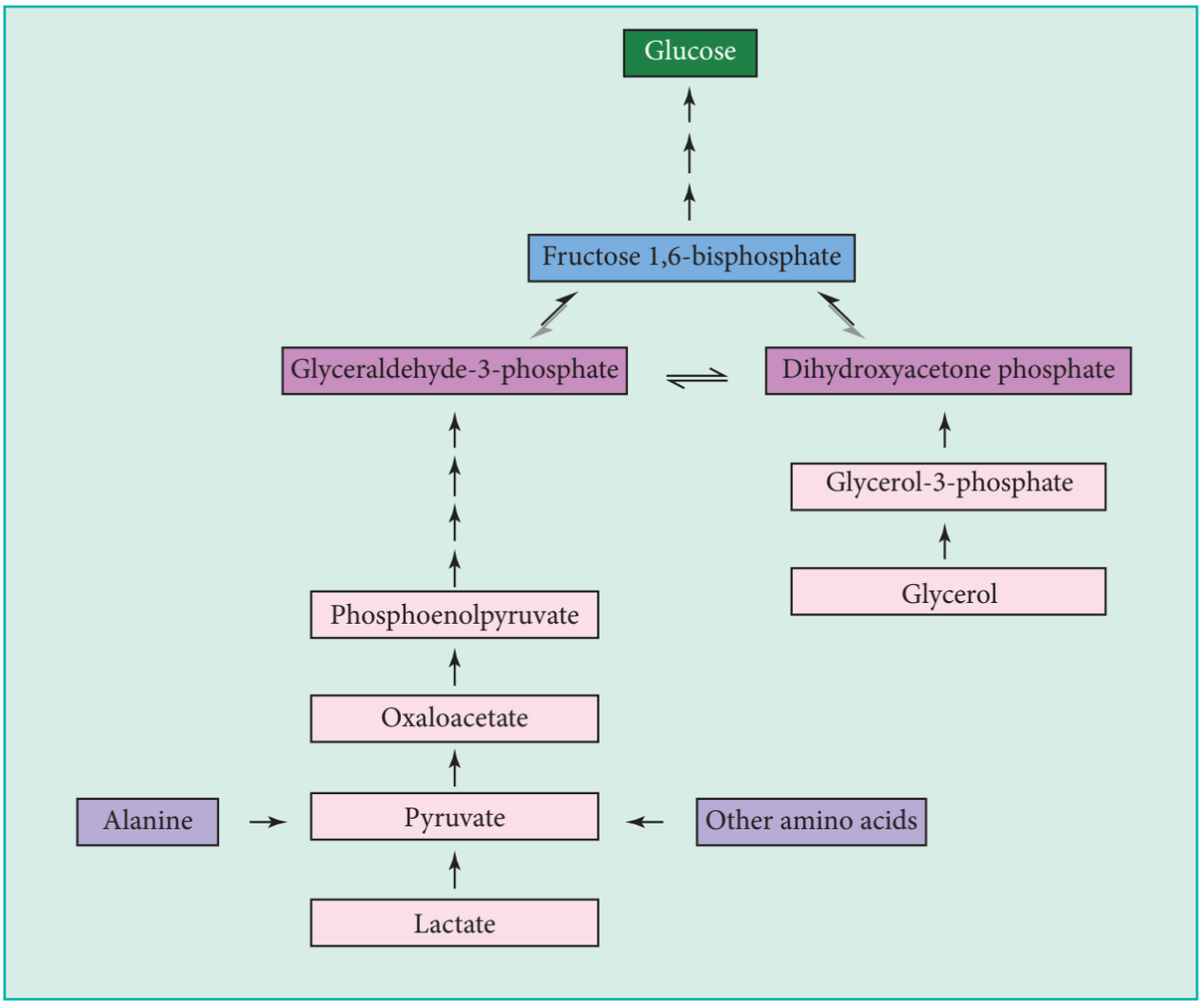

## 3.7. GLUCONEOGENESIS

Gluconeogenesis is a process through which noncarbohydrate precursors such as lactate, pyruvate, glycerol, and amino acids are converted to glucose.

Glucose occupies a central role in metabolism, both as a fuel and as a precursor of essential structural carbohydrates and other biomolecules. The brain and red blood cells are almost completely dependent on glucose as an energy source. Yet the liver's capacity to store glycogen is only sufficient to supply the brain with glucose for about half a day under fasting or starvation conditions. Thus, when fasting, most of the body's glucose needs must be met by gluconeogenesis (literally, new glucose synthesis), the biosynthesis of glucose from noncarbohydrate precursors.



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### 3.7.1 Key reactions in gluconeogenesis

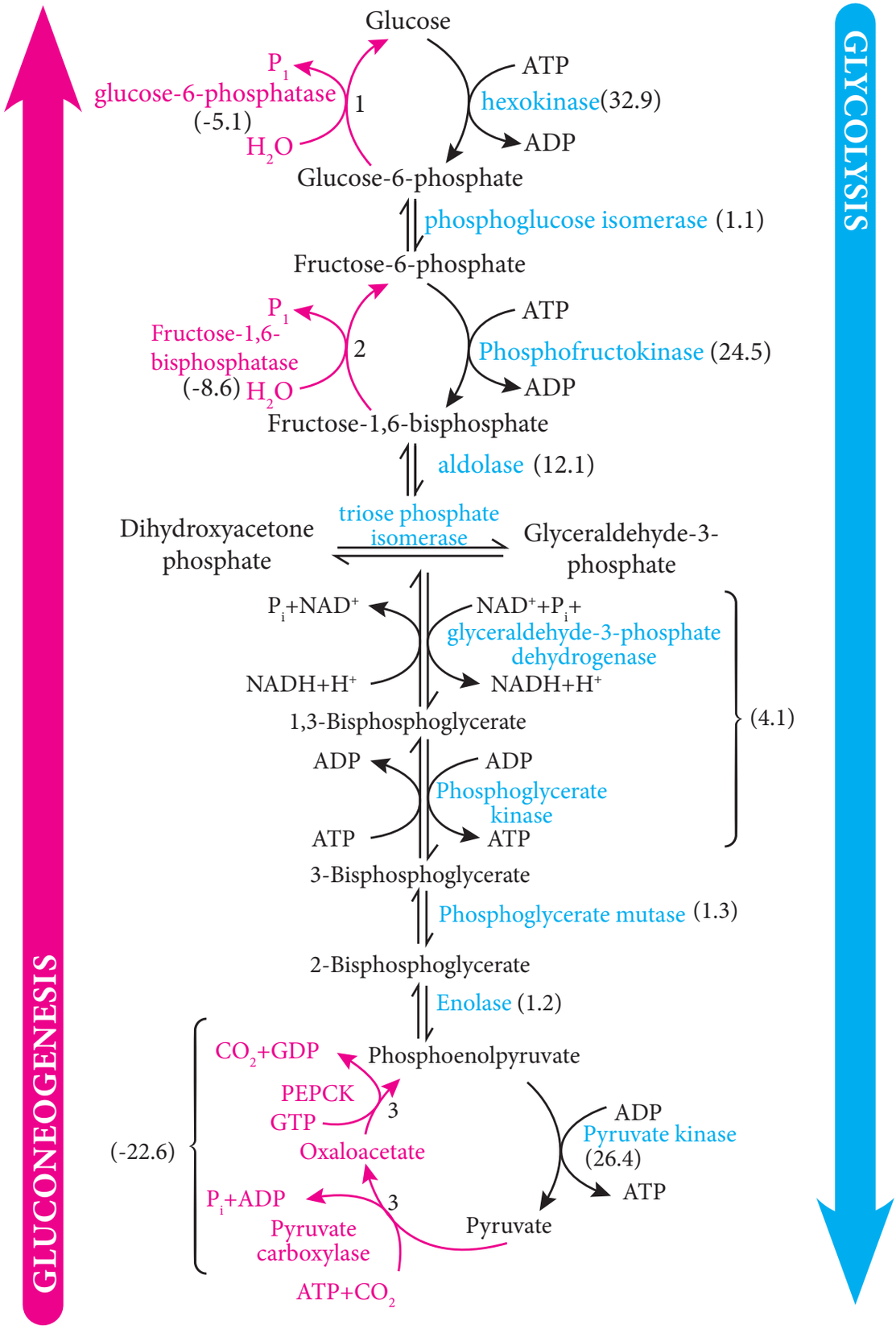
The noncarbohydrate precursors that can be converted to glucose include the glycolysis products lactate and pyruvate, citric acid cycle intermediates, and the carbon skeletons of most amino acids. First, however, all these substances must be converted to oxaloacetate, the starting material for gluconeogenesis.

### 3.7.2 Reactions of gluconeogenesis

Gluconeogenesis is more or less similar to the reverse reactions of the glycolysis. However, there are some differences between these two pathways. Biological reactions can occur in both the forward and reverse direction. If the reaction occurs in the reverse direction the energetics also reversed. i.e. in the forward reaction is exergonic then the reverse reaction should be endergonic. In glycolysis, there are three irreversible steps (1, 3, 10) catalysed by the enzymes hexokinase, phosphofructokinase, and pyruvate kinase respectively. If gluconeogenesis were to simply occur in reverse direction then the reverse of the above mentioned three reactions which would require three different enzymes. The beauty of the nature is that, the above reactions of gluconeogenesis are catalysed by different enzymes namely glucose-6-phosphatase, fructose-1,6-bisphosphatase and PEP carboxykinase.



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**The ΔG's for the reactions in the direction of gluconeogenesis under physiological conditions in liver are given in parentheses in kJ $mol^{-1}$.**



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**Step 1:** The first step in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvic acid (PEP). In order to convert pyruvate to PEP there are several steps and several enzymes required. Pyruvate carboxylase, PEP carboxykinase and malate dehydrogenase are the three enzymes responsible for this conversion. Pyruvate carboxylase is found on the mitochondria and converts pyruvate into oxaloacetate. Because oxaloacetate cannot pass through the mitochondrial membranes it must be first converted into malate by malate dehydrogenase. Malate can then cross the mitochondria membrane into the cytoplasm where it is then converted back into oxaloacetate with another malate dehydrogenase. Lastly, oxaloacetate is converted into PEP via PEP carboxykinase.

The next several steps are exactly the reverse reactions of glycolysis. These reactions are catalysed by the same glycolytic enzymes, except the following two reactions.

The second step that differs from glycolysis is the conversion of fructose-1,6-bisphosophate to fructose-6-phosphate with the use of the enzyme fructose-1,6-phosphatase.

The last step that differs from glycolysis is the conversion of glucose-6-P to glucose with the enzyme glucose-6-phosphatase. This enzyme is located in the endoplasmic reticulum.

Because it is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. In glycolysis and gluconeogenesis seven of the ten steps occur at or near equilibrium. In gluconeogenesis the conversion of pyruvate to PEP, the conversion of fructose-1,6-bisphosphate, and the conversion of glucose-6-phosphate to glucose all occur spontaneously and these reactions are highly regulated. It is important for the organism to conserve as much energy as possible. When there is an excess of energy available, gluconeogenesis is inhibited. When energy is required, gluconeogenesis is activated.

### 3.7.3. Precursors for glucose

**Gluconeogenesis from amino acids:** Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination

**Gluconeogenesis from Propionate:** Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl CoA.

**Gluconeogenesis from Glycerol:** At the time of starvation, glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolysed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it. Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.



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$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

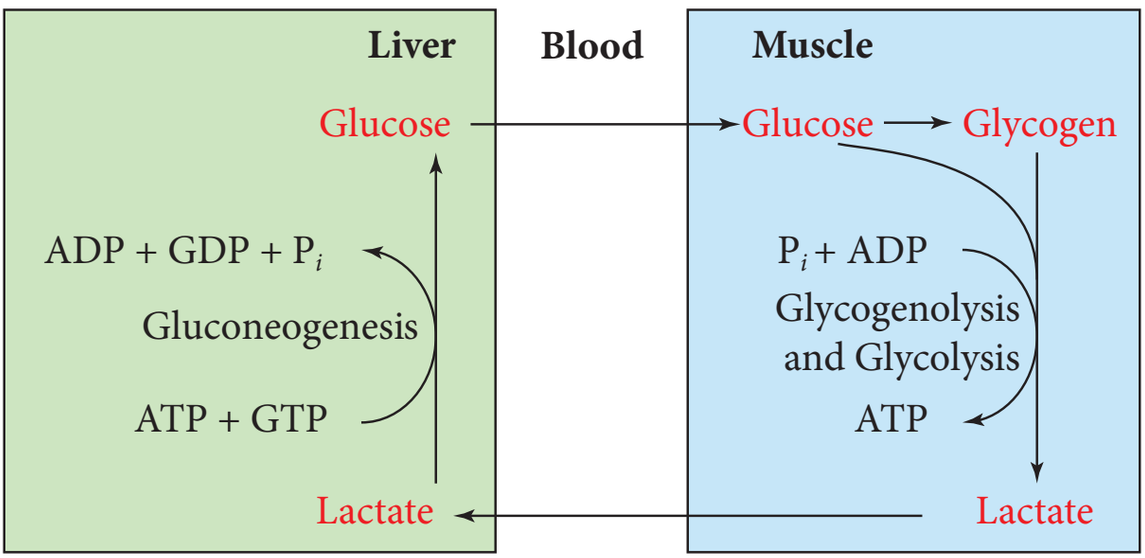
This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD+ and glycerol 3-phosphate dehydrogenase.

$$\text{Glycerol-3-phosphate} + NAD^{+} \xrightarrow{\text{Glycerol-3-phosphate dehydrogenase}} \text{Dihydroxyacetone phosphate} + NADH + H^{+}$$

This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose. Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

### 3.7.4. Cori cycle

Muscle contraction is powered by hydrolysis of ATP, which is then regenerated through oxidative phosphorylation in the mitochondria of slow-twitch (red) muscle fibers and by glycolysis yielding lactate in fast-twitch (white) muscle fibers. Slow-twitch fibers also produce lactate when ATP demand exceeds oxidative flux. The lactate is transferred, via the bloodstream, to the liver, where it is reconverted to pyruvate by lactate dehydrogenase and then to glucose by gluconeogenesis. Thus, through the intermediacy of the bloodstream, liver and muscle participate in a metabolic cycle known as the Cori cycle.




This is similar to ATP-consuming glycolysis/gluconeogenesis, however, instead of occurring in the same cell, the two pathways occur in different organs. Liver ATP is used to resynthesize glucose from lactate produced in muscle. The resynthesized glucose is returned to the muscle, where it is stored as glycogen and used, on demand, to generate ATP for muscle contraction. The ATP utilized by the liver for this process is regenerated by oxidative phosphorylation. After vigorous exertion, it often takes at least 30 min for all



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of the lactate so produced to be converted to glycogen and the oxygen consumption rate to return to its resting level, a phenomenon known as oxygen debt.

## 3.8. DIABETES MELLITUS

Diabetes mellitus is the most common metabolic disease, caused by the lack of insulin activity (not secreted in sufficient amounts or does not efficiently stimulate its target cells) and results in chronic excretion of large volume of urine containing glucose.

The β cells of the pancreas produces a hormone insulin, a 51 amino acid protein in response to high blood glucose levels. Insulin acts mainly on muscle, liver, and adipose tissue cells to stimulate the synthesis of glycogen, fats, and proteins while inhibiting the breakdown of these metabolic fuels.

Insulin functions by binding to the protein receptors on the outer cell surfaces and facilitate the entry of glucose into the cells. Insulin stimulates the uptake and utilization of glucose by most cells and also elevates the rate of glycolysis, glycogen synthesis and fatty acid synthesis. Thus, it lowers the blood glucose level and together with glucagon, insulin acts to maintain the proper level of blood glucose.

The lack of insulin disrupts the regulation and balance of many metabolic pathways. As a consequence, blood glucose levels become so elevated. Yet, despite these high blood glucose levels, cells "starve" since insulin-stimulated glucose entry into cells is impaired.

### 3.8.1. Types

**There are two major forms of diabetes mellitus:**

1. **Insulin-dependent, Type 1,** or **juvenile-onset diabetes mellitus**, which most often strikes suddenly in childhood. It is caused by deficiency of pancreatic β cells or inadequate insulin secretion of insulin by β cells.
2. **Noninsulin-dependent, Type 2,** or **maturity-onset diabetes mellitus**, which usually develops rather gradually after the age of 40, mostly in overweight individuals. It is characterized by insulin resistance as well as impaired insulin secretion, where some of the insulin receptors in the cell membrane fail to recognize insulin.

### 3.8.2. Symptoms

**The symptoms of diabetes mellitus include,**

- Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.
- **Polyuria**: This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).
- **Polydypsia**: The excessive thirst which leads to increased consumption of water.This condition is known as Polydypsia. This is to replace the volume of water excreted due to polyuria.



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- **Polyphagia**: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating, they lose weight and become lean and emaciated.
- Presence of ketones in the urine (ketones are a byproduct of the breakdown of muscle and fat that happens when there's not enough available insulin)
- Fatigue
- Irritability
- Blurred vision
- Slow-healing sores
- Frequent yeast or urinary tract infections

### 3.8.3. Diagnosis

Diabetes is diagnosed with fasting sugar blood tests or with A1C blood tests, also known as **glycated hemoglobin tests**. You do not have to be in fasting for an A1C blood test. A fasting blood sugar test is performed after you have had nothing to eat or drink for at least eight hours. Normal fasting blood sugar is less than **100 mg/dl (5.6 mmol/l)**.

| Test | Normal glucose level | Level of glucose in diabetes patient |
|---|---|---|
| **Fasting Glucose Test** | Less than 100 mg/dl | 126 mg/dl or higher |
| **Random (anytime) Glucose Test** | Less than 140 mg/dl | 200 mg/dl or higher |
| **A1C Test** | Less than 5.7% | 6.5% or higher |

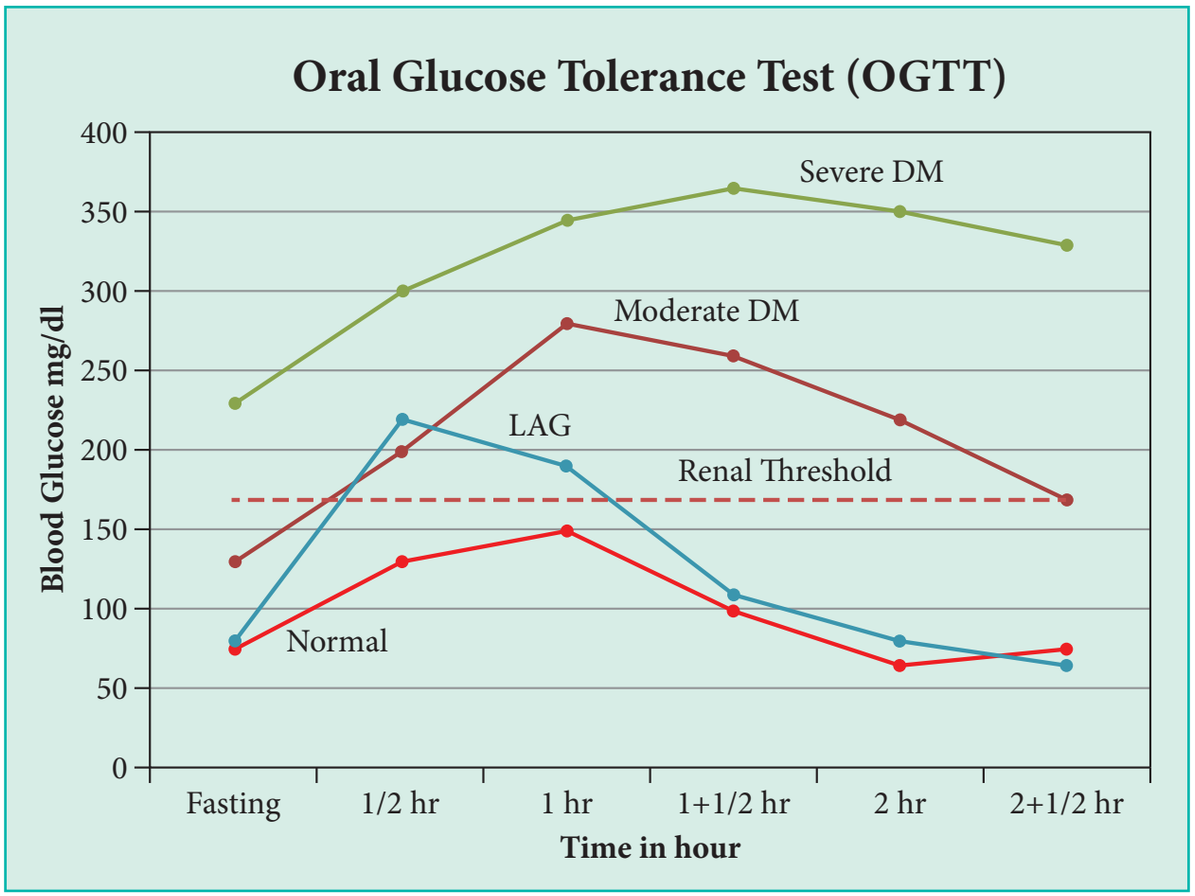
#### 3.8.3.1. Glucose tolerance test

The glucose tolerance test, also known as the **oral glucose tolerance test** (OGTT), measures your body's response to sugar (glucose). The glucose tolerance test can be used to screen for type 2 diabetes.

Before the test begins, a sample of blood will be taken.



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You will then be asked to drink a liquid containing a certain amount of glucose (usually 75 grams). Your blood will be taken again every 30 to 60 minutes after you drink the solution.

The test may take up to 3 hours.

More commonly, a modified version of the glucose tolerance test is used to diagnose gestational diabetes — a type of diabetes that develops during pregnancy.

## EVALUATION

### I. Choose the correct answer from the given four alternatives

1. How many NADHs are produced from 7.5 mol of acetyl CoA by the citiric acid cycle?

   a. 15 mol  b. 7.5 mol

   c. 75 mol  d. none of these

2. Blood sugar is

   a. Sucrose  b. Lactose

   c. Glucose  d. Fructose



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3. How many ATP molecules are generated during anaerobic glycolysis

   a. 2 b. 10

   c. 6 d. 8

4. Which one of the following enzyme is involved in substrate level phosphorylation

   a. Citrate synthase b. Isocitrate dehydrogenase

   c. Succinyl CoA synthetase d. Fumarase

5. The glucose renal threshold is reached when

   a. a condition of hyper glycemia occurs

   b. blood glucose levels are too low

   c. insulin levels are to high

   d. a fasting state occurs

6. The end product of aerobic glycolysis is

   a. Pyruvate b. Citrate

   c. Acetyl CoA d. Lactate

7. The important reducing power produced in HMP shunt pathway is

   a. NADH b. NADPH

   c. FAD d. FADH2

8. Pyruvate is converted to oxaloacetate by

   a. Pyruvate carboxylase b. Pyruvate kinase

   c. PFK d. Phosphoenol pyruvate carboxylase

9. Lactate is converted to glucose in

   a. Skeletal muscle b. liver

   c. Kidney d. lung

10. How many irreversible steps occurs in glycolysis?

    a. 2 b. 4 c.3 d. 5

11. Insulin is secreted by

    a. Liver b. Kidney

    c. Pancreas d. Thyroid

12. Glycolysis converts glucose to two pyruvate, the sum of the oxidation numbers of six carbon atoms is changed from 'x' (glucose) to 'y' (pyruvate) x and y are

    a. 0 and 4 b. 4 and 0

    c. 0 and 2 d. none of these



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13. Glycolysis is termed anerobic because

    a. molecular oxygen is required

    b. only small amounts of ATP are formed

    c. molecular oxygen is not required

    d. NADH is produced

14. In citric acid cycle the acetyl group of acetyl CoA is

    a. reduced to two carbondioxide

    b. oxidised to two carbondioxide

    c. oxidised to two NADGHs

    d. reduced to two NADHs

15. Which of the following enzyme links glycolysis and TCA cycle?

    a. Glucokinase  b. PFK

    c. LDH  d. Pyruvate dehydrogenase

16. Which of the following compounds cannot be used to synthesis glucose in gluconeogenisis

    a. glycerol  d. lactate

    c. acetyl CoA  d. aminoacid

## II. Fill up the blanks

1. Glucokinase acts on glucose to form _____
2. 2 - phosphoglycerate is converted to _____ by the enzyme enolase
3. In the anaerobic phase one molecule of glucose produces _____ molecules of ATP
4. Tricarboylic acid cycle occurs in _____
5. Glycogen biosynthesis is known as _____
6. The major source of glucose in ruminants is

## III. Say true of false

1. Phosphoglycerate kinase converts 1,3 bisphosphoglycerate to 3 - phosphoglycerate
2. Pyruvate kinase acts reversibly
3. 24 molecules of ATP are formed in TCA cycle
4. UDP glucose pyrophosphorylase is involved in the synthesis of glycogen
5. Degradation of glucose is also known as glycolysis
6. Pyruvate is the end product of glycolysis



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## IV. Match the following

| | | | |
|---|---|---|---|
| 1. | Glycolysis | - | Ribose 5 - phosphate |
| 2. | PDH | - | Insulin |
| 3. | HMP shunt pathway | - | Cytosol |
| 4. | Debranchin enzyme | - | Acetyl CoA |
| 5. | Diabetes mellitus | - | Glycogenolysis |
| 6. | TCA | - | Glycerol |
| 7. | Lipid | - | Mitochondira |

## V. Answer the following

1. Give short note on cori cycle.
2. Briefly discuss several common fats for glucose produced through glucoreogenesis in the liver.
3. Name the enzymes which are involved for NADH formation in TCA cycle?
4. What is the mean by aerobic and anaerobic phases?
5. What is the difference between NADH and NADPH?
6. Describe the steps involved in TCA cycle.
7. Write a short notes on GTT.
8. What are glucogenic amino acids?
9. What are the reaction sequences of glycolysis ?
10. How many moles of ATP can be formed form 0.5 mol of acetyl CoA using the citric acid cycle, electron transport and oxidative phosphorylation?
11. What are the steps involved in glycogen metabolism?
12. What is glycogenesis?
13. Would you define glycogenesis as anabolic or catabolic? Explain briefly.
14. If 2.5 mol of glucose is partially oxidised in glycolysis
    a. how many moles of pyruvate are produced
    b. how many moles of ATP are produced
15. Explain the glycogenolysis.
16. How pyruvate is converted to lactate?
17. Explain the HMP shunt pathway.
18. How pyruvate is converted to glucose?



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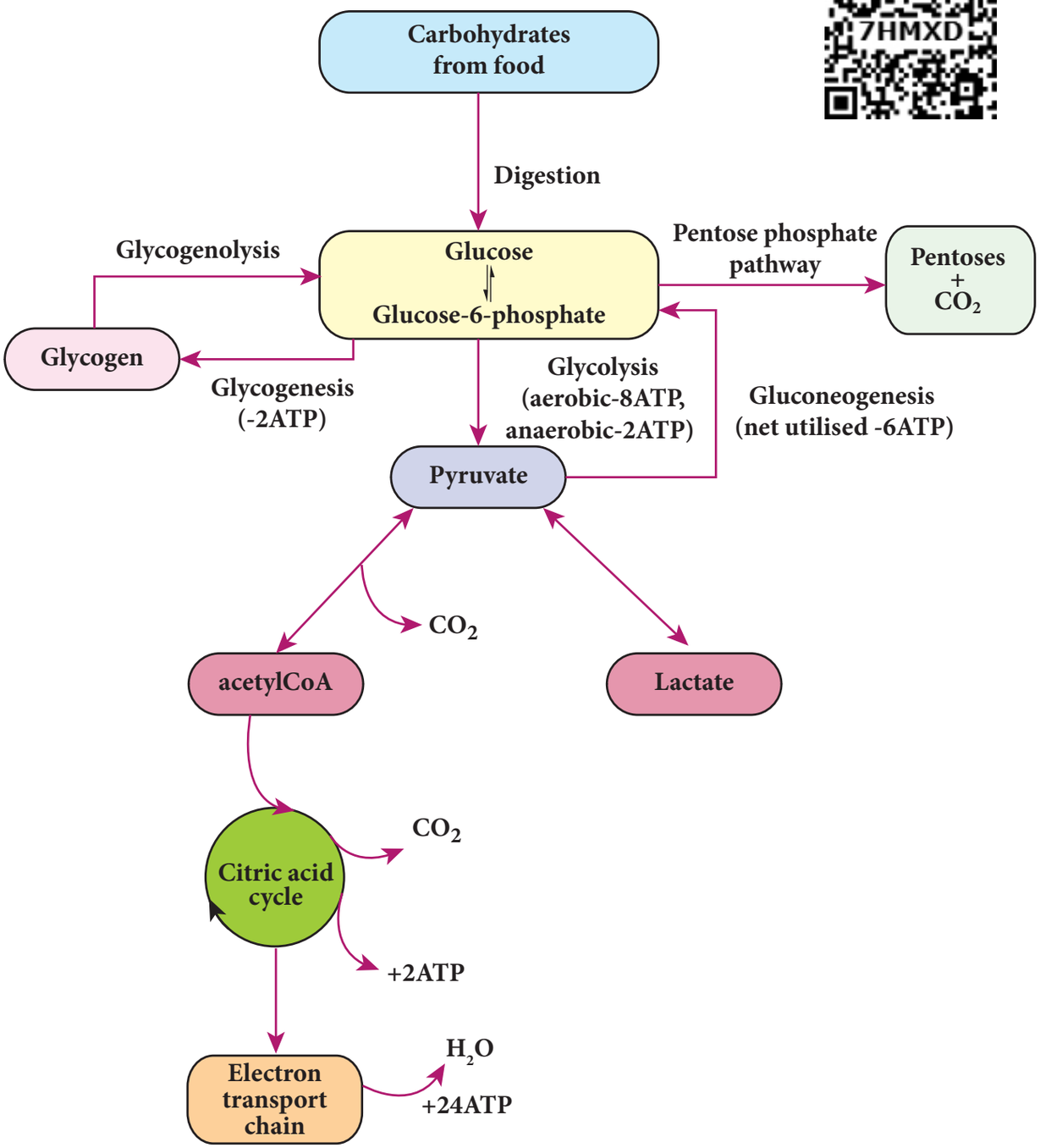
19. Explain the diabetes mellitus.
20. Whether the ATP productions that is associated with the citric acid cycle is substrate level phosphorylation (or) oxidative phosphorylation?
21. After meal, have a blood glucose concentration of 250 mg/dl of blood. Is this hyper glycemic (or) hypoglycemic?
22. What are the end products of the anerobic catabolism of glucose in muscle tissue.
23. Write the three important irreversible reactions in glycolysis?
24. How does gluconeogenesis get around the three irreversible steps of glycolysis.



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# CONCEPT MAP

## Carbohydrate metabolism






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UNIT 4

# PROTEIN METABOLISM



Sir Hans Adolf Krebs

"Krebs, a German-born Jew was trained in medicine and chemistry. When, he was forced out of his position at the University of Freiburg as the Nazis rose to power in the early 1930s, he went to England and joined University of Sheffield in 1935, where he achieved his ground breaking discoveries of TCA cycle and Urea cycle. He was awarded with Nobel prize for his discovery of TCA cycle.

W268G

## Learning Objectives

After studying this unit the students will be able to

- Understand the general reactions concerned with the catabolism of dietary amino acids
- Comprehend the various ways in which the amino groups of the amino acids are removed
- Reciprocate the removal of carboxyl groups and fate of carbon skeletons of the amino acids
- Emulate (Reproduce) the reactions of Urea cycle and realize that the amino groups of amino acids are removed in the form of urea by liver
- Understand that the amino acids also serve as precursors for many biologically important compounds



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## INTRODUCTION

We already know that proteins are made up of amino acids and have many functions in all living system. The 20 amino acids are obtained from the diet or from turnover of proteins. The major part of amino acid content of the cell exists in the form of proteins which is constantly being synthesized and degraded. Amino acids cannot be stored in an analogous form of glycogen (glucose) or triglycerides (fatty acids) leaving a small pool of amino acids in the cells as per the needs for protein synthesis. Excess amino acids are broken down into the carbon skeleton and the nitrogen amino group. The carbon skeleton is converted into common metabolites that can be used for the synthesis of other biomolecules or for further oxidation to generate ATP, while their amino group is converted to urea.

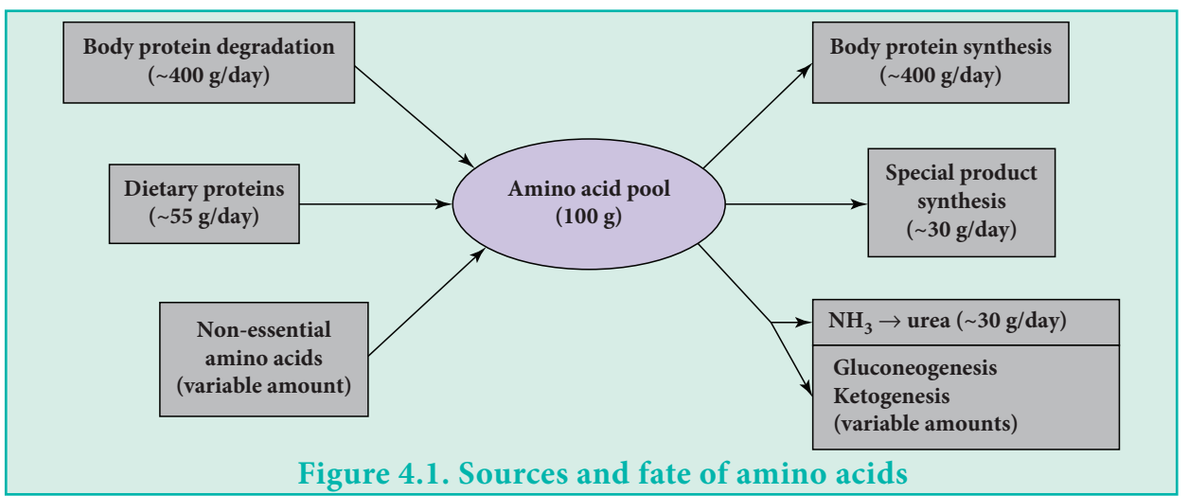



**Figure 4.1. Sources and fate of amino acids**

Amino acids are not only used to synthesize proteins, but also they act as precursors for specialized products of amino acid derivatives that play biologically significant roles in metabolism and homeostasis (Figure 4.1).

## 4.1 GENERAL REACTIONS OF AMINO ACID

Non-essential amino acids are synthesized from α-keto acids by transfer of amino groups with the help of transaminases. However, essential amino acids cannot be synthesized from α-keto acids. Transfer of amino groups also occurs during degradation of amino acids. Amino groups can be removed from amino acids by transamination as well as by deamination. Removal of carboxyl group (Decarboxylation) from amino acids results in the production of biologically active amines. The carbon skeletons of amino acids are degraded on entry into TCA cycle.

### 4.1.1 Catabolism of amino acid

Even though, each amino acid has a specific pathway for its catabolism, the first step in the catabolism of amino acids is the removal of its amino group. This can happen by two different ways namely, transamination and deamination.



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### 4.1.2. Transamination (AST and ALT)

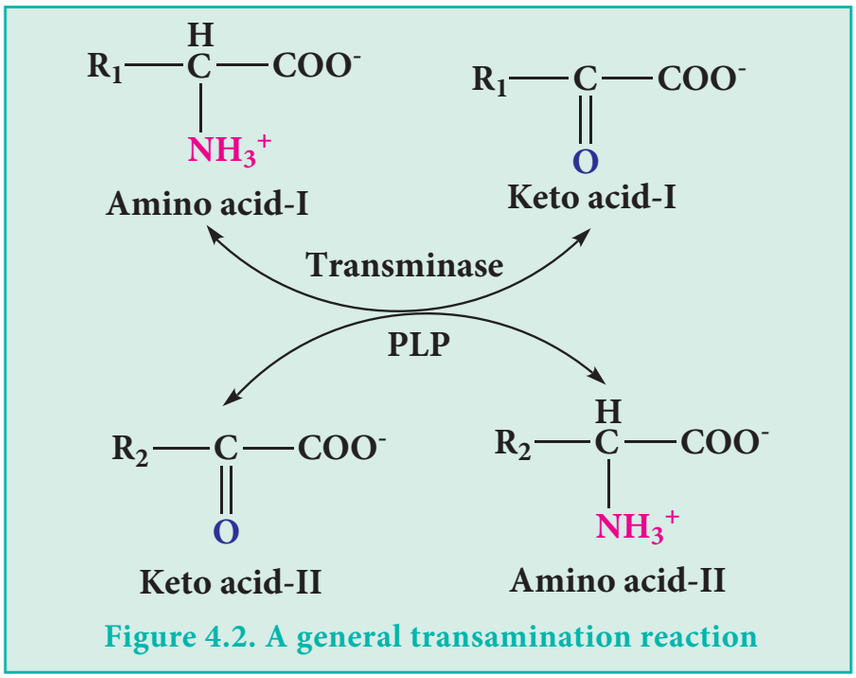



Figure 4.2. A general transamination reaction

**Transamination** is a process, in which transfer of amino groups occur between a keto acid and an amino acid. It is just shuffling of amino groups and not removal of amino groups. Each amino acid has a specific transaminase (Figure 4.2). However, Alanine transaminase (ALT) and Aspartate transaminase (AST) are the important transaminases.

**The reactions catalysed by ALT and AST are :**

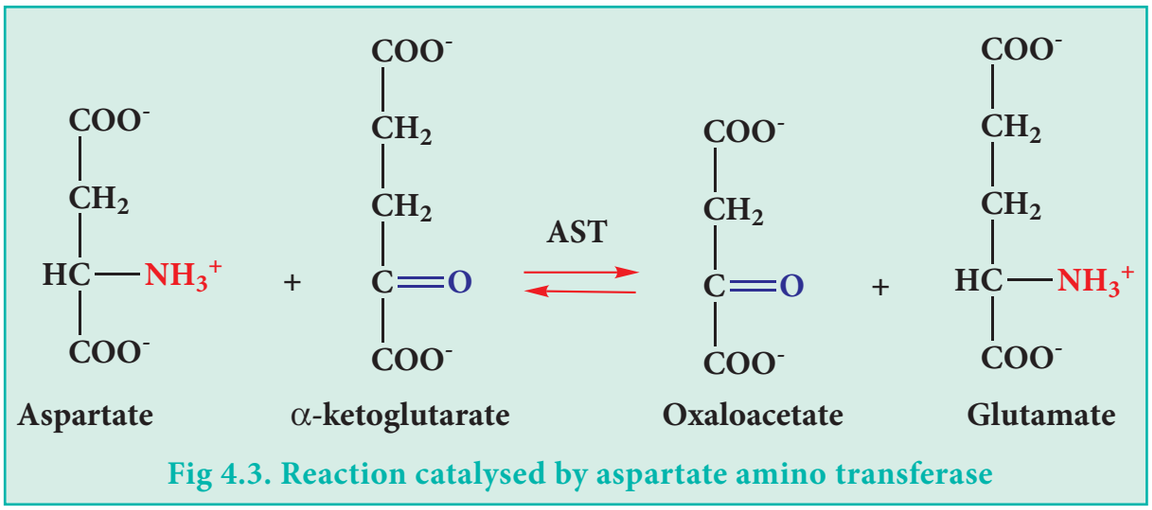



Fig 4.3. Reaction catalysed by aspartate amino transferase

Aspartate transaminase catalyses the inter-conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate (Figure 4.3), while alanine transaminase catalyses the conversion of alanine and α- ketoglutarate to pyruvate and glutamate (Figure 4.4).



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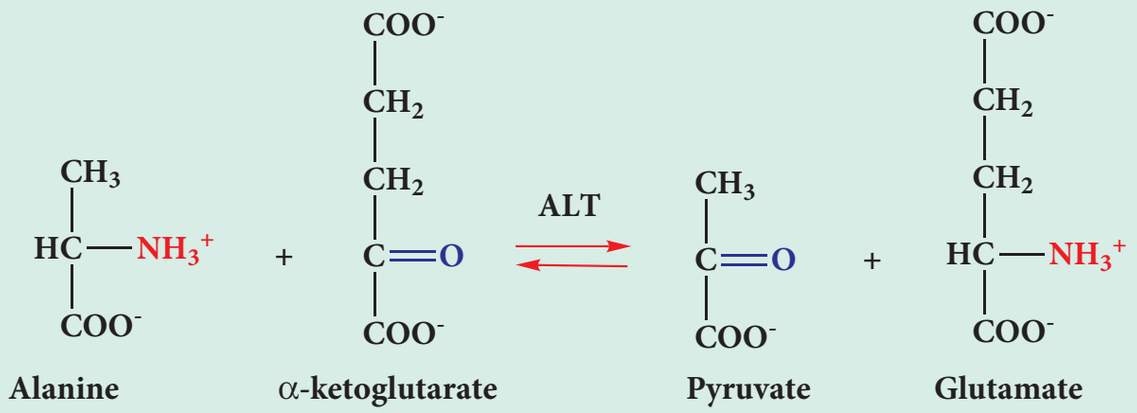



**Fig 4.4. Reaction catalysed by Alanine amino transferase or Alanine transaminase**

Transamination involves the coenzyme pyridoxal phosphate for the shuttling of amino groups. During this process, pyridoxamine phosphate is formed as an intermediate. The amino group of the amino acid reacts with pyridoxal phosphate bound to the enzyme to form a Schiff's base. As the alpha ketoglutarate is involved in all the transamination reactions, glutamate acts as a sink for the amino groups for most of the amino acids (Figure 4.5).

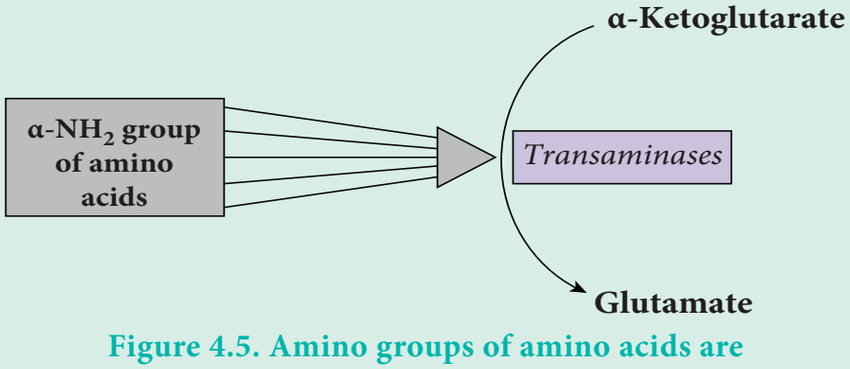



**Figure 4.5. Amino groups of amino acids are accepted by alpha ketoglutarate**

### Features of Transamination

- Transamination reactions are reversible reactions.
- All amino acids except lysine, threonine, proline and hydroxyl proline undergo transamination.
- Serum transaminases are significant markers for diagnosis of liver and heart diseases.
- Many non-essential amino acids are synthesized using transamination reactions.

### 4.1.3 Deamination

Deamination is the process of removal of amino groups from amino acids as ammonia. Deamination may be or may not be coupled with oxidation. Depending upon this, deamination is classified as oxidative deamination and non-oxidative deamination.



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## Oxidative Deamination

Oxidative Deamination is a process in which the alpha amino group of the amino acid is removed as ammonia, coupled with oxidation. Most of the amino acids dump their amino groups to alpha ketoglutarate and pyruvate forming glutamate and alanine, respectively.

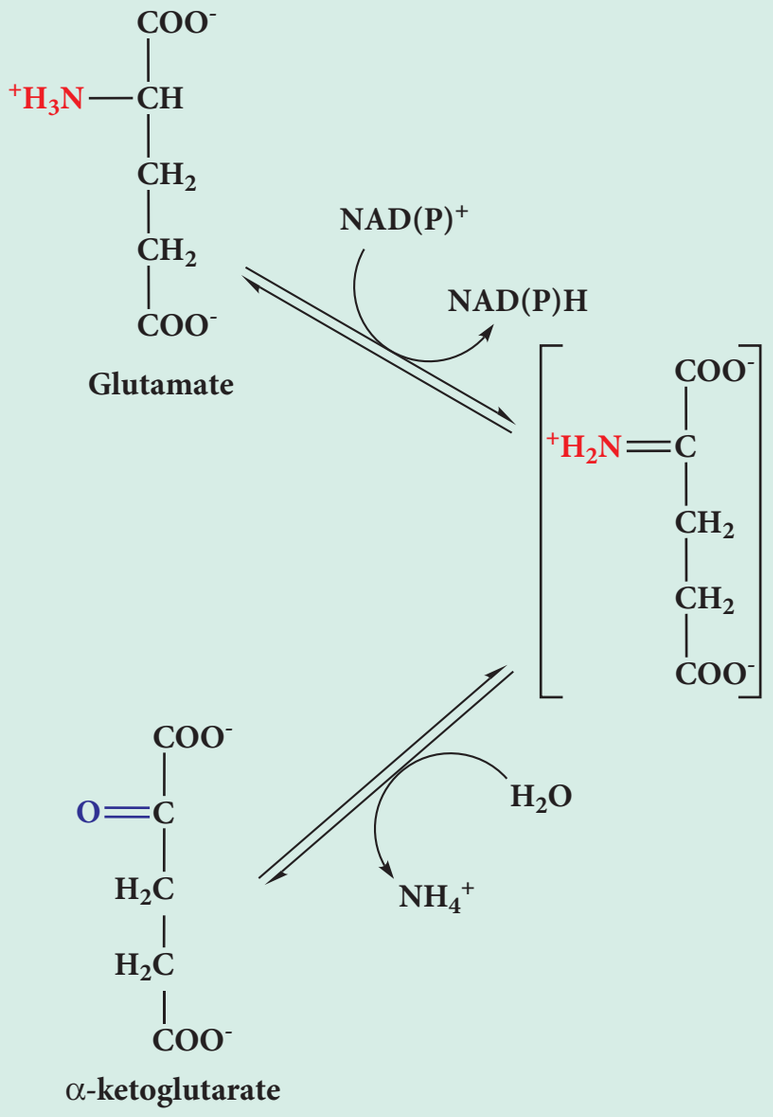



**Figure 4.6. Oxidative deamination of glutamate**

Oxidative deamination involving the removal of amino group of glutamate as ammonia is the key reaction involved in delivery of amino groups to the liver for urea synthesis. The reaction is catalysed by glutamate dehydrogenase (GDH) with $NAD^+$/ $NADP^+$ as the co-enzyme and this reaction is freely reversible. The enzyme is present in liver and kidneys and it plays a central role in nitrogen metabolism. Liver GDH activity is allosterically inhibited by ATP and GTP (Fig 4.6).

As glutamate acts as a collection centre for all alpha amino groups by transamination and subsequently glutamate liberates ammonia, the process is collectively called as trans-deamination.

L-amino acid oxidases and D-amino acid oxidases are also involved in oxidative deamination.



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### Steps involved in oxidative deamination:

1. In the first step, L-Amino acid oxidases convert the L-amino acid into L-imino acid, which involves FMN as the coenzyme. The resulting peroxide that is formed is detoxified by catalase (Figure 4.7).

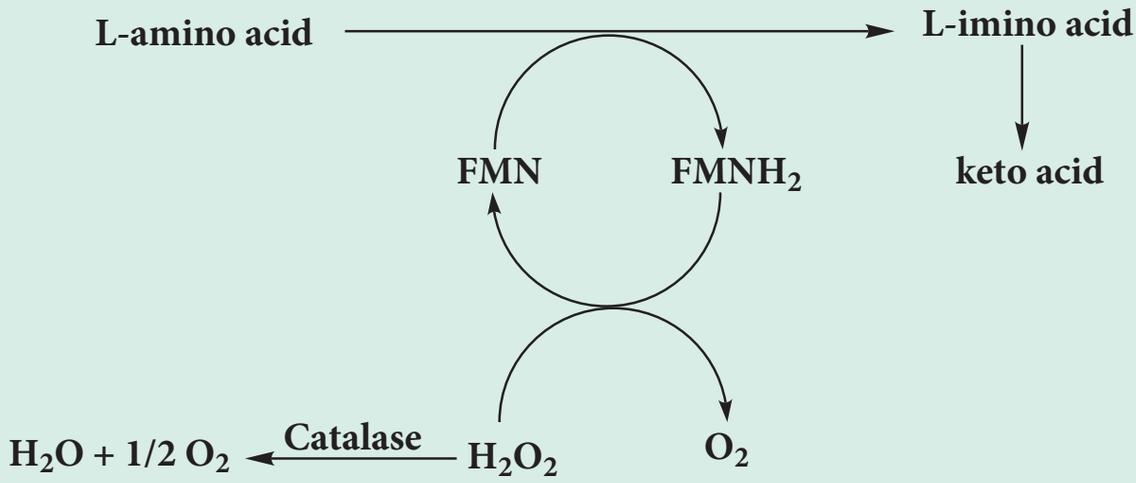



**Figure 4.7 Enzymatic reaction catalyzed by L-amino acid oxidase**

2. In the second step, the imino acid undergoes hydrolytic cleavage to keto acid and ammonia.

All L-amino acids except hydroxyl amino acids and dicarboxy amino acids can be acted upon by L-amino acid oxidases.

D-amino acid oxidases utilize FAD as the coenzyme.

### Non-oxidative deamination

Dehydratases act on hydroxyl amino acids like serine and threonine to form pyruvate and alpha keto butyric acid, respectively.

Cysteine desulfurase removes the sulfydryl group of cysteine to form pyruvate.

The amide groups of glutamine and asparagine are removed by glutaminase and asparaginase, respectively.

### 4.1.4 Decarboxylation

The removal of carboxyl group from the amino acid in the form of $CO_2$ is called as decarboxylation. Such decarboxylation reactions are involved in the formation of biologically important amines (Figure 4.8). Several amino acid decarboxylases are present in tissues like liver, kidney, lungs and brain that decarboxylate the respective amino acids to form amines.



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**For example,**

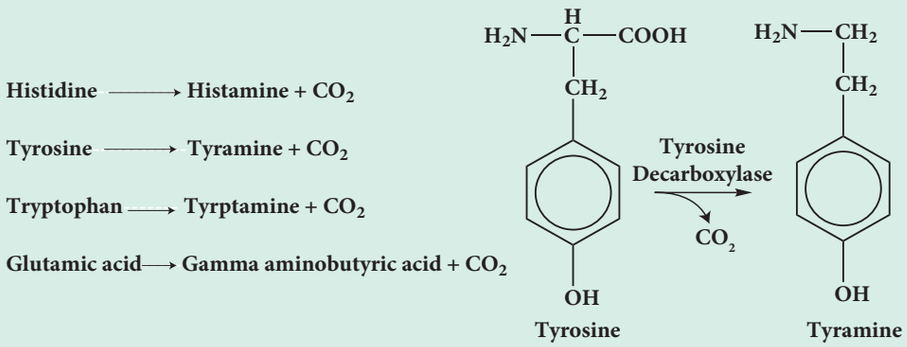



**Figure 4.8. Decarboxylation reactions and formation of amines**

### 4.1.5 Fate of carbon skeleton of amino acids

**Entry into TCA cycle**

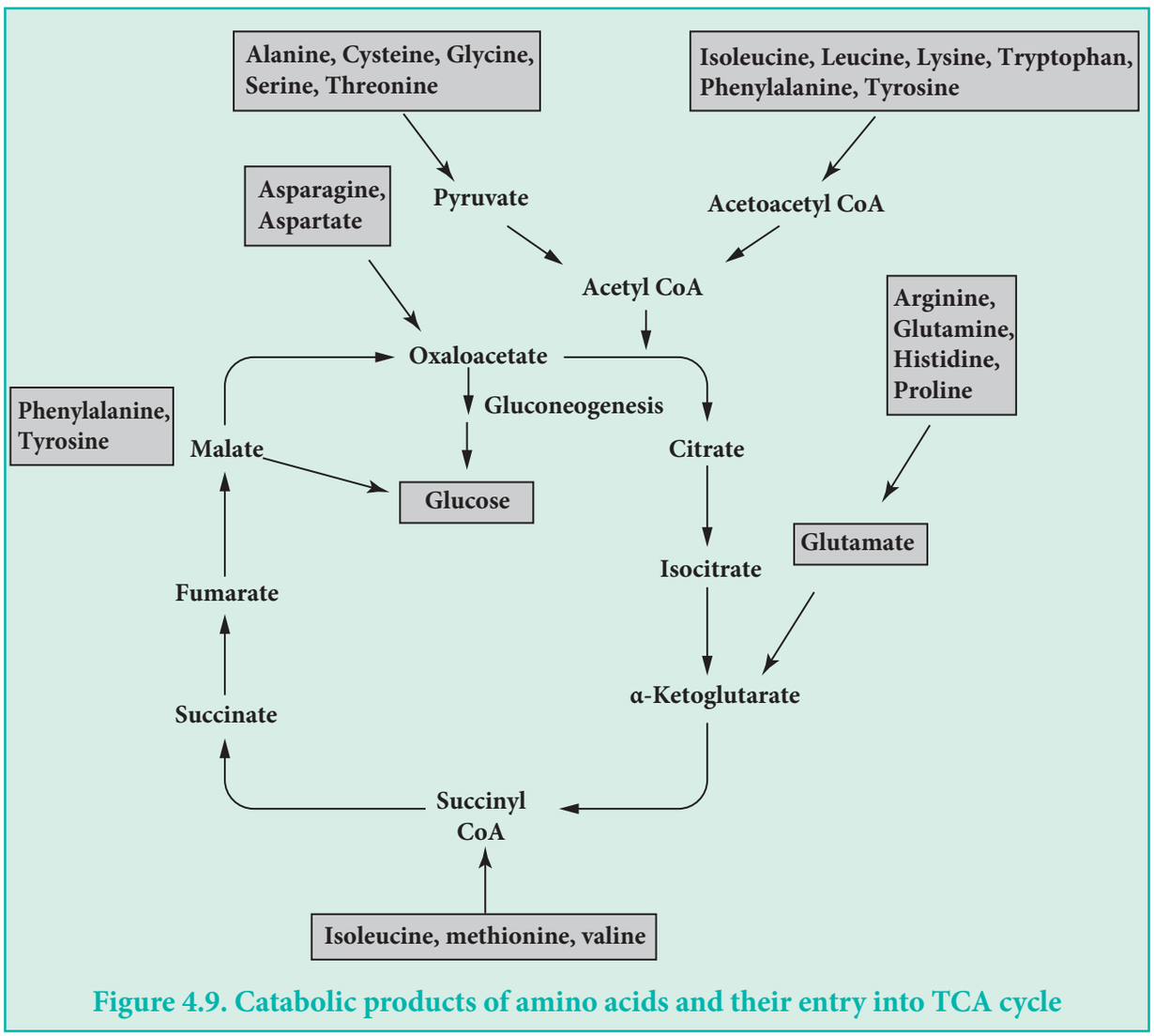



**Figure 4.9. Catabolic products of amino acids and their entry into TCA cycle**

After the amino group is lost, the keto acids formed from the amino acids like pyruvate, alpha ketoglutarate will enter into the TCA cycle and are utilized for energy purposes.



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Catabolism of phenylalanine and tyrosine yields fumarate that also enters TCA cycle for further catabolism. These intermediates that enter into TCA cycle can act as precursors of gluconeogenesis (Figure 4.9). Hence, the amino acids that can contribute their carbon skeletons for the synthesis of glucose are called as glucogenic amino acids. eg. glycine, alanine, aspartate, glutamate, etc. However, certain amino acids yield acetyl CoA and acetoacetyl CoA that can act as substrates of fatty acid synthesis or give rise to ketone bodies. These amino acids are called as ketogenic amino acids. Eg. Leucine and Lysine. Certain amino acids like phenylalanine, isoleucine and tyrosine are both glucogenic and ketogenic.

### Regeneration of amino acids

By reductive amination or by reversal of trans-deamination, the amino acids can be re-synthesized from their respective keto-acids.

### 4.1.6 Transmethylation

**One carbon metabolism**

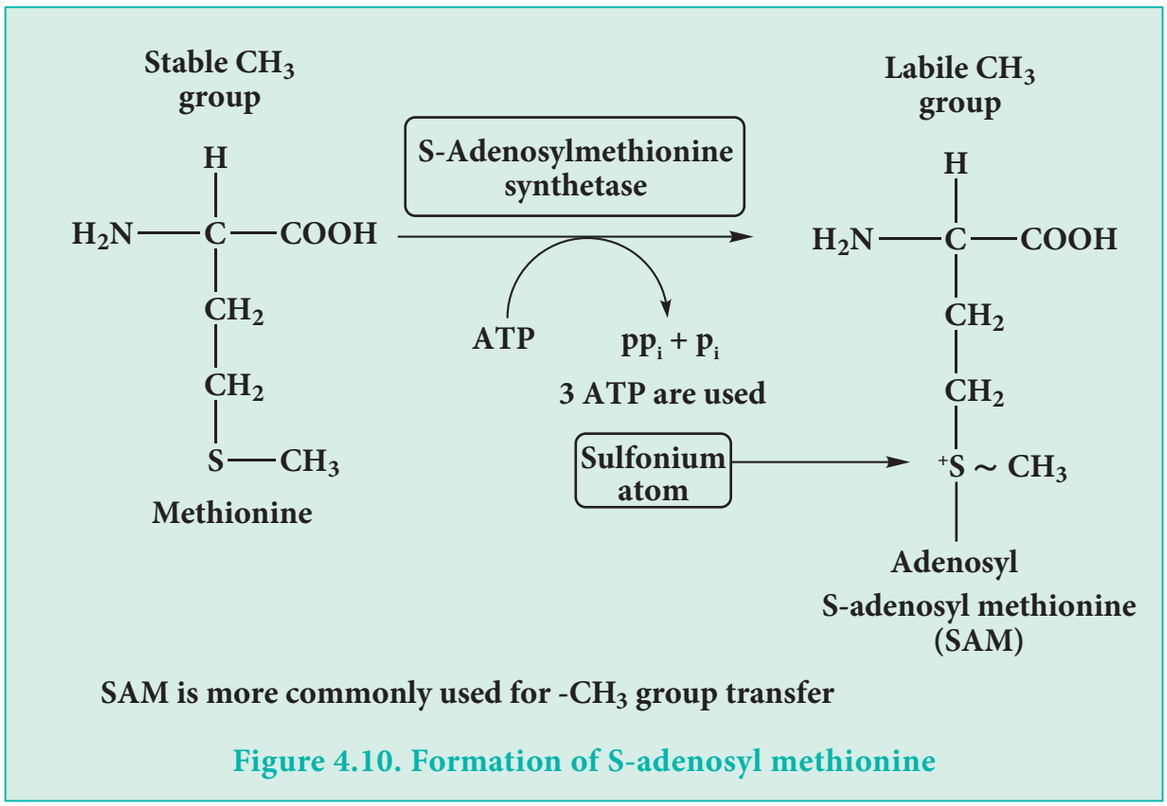



**Figure 4.10. Formation of S-adenosyl methionine**

One carbon metabolism plays an important role in the synthesis of many compounds like purines and pyrimidines. One carbon pool includes formyl, methyl, methylene, methenyl, formimino and hydroxymethyl groups. Except methyl group, all the other one carbon transfers are mediated by Tetrahydrofolate that bears one carbon moieties at N5 and N10 positions. Formate, tryptophan, glycine, serine and choline are the methyl group donors to tetrahydrofolate. Transfer of methyl group involves S-adenosyl methionine (Figure 4.10), which is formed from methionine



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S-Adenosyl methionine thus formed acts as a methyl group donor and is involved in the synthesis of epinephrine, creatine and thymine.

## 4.2. UREA CYCLE

### Toxicity of Ammonia

The ammonia formed by trans-deamination and from other sources like bacteria in the gut are transported to the liver and converted to urea. Under physiological conditions, the level of ammonia in the blood is kept under control stringently as the central nervous system is highly susceptible to ammonia intoxication. The major symptoms of ammonia intoxication include slurred speech, tremor, blurred vision, coma and finally death. Ammonia toxicity is because it reacts with α-ketoglutarate to form glutamate, thus depleting the levels of α-ketoglutarate and impairing the function of TCA cycle in neurons.

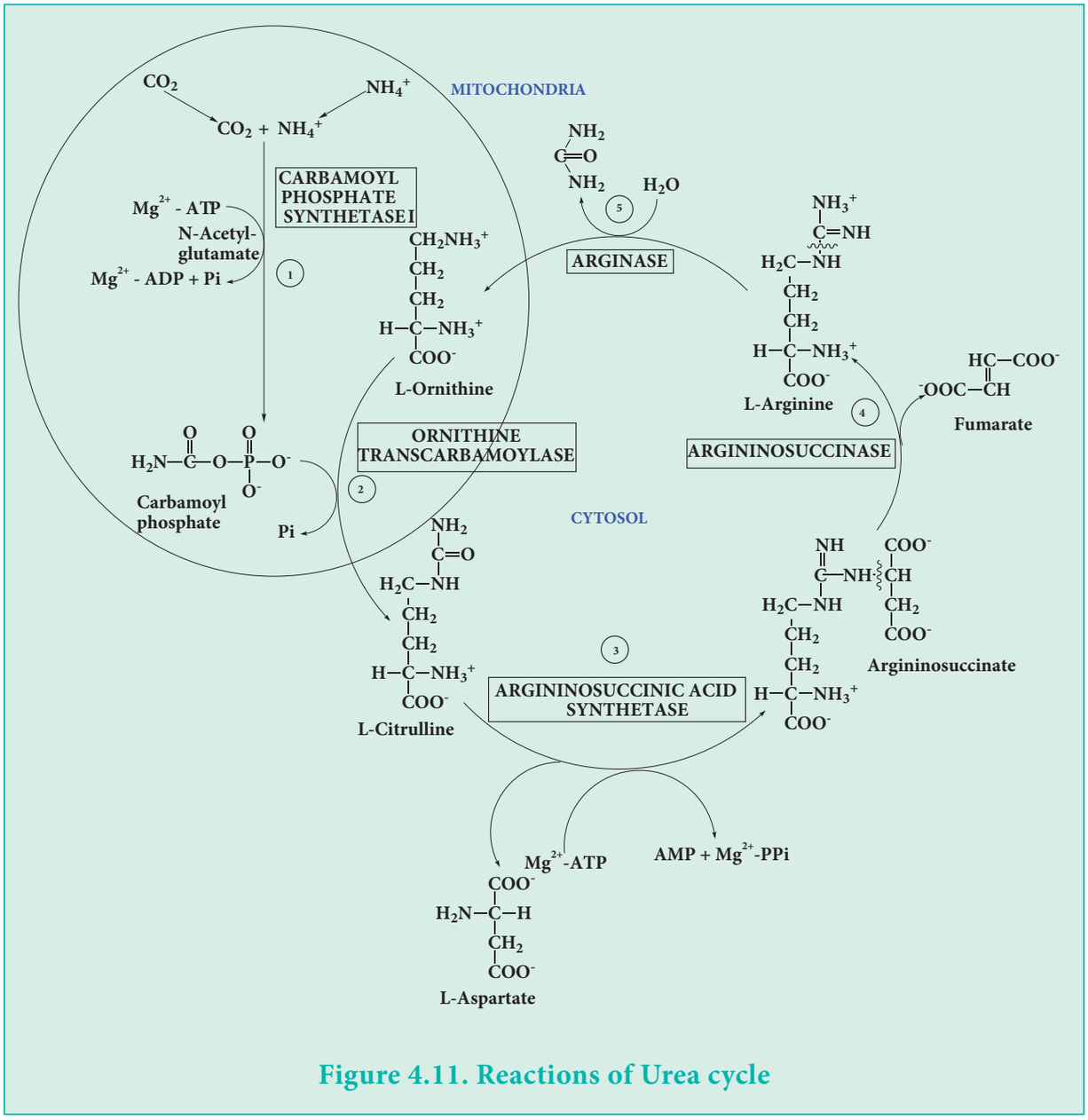



**Figure 4.11. Reactions of Urea cycle**



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The amino group of proteins can be excreted in three forms in living organisms – ammonia, urea or uric acid. Based on the product of elimination, the living organisms can be classified as ammonotelic, ureotelic and uricotelic respectively. Many terrestrial vertebrates excrete the amino nitrogen as urea, while birds and terrestrial reptiles excrete it in the form of uric acid.

In ureotelic animals, the ammonia that is released in the liver is converted to urea and excreted by the kidneys. The pathway of conversion of ammonia to urea involves ornithine in a cyclic fashion. Hence, the pathway is called as urea cycle or Ornithine cycle or Krebs Henseleit cycle after its discoverers Hans Krebs and a medical student, Kurt Henseleit in 1932.

Urea is produced from ammonia with the help of five enzymes and two of these enzymes are present in the mitochondria, while, the rest are present in the cytosol. Out of the two amino groups of urea, one amino group enters the urea cycle as carbamoyl phosphate, and the other enters as aspartate (Figure 4.11).

### The five steps of Urea cycle:

1. **Formation of carbamoyl phosphate:** Carbamoyl phosphate synthetase I present in the mitochondria facilitates the synthesis of carbamoyl phosphate from carbon dioxide, ammonia and ATP. This reaction consumes two ATPs and is irreversible. It is different from the Carbamoyl phosphate synthetase II present in the cytosol, which is involved in pyrimidine biosynthesis, and uses glutamine as the substrate.

2. **Formation of Citrulline:** Carbamoyl phosphate reacts with ornithine to form citrulline and the reaction is catalysed by Ornithine transcarbamoylase. The other three enzymes of urea cycle are present in the cytosol. Hence, Citrulline is transported out of the mitochondria by a transporter.

3. **Formation of argininosuccinate:** Citrulline and aspartate condense together to form argininosuccinate in the presence of the enzyme Argininosuccinate synthetase. This reaction requires ATP and is catalyzed by a citrullyl-AMP intermediate (the amino group of Aspartate provides the second nitrogen atom for Urea synthesis).

4. **Formation of arginine from argininosuccinate:** This reaction catalysed by argininosuccinase releases arginine and fumarate (which enters the citric acid cycle).

5. **Formation of urea and regeneration of ornithine:** The final step in urea synthesis is the release of urea from arginine by the enzyme arginase and this step regenerates ornithine. The ornithine thus formed enters into the mitochondria with the help of a transporter.

One molecule of Urea is synthesized from one molecule of carbondioxide, one molecule of ammonia and the amino group of aspartate. The reactions consume four high energy bonds equivalent to four ATPs.



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## 4.3 Formation of Niacin

Niacin (pyridine-3-carboxylic acid) is formed from the amino acid tryptophan. Niacin is the precursor for the synthesis of nicotinamide coenzymes. Only 3% of the tryptophan is converted to niacin in the liver. Maize eating population demonstrate niacin deficiency.

The steps involved in the synthesis of niacin (Figure 4.12) from tryptophan are:

1. Tryptophan is oxidized to N-formyl kynurenine.
2. N-formyl kynurenine transfers its formyl group to Tetrahydrofolate and results in the formation of Kynurenine
3. Kynurenine is hydroxylated at the 3rd position to form 3-hydroxy kynurenine.
4. Alanine is removed from 3-hydroxy kynurenine in the presence of pyridoxal phosphate to form 3-hydroxy anthranilic acid.
5. 3-hydroxy anthranilic acid is converted to quinolinic acid
6. Quinolinic acid on decarboxylation yields niacin.

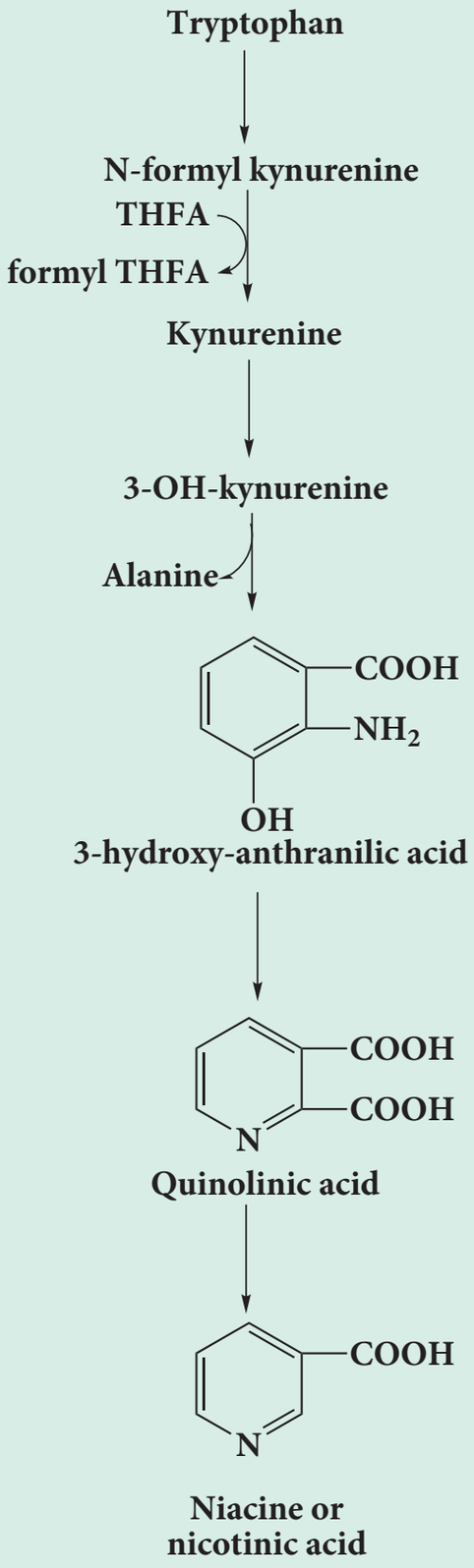



**Figure 4.12. Biosynthesis of Niacin**



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### Specialized products formed from tyrosine

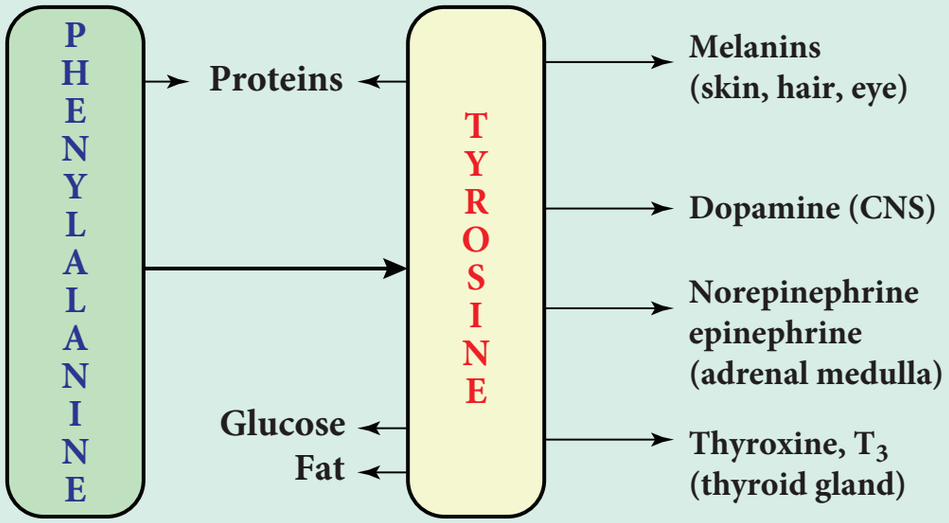



**Figure 4.13 Specialized products formed form amino acids**

Many specialized products are formed from tyrosine in different tissues (Figure 4.13). In the nervous system, tyrosine serves as the precursor for dopamine. In the adrenal glands, it is converted to stress hormones namely, epinephrine and nor-epinephrine. In the thyroid gland, tyrosine is iodinated and converted to thyroid hormones namely tri-iodo thyronine (T3) and tetraiodothyronine (T4-thyroxine)

## 4.4 Formation of Melanin

Melan means black. Melanin is the pigment that is responsible for the colour of the skin, hair and eyes. Melanin is a group of random polymers of indole quinone formed from tyrosine in the melanocytes. The major enzyme involved in its synthesis is Tyrosinase, which is a copper dependent enzyme (Figure 4.14).

1. Tyrosine is hydroxylated to form dihydroxyphenyl alanine (DOPA)

2. DOPA is decarboxylated to DOPA quinone.

3. DOPA quinone is converted to indole quinone by a series of reactions.

4. Indole quinone polymerizes to melanin.



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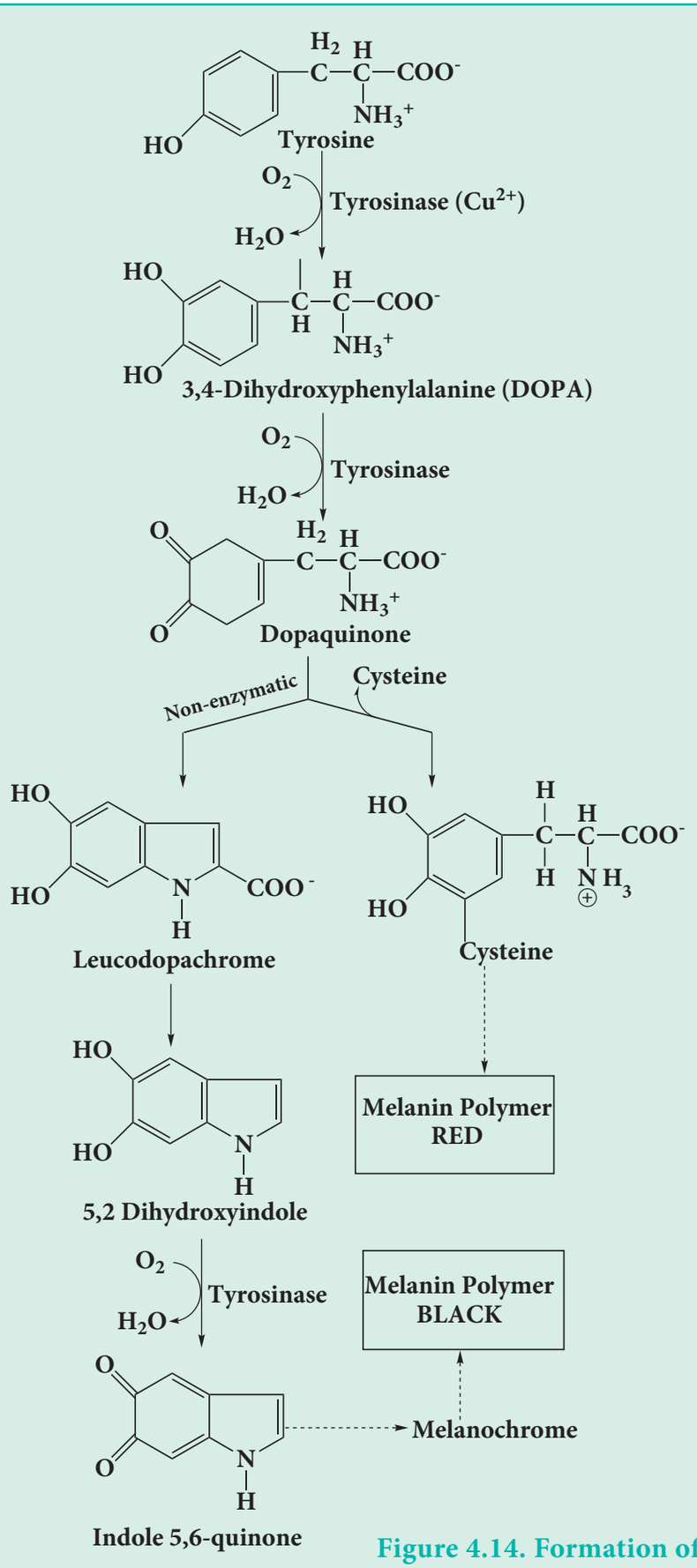



Figure 4.14. Formation of melanin



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## 4.5 Formation of thyroid hormones

Thyroid gland, a bilobed organ weighing about 20 – 25 grams, is responsible for the synthesis of thyroid hormones. Thyroid hormones are responsible for maintenance of basal metabolic rate. Tri-iodothyronine and tetraiodothyronine are the two important thyroid hormones (Fig 4.15).

**The steps involved in the synthesis of thyroid hormones are:**

1. Conversion of inorganic iodine to iodide (activation of iodine).
2. Iodination of tyrosine rings in the protein thyroglobulin to form di-iodotyrosine
3. Formation of di-iodothyronine and tetraiodothyronine by coupling and removal of alanine. Thyroglobulin bearing iodinated residues is stored in the thyroid gland.
4. Depending upon the needs of the body, TSH signals the thyroid gland resulting in the proteolysis of thyroglobulin and release of T3 and T4.

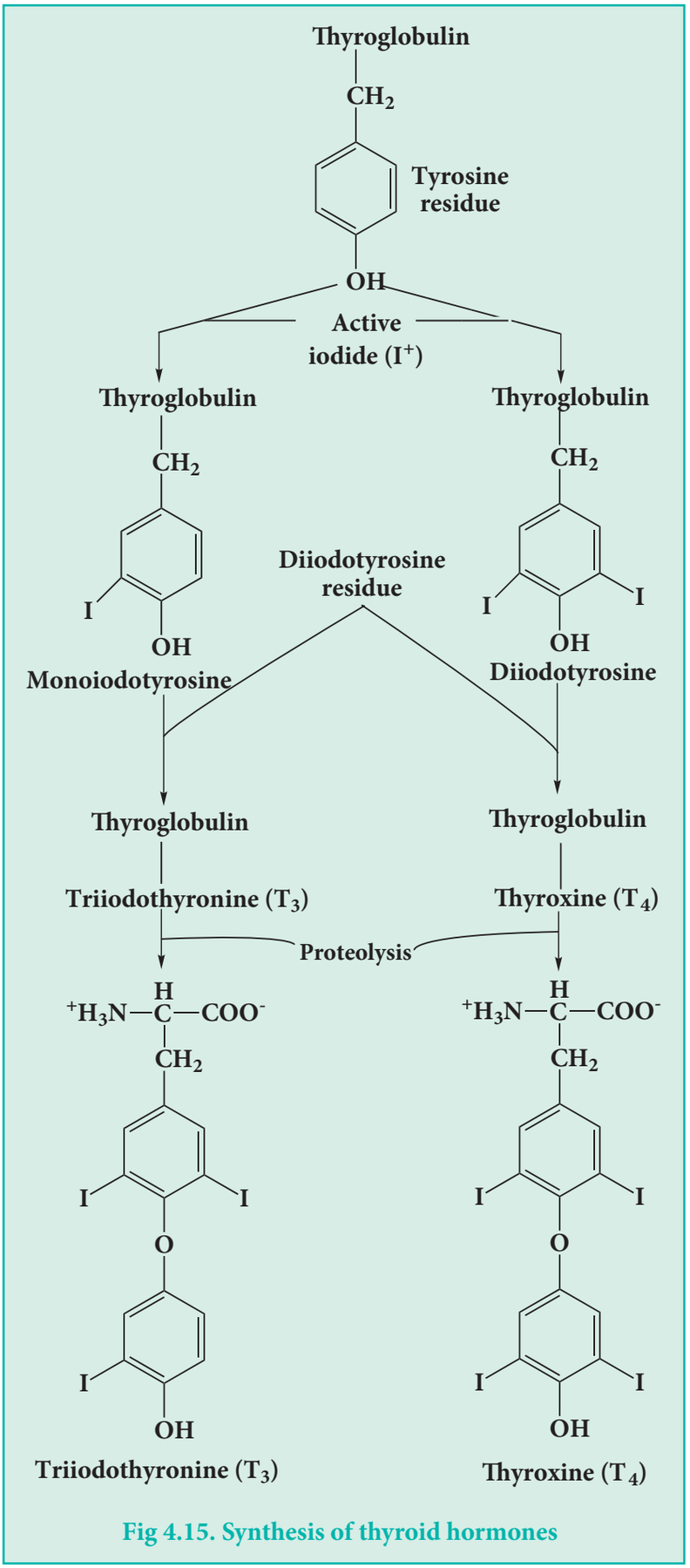



Fig 4.15. Synthesis of thyroid hormones



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## 4.6 Formation of Catecholamines

Adrenal glands present in the top of the kidneys are responsible for the secretion of hormones that regulate mineral metabolism (secreted from the adrenal cortex) along with hormones that are secreted in response to stress (secreted by the adrenal medulla), such as epinephrine and nor-epinephrine, that are specialized products of tyrosine (Fig 4.16).

**The functions of catecholamines (epinephrine and nor-epinephrine) are:**

1. They influence the metabolism of glucose and have an anti-insulin action, i.e. they raise the blood glucose levels.
2. They are responsible for the flight and fight response.
3. These hormones tend to increase the blood pressure by constricting the blood vessels and increasing the force by which heart muscles contract.

**The steps involved in the synthesis of catecholamines are:**

1. Tyrosine is acted upon by the enzyme tyrosine hydroxylase in the adrenal medulla to form dihydroxy phenyl alanine (DOPA).
2. DOPA is decarboxylated to Dopamine by DOPA decarboxylase.

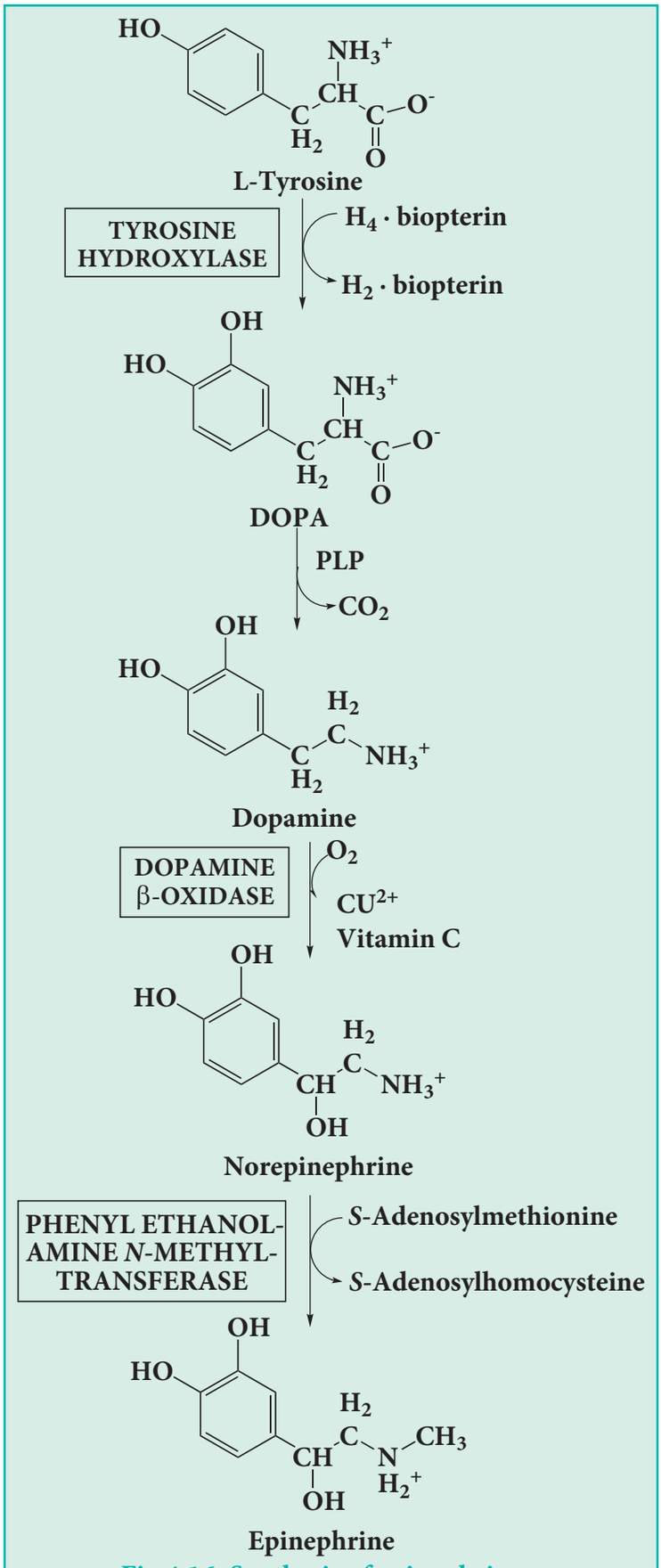



Fig 4.16. Synthesis of epinephrine

3. Dopamine is converted to nor-epinephrine (Nor means No 'R' group, ie. Methyl group) by a reaction catalyzed by DOPA hydroxylase.
4. Nor-epinephrine is methylated to Epinephrine. The methyl group donor is S-adenosyl Methionine and the enzyme is N-methyl transferase.

## Activity

1. Prepare a chart to explore the link between amino acid metabolism and TCA cycle
2. Prepare a chart to explain urea cycle.
3. With the help of e-books, try to prepare a list of syndromes associated with urea biosynthesis.




- Transaminases AST and ALT are important markers to assess liver damage. Many occupational hazards occur on exposure to solvents like $CCl_4$ and $CHCl_3$. **Example:** Liver damage.
- Insulin, Insulin like Growth factor-1 and Growth hormone have anabolic effects on protein metabolism.
- Ingestion of proteins or infusion with amino acids also have an anabolic effect on protein metabolism.

## Summary

- Excess intake of amino acids are used for energy purposes, gluconeogenesis or ketogenesis, while their amino group is converted to urea.
- The carbon skeletons of amino acids are degraded on entry into TCA cycle.
- The first step in the catabolism of amino acids is the removal of its amino group. This removal can happen in two different ways - Transamination and Deamination.
- Transamination is a process, in which transfer of amino groups occur between a keto acid and an amino acid. Alanine transaminase (ALT) and Aspartate transaminase (AST) are the important transaminases.
- Aspartate transaminase catalyses the inter-conversion of aspartate and alpha ketoglutarate to oxaloacetate and glutamate, while alanine transaminase catalyses the conversion of alanine and alpha ketoglutarate to pyruvate and glutamate.



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- Serum transaminases are significant markers for diagnosis of liver and heart diseases.
- Many non-essential amino acids are synthesized using transamination reactions.
- Deamination is classified into oxidative deamination and non-oxidative deamination.
- Oxidative Deamination is a process in which the alpha amino group of the amino acid is removed as ammonia, coupled with oxidation.
- Oxidative deamination involving the removal of amino group of glutamate as ammonia is the key reaction involved in delivery of amino groups to the liver for urea synthesis.
- L-amino acid oxidases and D-amino acid oxidases are also involved in oxidative deamination.
- Glutaminase and asparaginase remove the amino groups of glutamine and asparagine.
- Decarboxylation reactions are involved in the formation of biologically important amines.
- After the amino group is lost, the keto acids formed from the amino acids like pyruvate, alpha keto glutarate will enter into the TCA cycle and are utilized for energy purposes.
- Amino acids can be glucogenic and /or ketogenic.
- Urea is produced from ammonia with the help of five enzymes and two of these enzymes are present in the mitochondria, while, the rest are in the cytosol. Out of the two amino groups of the urea, one amino group enters the urea cycle as carbamoyl phosphate, and the other enters as aspartate.
- Niacin (pyridine 3 carboxylic acid) is formed from the amino acid tryptophan.
- Many specialized products are formed from tyrosine in different tissues. In the nervous system, tyrosine serves as the precursor for dopamine. In the adrenal glands, it is converted to stress hormones namely, epinephrine and nor-epinephrine. In the thyroid gland, tyrosine is iodinated and converted to Thyroid hormones namely tri-iodothyronine and tetraiodothyronine.



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# EVALUATION

## I Multiple choice questions

1. **Generally, Non-essential amino acids are synthesized by _____ reactions.**
   a) Oxidative deamination
   b) Oxidative decarboxylation
   c) **Transamination**
   d) Non-oxidative deamination
2. Essential Aminoacids
   a) are synthesized in the liver
   b) are only used in the synthesis of proteins
   c) **cannot be synthesized in the body**
   d) are only catabolized to urea
3. Transamination involves
   a) **exchange of amino groups between a ketoacid and an aminoacid**
   b) addition of keto groups from a ketoacid to an aminoacid
   c) transfer of amino groups from a ketoacid to an aminoacid
   d) all the above
4. The coenzyme involved in transamination is
   a) **Pyridoxal phosphate**
   b) Coenzyme Q
   c) Thiamine
   d) Pyridoxamine Phosphate
5. Serum Transaminases are significant markers for
   a) Liver diseases
   b) Heart Diseases
   c) **Both a and b**
   d) None of the above
6. In ureotelic animals, the amino group is excreted as
   a) Ammonia
   b) Urea and Uric Acid



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c) **Urea**

d) Uric acid

7. Carbamoyl Phosphate Synthetase –II is present in the

a) Mitochondria

b) **Cytosol**

c) Partly mitochondria and partly in the cytosol

d) Nucleus

8. DOPA is

a) Dehydroxyphenylalanine

b) Dihydroxyphenylacetate

c) **Dihydroxyphenylalanine**

d) Dehydrophenylalanine

9. The first step in the catabolism of amino acids is

a) **Removal of its amino group**

b) Removal of the carboxyl group

c) Removal of the carbon skeleton

d) Removal of methyl group

10. _____ serves as a precursor for dopamine

a) Tryptophan

b) Hydroxyproline

c) **Tyrosine**

d) Proline

11. The _____ reaction is catalyzed by the glutamate dehydrogenase with _____ as the co-enzyme.

a) Irreversible and $NADH^+/NADPH^+$

b) Irreversible and $NAD^+/NADP^+$

c) **Reversible and $NAD^+/NADP^+$**

d) Reversible and $NADH^+/NADPH^+$

12. _____ amino acids are both glucogenic and ketogenic in nature.

a) **Isoleucine and tyrosine**

b) Glycine and alanine

c) Leucine and lysine



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d) Aspartate and glutamate

13. _____ acts as a methyl donor for the synthesis of epinephrine, creatine and thymine.

a) Methotrexate

b) **S- adenosyl methionine**

c) Tetrahydrofolate

d) Biotin

14. Catabolism of phenylalanine and tyrosine yield _____.

a) Succinate

b) α-ketoglutarate

c) Malate

d) **Fumarate**

15. Co-enzyme required for the conversion of L-Amino acid into L-Imino acid.

a) $FAD^+$

b) **FMN**

c) $NAD^+$

d) $NADP^+$

## II Give short answer for the following

**1.** What is the fate of excess dietary amino acids?

**2.** What are non-essential amino acids? How are they synthesized?

**3.** Give the reaction catalyzed by AST.

**4.** What are glucogenic amino acids and ketogenic amino acids? Give examples.

**5.** What is decarboxylation? Give example.

**6.** Name the enzymes involved in urea cycle.

**7.** Mention any four amino acids whose carbon skeletons enter into TCA cycle.

**8.** How is citrulline formed in the mitochondria converted to argininosuccinate in the cytosol?

**9.** How is tyrosine converted to dopaquinone?

**10.** What is the role of S-adenosyl methionine in conversion of norepinephrine to epinephrine?

## III Give short answer for the following

**1.** What is transamination? Add a note on its features.

**2.** Explain the different types of deamination reactions with examples.

**3.** Write a note on the formation of amines from amino acids.



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4. Describe one carbon metabolism and write its significance.
5. What is ammonia intoxication? Give its symptoms.
6. How is tryptophan converted to niacin?

## III Answer the following

1. Write in detail about oxidative and Non-oxidative deamination.
2. Elaborate on Urea Cycle and its significance.
3. Write a note on specialized products formed from tyrosine.
4. Briefly discuss on the synthesis and functions of thyroid hormones.
5. Enumerate the steps involved in the formation of catecholamines.

## Analyze the table and match the following:

| Amino acids | Entry into TCA cycle | Glucogenic or Ketogenic |
|---|---|---|
| Glutamate | Succinyl Co-A | Ketogenic |
| Aspartate | Oxaloacetate | Both glucogenic and ketogenic |
| Tyrosine | Acetyl Co-A | Glucogenic |
| Isoleucine | Alpha ketoglutarate | Both glucogenic and ketogenic |
| Leucine | Fumarate | Glucogenic |

## Answer Table:

| Amino acids | Entry into TCA cycle | Glucogenic or Ketogenic |
|---|---|---|
| Glutamate | Alpha ketoglutarate | Glucogenic |
| Aspartate | Oxaloacetate | Glucogenic |
| Tyrosine | Fumarate | Both glucogenic and ketogenic |
| Isoleucine | Succinyl Co-A | Both glucogenic and ketogenic |
| Leucine | Acetyl Co-A | Ketogenic |

## Assertion and Reason:

**Direction:** In each of the following questions a statement of assertion (A) is given and a corresponding statement of reason (R) is given just below it. Mark the correct statement as.

a) If both A and R are true and R is correct explanation of A

b) If both A and R are true but R is not the correct explanation of A

c) If R is true but A is false

d) If both A and R are false.



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**1. Assertion:** GDH plays a major role in nitrogen metabolism.

**Reason** : Glutamate acts as a sink for amino groups of amino acid.

**2. Assertion:** Defects in urea cycle will affect brain.

**Reason** : Depletion of α-ketoglutarate occurs due to its conversion to glutamate.

**3. Assertion:** α-ketobutyric acid is formed by transamination reaction.

**Reason** : Transamination reaction catalyses the inter-conversion of ketoacid and amino acid.

**4. Assertion:** Essential amino acid are synthesized from α-keto acid by the transfer of amino group with the help of transaminases.

**Reason** : Transfer of amino group occurs during synthesis and degradation of amino acid.

**5. Assertion:** Phenyl alanine is a ketogenic amino acid.

**Reason** : Catabolism of phenylalanine yields fumarate that enters into TCA cycle.

**Answer:**

**1.** a) Both A and R are true and R is correct explanation of A

**2.** a) Both A and R are true and R is correct explanation of A

**3.** c) R is true but A is false

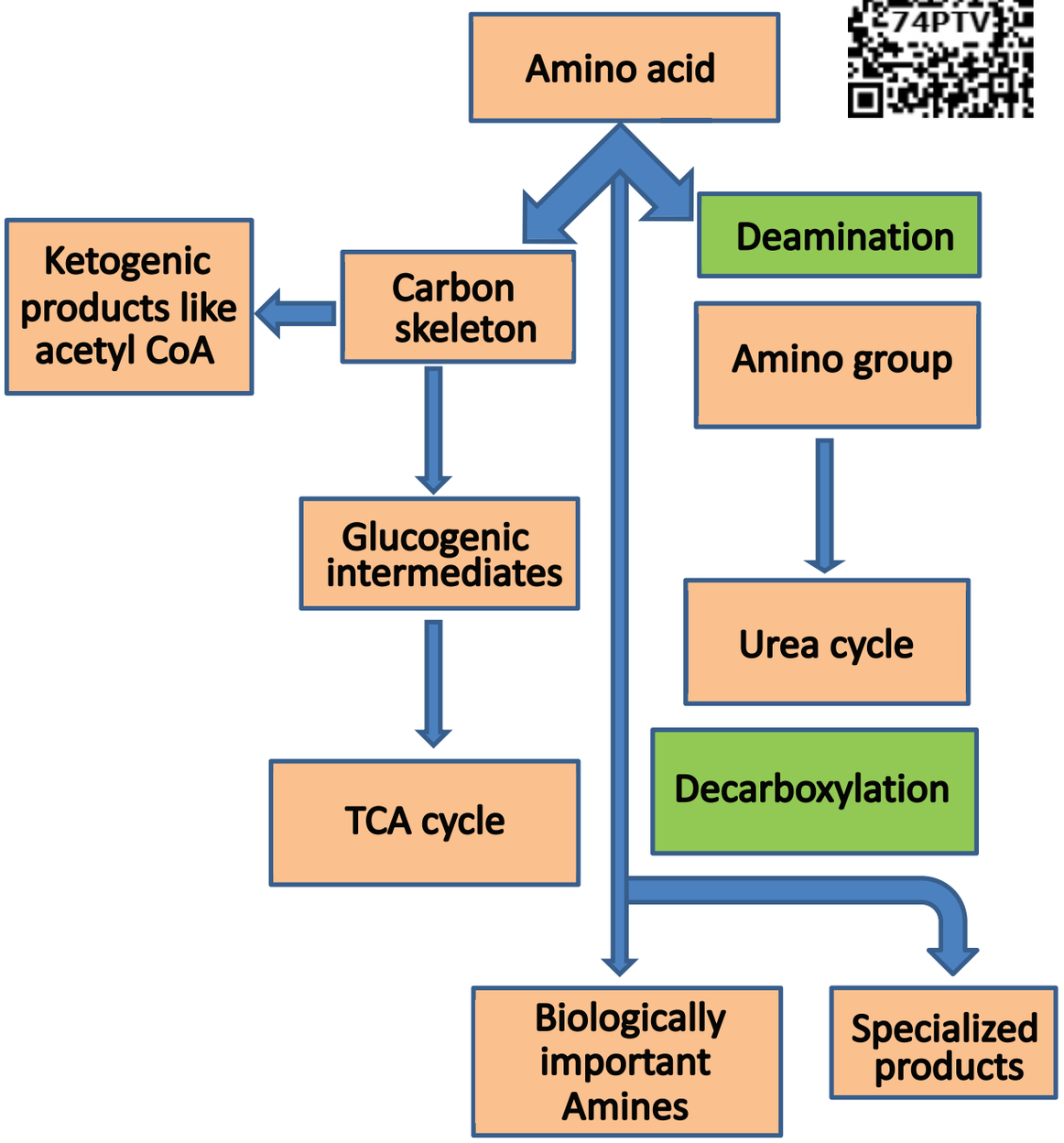
**4.** c) R is true but A is false

**5.** b) Both A and R are true but R is not the correct explanation of A



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## Concept Map



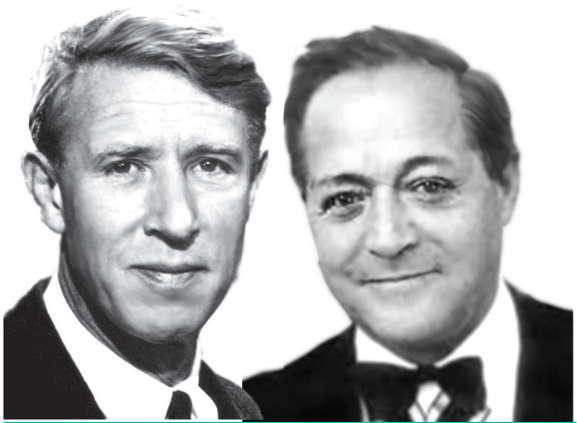



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UNIT 5

# LIPID METABOLISM



**Konrad Emil Bloch and Feodor Lynen**

Konrad Emil Bloch (German American Biochemist) and Feodor Lynen (German Biochemist) both in 1964 received Nobel Prize in Physiology or Medicine for discoveries concerning the mechanism and regulation of the cholesterol and fatty acid metabolism. Bloch and Lynen studied the biochemistry of cholesterol by using a method developed in Rudolf Schoenheimer's lab that used radio isotopes as tracers to chart the path of particular molecules in cells and living organisms.

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## Learning Objectives

After studying this unit the students will be able to

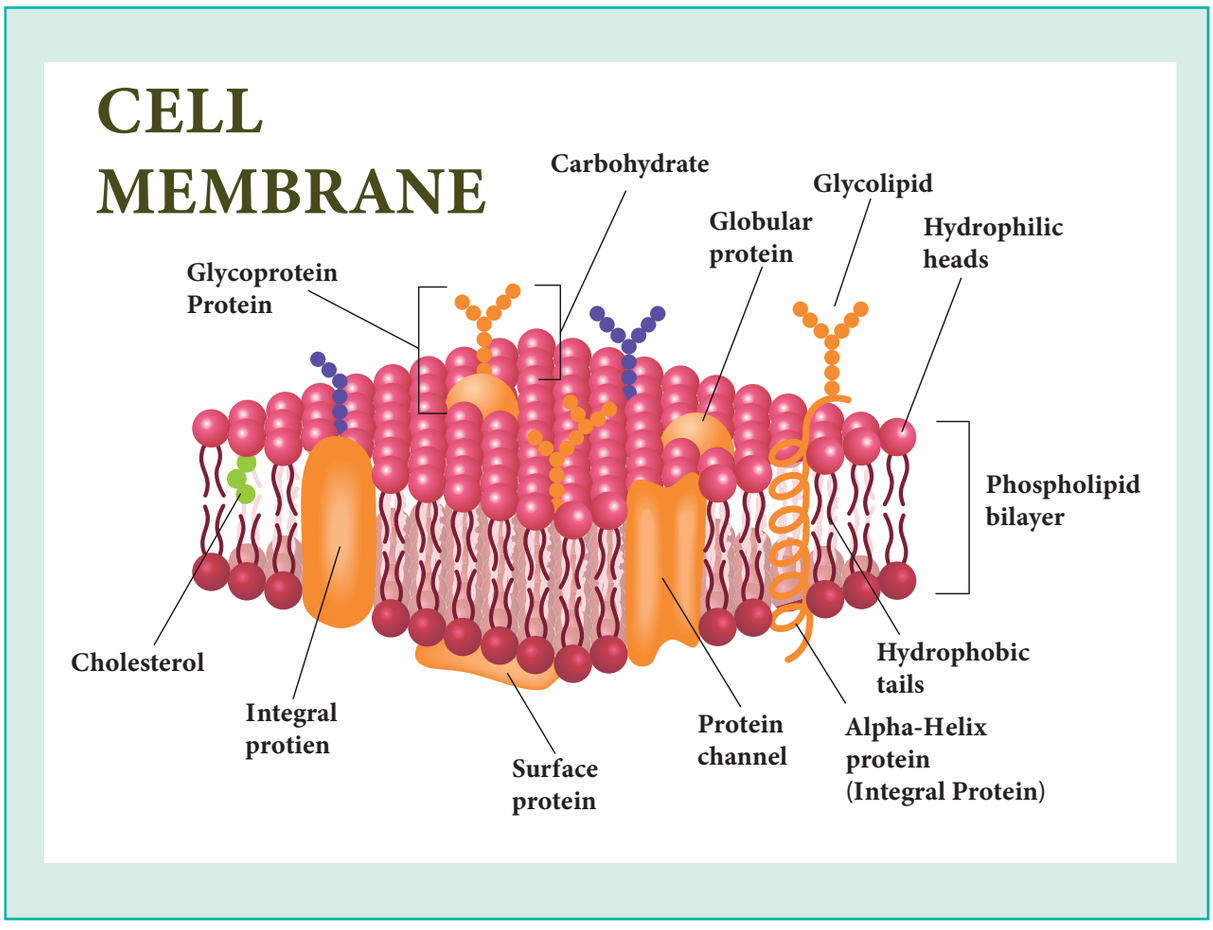
- recognise the nutritional importance of lipids
- explain the β -Oxidation of fatty acid.
- describe the cholesterol synthesis.
- recall various products of cholesterol
- describe phospholipids synthesis
- Understands enzymatic hydralyse by phospholipids
- recognise alter Atherosclerosis



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## 5.1 INTRODUCTION

Lipids (Greek: lipos-fat) are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. According to Bloor's criteria lipids are compounds having the following characteristics. They are insoluble in water and soluble in organic solvents (Chloroform, Benzene, Acetone etc.). Unlike polysaccharides, proteins and nucleic acids, lipids are not polymers. Further, lipids are mostly small molecules. Examples include fats, oils, waxes, sterols and fat-soluble vitamins.




### 5.1.1 Biological functions of Lipids

**Lipids perform several important functions:**

i. They are the concentrated fuel reserve of the body (triacylglycerols).

ii. Lipids are the constituents of biological membranes and regulate membrane permeability (phospholipids and cholesterol).

iii. They serve as a source of fat-soluble vitamins (A, D, E and K).

iv. Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).

v. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.

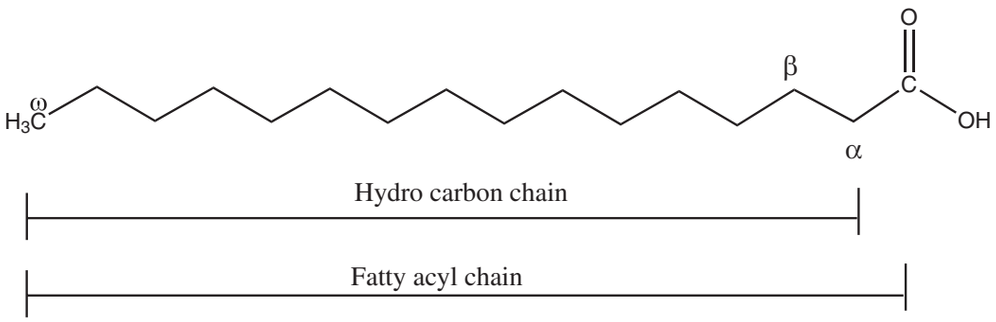


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vi. Apart from the general functions lipids serve as pigments (carotene), hormones, signaling molecules (eicosanoids), cofactors (vitamin K), detergents (bile salts) and many other functions.

## 5.2 BIOSYNTHESIS OF FATTY ACIDS

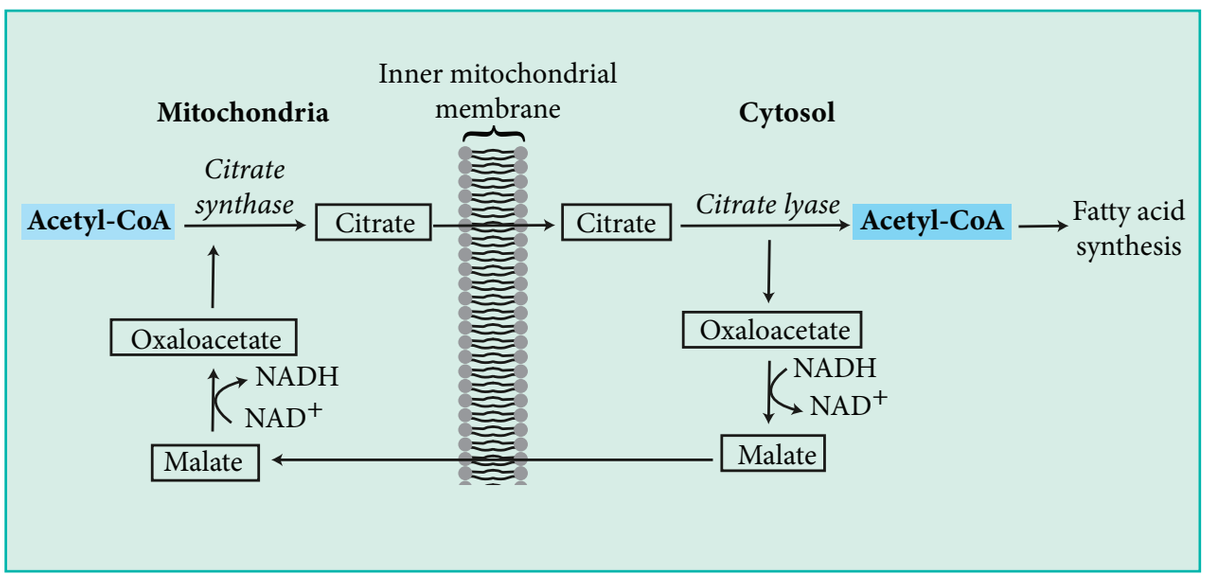
Fatty acids are the simplest form of lipids and they serve as the constituent in a large number of complex form of lipids. They are long chain hydrocarbons (4 to 36 carbons long) with one carboxyl group. Fatty acids are amphipathic in nature; that is, they have both polar and non-polar ends.




In a fatty acid the second carbon is referred as α carbon, third carbon as β carbon and the methyl carbon is called as ω carbon. The alkyl chain in fatty acids may be saturated or unsaturated.

Biosynthesis of fatty acids in animals occurs mainly in the cytosol of adipose tissue, mammary glands, and liver. In case of plants, it occurs in the stroma of chloroplast.

Biosynthesis of fatty acids involves stepwise addition of 2 carbon units, which is supplied by Acetyl CoA. Acetyl CoA is a product of catabolism of carbohydrates, fatty acids and many amino acids. Acetyl CoA formed in the matrix of mitochondria cannot cross the inner mitochondrial membrane and a shuttle system is required to transport the 2-carbon units out of the mitochondria into the cytosol. This is called Acetyl CoA shuttle system.






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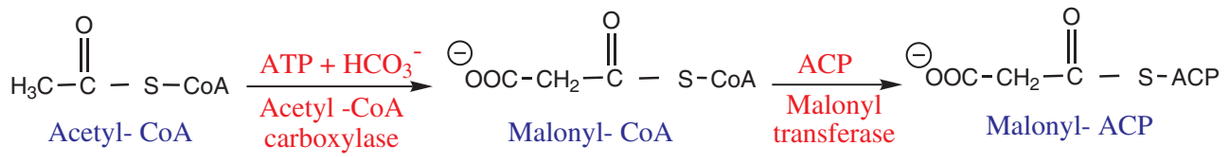
Within the mitochondria, the acetyl CoA reacts with oxaloacetate in a reaction catalyzed by citrate synthase to form citrate. The acetyl group passes out of the mitochondria as citrate. In the cytosol, the citrate reacts with cytosolic CoA to form acetyl CoA and oxaloacetate. Oxaloacetate is reduced to malate and then transported to mitochondrial matrix.

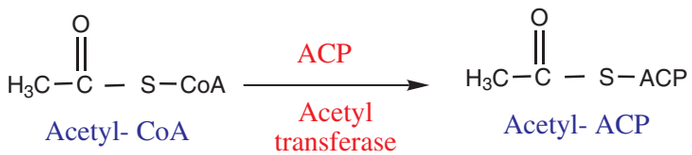
**Fatty acid synthesis takes place in the cytosol in two steps.**

1. Synthesis of palmitic acid
2. Elongation of fatty acid

### Synthesis of palmitic acid:

Palmitic acid is a 16-carbon saturated fatty acid. A sequence of 7 enzyme catalyzed reactions converts 2 carbons unit to 4 carbons unit. First three steps of biosynthesis involve formation of acetyl ACP and malonyl ACP from acetyl CoA.




The last four enzymatic reactions of fatty acid biosynthesis involve the elongation step which includes condensation, reduction, dehydration and reduction. Repetition of this elongation step, increases the chain length by 2 carbon atoms. 7 elongation steps produce palmitic acid.

### Elongation of fatty acids:

The normal product of fatty acid biosynthesis at cytosol is palmitic acid. Fatty acids longer than 16 C are formed through the addition of extra 2 carbon units by elongation reaction. The fatty acids elongation happens in both mitochondria and endoplasmic reticulum.

## 5.3 OXIDATION OF FATTY ACIDS

The digestion of fats starts in the small intestine. Fats are emulsified by bile salts and hydrolyzed by pancreatic lipases to form free fatty acids. These free fatty acids combine with glycerol phosphate (derived from dihydroxy acetone phosphate produced by the glycolytic process) to form triglycerides. They combine with proteins to form lipoproteins and enter circulation to perform various biological functions such as oxidation, storage and formation of new lipids. Thus, the various fatty acids may exist in the free form as well



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as in the esterified form (Triglyceride) in blood. Fatty acids are the immediate source for oxidation of fats in various tissues viz. liver, adipose tissue, muscles, heart, kidney, brain, lungs and testes.

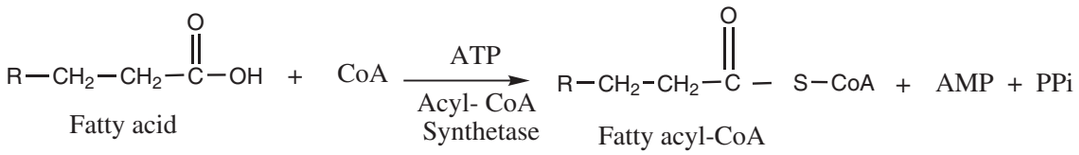
The fatty acids in the body are mostly oxidized by β-oxidation in the mitochondria. β-oxidation may be defined as the oxidation of fatty acids on the β-carbon atom. This results in the sequential removal of a two-carbon fragment, acetyl CoA as described below.

### 5.3.1 β-Oxidation

The β-oxidation of fatty acids involves three stages namely activation of fatty acids, transport of activated fatty acids to mitochondria followed by and the oxidation of the activated fatty acids:

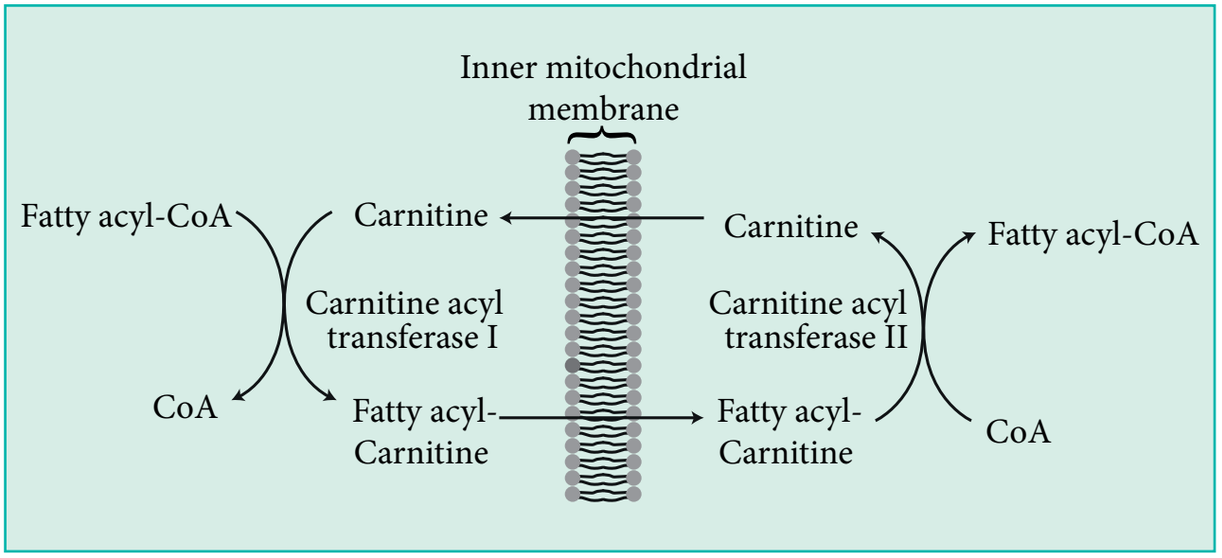
#### i. Activation of fatty acids occurring in the cytosol

Before the β-oxidation begins, the fatty acids are activated in a reaction with ATP and CoA to form fatty acyl CoA.




#### ii. Transport of fatty acids into mitochondria

Though fatty acids are activated for oxidation in cytosol, they are oxidized in the mitochondria. Since the mitochondrial membrane is impermeable for long chain fatty acids, they are transported by a special carrier molecule called carnitine.



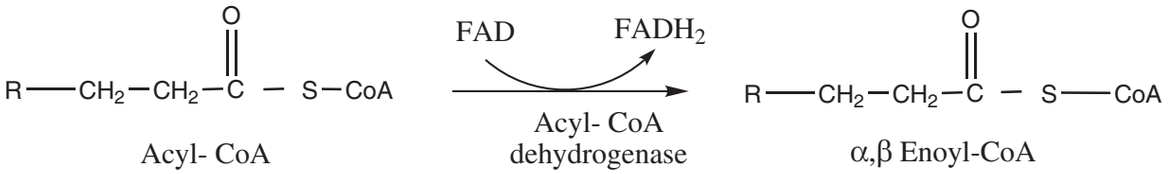

#### iii. β-Oxidation in the mitochondrial matrix

Once inside the mitochondrial matrix, fatty acyl CoA is oxidized with β-carbon oxidation and the long chain acyl CoA is subjected to a repeated 4 step process which removes two carbon chains from the chain until last two carbon fragment is obtained.



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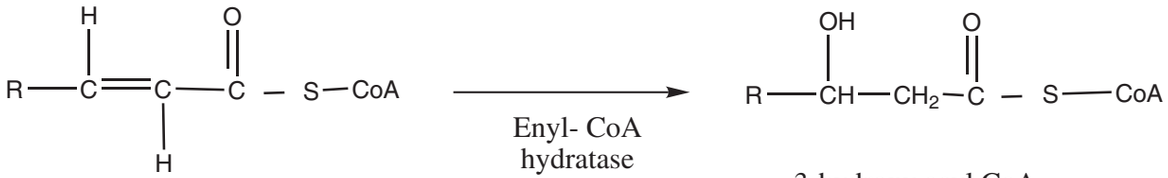
Oxidation




Acyl- CoA

α,β Enoyl-CoA

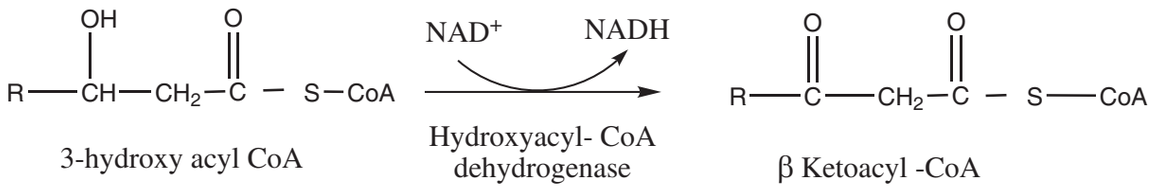
Hydrolysis




α,β Enoyl-CoA

3-hydroxy acyl CoA

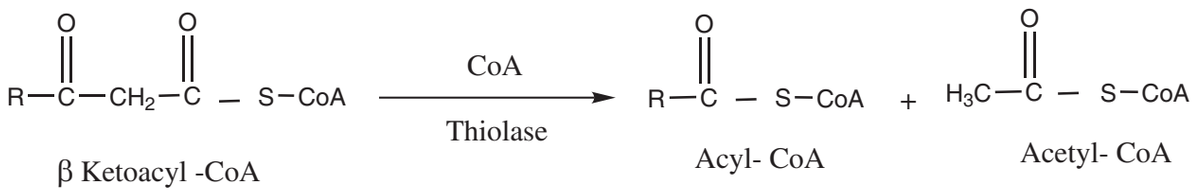
Oxidation




3-hydroxy acyl CoA

β Ketoacyl -CoA

Thiolysis




β Ketoacyl -CoA

Acyl- CoA

Acetyl- CoA

Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.

## 5.4 CHOLESTEROL

Cholesterol is widely distributed in all cells and is a major component of the cell membrane and lipoproteins. Cholesterol is found exclusively in animals; hence it is often called as animal sterol. The total body content of cholesterol in an adult man weighing 70 kg is about 140 g i.e., around 2g/ kg body weight. Cholesterol is amphipathic in nature, since it possesses both hydrophilic and hydrophobic regions in the structure.

Cholesterol is a $C_{27}$ alcohol with molecular formula $C_{27}H_{46}O$. It has a structure consisting of four fused hydrocarbon rings forming the bulky steroid structure. There is a



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hydrocarbon tail linked to one end of the steroid and a hydroxyl group at C3 and a double bond between C5 and C6. The hydroxyl group at C3 constitutes the polar head. The rest of the molecule is hydrophobic.

*Normal value of total blood cholesterol level is < 200 mg/dL.

### Properties of cholesterol:

i. Cholesterol exists as crystals that are white, shiny and rhombic in nature

ii. It is tasteless and odorless

iii. It has a high melting point of 150°C

iv. It is insoluble in water and soluble in fat solvents

v. It is a poor conductor of heat and electricity and serves as an insulator. In brain, where it is present abundantly, it acts as an insulator against nerve impulse which is electric in nature

vi. Cholesterol, when oxidized under suitable conditions, undergoes rapid oxidation to form a ketone called cholestenone

vii. The hydroxyl group of cholesterol readily forms ester with fatty acids like stearic acid

### Cholesterol is essential to life, as it performs several important functions

i. lt is a structural component of the cell membrane

ii. Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids

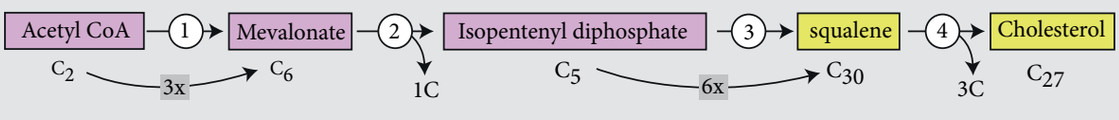
iii. lt is an essential ingredient in the structure of lipoproteins which transport lipids in the body. Fatty acids are transported to liver as cholesteryl esters for oxidation.

## 5.4.1 Biosynthesis of cholesterol

About 1 g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by liver (50%), intestine (15%), skin, adrenal cortex and reproductive tissue.

The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by NADPH while ATP provides energy.

For the production of one mole of cholesterol, 18 moles of acetyl CoA, 36 moles of ATP and 16 moles of NADPH are required.




The four basic steps in cholesterol synthesis are,



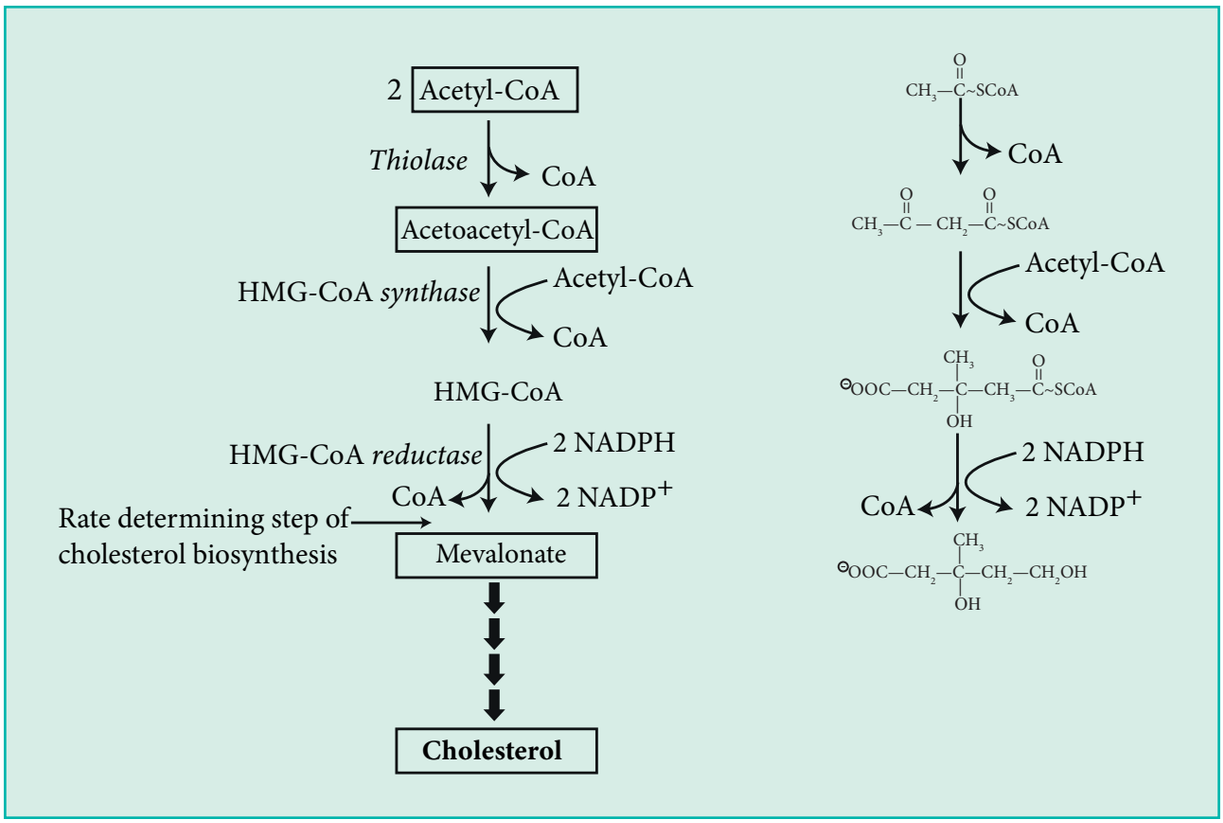
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i. Formation of acetyl CoA: A molecule of acetate combines with coenzyme A (CoA) to produce Acetyl CoA in the presence of an enzyme Acetyl CoA synthetase. Acetate is the source of all carbon atoms in cholesterol.

ii. Formation of acetoacetyl CoA: Two molecules of acetyl-CoA condense to form an acetoacetyl-CoA molecule, catalyzed by the enzyme thiolase

iii. Formation of HMG CoA: The acetoacetyl-CoA further undergoes condensation with one more molecule of acetyl-CoA to form HMG-CoA (3-Hydroxy 3-Methyl Glutaryl-CoA). The enzyme which mediates this reaction is called HMG-CoA synthetase.

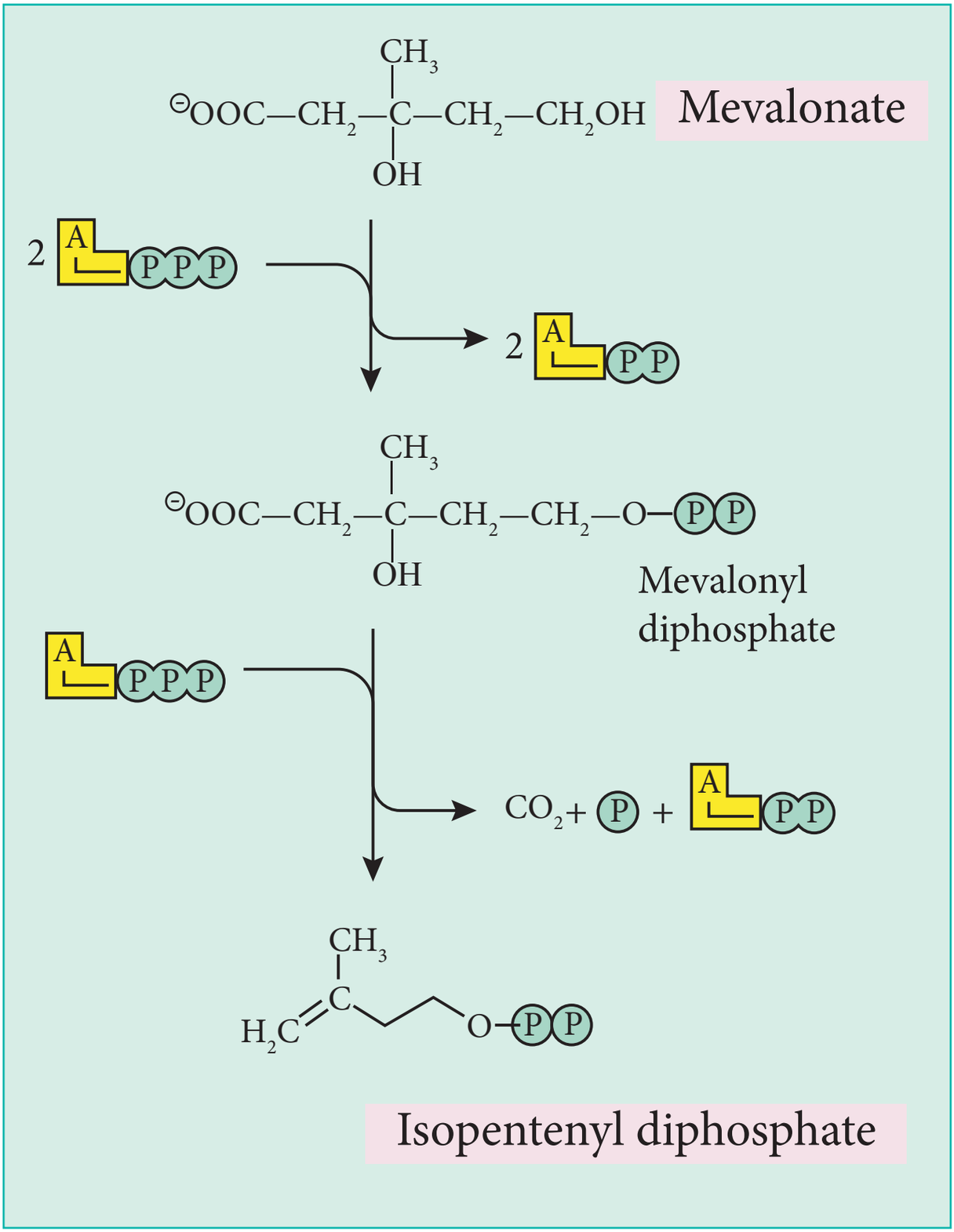
iv. Formation of mevalonate: The HMG-CoA is reduced to form mevalonate by NADPH dependent reductase (HMG-CoA reductase). This is the rate limiting enzyme in the pathway of cholesterol biosynthesis.




Mevalonate thus formed is then converted to squalene through various steps. Squalene, with the formation of various intermediates finally gives rise to the end-product cholesterol.



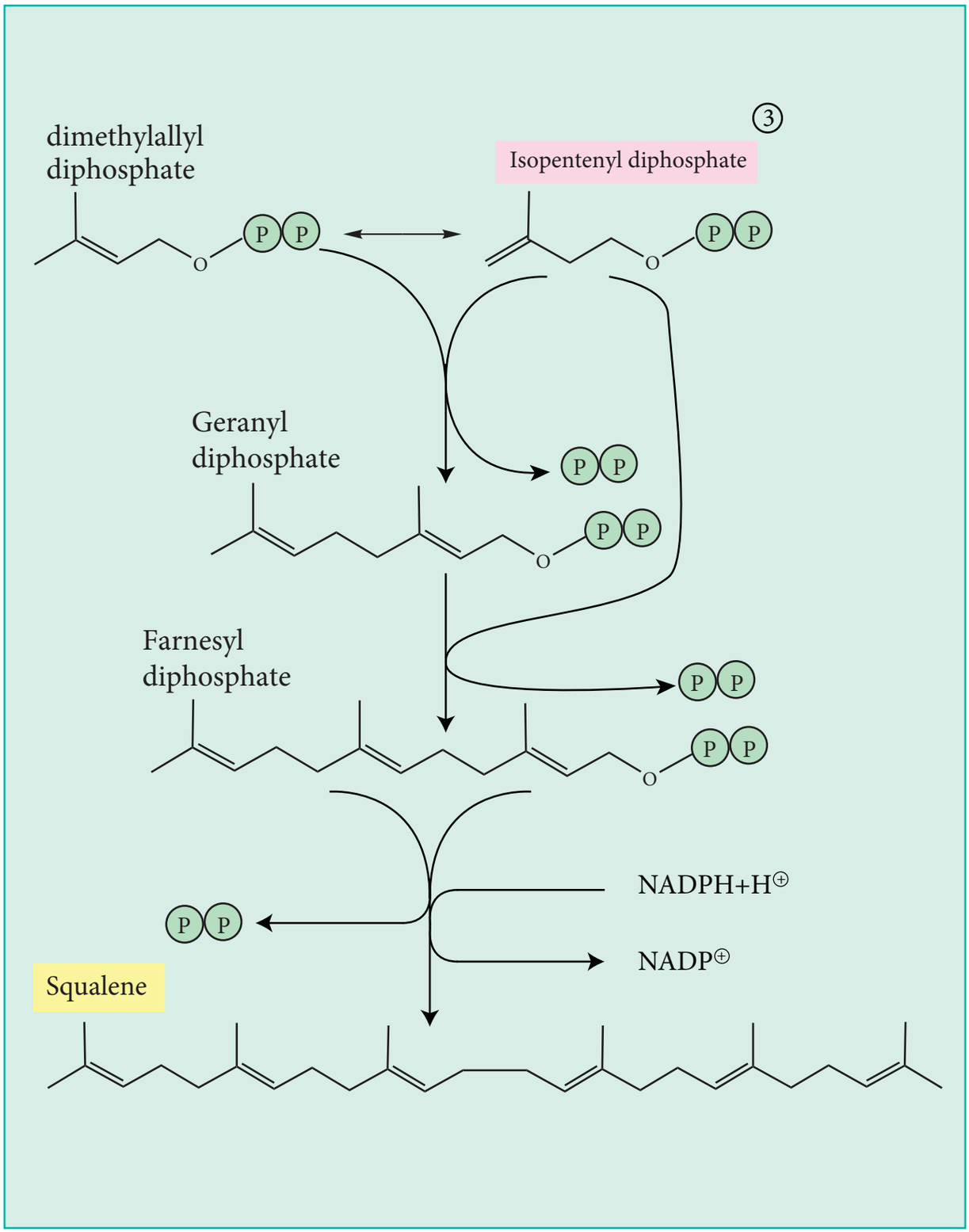
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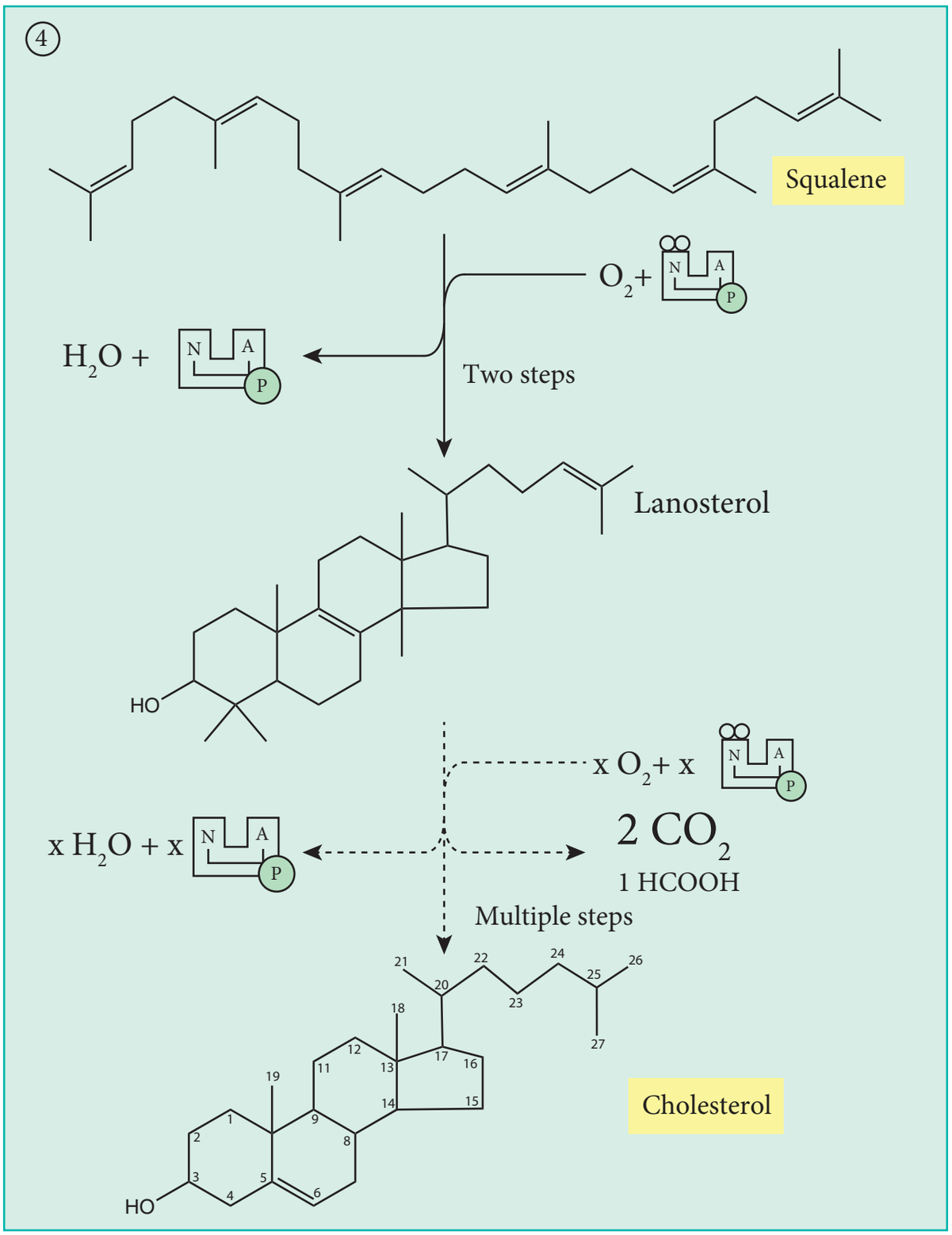


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### 5.4.2 Important derivatives of cholesterol

Cholesterol is a precursor for other important steroid molecules such as the bile salts, steroid hormones, and vitamin D etc....

#### 5.4.2.1 Bile salts

As polar derivatives of cholesterol, bile salts are highly effective detergents because they contain both polar and nonpolar regions. Bile salts are synthesized in the liver, stored and concentrated in the gall bladder, and then released into the small intestine. They help in the absorption of dietary forms of vitamin D in small intestine. Four bile acids are present in human bile, they are cholic acid, deoxycholic acid, chenodeoxycholic acid and lithocholic acid.

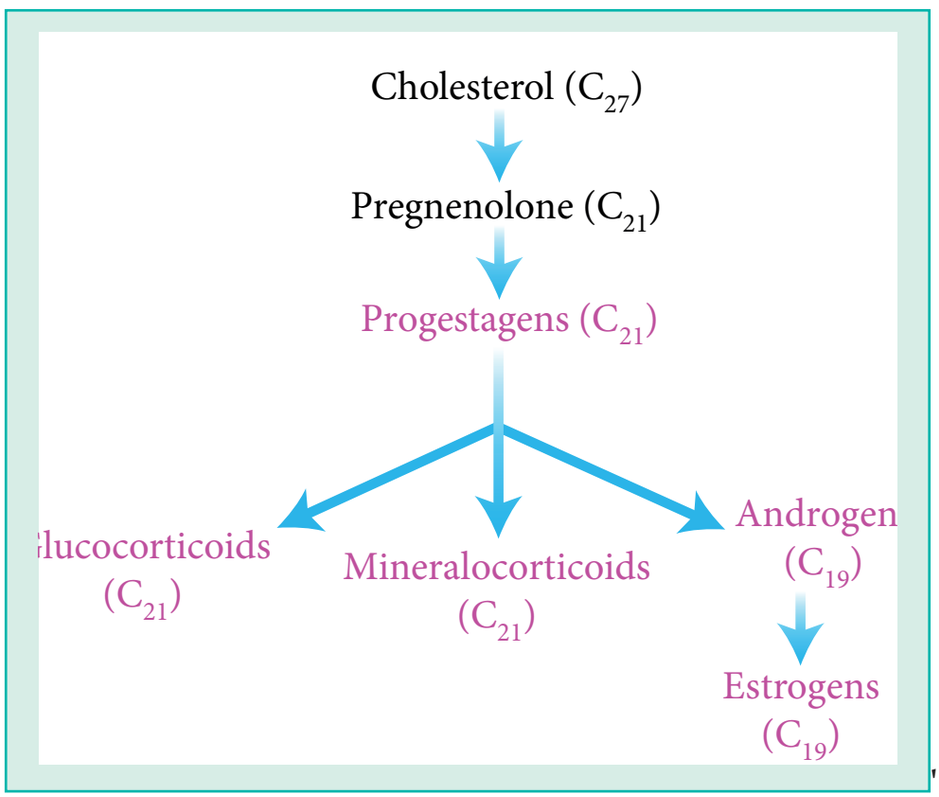
An efficient recycling system allows the bile salts to re-enter the bloodstream and return to the liver for reuse and only very small amount of bile salts escape this recycle system and this is the body's only route for cholesterol excretion.

#### 5.4.2.2 Steroid hormones

Cholesterol is the precursor of the five major classes of steroid hormones: progestagen, glucocorticoids, mineralocorticoids, androgen, and estrogen.

#### 5.4.2.3 Vitamin D

Cholesterol is also the precursor of vitamin D, which plays an essential role in the control of calcium and phosphorus metabolism. Vitamin D also serves as precursor for hormone synthesis.






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## 5.5 PHOSPHOLIPIDS

Phospholipids are a characteristic constituent of cellular membranes. A phospholipid is an amphipathic molecule constructed from four components: fatty acids, a platform to which the fatty acids are attached, a phosphate and an alcohol attached to the phosphate. The platform on which phospholipids are built may be glycerol or sphingosine.

Phospholipids derived from glycerol are called phosphoglycerides. The common alcohol moieties of phosphoglycerides are the amino acid serine, ethanolamine, choline, glycerol, and the inositol.

Sphingomyelin is a phospholipid whose backbone sphingosine, an amino alcohol that contains a long, unsaturated hydrocarbon chain.

### 5.5.1 Types of Phospholipids

There are five general classes of phospholipids based on the type of molecule that is attached to the phosphate group. They are,

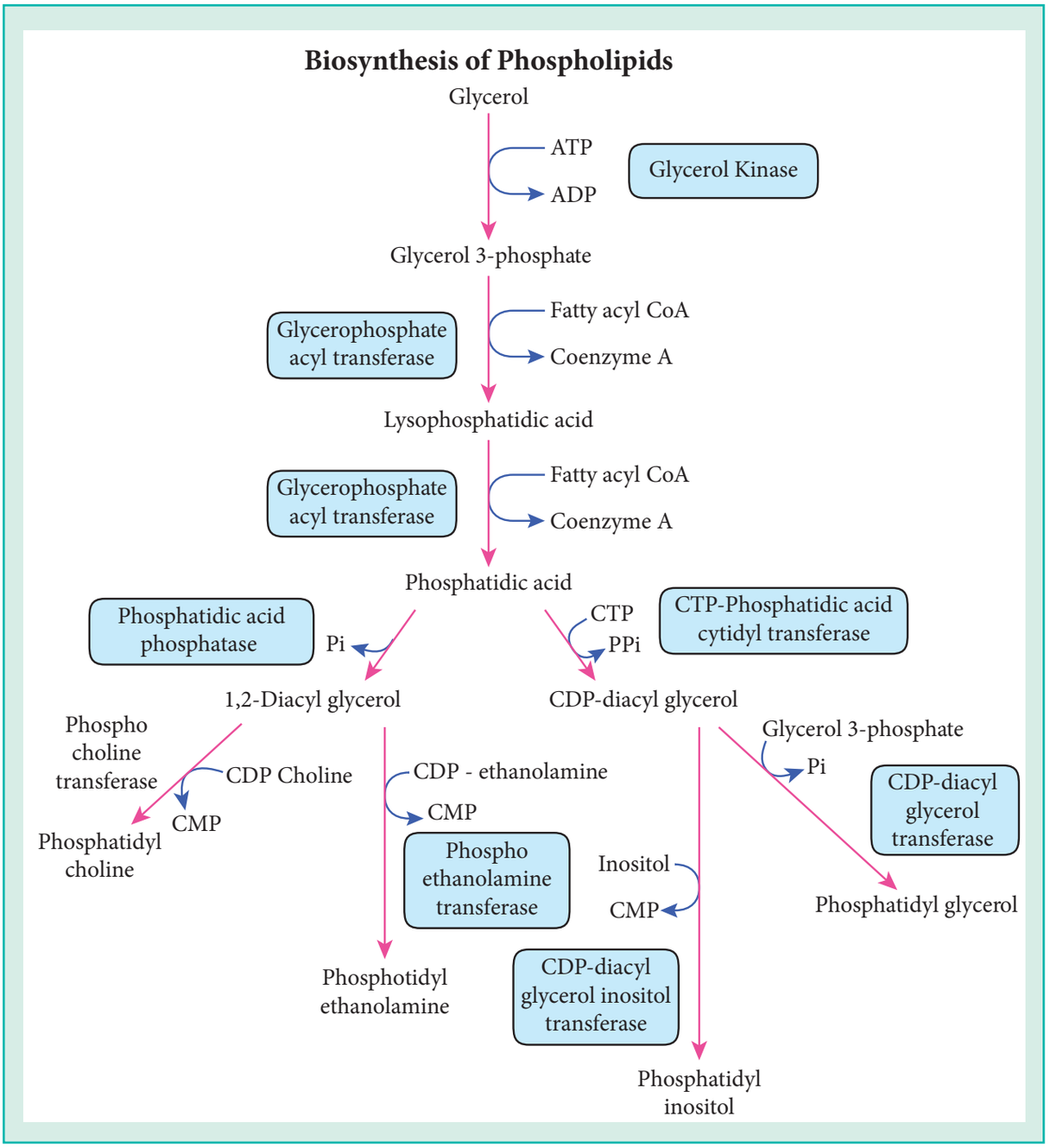
1. Phosphatidylserine
2. Phosphatidylcholine
3. Phosphatidylethanolamine
4. Phosphatidylinositol
5. Sphingomyelin

### 5.5.2 Biosynthesis of phospholipids

Phospholipids can be synthesized by two mechanisms. One utilizes a CDP-activated polar head group for attachment to the phosphate of phosphatidic acid. The other utilizes CDP-activated 1,2-diacylglycerol and an inactivated polar head group. Two fatty acids linked to coenzyme A (CoA) carriers are first joined to glycerol-3-phosphate, yielding phosphatidic acid. A phosphatase then converts phosphatidic acid to diacylglycerol. The attachment of different polar head groups to diacylglycerol then results in formation of phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine.



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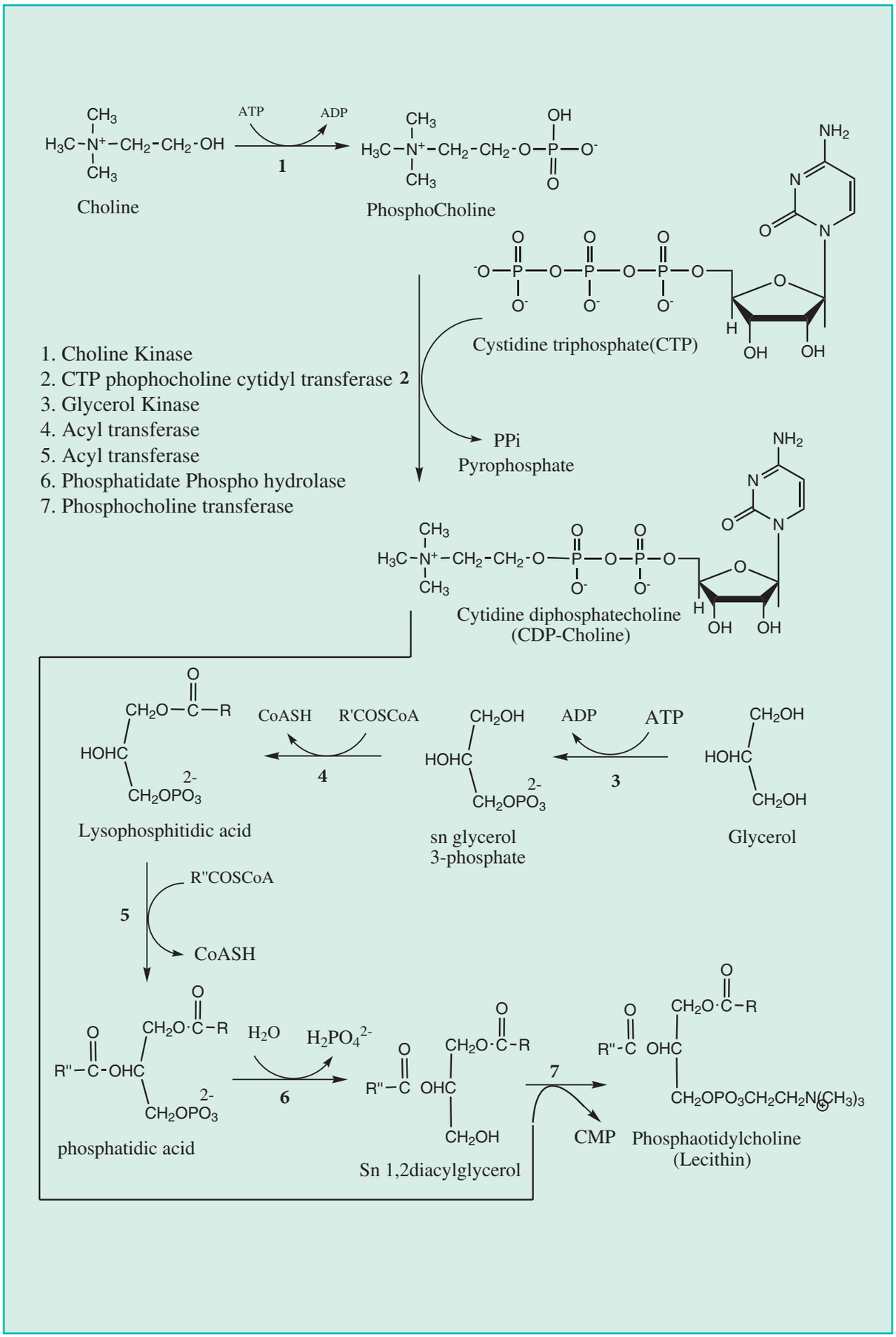

### 5.5.3 Biosynthesis of lecithin

Lecithin (phosphatidylcholine) is a precursor for choline. It is involved in the synthesis of the neurotransmitter acetylcholine. Lecithin is also involved in complex intracellular processes, including the regulation of cellular membrane permeability.

Most of the reactions of lecithin biosynthesis occurs in the endoplasmic reticulum. The principal pathway of lecithin biosynthesis uses cytidine diphosphate choline(CDP-choline). CDP-choline is formed from the reaction of CTP with phosphocholine, which is obtained by the phosphorylation of choline.



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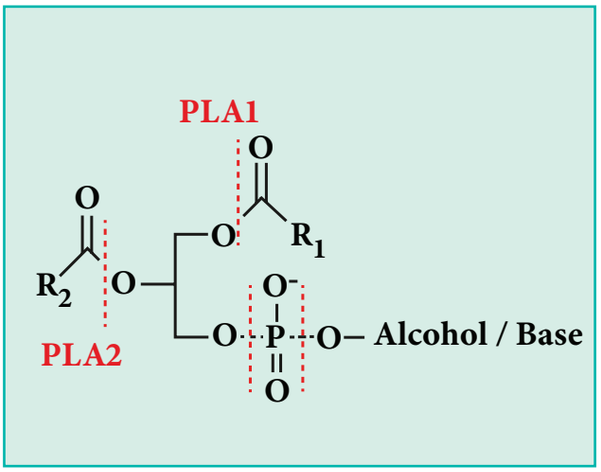





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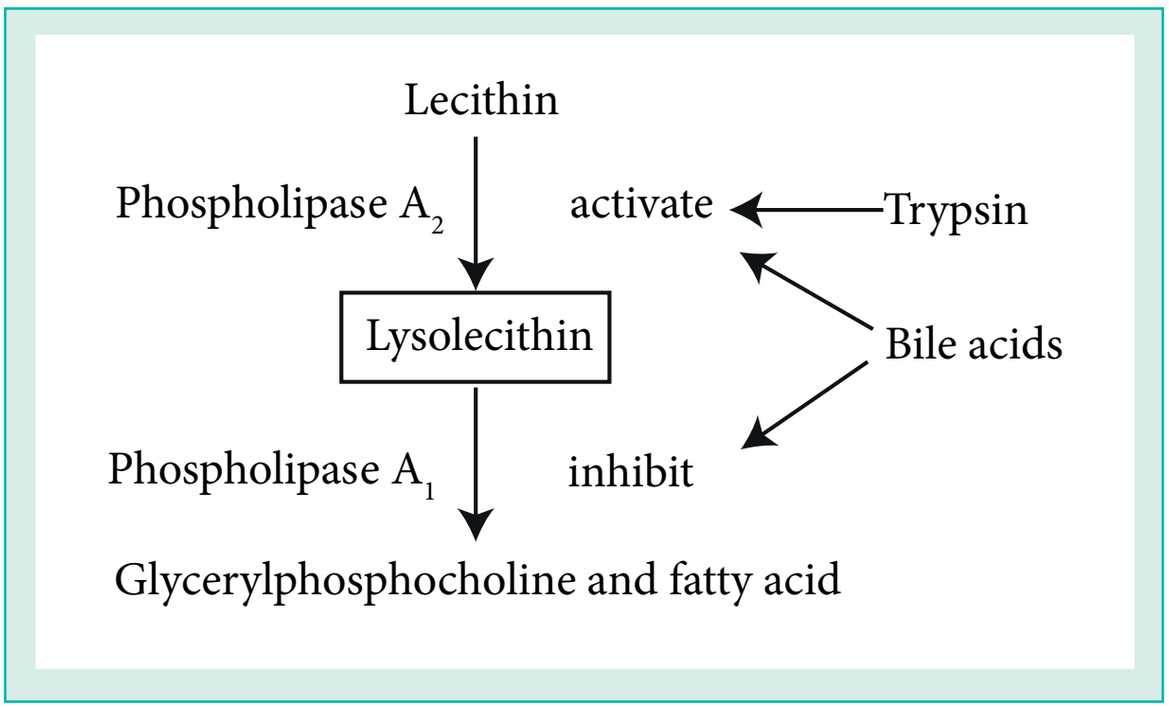
### 5.5.4 Degradation of Phospholipids

Phospholipases are enzymes that degrade phospholipids through hydrolytic cleavage of carboxy- and phospho-diester bonds. The enzymes are classified as phospholipases A1, A2, C or D depending on the site of hydrolysis at the sn-1 or sn-2 acyl ester bond, at the glycerol phosphate bond or at the glycerol phosphate-base phosphodiester bond, respectively.




### 5.5.5 Lysolecithins formation

Lysophosphatidylcholines (LPC, lysoPC), also called lysolecithins, are a class of chemical compounds which are derived from phosphatidylcholines. They are formed in the duodenum by the partial hydrolysis of lecithin in bile by phospholipase $A_2$ of pancreatic juice, which removes one of the terminal fatty acid groups. This reaction is activated by acids and trypsin. Lysolecithin may be further hydrolyzed to glyceryl phosphorylcholine by phospholipase $A_1$, but this reaction is inhibited by bile acids.




### 5.5.6 Effects of lysolecithin

Lysolecithin is highly toxic to cell membranes and it is a strong detergent similar to bile salts. It can demyelinate nerves and destroy red blood cells. Due to its emulsification properties, it helps to make the consistency of products smooth and easy to spread. Because of this, LPC is widely used in food and pharmaceutical industry and also in livestock and poultry feeds.



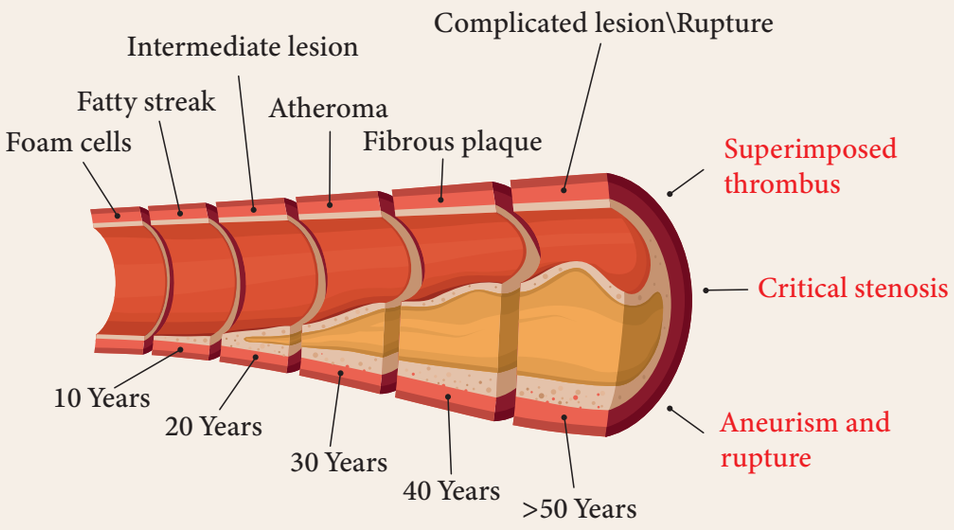
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## 5.6 CEPHALIN

Cephalin is a group of phospholipid compounds, which includes phosphatidyl ethanolamine and phosphatidyl serine. These esters are widely distributed in the body, especially in the brain and spinal cord, and are used as local hemostatic and as reagents in liver function test. They are found abundant in the white matter of the brain and spinal cord.

## 5.7 ATHEROSCLEROSIS

Atherosclerosis is a disease in which plaque made of fat, cholesterol, calcium found in blood builds up inside the arteries. Over time, this plaque hardens and narrows the arteries, limiting the flow of oxygen-rich blood to various organs. This could lead to various other problems like stroke, heart attack or even death.




### Summary

- Lipids (Greek: lipos-fat) are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions.
- They are the concentrated fuel reserve of the body (triacylglycerols).
- Lipids are the constituents of biological membranes and regulate membrane permeability (phospholipids and cholesterol).



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- Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.
- Fatty acids are the simplest form of lipids and they serve as the constituent in a large number of complex form of lipids.
- Biosynthesis of fatty acids in animals occurs mainly in the cytosol of adipose tissue, mammary glands, and liver. In case of plants, it occurs in the stroma of chloroplast.
- Palmitic acid is a 16-carbon saturated fatty acid. A sequence of 7 enzyme catalyzed reactions converts 2 carbons unit to 4 carbons unit. First three steps of biosynthesis involve formation of acetyl ACP and malonyl ACP from acetyl CoA.
- The digestion of fats starts in the small intestine. Fats are emulsified by bile salts and hydrolyzed by pancreatic lipases to form free fatty acids.
- The β-oxidation of fatty acids involves three stages namely activation of fatty acids, transport of activated fatty acids to mitochondria followed and the oxidation of the activated fatty acids.
- Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.
- Cholesterol is widely distributed in all cells and is a major component of the cell membrane and lipoproteins. Cholesterol is found exclusively in animals; hence it is often called as animal sterol.
- Cholesterol, when oxidized under suitable conditions, undergoes rapid oxidation to form a ketone called cholestenone.
- In the cholesterol biosynthetic pathway, the HMG-CoA is reduced to form mevalonate by NADPH dependent reductase (HMG-CoA reductase). This is the rate limiting enzyme in the pathway of cholesterol biosynthesis.
- Cholesterol is a precursor for other important steroid molecules such as the bile salts, steroid hormones, and vitamin D etc..
- Phospholipids are a characteristic constituent of cellular membranes. A phospholipid is an amphipathic molecule constructed from four components: fatty acids, a platform to which the fatty acids are attached, a phosphate and an alcohol attached to the phosphate. The platform on which phospholipids are built may be glycerol or sphingosine.
- Phospholipids can be synthesized by two mechanisms. One utilizes a CDP-activated polar head group for attachment to the phosphate of phosphatidic acid. The other utilizes CDP-activated 1,2-diacylglycerol and an inactivated polar head group.



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- Lecithin (phosphatidylcholine) is a precursor for choline. It is involved in the synthesis of the neurotransmitter acetylcholine.
- Phospholipases are enzymes that degrade phospholipids through hydrolytic cleavage of carboxy- and phospho-diester bonds.
- Lysolecithin is highly toxic to cell membranes and it is a strong detergent similar to bile salts. It can demyelinate nerves and destroy red blood cells.
- Cephalin is a group of phospholipid compounds, which includes phosphatidyl ethanolamine and phosphatidyl serine.
- Atherosclerosis is a disease in which plaque made of fat, cholesterol, calcium found in blood builds up inside the arteries.

## EVALUATION

### I. Choose the correct answer from the given four alternatives

1. _____ is not an essential fatty acid.

   a. Linoleic acid  b. Linolenic acid

   c. Arachidonic aicd  d. Oleic acid

2. How many ATP's are produced in beta oxidation of one stearic acid molecule

   a. 2  b. 98

   c. 0  d. none of these

3. Which one is a saturated acid?

   a. Oleic acid  b. Cerebronic acid

   c. Nervonic acid  d. Stearic acid

4. Biosynthesis of fatty acids from acetyl CoA is called

   a. gluconeogenesis  b. lipogenesis

   c. beta oxidation  d. both (b) and (c)

5. _____ is a derivative of cholesterol

   a. Vitamin A  b. Vitamin C

   c. Vitamin E  d. Vitamin D

6. Which of the following is an intermediate in the process of lipogenesis?

   a. isopentenyl pyrophosphate  b. Molonyl ACP

   c. Oxaloacetate  d. Acetoacetate



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7. Which of the following is a ketone body?

   a. Carnitine  b. Acetoacetate

   c. Oxaloacetate  d. Acetyl CoA

8. Lysolectithin is formed by the action of _____ on lecithin

   a. Lecithinase A  b. Lecithinase A2

   c. Lecithinase C  d. Lecithinase D

9. How many cycles of the β- oxidation pathway are needed to process a $C_{18}$ fatty acid molecule?

   a. Seven  b. eighteen

   c. nine  d. eight

10. What is the first functional group change that occurs in the $\beta$ - oxidation pathway?

    a. alkane to alkene  b. $2^0$ alcohol to ketone

    c. Alkene to $2^0$ alcohol  d. Alkane to $2^0$ alcohol

## II. Fill up the blanks

1. The active intermediate form of fatty acids to undergo β oxidation are _____
2. _____ is the amino alcohol present in sphingolipids.
3. The rate limiting step of cholesterol biosynthesis is _____
4. Atherosclerotic plaque consists of _____ and _____ in plasma.

## III. Say true of false

1. Lipids can be stored in the body in almost unlimited amounts.
2. Acyl CoA dehydrogenase is an enzyme involved in fatty acid biosynthesis
3. Cephalin is also called as phosphatidyl ethanolamine.
4. Obesity is one of the causative factor of atherosclerosis.
5. Acyl carrier protein is involved in fatty acid degradation.

## IV. Match the following

| | | | |
|---|---|---|---|
| 1. | Lecithin | - | Cholesterol biosynthesis |
| 2. | Cephalin | - | Bile salt |
| 3. | Acetyl CoA carboxylase | - | Phosphatidyl choline |
| 4. | Cholic acid | - | Fatty acid biosynthesis |
| 5. | HMG CoA reductase | - | Phosphatidyl ethanolamine |



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## V. Give short answer for the following

1. Is fatty acid synthesis simply the reverse of betaoxidation? Explain briefly?
2. Briefly discuss the various steps involved in cholesterol biosynthesis.
3. Give an account on atherosclerosis.
4. How fatty acids are synthesized in our body?
5. Give an account on oxidation of fatty acids.
6. Name the cyclic hexahydric alcohol present in phospholipids.
7. What is meant by the terms betaoxidation and beta cleavage in relation to fatty acid catabolism?
8. Coenzyme A plays an important role in fatty acid degradation what is its counterpart in fatty acid bio synthesis?
9. How many cycles of β oxidation are needed to convert palmitic acid to acetyl CoA?
10. Name the enzyme that coverts acetyl CoA to malonyl CoA.
11. List the biological function of lipids.
12. In general terms, how is citric acid cycle involved in obtaining energy from fats?
13. What is the starting material for the biosynthesis of cholesterol?

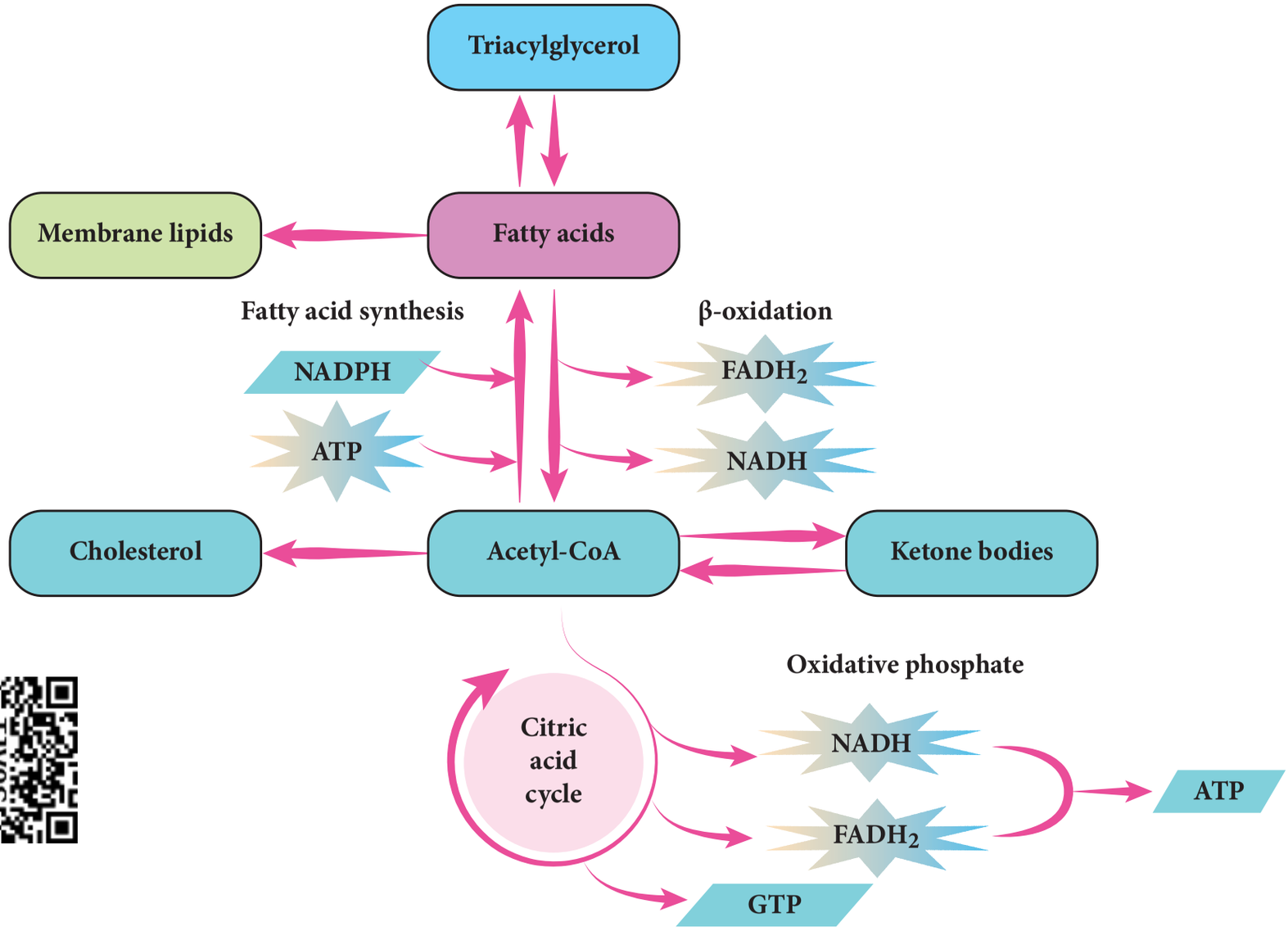


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## CONCEPT MAP

# Lipid metapolism



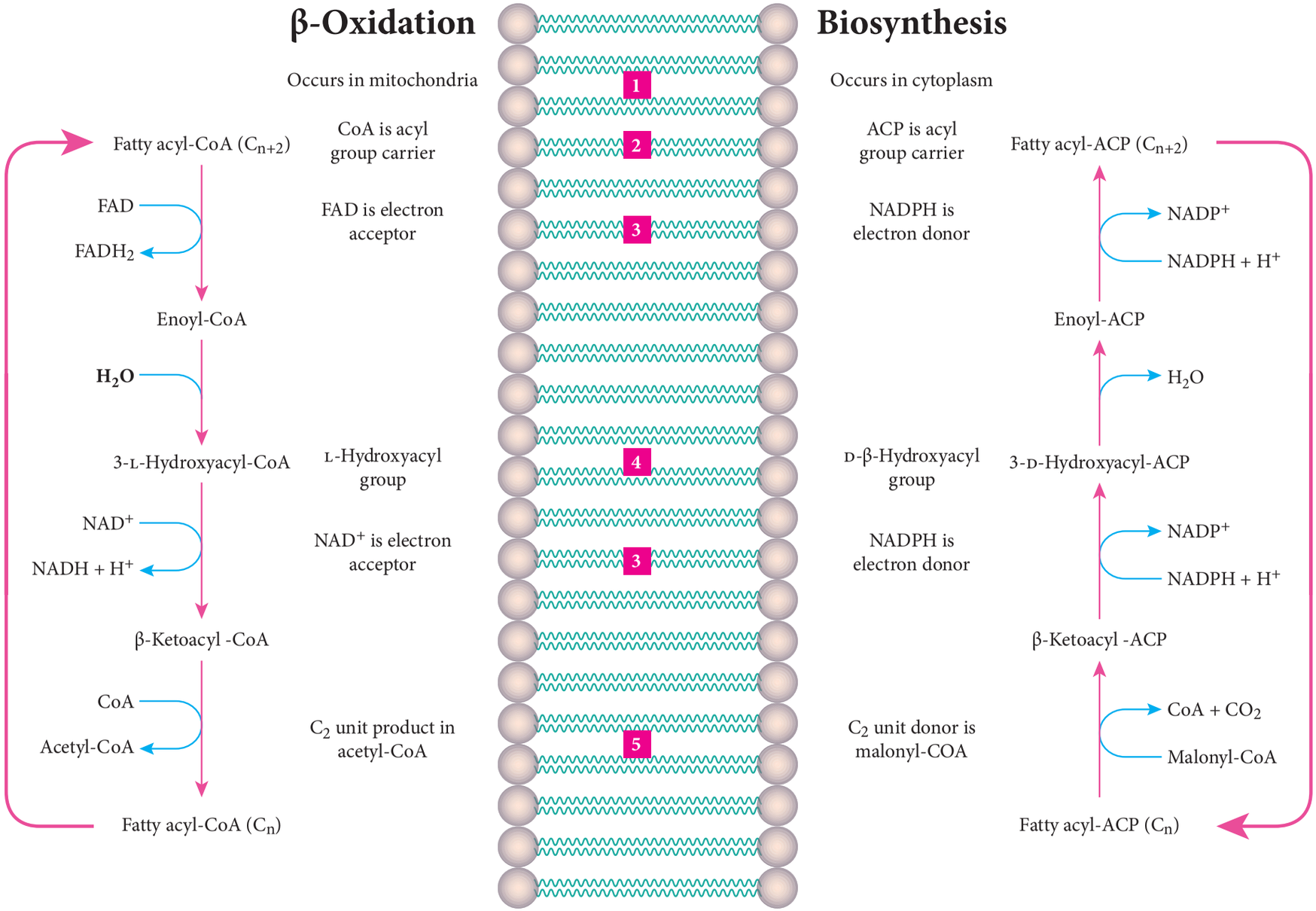



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UNIT 6

# MOLECULAR BIOLOGY



Karry Mullis

Modern biology can be divided into two epochs, the one before the invention of the Polymerase Chain Reaction (PCR) procedure and the one after it. This method is now routinely used in all molecular biology labs and also for medical diagnosis and forensic investigation. Karry Mullis is an American biochemist who was responsible for this invention and was duly awarded Nobel Prize in chemistry in 1993.

QO67M

## Learning Objectives

After studying this unit the students will be able to

- Explain the central dogma of Molecular Biology.
- Explain the replication process.
- Describe the mechanism of RNA synthesis.
- Explain the structure of activated tRNA.
- Describe the mechanism of protein synthesis.



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## INTRODUCTION

Molecular biology is a discipline of biology which aims to understand the basic cellular functions such as growth, division, specialization, movement, interaction etc... in terms of the cell's macro molecular components. The term molecular biology was coined in 1938 by Warren Weaver, who was the director of natural sciences department at Rokefeller foundation. Though DNA was identified to be the genetic material as early as 1869, the exact nature of its function was understood only after the discovery of the molecular structure of DNA by J.D Watson and F.H.C Crick in 1952. The concept of base pairing introduced by them directly lead to the understanding DNA replication, RNA synthesis etc. Following these discoveries, the new field of molecular biology progressed rapidly allowing scientists to map out the fundamental biological principles behind macromolecular synthesis and function inside the cell. Today molecular biology has evolved into a very important field of biology with important applications like disease diagnosis and therapy, industrial applications like biotechnology products and to improve agricultural yields.

## 6.1 THE CENTRAL DOGMA OF MOLECULAR BIOLOGY

The central dogma of molecular biology tries to explain how the genetic information contained in the DNA is conveyed or expressed in the biological system. This dogma was first stated by Crick in 1958 and later revised by Watson in 1965. This describes the detailed residue by residue transfer of the genetic information from DNA to Protein. This dogma is the frame work for understanding the information transfer between the important biological macromolecules: the DNA, RNA and proteins. When cells divide, a new copy of DNA has to be synthesised from the existing DNA. This process where a new DNA molecule is synthesised with the existing DNA as a template is known as replication. The information in the DNA can be copied to RNA by a process called transcription. RNA molecules can act as templates to produce protein molecules by a process called translation.

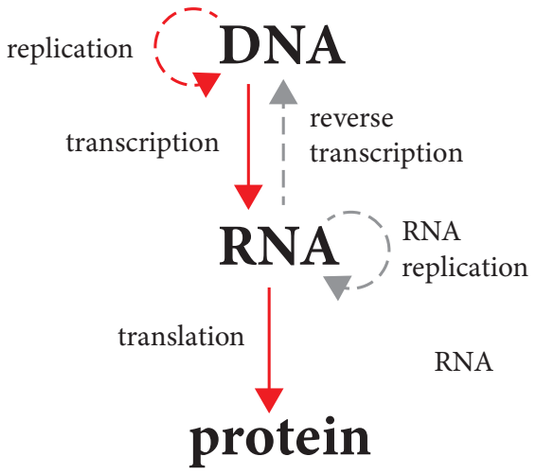



**Figure 1: The central dogma of molecular biology**

This simple information flow from DNA to RNA to Protein is the common mechanism in most organisms. But rarely, as in the case of retroviruses DNA molecule can be synthesised from RNA molecules (reverse transcription). Similarly RNA molecules are also known to replicate ie synthesise new RNA molecules in RNA viruses such as polio virus and mengoviruses.



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## 6.2 DNA replication

Genetic information has to flow from the parental cells or organisms to progeny by a very faithful mechanism. This copy of DNA from DNA known as replication is the first and fundamental step in the central dogma. Any DNA replication mechanisms should consider and overcome the following complexities

1. As we have studied already the strands of DNA run in opposite directions (5'->3') on both strands. So the new nucleotides added should maintain that anti parallel directionality.
2. DNA is helical and a supply of energy is required to unwind it.
3. Such unwound single strands should be prevented from base pairing once again (re-annealing)
4. The DNA molecule is large and so the replication process should start and proceed at several points.
5. There should be a special mechanism to identify and correct errors during replication.

The replication of DNA molecule is an enzymatic reaction catalyzed by the enzyme DNA polymerase. However several other protein molecules are also associated with the replication process considering the above mentioned complexities.

### 6.2.1 The models of DNA replication

After the discovery of Watson and Crick double helical DNA structure, three different types of models for DNA replication were proposed, which were the conservative, semi conservative and dispersal models.

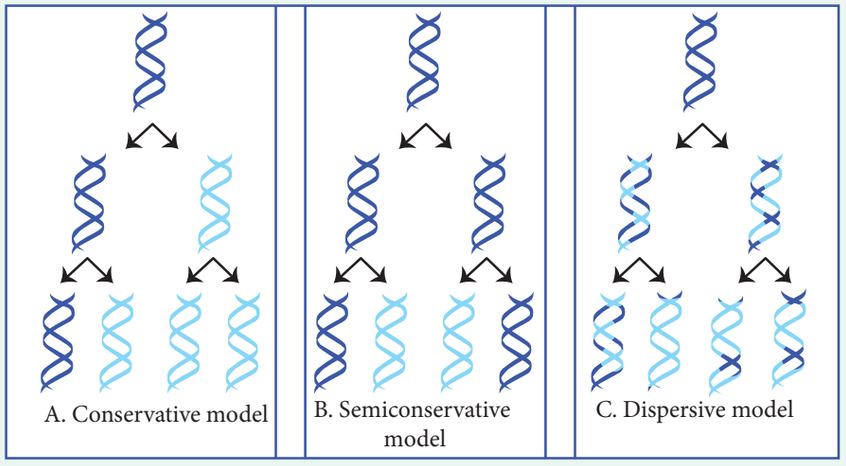



**Figure 2 Different models of DNA replication**

### 6.2.2 The conservative model

In this model the two parental strands base pair once again after forming a new copy. The newly synthesized strands combine together to form another daughter molecule. So in effect at the end of one cycle of replication an intact parental DNA and a new daughter DNA are obtained.

### 6.2.3 The Semiconservative model

According to this model the two strands separate in parental DNA and each of these strands act as a template to form a new DNA molecule. Thereby, after a cycle of replication



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two DNA molecules are formed and each of these two molecules will have one parental strand and one newly synthesized strand.

### 6.2.4 The dispersive model

This model states that the parental DNA is randomly segmented into multiple double stranded DNA fragments. These fragments undergo replication similar to conservative model. The fragments later reassemble into complete DNA molecules. This model of replication will result in synthesis of two DNA molecules with interspersed parental and newly synthesized double helix.

### 6.2.5 The Meselson-Stahl experiment and the conformation of semiconservative model

The semiconservative model of DNA replication was confirmed by Matthew Meselson and Franklin Stahl in 1958. They took advantage of the fact that though $^{14}N$ isotope of nitrogen is normally present in DNA, the presence of the heavier $^{15}N$ isotope doesn't affect the function of DNA.

In this experiment, three different batches of the bacterium *E. coli* were grown. The first batch was grown with the medium containing $^{15}N$ as the only source of nitrogen for several generations. The bacteria took up this $^{15}N$ for growth and eventually all the nitrogen in the DNA were $^{15}N$ isotope. The *E. coli* were then switched suddenly to the medium containing the lighter $^{14}N$ as the only source of nitrogen, so as the bacterium divides these lighter nitrogen gets incorporated into the DNA. After switching to the light $^{14}N$ medium, sample DNA were isolated for every generation of *E. coli*, starting from generation zero (ie., every 20 mins). The DNA extracted subjected to caesium chloride (CsCl) density gradient centrifugation. This method of centrifugation separates the DNA into bands according to their density, with heavier DNA band at the bottom and progressively lighter DNA bands at the top.

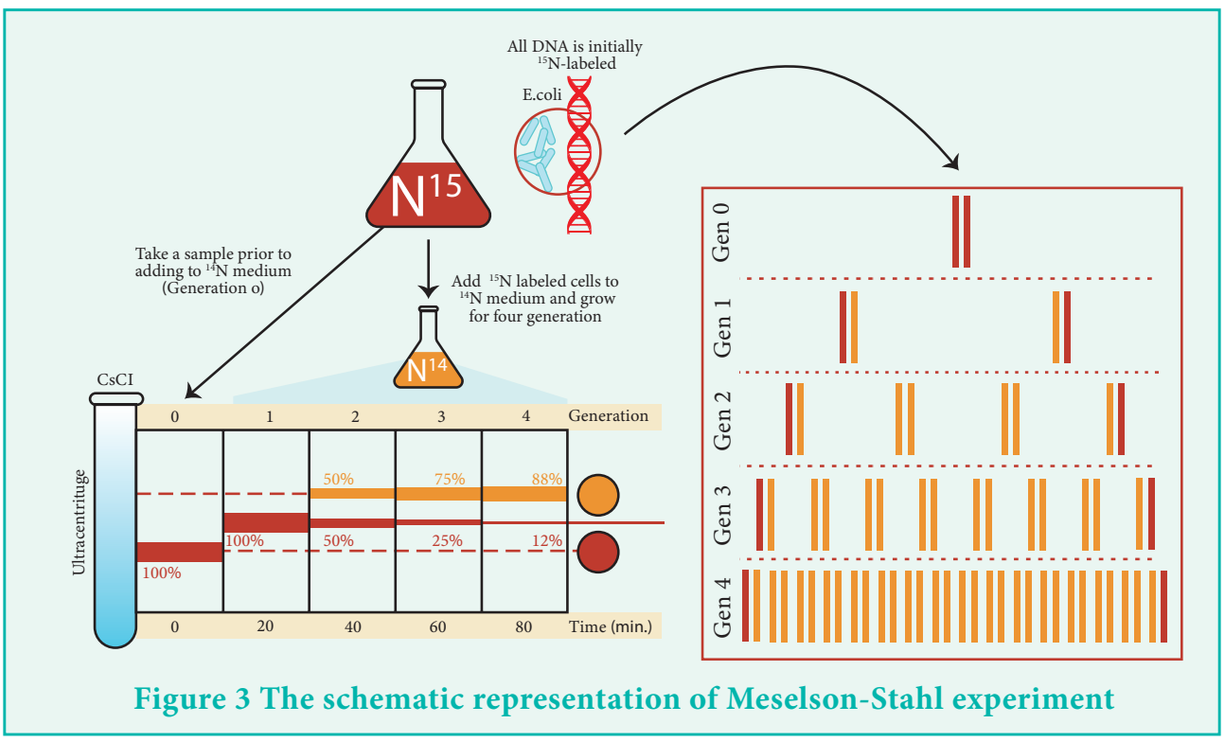



**Figure 3 The schematic representation of Meselson-Stahl experiment**



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The experiment and the results are shown in figure 3. It could be seen that at generation zero, a single band at the bottom of the tube was produced. This is due to the fact that all the nitrogen in the DNA were the heavy $^{15}N$ isotope. At generation one, a single band was produced but in the intermediary position between where $^{15}N$ and $^{14}N$ isotopes were expected. This directly proves semi conservative replication as one strand is made of lighter isotope of nitrogen and the other strand is made of heavy isotope of nitrogen. Similarly the DNA at generation two showed two bands one at the intermediary region and another band at the lighter $^{14}N$ region. Subsequent generations of DNA showed these two bands, however the intermediary band was shown to become thinner and the $^{14}N$ band becoming broader. This is due to the fact that the strands containing $^{14}N$ isotope increases as the DNA replicates in $^{14}N$ medium.

### 6.2.6 Overview of DNA Replication

1. DNA replication is the process by which DNA makes a copy of itself during cell division.
2. The first step in DNA replication is to denature or 'unzip' the double helix structure of the DNA molecule, where the two strands of DNA are seperated.
3. DNA denaturation is carried out by an enzyme called helicase which breaks the hydrogen bonds holding the complementary bases of DNA together. Single strand binding (SSB) proteins bind to these strands to prevent them from annealing together.
4. Since the DNA is a very large molecule, the separation of the two single strands of DNA creates a 'Y' shape called a replication 'fork'.
5. The two separated strands will act as templates for synthesis of the new strands of DNA.
6. One of the strands is oriented in the 3' to 5' direction (towards the replication fork), this is the leading strand.
7. The other strand is oriented in the 5' to 3' direction (away from the replication fork), this is the lagging strand. As a result of their different orientations, the two strands are replicated differently.
8. Leading Strand replication: A short piece of RNA called a primer (produced by an enzyme called primase) comes along and binds to the end of the leading strand. The primer acts as the starting point for DNA synthesis. An enzyme called DNA polymerase binds to the leading strand and then 'walks' along it, adding new complementary nucleotide bases (A, C, G and T) to the strand of DNA in the 5' to 3' direction in a continuous fashion.
9. Lagging strand replication: Numerous RNA primers are made by the primase enzyme and bind at various points along the lagging strand. Chunks of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5′ to 3′ direction, by DNA polymerase. This type of replication is called discontinuous as the Okazaki fragments will need to be joined up later.
10. Once all of the bases are matched up (A with T, C with G), an enzyme called exonuclease strips away the primer(s). The gaps where the primer(s) were are then filled by yet more complementary nucleotides.



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### 6.2.7 The DNA polymerase

The DNA polymerase or the DNA dependent DNA synthetase is a large multi subunit enzyme which adds deoxyribonucleotides to the 3' end of the growing strand one by one. Hence it could be thought of an enzyme that elongates the existing nucleic acid (DNA or RNA primer) in 5'->3' direction. The DNA polymerase enzyme can perform two other activities which are the 3'→5' exonuclease activity and 5'→3' exonuclease activity. Exonucleases are enzymes which can remove bases one by one from the ends of a DNA molecule. The 3'→5' exonuclease activity of DNA polymerases provides as an error correction or proof reading action by removing incorrectly incorporated bases during replication. There are different types of DNA polymerases adapted to different types of cells/organisms and the features described above are of the most predominate enzyme called DNA polymerase I. There are other DNA polymerases like DNA polymerase II, III, alpha, beta, delta etc.,

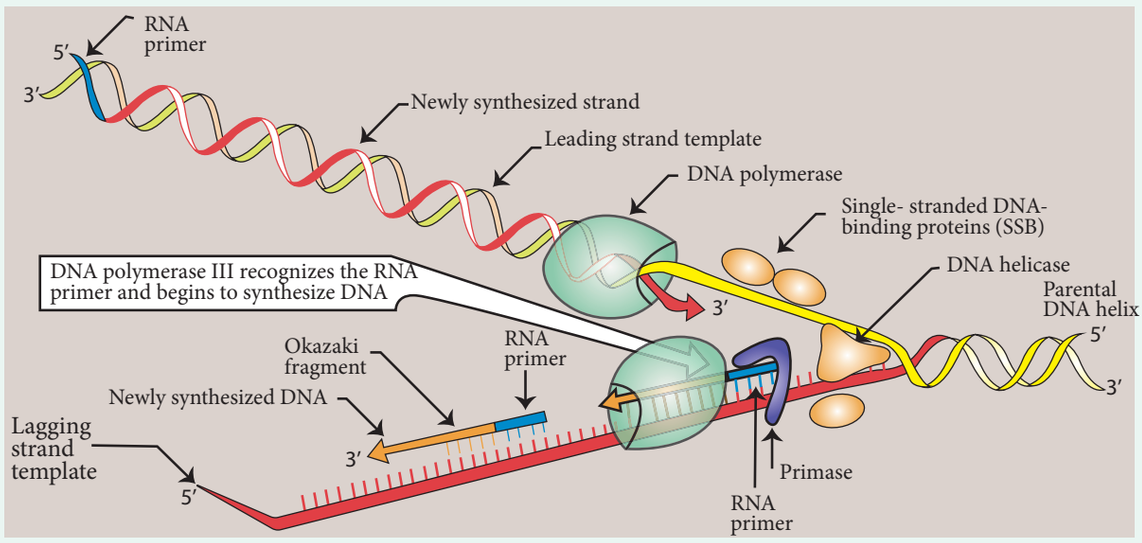



**Figure 4 Overview of the DNA replication process**

### 6.2.8 Difference between prokaryotes and eukaryotes in DNA replication

| S. No. | Prokaryotes | Eukaryotes |
|---|---|---|
| 1 | It occurs inside the cytoplasm | It occurs inside the nucleus |
| 2 | There is a single origin of replication | Origin of replication are numerous |
| 3 | DNA polymerase III carries out both initiation and elongation | Initiation is carried out by DNA polymerase alpha while elongation by DNA polymerase by delta and epsilon |
| 4 | DNA repair and gap filling are done by polymerase I | The same are performed by DNA polymerase beta |



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| | | |
|---|---|---|
| 5 | RNA primer is removed by DNA polymerase I (5'->3' exonuclease activity) | RNA primer is removed by DNA polymerase beta |
| 6 | Okazaki fragments are long | Okazaki fragments are short |
| 7 | Replication is very rapid | Replication is very slow |
| 8 | DNA gyrase is needed to remove the supercoils in the circular bacterial DNA | DNA gyrase is not needed |

### 6.2.9 The polymerase chain reaction – an essential tool for molecular biology

Polymerase chain reaction (PCR) is a method used to make billions of copies of a small region of DNA. This process is also known as PCR amplification. Depending on the application the DNA region being amplified can vary. Some examples include

1. A gene whose function a researcher wants to understand
2. A genetic marker used by forensic scientists to match crime scene DNA with suspects.
3. A genetic marker region in DNA which could be used for diagnosis of a disease like cancer

The goal of PCR amplification is to make enough quantity of the target DNA so that it could be processed for further analysis including sequencing, electrophoresis or plasmid cloning. PCR is routinely used in many areas of biology and medicine, including molecular biology research, medical diagnostics, ecology etc.,

#### 6.2.9.1 The steps involved in PCR amplification

This reaction is essentially a DNA replication carried out in a tube. The key ingredients of a PCR reaction are the enzyme Taq polymerase, primers, template DNA, and deoxy ribo nucleotides (DNA building blocks). The Taq Polymerase is the DNA polymerase enzyme of the heat tolerant bacterium *Thermus aquaticus*. This enzyme can be functional even in temperatures around 70°C. The ingredients are assembled in a tube, along with cofactors needed by the enzyme and are subjected to repeated cycles of heating and cooling that allow DNA to be synthesized. Each cycle of amplification consists of three steps, which are

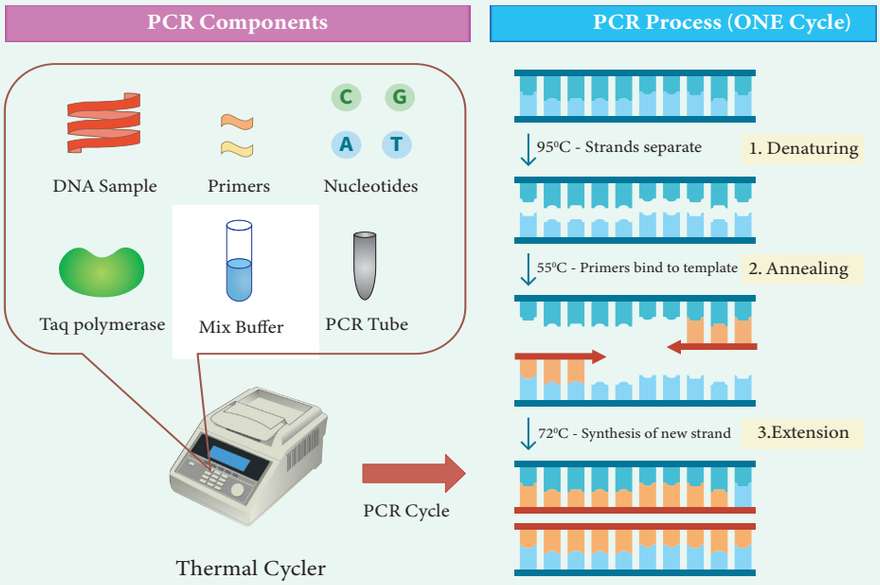
i. Denaturation (96°C): Heat the reaction strongly to separate or denature the DNA strands. This provides single-stranded template for the next step.

ii. Annealing (55 - 65°C): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.

iii. Extension (72°C): Raise the reaction temperatures so Taq polymerase extends the primers, synthesizing new strands of DNA.



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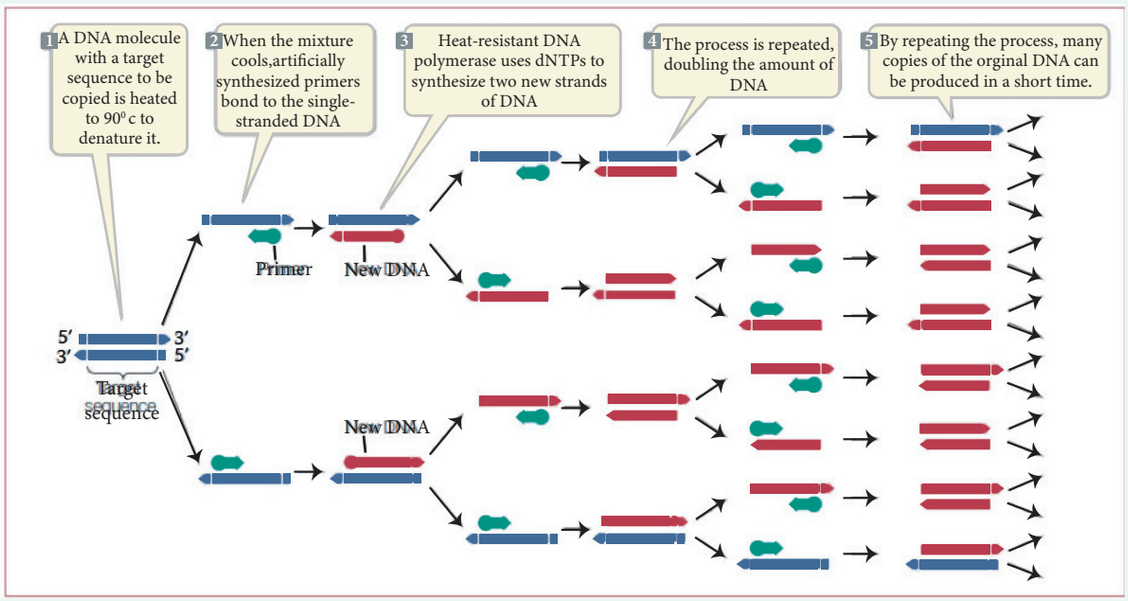




**Figure 5 The polymerase chain reaction (PCR) process**

## 6.3 TRANSCRIPTION

As described earlier transcription is the process by which the information in a strand of DNA (deoxyribonucleic acid) is copied into a new molecule of RNA (ribonucleic acid) with the help of the enzyme called RNA polymerase and a few other proteins.

### 6.3.1 Genes and Gene expression

Genes are described as units of heredity in classical genetics. However in molecular biology terms, genes can be considered as a region in DNA which acts as an information



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template for a protein or RNA molecule (in case of tRNA or rRNA genes). Each gene contain a distinct sequence of nucleotides which determines the aminoacid sequence or nucleotide sequence a cell might synthesize. If a gene i.e, a segment of DNA can act as a template to synthesize an RNA transcript by the process of translation, that gene is considered to be expressed.

### 6.3.2 Overview of transcription

1. It is a process where a DNA sequence is read by an enzyme called RNA polymerase and a complementary, antiparallel RNA strand is synthesized.
2. Apart from RNA polymerase many proteins called transcription factors control the rate of transcription by binding to specific DNA sequence.
3. Transcription between eukaryotes and prokaryotes will vary slightly.
4. Some viruses (eg. HIV) have the ability to transcribe RNA into DNA. HIV has an RNA genome that is reverse transcribed into DNA.
5. RNA polymerase, together with one or more general transcription factors, binds to promoter DNA.
6. RNA polymerase creates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.

**There are three stages in transcription which are initiation, elongation and termination.**

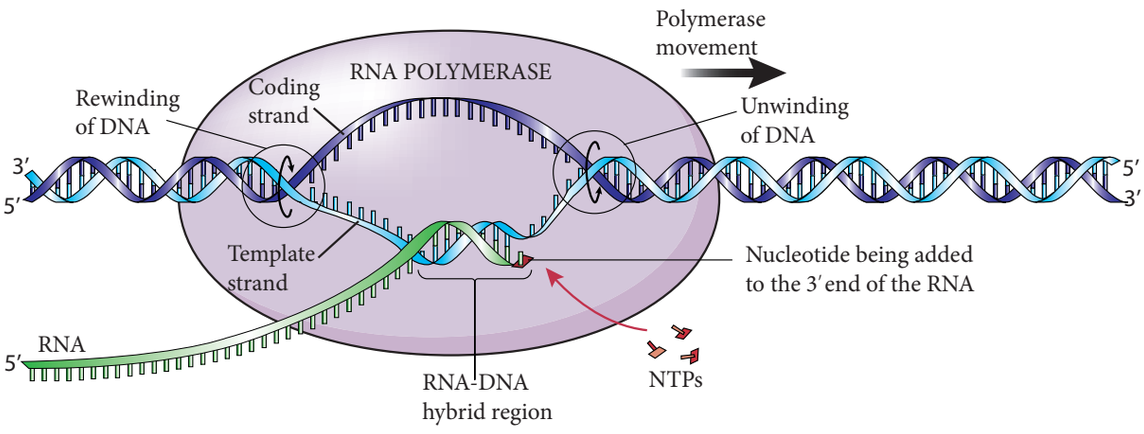



**Figure 6. Representation of the transcription process**

#### 6.3.2.1 Initiation of transcription

1. RNA polymerase, together with one or more general transcription factors, binds to promoter DNA.
2. RNA polymerase, along with transcription factor sigma, binds to a sequence of DNA called the promoter, found near the beginning of a gene.



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3. Each gene (or group of co-transcribed genes, in bacteria) has its own promoter.
4. Once bound, RNA polymerase separates the DNA strands, providing the single-stranded template needed for transcription.
5. RNA polymerase creates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.

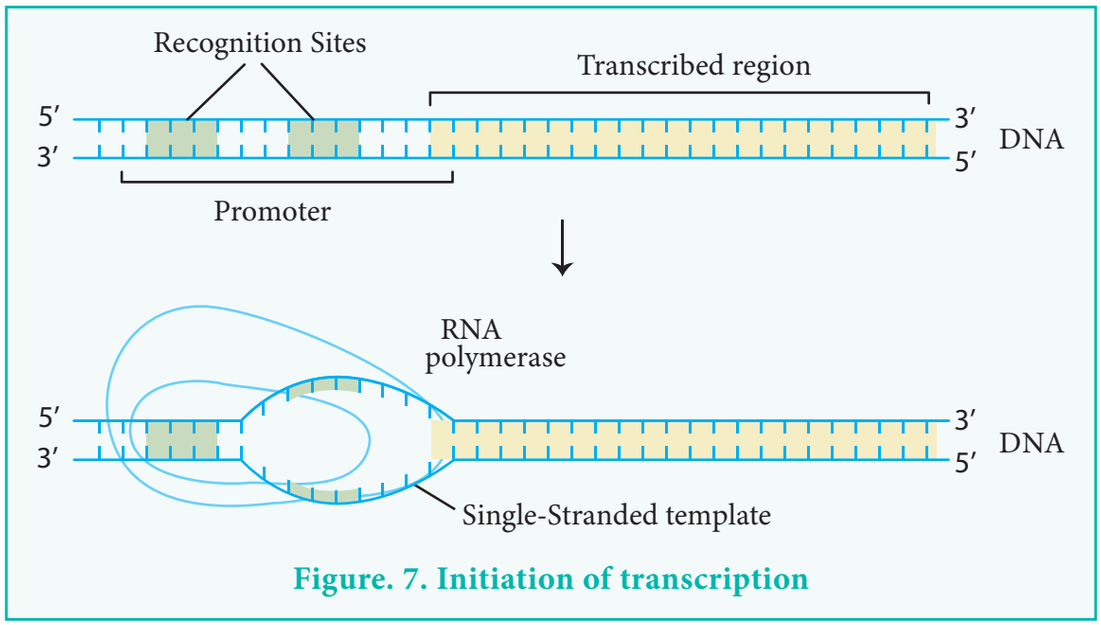



**Figure. 7. Initiation of transcription**

### 6.3.2.2 Elongation of transcription

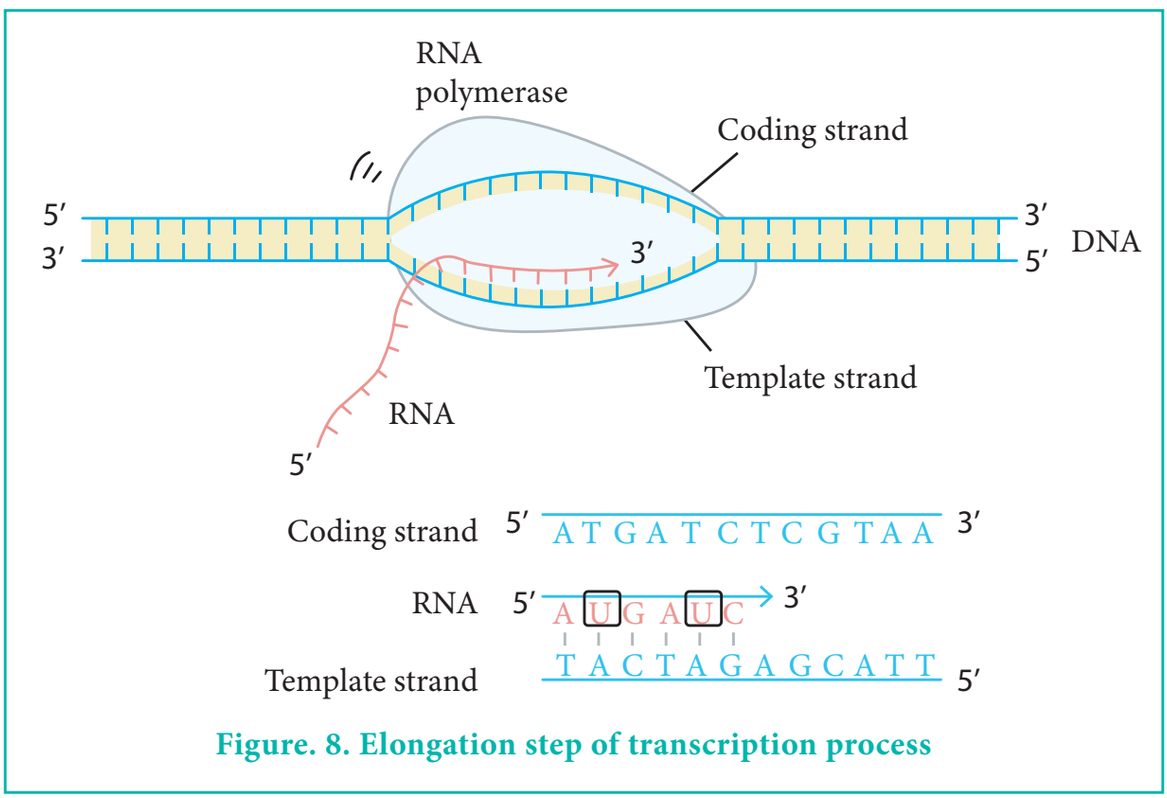



**Figure. 8. Elongation step of transcription process**



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1. After separation of the DNA strands, one strand of DNA, the template strand, acts as a template for RNA polymerase.
2. The enzyme RNA polymerase reads this template strand one base at a time and synthesizes an RNA molecule out of complementary nucleotides always in 5' to 3' direction.
3. The RNA transcript carries the same information as the non-template (coding) strand of DNA the base thymine (T) is replaced by uracil (U).

### 6.3.2.3 Termination of transcription

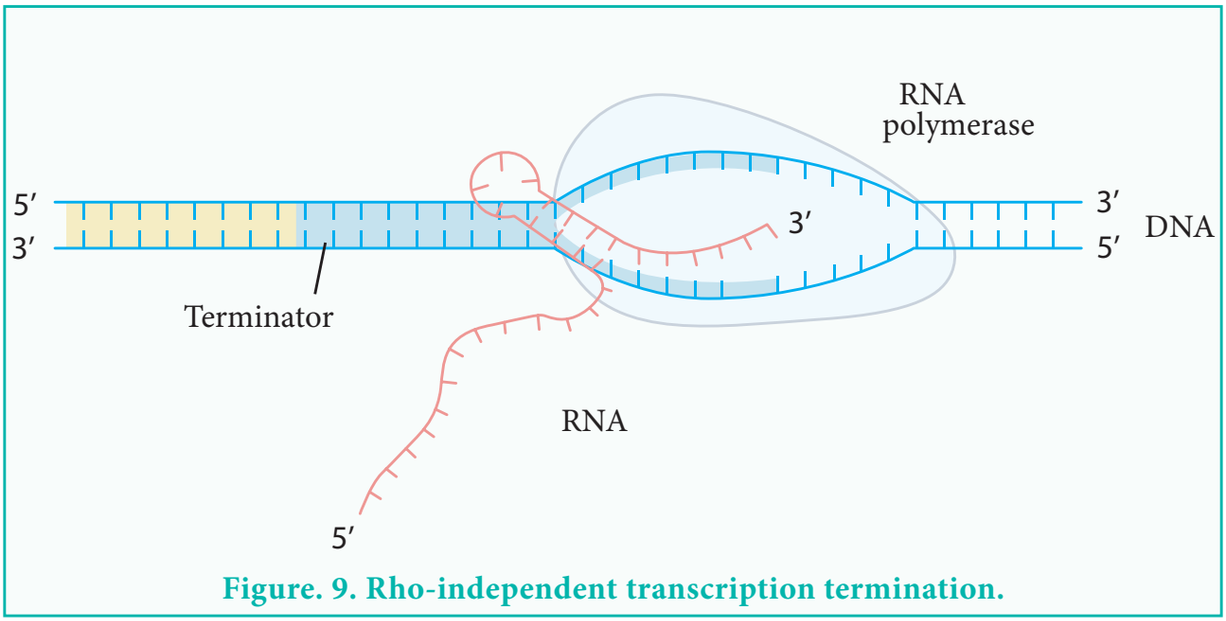



**Figure. 9. Rho-independent transcription termination.**

1. Two different types of transcription termination are observed in bacteria: the Rho-independent termination and Rho-dependent termination.
2. In Rho-independent termination, the RNA transcript forms a hairpin loop structure rich in GC base pairs, followed by a poly U region.
3. The hair pin structure of the newly synthesized RNA transcript produces a mechanical stress in the RNA polymerase and stalls it from moving. At the same time the relatively weak hydrogen bonds made by the poly U region with template DNA pulls out of the RNA polymerase, terminating transcription.
4. In the "Rho-dependent" type of termination, a protein called Rho destabilizes the interaction between the template and the mRNA and releases the newly synthesized mRNA from the elongation complex.

### 6.3.3 Types of RNA, synthesis and processing

There are three types of RNA which play a major role in protein synthesis, which are the ribosomal RNA or rRNA, the transfer RNA ot tRNA and the messenger RNA or mRNA



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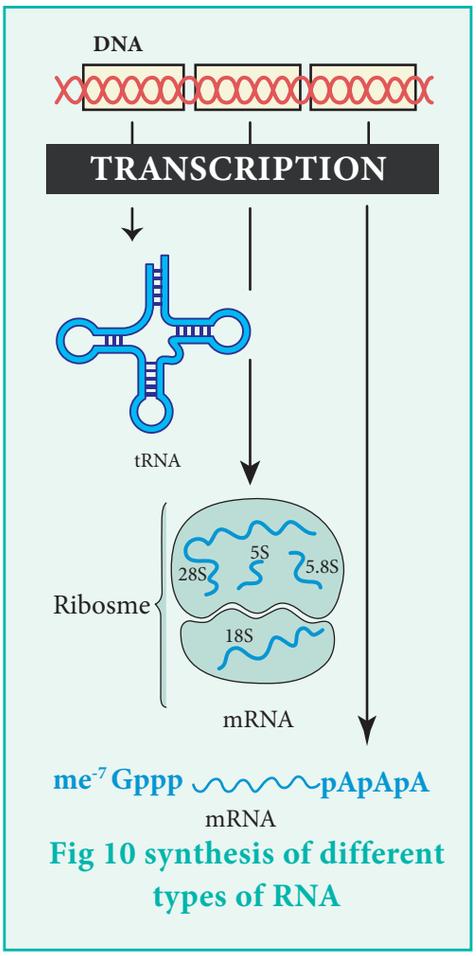



**Fig 10 synthesis of different types of RNA**

### 6.3.3.1 The Ribosomal RNA

These molecules are components of ribosomes. They form complexes with ribosomal proteins and act as important machinery for protein synthesis. There are three different species of rRNA, the 5S, 16S and 23S rRNA in prokaryotes and four in eukaryotes, the 5S, 5.8S, 18S and 28S rRNA. Here S represents the svedbergs unit or the sedimentation rate during centrifugation.

### 6.3.3.2 The Transfer RNA

These are the smallest among the three RNA types. Their primary function is to transfer aminoacids during protein synthesis. So there is atleast one tRNA molecule for every aminoacid. Figure 11 shows the secondary and tertiary structure of a typical tRNA molecule. The secondary structure is like a clover leaf and aminoacids are covalently linked to the 5'end. It could also be noted that there are many unusual bases in the tRNA like dihydrouracil. They also form extensive intra chain base pairing forming different loops in the RNA structure like the TψC loop, anticodon loop and D loop.

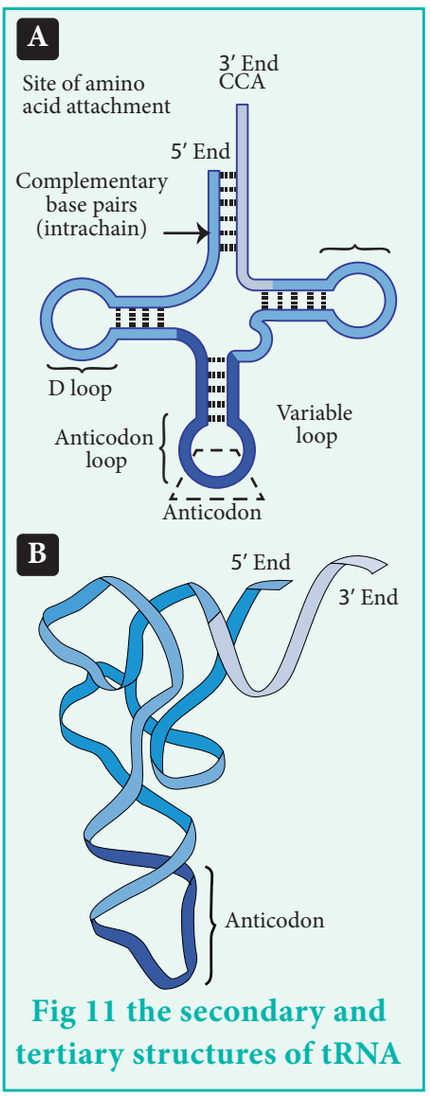



**Fig 11 the secondary and tertiary structures of tRNA**

### 6.3.3.3 The messenger RNA

This carries the genetic information from the DNA to the cytoplasam and hence the name messenger RNA. They are highly heterogeneous varying in size and sequence content. They act as template for protein synthesis. If the mRNA contains information for only one protein it is called monocistronic. The eukaryotic mRNAs are mostly monocistronic. However prokaryotic mRNA can contain information for encoding more than one mRNA and are called polycistronic.

### 6.3.4 Post transcriptional modification of RNA

In eukaryotes the RNA molecules are further processed. The most important modificatios are



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1. A 5-methyl guanosine base is added to the 5' end of the transcript
2. The 3' end is added with poly Adenine bases known as the poly A tail.
3. The eukaryotic genes and there by their corresponding RNA transcripts can have multiple non coding regions in between known as *introns*. The coding regions are known as exons. The process by which the noncoding *intron* regions are removed from the RNA transcript is known as RNA splicing. This processed RNA molecule which is ready for translation is known as an mRNA or a messenger RNA.

### 6.3.5 Difference between Prokaryotic and Eukaryotic in Transcription

| Sl. No | Prokaryotic Transcription | Eukaryotic Transcription |
|---|---|---|
| 1 | Coupled transcription-translation is the rule | Coupled transcription translation is not possible. |
| 2 | Occurs in the cytoplasm | Occurs in the nucleus |
| 3 | There is no definite phase for occurrence | Majority of transcription takes place in the G1 and G2 phase of the cell cycle |
| 4 | A single RNA polymerase synthesis all the three types of RNA(mRNA,tRNA,rRNA) | Different types RNA polymerases (I,II,III etc) synthesize different RNA molecules such as rRNA,mRNA,tRNA etc... |
| 5 | Initiation of transcription factor does not need any proteins or initiation factors | Initiation of transcription proteins requires transcription factors.These are TFIIA,TFIIB,TFIID,TFIIE,TFIIH.These recognises the TATA box. |
| 6 | Transcription usually involves more than one gene (polycistronic) | Transcription normally involves only one gene (monocistronic) |
| 7 | Usually there is no post transcriptional modification of the primary transcript. | Primary transcript undergo post translational modification(RNA editing) |
| 8 | Introns absent in the primary transcript. | Introns present in the primary transcript. |

## 6.4 TRANSLATION

As we have already studied, Proteins are the key molecules which literally carry out every single molecular process inside our cell. The instructions about how these proteins should be made is encoded in the DNA and is transcribed from the chromosome in the form RNA by a process which we saw as transcription. Similalry the process by which a new protein molecule is synthesized using this mRNA as a template is known as translation.



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### 6.4.1 The genetic code

During translation, a cellular organelle called ribosome "reads" the information in a messenger RNA (mRNA) and uses it to build a polypeptide or chain of amino acids. In an mRNA, three consecutive nucleotides are bases known as codons provide the instructions for building a polypeptide. Each codon codes for a single aminoacid. There are totally 61 codons which codes for amino acids and there are three more codons UAA, UAG and UGA which are known as stop codons as they are signals for termination of translation. The codon AUG codes for the aminoacid methionine and it also acts as a start codon. The genetic code is universal i.e, baring a few rare instances it is followed by all organisms. Similalry the genetic code is degenerate, meaning that one codon can only represent one aminoacid but one aminoacid can be represented by more than one codon. For example the codon UUU represents only phenylalanine. However phenylalanine can be represented by two codons UUU and UUC.

Second letter

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

**Figure 12 The universal genetic code**

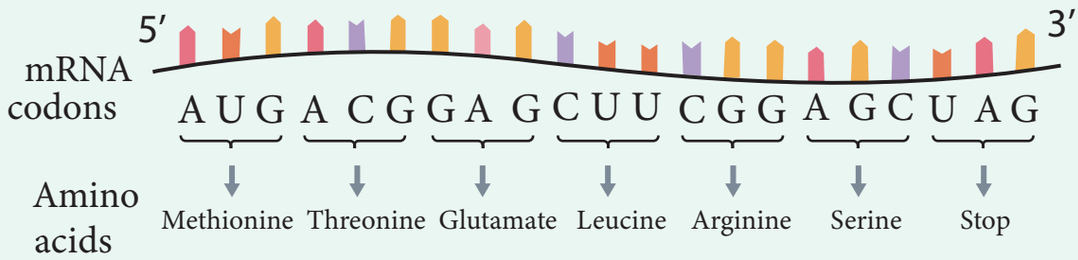



**Figure 13 Representational decoding of codons in a sample mRNA to corresponding amino acid sequence.**

### 6.4.2 Overview of translation

How is an mRNA molecule "read" to make a polypeptide? Two important components act as a machinery for this which are the tRNAs and ribosomes. As we have seen already



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the tRNAs paly a very important role in protein synthesis. They act as molecular "bridges" that connect mRNA codons to the amino acids they encode. Each tRNA has an anticodon loop, which has a sequence of three nucleotides called an anticodon. They can bind to specific codon in the mRNA. The other end of the tRNA carries the amino acid specified by the codons.

### 6.4.3 Ribosomes

Ribosomes are the cellular organelles where polypeptides (proteins) are built. They themselves are made up of different proteins and rRNA. Each ribosome has two subunits, a large one and a small one, which sandwiches the mRNA as shown in figure 14

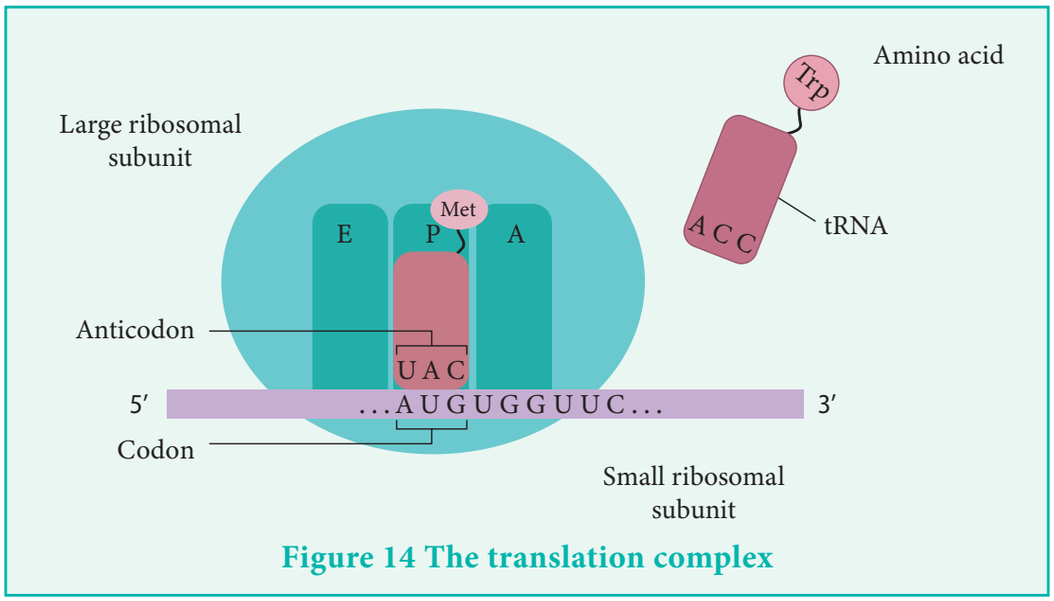



**Figure 14 The translation complex**

The ribosome provides three different structural packets where tRNA can enter and transfer its amnoacid to the growing polypeptide chain. These slots are called the A, P, and E sites. The ribosome itself can act as an enzyme, catalyzing the chemical reaction that links amino acids together to make a chain.

### 6.4.4 Molecular events in translation

The translation process can be divided into three different stages: initiation, elongation, and termination.

#### 6.4.4.1 Translation Initiation

Inititaion require three important components

- A ribosome – the large subunit and the small subunit
- An mRNA with instructions for the encoding protein.
- An "initiator" tRNA carrying the first amino acid in the protein, which is almost always methionine (Met)

The methonine tRNA attaches to the small ribosomal subunit. Together, they bind to the 5' end of the mRNA by recognizing the 5' cap in eukaryotes and then they move along the mRNA in the 3' direction, stopping when they reach the start codon AUG. This is later



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joined by the large subunit of the ribosome and this complex is known as the translation initiation complex.

In bacteria the small ribosomal subunit attaches directly to a pattern of sequence in the mRNA known as Shine-Dalgarno sequences which are present just before start codons and "point them out" to the ribosome.

Since the bacterial mRNA are polycistronic, they are transcribed in groups (called operons), so one bacterial mRNA can contain the coding sequences for several genes. A Shine-Dalgarno sequence marks the start of each coding sequence, letting the ribosome find the right start codon for each gene.

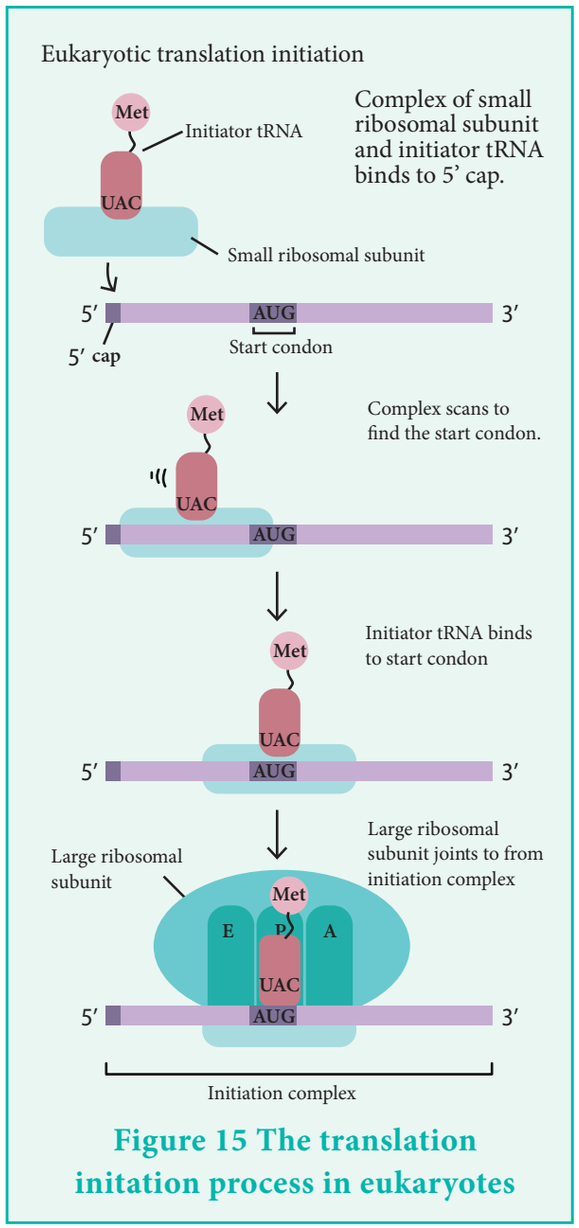



**Figure 15 The translation initation process in eukaryotes**

### 6.4.4.2 Translation Elongation

1. The first methionine-carrying tRNA starts in the middle slot or the P site of the ribosome. The subsequent codon is open to the A site.
2. The next tRNA with the anticodon which is complementary to this codon occupies the A site of the ribosome.
3. Once the matching tRNA has landed in the A site the first amino acid methionine from the first tRNA is covalently bonded (peptide bond) to the amino acid of the second tRNA in the A site.
4. Thus a dipeptide is formed with the methionine as N-terminus and the other amino acid is the C-terminus.

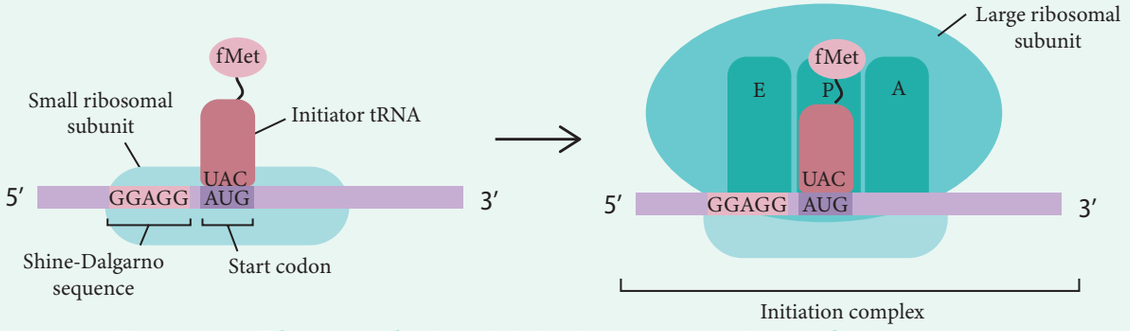



**Figure 16 The translation initiation process in prokaryotes**



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5. Once the peptide bond is formed, the mRNA is pulled onward through the ribosome by exactly one codon. This shift allows the first, empty tRNA to drift out via the E site at the same time exposing the a new and subsequent codon to the A site.

6. The entire cycle repeats and every time a new aminoacid is added as per the information in the codons being exposed to the A site. Thus a polypeptide stats growing in the ribosome.

### 6.4.4.3 Termination of translation.

The growing polypeptide is stoped when the A site of the ribosome encounters one of the stop codons in the mRNA which are UAA, UAG, or UGA.

Stop codons are recognized by proteins called release factors, which fit neatly into the P site helping the synthesized polypeptide to be released.

The small and large ribosomal subunits separate from the mRNA and from each other and are free to take part in another different translation process.

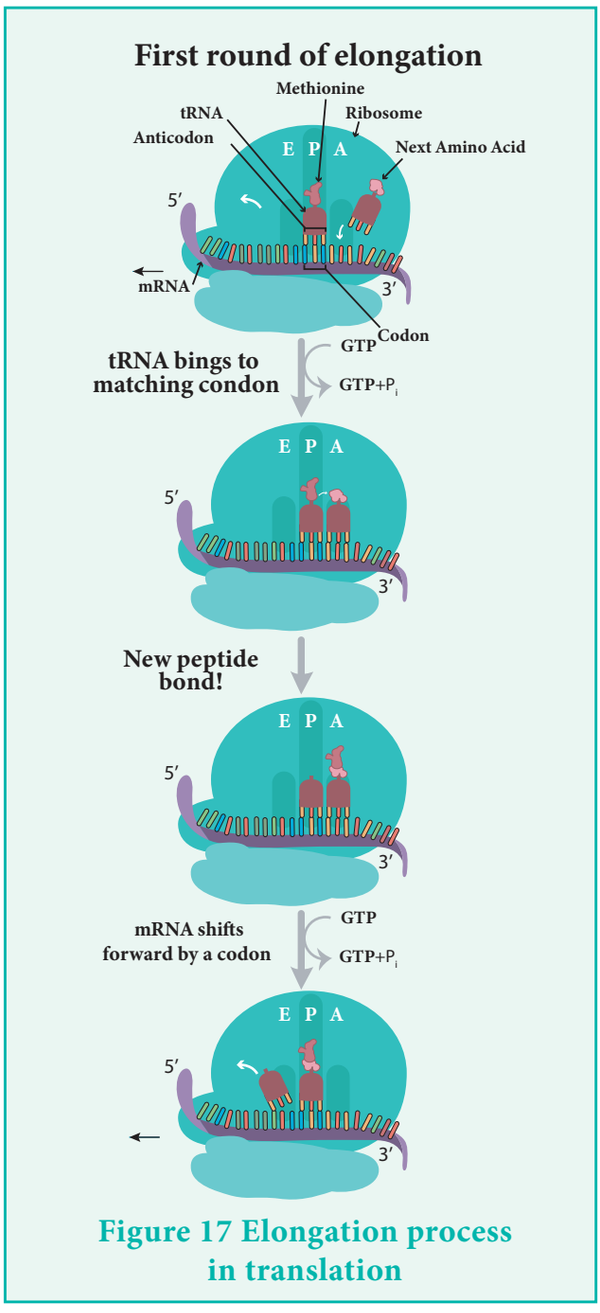



**Figure 17 Elongation process in translation**

### 6.4.5 Post-Translational Modifications

1. After translation, amino acids may be chemically altered or removed.

2. The chemical modifications include phosphorylation, glycosylation, glycation, nitrosylation, methylation, acetylation, lipidation etc

3. The new polypeptide will also fold into a distinct 3D structure and may join with other polypeptides to make a multi-subunit protein.



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### 6.4.6 Difference between prokaryotic and eukaryotic translation

| S. No | Prokaryotic translation | Eukaryotic translation |
|---|---|---|
| 1 | Transcription and translation are part of a continuous process and occurs simultaneously | Transcription and translation are separate process |
| 2 | The 5' end of the transcribed mRNA is immediately available for translation without any modification | The primary transcript is subjected to post transcriptional modifications and has to cross the nuclear membrane to reach the cytoplasam for translation |
| 3 | 70S ribosome is involved, with a 50S larger subunit and a 30S smaller sub-unit | 80S ribosome is involved with a 60S larger subunit and a 40S smaller subunit |
| 4 | Larger subunit of ribosome is made of 23S, 5S rRNA and 36 different proteins | Larger subunit is made of 28S, 5S, 5.8S rRNA and 40 different proteins |
| 5 | Smaller subunit of ribosome is composed of 16S rRNA and 21 different proteins | Smaller subunit of ribosome is made of 18S rRNA and 33 proteins |
| 6 | mRNA is polycistronic | mRNA is monocystronic |

## 6.5 RECOMBINANT DNA TECHNOLOGY

This technology is also known as DNA cloning allows us to insert a DNA fragment of interest such as a new gene into an organism, which doesn't contain that fragment of DNA. Though there are a variety of strategies to achieve this, the simplest of it is introducing a new gene into bacteria through plasmid vectors. Plasmids are small circular DNA molecules present in bacteria. They can be used to carry a foreign gene into the bacteria and hence the name vectors. The entire process is explained in figure 18. and consists of the following important steps

1. A bacterial plasmid designed with an antibiotic resistant marker gene is selected as a vector.
2. The circular plasmid vector is cleaved at specific sites using special enzymes called restriction endonuclease which are enzymes that cleave DNA at specific sequence patterns. For example EcoRI is a restriction enzyme which cleaves at the sequence GAATC.
3. The gene to be cloned is inserted in the gap produced by the endonuclease and an enzyme called DNA ligase is used to join the DNA pieces. This results in a circular plasmid vector containing the gene of interest or the recombinant plasmid.
4. This recombinant plasmid vector is now transferred into the cell by means of a process called transformation.



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5. These bacteria can be grown in a media containing specific antibiotics as per the resistant gene present in the plasmid vector. The bacterial cells which have taken up the recombinant plasmid vector can only grow in the presence of antibiotics (positive clones).
6. These positive clones can then be subjected to further applications like production of recombinant proteins, studying expression etc.,
7. This method has been successfully used for commercial production of many proteins like Human Insulin, antibodies, enzymes of medical and industrial interests etc.,

## 6.6 GENOME SEQUENCING AND THE BIRTH OF GENOMICS ERA

The birth of the 21$^{st}$ century has seen many revolutions in the field of molecular biology. Today molecular biologists are more interested in studying the collective behaviour of the genome rather than studying one individual gene. The entire information present in the DNA of an organism can be considered as its genome. Genomics can be defined as a study of whole genomes of organisms and uses a combination of techniques such as recombinant DNA technology, PCR amplification, Advanced DNA sequencing methods and bioinformatics analysis. Genomics experiments tries to harness the availability of entire sequence of genome which is possible today by the pioneering sequencing experiment by Fredrick Sanger and the recent next generation sequencing technologies most of which are build upon Sanger sequencing procedure

### 6.6.1 The Sanger sequencing procedure

1. It is also known as dideoxy chain termination method.
2. It sequence is generated by replicating a template DNA strand (the region to be sequenced) and interrupting the replication process at one of the four bases.
3. Four different reaction mixtures are produced such that the growing chain terminates in A,T, G or C
4. The reaction mixture also contains dideoxynucleoside triphosphates (ddNTPs) along with usual dNTPs.
5. If during replication ddNTPs is incorporated instead of usual dNTPs in the growing DNA strand then the replication stops at that nucleotide.
6. The ddNTPs are radio labelled (with $P_{32}$)or fluorescently labelled so the chain can be visualized in a gel electrophoresis procedure.

The details of Sanger sequencing procedure is depicted in figure 19 This sequencing procedure was performed in a massively parallel fashion and the human genome was sequenced in the year 2000.



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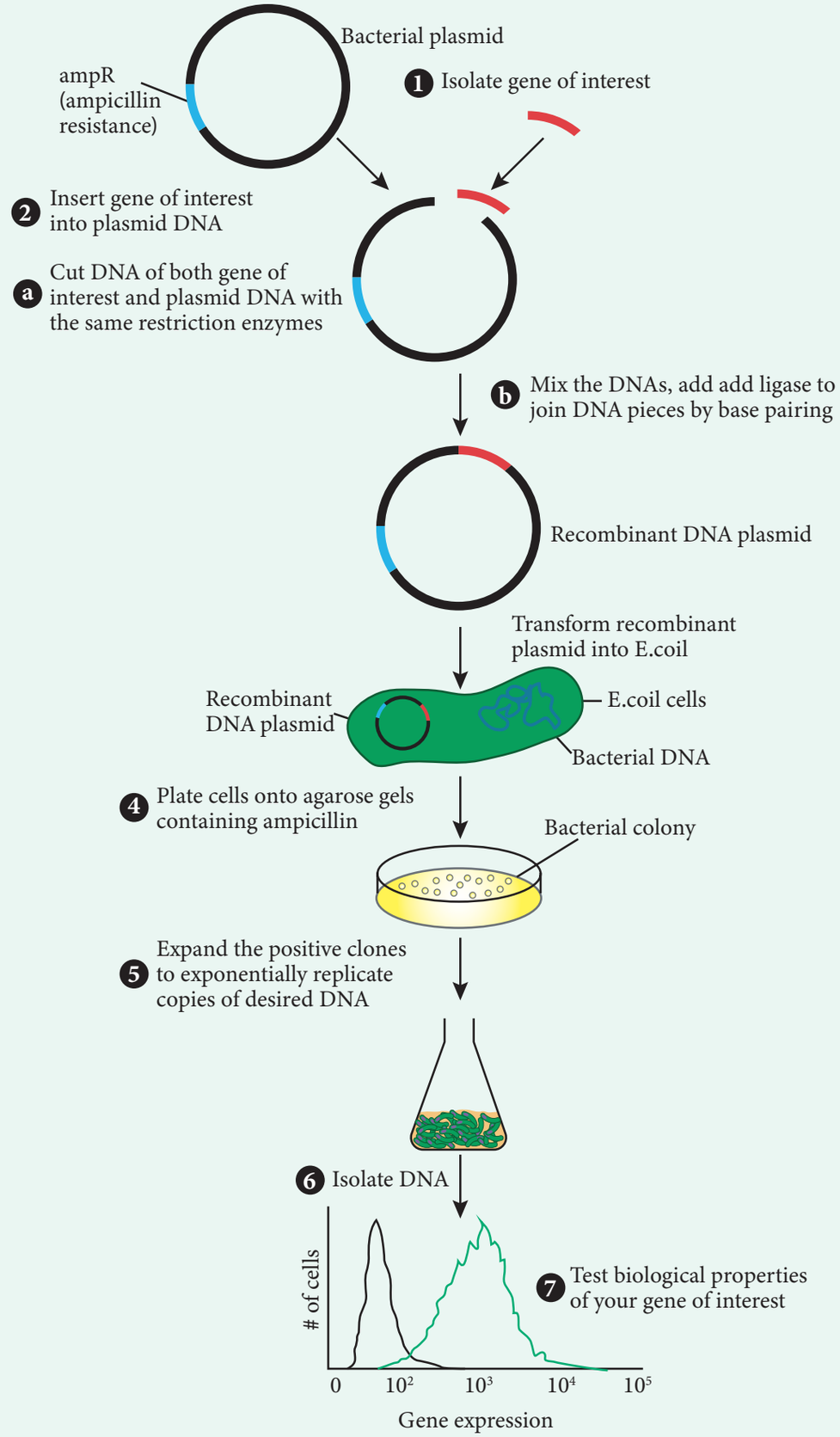



**Figure 18 The steps in recombinant DNA technology (bacterial gene cloning)**



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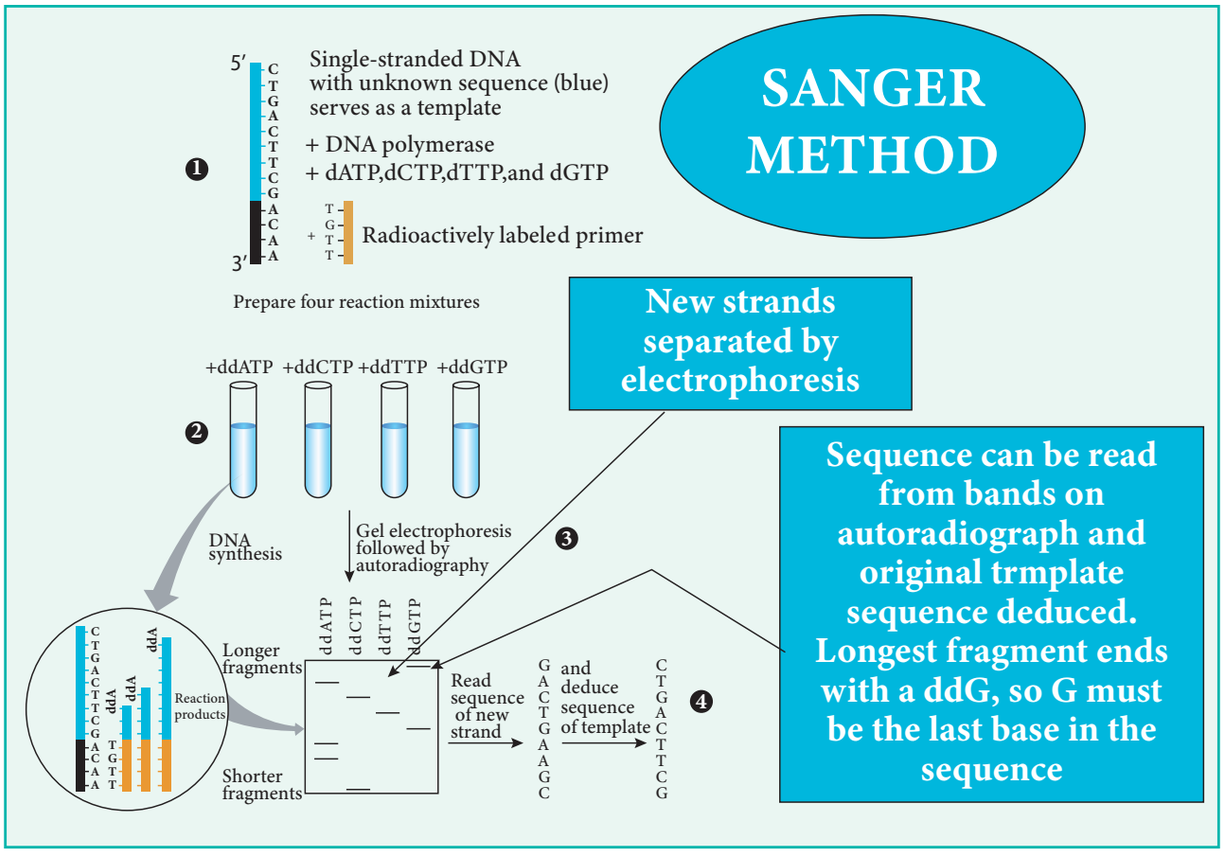



**Figure 19 Schematic representation of Sanger sequencing procedure**

### 6.6.2 The Next Generation Sequencing (NGS) Technologies and their role in genomics.

All the Next Generation Sequencing (NGS) methods adopt the principle of Sanger's method i.e., sequencing-by-synthesis. However they all use technologies that do not permenantly terminate the growing chain, so that the synthesis can be monitored as each base is incorporated. This improvement allows us to conduct sequencing of millions of DNA fragments simultaneously and hence achieving high throughput, which was not possible in Sanger's method. Moreover the sequencing costs has become much lower using these technologies and today a human genome can be sequenced at around $200 and within 48 hours. Some of the popular methods are the Illumina reversible dye terminator method, ion torrent method and nano pore DNA sequencing methods.

The advancements in genome sequencing has led to more ambitious projects like the 1000 genomes project or 1KGP. It was an international collaboratory effort to sequence 1000 different human genomes and to catalogue their genetic variations. This project was started in 2008 and was completed in 2012 with the complete genome sequence of 1098 humans. With the help of this data, so far about 100 regions in human genome has been identified to have association with diseases like coronary heart disease, diabetes mellitus etc.,

The Cancer Genome Atlas program (TCGA) is another ambitious project which was started in 2007 and so far has collected genomics data from 20,000 cancer patients and includes 33 types of cancer. This data is freely available for researchers around the world



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and act as a potential resource for combating caner.

### 6.6.3 Applications of NGS technologies

Apart from obtaining the sequence of DNA or genomes, the current sequencing strategies can be tailored for different applications. A few important applications are discussed below.

#### Transcriptomic Profiling (RNA-Seq)

In this method the RNA content in a cell can be isolated and sequenced in a quantitative fashion. As a result this method can profile the genes that are expressed in a cell in a given condition.

#### Polymorphism and Variation Discovery

This experiment identifies the mutations or variations in a genome sequence and associates them with phenotypes such as diseases.

#### Protein-DNA Interaction Analysis (ChIP-Seq)

This method is also known as Chromatin Immuno Precipitation (ChIP), which precipitates the protein (histone) DNA complexes alone, by means of specific antibodies and then that region of DNA alone is sequenced. This method helps us to identify the genome regions which can interact with proteins like histones.

#### Metagenomics

Metagenomics can be defined as the study of genetic material recovered directly from environmental samples. For example, the study of the microbiome in the gut of an individual. These samples contain extremely large but unknown numbers of unculturable microbial species. So such samples are directly subjected to DNA isolation and sequencing (without culturing) and considered as the genome of an entire community.



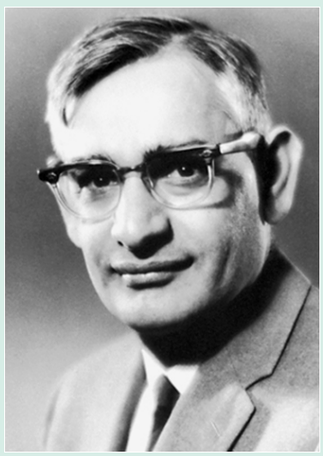
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Scientist Steve Howell in 1986 created a tobacco plant that can glow in dark like a firefly. He isolated a gene called luciferase which are present in fireflies and are the reason for the fireflies to glow in dark. He introduced this gene into toboco plants and showed that they can also glow in dark. These type of plants which contains foreign genes are known as transgenic plants or genetically modified (GM) plants. The methods used for producing such organisms are collectively known as genetic engineering. These methods promise increased agricultural yields by producing transgenic plants which can be pest resistant, drought resistant, saline resistant etc., However extreme caution has to be employed when introducing these plants to main stream agriculture as several factors has to be considered such as the balance in eco system, cost and effectiveness of the GM variety while cultivating in the field etc., Some countries have also banned GM crops and many countries follow the policies of strictly regulated use.



Har Gobind Khorana was an Indian-American biochemist who did pioneering experiments to reveal the genetic code. He was born to Krishna Devi Khorana and Ghanpat Rai Khorana in Punjab during 1922. He finished his Bachelaor's and Master's degrees in Punjab University (Lahore). He lived in pre independent India until 1945, after which he moved to England and obtained his PhD in organic chemistry from The University of Liverpool. In 1960 he was appointed as a professor in University of Wisconsin (USA) where he did a serried of experiments to understand how RNA codes for proteins. He synthesized repeating units of nucleotides 'UCU CUC UCU ...' codes for the amino acids serine and leucine repeatedly. By this way he was able to show that three RNA nucleotides were responsible for an amino acid. He also identified stop codons. In short he was able to synthesize a gene or in other words 'a nucleotide sequences which could code for a desired aminoacid sequence' He was awarded Nobel prize in 1968 as recognition for his works in understanding the molecular biology of protein synthesis.



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## Summary

- DNA Replication : Each strand of the DNA double helix serves as a template for the synthesis of a complementary daughter strand by the semiconservative mode of replication which was confirmed by the Meselson Stahl experiment.
- The two strands of the double helix unwind with the help of helicase. DNA, being a large molecule, the separation of the two single strands creates a 'Y' shaped structure called a 'replication fork'. DNA polymerases synthesize the new DNA strands only in the 5' to 3' direction.Therefore, one of the newly synthesized strands grows in the 5' to 3' direction towards the replication fork (leading strand) and and one in the 5' to 3' direction away from the replication fork (lagging strand).
- A short stretch of RNA acts as a primer for DNA polymerase and it is synthesized by RNA primase.The leading strand needs only one primer, but the lagging strand needs many and short fragments of DNA called Okazaki fragments are synthesized, which are eventually ligated.
- In *E.coli*, DNA polymerase III elongates a DNA chain and proofreads mismatched bases with its 3' to 5' exonuclease activity. DNA polymerase I removes RNA primers using its 5' to 3' exonuclease activity and fills the gaps.
- Transcription: The process of RNA synthesis directed by a DNA template is called transcription and it is performed by RNA polymerase. Transcription occurs in 3 steps: initiation, elongation and termination.
- RNA polymerase binds to a sequence of DNA called the promoter, found near the start of a gene. Once bound, RNA polymerase separates the DNA strands, exposing the single-stranded DNA template. Complementary bases are added (instead of T, U is added) one at a time by RNA polymerase.The newly synthesized RNA strand grows from 5' to 3'. Termination occurs with the help of a rho factor or with specific terminator sequences.
- In eukaryotes post transcriptional modifications occur by which, a methyl guanosine cap and a poly A tail are added to 5' and 3' ends, respectively, to mRNA. Introns are spliced out of mRNA, tRNA and rRNA primary transcripts.
- Translation: The three major components in translation are mRNA, ribosomes and tRNA. The mRNA code is read in the 5' to 3' direction, and the protein is synthesized from the amino terminus to the carboxyl terminus.Three steps occur during translation: Initiation, elongation and termination.
- There is at least one specific tRNA and one aminoacyl tRNA synthetase for each amino acid. An anticodon in tRNA recognizes the codon present in mRNA.
- Initiation of translation: mRNA associates with the small ribosomal subunit and this process requires a sequence called Shine-Dalgarno sequence on mRNA.



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The initiating codon is AUG and the initiating amino acid is formyl methionine.

- Elongation is facilitated by factors that facilitate the binding of aminoacyl tRNA to the A site on the ribosome as well as the movement of the ribosome along the mRNA.
- Peptide bond formation is catalyzed by peptidyl transferase and after peptide bond formation, it moves forward along the mRNA in 5' to 3' direction to the next codon.
- Termination begins when one of the stop codons moves into the A site. The recognition of these codons by release factors releases the newly formed protein from the m-RNA-ribosome complex.
- Post-translational modifications like removal of amino acids, phosphorylation, glycosylation, hydroxylation and protein folding also occur after translation in eukaryotes.
- Recombinant DNA technology: This technology, is also known as DNA cloning,allows insertion of a DNA fragment of interest, such as a new gene, into an organism. The simplest strategy is introducing a new gene into bacteria through plasmid vectors. An outline of gene cloning using plasmid vectors is given.
- PCR amplification of DNA, sequencing of DNA using Sanger's dideoxy chain termination method and the latest Next Generation Sequencing (NGS) methods are explained along with their applications.

## EVALUATION

### I Fill in the Blanks

1. The information in the DNA can be copied to RNA by a process called _____
2. Name the three different models for DNA replication.
3. A short piece of RNA produced by primase enzyme is called as _____
4. The fragments of DNA in the lagging strand during DNA replication are called _____
5. _____ is an enzyme that strips away the primer(s).

### II State True or False

6. In Prokaryotes, DNA replication occurs inside the nucleus.
7. Replication is very slow in Eukaryotes.
8. The eukaryotic mRNAs are mostly monocistronic.



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9. In Prokaryotes, introns are absent in the primary transcript.

10. The reaction that makes billions of copies of a small region of DNA is known as _____

11. Transcription is a process by which DNA sequence is read by an enzyme called _____ and a complementary, antiparallel RNA strand is synthesized.

12. RNA polymerase, along with transcription factor sigma, binds to a sequence of DNA called the _____

13. While comparing the coding strand of DNA and a transcribed RNA the base _____is replaced by _____.

14. Name the three types of RNA.

15. _____is type of RNA carries the genetic information from the DNA to the cytoplasm.

16. An mRNA that can contain information for encoding more than one mRNA and are called _____.

17. The cytoplasmic cellular organelle involved in translation process is _____.

18. _____ play a very important role in protein synthesis and acts as molecular "bridges" connecting the mRNA and the growing polypeptide chain.

19. State the different stages of translation process.

20. _____ is also known as DNA cloning which allows us to insert a new gene into an organism.

## III Match the following

| | | | |
|---|---|---|---|
| 21. | Sanger's Method | - | Bacterial Translation |
| 22. | Shine Dalgarno sequence | - | UAG |
| 23. | Stop codon | - | AUG |
| 24. | Taq polymerase | - | DNA sequencing |
| 25. | Start Codon | - | Polymerase Chain Reaction |



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## IV Answer the following questions

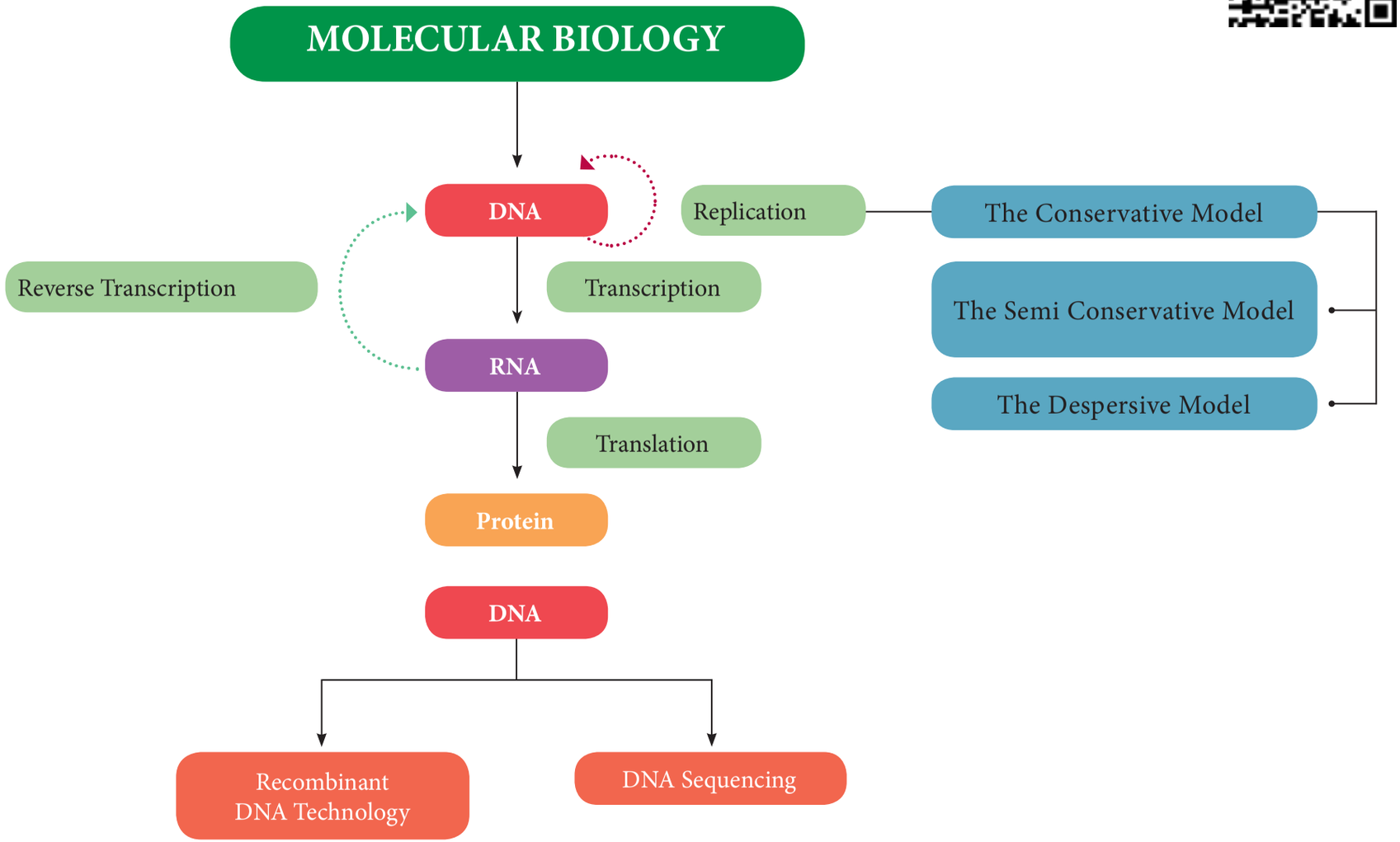
1. Write in brief about the central dogma of molecular biology.
2. Describe the Meselson-Stahl experiment and the conformation of semi-conservative model.
3. Explain the events involved in DNA Replication briefly.
4. Compare the different models of DNA replication.
5. Differentiate between the process of DNA replication in prokaryotes and eukaryotes.
6. Explain the role of the DNA polymerase in DNA replication.
7. Describe the steps involved in PCR amplification.
8. Give an Overview of transcription.
9. Compare Prokaryotes and Eukaryotes with respect to Transcription
10. Write briefly about the Molecular events in translation.
11. Write short notes on Recombinant DNA technology.
12. Explain the Sanger sequencing procedure in detail.
13. Discuss about the Post transcriptional modification and Post-translational modifications events.
14. Compare the three main types of RNA in living organisms.
15. Discuss about Next Generation Sequencing (NGS) Technologies and their role in genomics.
16. Explain the different applications of NGS technologies in genomics.



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# CONCEPT MAP



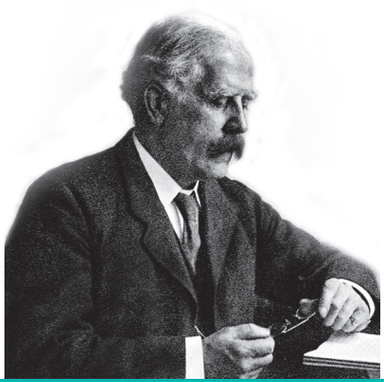



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UNIT 7

# INBORN ERRORS OF METABOLISM



**Sir Archibald Garrod**

Sir Archibald Garrod, an English physician, introduced the term "Inborn Errors of Metabolism" in 1908. He postulated that inherited disorders result from reduced activity or complete absence of an enzymes involved in biochemical pathways, causing a block in the metabolic pathway. His study of alkaptonuria led to the finding that a mutation in a gene resulted in the accumulation of homogentisic acid and the presence of black urine on exposure to air. Following this, in 1941, George Beadle and Edward Tatum, using the bread mould *Neurospora*, confirmed Garrod's hypothesis and proposed the 'One gene one enzyme' theory. This hypothesis was further modified in 1957 by Vernom Ingram, as 'One gene one polypeptide' since many proteins are made up of more than one polypeptide chain.

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## Learning Objectives

After studying this unit you will be able to understand the following:

- Biochemical basis of Inborn errors of metabolism
- Types of inborn errors of metabolism
- Causes and symptoms of Galactosemia
- Causative factors of Von Gierke disease
- Various types of haemophilia and clotting factors
- Ocular and oculo-cutaneous albinism
- Causes and symptoms of Alkaptonuria
- Causative factors of Tay – Sachs disease.



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## INTRODUCTION

Inborn errors of metabolism (IEM) or inherited metabolic disorders are a group of disorders with specific enzyme defects that impede with normal metabolism of protein, fat or carbohydrate. As its name implies, inborn errors mean birthdefects in young infants which are inherited. IEM like galactosemia, phenylketonuria, alkaptonuria, albinism, Tay -Sachs disease and von Gierke disease, can appear atbirth or later in life .

A food product that is not broken down into energy can build up in the body and cause a wide range of symptoms. Several inborn errors of metabolism cause developmental delays or other medical problems if they are not controlled. The main indication of IEM is an excess storage or accumulation of specific metabolites in tissues, organs and blood which further manifest to health diseases. Most IEM are rare but some are life threatening.

The metabolism of our body comprises two major balanced activities: anabolism (synthesis) and catabolism (degradation). The absence or deficiency of an enzyme will cause an abnormal accumulation of the intermediate products of metabolism in the body and increased excretion in urine as such, or, as their degradation products. Some of the intermediates could even be toxic. For example, in the following reaction

$$R \xrightarrow{a} B \xrightarrow{b} C \xrightarrow{c} D \xrightarrow{d} P$$

- R is the reactant,
- B, C and D are intermediates, P is the product
- a, b, c and d are enzymes catalyzing the indicated steps of the reactions.
- In this pathway, if any enzyme is deficient or absent, the previous intermediate accumulates and produces toxicity. It also affects the amount of product (P) formed and thereby leads to a disease.

**The classification of IEM is presented in the following table**

**A few important diseases associated with IEM are given below:**

| Disorders | Examples |
|---|---|
| Disorders of aminoacid metabolism | Phenylketonuria, Alkaptonuria, Albinism Homocysteinuria |
| Disorders of carbohydrate metabolism | Galactosemia, hereditary fructose intolerance, Glycogen storage disorders (Von Gierke disease) |
| Lysosomal storage disorders | Mucopolysaccharidosis, Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease |
| Organic acidemia | Methyl malonic acidemia |



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| | |
|---|---|
| Disorders of purine or pyrimidine metabolism | Lesch–Nyhan syndrome |
| Transport disorders | Cystinuria<br>Hypercholesterolemia |
| Peroxisomal disorders | Adrenoleukodystrophy |
| Urea cycle disorders | Citrullinemia, Ornithine transcarbomylase deficiency |
| Metal metabolic disorders | Wilson's disease |

## 7.1 GALACTOSEMIA

Galactosemia, first described in 1908 by Von Reuss, is a genetic disease, characterized by the inability to process galactose, which is found in many foods. galactosemia implies 'galactose in the blood'. Galactose, a sugar byproduct of lactose, is present in cow's milk, breast milk and dairy products. As a result, if not treated , it builds up in the tissues and blood causing life threatening problems. The incidence of this disease is about 1 in 18,000 livebirths.

### 7.1.1 Causes

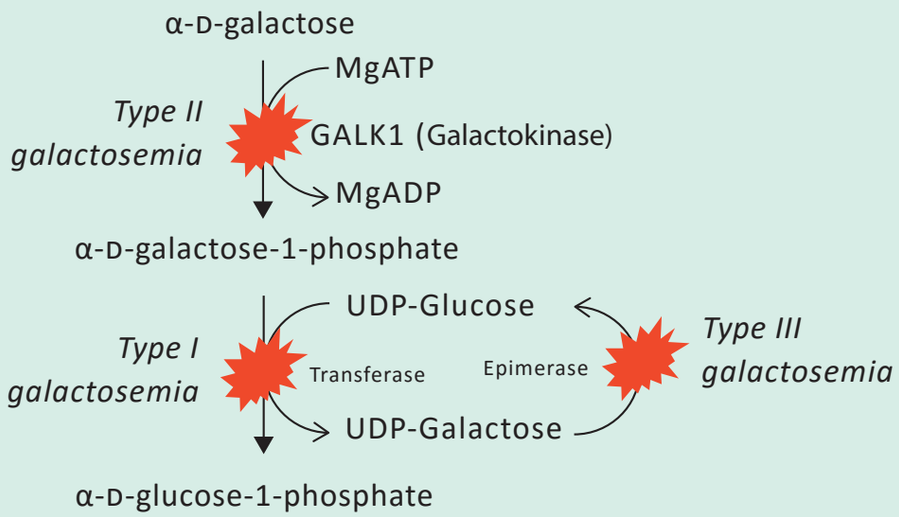



**Figure 7.1 Pathways of Galactose metabolism**

Galactosemia occurs when a specific enzyme known as galactose-1-phosphate uridyltransferase (GALT) is absent. This enzyme present in liver is responsible for breaking down galactose into glucose. In normal subjects, GALT binds to galactose, and converts it into glucose. However, in galasctosemia, due to the absence of GALT, galactose-1-



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phosphate, is not converted to glucose 1 phosphate and accumulates in tissues and blood. The toxic galactose levels lead to enlarged liver, kidney failure and cataract.

The pathway of galactose metabolism is shown in figure 7.1.

Three types of galactosemia are known which are caused by mutations in GALT, GALK1 (galactokinase) and GALE (UDP-galactose 4′-epimerase) genes. However, GALT deficiency is the most severe and life threatening form of the disease (Figure.7.2).

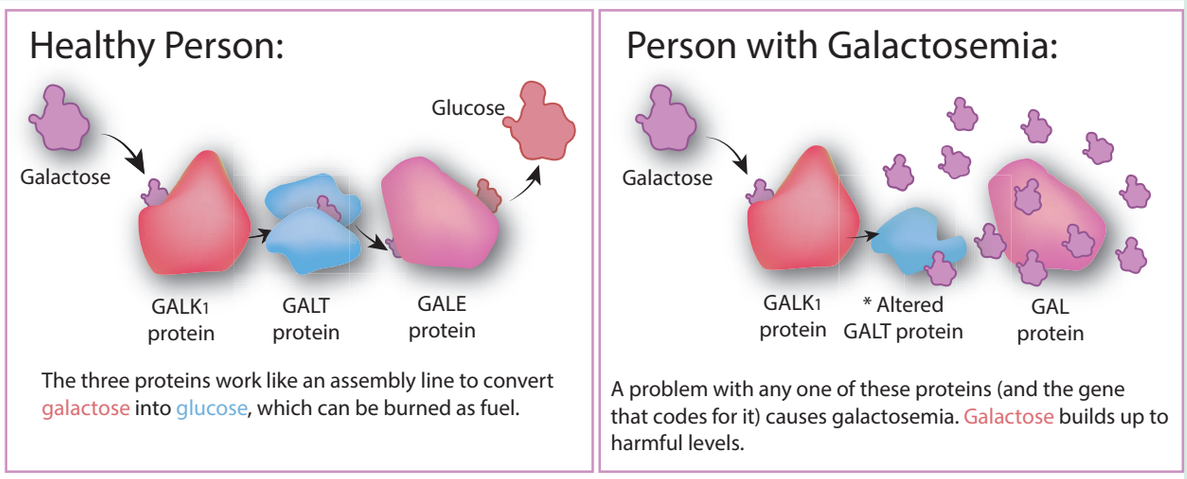



**Figure.7.2 HealthyVs Galactosemia subjects**

### 7.1.2 Symptoms

The deficiency of GALT is clinically important. Due to the enzyme defect, galactose accumulates in blood and is reduced by aldose reductase in the eye to the corresponding galactitol which causes cataract. The general condition is more severe, since, due to a deficiency in GALT, galactose 1- phosphate accumulates and injures the liver leading to its failure. Mental deterioration is caused due to accumulation of direct bilurubin, as it moves from blood to brain .

If an infant is breast feeding, but still experiences poor weight gain, it may due to galactosemia. Infants appear normal at birth but later they show failure to thrive and become lethargic. They have frequent vomiting and hypoglycemia. After 2 - 3 months of age, the liver may show fatty infiltration and lead to cirrhosis (nonfunctioning of liver cells). Galactosemia at this age is associated with mental retardation due to accumulation of galactose and galactose 1 - phosphate in cerebral cortex. Other symptoms include, jaundice, vomiting, hepatomegaly, *E.coli* sepsis and irritability.

## 7.2 VON GIERKE DISEASE

Glycogen storage diseases are a group of inherited disorders associated with glycogen metabolism. Von Gierke disease, also known as glycogen storage disease(GSD) type I, is one of the groups of rare genetic disorders due to the defect in one or more of the enzymes involved in glycogen metabolism leading to excessive accumulation of glycogen in the



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tissue, especially in liver, muscle and heart. In 1929, Von Gierke identified the first patient with GSD type I and named it as hepatonephromegalia glycogenica.The enzymatic defect, namely glucose 6 phosphatase deficiency, that caused type I GSD was elucidated in 1952 byCarl and Gerty Cori. They found that glucose 6-phosphatase was missing from the liver of a patient with this disease. This was the first demonstration of an inherited deficiency of a liver enzyme. The liver glycogen is normal in structure but present in abnormally large amounts.

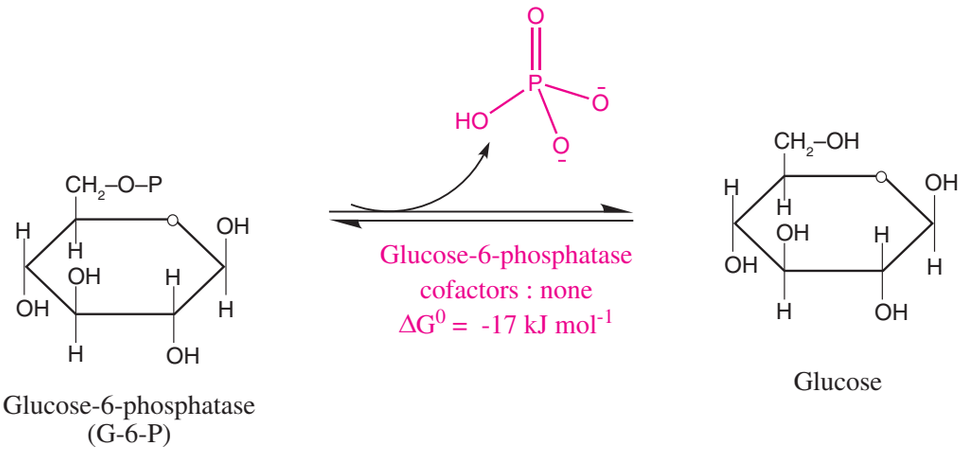



**Figure.7.3 Action of glucose-6-phosphatase**

Glucose -6-phophatase catalyzes the final step leading to the release of glucose in blood stream by the liver **(Figure.7.3)**. Deficiency of this enzyme results in an increase in intracellular glucose -6-phosphate. This phosphorylated sugar cannot leave the liver, because it cannot cross the plasma membrane, which leads to a large accumulation of glycogen in the liver and inability to increase blood glucose concentration in response to glucagon or epinephrine. Since glucose6- phosphate cannot leave liver cells, there is compensatory increase in glycolysis leading to increased levels of pyruvic acid and lactic acid.

Patients who have Von Gierke disease also have an increased dependence on fat metabolism. This disease can also be produced by a mutation in the gene that encodes the glucose 6-phosphate transporter.

### 7.2.1 Clinical manifestations

The clinical manifestations of Von Gierke disease, as shown in Figure.7.4, include hypoglycemia with lactic acidosis during fasting, enlarged liver, distended abdomen, cherubic or doll like face and seizures.



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### 7.2.2 Symptoms

Signs and symptoms of this disease appear around 3-4 years of age. Affected infants may have hypoglycemia associated with seizures. High level of blood uric acid and hyperlipidemia are evident. Symptoms include low blood sugar, intolerance of fasting, frequent nose bleeds. Both liver cells and the cells of renal convoluted tubules are loaded with glycogen. Ketosis and hyperlipidemia are also present. Treatment includes intake of food high in starch



Figure.7.4 Von Gierke affected child

## 7.3 HEMOPHILIA

Clotting factors are a set of proteins that control bleeding. When a blood vessel is affected, the lining of the blood vessels contract to reduce the blood loss at the site of injury. Small blood cells called as platelets stick to the injured site and prevent blood loss. Inside the platelets, chemicals that attract other cells and make up a clump called as a platelet plug. At the site of injury, in the surface of platelets, clotting factors orchestrated by a set of proteins which form a coagulation cascade leading to the formation of fibrin clot. The fibrin clot acts as a mesh and stops bleeding.



Figure 7.5 Hemophilia

The term Hemophilia was first coined by Dr. John Conrad Otto, in the early 19th century. Hemophilia is an inherited bleeding disorder in which a person lacks or has low levels of certain proteins called "clotting factors" and the blood does not clot properly. There are nearly 13 factors involved in the mechanism of blood clotting. If any one or more of these factors are not synthesized adequately that results in the defect in blood clotting and thereby haemorrhage. A number of inherited deficiencies of the blood clotting factors are found in human and are collectively called as hemophilias. The clotting factors are presented in Table 7.1.

| Clotting factor | Factor name | Source |
|---|---|---|
| I | Fibrinogen | Liver |
| II | prothrombin | Liver |



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| | | |
|---|---|---|
| III | Tissue thromboplastin (Tissue factor) | Tissue cells |
| IV | Calcium ions | Plasma |
| V | Labile factor (pro-accelerin) | Liver, platelets |
| VII | Stable factor (pro-convertin) | Liver |
| VIII | Anti-hemophilic factor | Liver, lung capillaries |
| IX | Christmas factor Plasma thromboplastin component | Liver |
| X | Stuart-prower factor | Liver |
| XI | Plasma thromboplastin anticedent | Liver |
| XII | Hageman factor | Liver |
| XIII | Fibrin stabilizing factor | Liver, bone marrow |

### 7.3.1 Causes

Hemophilia is an inherited disease, where clotting occurs at an abnormally slow rate due to the absence of one or more of the blood clotting factors. The sufferers are known as 'hemophiliacs' or 'bleeders'. It is peculiar that it mainly affects males, but in rare circumstances, females can also be affected. There are several types of hemophilia and most forms are inherited. Three major forms of hemophilia namely hemophilia A, B, and C are important.

(i) *Hemophilia A*: Classic hemophilia or Hemophilia A, is a sex linked recessive disorder characterized by a deficiency of factor VIII. About 1 in 10,000 males is born with a deficiency of factor VIII.

(ii) *Hemophilia B*: Hemophilia B is the second most common form of hemophilia. It is due to a dysfunction in factor IX, which is also called as Christmas disease

(iii) *Hemophilia C* : It is mild and is caused by a deficiency of factor XI.

### 7.3.2 Symptoms

People with hemophilia may lose large amounts of blood from even the smallest injury, and the blood takes a longer time to clot. They will experience spontaneous or internal bleeding and often have painful, swollen joints due to bleeding into the joints. Unusual bleeding after vaccination is also evident. This rare but serious condition can have life-threatening complications. Symptoms of hemophilia vary with the level of clotting factors. If blood clotting-factor level is not very low, bleeding will occur only after surgery or trauma. If blood clotting factor deficiency is severe, it causes spontaneous bleeding.



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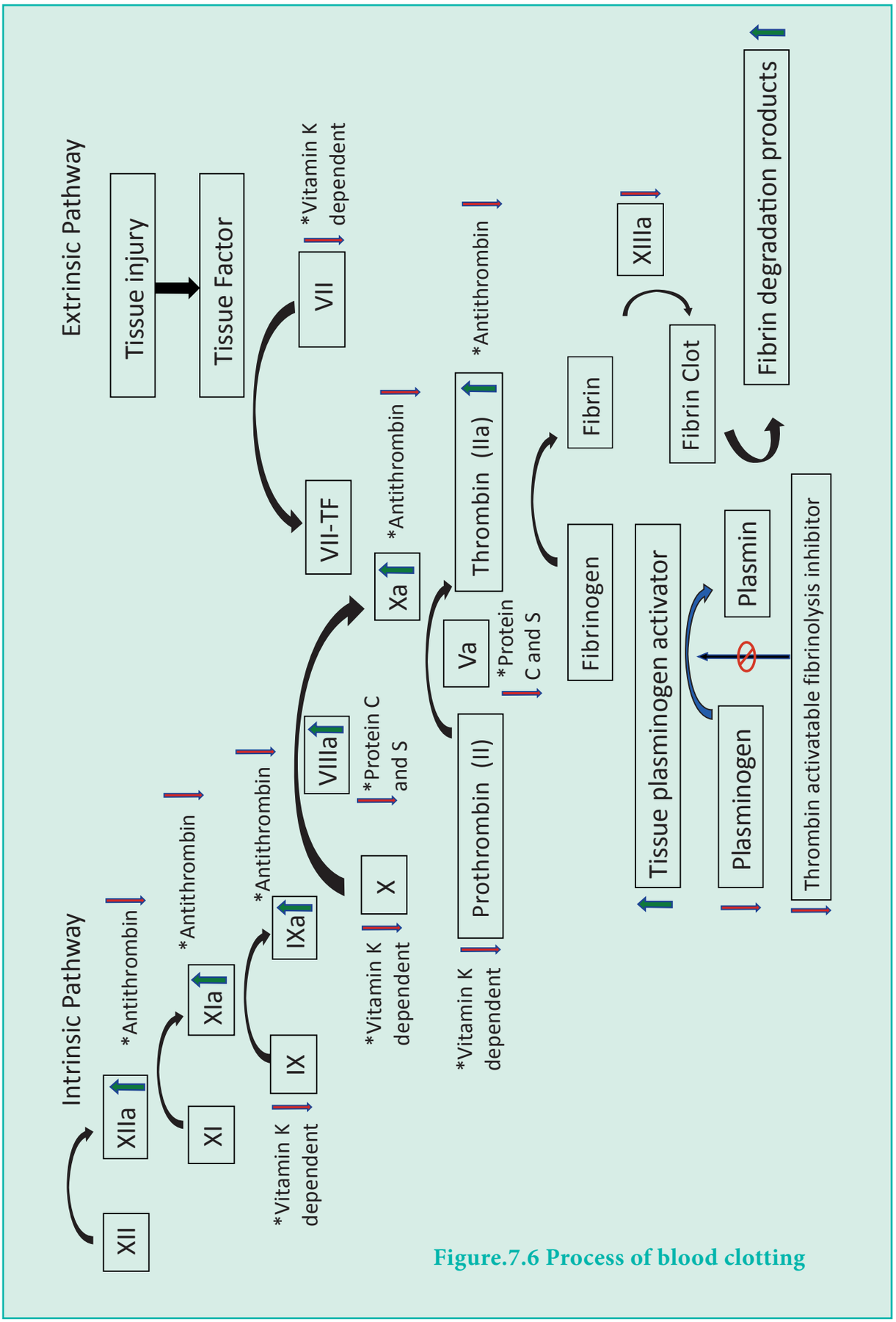



**Figure.7.6 Process of blood clotting**



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In certain cases, the immune system gives a negative reaction, in response to the clotting factors when used as a treatment for bleeding. The immune system develops proteins (inhibitors) that inactivate the clotting factors, making treatment less effective.

## 7.4 ALBINISM

Albinism is a rare group of congenital disorders that cause the skin, hair, or eyes to have little or no colour. Melanin is the pigment responsible for the colour of the skin, hair, and eyes. The term albinism is from the Latin *albus*, means "white". Albinism is associated with a number of vision defects, such as photophobia (abnormal intolerance to visual perception of light), nystagmus(involuntary eye movement, acquired in infancy or later in life, that may result in reduced or limited vision) and amblyopia (disorder of eye sight due to the eye and brain not working well together). Lack of skin pigmentation makes the individual for more susceptibile to sunburn and skin cancers.

There are different types of albinism. Defects in different gene characterize its types.

**OCA1 :** Defect in tyrosinase and possesses two subtypes , OCA1a and OCA1b.

**OCA1a:** Subjects with OCA1a have a total absence of the pigment melanin. Therefore the affected individuals have white hair, very pale skin, and light coloured eyes.

**OCA1b:** Subjects with OCA1b make some melanin. They have light-colored skin, hair, and eyes.

**OCA2:** This type is common in African descendants and Native American populations. It is less severe than OCA1 and there is reduced melanin production. Individuals with OCA2 are born with light colored skin. Their hair may be yellow, blonde, or light brown.

**OCA3:** A defect in the TYRP1 gene. This type affects individual with dark skin especially, South African populations. It usually affects people with dark skin, particularly black South Africans. These individuals have reddish-brown skin, reddish hair, and brown eyes.

**OCA4:** It is due to a defect in the SLC45A2 protein. Like other types, it results in impeded melanin production and common in East Asian descendants.

### 7.4.1 Causes

A defect in one of several genes described above that produces melanin causes albinism. Melanin are natural pigments found in humans and animals. In the human eye, melanin is present in the uveal tract and the pigmented epithelial layer of the retina. It protects eyes by absorbing visible light that penetrate the lens through binding free radicals. The defective gene passes down from both parents to the child and leads to albininism.

The synthesis of melanin from tyrosine is as shown in the figure 7.7. The figure depicts that melanin is synthesized from tyrosine through DOPA. DOPA is the product of the enzyme, tyrosinase (diphenol oxidase). It is a copper containing enzyme and uses



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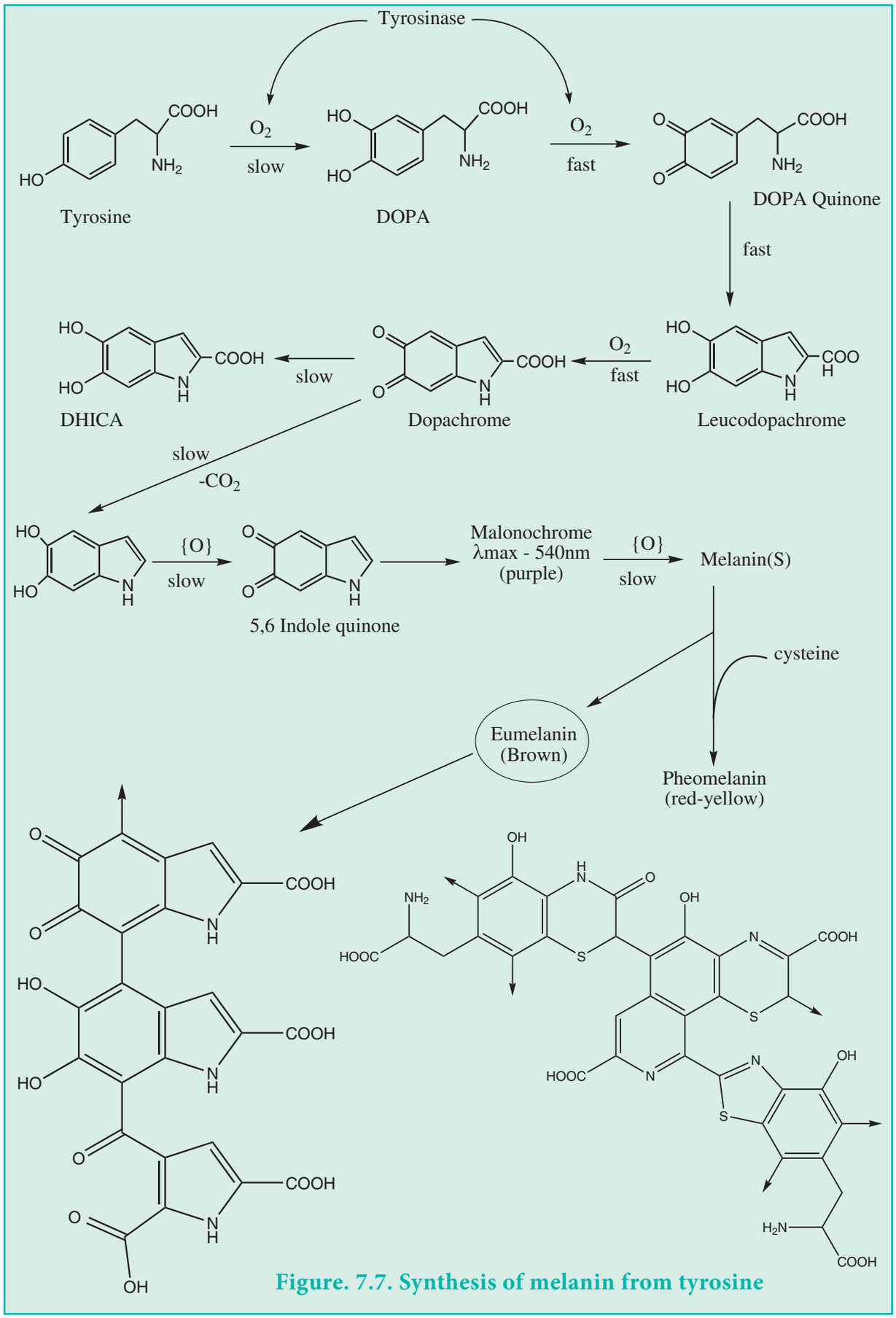



**Figure. 7.7. Synthesis of melanin from tyrosine**



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oxygen directly to form DOPA. The synthesis of melanin occurs in the melanocyte, and the reactions starting with tyrosine are shown in **Figure.7.7**.

DOPA is converted to DOPA quinone. A number of intermediates are formed giving melanin. The more common product is eumelanin, brown in appearance. However, in the presence of cysteine, pheomelanin can be formed (red to yellow). Melanin is formed primarily in the melanocyte, located in the inner layers of the skin where melanin and carotene blend to produce the skin colour as well as the colour in the eyes and hair (Figure.7.8).

### 7.4.2 Symptoms

In Oculo-cutaneous albinism, there is decreased pigmentation of skin and eyes, whereas in Ocular albinism, there is decreased pigmentation of only eyes and not the skin. The common symptoms include the following

- Absence of colour in the skin or iris of the eye
- Patchy and missing skin colour
- Crossed eyes (Strabismus)
- Light sensitivity (photophobia)
- Rapid eye movements (Nystagmus)



**Figure 7.8. A woman affected with albinism**

## 7.5 ALKAPTONURIA

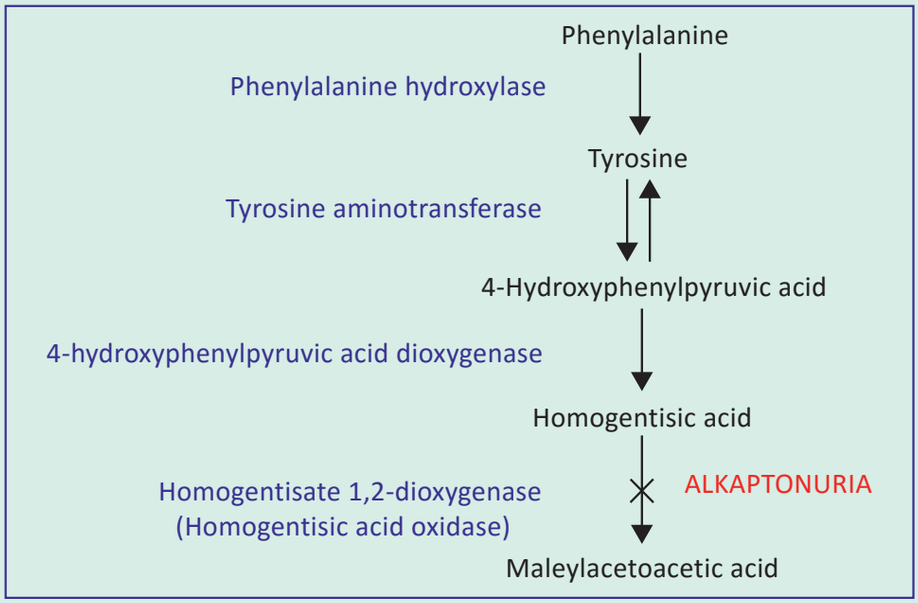



**Figure 7.9 Metabolism of Phenyl alanine and Tyrosine**



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In 1584, Scribonius was the first to describe dark coloured urine, looking at the caseof a child, which excreted urine which turned "black as ink" on exposure to air. In 1859, Boedekerused, for the first time, the term alkaptonuria. It is a rare congenital metabolic abnormality where urine contains large quantities of homogentisic acid. Homogentisic acid (2,5, dihydroxyphenylacetic acid), is the key intermediate in the metabolism of phenyalanine and tyrosine.. A block in the conversion of homogentisic acid to maleyl aceto acetic acid is due to deficiency of the enzyme homogentisic oxidase. Consequently, homogentisic acid (called Alkapton) is excreted in the urine and the defect is called Alkaptonuria. The urine becomes dark brown or black on standing through oxidation by air.

### 7.5.1 Causes

Alkaptonuria is characterized by the deficiency of homogentisate 1, 2 dioxidasewhich catalyses the conversion of homogentisic acid to Maleylacetoacetic acid. Ochronosis,a dark pigment in connective tissue,is characteristic of this disorder. Ochronotic pigment may be sedimented in any of the outer structures of the eye: sclera, cornea, conjunctiva. The pigment can be seen in the nasal and temporal aspects of the sclera (Fig.7.8).



**Figure. 7.10 Symptoms of alkaptonuria**

Alkaptonuria does not show any ill effects in early life but it leads to degenerative arthritis in old age. This is because of the crystallization of the homogentisic acid derivatives in cartilages of ears and other exposed places. This results in generalized pigmentation of connective tissues and deposition in joints leading to arthritis.

### 7.5.2 Symptoms

It has a very low prevalence (1:100,000-250,000) in most ethnic groups. Two countries, Slovakia and Dominican republic, exhibit an increased incidence of this disorder. The first clinical sign is the appearance of dark spots in nappies of the affected children, due to darkening of urine owing to oxidation of the HGA. Till now no treatment modalities are known to treat this disease. The best way is to reduce the intake of tyrosine and



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phenylalanine and intake of ascorbic acid. It must be noted that dietary restriction may be promising for children but not for adults.

## 7.6 TAY-SACHS DISEASE

Gangliosides are glycosphingolipids which are main components of cell membranes. Nervous tissues are particularly rich in gangliosides. They constitute a significant fraction (6%) of brain lipids. Other tissues also contain gangliosides but in lesser amounts. Their complex carbohydrate head groups, which extend beyond the surfaces of cell membrane, act as specific receptors for certain glycoprotein hormones. They act as receptors for some bacterial toxins. Gangliosides are also involved in cell – cell recognition. Generally, the carbohydrate segments of glycolipids are removed bylysosomal hydrolases in the early phases of the turnover of these compounds. Disorders of ganglioside break down are responsible for several hereditary sphingolipid storage diseases including Tay – Sachs disease.

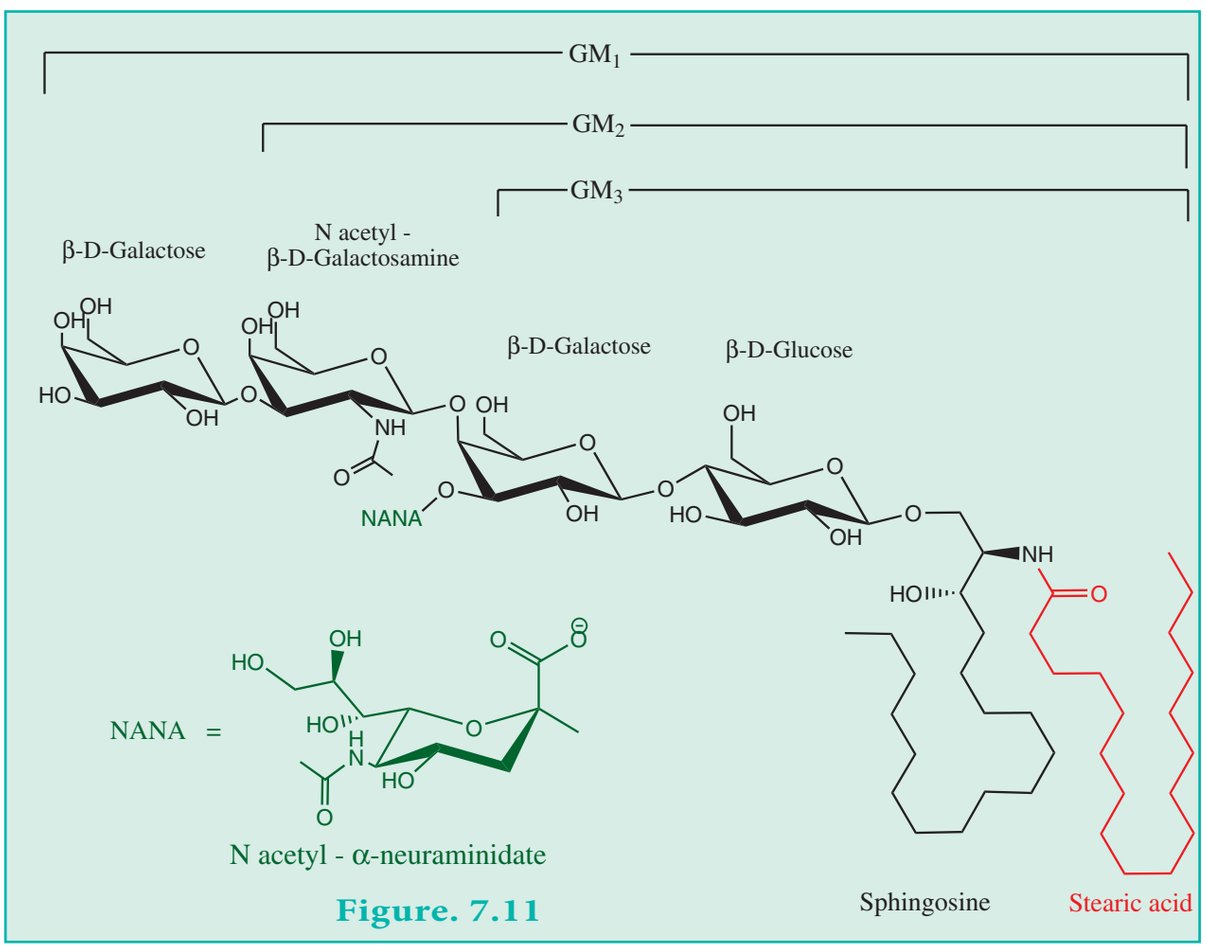



**Figure. 7.11**

$$\textbf{GM1} \xrightarrow{\beta\text{-galactosidase}} \textbf{GM2} \xrightarrow{\text{hexaminadase-A}} \textbf{GM3}$$

Tay-Sachs Disease is an autosomal recessive neurodegenerative disorder. It is common in Jewish population which is fatal within the first three to four years of life. A British physician, Warren Tay, and an American physician, Bernard Sachs independently worked on this disease.



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Tay noticed the characteristic red spot in the eye that is the earlier symptom of this disease due to degeneration in the central nervous system. Sach characterized this syndrome and identifying its familial characteristics, like mental disturbances, a deficiency in normal reflexes, progressive blindness and mortality, in Ashkenazi Jews (A Jewish ethnic group).

### 7.6.1 Causes

Tay-Sachs disease is classified as a lysosomal storage disease (Figure.7.9). In a healthy child, a lipid known as GM2 ganglioside, enter the nerve cell as a source of food. Among the components of the cell are lysosomal bags, which contain hexoaminidase A to digest this GM2 gangliosides. However, in Tay-Sachs disease, due to a failure in this degradation, levels of GM2-ganglioside increases in the lysosomes of neuronal tissue, thereby worsening the condition.

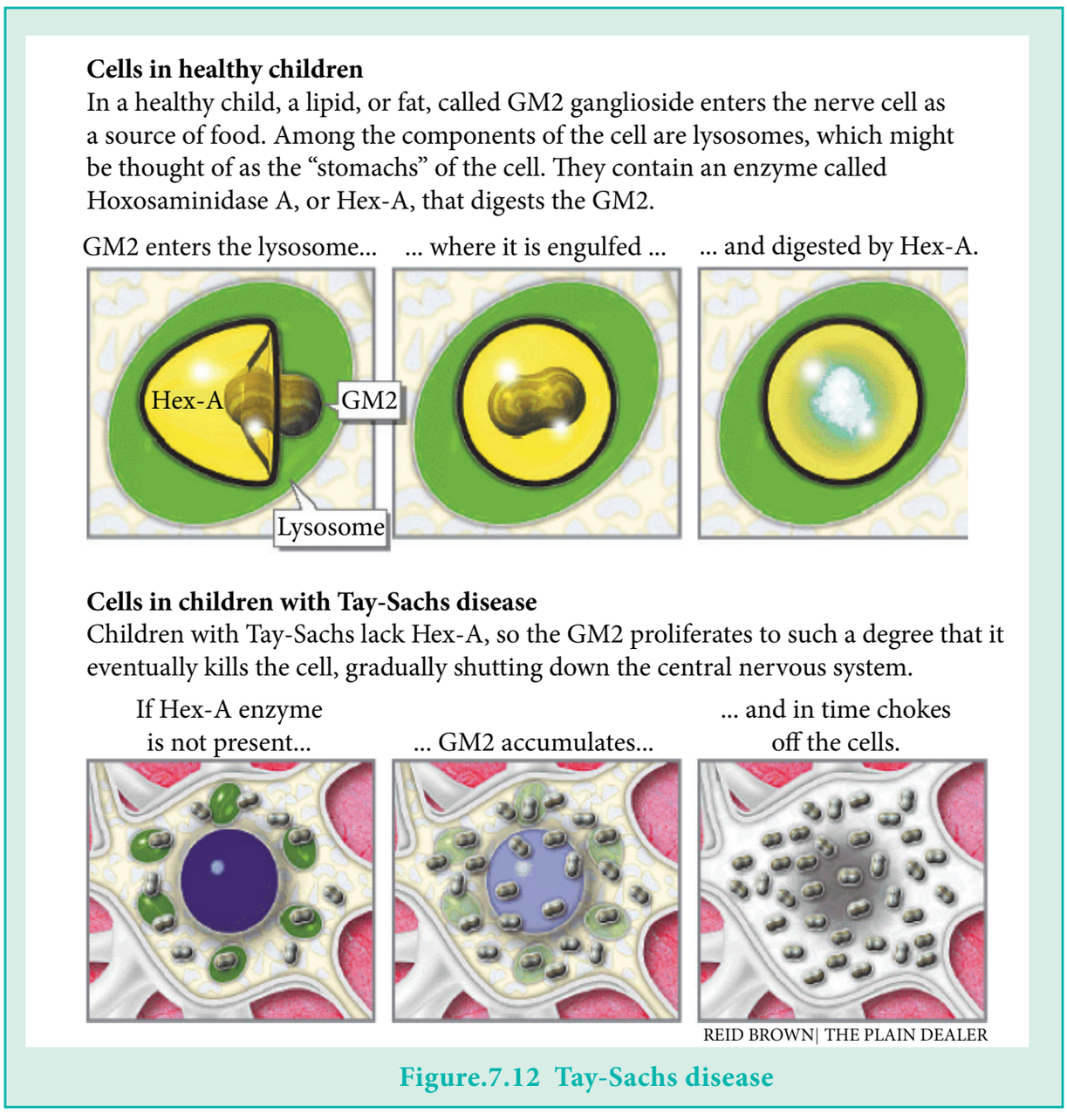



**Figure.7.12 Tay-Sachs disease**



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### 7.6.2 Symptoms

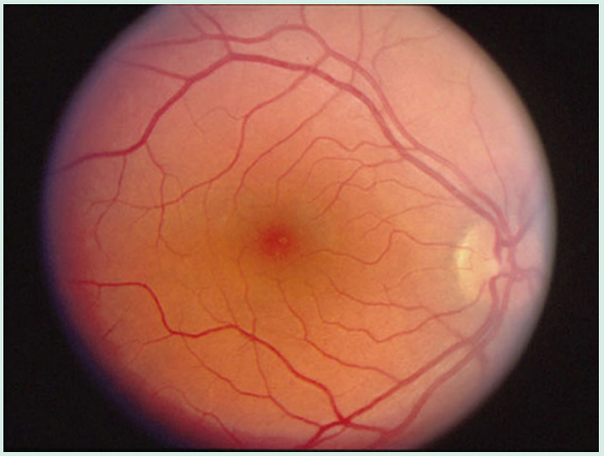


Figure 7.13 Cherry red spot in the retina

Muscle weakness, progressive retardation in development and difficulty in eating are typical early symptoms. Mental retardation and blindness are the characteristic symptoms in this rare genetic disorder. Death between 3 -4 years is unavoidable. More than 90 % of the patients have a characteristic cherry red spot in the retina. Tay-Sachs disease can be diagnosed by taking amniotic fluid from the motherand assaying the hexosaminidase A activity. Bad memory, defective learning skills, poor decision making, eventually the child goes blind, deaf, paralyzed, and dies within 3 years.

Till now, there is no cure for Tay-Sachs disease. Treatments include gene therapy or enzyme replacement therapy. They are not promising cure for the disease but could slow down disease progression.

## SUMMARY

Metabolic processes, including transport of molecules across the membrane, are coordinated by a series of biochemical reactions and each reaction is controlled by an enzyme. One or both of a pair of genes involved in the production of a particular enzyme may be defective. The production of an enzyme, be it normal or low, depends on whether the gene is dominant or recessive, which is inherited from the parents. Inborn errors of metabolism, are fortunately, linked to recessive traits. Though, the consequences of this disease are extremely rare, they are collectively common and presented in new born infants or shortly thereafter.

The basis of IEM is the mutation in a gene that codes for a particular enzyme in a specific pathway, involving either the break-down or storage of carbohydrates, fats and proteins.

1. The term 'inborn errors of metabolism' was first proposed by Garrod followed by Beadle and Tatum
2. Common defects in biochemical pathways include, defects in the transport, excessive accumulation of substrate, deficiency of product and secondary inhibition.
3. Enzymes play a major role in performing the anabolism and catabolism of reactive pathways and serve as catalysts in the conversion of one compound to the other.
4. The major classification of inborn errors of metabolism includes protein, carbohydrate,



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lipid disorders.

5. Galactosemia is an inherited disorder characterized by inability of the body to metabilize galactose. The major deficient enzyme is galactose 1 phosphate uridyl transferase (GALT).

6. Von Gierke disease is a type I glycogen storage disease characterized by deficiency of glucose 6 phosphatase, that hydrolyzes glucose 6 phosphate. This impairs the ability of the liver to produce glucose from glycogen.

7. A deficiency in clotting factors causes hemophilia, with several types described.

8. Alkaptonuria or black urine disease is a rare genetic inherited disorder, characterized by deficiency in homogetisate 1,2 dioxygenase, which takes part in the degradation of tyrosine.

9. Albinism is characterized by pigmentation of skin due to impairment in the melanin synthesis.

10. Tay-Sachs disease, a lysosomal disorder, is characterized by the inability of hexosaminidase A to degrade the GM2 gangliosides in brain. This causes neurotoxicity and death of child.

## EVALUATION

### I. Choose the correct answer

1. Which of the following enzyme deficiency causes galactosemia
   a. gluco kinase
   b. galacto kinase
   c. galactose 1 phosphate uridyl transferase
   d. phosphoglucomutase
2. Liver failure and mental retardation are symptoms of
   a. von-gierke diseases
   b. galactosemia
   c. albinism
   d. all of these
3. glucose 6-phosphatase deficiency causes
   a. galactosemia
   b. albinism



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c. von-gierke diseases

d. hemophilia

4. The absence of glucose 6-phosphatase in liver causes

a. hypoglycemia

b. hyperglycemia

c. depletion of glycogen in liver

d. none of the above

5. Which of the following is not the symptoms of Von-Gierke diseases?

a. ketosis

b. Hyperlipidemia

c. Hypoglycemia

d. Hyperglycemia

6. How many factors are involved in blood clotting process?

a. 10

b. 8

c. 12

d. 13

7. Hemophilia A is caused by the following factor

a. Factor VIII

b. Factor VII

c. Factor VI

d. Factor V

8. Blood clotting factor IV is

a. Fibrinogen

b. Calcium ions

c. Christmas factor

d. Hageman factor

9. Albinism is due to deficiency of

a. Tyrosinase

b. Hexokinase

c. DOPA hydroxylase



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d. All of these

10. The decreased pigmentation in eyes are called as

a. Ocular albinism

b. Oculo-cutaneous albinism

c. Alkaponuria

d. Hemophilia

11. _____ amino acids are involved in alkaptonuria

a. Glycine & alanine

b. Cysteine & methionine

c. Tryptophan & lysine

d. Phenyl alanine & tyrosine

12. Deficiency of hexaminIdase-A leads to accumulation of

a. GM3 b. GM2

c. GM1 d. GM1 and GM3

13. The first condition identified as an inborn error of metabolism was

a. Albinism b. Alkaptonuria

c. Phenylketonuria d. Hemophilia

14. Most IEMs have autosomal recessive modes of inheritance. It means _____?

a. Two copies of the defective gene must be passed on to the child for the disease to develop

b. The defective gene is passed down to the child from the mother.

c. The defective gene is passed down to the child from the father.

d. The defective gene is not present on the mother or father's chromosomes.

15. One gene one enzyme theory was proposed by

a. Beadle and Tatum

b. Garrod

c. Ingram

d. Von gierke

16. Deficiency in homogentisic acid oxidase causes

a. Phenylketonuria

b. Alkaptonuria



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c. Tyrosinemia

d. Galactosemia

17. A child's diapers on exposure to air became dark coloured. What is your probable diagnosis?

a. Tay-Sachs disease

b. Glycogen storage disease

c. Lysosomal storage disease

d. Alkaptonuria

18. ---------- clot acts as a mesh and stops bleeding

a. fibrogen

b. prothrombin

c. christmas factor

d. fibrin

## II. Answer the following:

1. Classify inborn errors of metabolism.
2. Give the metabolism of galactose.
3. Comment on the symptoms of Von Gierke disease.
4. Describe lysosomal storage disease.
5. Write a note on hemophilia.
6. Give an account on the deficient enzyme involved in alkaptonuria.
7. What are symptoms of galactosemia?
8. Describe the types of albinism.
9. Give an account on alkaptonuria.
10. How GM1 is converted into GM2?
11. Write the conversion reaction of glucose-6-phosphate to glucose.
12. Describe the causes and symptoms of Tay-Sachs disease.

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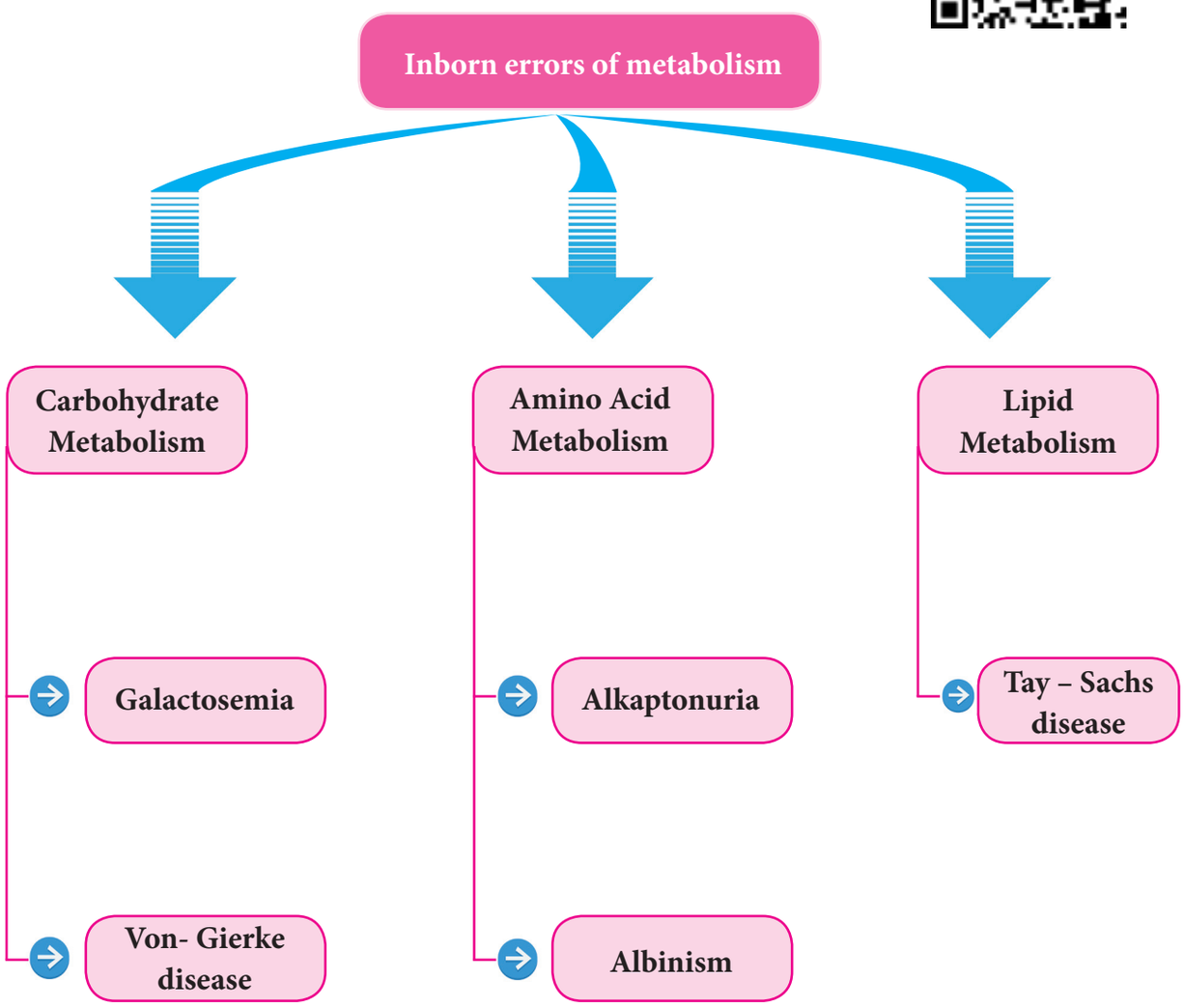
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## CONCEPT MAP

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UNIT 8

# BIOLOGICAL OXIDATION



**Albert Lester Lehninger and Eugene Patrick Kennedy**

Albert Lester Lehninger was an American Biochemist in the field of bioenergetics. He made fundamental contributions to the current understanding of metabolism at a molecular level. In 1948, he discovered with Eugene Patrick Kennedy (American Biochemist known for his work on lipid metabolism and membrane function) that mitochondria are the site of oxidative phosphorylation in eukaryotes, which introduced in the modern study of energy transduction.

SMYKA

## Learning Objectives

After studying this unit the students will be able to

- Explain the cellular respiration and how cells make ATP
- The basis of redox reaction and redox potential
- Structural insights of mitochondria
- Components of Electron transport chain
- Inhibitors of the electron transport chain
- The basis of oxidative phosphorylation
- ATP synthesis and mechanics of ATP synthase
- Uncouplers of oxidative phosphorylation



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## INTRODUCTION

Among the pioneering works involving biological oxidation, Antoine Lavoisier, who is known as the father of modern chemistry, in 1789 demonstrated that aerobic systems consume oxygen and generate carbon dioxide to obtain enormous amount of energy as compared to anaerobic systems.

Aerobic organisms achieve it through oxidation of glucose and fatty acids. Oxygen is a highly reactive molecule and therefore its use is tightly regulated. Inappropriate use of oxygen accumulates toxic products and therefore aerobic organisms possess multiple mechanisms to protect themselves against the deleterious effects of oxygen. Antioxidant enzymes such as superoxide dismutase and catalase protect cells against oxidative damage. However, despite this precision, oxygen metabolites sometimes cause damage to living cells and collapse the antioxidant mechanisms. Life threatening disorders such as cancer, neuro-degeneration, diabetes are known to be caused in part due to oxygen metabolites.

The complexity of aerobic cells is due to the presence of thousands of small molecules organized as molecular machines. These molecular machines are proteins that use chemical bond energy of nucleotides to attain energy. The structure of the machine is designed to bind Adenosine tri phosphate (ATP) in a specific orientation. Aerobic eukaryotic cells possess variety of molecular machines. Higher animals are absolutely dependent upon a steady supply of oxygen for respiration, the process through which cells derive energy in the form of ATP.

Otto Warburg in his studies demonstrated that biological oxidations are catalyzed by enzymes designated as oxygenases. Many drugs and toxic metabolites are metabolized by a specialized enzyme class known as mixed function oxidases (cytochrome P450 system).

Harvesting of energy from glucose has three stages (1) Glycolysis (breaks down glucose into two molecules of pyruvate), (2) The citric acid cycle (completes the breakdown of glucose) and (3) Oxidative phosphorylation (accounts for most of the ATP synthesis).

## 8.1 REDOX REACTION

Biological electron transport is a series of coupled oxidation and reduction reactions. The oxidation of a molecule involves the loss of electrons and reduction involves gain of electrons.

$$\text{Reduced compound } (e^- \text{ donor}) \rightleftharpoons \text{Oxidized compound} + e^-$$

$$\text{Eg.} \qquad Fe^{3+} \rightleftharpoons Fe^{2+} + e^-$$

A complete redox reaction must have one reactant, an electron acceptor, which gets reduced by gaining electrons. The electron donor is called as reductant which becomes oxidized while transfering electrons to the other substrate which is called as the oxidant.



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$$\text{Reductant + Oxidant} \rightleftharpoons \text{Oxidized reductant + reduced oxidant}$$

Example : oxidation of copper by iron

$$Cu^{+} + Fe^{3+} \rightleftharpoons Cu^{2+} + Fe^{2+}$$

Electrons cannot be created or destroyed in a chemical reaction and therefore if one molecule is oxidized the other one must be reduced, known as a redox reaction. The complete oxidation of glucose by oxygen is described in the following reaction.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad \Delta G^{\circ\prime} = -2823\ kJ.mol^{-1}$$

The above reaction can be divided into two half reactions. In the first reaction, the carbon atoms of glucose are oxidized as follows:

$$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^{+} + 24e^{-}$$

In the second half reaction, molecular oxygen is reduced

$$6O_2 + 24H^{+} + 24e^{-} \longrightarrow 12H_2O$$

In living organisms, these half reactions occur through coordinated multistep pathways that harness the liberated free energy in the form of ATP. In the above reaction, the electrons involved are not transferred directly to oxygen, whereas the transfer process occurs through coenzymes $NAD^{+}$ and FAD to form 10NADH and $2FADH_2$ through several glycolytic enzymes such as glyceraldehyde 3 phosphate dehydrogenase, pyruvate dehydrogenase and citric acid cycle enzymes – isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. The stepwise passage of electrons from NADH and $FADH_2$ to $O_2$ through a series of electron carriers is coupled to the translocation of protons from the mitochondrial matrix to the intermembrane space. The free energy stored in the resulting pH gradient drives the synthesis of ATP from ADP and Pi through oxidative phosphorylation.

### 8.1.1 Redox potential

The oxidation reduction potential, E, in general, redox potential, is a measure of the affinity of a substance for electrons and is measured relative to hydrogen. In principle, a positive redox potential implies that the substance has higher affinity for electrons than does hydrogen and is anticipated to accept electrons from hydrogen. In negative redox potential, the substance has lower affinity for electrons than hydrogen and therefore would donate electrons to $H^{+}$, forming hydrogen. Any atom or molecule, which is more electronegative than hydrogen is assigned a positive (+) redox potential; those less electronegative a negative (–) redox potential. Greater the difference between the redox potentials of two substances (ΔE), greater the electrons flow spontaneously from the less positive to the more positive side. ΔE is expressed in volts.



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The free energy change at the standard state of pH 7 is represented as $\Delta G^{\circ\prime}$. The standard free energy change can be calculated from the change in redox potential $\Delta E^{o\prime}$ of the substrate and products as represented below, where n denotes the number of electrons transferred per mole of reactants, $\Delta E^{o\prime}$ in Volts (V) and $\Delta G^{\circ\prime}$ in kilocalories per mole (kcal $mol^{-1}$), F is Faraday's constant (96,494°C $mol^{-1}$). The standard free energy for the reaction is: $\Delta G^{\circ\prime} = -nF\Delta E^{o\prime}$

## 8.2 ELECTRON TRANSPORT CHAIN

To understand biological oxidation and the electron transport chain, an understanding of oxidation - reduction reactions and the biology of mitochondria are essential. In eukaryotic cells, cellular metabolism takes place in major compartments of the cell. For instance, glycolysis takes place in the cytosol, whereas oxidation of pyruvate, β oxidation of fatty acids and the citric acid cycle take place in the mitochondrial matrix.

The mitochondrial electron transport chain (ETC) is a complicated system, where a series of electron carriers are arranged in the inner membrane of the mitochondria in the order of increasing electron affinity. They transfer the electrons derived from reduced coenzymes to oxygen.

### 8.2.1 Structure of mitochondria

The size of a mitochondrion is close to the size of a bacterium (ranging between 1 to 2μm). Mitochondria are believed to be developed during an early phase of evolution from aerobic bacteria that entered into symbiosis with primeval anaerobic eukaryotes. This is known as endosymbiosis.

The mitochondria has two membranes, a smooth outer membrane and an inner membrane, which forms numerous folds called as cristae that project into the interior of the mitochondria and enclose the matrix (Fig.8.1). Since the respiratory proteins are embedded in the inner membrane, the density of the cristae is related to the respiratory activity of the cell. For example, the heart muscle with rigorous oxidative metabolism has greater numbers of mitochondria as compared to the liver. Even within one type of tissue, the shape of the mitochondrion varies depending on the functional status of the cell.

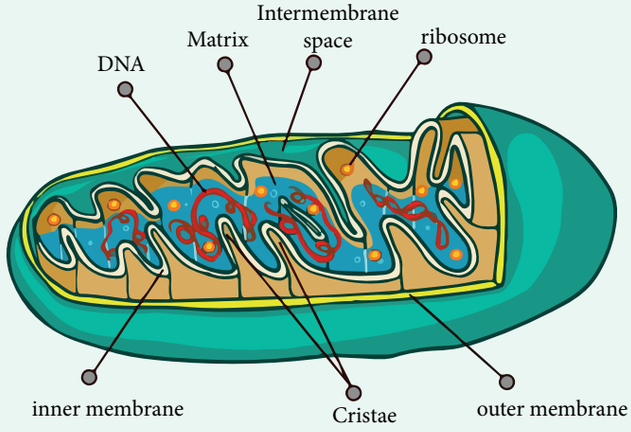



**Figure 8.1. Structure of Mitochondria**

The mitochondrial membrane is rich in proteins. Porins present in the outermembrane of the mitochondria allow small molecules to be exchanged between the cytoplasm and the inter membrane space. However the inner



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mitochondrial membrane is impermeable to small molecules, with the exception of oxygen, carbon-dioxide and water. All of the other substrates of mitochondrial metabolism, as well as its products, therefore have to be moved through the innermembrane with the help of special transporters. Embedded within the innermembrane of the mitochondria are various protein carriers, principally cytochromes.

The respiratory chain is organized in the form of multi-protein complexes. The inner space also contains ATP synthase and other enzymes. The inner membrane plays a major role in oxidative phosphorylation. The matrix of the mitochondrion is like a gel with ~50% protein. The matrix contains enzymes involved in the oxidation of pyruvate, amino acids, fatty acids, TCA cycle, as well as $NAD^+$, FAD, ADP and Pi.

The energy liberated during the oxidation of carbohydrate and food is available in mitochondria as reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP (Figure 8.2).

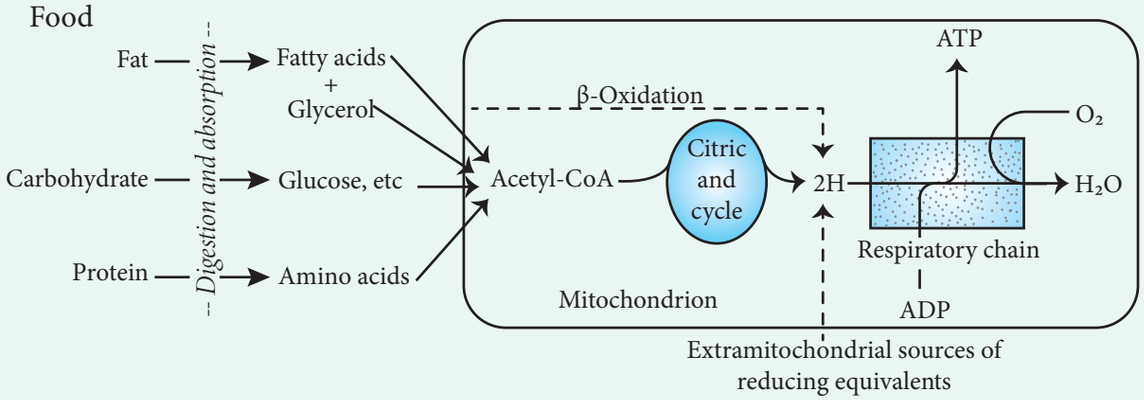



**Figure 8.2. Digestion and absorption of food and production of energy**

### 8.2.2 Components of the electron transport chain

The components of the ETC in eukaryotes are located in the inner mitochondrial membrane and organized into four large protein complexes (Figure 8.3). These complexes consist of several proteins and prosthetic groups. Apart from these, two other molecules, coenzyme Q and cytochrome c also play a significant role in the ETC. Complex I, also known as NADH-coenzyme Q oxidoreductase, or NADH dehydrogenase, catalyses the transfer of electrons from NADH to Coenzyme Q (CoQ or Ubiquinone UQ), because it is ubiquitous in biological systems). It contains one molecule of flavin mononucleotide (FMN) and 8 iron-sulfur clusters that participate in electron transfer. Complex II [Succinate Dehydrogenase or Succinate–Coenzyme Q Oxidoreductase] contains succinate dehydrogenase and three small hydrophobic subunits, and pass electrons from succinate to CoQ. Complex III is Coenzyme $QH_2$-cytochrome c oxidoreductase. It passes electrons from reduced CoQ to cytochrome c. It contains cytochromes b, c1 and one [2Fe-2S] cluster. Complex IV is cytochrome c oxidase. It catalyses the one-electron oxidation of four consecutive reduced cytochrome c molecules and four-electron reduction of



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one oxygen molecule to yield 2 molecules of water. The flow of electrons through the complexes spanning I to IV results in pumping of protons from the matrix across the inner mitochondrial membrane into the intermembrane space. The proton-motive force that is generated powers ATP synthesis. Complex V catalyzes ATP synthesis.

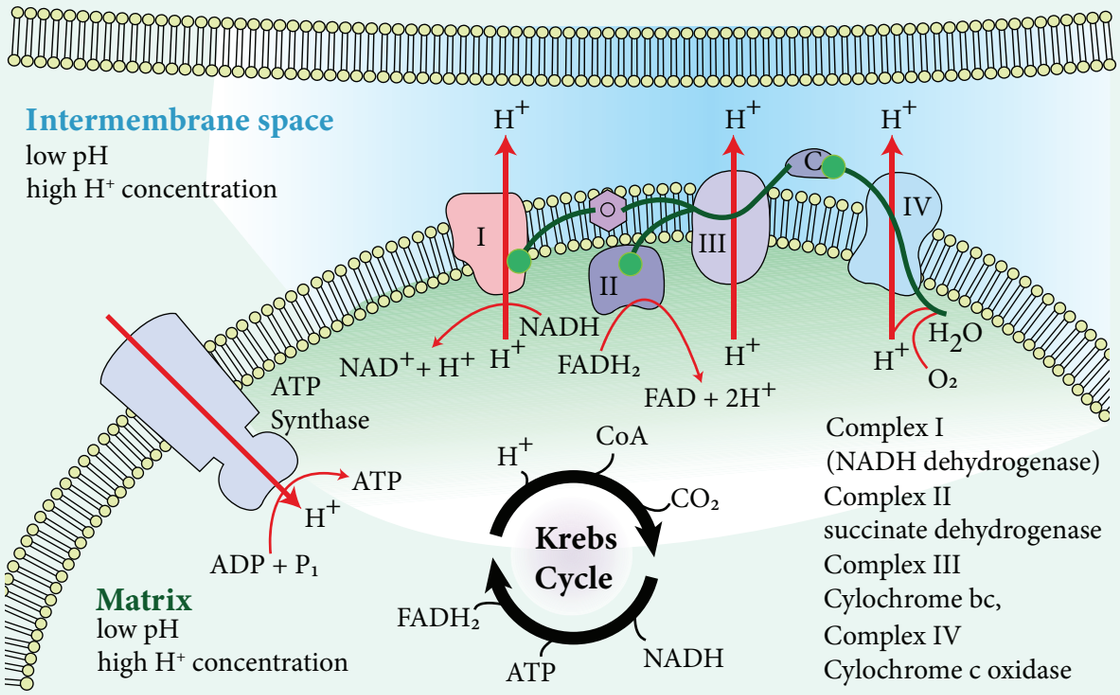



**Figure 8.3. Overview of electron transport chain**

A pair of electrons flowing from Complex I to Complex IV yields 3ATP, while a pair of electrons moving from Complex II to Complex IV yields 2ATP.

### 8.2.3 Reactions of Electron transport chain

Except coenzyme Q, all members of the respiratory chain are proteins. These proteins function as enzymes, for example, dehydrogenases. They may contain iron as part of an iron-sulfur center. Also, they may contain iron co-ordinated to a porphyrin ring as in the cytochromes, or they may contain copper, as in cytochrome a + a3 complex.

**Formation of NADH**: $NAD^+$ is reduced to NADH by dehydrogenases. which remove two hydrogen atoms from the substrate. The major sources of NADH include several reactions of the citric acid cycle, fatty acid oxidation etc. Both electrons and one proton are transferred to the $NAD^+$ forming NADH and a free proton $H^+$.

**NADH dehydrogenase:** The NADH and $H^+$ formed are transferred to NADH dehydrogenase. This complex I, is the large protein complex comprises of 46 polypeptides. is burried in the mitochondrial inner membrane and has a bound molecule of flavin mononucleotide (FMN). NADH dehydrogenase reduces FMN to $FMNH_2$. The sequential transfer of two electrons to the iron sulfur center composed of iron atoms complexed with an equal number of sulfide ions releases four protons into the intermembrane space (Fig.8.4).



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NADH dehydrogenase complex functions to accept high energy electrons from NADH. It acts as a proton pump that uses the movement of electrons to move hydrogen ions into the innermembrane space. These clusters are essential for the transfer of hydrogen to the next segment of the chain, Coenzyme Q.

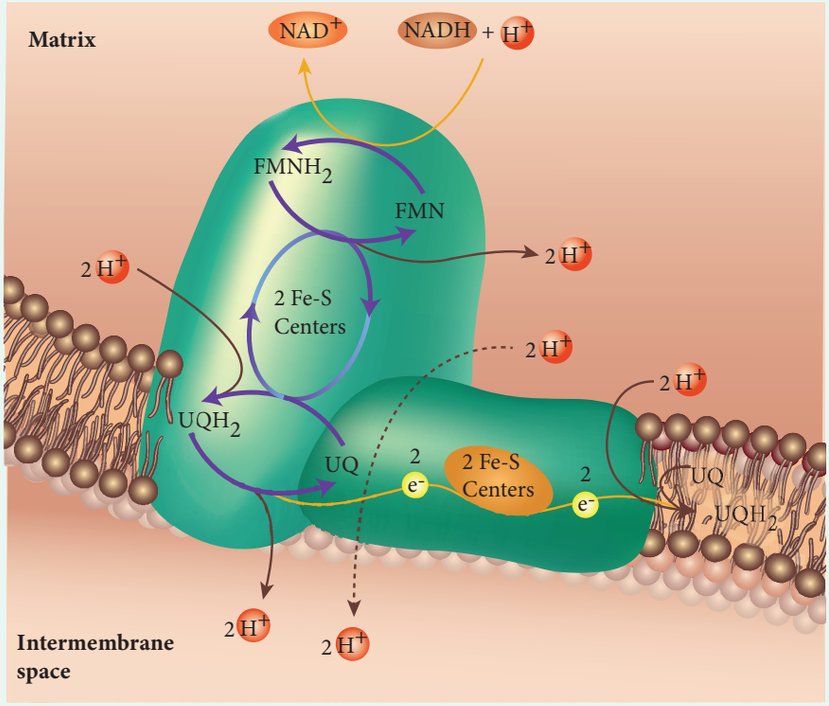



**Figure 8.4 NADH dehydrogenase complex**

Succinate dehydrogenase complex : This complex II contains the succinate dehydrogenase enzyme used in TCA cycle to transform succinate to fumarate and in the process form $FADH_2$. The $FADH_2$ stays in the complex, and gives two electrons, to a series of Fe-S cluster, that is transferred to ubiquionone (Fig.8.5). This complex is not a proton pump, it does not move the hydrogen ions across the membrane from matrix side to the intermembrane side of mitochondria.

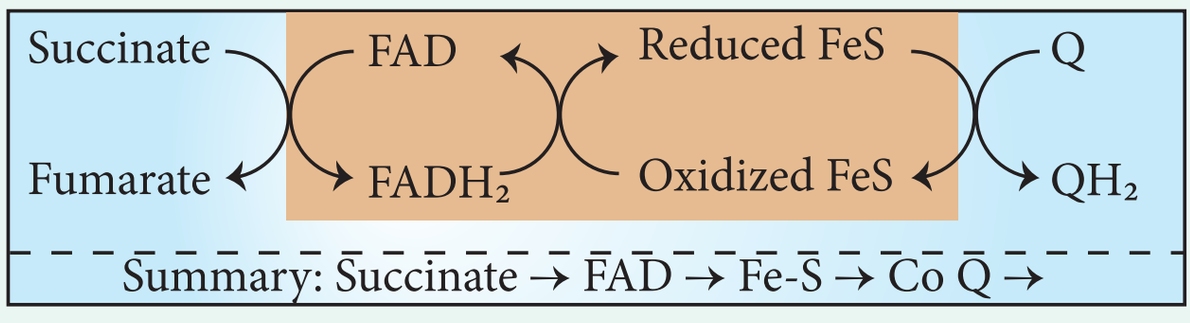



**Figure 8.5 Reactions involved in complex II**

Coenzyme Q : The succinate dehydrogenase complex of Complex II consists of the TCA cycle enzyme succinate dehydrogenase and two iron sulphur centers. The transfer of electrons from succinate to Coenzyme Q (UQ) is mediated via the iron sulphur centers. Co Q can accept hydrogen atoms from $FMNH_2$ of NADH dehydrogenase (Complex I) and $FADH_2$ from succinate dehydrogenase (Complex II). Further, electrons from cytoplasmic NADH are also transferred to ubiquinone (UQ) through glycerol 3 phosphate dehydrogenase and acyl coA dehydrogenase (Fig.8.6).



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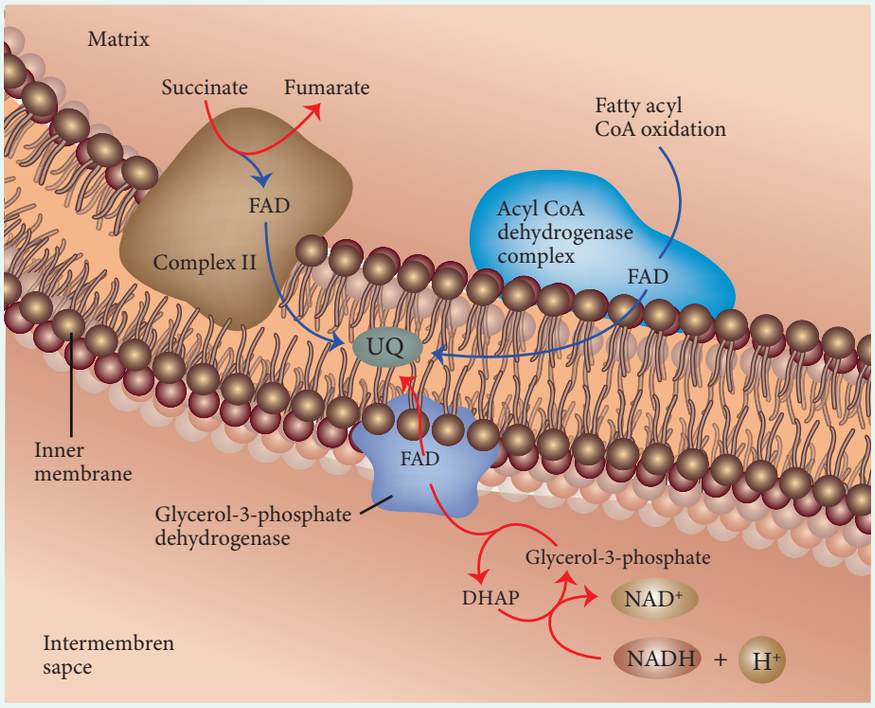



**Figure 8.6. Succinate dehydrogenase complex**

Cytochrome reductase : Electrons from reduced ubiquinone ($UQH_2$) are passed to cytochrome c via Complex III. Cytochromes are proteins with heme prosthetic group. All cytochromes have the tendency to act as electron carriers. Once the electron is accepted, the iron atom of heme group changes from $Fe^{3+}$ to $Fe^{2+}$ state. The pathways of electrons transfer from $UQH_2$ to cytochrome c is quite complex. As represented in Fig.8.7, $UQH_2$ is oxidized to UQ in a multistep process. One electron is transferred to cytochrome c via Rieske Fe-S protein / cyt c1 and the second electron is transferred to Cyt b.

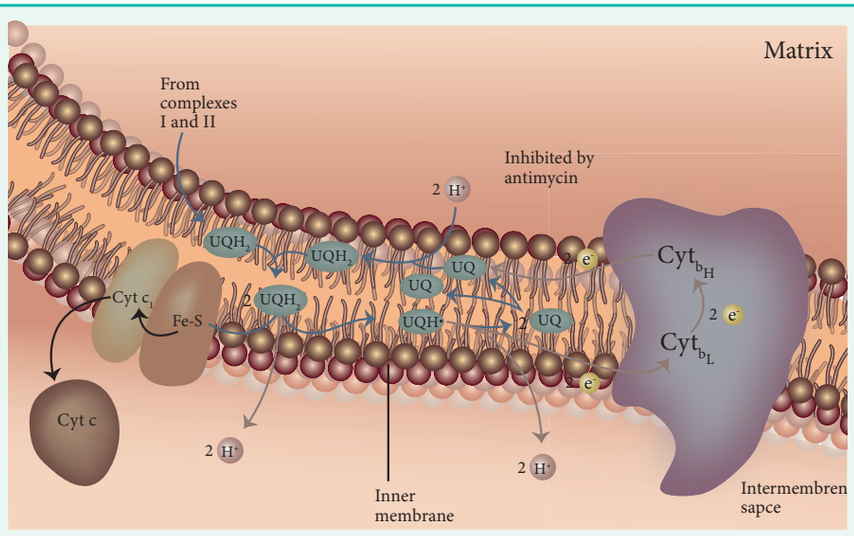



**Figure 8.7 Overview of reactions in complex III**



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One of the two molecules of UQ produced diffuses to the matrix side of mitochondria where it is reduced to form $UQH_2$. The so formed $UQH_2$ diffuses to its oxidation site and joins the pool of $UQH_2$ from complexes I and II. During this process, 2H is released into the intermembrane space.

Cytochrome c oxidase: Cytochrome c oxidase of Complex IV catalyzes the one electron oxidation of four consecutive reduced cytochrome c molecules and the concomitant four electron reduction of one oxygen molecule. Cytochrome c oxidase contains two cytochromes (a and b). Cyt a is paired with copper atom, (CuA) and Cyt a3 is paired with a different copper atom, (CuB). Each of the reduced Cytc molecules donates two electrons, one at a time to CuA. The electrons are further transferred to cyt a, cyt $a_3$, CuB. The transfer of four electrons from cyt c converts oxygen and four protons to two molecule of water (Fig.8.8).

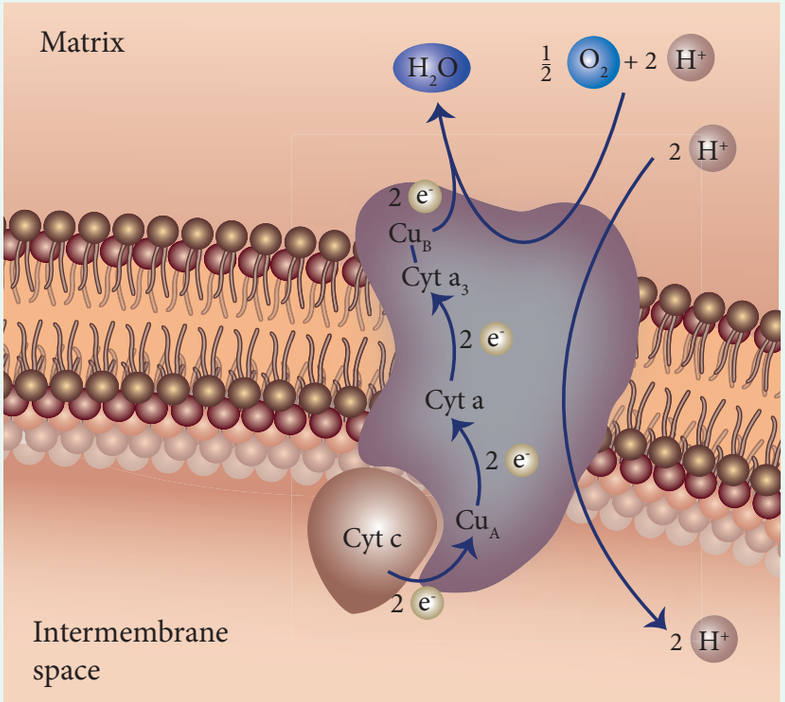



**Figure 8.8 Overview of reactions in Complex IV**

| Complex No. | Complex Name | Polypeptides | Prosthetic groups |
|---|---|---|---|
| Complex I | NADH dehyrogenase | Around 46 | FMN, Fe-S Centres |
| Complex II | Succinate dehydrogense | 2 Fe-S Proteins | FAD, Fe-S Centres |
| Complex III | Cytochrome b $c_1$ complex | 2 Cytochromes<br>1 Fe-S protein<br>6-8 other proteins | b and c type hemes (cyt $b_1$, $b_H$, $c_1$)<br>Fe-S centres |
| Complex IV | Cytochrome oxidase | Around 13 | a type hemes<br>(cyt a,$a_3$)<br>2 Cu |



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### 8.2.4 Inhibitors of the Electron transport chain

ETC may be blocked by many inhibitors. ETC inhibitors act by binding a suitable area in the ETC. They prevent electrons from being passed from one carrier to the next one. Each inhibitor binds a particular carrier For example, rotenone and amytal inhibits complex I at NADH dehydrogenase and prevent NADH oxidation. Antimycin A and dimercaprol inhibit ETC at complex III. Poisons such as hydrogen sulphide, Cyanide, azide and carbon monoxide inhibit Complex IV. Oligomycin is a ATP synthase inhibitor. Many of the details of respiratory chain were obtained using inhibitors of ETC. The correct order of the ETC components was determined using inhibitors. Using oxygen electrode, the extent of electron transport was measured. During this process, when electron transport is inhibited, oxygen consumption is diminished. (Fig.8.9).

## 8.3 OXIDATIVE PHOSPHORYLATION

During aerobic cellular respiration, most of the ATP is generated by oxidative phosphorylation where electrons derived from NADH and $FADH_2$ are oxidised by electron transport through the respiratory chain. In oxidative phosphorylation, carbon fuels are oxidized in TCA cycle to give electrons with high transfer potential. Eventually, this electro-motive force is converted into a proton-motive force and finally into phosphoryl transfer potential.

The conversion of electro-motive force into proton-motive force is carried out by three electron-driven proton pumps: NADH-UQ oxidoreductase, UQ-cytochrome c oxidoreductase, and cytochrome c oxidase. These large transmembrane complexes contain multiple oxidation-reduction centers, including quinones, flavins, iron-sulfur clusters, hemes, and copper ions. The final phase of oxidative phosphorylation is carried out by ATP synthase, an ATP-synthesizing assembly that is driven by the flow of protons back into the mitochondrial matrix. Components of this remarkable enzyme rotate as part of its catalytic mechanism.

Oxidative phosphorylation clearly shows that proton gradients are an interconvertible currency of free energy in biological systems. Oxidative phosphorylation is different from substrtate level phosphorylation, as it does not involve phosphorylated chemical intermediates and uses a different mechanism known as chemiosmotic hypothesis.

### 8.3.1 Chemiosmotic theory

In 1961, Peter Mitchell proposed the Chemiosmotic theory which demonstrates that the energy derived from oxidation of components in the ETC is coupled to the translocation of protons from the inside to the outside of the inner surface of the mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP. As explained earlier, the complexes of the respiratory chain act as proton pumps.



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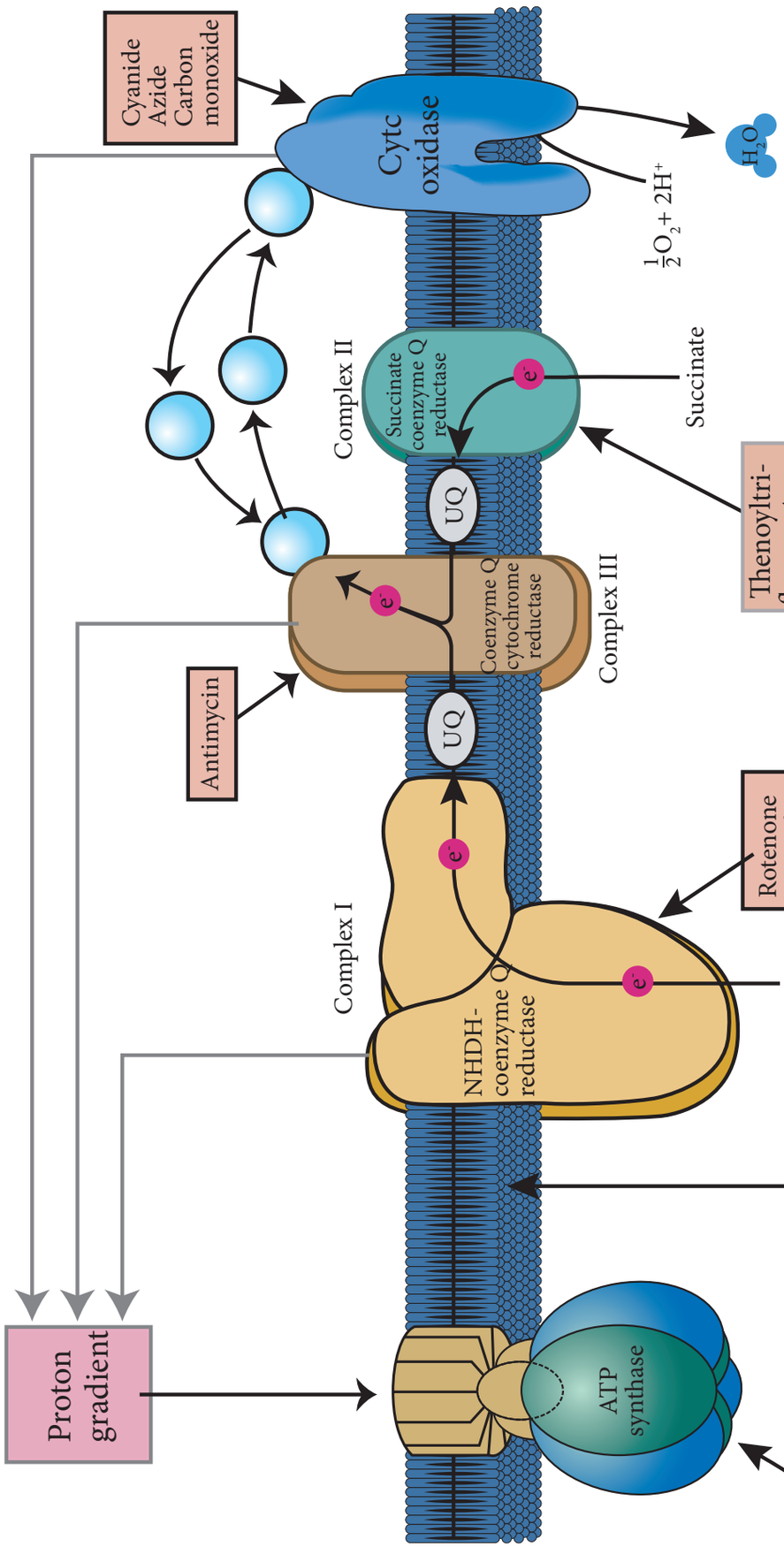



**Figure 8.9 Inhibitors of electron transport chain**



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The inner membrane of mitochondria is impermeable to ions, particularly to protons, which accumulate outside the membrane, thereby creating an electrochemical potential difference across the membrane ($\Delta\mu H^+$). This is called as chemical potential and electric potential.

The chemiosmotic coupling theory has the following features:

1. As electrons pass through the ETC, protons are transported from the matrix and released into the intermembrane space. An electrical potential ($\psi$) and proton gradient ($\Delta pH$) arise across the inner membrane. The electrochemical proton gradient is called as proton motive force.
2. Protons, now in greater excess in the intermembrane space, pass directly through the inner membrane and back to the matrix, down the concentration gradient, via specific channels, thereby producing ATP.

### 8.3.2 Role of ATP synthase

The enzyme complex ATP synthase is known as Complex V. It synthesizes ATP using the energy of the proton gradient generated by the electron transport chain. The role of this ATPase is to bring back the protons to the mitochondria. The interior of a mitochondrion is alkaline and the reaction is favourable to drive the synthesis of ATP.

The proton-flow through the $F_1F_0$ ATPase is required to release ATP from the active site where it was synthesized from ADP and $P_i$. The ATP made in the interior of the mitochondria must be exchanged for ADP outside the mitochondria to keep the cytosol supplied with ATP. The exchange of mitochondrial ATP for cytoplasmic ADP is catalyzed by the ATP/ADP translocase. The complete transfer of 2 electrons from NADH through the entire ETC to oxygen generates 3 ATPs. One $FADH_2$ feeds electrons into coenzyme Q after the first ATP-generating step. Flavin-linked substrates generate only 2 ATPs per 2 electrons transferred down the chain. Flavin-linked substrates generate only 2ATPs, not only because they feed electrons after the first ATP has already been made, also because $FADH_2$ is not as strong a reducing agent as NADH. There is not enough energy in the oxidation of $FADH_2$ to generate 3 ATPs.

## 8.4 HIGH ENERGY COMPOUNDS

High energy compounds are usually referred to as high-energy phosphates. Inorganic phosphate groups form high energy bonds which have the ability to break, provide energy, and run the metabolic processes of life. The hydrolysis of high energy phosphate bonds releases energy. For a reaction to be feasible, $\Delta G$ (Change in Gibbs free energy) value must be negative. Compounds that contain phosphate are known as high-energy entities with a large negative value of $\Delta G$ of the order of $-25$ or $-30$ kJ/mol.



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### 8.4.1 Storage form of high energy compounds

Living organisms use two main types of energy storage. First one is energy-rich molecules such as glycogen and triglycerides. They store energy in the form of covalent chemical bonds. Cells synthesize such molecules and store them for later release of the energy. The second form of biological energy storage is electrochemical reactions and takes the form of gradients of charged ions across cell membranes.

### 8.4.2 ATP as a high energy compound

Adenosine triphosphate (ATP) is the chief carrier of energy in cells. Hydrolysis releases energy from the chemical bonds in ATP to fuel cellular processes. When energy is needed by the cell, it is converted from storage molecules into ATP. The high energy of ATP is because of its two high-energy phosphate bonds. The bonds between phosphate molecules are called phosphoanhydride bonds. Structure of ATP is represented in Figure 8.10.

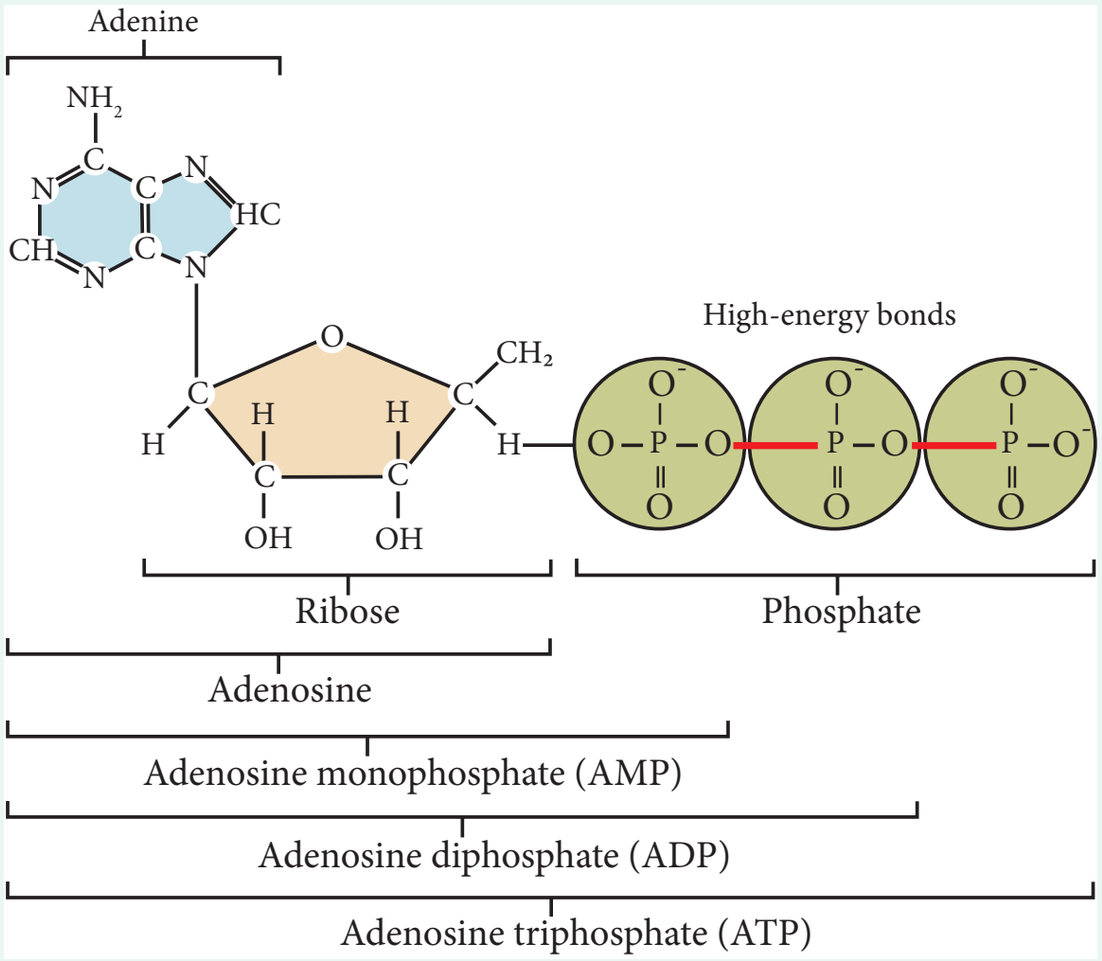



**Figure 8.10 Structure of Adenosine phosphates**



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### 8.4.2.1 Structure of ATPase ($F_1F_0$ ATPase)

Mitochondrial ATP synthase is an F-type ATPase. It is quite unique in its structure compared to other ATP synthases derived from chloroplast and eubacteria. Looking into the structure, it is a large enzyme complex found in the inner mitochondrial membrane, which catalyzes the formation of ATP from ADP and Pi. As demonstrated in Fig.8.11, ATP synthase has two discrete components: $F_1$ (catalytic unit), a peripheral membrane protein, that lies in the mitochondrial matrix and is made up of five type of polypeptide chains ($\alpha_3$, $\beta_3$, γ, ε and δ chains), and $F_o$ (o denoting oligomycin-sensitive), is an integral membrane protein which is composed of three subunits, a, b, and c, in the proportion $ab_2c_{10-12}$.

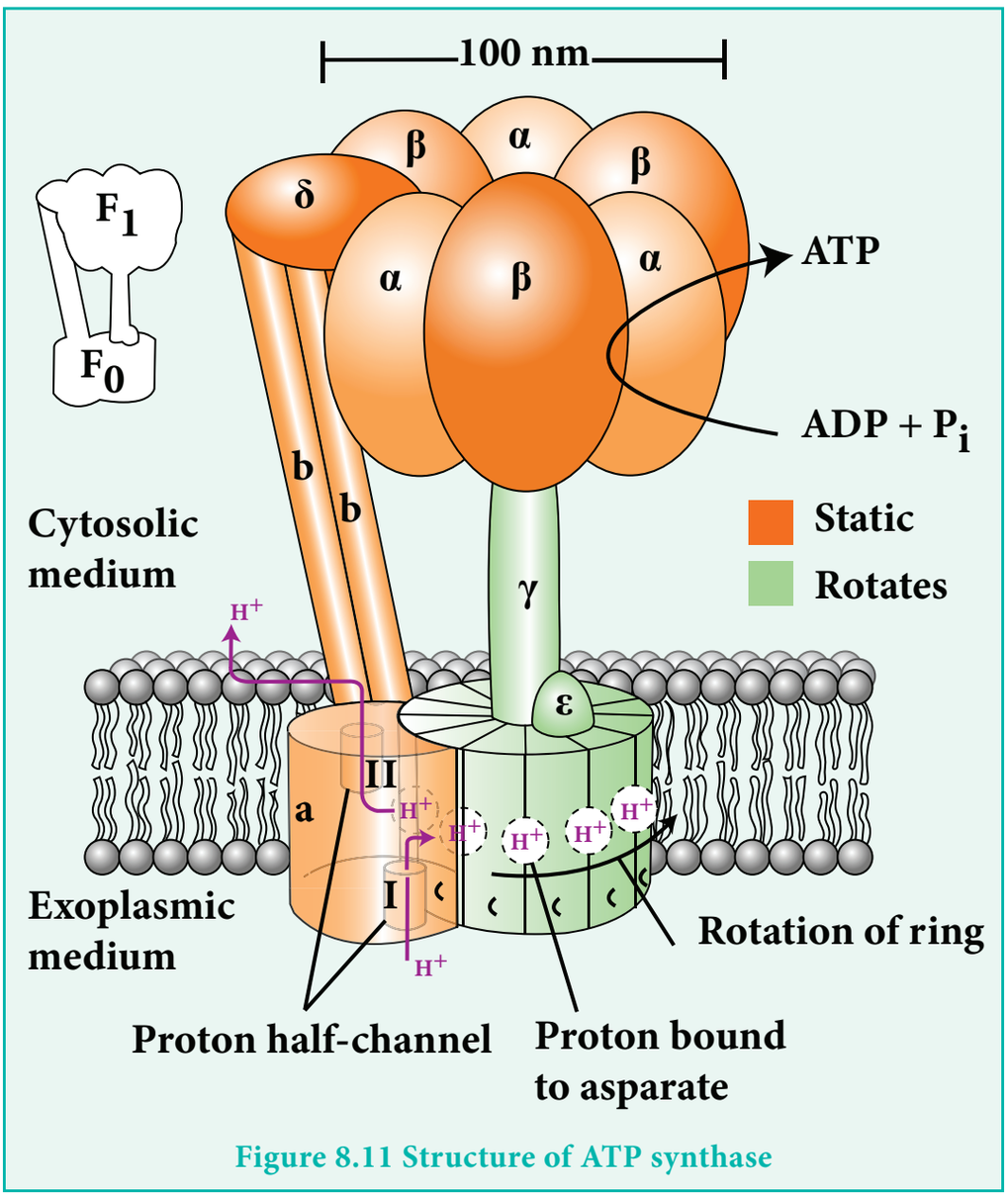



Figure 8.11 Structure of ATP synthase

The critical subunits namely, β possesses ADP / $P_i$ binding sites, α subunit can bind ATP; γ subunit rotates to change to alpha and beta conformations. c subunit of $F_0$ contains proton half channels; 'a' is the site of proton entry into 'c' subunits.



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$F_1$ catalytic unit: Three α and three β chains combine to form hexameric $\alpha_3\beta_3$ that is responsible for catalyzing the synthesis of ATP. The γ and ε polypeptide chains organize to form the central stalk and run to the inner cavity of the hexameric ring. The γ subunit helps in holding the $\alpha_3\beta_3$ and manifests rotating of the machine. This complex harness energy released by the electron transport to drive the synthesis of ATP, whereas, while alone, the $F_1$ component hydrolyses ATP.

$F_0$ region of proton pump: It consists of 10-14 c subunits organized into a ring-like structure and acts as a proton channel. A single 'c' unit connects $F_0$ to $F_1$ unit. $F_0$ and $F_1$ are connected at two points through γε central stalk and through the arm formed by 'a' subunit, two b subunits and one δ subunit.

### 8.4.2.2 Free energy of hydrolysis of ATP

Among phosphate-containing molecules, ATP is an important molecule. It exchanges energy between enzymes. ATP is an unstable molecule which hydrolyzes to ADP and inorganic phosphate. The high energy of this molecule comes from the two high-energy phosphoanhydride bonds.

The hydrolysis of ATP has a $\Delta G^{\circ\prime}$ of –30.5 kJ/mol. In addition, since the cells contain higher concentration of ATP than ADP, the ΔG for the reaction is more negative than the $\Delta G^{\circ\prime}$ value. ATP hydrolysis can therefore donate energy to other systems to allow those systems to perform reactions that would otherwise be thermodynamically unfavorable.

The phosphate bond holds usable energy. The hydrolysis of a phosphate which is responsible for release of free adenosine from AMP has a $\Delta G^{\circ\prime}$ of –14 kJ/mol; this is not considered to be a high-energy phosphate bond; and is emphasized by drawing the bond as a straight line.

The net result is that ATP hydrolysis can act as a thermodynamic driving force to allow thermodynamically unfavorable reactions that do not involve phosphate transfer to proceed. Though ATP is the most widely used high-energy compound, there are other high-energy compounds of physiological significance. An example is the high-energy thioester bond of acetyl-CoA.

## 8.5 UNCOUPLERS

Chemicals such as 2,4 dinitrophenol (DNP) act as uncoupling agents, which means they stop the synthesis of ATP, but ETC still continues and so oxygen is still consumed. DNP and other uncouplers are lipid soluble small moieties and can bind $H^+$ ions and transport them across membranes.



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## Summary

In eukaryotes, reactions of aerobic energy occur in mitochondria. An inner membrane separates the mitochondria into two spaces: matrix and intermembrane space. ETC process in the innermembrane oxidises NADH and succinate, utilizing oxygen and generates ATP. The scheme of respiratory chain and its coupling to ATP production can be summarized as follows:

1. Chemical reactions that transfer electrons between reactants are called oxidation-reduction reactions, or redox reactions.

2. During oxidation, a substance loses electrons, or is oxidized. In reduction, a substance gains electrons, or is reduced.

3. Electron transport chain is a step wise process comprising of four complexes. A series of flavoproteins, cytochromes, iron-sulfur clusters and quinone constitute the electron transport chain.

4. Complex I transfers electron from NADH to the electron carrier ubiquinone and complex II transfers electrons from succinate to ubiquinone

5. Complex III is comprised of three important cytochromes known as cytochromes bL, bH and c plus an iron sulphur center. These transfer electrons from reduced ubiquinone (UQH2) to cytochrome c

6. Complex IV contain two cytochromes (a and a3), two copper atoms, and transfers electrons to oxygen

7. Several poisons such as MPTP, rotenone, azide and cyanide inhibit the electron transport chain

8. During oxidative phosphorylation electrons derived from NADH and FADH2 combine with oxygen and the energy released is used to drive the synthesis of ATP from ADP.

9. ATP synthase is a reversible coupling device that can convert the energy of the electrochemical proton gradient into chemical bond energy or vice versa.

10. The transfer of energy from $NADH/FADH_2$ to $O_2$ yields high energy of $\Delta G$ equal to -52.5kcal/mol from each pair of electrons transferred.



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# EVALUATION

## I Multiple Choice Questions

1. During aerobic cellular respiration, most of the ATP made is generated by

   a. Substrate level phosphorylation
   b. Pyruvate kinase
   c. Glycolysis
   d. Oxidative phosphorylation

2. The prosthetic group of NADH dehydrogenase is

   a. FMN
   b. NADH
   c. FAD
   d. NADPH

3. The chemiosmotic theory involves all except

   a. A membrane impermeable to protons
   b. Electron transport by the respiratory chain pumps protons out of mitochondria
   c. Proton flow into the mitochondria depends on ADP + Pi
   d. Only proton transport is strictly regulated. other positively charged ions can easily diffuse freely across the mitochondrial membrane

4. Which of the following accepts only one electron?

   a. Cytochrome b
   b. Coenzyme Q
   c. FMN
   d. FAD

5. Loss of electrons can be termed as

   a. Metabolism
   b. Anabolism
   c. Oxidation
   d. Reduction

6. Which complex does not pump protons?

   a. NADH dehydrogenase complex I
   b. Succinate dehydrogenase complex II
   c. cytochrome reductase complex III
   d. cytochrome oxidase complex IV

7. An uncoupler of oxidative phosphorylation is

   a. DNP
   b. DTT
   c. azide
   d. Rotenone

## II Give short answer for the following

1. Explain Redox reactions.
2. Outline the structure of mitochondria.
3. Describe the inhibitors of electron transport chain.
4. What do you understand by oxidative phosphorylation?



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5. Describe high energy phosphate bonds.

## III Answer the following

1. Give a diagrammatic representation of electron transport chain showing the flow of electrons
2. Describe the structural features of ATP synthase.

### Students Activity



Using a cardboard, prepare a mitochondrion and label its parts.

Using a chart, schematically present the components of electron transport chain.

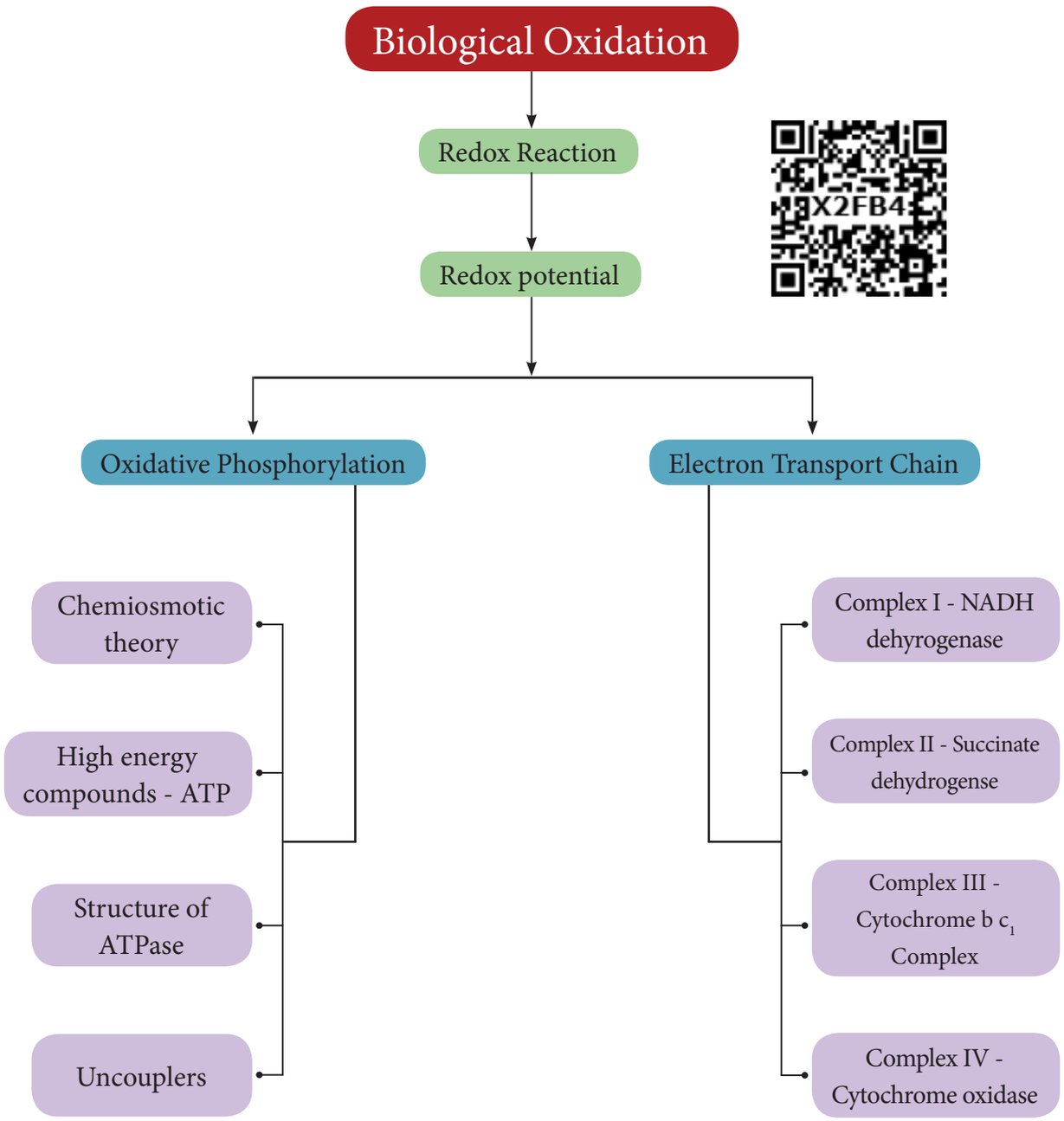
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# CONCEPT MAP






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UNIT 9

# ENZYME KINETICS



Jon Jakob Berzelius James B. Sumner

In 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalytic. It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the Nobel Prize in 1947.

SW5H3

## Learning Objectives

After studying this unit the students will be able to

- Derive the Michaelis – Menten equation.
- Understand the types of enzyme inhibitors.
- Explain the differences between competitive, non-competitive and un-competitive inhibition



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## INTRODUCTION

In Class XI you have already learnt about enzymes as biocatalysts. Enzymes are generally considered as essential components for biological reactions.

One of the most captivating areas of study in chemical kinetics is enzyme catalysis. The phenomenon of enzyme catalysis usually results in a very large increase in reaction rate and high specificity. By specificity, we mean that an enzyme is capable of selectively catalysing certain reactants, called substrates.

The specificity of an enzyme for one of the two enantiomers depends on which one fits better than the other into the active site of the enzyme (Figure 9.1).

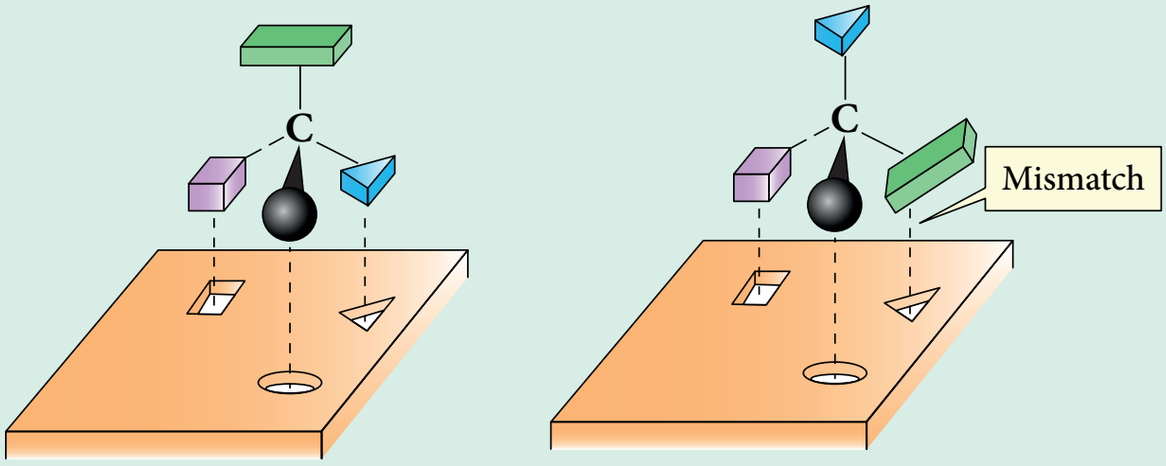



**Figure 9.1 Specificity of an enzyme for its substrate**

Activation energy is a term coined in 1889 by the Swedish scientist Arrhenius to describe the minimum energy which must be available to the reactants to result in a chemical reaction. An uncatalyzed reaction requires a higher activation energy than does an enzyme-catalyzed reaction (Figure 9.2)

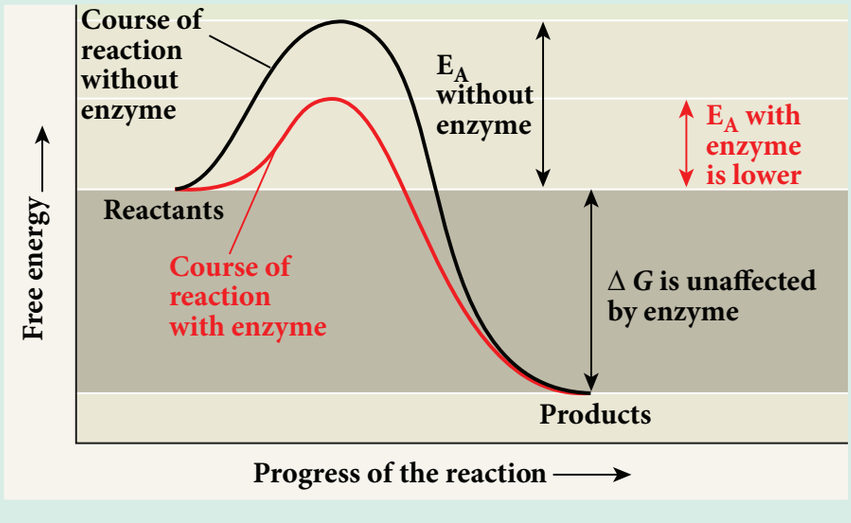



**Figure 9.2 Enzymes lower the activation energy barriers.**

One important factor which affects the enzyme activity is temperature. All enzymes have the highest activity at a particular temperature which is known as optimum



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temperature. Above this temperature enzymes get destroyed and below this they remain inactive (Figure 9.3a). The second important factor is pH. Enzymes work at a specific pH known as optimum pH. For example, digestive enzymes work well at acidic pH and intracellular enzymes work better at neutral pH (Figure 9.3b). The other factors are enzyme and substrate concentration, if the initial concentration is high it gradually increases the reaction rate (Figure 9.3c).

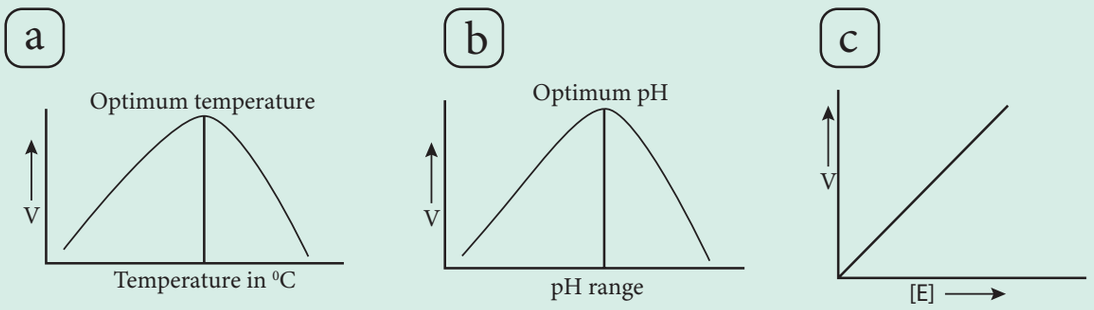



**Figure 9.3 Factors affecting enzyme activity**

## 9.1 DERIVATION OF MICHAELIS - MENTEN EQUATION

To understand the mechanism of any catalytic reaction, one should study the kinetic behaviour of the reaction systems, where the rate of the reaction can be obtained at various concentrations of the enzyme and the substrate. Formation of enzyme substrate complex is a critical event in an enzyme-catalyzed reaction. Michaelis and Menten proposed that the binding of the substrate and the enzyme is reversible and derived a kinetic model for a simple single substrate enzymatic reaction. This laid the basis for understanding the various factors that influence the rate of the reaction, such as concentration of substrate, temperature and pH of the system.

The kinetics of enzyme catalyzed reactions is explained by Michaelis- Menten equation. It can be derived with the following assumptions:

1. The concentration of enzyme is much lower than the concentration of the substrate.
2. The initial rate (or initial velocity), designated $v_0$, when [S] is much greater than the concentration of enzyme [E], is measured
3. None of the products formed revert to the initial substrate.
4. A steady-state condition is reached between the formation of ES complex and its decomposition.

The enzyme (E) combines with the substrate (S) to form ES complex with the rate constant $k_1$. The ES complex can dissociate either into 'E' and 'S' with the rate constant $k_2$ or it can form the product (P) with the rate constant $k_3$.



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$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

If, only the initial period of the reaction is considered, the product concentration [P] is negligible and the formation of ES from product can be ignored.

The velocity of the overall enzymic process

$$v = k_3[ES] \quad ...(1)$$

Rate of formation of ES = $k_1[E]\,[S]$

Rate of breakdown of ES = $[k_2+k_3]\,[ES]$

Under steady state conditions,

Rate of formation of ES = Rate of breakdown of ES

(i.e) $k_1[E][S] = [k_2 + k_3]\,[ES]$

$$[ES] = \frac{(k_1[E][S])}{(k_2 + k_3)}$$

$$[ES] = \frac{([E][S])}{\frac{(k_2 + k_3)}{k_1}} = \quad ...(2)$$

Where, $K_m = \frac{(k_2 + k_3)}{k_1}$, is a constant known as Michaelis constant.

The concentration of uncombined enzyme [E], is equal to the total enzyme concentration [ET] minus concentration of ES complex, (i.e) [E] = [ET] – [ES]

Substitute this value of [E] in equation (2), we get

$$[ES] = \frac{(\{[E_T]-[ES]\}\,[S])}{K_m} \quad ...(3)$$

$$[ES]\,K_m = [E_T]\,[S] - [ES]\,[S]$$

$$[ES]K_m + [ES]\,[S] = [E_T]\,[S]$$

$$[ES]\,(K_m + [S]) = [E_T]\,[S]$$

$$\therefore [ES] = \frac{([E_T][S])}{(K_m + [S])}$$

Substitute this value of [ES] in equation (1)

$$v = \frac{(k_3[E_T][S])}{(K_m + [S])} \quad ...(4)$$

The velocity becomes maximum velocity ($V_{max}$) when the catalytic sites on the enzyme are saturated with substrate.



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(i.e) when [S] >>> $K_m$;

$K_m$ + [S] = [S]

Therefore, $V_{max} = \frac{(k_3[E_T][S])}{([S])} = k_3$ ET

Substitute this value of $k_3$[ET] in equation (4)

$$V = \frac{(V_{max}[S])}{(K_m + [S])} \quad ...(5)$$

This equation is known as Michaelis - Menten equation.




Enzymes may be denatured by excessive heat, extreme pH or various chemicals. Such extremities or chemicals cause enzymes to denature by the disruption and possible destruction of both the secondary and tertiary structures. Such changes are irreversible.

### 9.1.1. Significance of Michaelis - Menten Equation:

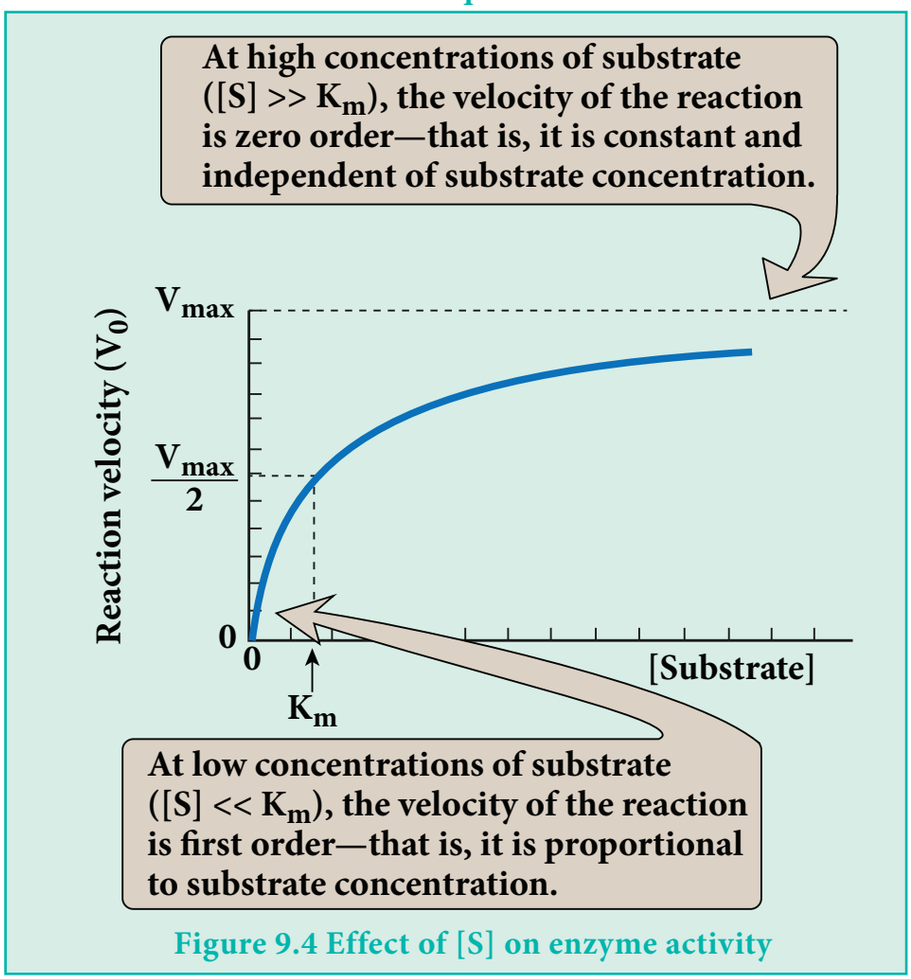



Figure 9.4 Effect of [S] on enzyme activity

Michaelis - Menten equation accounts for the three different phases of the substrate - concentration graph.



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i) When [S] in very much less than $K_m$:

The Michaelis-Menten equation is

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

When [S] << $K_m$; the equation becomes

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

When both the constants '$V_{max}$' and '$K_m$' are replaced by another constant 'K' the above equation becomes v = K[S]

i.e. the velocity is directly proportional to the substrate concentration (first order) (Fig 9.4)

ii) When [S] is very much greater than $K_m$:

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

When [S] >> $K_m$ the equation becomes,

$$v = \frac{(V_{max}[S])}{([S])} = V_{max}$$

The velocity is independent of substrate concentration (zero order) (Fig 9.4)

iii) When [S] = $K_m$ : (Derivation of $K_m$):

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

When $K_m$ = [S]

$$v = \frac{(V_{max}[S])}{([S]+[S])} = \frac{(V_{max}[S])}{(2[S])} = ½\ V_{max}$$

Thus $K_m$ = [S] at ½ $V_{max}$

### 9.1.2. Significance of $K_m$ : (Michaelis-Menten Constant)

$K_m$ is defined as the substrate concentration at half maximal velocity.

At ½ $V_{max}$, $K_m$ = [S]

$K_m$ is also defined as

$$K_m = \frac{(k_2 + k_3)}{k_1}$$

$k_1$ is the rate constant for the formation of ES complex,



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$k_2$ is the rate constant for the dissociation of ES complex into E & S and

$k_3$ is the rate constant for the dissociation of ES complex into P.

$K_m$ is a measure of the strength of ES complex. A high value of $K_m$ indicates weak binding and a low value of $K_m$ indicates strong binding between enzyme and substrate. The $K_m$ values of a few enzymes are given below.

| ENZYME | SUBSTRATE | $K_m$(mM) |
|---|---|---|
| Aspartate aminotransferase | Aspartate<br>$\alpha$-Ketoglutarate<br>Oxaloacetate<br>Glutamate | 0.9<br>0.1<br>0.04<br>4 |
| Threonine deaminase | Threonine | 5 |
| Arginyl-tRNA synthetase | Arginine<br>$tRNA^{Arg}$<br>ATP | 0.003<br>0.0004<br>0.3 |
| Pyruvate carboxylase | $HCO_3^-$<br>Pyruvate<br>ATP | 1.0<br>0.4<br>0.06 |
| Penicillinase | Benzylpenicillin | 0.05 |
| Lysozyme | Hexa-*N*-acetylglucosamine | 0.006 |

Note: For a given enzyme $K_m$ is a constant for a specific substrate.

### 9.1.3. Lineweaver - Burk Equation:

The Michaelis - Menten equation is

$$v = \frac{(V_{max}[S])}{(K_m + [S])} \qquad ...(1)$$

Taking reciprocals on both sides of equation (1)

$$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{([S])} + \frac{1}{V_{max}} \qquad ... (2)$$

This is the equation for a straight line., $y = mx + c$

Equation (2) is known as Lineweaver - Burk equation.

A plot of $\frac{1}{v}$ versus $\frac{1}{([S])}$ is called a double - reciprocal plot (or) Lineweaver - Burk plot (LB plot) which yields a straight line.



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This line, on extrapolation, cuts the 'X' axis at $\frac{-1}{K_m}$ and 'Y' axis at $\frac{1}{V_{max}}$.

The slope = $\frac{K_m}{V_{max}}$.

From these data both $K_m$ and $V_{max}$ can be determined (Figure 9.5)

For most of the enzymes, $K_m$ lies between $10^{-1}$ and $10^{-7}$ M.

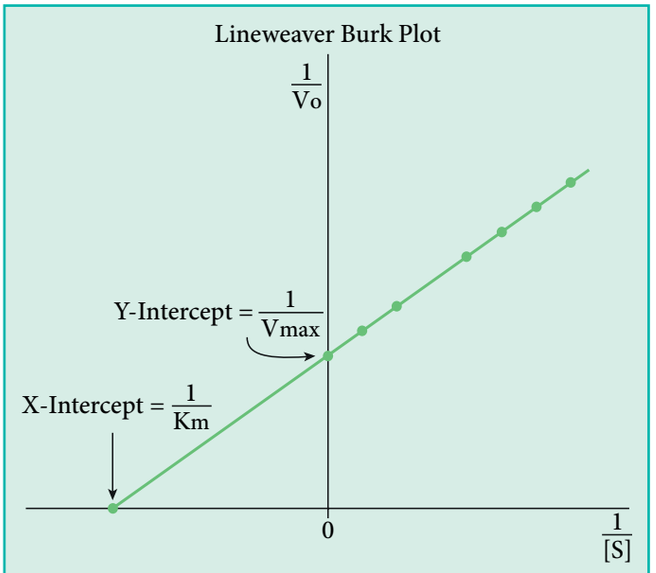



**Figure 9.5 Lineweaver Burk double reciprocal plot**

### Significances of $V_{max}$ :

1) $V_{max}$ is the maximal velocity of the enzyme catalysed reaction.
2) This condition is attained when the catalytic sites on the enzyme are saturated with substrate. (i.e) when [S] >>> $K_m$.
3) $V_{max} = k_3$ [ET], where $k_3$ = rate constant for the dissociation of ES complex into product; [ET] = Total enzyme concentration.
4) $k_3$ = kcat = $\frac{V_{max}}{[ET]}$

The turnover number of an enzyme (kcat) is defined as the number of substrate molecules converted into product by an enzyme molecule in unit time when the enzyme is fully saturated with the substrate. The turnover numbers of most enzymes fall in the range of 1 to $10^4$ $sec^{-1}$. The turnover number of different enzymes are given as follows :

| S. NO. | ENZYME | TURNOVER NUMBER ( $SEC^{-1}$) |
|---|---|---|
| 1 | Carbonic anhydrase | 6,00,000 |
| 2 | Penicillinase | 2,000 |
| 3 | DNA polymerase I | 15 |
| 4 | Lysozyme | 0.5 |




Haem is an iron-containing prosthetic group. It may function as an electron carrier and oxygen carrier in hemoglobin. It is also found in catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide to water and oxygen.



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## 9.2. ENZYME ACTION

### Active Site:

It is a three dimensional area or region on the surface of the enzyme molecule where the substrate binds. It is also known as 'catalytic site' or 'substrate site'

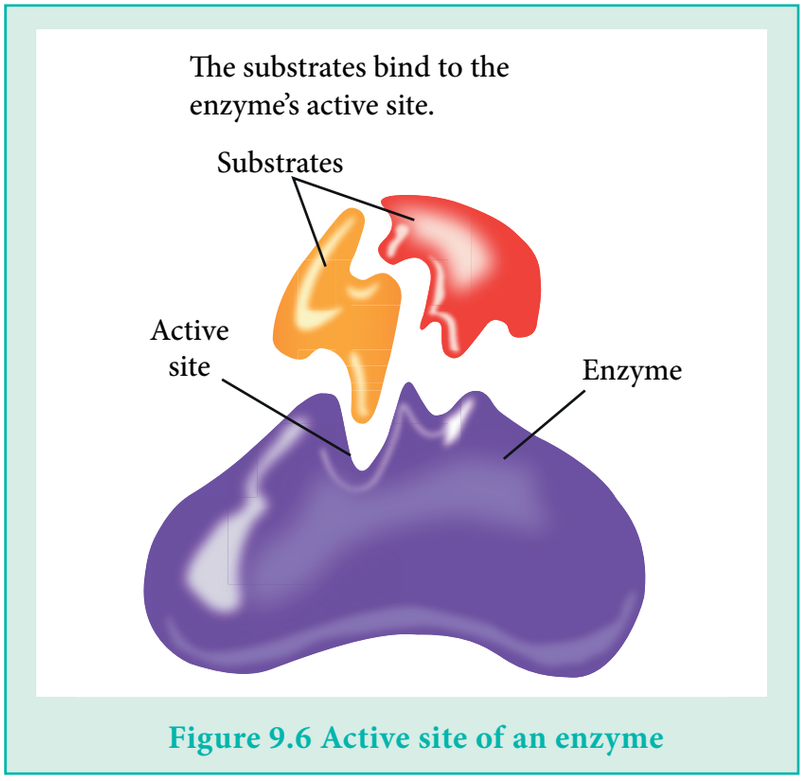



**Figure 9.6 Active site of an enzyme**

Although the enzymes differ widely in their properties, the active site present in their molecule possesses some common features. These are listed below :

The active site occupies a relatively small portion of the enzyme molecule.

The active site is neither a point nor a line or even a plane but is a 3- dimensional entity. It is made up of groups that come from different parts of the linear amino acid sequence.

Usually, the arrangement of atoms in the active site is well defined, resulting in a marked specificity of the enzymes. Although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in structure from its own substrate.

The active site binds the substrate molecule by relatively weak forces.

The active sites in the enzyme molecules are grooves or crevices from which water is largely excluded. It contains amino acids such as aspartic acid, glutamic acid, lysine, serine etc. The side chain groups like -COOH, $-NH_2$, $-CH_2OH$ etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which is essential for catalysis.

### (i) Mode of enzyme action

In order to explain the mode of enzyme action, the following models have been proposed.

i) Fischer's lock and key model: According to this model the substrate binds with the enzyme at the active site just like a key fits into a lock. This leads to the formation of enzyme-substrate complex (ES complex). This complex decomposes to give the product and free enzyme.



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In fact, the enzyme-substrate union depends on a reciprocal fit between the structure of the enzyme and the substrate. Since two molecules (the substrate and the enzyme) are involved, this hypothesis is also known as the concept of intermolecular fit. The enzyme-substrate complex is highly unstable and almost immediately this complex decomposes to form the product. (Fig 9.7).

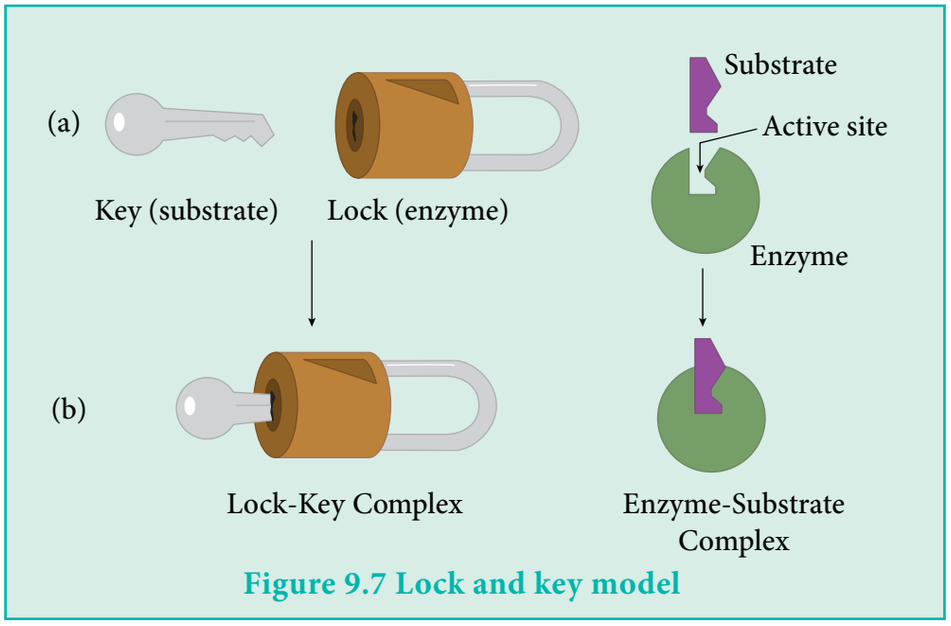



**Figure 9.7 Lock and key model**

(ii) Koshland's induced fit model: According to this model, the initial interaction between enzyme and substrate is relatively weak. These weak interactions quickly induce conformational changes in the enzyme that strengthen binding. This is the more accepted model for enzyme-substrate complex than the lock-and-key model. The failure of Fischer's model is the rigidity of the active site. Unlike the lock-and-key model, the induced fit model shows that enzymes are flexible structures in which the active site continually changes by interacting with the substrate (Fig 9.8).

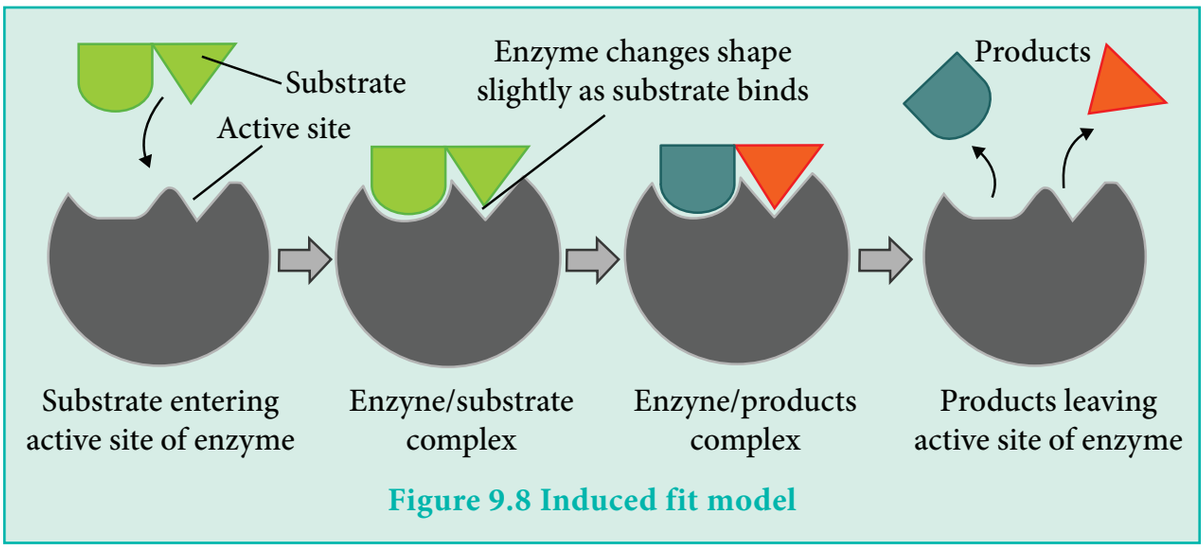



**Figure 9.8 Induced fit model**



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### (ii) Mechanism of enzyme action :

The enzyme-substrate union results in the release of energy. It is this energy which, in fact, raises the energy level of the substrate molecule, thus inducing the activated state (Figure 9.2). In this activated state, certain bonds of the substrate molecule become more susceptible to cleavage.

Evidences Proving the Existence of an ES Complex: The existence of an ES complex during enzymatically catalyzed reaction has been shown in many ways :

1. The ES complexes have been directly observed by electron microscopy and x-ray crystallography.
2. The physical properties of enzymes (solubility, heat sensitivity) change frequently upon formation of an ES complex.
3. The spectroscopic characteristics of many enzymes and substrates change upon formation of an ES complex. It is a case parallel to the one in which the absorption spectrum of deoxyhemoglobin changes markedly, when it binds oxygen or when it is oxidized to ferric state.
4. Stereospecificity of highest order is exhibited in the formation of ES complexes. For example, D-serine is not a substrate of tryptophan synthetase. As a matter of fact, the D-isomer does not even bind to the enzyme.
5. The ES complexes can be isolated in pure form. This may happen if in the reaction, $A + B \rightarrow C$, the enzyme has a high affinity for the substrate A and also if the other reactant B is absent from the mixture.
6. A most general evidence for the existence of ES complexes is the fact that at a constant concentration of enzyme, the reaction rate increases with increase in the substrate concentration until a maximal velocity is reached.

## 9.3. Enzyme Inhibition

A substance which binds with an enzyme and brings about a decrease in catalytic activity of that enzyme is called an enzyme inhibitor. This process is known as enzyme inhibition. The enzyme inhibitor may be organic or inorganic in nature. Enzyme inhibition is classified into three types as follows :

1) Reversible inhibition
2) Irrerversible inhibition.
3) Allosteric inhibition

### 9.3.1. Reversible inhibition :

When the inhibitor binds noncovalently with the enzyme and the inhibitor dissociates rapidly from the enzyme inhibitor complexes, it is known as reversible inhibition. It is further subdivided into



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1. Competitive inhibition
2. Noncompetitive inhibition
3. Uncompetitive inhibition.

### 9.3.1.1. Competitive inhibition (or) substrate analogue inhibition :

It is a type of reversible inhibition in which the inhibitor resembles the substrate and binds to the active site of the enzyme.

Since the competitive inhibitor resembles the substrate, it is otherwise known as substrate analogue.

In competitive inhibition, the enzyme can bind substrate (ES complex) or inhibitor (EI)

The inhibitor thus competes with the substrate to combine with the enzyme.

A competitive inhibitor decreases the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate.

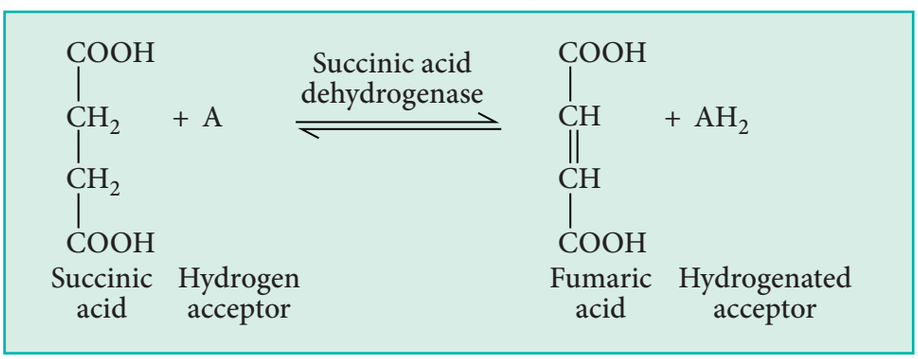
The degree of inhibition depends upon the relative concentrations of both substrate and inhibitor.

Competitive inhibition can be overcome by a sufficiently high concentration of substrate.

In competitive inhibition $K_m$ value increases, whereas $V_{max}$ remains unchanged.

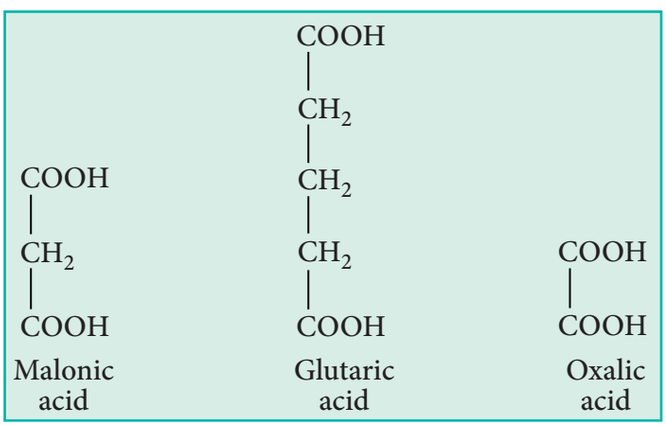
**Example :**

The action of malonate on succinate dehydrogenase (SDH)




Oxalic, malonic and glutaric acids are competitive inhibitors of SDH. Among them, malonic acid is the most potent competitive inhibitor.

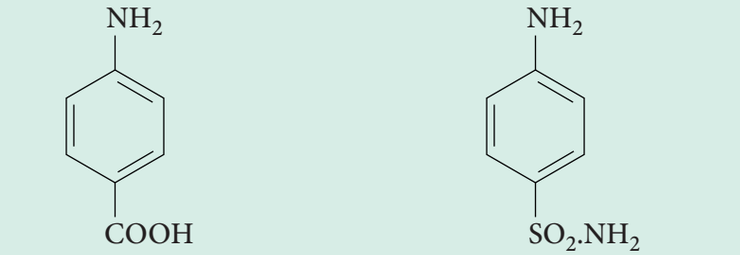
The pharmacological action of many drugs may be explained by the principle of competitive inhibition. For example, sulphonamides are very commonly used antibacterial drugs.






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Bacteria synthesise the vitamin folic acid from P-aminobenzoicacid (PABA). Bacterial cell wall is impermeable to folic acid. Sulphanilamides and other sulpha drugs are structural analogues of PABA and hence inhibit folic acid synthesis in bacteria and they die. This drug is nontoxic to human cells, because human beings lack the enzymes necessary for folic acid synthesis and it is needed as a vitamin in the diet.



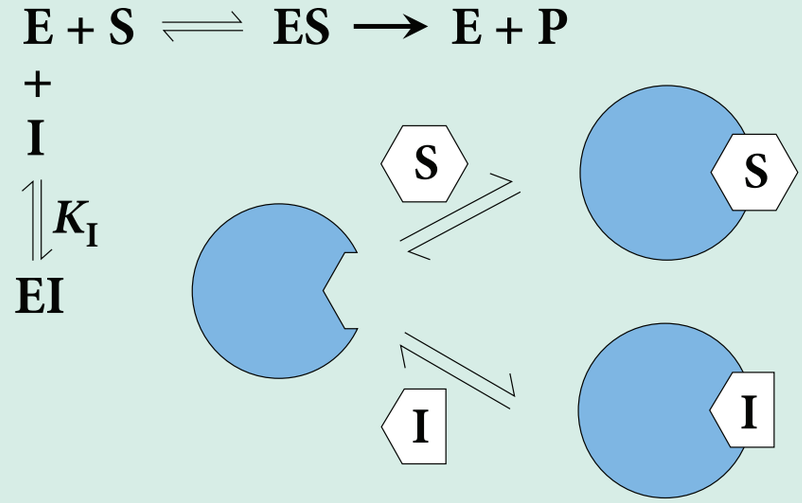




**Figure 9.9 Competitive inhibition**

The action of competitive inhibitor may be given as follows (Figure 9.9)

The inhibitor constant ($K_i$) for competitive inhibition is

$$K_i = \frac{([E]\ [I])}{([EI])}$$

### 9.3.1.2. Noncompetitive inhibiton

It is also a reversible inhibition in which the inhibitor has no structural resemblance with the substrate and binds at a site other than the active site on the enzyme surface.

In noncompetitive inhibition both the substrate and inhibitor can bind simultaneously to an enzyme molecule.

The inhibitor binds with the enzyme as well as ES complex which leads to the formation of EI and ESI complexes, respectively.

Noncompetitive inhibition cannot be overcome by increasing the substrate concentration.

In noncompetitive inhibition $K_m$ value is unchanged while $V_{max}$ is decreased.



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**Example** :

i) Various heavy metals ($Ag^{+}$, $Pb^{2+}$, $Hg^{2+}$) can non-competitively inhibit various enzymes. For example, urease is highly sensitive to all these heavy metal ions.

ii) $H_2S$ and cyanide strongly inhibit the action of iron containing enzymes like cytochrome oxidase.

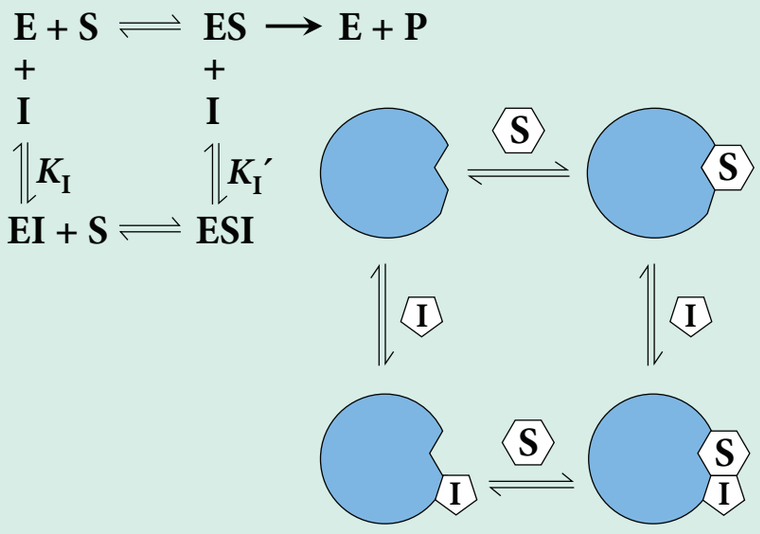



**Figure 9.10 Non-competitive inhibition**

The action of a non-competitive inhibitor is given as follows (Figure 9.10)

The noncompetitive inhibitor gives two $K_i$ values as follows.

$$K_i = \frac{([E]\ [I])}{([EI])}$$

$$K_i = \frac{([ES]\ [I])}{([ESI])}$$

### 9.3.1.3. Uncompetitive inhibition :

It is also a type of reversible inhibition in which the inhibitor does not have any affinity for free enzyme, but binds only with the enzyme-substrate complex.

Uncompetitive inhibitor decreases both $K_m$ and $V_{max}$ values of the enzyme.

**Example :** Inhibition of placental alkaline phosphatase by phenyl alanine is an example of uncompetitive inhibition.

The action of uncompetitive inhibitor may be given as follows (Figure 9.11)

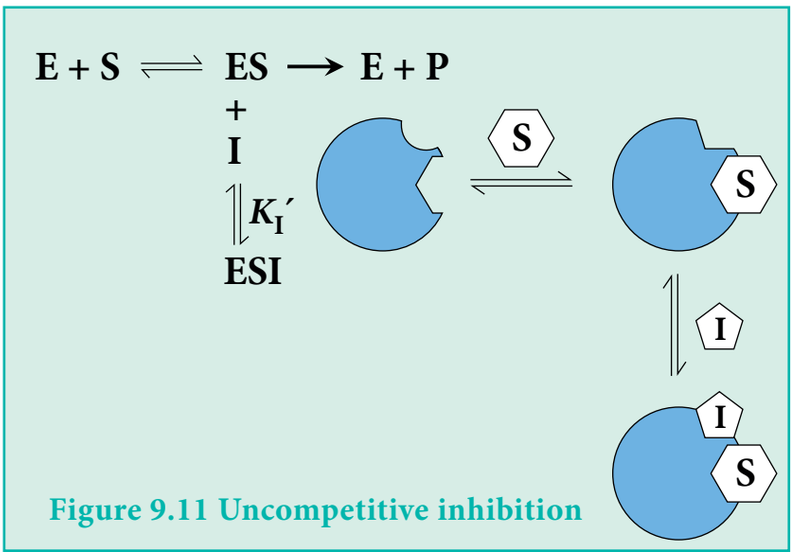



**Figure 9.11 Uncompetitive inhibition**



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Uncompetitive inhibition is rare in one-substrate reactions but common in two-substrate reactions.

The degree of uncompetitive inhibition may increase with increase in substrate concentration.

The inhibitor constant ($K_i$) of uncompetitive inhibition is

$$K_i = \frac{([ES]\ [I])}{([ESI])}$$

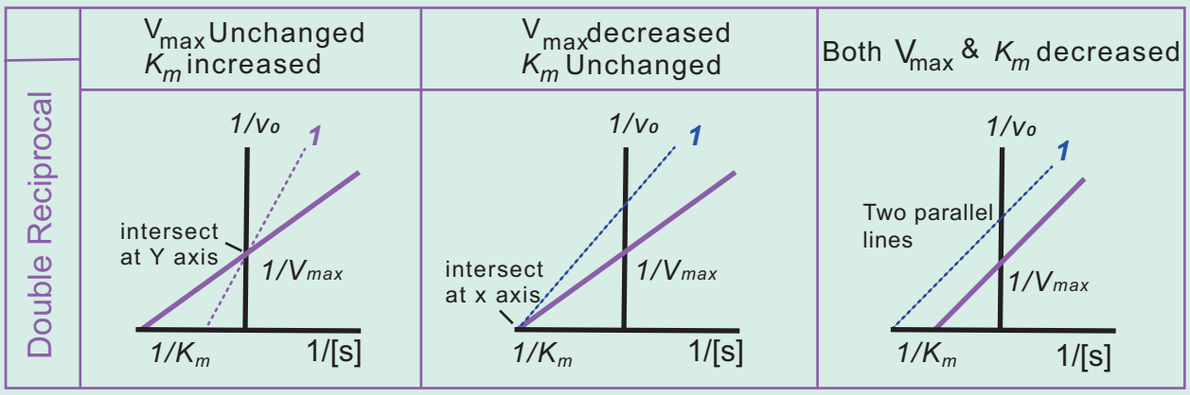



**Figure 9.12 Lineweaver Burk plots for competitive, non-competitive and uncompetitive inhibition.**

## Differences Between Competitive And Noncompetitive Inhibition

| COMPETITIVE INHIBITOR | NON-COMPETITIVE INHIBITOR |
|---|---|
| Inhibitor resembles the substrate (substrate analogue) | Inhibitor has no structural resemblance with the substrate. |
| Inhibitor binds at the active site. | Inhibitor binds at a site other than active site |
| Enzyme binds either with substrate or inhibitor | Enzyme binds with both substrate and inhibitor |
| Reversible | Irreversible |
| Can be overcome by increasing substrate concentration. | Cannot be overcome by increasing substrate concentration |
| $K_m$ value will be increased | $K_m$ remains unchanged |
| $V_{max}$ remains unchanged. | $V_{max}$ value will be decreased |



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| | |
|---|---|
| Example: Action of malonate on succinate dehydrogenase. | Example: Action of cyanide on cytochrome oxidase. |
| Applied in drug action | Applied in toxicological studies. |

### 9.3.2 Allosteric inhibition: (Greek allo = 'other'; stereos = 'space' or 'site')

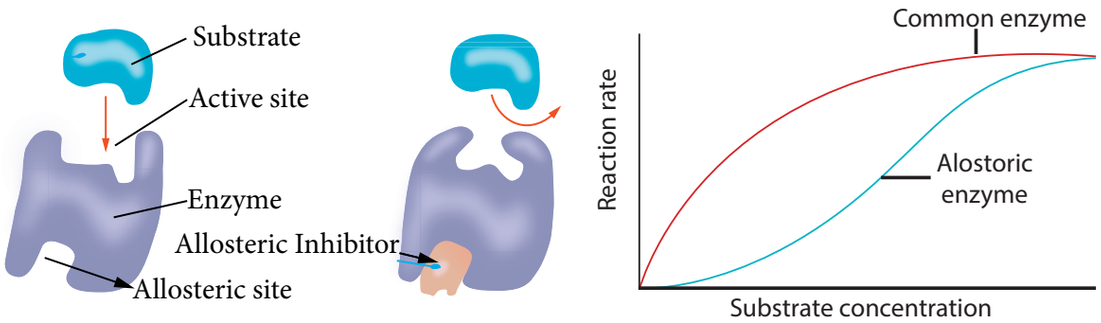



**Figure 9.13 Allosteric inhibition**

The allosteric inhibitor binds to an enzyme at a site other than the active site (Allosteric site). This leads to a conformational change which reduces the affinity of the enzyme for substrate (Figure 9.13). Allosteric enzymes do not exhibit Michaelis Menten kinetics. A graph of $v_0$ versus [S] gives a sigmoidal curve.

### 9.3.3. Irrversible enzyme inhibition :

When the inhibitor binds tightly to the enzyme either covalently or noncovalently and dissociates very slowly from the enzyme - inhibitor complex, it is known as irreversible inhibition (Figure 9.14)

**Example :**

1) Action of nerve gases on acetylcholine esterase.
2) Action of iodoacetamide on enzymes containing –SH group by modifying cysteine.

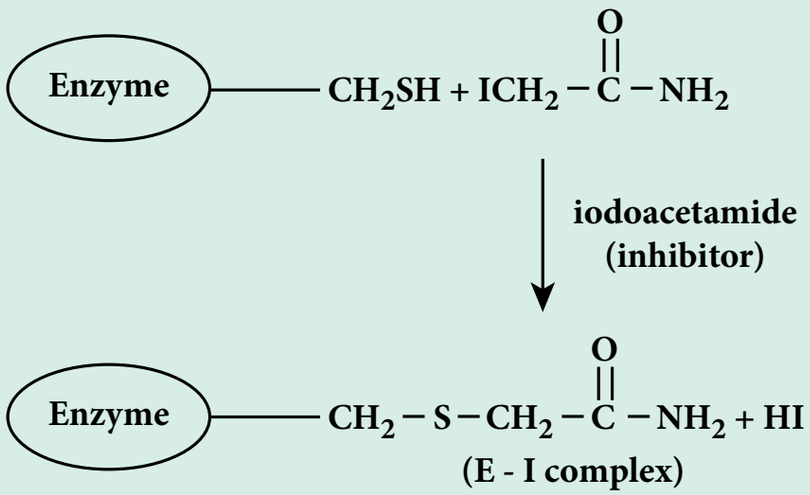



**Figure 9.14 Irreversible Inhibition**



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## Summary

1. Enzymes increase the rate of a reaction by lowering the energy of activation.
2. The active site of an enzyme is a pocket or cleft containing aminoacid side chains that participate in the binding of substrate and the catalytic reaction.
3. Binding of substrate causes a conformational change in the enzyme (induced fit) that favours catalysis.
4. When a substrate binds to the active site, an enzyme–substrate (ES) complex is formed which subsequently dissociates to enzyme and product.
5. Most enzymes show Michaelis-Menten kinetics, and a plot of the initial reaction velocity (v) against substrate concentration([S] has a hyperbolic graph.
6. Michaelis Menten derived the equation for a single substrate enzyme catalyzed reaction assuming steady state conditions where there is no change in [ES].
7. Lineweaver Burk used the double reciprocal linear plot to determine $V_{max}$ and $K_m$ more accurately.
8. Inhibitors are molecules that can reduce the rate of an enzyme catalyzed reaction. There are two types of inhibitors; reversible and irreversible inhibitors.
9. Competitive ($V_{max}$ unchanged, $K_m$ increased), non-competitive ($V_{max}$ decreased, $K_m$ unchanged) and uncompetitive inhibitors (Both $V_{max}$ and $K_m$ decreased) are reversible inhibitors.
10. Allosteric enzymes do not show Michaelis Menten kinetics, instead they show sigmoidal kinetics. Allosteric inhibitors bind to a site other than the active site.
11. Irreversible inhibitors bind tightly to the enzyme, either covalently or noncovalently, and dissociates very slowly from the enzyme - inhibitor complex.



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**Hirudin:** An enzyme from leech saliva which keep blood flowing without being clotted from the host body. This helps leech suck as much blood as it needs. Hence leech therapy uses them to minimize pain of varicose veins by sucking accumulated blood.

## EVALUATION

### I. Choose the correct answer from the four given alternatives

1. ES complex formation is
   a) a reversible reaction
   b) an irreversible reaction
   c) an energy consuming reaction
   d) a complete reaction
2. According to Michaelis Menten theory
   a) only a single substrate is involved
   b) the concentration of substrate is much greater than that of enzyme
   c) an intermediate enzyme substrate complex is formed
   d) all the above
3. The reciprocal form of M-M equation was considered by
   a) Lineweaver – Burk
   b) Fischer
   c) Koshland
   d) Dixon
4. Lock and Key theory was proposed by
   a) Dixon
   b) Fischer
   c) Koshland
   d) Michaelis Menten
5. An exact structural similarity with the substrate is needed for a
   a) competitive inhibitor
   b) uncompetitive inhibitor
   c) noncompetitive inhibitor
   d) irreversible inhibitor
6. In uncompetitive inhibition inhibitor binds only to
   a) enzyme
   b) substrate
   c) ES-complex
   d) Active site
7. The number of molecules of substrate converted to product per enzyme molecule per second is called the …
   a) Turnover number
   b) Optimum number
   c) Maximum reaction rate
   d) $K_m$



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8. The active site of an enzyme is generally a

a) cleft b) Indentation

c) Hole d) Tube

9. Which one of the following conditions is least likely to denature an enzyme?

a) a high temperature b) an extreme pH

c) heavy metal ions d) a low temperature

10. According to lock and key & induced fit models which of the following is not true?

a) Induced fit model is more specific than lock and key model

b) In lock and key model the active site is more RIGID than induced fit model

c) In induced fit model the active site can undergo conformational change

d) In lock and key model, the enzyme is considered as a lock and substrate is considered as a key

11. Which of the following is the best description of an enzyme?

a) they allow chemical reactions to proceed very quickly

b) they increase the rate at which a chemical reaction approaches equilibrium

c) they make a reaction thermodynamically favorable

d) all of the above e. none of the above

12. Given an enzyme with a $K_m$ = 10m M and $V_{max}$ = 100 m mol/min. If [S] = 100 m M, which of the following will be true?

a) A 10 fold increase in $V_{max}$ would increase velocity 10 fold y

b) A 10 fold decrease in $K_m$ would increase velocity

c) Both (a) and (b)

d) A 10 fold increase in $V_{max}$ would decrease velocity 20 fold.

13. The relationship between $K_m$ and $V_{max}$ is known as

a) Haldane equation

b) Michaelis Menten equation

c) Numerical solution approach

d) Gibbs-Helmholtz equation

14. A competitive inhibitor of an enzyme is usually

a) a highly reactive compound b) a metal ion such as $Hg^{2+}$ or $Pb^{2+}$

c) structurally similar to the substrate. d) water insoluble



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15. The types of inhibition pattern based on Michaelis-Menten equation are
    a) competitive
    b) non-competitive
    c) uncompetitive
    d) all of the above
16. In a Lineweaver-Burk Plot, competitive inhibitor shows which of the following effect?
    a) It moves the entire curve to right
    b) It moves the entire curve to left
    c) It changes the x-intercept
    d) It has no effect on the slope
17. An allosteric inhibitor of an enzyme usually
    a) participates in feedback regulation
    b) denatures the enzyme
    c) is a hydrophobic compound
    d) causes the enzyme to work faster
18. The enzyme inhibition can occur by
    a) reversible inhibitors
    b) irreversible inhibitors
    c) Both (a) and (b)
    d) None of these
19. Non-competitive inhibitor of an enzyme catalyzed reaction
    a) decreases $V_{max}$
    b) binds to Michaelis complex (ES)
    c) both (a) and (b)
    d) can actually increase reaction velocity in rare cases
20. The active site of an enzyme remains
    a) at the center of globular proteins
    b) rigid and does not change shape
    c) complementary to the rest of the molecule
    d) none of the above



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## II. Say True or False.

1. Enzyme substrate complex is a permanent stable complex.
2. Malonate is the competitive inhibitor of succinate dehydrogenase.
3. An enzyme substrate complex is formed in all the enzymatic reactions.
4. The degree of competitive inhibition cannot be decreased by increasing the concentration of the substrate.
5. An uncompetitive inhibitor has affinity towards ES complex.

## III. Match the following

1. Enzymes - $\frac{1}{V}$ vs $\frac{1}{S}$
2. ES complex – affinity of enzyme for substrate
3. $k_m$ - Unstable and highly energetic
4. Line weaver Burk plot - Biocatalysts

## IV. Give short answers for the following

1. Define $K_m$
2. What is the nature of active site according to lock and key theory ?
3. What is competitive inhibition ?
4. What is induced fit theory ?
5. What is irreversible enzyme inhibition ?

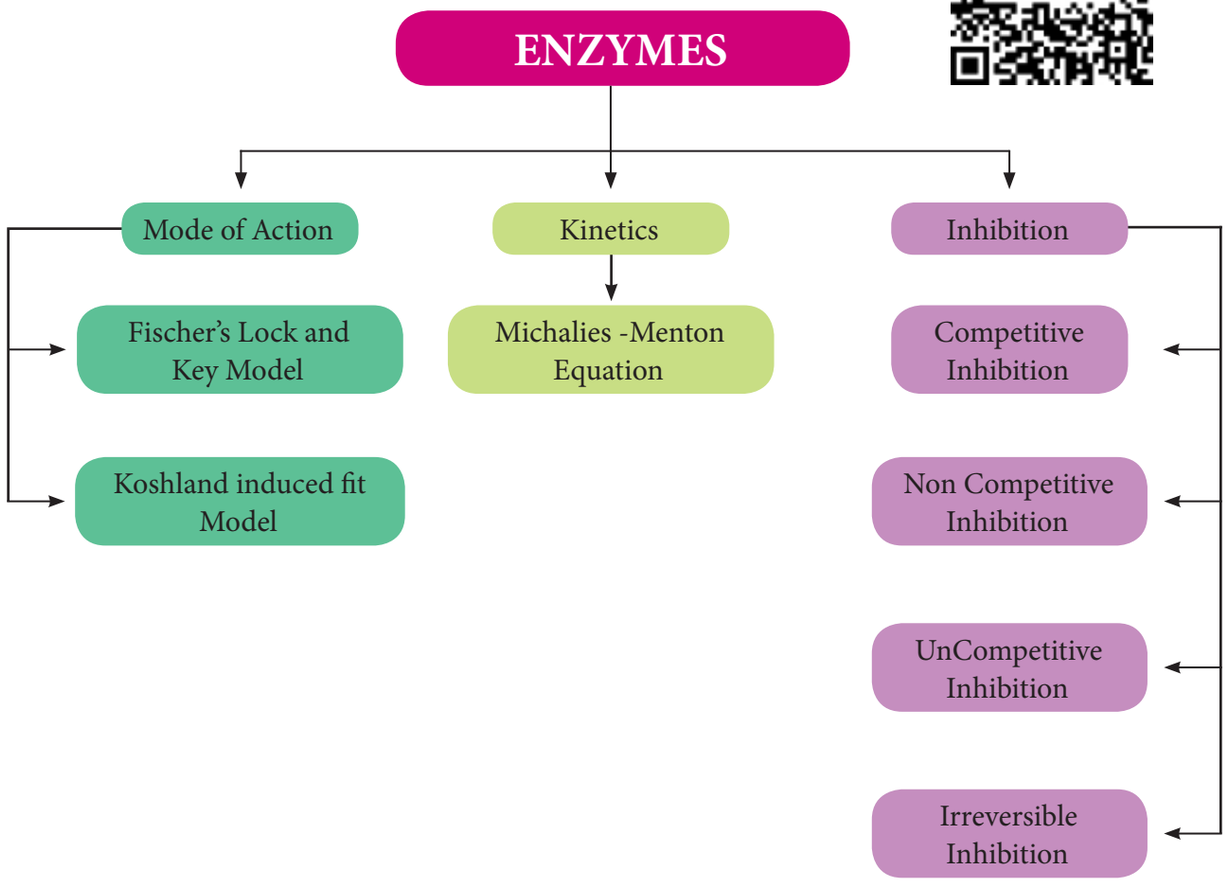
## V. Answer the following

1. Derive MM equation.
2. How is the LineWeaver Burk plot arrived at?
3. Explain the concept of competitive inhibition
4. What is the action of malonate on succinate dehydrogenase ?
5. Compare competitive and non-competitive inhibition.



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# CONCEPT MAP






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UNIT 10

# IMMUNOLOGY



**Edward Jenner,**

Edward Jenner, an English Physician and Scientist, is known as the Father of Immunology because of his contribution to the invention of a vaccine against small pox which saved many lives.

RI591

## Learning Objectives

After studying this chapter, one should be able to

- Understand the basic concepts of infection.
- Identify few infectious diseases.
- Understand the differences between innate and adaptive immunity.
- List out the functions of antibodies.
- Carry out blood grouping tests



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## 10.1. INTRODUCTION TO IMMUNOLOGY

"A healthy life is a wealthy life" and "Prevention is better than cure" are the proverbs frequently used to define the status of human health which is closely related to immunity and the immune system. Immunity is defined as the state of resistance to disease caused by specific microorganism or their toxic products. The immune system is the system of specialized cells and organs that protect an organism from diseases and infectious organisms. It is also called as the host defense system. Immunology is the study of all aspects of the immune system in an organism.

The concept of immunity can be traced back to 430BC, when Thucydides observed that individuals who had recovered from the plague would not get it a second time and could nurse other affected patients. The earliest recognised and written evidence of inducing immunity was practiced by the Chinese and Turks in the 15$^{th}$ Century. They either inhaled the dried crusts derived from small pox pustules or inserted them into wounds in the skin. Lady M.W. Montagu, the wife of the British ambassador to Constantinople, applied this technique on her own children and found the results to be positive. In 1798, a vaccine ( from the Latin word 'vacca' , meaning 'cow') for small pox was developed by Edward Jenner. He inoculated an 8-year old boy James Phipps with material obtained from a cowpox lesion. The results were conclusive of prevention of small pox infection. Friedrich Henle was the first to discover that germs caused disease and the isolation of infectious bacteria was done by his pupil Robert Koch. Louis Pasteur developed vaccine for chicken cholera, anthrax and rabies. Modern immunology begins with the research of Metchnikoff, who discovered the phenomenon of phagocytosis in starfish and extrapolated it to macrophages in humans as cells that engulf infectious agents.




Variolation was the method used to immunize individuals against smallpox by infecting them with substance from the pustules of patients.

## 10.2. INFECTION

Infection is defined as the invasion and multiplication of pathogenic organisms in the host. An infection without symptoms is represented as subclinical; and with symptoms it is represented as clinically apparent. Illness caused through pathogenic organisms is termed as infectious disease or communicable disease or transmissible disease.

### Epidemiology

Epidemiology is the branch of medical science that deals with the geographical distribution and timing of infectious disease occurrences. The study also includes the modes of transmission and maintenance in nature, with the goal of recognizing and controlling outbreaks.



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The spectrum of occurrence or prevalence of disease in a defined population includes sporadic, endemic, epidemic and pandemic.

**Sporadic** refers to a disease that occurs infrequently and irregularly without a geographic focus. Examples of sporadic diseases include tetanus, rabies, and plague.

**Endemic** disease is an infectious disease which is restricted to a population in a given geographical region only and the constant rate of presence for years.

**Epidemic** refers to an increase in the number of cases of a disease in a particular geographical region within a short span of time when compared to the previous year infection rate. Influenza (common cold) is a good example of a common epidemic disease.

**Pandemic** refers to an epidemic that has spread over several countries or continents, usually affecting a large number of people i.e. a wide geographical region. AIDS is an example for pandemic since it is present in many countries.

## Transmission

Transmission of an agent causing an infectious disease can be direct or indirect. The transfer of an infectious agent directly into the body is known as direct transmission. There are four types of **direct contact transmission**.

1. **Physical contact** between hosts (Influenza, Skin infections).
2. **Direct contact** with body fluids or tissues of an infected individual (HIV, HPV).
3. **Droplet contact** in which large infectious particles sprayed into the air from the respiratory tract of an infected individual (pneumonia, mumps, measles).
4. **Droplet nuclei contact** in which small infective dried droplet particles that are suspended in the air are taken in by a host, and are capable of traveling to the lung (TB, chickenpox).

**Indirect transmission** is the transfer of a pathogen by a vector or vehicle. Malaria is an example of a vector borne disease. Examples of diseases spread through vehicle-borne transmission are food-borne diseases and waterborne diseases eg: Cholera. Zoonosis occurs when diseases are transferred from animals to people. Zoonotic diseases include anthrax from sheep and plague from rodents.

## Etiology

Etiology is the study of cause or origin of disease. The etiologic agent or causative agent is responsible for the cause of a disease. A pathogen or infectious agent is a biological agent that causes disease or illness to its host organism. Pathogenic organisms are of five major types - bacteria, virus, fungi, worms, and protozoa.

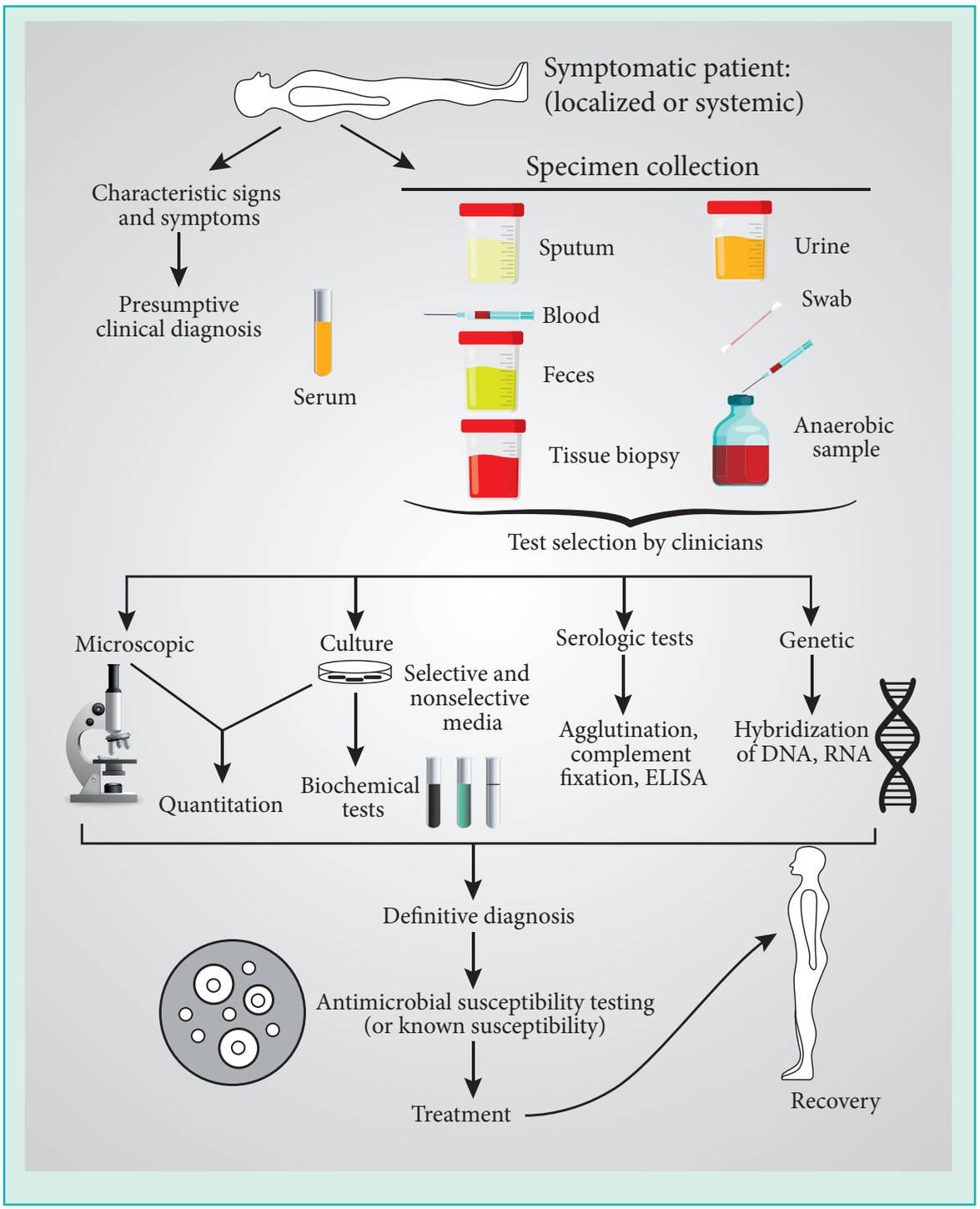
## Diagnosis

Laboratory tests may identify organisms directly (e.g., visually, using a microscope, growing the organism in culture) or indirectly (e.g., identifying antibodies to the organism). They use a sample of blood, urine, sputum, stool, throat swab or other fluid or tissue from



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the infected individual. This sample may be stained and examined under a microscope, cultured, tested for antibodies, tested for a microorganism's antigens or tested for genetic material (such as DNA or RNA) from the microorganism.




## Treatment and Prevention

Antibiotics, anti-virals, anti-fungals, and anti-parasitic agents along with quorum quenching methods are being used to treat infectious diseases depending upon the nature of infection. Many infectious diseases can be prevented by personal hygiene and vaccines.



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Pus is a thick protein rich fluid called as liquor puris. It consists of dead white blood cells and infected agents. It is a natural product formed during immunological reactions against infecting agents. It might be yellow or green or brown in colour and with foul odour. Appearance of pus at the site of surgery indicates an infection.

### 10.2.1. Bacterial Infections

Bacterial infections include any type of illness caused by bacteria. Based on the structure and shape,there are three major groups of bacteria namely, Bacillus (cylindrical forms), Coccus (spherical forms) and Spiral. Humans and animals have abundant normal flora (microbes) that usually do not produce disease under normal healthy condition. These bacteria are referred to as good bacteria or healthy bacteria or normal flora. Harmful bacteria that cause bacterial infections and disease are called pathogenic bacteria. Bacterial diseases occur when pathogenic bacteria enter into the body and begin to reproduce and to grow in tissues that are normally sterile. Harmful bacteria may also emit toxins that can damage the body.

| Name of Disease | Pathogen | Mode of transmission | Incubation Period | Symptoms | Therapy |
|---|---|---|---|---|---|
| Pulmonary Tuberculosis | *Mycobacterium tuberculosis* | Airborne and Droplet infection | 2-10 weeks | Coughing; chest pain and bloody sputum | Streptomycin, para-amino salicylic acid, rifampicin |
| Diphtheria | *Corynebacterium diphtheriae* | Airborne and Droplet infection | 2-6 days | Inflammation of mucosa of nasal chamber, throat etc. respiratory tract blocked | Diphtheria antitoxins, Penicillin, Erythromycin |
| Cholera | *Vibrio cholerae* | Direct and oral with contaminated food and water | 6 hours to 2 – 3 days | Acute diarrhoea and dehydration | Oral rehydration therapy and tetracycline |
| Leprosy | *Mycobacterium leprae* | Slowest, infectious and contagious | 2-5 years | Skin hypopigmentation, nodulated skin, deformity of fingers and toes. | Dapsone, rifampicin, Clofazimine |

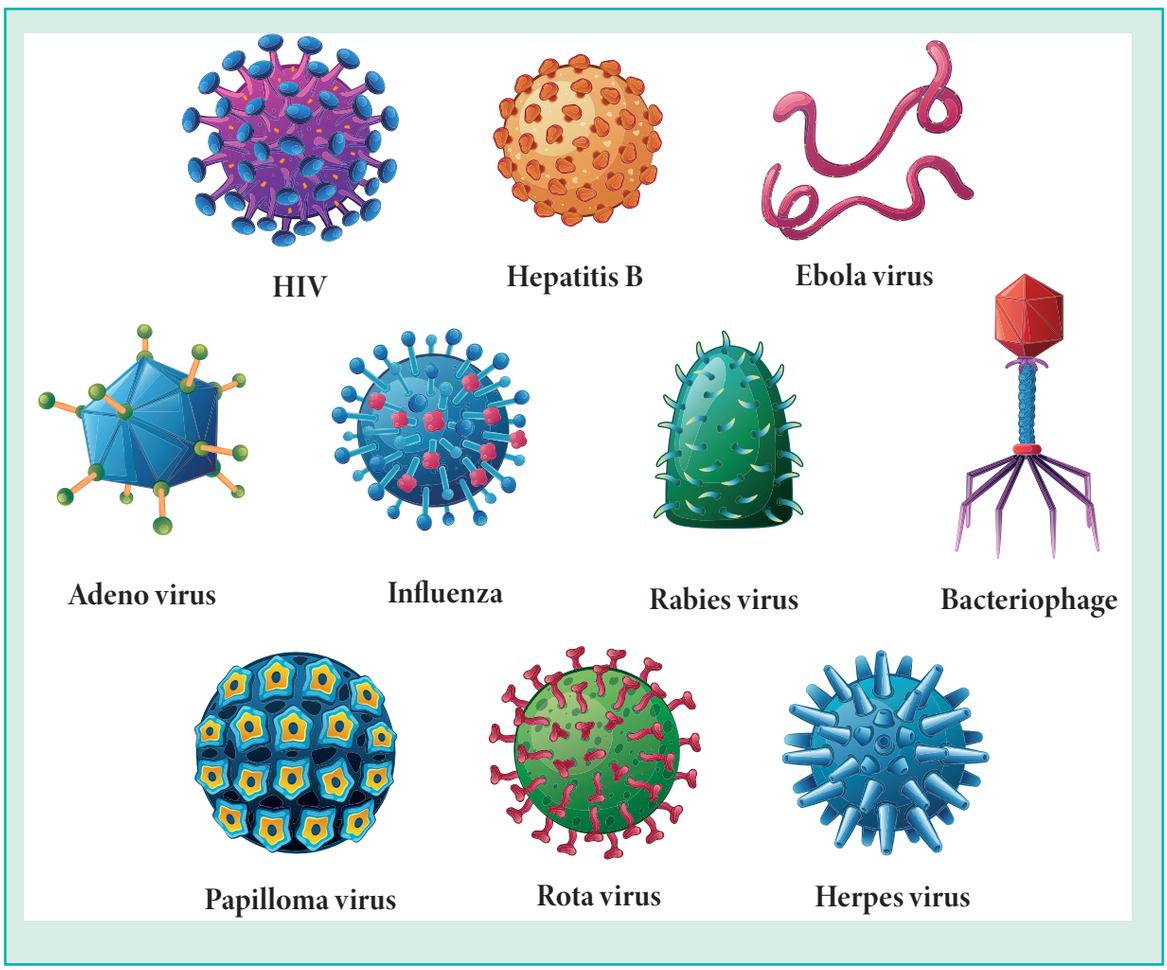


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| | | | | | |
|---|---|---|---|---|---|
| Tetanus (Lock Jaw) | *Clostridium tetani* | Through injury | 3-21 days | Degeneration of motor neurons, rigid jaw muscles, spasm , paralysis | Tetanus-antitoxins |
| Plague | *Yersinia pestis* | Indirect and inoculative (vector is rat flea) | 2-6 days | Bubonic plague affects lymph nodes; Pneumonic plague affects lungs and Septicemic plague causes anaemia | Tetracycline, streptomycin, Chloromycetin |

### 10.2.2. Viral Infections

Viruses are acellular obligate intracellular parasites. They contain only one type of nucleic acid, it may be either single or double stranded DNA or RNA. Viral diseases range from minor ailments such as the common cold to severe diseases such as Rabies and Acquired Immune Deficiency Syndrome (AIDS). They may be sporadic like Mumps, endemic like Infectious hepatitis, epidemic like Dengue fever or pandemic like Influenza.






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| Name of disesae | Pathogen | Mode of transmission | Incubation period | Symptoms | Therapy |
|---|---|---|---|---|---|
| Poliomyelitis | Polio-virus | Direct and oral | 7-14 days | Damages motor neurons causing stiffness of neck, convulsion, paralysis of generally legs | Physiotherapy |
| Measles | Rubella-virus | Contagious and Droplet infection | 10 days | Rubeola (skin eruptions), coughing, sneezing | Antibiotics and sulpha drugs |
| Mumps | Mumps-virus | Contagious and Droplet infection | 12-26 days | Painful enlargement of parotid salivary glands | Antibiotics |
| Rabies (Hydrophobia) | Rabies-virus | Indirect and inoculative | 10 days to 1- 3 months | Spasm of throat and chest muscles, fears from water, paralysis and death | Pasteur-treatment |
| Influenza (Flu) | Myxovirus influenzae | Air borne and pandemic | 24-48 Hours Lasts for 4-5 days | Bronchitis, sneezing bronchopneumonia, leucopenia, coughing | Antibiotic therapy |
| Hepatitis (Epidemic Jaundice) | Hepatitis-B virus | Direct and oral | 20-35 days | Damage to liver cells releasing bilirubin, jaundice | Hepatitis-B vaccine |
| Chikungunya | chikungunya virus | infected *Aedes aegypti* mosquitoes (vector) | 3 weeks | rash, muscle pain, fever and severe joint pain | Antipyretics, analgesics, fluids, and rest |
| Dengue fever | *Dengue virus* | infected *Aedes aegypti and Aedes albopictus* mosquitoes (vector) | 3 to 14 days | Fever, Headache, Rash appearing between the second and fifth day of fever, Platelets reduction | Intravenous (IV) fluids, acetaminophen |



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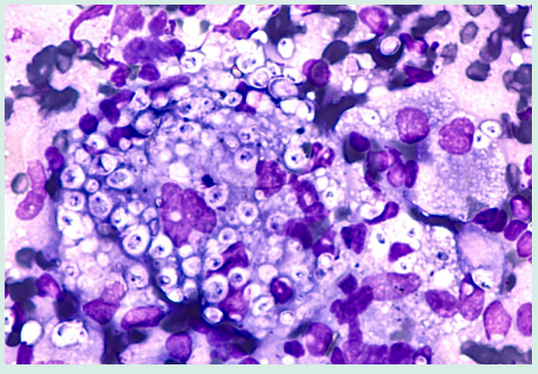
| | | | | | |
|---|---|---|---|---|---|
| AIDS | Human Immuno deficiency Virus (HIV) | Direct contact with infected blood, semen or vaginal fluids | 12 years | flu-like symptoms such as fever, sore throat and fatigue after few weeks of infection | anti-retroviral treatment (ARVT) |

### 10.2.3. Fungal Infections

Fungi are eukaryotic protista, recognized as causative agents of human disease earlier than bacteria. Fungal infections (mycosis) are most common among those patients who use antibiotics for prolonged period of time. These antibiotics not only kill pathogenic bacteria but also target the normal flora of human body (useful bacteria) and give rise to fungal growth. Human fungal infections are usually of two types: superficial and deep infection. Fungi causing superficial mycoses are specialized saprophytes, with the capacity to digest keratin. Superficial mycoses are of two types - surface infections (only on dead layers of skin) and cutaneous infections (cornified layer).



**Mucormycosis molds**



**Histoplasma fungi**

| Disease Name | Pathogen | Mode of transmission | Symptoms | Therapy |
|---|---|---|---|---|
| Candidiasis or yeast infections | *Candida* yeasts *Candida albicans* | Direct contact | itching and swelling, redness and soreness | Nystatin , Clotrimazole and fluconazole |
| Jock itch or Tinea cruris (Dermatophytoses) | *Trichophyton rubrum* and *T. mentagrophytes* | direct contact with an infected person | redness in the groin, buttocks, or thighs, itching | miconazole, clotrimazole, ketoconazole |



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| | | | | |
|---|---|---|---|---|
| Athlete's foot or Tinea pedis (Dermatophytoses) | *T. mentagrophytes, Trichophyton rubrum* | Direct contact | redness or blisters, peeling or cracking skin | topical antifungal ointments, itraconazole, terbinafine |
| Tinea capitis or scalp ringworm (Dermatophytoses) | *Trichophyton tonsurans, T. schoenleinii, T. violaceum* | Direct contact | round patches of dry scale, alopecia | Griseofulvin, Terbinafine |
| Mucormycosis | *Mucor and Rhizopus* | Air borne | Opportunistic pathogen | Amphotericin B |

## 10.3 IMMUNITY

Immunity refers to the ability of the immune system to defend against diseases caused by microbes or foreign substances which are products like toxins from microbes. Immunity depends upon various factors like host resistance, dosage of organism injected and virulence of the organisms.

### 10.3.1. Classification

Immunity is mainly classified into innate and acquired immunity. Innate or nonspecific or natural immunity refers to the basic resistance to disease that an individual is born with. Acquired or specific or adaptive immunity requires the activity of a functional immune system, involving cells called lymphocytes and their products. Innate defense mechanisms provide the first line of defense against invading pathogens until an acquired immune response develops. In general, most of the microorganisms encountered by healthy individual are readily cleared within a few days by non-specific defense mechanisms without enlisting a specific immune response. When an invading microorganism eludes the nonspecific host defense mechanism, a specific immune response occurs.

#### 10.3.1.1. Innate (Natural) Immunity

Innate immunity may be considered at the level of the species, race or individual. Species immunity refers to the total or relative refractoriness to a pathogen, shown by all members of a species. For instance, all human beings are totally insusceptible to plant pathogens and to many pathogens of animals such as rinderpest.. This immunity is something a person obtains by birth, for the reason that he belongs to the human species. The mechanisms of species immunity may be due to physiological and biochemical differences between the tissues of the different host species, which determine whether or not a pathogen can multiply in them.



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Within a species, different races may show differences in susceptibility to infections. This is known as racial immunity. It has been reported that the African-descendants in the USA are more susceptible to tuberculosis than the Americans. The differences in innate immunity exhibited by different individuals in a race are known as individual immunity. It is well documented that homozygous twins exhibit similar degrees of resistance or susceptibility to lepromatous leprosy and tuberculosis.

### 10.3.1.2. Components involved in Innate Immunity

Components involved in innate immunity include skin, mucus, cells like neutrophils, macrophages, natural killer cells and soluble factors like complements, cytokines, and acute phase proteins.

Skin and mucus provide anatomical barrier, cells like macrophages and neutrophils provide phagocytic barrier and soluble factors like complement, acute phase proteins provides physiological and inflammatory barriers.

### 10.3.1.3 Mechanisms involved in Innate immunity

Innate immunity is provided by four types of defensive barriers namely, anatomical, physiological, phagocytic and inflammatory barriers.

#### Anatomical Barriers

Anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms. The outer epidermis of skin contains several layers of tightly packed epithelial cells which prevents entry of pathogens. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The inner dermis layer of skin, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called sebum. Sebum consists of lactic acid and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms.

The conjunctivae, the alimentary, respiratory, and urogenital tracts are lined by mucous membranes. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. The viscous fluid called mucus, which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract, the mucous membrane is covered by cilia, the hair like protrusions of the epithelial cell membranes. The synchronous movement of cilia propels mucus-entrapped microorganisms from these tracts.

#### Physiological Barriers

The physiological barriers that contribute to innate immunity include temperature, pH, and various soluble and cell associated molecules. Many species are not susceptible



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to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents.

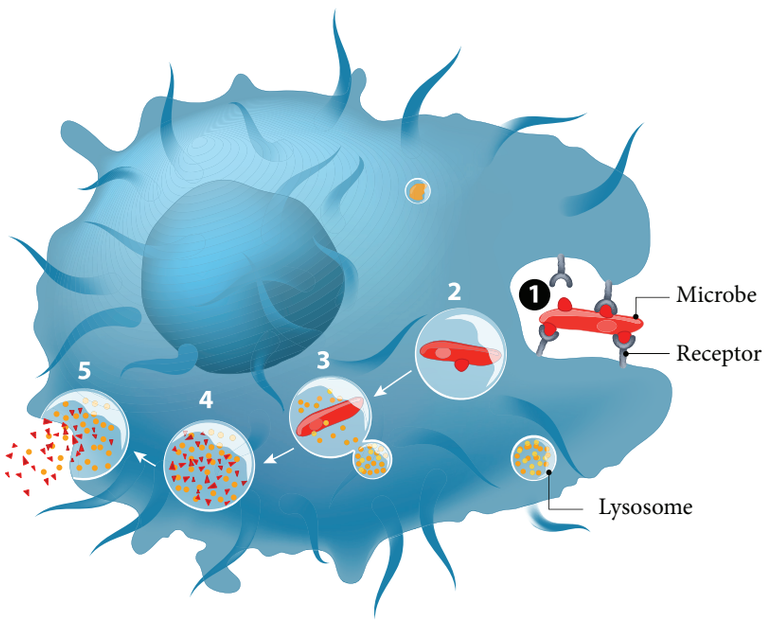
A variety of soluble factors contribute to innate immunity, among them are the soluble proteins lysozyme, interferon, and complement. Lysozyme, a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. Interferon comprises a group of proteins produced by virus-infected cells which prevents viral infection of neighbouring cells. Complements lyse bacteria by forming membrane attack complex.




The newborn babies (neonates) are susceptible to some infections that do not affect adults. The reason is that their stomach contents are less acidic i.e higher pH than those of adults. The pH variation provides optimum medium for the growth of pathogenic microorganisms.

## Phagocytic Barriers

Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis. The process of phagocytosis was discovered by Metchnikoff. The term phagocytic denotes the engulfment and digestion of whole cells. The two major cell types in the body which are associated with the engulfment and digestion of microorganism are the polymorphonuclear leucocytes and the macrophages. Minor cell types are the eosinophils. The process of phagocytosis involves the following steps:




1. Binding and absorption
2. Phagosome formation
3. Phagosome and lysosome fuse to form a phagolysosome
4. Digestion
5. Release of microbial products



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1. Attachment

   Attachment is the adherence of a bacterium to the cell membrane of the phagocytic cell. Some bacteria are easily attached to the phagocytic cell. Example , *Mycobacterium tuberculosis*.

2. Phagosome formation

   After attachment the phagocyte extend small pseudopodia around the infecting bacterium. The pseudopodia fuse to form an endosome which contains a bacterium surrounded by the cell membrane. This structure is called as phagosome.

3. Phagolysosome formation

   After engulfment, the lysosome containing the hydrolytic enzymes fuses with phagosome to form phagolysosome. In this step, lysosomal enzymes are discharged to phagosome which is vital for the lysis of bacteria.

4. Lysis

   A number of antimicrobial and cytotoxic substances produced by phagocytes can destroy phagocytosed microorganisms.

### Oxygen dependent killing mechanisms

During phagocytosis, a metabolic process known as the 'respiratory burst' occurs in activated phagocytes. This process results in the activation of a membrane bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. Activated phagocytes begin to express high levels of nitric oxide synthase (NOS), an enzyme that oxidizes L-arginine to yield L-citrulline and nitric oxide (NO). Much of the antimicrobial activity of phagocytes against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

### Oxygen independent killing mechanisms

Activated phagocytes also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated phagocytes produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins. Cathepsin G is an example for defensins. These molecules are cysteine-rich cationic peptides that form circularized defensin which inturn form ion-permeable channels in bacterial cell membranes to lyse bacteria. Lactoferrin chelates iron from the medium and prevents the growth and proliferation of iron dependent bacteria. Lysozyme splits mucopeptide in bacterial cell wall and lysed bacteria.

5. Exocytosis

   Finally the killed organisms are digested by hydrolytic enzymes and the degraded products are released to the exterior by the process of exocytosis.



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## Inflammatory Barriers

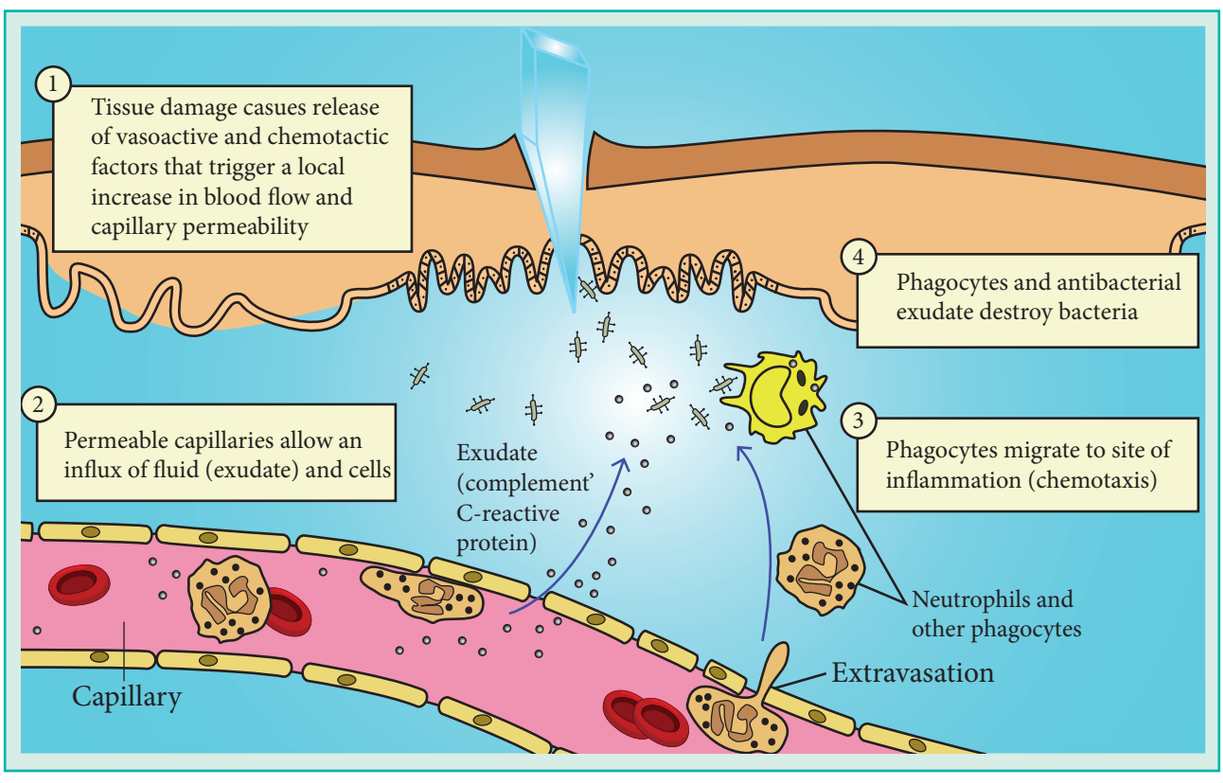
This barrier is created by the inflammatory response. Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. Inflammatory response is described by the "four cardinal signs of inflammation" as rubor (redness), tumor (swelling), calor (heat) and dolor (pain). Presently a fifth sign, functio laesa (loss of function),is included.

**The following are the sequential steps that occur during inflammation:**

Vasodilation occurs in nearby capillaries resulting in enlargement of the capillary network. The enlarged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.

An increase in capillary permeability facilitates an influx of fluid and cells from the enlarged capillaries into the tissue. The fluid that accumulates (exudate) has much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling (edema).

Influx of phagocytes from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels (margination), followed by their emigration between the capillary endothelial cells into the tissue (diapedesis or extravasation), and finally, their migration through the tissue to the site of the invasion (chemotaxis). As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.






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Inflammation that develops at the site of infection induces the acute phase response. This generalized response is characterized by fever, changes in vascular permeability and changes in biosynthesis, metabolism and catabolism in many organs. These changes result in a rise in the concentration of certain proteins in the blood and a drop in the concentration of other proteins. These proteins that are elevated termed as acute phase proteins or acute phase reactants. These include C- reactive protein (CRP), fibrinogen and serum amyloid A protein. The concentration of CRP in the blood increases from a normal level of 1 mg / ml to as much as 1000 mg / ml during the acute phase response. It functions in clearance of nuclear material released from killed microbes and killed host cells during inflammation by binding to DNA, chromatin and histones.

### 10.3.2. Acquired (Adaptive) Immunity

The form of immunity that is mediated by lymphocytes and stimulated by exposure to infectious agents is adaptive immunity. It reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign microorganisms and molecules. It is characterized by four characteristics namely antigenic specificity, diversity, immunologic memory, and self and non-self recognition.

#### Antigenic specificity

Immune responses are directed toward and able to distinguish between distinct antigens or small parts of macromolecular antigens. This fine specificity is attributed to lymphocyte antigen receptors that may bind to one molecule but not to another with only minor structural differences from the first. Antibodies can differentiate between two molecules that differ by only a single aminoacid.

#### Diversity

Diversity is the result of variability in the structure of the antigen binding sites of lymphocyte receptors for antigens. Diversity allows the adaptive immune system to specifically recognize billions of uniquely different structures on foreign antigens.

#### Immunologic memory

Immunologic memory is mediated by memory cells. Memory cells are clonally expanded progeny of T and B cells formed during the primary response following initial exposure to antigen. Memory cells are more easily activated than naive lymphocytes and mediate secondary response on subsequent exposure to antigen. They survive in a functionally quiescent state for many years, even after the elimination of the antigen. Due to this attribute, the immune system can confer life long immunity to many infectious agents.

#### Self and non-self recognition

The immune system is able to distinguish self (host) from nonself (foriegn) antigens and respond only to non-self molecules. The production of immune cells only against non- self molecules, is achieved by selection procedure like positive selection and negative



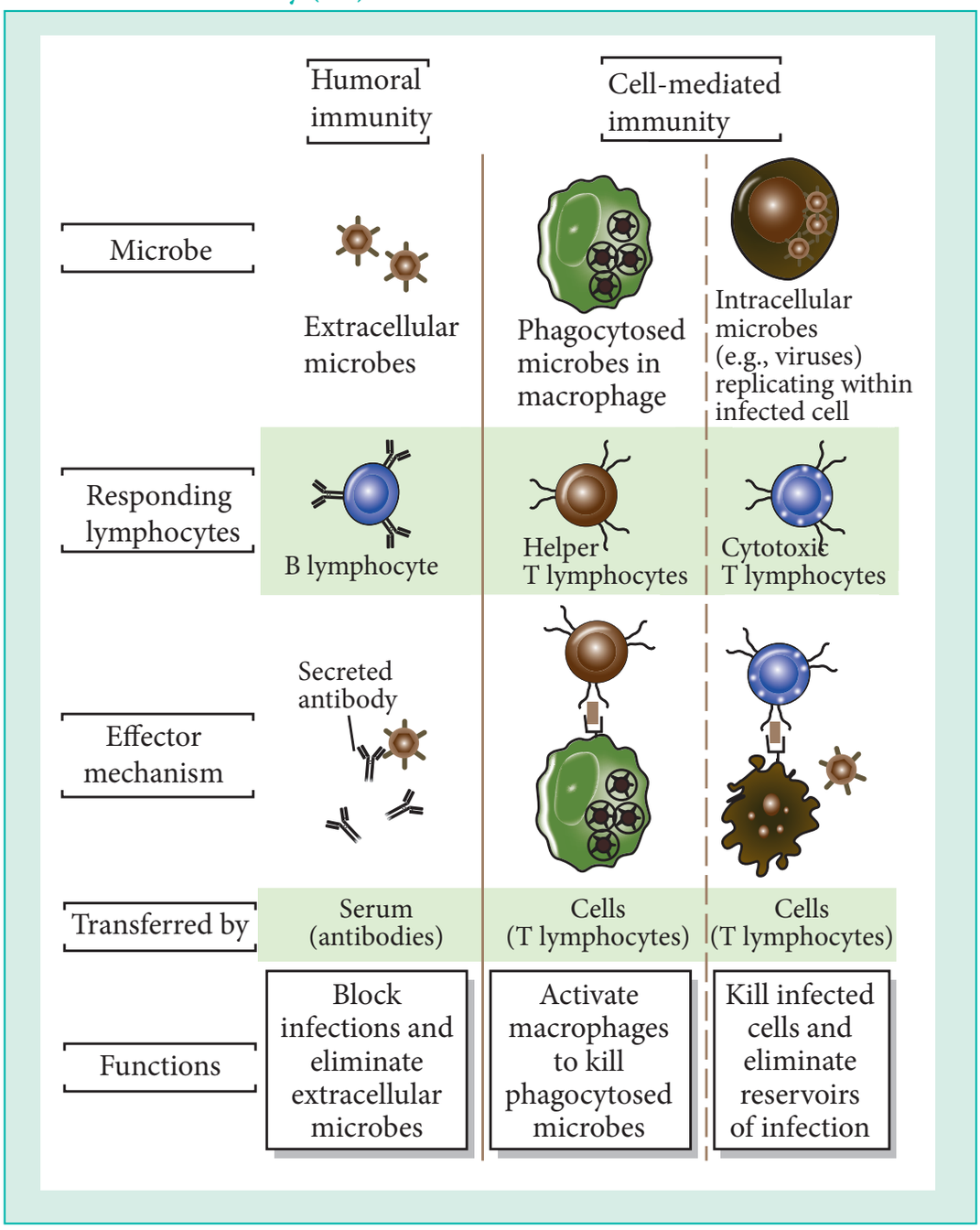
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selection during the maturation process of lymphocytes in bone marrow and thymus.

Acquired immunity does not occur independent of innate immunity and vice versa. For example, the phagocytic cells crucial to nonspecific immune responses are intimately involved in activation of the specific immune response. Similarly, the soluble factors produced during a specific immune response have been shown to augment the activity of these phagocytic cells.

Acquired immunity, on the basis of components involved in immunity is classified into two types namely, Humoral immunity and Cell mediated immunity.

### 10.3.2.1 Humoral Immunity (HI)






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**An overview of Acquired Immunity - Humoral and Cell mediated Immunity**

The term humoral is derived from the Latin word humor meaning "body fluid". Thus humoral immunity refers to immunity that can be conferred on a non-immune individual by administration of serum antibodies from an immune individual. Humoral immunity is mediated by molecules in blood and mucosal secretions containing antibodies that are produced by cells called B lymphocytes.

Antibodies recognize microbial antigens, neutralize infectivity of the microbes and target microbes for elimination by various effector mechanisms. Antibodies themselves are specialized and different types of antibodies may activate by different mechanisms, for example, IgG and IgM antibodies promote phagocytosis and IgE antibodies trigger the release of inflammatory mediators from leukocytes such as mast cells. Binding of antibody to antigen on a microorganism also can activate the complement system, resulting in lysis of the microbes.

Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination.

### 10.3.2.2 Cell Mediated Immunity (CMI)

Cell Mediated immunity (cellular immunity) is mediated by T lymphocytes. There are two types of T-cells mainly TH and TC which differ by their surface marker CD4 and CD8, respectively. T-cells are activated by antigen presenting cells (APC) after processing antigens. Both activated TH cells and TC cells serve as effector cells in CMI. Cytokines secreted by TH cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. Cytotoxic T lymphocytes (CTL) participate in CMI by killing altered self cells. They also play an important role in the killing of virus infected cells and tumor cells.

Intracellular microbes such as virus and some bacteria survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell mediated immunity.




Antigen presenting cells are a functionally defined group of cells which are able to take up antigens and present them to T lymphocytes. Eg: Dendritic cells, activated macrophages, and activated B-cells.

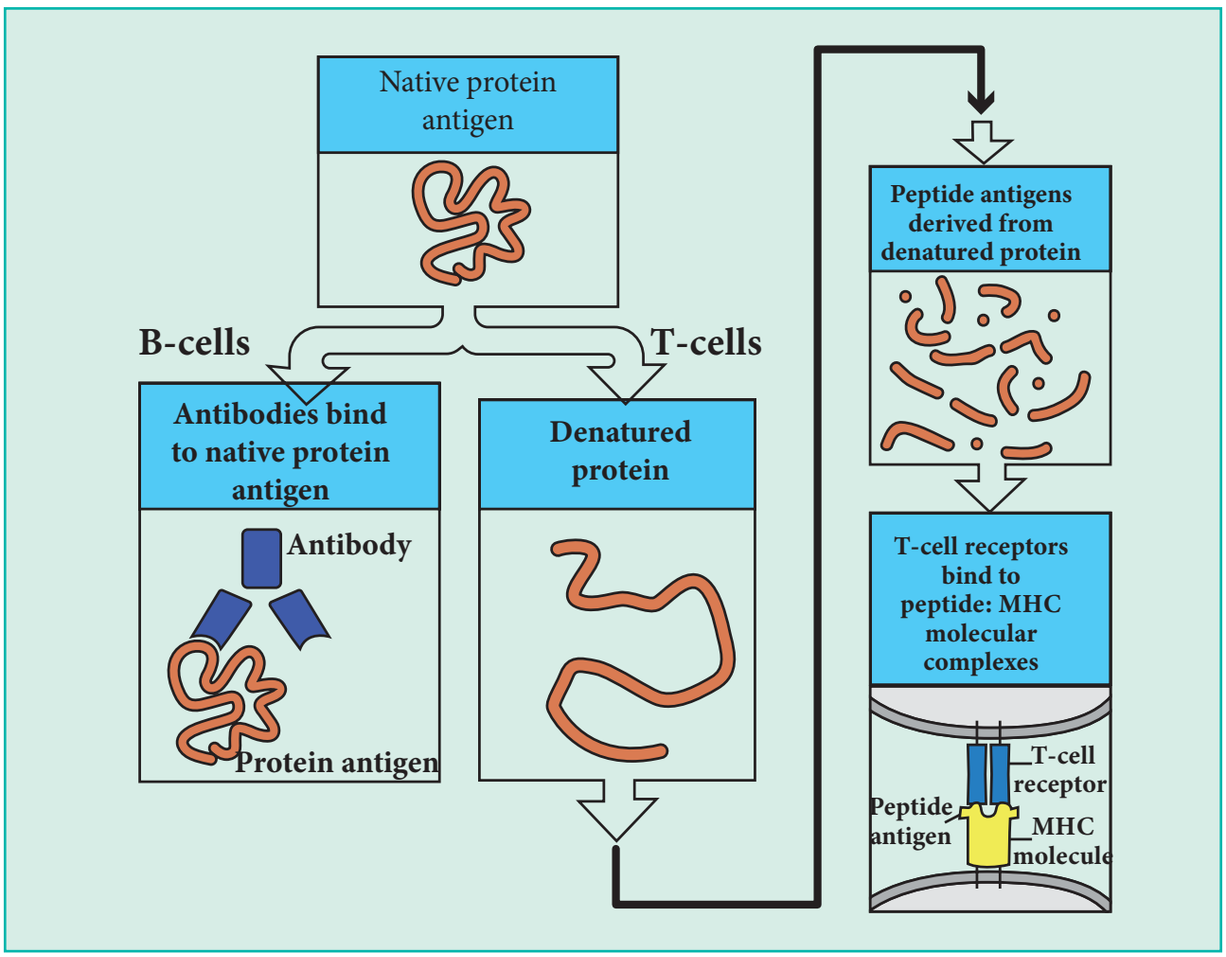
A collective and coordinated response to the introduction of foreign substances in an individual, mediated by the cells and molecules of the immune system is referred to as immune response. An adaptive immune response that occurs upon the first exposure of native lymphocytes with a foreign antigen is known as primary immune response. An adaptive immune response that occurs upon the second or subsequent encounter of primed lymphocytes with a given antigen is known as secondary immune response.



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## 10.4. ANTIGENS

Antigens are foreign substances that have an ability to induce antibody generation. Antigenic determinant or epitope is the region of the antigen recognized by antibodies or T-Cell Receptor (TCR) of T cells. There are two types of epitopes namely B cell epitope and T cell epitope. B cell epitope is the region of antigen recognized by antibodies. T cell epitope is the region of antigen recognized by TCR of T cells.




### 10.4.1. Types of antigens

| Sl. No. | Types of Antigens | Nature | Example |
|---|---|---|---|
| 1. | Sequestered antigens | These antigens are secluded or sequestered in capsule i.e. it is not exposed to immune system during development or when produced. | Lens proteins and sperm proteins. |
| 2. | Neoantigens | They are newly produced antigens i.e. normal agents become antigens. They are formed due to the change in the chemical, physical and biological status of the agents. | Penicillin can be converted to neoantigen when it is bound with protein. |



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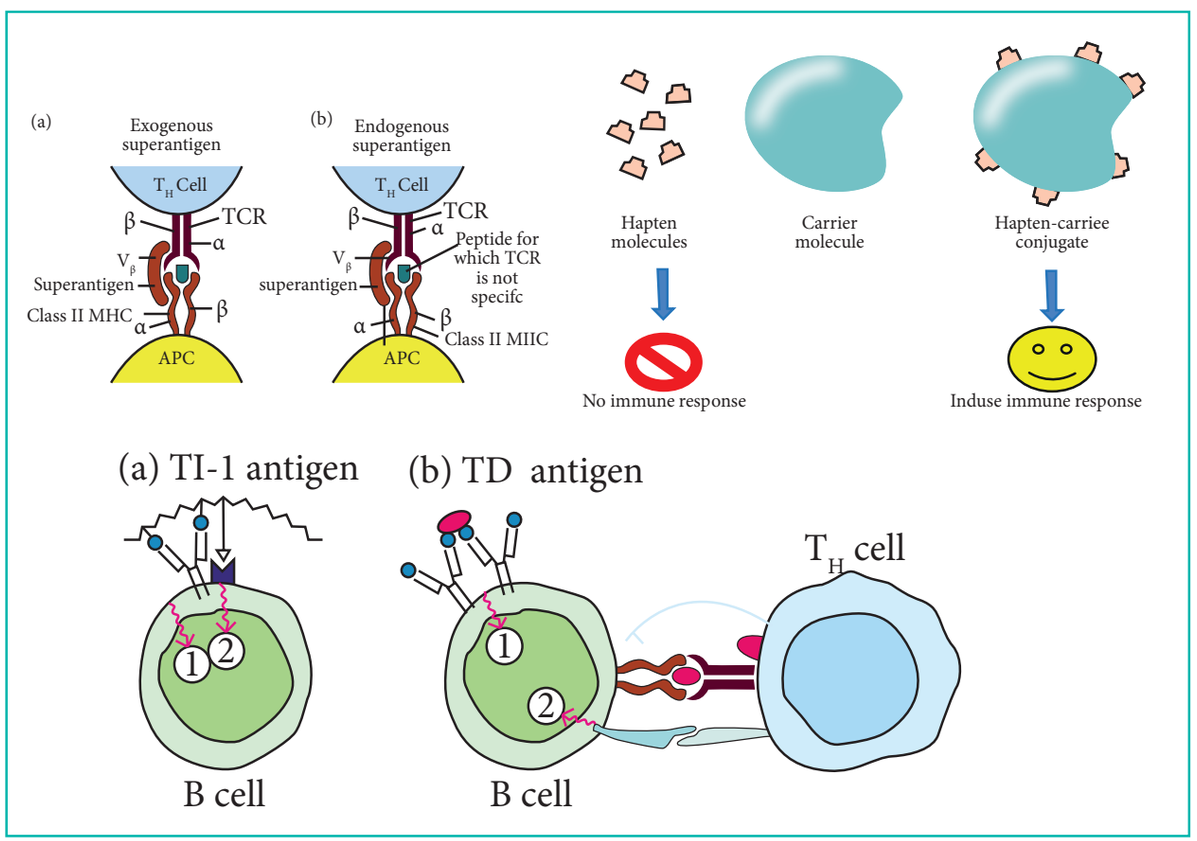
| | | | |
|---|---|---|---|
| **3.** | Heterophile or Heterogenic or Cross reactive antigen | These antigens interact with antibodies produced against another antigen. | Forssman antigen. These antigens are present on mucosal cells of GI tract of human and RBCs of horse. |
| **4.** | Mitogenic antigens | Since some mitogens (they induce cell division) activate immune cells, they can be considered as mitogenic antigens. | Lipopolysaccharides induce B cell proliferation, Concanavalin – A (CON A) and Phytohemagglutinin (PHA) induce T cell proliferation and Pokeweed mitogen (PWM) induces both T and B cell proliferation. |
| **5.** | Superantigens | These antigens activate T cells non-specifically. | TSST-1 (Toxic shock syndrome toxin – 1) produced from *Staphylococcus sp*. . - Exogenous superantigens<br><br>Mouse mammary tumor viral (MMTV) antigens - Endogenous superantigens |
| **6.** | Exogenous antigens | These antigens are usually secretory products of microbes or soluble antigens. They are usually present outside the host cell | Diphtheria toxin from *Cornebacterium diptheriae* |
| **7.** | Endogenous antigens | They are usually microbes which are present inside the cell or particulate antigen. | Virus and endotoxins |

| **Sl. No.** | **Types of Antigens** | **Nature** | **Example** |
|---|---|---|---|
| **8.** | T cell dependent antigens | These antigens require T cells especially $T_H$ cells to induce immune response. | Soluble antigens |



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| | | | |
|---|---|---|---|
| **9.** | T cell independent antigens | These require T cells partially or not to induce immune response. T cell independent antigen – I (TI-1) does not require T cell to induce immune response. T cell independent antigen – 2 (TI-II) does not require direct contact of T cells but cytokines produced by them to induce immune response. | Lipopolysaccharides – Example for TI-I Cell wall polysaccharides and glycoproteins – Example for TI-II. |
| **10.** | Allergens | Antigens responsible for allergic response are called allergens. | Pollens |
| **11.** | Autogens | Those antigens which are capable of inducing autoimmune disorders are known as autogens. | Immunoglobulins for Rheumatoid Arthritis. |
| **12.** | Self antigens | They are not originally antigens. They are normal cell surface components and proteins of normal host. | Host proteins |
| **13.** | Haptens | Haptens are otherwise known as incomplete antigens or partial antigens because they are unable to elicit immune response by itself, but they can gain this ability when they bind with a carrier molecule. | Dinitrophenol (DNP) |






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### 10.4.2. Factors influencing the antigenicity of antigens

Ability of an antigen to induce an immune response is known as antigenicity. The factors that influence antigenicity are mainly of two types i.e. factors contributed by antigens and factors contributed by host cells.

### Factors contributed by Antigens:

The factors are: size, foreignness, chemical nature, complexity, heterogeneity and susceptibility for antigen processing.

### Size

Antigens with greater than 10,000 Daltons molecular weight are found to be effective antigens. For example: Hemoglobin is more antigenic than penicillin.

### Foreignness (Alien)

It refers to the distance in the phylogenetic tree i.e. evolutionary distance. Highly distant organisms are found to have more antigenicity than less distant ones. For example: Protein from gorilla is found to have less antigenicity than the protein from fish to humans because fish is more evolutionarily distant from gorilla.

### Chemical Nature

Depending on the chemical nature, antigenicity varies.; For example, proteins have greater antigenicity than carbohydrates, lipids and nucleicacids. Solubility also plays an important role. Less soluble antigens have more antigenicity.

### Complexity

As the complexity of the antigens increase antigenicity also increases. For example, Primary structure of proteins has lesser antigenicity than tertiary structure of the same protein.

### Heterogeneity

In the case of multimeric proteins, heteromultimeric proteins have more antigenicity than homomultimeric proteins. This is mainly because of presence of the different types of epitopes in heteromultimeric proteins.

### Susceptibility for Antigen Processing

Those antigens which are found to be easily processed by Antigen Presenting Cells (APCs), have greater antigenicity than those antigens which are not easily processed. For example: Horse RBCs have greater antigenicity than asbestos.

### Factors contributed by Host

These factors include route of entry, genotype and dose.



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### Route of entry

Route of entry is also important to provide antigenicity and disease. If a microorganism enters through an adverse route, it is degraded by the immune mechanism and is less antigenic. When it enters through its natural route, it will cause disease and it also escapes from the immune system partially. For example: *Vibrio cholerae* entering through the circulatory system will not cause diarrhea; but if it enters through GI tract, it causes diarrhea.

### Genotype

The genetic constitution (genotype) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For instance, human beings are protected against some diseases, yet affected by other diseases. It purely depends upon the genotype.

### Dose

To induce antigenicity, an optimum amount of infecting agent or antigen is required and this optimum amount is known as optimum dose. When microbes enter above and below this optimum dose level, fluctuation occurs in antigenicity.

## 10.5. ANTIBODIES (IMMUNOGLOBULINS)

Antibodies are proteins secretedby B-cells. Antibodies combine chemically with substances which the body recognizes as alien, such as bacteria, virus, and foreign substances in the blood.

Antibodies protect the host against potential parasites by:

1) directly inhibiting binding sites of virus or various enzymes and toxins produced by bacteria,
2) agglutination,
3) opsonization which facilitates removal by phagocytes,
4) lysis of susceptible organisms via complement fixation, and
5) inducing inflammation.

### 10.5.1. Antibody structure

Antibodies are classified into five types. They are IgG, IgA, IgM, IgD and IgE.

The general structure of antibodies are explained with the example of IgG. The IgG molecule is composed of two identical heavy (H) chains and two identical light (L) chains. The H and L chains are linked by disulfide bridges.

The H chain contains four or five domains. Each domain of immunoglobulin molecule consists of 110 amino acid residues. The amino terminal of heavy chain , shows great sequence variation to other antibodies for different antigens and was therefore called the variable (V) region. The remaining part of the heavy chain is called as constant (C) region. Each of the different heavy chain constant region sequences is called an isotype. The



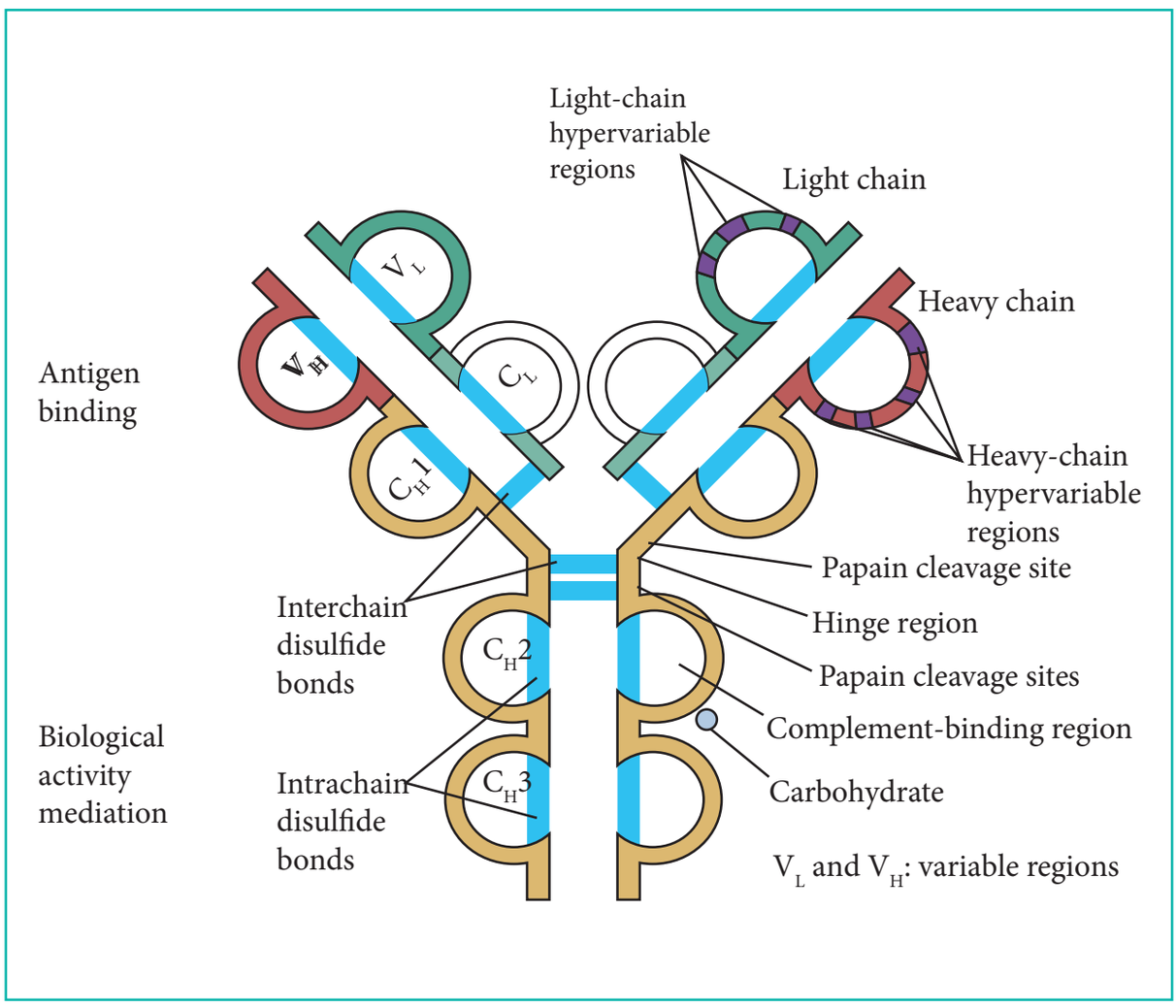
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length of the constant region is approximately 330 amino acids for gamma (IgG), delta (IgD) and alpha (IgA) and 440 amino acids for mu (IgM) and epsilon (IgE). In humans there are two subclasses of alpha heavy chains and four subclasses of gamma chains .

Light chain domain consisting of 110 amino acids was found to have varying amino acid sequences in different antibodies for different antigens at amino terminal end. This region was called as the variable (V) region. The carboxy terminal half of the molecule, is called the constant (C) region. There are two types of light chains , kappa and Lambda . In humans 60% of the light chains are kappa and 40% are lambda. A single antibody molecule contains either kappa chains or lambda chains, but never both.

The gamma, delta, and alpha heavy chains contain an extended peptide sequence between the $C_{H1}$ and $C_{H2}$ domains. This region, called the hinge region**,** is rich in proline residues and is flexible.

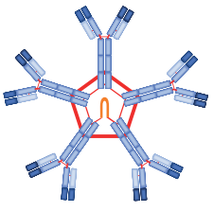
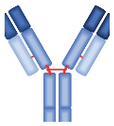
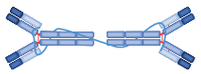
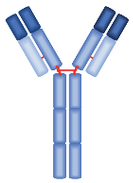
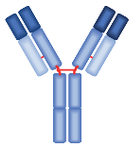
A paratope (antigen-binding site) is a part of an antibody which recognizes and binds to an antigen. It is a small region of the antibody's variable region, and contains parts of the antibody's $V_H$ domain of heavy and $V_L$ domain of light chains. This region is also called as complementarity determining region (CDR).






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### 10.5.2. Types of Antibodies - Classification

| The Five Immunoglobulin(Ig) Classes | | | | | |
|---|---|---|---|---|---|
| | IgM Pentamer | IgG Monomer | Secretory IgA Dimer | IgE Monomer | IgD Monomer |
| |  |  |  Secretory Component |  |  |
| Heavy chains | μ | γ | α | ε | δ |
| Number of antigen binding sites | 10 | 2 | 4 | 2 | 2 |
| Molecular weight (Daltons) | 900000 | 150000 | 385000 | 200000 | 180000 |
| Percentage of total antibody in serum | 6% | 80% | 13% | 0.002% | 1% |
| Crosses placenta | No | Yes | No | No | No |
| Fixes complement | Yes | Yes | No | No | No |
| Fc binds to | | Phagocytes | | Mast cells land basophils | |
| Function | Main antibody of primary responses, best at fixing complement, the monomer form of IgM serves as the B cell receptor. | Main blood antibody of secondary responses, neutralizes toxins, opsonisation | Secreted into mucus, tears, Saliva, Colostrum | Antibody of allergy and antiparasitic activity | B Cell receptor |

## 10.6 ANTIGEN-ANTIBODY REACTIONS

Antigen-Antibody reactions refer to the interaction between antigens and antibodies. The reactions between antigens and antibodies occur in three stages.

The primary stage is the initial interaction between antigen and antibody without any visible effects.



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The secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization of toxins and other biologically active antigens, fixation of complement, immobilization of motile organisms and enhancement of phagocytosis.

The tertiary stage reactions lead to neutralization or destruction of antigens which might lead to tissue damage. They include humoral immunity against infectious disease as well as clinical allergy and other immunological disease.

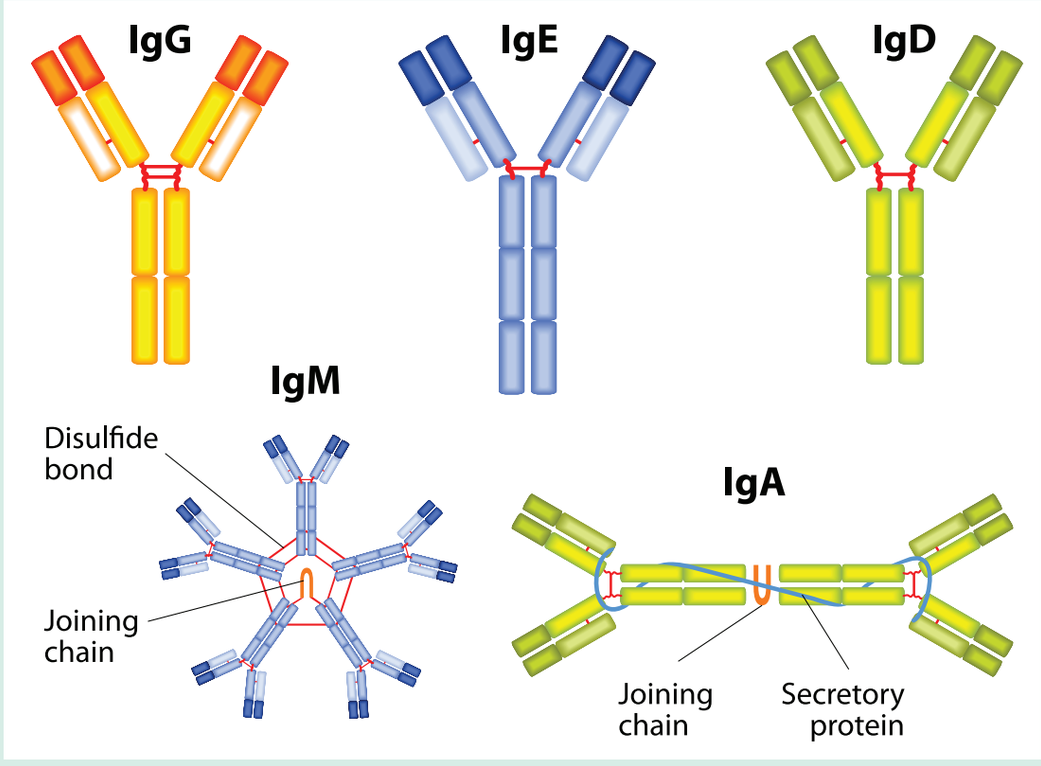
Antigen-antibody reactions have the following general characteristics:

1. The reaction is specific. However cross reaction may occur.
2. Entire molecules react and not fragment.
2. There is no denaturation of the antigen or the antibody during the reaction.
3. The combination occurs at the surface.
4. The combination is firm but reversible. The firmness of the union is influenced by the affinity and avidity of the reaction.

Affinity refers to the intensity of attraction between single epitope of the antigen and paratope of antibody molecules.

Avidity is the total strength of the bond after the formation of the antigen antibody complexes. Generally IgG possess greater affinity and IgM possess higher avidity

5. Both antigen and antibodies participate in the formation of agglutinates or precipitates.
6. Antigens and antibodies can combine in varying proportions.



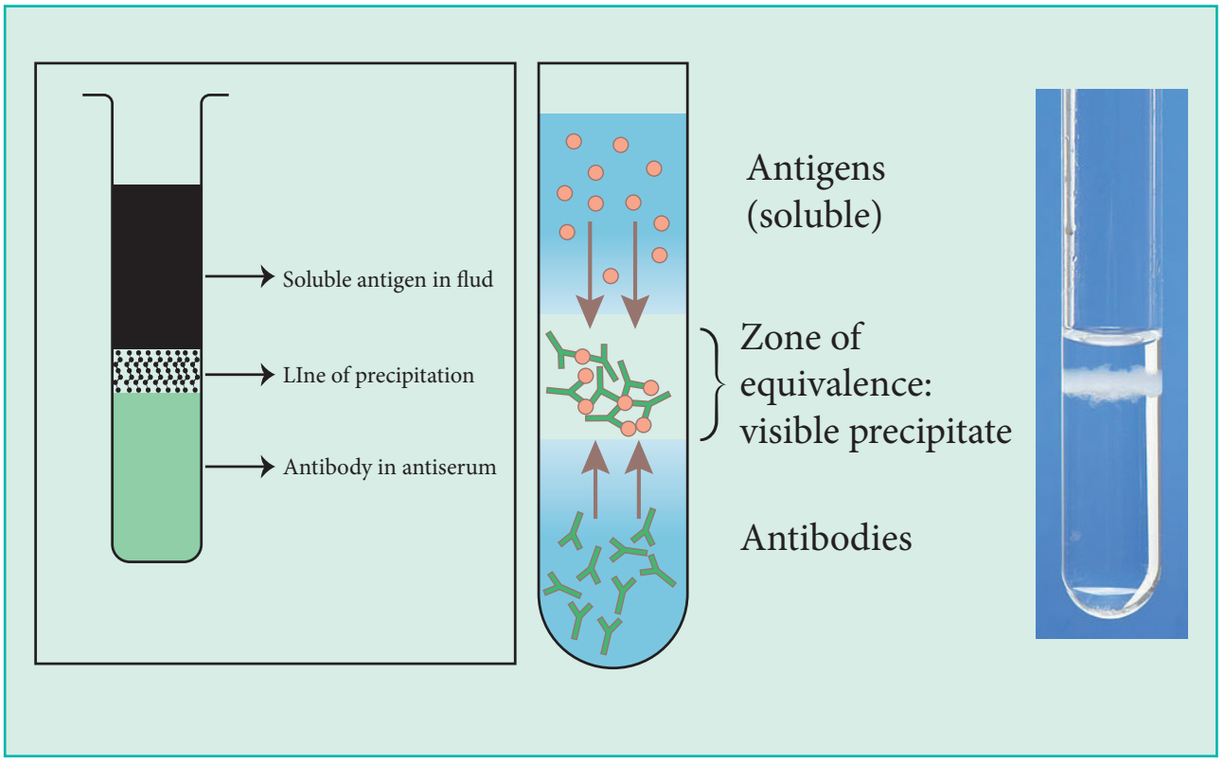



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### 10.6.1. Precipitation

The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops a visible precipitation. Antibody that forms precipitation is known as precipitin. This process is called as precipitation reaction. Formation of an Ag-Ab lattice depends on the valency of both antigen and antibody. Zone of equivalence is a point at which the maximum precipitation occurs. This reaction is widely used in several immunological techniques.

**Ring test**: In this test, over a column of antiserum (antibody), the antigen solution is layered. A precipitate forms at the junction of the two liquids.




### 10.6.2. Agglutination

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the cross linking of polyvalent antigens. Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the prozone effect.

#### Haemagglutination (Slide Agglutination)

In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions. At neutral pH, red blood cells are surrounded by a negative ion cloud that makes the cells repel one



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another.This repulsive force is called zeta potential. Because of its size and pentameric in nature, IgM can overcome the zeta potential and cross link red blood cells, leading to agglutination. The smaller size and bivalency of IgG makes it less able to overcome the zeta potential. For this reason, IgM is more effective than IgG in agglutinating red blood cells.

### Bacterial Agglutination (Tube Agglutination)

A bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions.

Widal test is used for the diagnosis of typhoid fever. In typhoid patients, the serum contains antibodies to *Salmonella typhi*. In Widal test, two antigens are used. They are antigen H, the flagellar antigen and O antigen, the somatic antigen. When antiserum of the patient is added to the antigens, the antigens are clumped and identified.

### Antiglobulin Test (Coombs Test):

The antiglobulin test was devised by Coombs, Mourant and Race for the detection of anti-Rh antibodies that do not agglutinate Rh positive erythrocytes in saline. When sera containing incomplete anti-Rh antibodies are mixed with Rh positive red cells, the antibody globulin coats the surface of the erythrocytes, though they are not agglutinated. When such erythrocytes coated with the antibody globulin are treated with a rabbit antiserum against human gammaglobulin (antiglobulin or Coombs serum), the cells are agglutinated. This is the principle of the antiglobulin test.

## 10.7. BLOOD GROUPS

A blood type is a classification of blood, based on the presence and absence of antibodies in blood and inherited antigenic substances on the surface of red blood cells. These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system.

### 10.7.1. ABO System

Karl Landsteiner discovered ABO blood group system. He was awarded Noble Prize in 1930 for his discovery. The membranes of human red cells contain a variety of blood group antigens,which are also called **agglutinogens.** The most important and best known of these are the A and B antigens. Antibodies against red cell agglutinogens are called **agglutinins.** Antibody-A and Antibody-B are examples of agglutinins. Landsteiner law states that if an agglutinogen is present on the RBC of an individual, the corresponding agglutinin must be absent in the plasma of that individual and vice-versa.

There are four main blood groups defined by the ABO system:

Blood group A people have A antigens on the red blood cells with anti-B antibodies in the plasma. Blood group B people have B antigens with anti-A antibodies in the plasma



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Blood group O people have no antigens, but both anti-A and anti-B antibodies in the plasma. Blood group AB people have both A and B antigens, but no antibodies

### Rh System

Another group of antigens found on the red blood cells of people is the Rh factor (Rhesus monkey, in which these antigens were first discovered). The Rh blood group system was discovered in 1940 by Karl Landsteiner and A.S. Weiner. It is the second most important blood group system, after the ABO blood group system. There are five different antigens (C, D, E, c and e) in this group, but D antigen predominantly presents and is medically important. Thus Rh antigen is always referred to as the Antigen-D. If this Rh antigen is present on a person's red blood cells, the person is Rh positive; if it is absent, the person is Rh negative.

Thus the common major blood group systems are of eight blood groups:

A RhD positive (A+)

A RhD negative (A-)

B RhD positive (B+)

B RhD negative (B-)

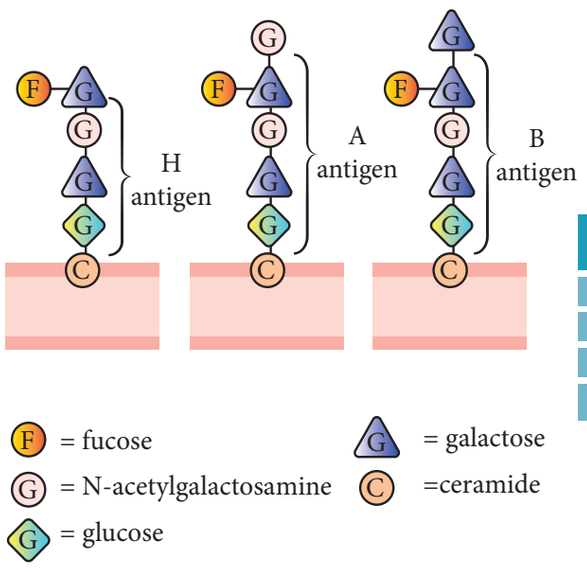
O RhD positive (O+)

O RhD negative (O-)

AB RhD positive (AB+)

AB RhD negative (AB-)

### 10.7.2. Antigens and Natural antibodies of ABO blood groups




| Genotype | Antigen on RBCs | Antibody in Plasma |
|---|---|---|
| $I^AI^A$, $I^Ai$ | A | Anti-B |
| $I^BI^B$, $I^Bi$ | B | Anti-A |
| ii | O | Anti-A and anti-B |
| $I^AI^B$ | AB | Neither anti-A and anti-B |



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Each person inherits two genes (one from each parent) that control the production of the ABO antigens. The genes for A or B antigens are dominant to the gene for O. The O gene is recessive, simply because it does not code for either the A or the B red blood cell antigens. The genes for A and B are often shown as $I^A$ and $I^B$ and the recessive gene for O is shown as the lowercase i. A person who is type A, therefore, may have inherited the A gene from each parent ($I^A$ $I^A$), or the A gene from one parent and the O gene from the other parent ($I^A$i). Likewise, a person who is type B may have the genotype $I^BI^B$ or I $^B$i. It follows that a type O person inherited the O gene from each parent , whereas a type AB person inherited the A gene from one parent and the B gene from the other ($I^A$ $I^B$).

The immune system exhibits tolerance to its own red blood cell antigens. People who are type A, for example, do not produce anti-A antibodies. However, they do make antibodies against the B antigen and, conversely, people with blood type B make antibodies against the A antigen. This is believed to result from the fact that antibodies made in response to some common bacteria present in the gut (normal flora) which are similar to A or B antigens. People who are type A, therefore, acquire antibodies that can react with B antigens by exposure to these bacteria, but they do not develop antibodies that can react with A antigens because tolerance mechanisms prevent this. People who are type AB develop tolerance to both of these antigens, and thus do not produce either anti-A or anti-B antibodies. Those who are type O, by contrast, do not develop tolerance to either antigen; therefore, they have both anti- A and anti-B antibodies in their plasma.

**Isoantibodies:** Iso means belonging to the same species. Isoantibody is an antibody produced by one individual that reacts with the antigen of another individual of the same species. Antibody – A and Antibody– B are called Isoantibodies. Both of these anti-A and anti-B antibodies are of Ig M type. Rh antibody (Antibody - D) is of Ig G type.

**Isoantigens:** An antigen of an individual which is capable of eliciting an immune response in individuals of the same species who are genetically different and who do not possess that antigen is called isoantigen. It is also otherwise known as alloantigens. A-Antigen and B-Antigen are examples of isoantigens.

**Natural antibodies:** Humans form antibodies against the blood group antigens they do not express.These antibodies are called naturally occurring antibodies or isoagglutinins. Antibody production starts at 3 months of age, reaches its highest level during adult and decreases with advancing age.

## Test Procedure

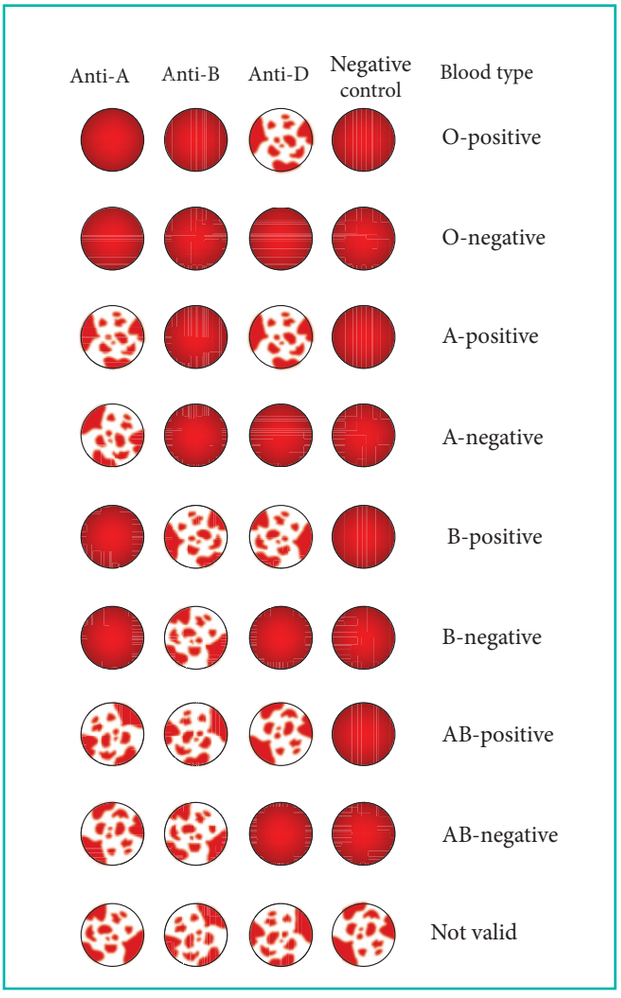
Basically, a sample of blood is mixed separately with anti-A antibodies, anti-B antibodies and Rh antibodies. If the red cells clump together with anti-A antibodies, then it indicates the presence of A antigens in the red blood cells and the person belongs to A group. Similarly, if agglutination reaction occurs with anti-B antibodies then it indicates the presence of B antigen.

When agglutination is found in both anti-A and anti-B antibodies it indicates that the person belongs to AB group. If no agglutination is found with both antibodies of A and B



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then it indicates the absence of antigens and the person belongs to O group. Similarly if an anti-Rh antibody shows agglutination with the given blood then it indicates the presence of Rh antigens on the blood cells. Hence the person is Rh positive. If no agglutination is seen then the person is Rh negative.




## Activity 10.1

Prepare the flow chart of the history of immunology

## Activity 10.2

Cut an apple in half. Cover one half of the apple with food wrapper and leave the other half uncovered. Using a dropper, release several drops of food coloring on each half of the apple. Answer the following questions: What happened to the uncovered half of the apple and to the covered half? How does the food wrapper provide a model of the human skin?

## Activity 10.3 Creating Personal Health Record

You can check on the status of your health habits by creating a personal health record for your own use.



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Taking Care of Your Immune System

| S.No. | Activities |
|---|---|
| 1. | Eat a well-balanced and healthy diet. |
| 2. | Get plenty of exercise and rest. |
| 3. | Brush your teeth and bathe or shower regularly |
| 4. | Keep your home clean. |
| 5. | Avoid tobacco, drugs and alcohol. |
| 6. | Get vaccinations that prevent diseases. |

**Do the following activities:** Look at the list of behaviors in the table above. Write each of the behaviors at the top of a separate piece of paper. Write down your habits related to each behavior during a typical week. Do you think that your weekly habits are healthy?

## Activity 10.4

On a separate sheet of graph paper, graph the data of antibody production given below. You should assume that on 0th day, the body was invaded by an unknown antigen. Then you can also assume that the person was exposed for a second time to the same antigen on day 40.

| Time | Antibody Units | Time | Antibody Units | Time | Antibody Units | Time | Antibody Units |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 16 | 80 | 32 | 0 | 48 | 150 |
| 4 | 10 | 20 | 20 | 36 | 0 | 52 | 300 |
| 8 | 70 | 24 | 24 | 40 | 0 | 56 | 260 |
| 12 | 120 | 28 | 0 | 44 | 40 | 60 | 200 |

## Answer the following questions from the graph:

How does the first part of the graph (days 0-28) compare to the second part of the graph (days 28-60)?

Which do you think is the response being made by the memory cells?



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## Summary

The immune system protects the host from infectious agents. Entry, survival and proliferation of pathogenic microbes are referred to as infections. Bacterial, viral and fungal pathogens predominantly cause infectious diseases. Recovery from these infections is naturally achieved with the help of the natural defence system of the host , that is the immune system. The ability of the immune system to protect the host from infections is known as immunity.

There are two major types of immunity, namely innate and acquired immunity. Innate immunity is a type of immunity obtained by birth, but it is non-spectific in its action. Innate immunity is provided by four barrier systems namely anatomical, physiological, phagocytic and inflammatory barriers.

Acquired immunity is a specific type of immunity which is provided by immune cells like T-Cells, B-Cells, K-Cells, NK-Cells etc., and immune components like antibodies. Acquired immunity is further divided into humoral immunity and cell mediated immunity. Antibodies play a vital role in humoral immunity. Immune cells like T-Cells play a vital role in cell mediated immunity.

Antigens are foreign substances which have an ability to induce production of immune response products.Depending upon their nature and origin, there are about thirteen different types of antigens. Antigenic determinant or epitope is the region of the antigen recognized by antibodies or T-Cell Receptor (TCR) of T cells. The ability of an antigen to induce an immune response is known as antigenicity. The factors that influence antigenicity are mainly of two types i.e. factors contributed by antigens and factors contributed by host cells.

Antibodies are proteins produced by B cells in response to a specific antigen. The IgG molecule is composed of two identical heavy (H) chains and two identical light (L) chains. There are about five isotypes of antibodies namely Ig G, Ig M, Ig A, Ig E and Ig D. The H and L chains are linked by disulfide bridges. Antigen-Antibody reactions refer to the interaction between antigens and antibodies.

The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops a visible precipitation. Antibody that forms precipitation is known as precipitin. The interaction between antibody and a particulate antigen results in visible clumping called agglutination**.** Antibodies that produce such reactions are called agglutinins**.**

A blood type is a classification of blood, based on the presence and absence of antibodies in blood and inherited antigenic substances on the surface of red blood cells. Karl Landsteiner discovered ABO blood group system. The membranes of human red cells contain a variety of blood group antigens**,** which are also called agglutinogens. The most important and best known of these are the Aand B antigens.



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Antibodies against red cell agglutinogens are called agglutinins. Antibody-A and Antibody-B are examples of agglutinins. Another group of antigens found on the red blood cells of people is the Rh factor which leads to the determination of the second major blood group system i.e. Rh system. There are eight common types of blood groups in combination of ABO and Rh blood grouping systems. Blood grouping tests are used for avoiding transfusion reactions and transplantation rejections.

## EVALUATION

### I. Choose the correct Answer

1. Who developed first vaccine for Rabies?

   a. Edward Jenner  b. Rober Koch

   c. Louis Pasteur  d. Lady Montagu

2. Which one of the following diseases is a pandemic disease?

   a. AIDS  b. Common cold

   c. Rabies  d. plague

3. Find the suitable vaccine for tuberculosis from the following vaccines:

   a. DPT  b. MMR

   c. BCG  d. TDP

4. One of the four cardinal sign of inflammatory response “dolor” refers to

   a. Swelling  b. Redness

   c. Heat  d. Pain

5. Which type of the biomolecule is more antigenic in nature?

   a. Protein  b. Carbohydrates

   c. Lipid  d. Nucleic acid

6. Name of the protein produced in response to and counteracting a specific antigen from B-cells.

   a. antibody  b. Interferon

   c. complement  d. acute phase protein

7. What is the name of the region of antibody which recognizes and binds to antigen?

   a. paratope  b. agretope

   c. epitope  d. none



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8. Which test is used for the diagnosis of typhoid fever?

a. Widal test b. ring test

c. tube test d. none

9. Antibodies against red cell agglutinogens are called as _____.

a. precipitin b. agglutinin

c. hapten d. epitope

10. What will be the blood group of a person with the genotype $I^BI^B$ or $I^Bi$ in ABO system?

a. A Group b. B Group

c. O Group d. AB group

11. Who discovered Rh factor?

a. Landsteiner and Weiner b. Louis Pasteur

c. Landsteiner and Koch d. None

12. How many types of constant region of heavy chain identified?

a. 2 b. 3

c. 4 d.5

13. Which one of the following immunity is non-specific in nature?

a. acquired immunity b. humoral immunity

c. innate immunity d. None

14. Name the process carried out by macrophages to lyse bacteria.

a. pinocytosis b. phagocytosis

c. transcytosis d. oxidation

15. What is the alternative name for antiglobulin test?

a. VDRL test b. Cooms test

c. Rabies test d. Koch test

16. Number of antigen binding sites in IgM antibody are

a. 7 b. 8

c. 9 d. 10

17. Which antibody crosses placenta?

a. Ig G b. Ig A

c. Ig M d. Ig E



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18. Which type of light chain of antibody predominates?

a. Kappa b. Lamda

c. Gamma d. Alpha

19. What is the average number of amino acids present in a domain of antibody?

a. 440 b. 330

c. 220 d. 110

20. Which one of the following is a superantigen?

a. Autogen b. TSST-1

c. toxoid d. hapten

21. What is the causative agent causing Athlete's foot?

a. Bacteria b. virus

c. fungus d. helminthus

22. The causative agent for AIDS is

a. HPV b. Hepatitis virus

c. HIV d. SV

23. What is the name of the vaccine administered to prevent polio?

a. Salk vaccine b. Sabin vaccine

c. Both a and b d. None

24. Which vaccine used to prevent Tuberculosis?

a. TT b. DPT

c. BCG d. MMR

25. Who is the "Father of Immunology"?

a. Edward Jenner b. Rober Koch

c. Louis Pasteur d. Lady Montagu

## II. Match the following

| | | |
|---|---|---|
| 1. Ig G | - | Complement fixation |
| 2. Ig M | - | Colostrum |
| 3. RA Factor | - | Allergy |
| 4. Ig E | - | Opsonisation |
| 5. Ig A | - | Rheumatoid Arthritis |



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## III. Answer the following questions

1. Define epitope
2. What do you meant by phagocytosis?
3. What are haptens?
4. What is the significance of immunologic memory?
5. Give four examples for viral infections.
6. Write short notes on fungal infections.
7. Elaborate the events occuring during inflammatory response.
8. Compare the events of humoral and cell mediated immunity.
9. List out the general characteristics of antigen-antibody reactions.
10. How to identify blood group?
12. Define isoantibodies.
13. What are acute phase proteins?
14. What is the role of tears in immunology?
15. List out minor blood group systems.
16. Explain the tube agglutination test.
17. Define allergen.
18. Define Epidemiology.
19. List out five viral diseases with treatment.
20. Define etiology.
21. Write a note on different diagnostic procedures for infectious disease.
22. What are the different transmission modes of infections?
23. Define variolation.
24. Briefly explain the steps involved in phagocytosis.
25. Differentiate different types of antibodies with a neat table.

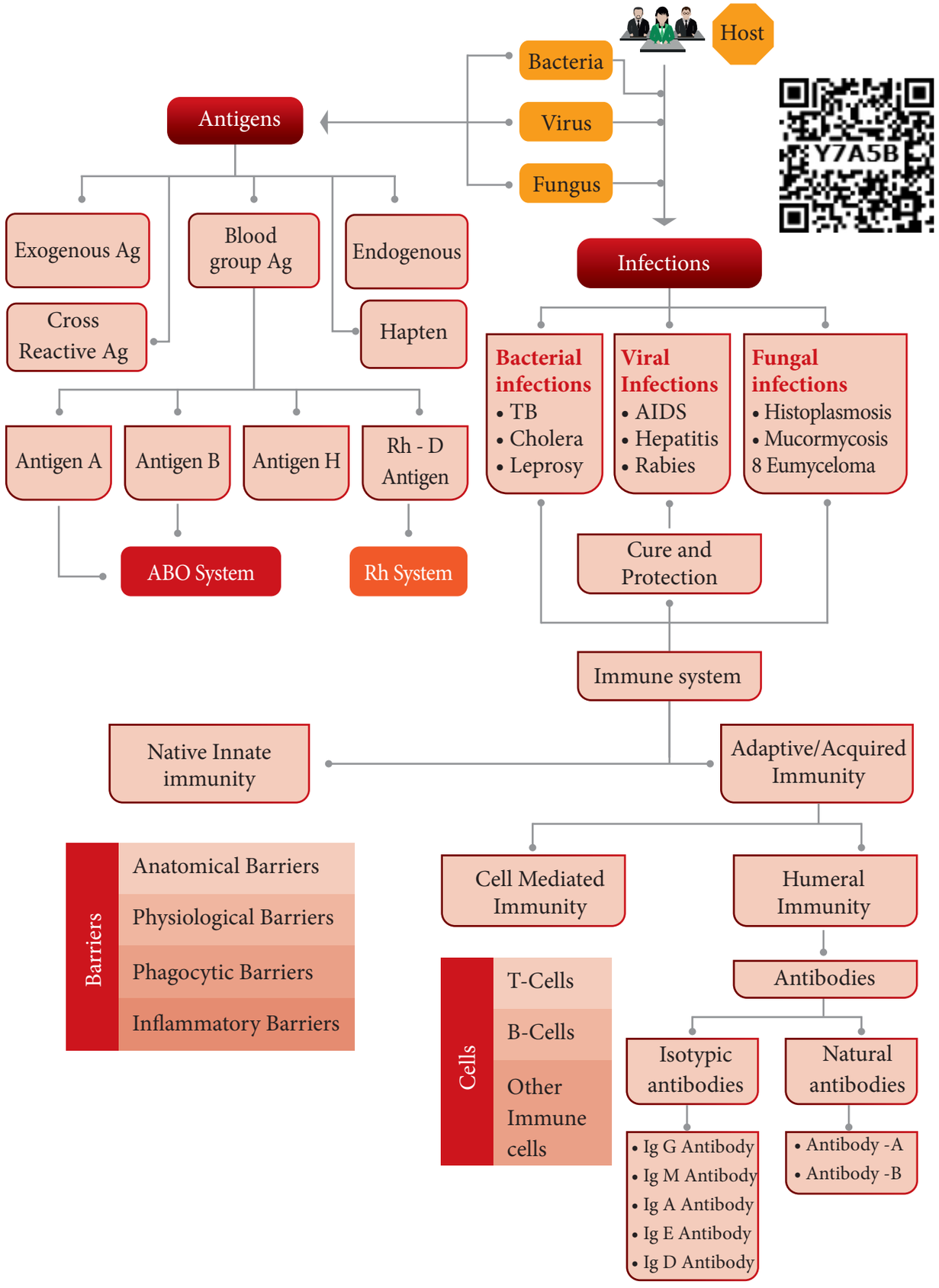
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# CONCEPT MAP






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+2

# PRACTICALS

## Important instructions

- The students are required to have an observation and a record note book
- The students are advised to use overcoat and safety glass in laboratory.
- They are not permitted to taste or touch any reagent. If any reagent falls on skin, it must be immediately washed with water.
- The students should not inhale any gas or vapour directly.
- To transfer any solutions use droppers and for salts use spatula. During heating of a test tube, the open end should not face any student.
- For any accident in lab, immediately report to the teacher - incharge.
- Follow the systematic procedure carefully during analysis.
- Try to understand the concept of Bio-chemistry in each test clearly.
- At the beginning and after finishing of each lab session wipe your bench tops using disinfectant solution.
- Never pipette any chemical reagents by mouth. Doing so is strictly prohibited .Pipetting is to be carried out with the aid of a mechanical pipetting device.
- Do not contaminate chemicals while doing experiments.
- Speak quietly and avoid unnecessary movement around the laboratory to avoid distractions that may cause accidents.



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# QUANTITATIVE ANALYSIS

## What is Titration?

To determine the concentration of an unknown sample, we use a common laboratory technique called volumetric analysis. In this quantitative technique, the concentration of the particular substance present in an unknown sample solution can be determined by measuring the volume of another solution that reacts with it. The solution that has been taken in the burette is called the titrant and the solution taken in the conical flask is called the analyte.

## What is end point of a titration?

When the reaction between fixed volume of the titrant and the analyte becomes complete, the titration is said to be reached the end point. This end point can be identified using suitable indicators.

## What are indicators?

An indicator is a chemical substance that undergoes a sharp colour change at the end point. In acid-base titrations, the end point is usually determined using acid-base indicators or pH indicators. Acid Base indicators are either weak organic acids or weak organic bases.

## What is a standard solution?

A solution whose concentration is known is called a standard solution. It can be prepared by dissolving a known quantity of the substance in a definite volume of the solvent.

## How do we determine the strength of a given acid or base?

Determination of the strength is based on the Law of Equivalents. According to this law, the number of equivalence of the substance to be titrated is equal to the number of equivalence of the titrant used.

Consider an acid-base titration in which $V_1$ ml of an acid solution of normality $N_1$ is required to neutralize $V_2$ ml of a base of normality $N_2$, then according to normality equation

$$V_1N_1=V_2N_2$$

From the above equation the normality of unknown solution can be calculated by measuring the volume of standard solution consumed.

## Apparatus Used in Volumetric Analysis

The following are the apparatus required in volumetric analysis.

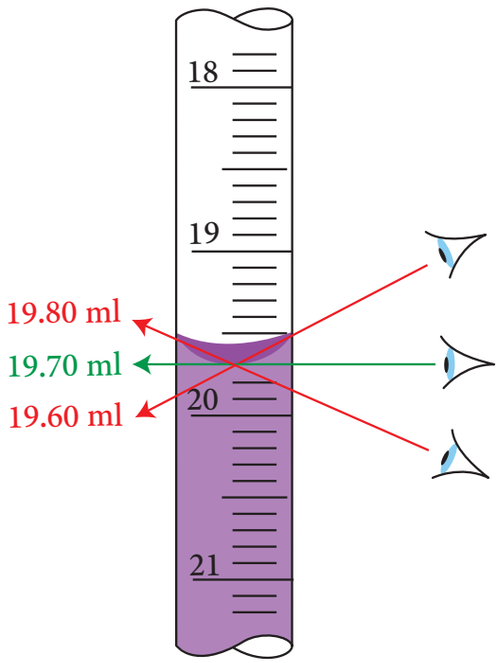
(i) Graduated-burette.
(ii) 20 ml pipette
(iii) Conical flask.
(iv) Funnel, weighing machine, wash bottle, etc.,.



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Before a burette is filled with the solution, it is thoroughly washed, so that no greasy matter is sticking inside or outside the burette. Then rinse the burette with a small volume of titrant solution to be taken in it. Discard this solution through the stop-cock. After that,the burette is filled with titrant solution with the help of a funnel inserted in the top. The funnel must then be taken out after filling the burette.Care must be taken that no air bubbles remain in the narrow bottom tip of the burette. If air bubbles found in it, should be removed by running out the solution rapidly into the reagent bottle. Burette reading forms the most important aspect of the experiment, therefore, burette reading should be read very carefully.

In case of colourless solutions lower meniscus is read, while in case of coloured solutions, level is read from the upper meniscus. This is due to the reason that in case of coloured solutions lower meniscus is not visible clearly.Take reading of the burette placing your eye exactly in front of meniscus (Fig.) of the solution.




## Pipette

The pipette is used for accurate measurements of definite volume of solution. It consists of a long narrow tube with cylindrical bulb in the middle and a jet at its lower end. On the upper part of the stem, there is an etched circular mark. Before a pipette is filled with the solution, it is washed and thoroughly rinsed with the solution to be measured with it. The upper part of pipette is then held by the thumb and middle finger of the right hand, the lower end is dipped into the liquid and the solution is sucked into the pipette until the liquid level is about 2 cm above the mark. The open end of pipette is then closed with index finger. The liquid is allowed to run slowly until the lower edge of meniscus just touches the mark. The solution is then allowed to run freely out of the pipette in the titration flask.



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# 1. DETERMINATION OF BLOOD GROUPING

## Aim

To determine the blood group of the given blood sample.

## Principle

This test is based on the antigen antibody complex formation between the antigen present on the RBC and the antibody present in the serum.

## Reagents and Equipments

1. Anti A, 2. Anti B, 3. Anti D, 4. Sterile lancet, 5. White marble tiles, 6. Sticks for mixing and 7. Alcohol

## Procedure

The middle finger of the individual to be tested for blood group is cleaned with alcohol and the excess of alcohol is wiped out with sterile cotton. A clean white tile is taken and it is divided into four columns marked as A, B, D and C.

A small drop of anti A is added in the portion A, anti B in the portion B and anti D in the portion D. The portion C is used for positive or negative control. A small prick is made on the cleaned finger, the first drop of blood coming out is wiped off and second drop of blood is collected directly on the region marked as A, B and D. Immediately, blood is mixed with the corresponding antibody by using the sterile stick and observed for any agglutination in the form of clump formation.

If the clump is observed in the region marked as A, the blood group is A and the formation of clump in B shows presence of B blood group. If the clump is formed in both A and B , the blood group is of AB type. If there is no clump formation in both A and B, the blood group, is of O type. The clump formation in D portion is observed carefully. The formation of clump in the D region shows presence of Rh positive blood group and if there is no clump formation it shows the presence of Rh negative blood group.

Clump formation in the regions A and D shows the presence of A+ blood group and clump formation in the regions B and D shows the presence of B+ blood group. If clump is formed in all the three regions it shows the presence of AB+ blood group. No clump formation in regions A & B and clump formation in region D shows O+ blood group. No clump formation in all the three regions shows the presence of O- blood group. The following chart shows the type of blood group and the agglutination with anti-bodies.

If the clump formation is observed immediately in A blood group type it can be denoted as $A_1$ type, if it is not immediate then the blood group is of $A_2$ type.



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| S.No. | Antibody | Clump formation | Type of blood group |
|---|---|---|---|
| 1. | Anti A | Yes | A Positive |
| | Anti D | Yes | |
| 2. | Anti A | Yes | A Negative |
| | Anti D | No | |
| 3. | Anti B | Yes | B Positive |
| | Anti D | Yes | |
| 4. | Anti B | Yes | B Negative |
| | Anti D | No | |
| 5. | Anti A & B | Yes | AB Positive |
| | Anti D | Yes | |
| 6. | Anti A & B | Yes | AB Negative |
| | Anti D | No | |
| 7. | Anti A & B | No | O Positive |
| | Anti D | Yes | |
| 8. | Anti A & B | No | O Negative |
| | Anti D | No | |

**Result:**

The blood group of the person is found to be _____



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## 2. ESTIMATION OF PROTEIN (BIURET METHOD)

### Aim

To estimate the amount of protein present in the given test sample.

### Principle

The-CO-NH- group of protein forms a purple coloured complex with copper ion in alkaline medium. The colour intensity is measured at 540nm. Since all proteins contain peptide bond, this method is fairly specific and there is little interference with other compounds.

### Reagents required

**Stock Biuret reagent**

Dissolve 45g of Rochelle's Salt (Sodium potassium tartarate ) in about 400ml of 0.2N sodium hydroxide and add 15g of copper sulphate. Stir it continuously until the solution is complete. Add 5g of potassium iodide and make upto 1 litre with 0.2N sodium hydroxide.

**Biuret solution for use**

Dilute 200ml of stock biuret reagent to 1 litre with 0.2N sodium hydroxide containing 5gm of potassium iodide.

**Stock Standard Solution**

1g of protein(egg albumin) is weighed and made upto 100ml with distilled water.

Concentration = 10mg/ml

**Working standard solution**

10ml of the stock is diluted to 100ml using distilled water.

Concentration = 1mg/ml

### Procedure

**Estimation of protein**

0.5-2.5ml of standard protein solution is pipette out into five different test tubes (S1-S5). The concentrations of protein in the tubes are 0.5-2.5mg. 0.5 and 1.0ml of test solution is taken in two test tubes labelled as $T_1$ and $T_2$. The volume in all the tubes are made upto 5ml using distilled water. A blank is also prepared simultaneously by adding 5ml of distilled water. Then 3ml of biuret reagent is added to all the test tubes including blank. The tubes are mixed well. The tubes are then maintained at room temperature for 10 minutes. The optical density is measured at 540nm.

From the values obtained, a standard graph is drawn using concentration of protein in X-axis and optical density in the Y-axis. From the graph the amount of protein present in the given test solution is calculated.



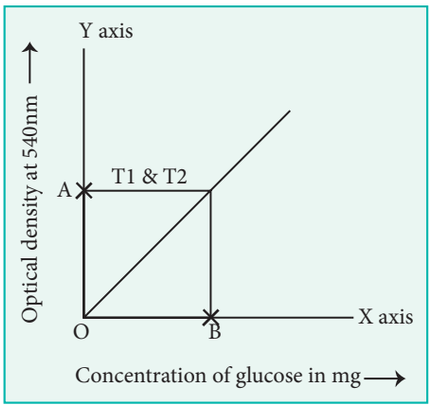
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**Protocol for protein estimation**

| S. No. | Reagents required | Blank B | Standard | | | | | Test solution | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S1 | S2 | S3 | S4 | S5 | $T_1$ | $T_2$ |
| 1. | Standard protein (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - | - |
| 2. | Concentration of protein (mg) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - | |
| 3. | Test solution (ml) | - | - | - | - | - | - | 0.5 | 1.0 |
| 4. | Distilled water(ml) | 5 | 4.5 | 4.0 | 3.5 | 3.0 | 2.5 | 4.5 | 4.0 |
| 5. | Biuret reagent (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 6. | Optical density at 540nm | | | | | | | | |

## Graph

Let the optical density of tubes $T_1$ & $T_2$ be A and the corresponding protein concentration is B as shown in the graph.




## Calculation

$T_1$

0.5ml of the test solution corresponds to _____ mg of protein.

Therefore, 100ml of the test solution will contain = $\frac{100 \times \text{\_\_\_\_\_ mg of protein}}{0.5}$

= Z mg of protein

$T_2$

1.0ml of the test solution corresponds to _____ mg of protein.

Therefore, 100ml of the test solution will contain = $\frac{100 \times \text{\_\_\_\_\_ mg of protein}}{1.0}$

= Z mg of protein

## Result:

The amount of protein present in 100ml of the given test solution = Z mg of protein.



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## 3. ESTIMATION OF GLUCOSE (ORTHOTOLUIDINE METHOD)

### Aim

To estimate the amount of glucose present in the given test solution.

### Principle

A solution of orthotoluidine in glacial acetic acid when treated with glucose produces a blue coloured product with an absorption maximum at about 640nm. The values obtained represent the true glucose level.

### Reagents required

**1. Stock Solution**

100mg of glucose is weighed and made upto 100ml with distilled water.

Concentration of glucose = 1mg/ml

**2. Working standard solution**

10ml of stock solution is diluted to 100ml with distilled water.

Concentration of glucose = 100µg/ml.

**3. Orthotoluidine reagent**

12.5mg of thiourea and 12g of boric acid are dissolved in 50ml of distilled water by heating over a mild flame. 75ml of redistilled orthotoluidine reagent and 375ml of analar acetic acid are mixed separately. The two solutions are mixed and the total volume is made upto 500ml with acetic acid. The reagent is kept overnight at 4˚C.

### Procedure

**Estimation of glucose**

0.5-2.5ml of standard glucose solutions are pipetted out into five different test tubes labelled S1-S5 with the concentration of 50-250µg. 0.5 and 1.0ml of the test solution is pipette out into two different test tubes labelled as $T_1$ and $T_2$. Final volume is made upto 2.5 ml using distilled water in all the standard tubes. 4ml of orthotoluidine reagent is added to all the test tubes. A blank is also prepared simultaneously comprising 2.5ml of distilled water and 4ml of orthotoluidine reagent. All the test tubes are heated for 20 minutes in a boiling water bath. The blue colour developed is measured at 640nm using a colorimeter.

A standard graph is drawn with optical density in Y- axis vs. Concentration of glucose in axis. The amount of glucose present in the given test solution is then calculated.

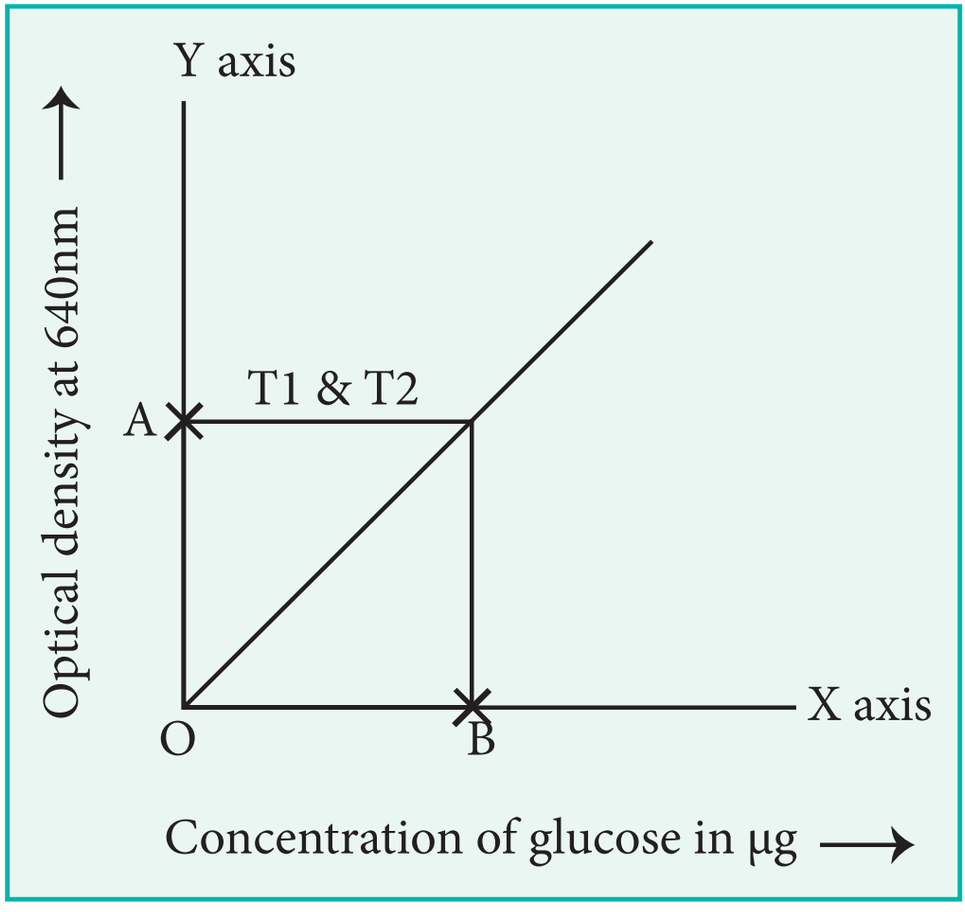


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**Protocol for glucose estimation**

| S. No. | Reagents required | Blank B | Standard | | | | | Plasma | |
|---|---|---|---|---|---|---|---|---|---|
| | | | S1 | S2 | S3 | S4 | S5 | $T_1$ | $T_2$ |
| 1. | Standard glucose (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - | - |
| 2. | Concentration of glucose (µg) | - | 50 | 100 | 150 | 200 | 250 | - | - |
| 3. | Test solution (ml) | - | - | - | - | - | - | 0.5 | 1.0 |
| 4. | Distilled water (ml) | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - | 2.0 | 1.5 |
| 5. | Orthotoluidine reagent (ml) | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Tubes are kept in boiling water for 20 minutes and cooled. | | | | | | | | | |
| 6. | Optical density at 640nm | | | | | | | | |

## Graph






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## Calculation

$T_1$

0.5ml of the test solution corresponds to _____ µg of glucose.

Therefore, 100ml of the test solution will contain = $100 \times \frac{\text{_____ µg of glucose}}{0.5}$

= Z µg of glucose

$T_2$

1.0ml of the test solution corresponds to _____ µg of glucose.

Therefore, 100ml of the test solution will contain = $100 \times \frac{\text{_____ µg of glucose}}{1.0}$

= Z µg of glucose

## Result:

The amount of glucose present in 100ml of the given test solution = _____ µg.



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## 4. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

### Aim:

To estimate the amount of total protein (BSA) in the given test solution.

### Principle:

It is the most commonly used method for determination of proteins in cell free extracts because of its high sensitivity and quantities as low as 20µg of proteins can be measured. The CO-NH (peptide bonds) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a blue coloured complex. In addition, tyrosine and tryptophan residues of proteins cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method

### Reagents:

1. 5% TCA
2. 0.1N sodium hydroxide
3. **Lowry's reagent:** Solution A: 2.0% sodium carbonate in 0.1N sodium hydroxide; Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate solution (freshly prepared); Mix 50ml of solution A and 1ml of solution B prior to use.
4. **Folin'sciocalteu reagent:** A commercial preparation is diluted with water in the ratio of 1:2 before use.
5. **Stock solution:** 100mg BSA is weighed and made upto 100ml of distilled water
6. **Working standard solution:** 10ml of the stock is diluted to 100ml using distilled water

   Concentration = 100 µg / ml

### Procedure:

0.5-2.5ml of working standard BSA solution is pipetted out into five different test tubes and labelled as S1-S5 with the concentration of 50-250µg. 0.5ml of the test solution is pipetted out into a test tube and labelled as $T_1$. Final volume is made upto 2.5 ml using 0.1N sodium hydroxide in all the tubes. A blank is also prepared simultaneously comprising 2.5ml of 0.1N sodium hydroxide. 4.5ml of lowry's reagent is added to all the test tubes followed by the addition of 0.5ml of Folin's reagent. All the test tubes are kept at room temperature for 20 minutes. The colour developed is measured at 660nm using a colorimeter.

A standard graph is drawn with optical density in Y- axis vs. concentration of BSA in X axis. The amount of BSA present in the given test solution is then calculated.



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| S.No. | Particular/ Reagents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | T |
|---|---|---|---|---|---|---|---|---|
| 1. | Standard solution | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - |
| 2. | Concentration (µg) | - | 50 | 100 | 150 | 200 | 250 | - |
| 3. | Volume of Test solution (ml) | - | - | - | - | - | - | 0.5 |
| 4. | Volume of NaOH (ml) | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | - | 2.0 |
| 5. | Lowry's reagent | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| Mix the contents in the test tubes | | | | | | | | |
| 6. | Volume of Folin's reagent (ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Incubate all the test tubes @ room temperature | | | | | | | | |
| 7. | Optical density @ 660nm | | | | | | | |

## Calculation:

0.5ml of the test solution corresponds to _____ µg of BSA

Therefore, 100ml of the test solution will contain $100 \times \frac{\_\_\_\_\_}{0.5}$ µg of BSA

= Z µg of BSA

## Result:

The amount of protein present in the given test solution was found to be _____ µg



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## 5. ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD

### Aim:

To determine the amount of cholesterol present in the given test solution by Zak's method

### Principle:

In this method, cholesterol is treated with concentrated sulphuric acid. Cholesterol in the presence of sulphuric acid undergoes dehydration to form 3, 5-cholestadiene. This is inturn oxidised and sulphonated to form red colouredCholestapolyenesulphonic acid in the presence of Fe3+ ions. The intensity of red colour formed is proportional to the amount of cholesterol present in the test solution. The colour intensity is measured by using a green filter at 540nm.

### Reagents:

1. **Stock ferric chloride reagent:** 840mg of was weighed and dissolved in 100 ml of glacial acetic acid.
2. **Ferric chloride diluting reagent:** 8.5ml of stock ferric chloride was diluted to 100ml with glacial acetic acid.
3. **Standard cholesterol solution:** 100mg pure dry cholesterol was dissolved in 100ml of acetic acid.
4. **Working standard solution:** 10ml of the stock is diluted to 100ml using acetic acid.

   Concentration = 100 µg / ml.

### Procedure:

0.5-2.5ml of working standard cholesterol solution is pipetted out into five different test tubes and labelled as S1-S5 with the concentration of 50-250µg. 1.0ml of the test solution is pipetted out into a test tube and labelled as $T_1$. Final volume is made upto 5.0 ml using $FeCl_3$ diluting reagent in all the tubes. A blank is also prepared simultaneously comprising of 5.0ml of $FeCl_3$ diluting reagent. 3.0ml of conc. $H_2SO_4$ is added to all the test tubes. The contents in the test tubes are mixed well and kept at room temperature for 20 minutes. The intensity of red colour developed is measured at 540nm using a colorimeter.

A standard graph is drawn with optical density in Y- axis vs. concentration of cholesterol in X axis. The amount of cholesterol present in the given test solution is then calculated.



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| S.No. | Particular/ Reagents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | T |
|---|---|---|---|---|---|---|---|---|
| 1. | Standard solution (ml) | - | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - |
| 2. | Concentration (µg) | - | 50 | 100 | 150 | 200 | 250 | - |
| 3. | Volume of Test solution (ml) | - | - | - | - | - | - | 1.0 |
| 4. | Volume of $FeCl_3$ diluting agent (ml) | 5 | 4.5 | 4.0 | 3.5 | 3.0 | 2.5 | 4 |
| Mix the contents in the test tubes | | | | | | | | |
| 5. | Volume of conc $H_2SO_4$ (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Mixall the test tubes and leave at room temperature for 10 mins | | | | | | | | |
| 6. | Optical density @ 540nm | | | | | | | |

**Calculation:**

1.0ml of the test solution corresponds to _____ µg of cholesterol

Therefore, 100ml of the test solution will contain 100 x $\frac{\text{\_\_\_\_\_ µg of cholesterol}}{1.0}$

= Z µg of cholesterol

**Result:**

The amount of cholesterol present in 100ml of the given test solution is _____(µg)



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## 6. ESTIMATION OF ASCORBIC ACID (VITAMIN C)

### Aim:

To estimate the vitamin C (ascorbic acid) content in the given test solution colorimetrically.

### Reagents:

1. **Indophenol reagent:** 20mg of 2,6–dichlorophenolindophenols was dissolved in 10ml of warm distilled water.
2. **DNPH-Thiourea mix reagent:** 2gm of 2,4 – dinitrophenyl hydrazine and 1gm of Thiourea was dissolved in 100ml of 9N sulphuric acid.
3. **5% TCA:** 5gm of Tricholoroacetic acid was dissolved in 100ml of distilled water.
4. **85% sulphuric acid:** 85ml of concentrated sulphuric acid is made up to 100 ml with distilled water.
5. **Stock solution:** 100mg of ascorbic acid dissolved in 100ml of distilled water.
6. **Working standard solution:** 10ml of the stock is diluted to 100ml using distilled water. Concentration = 100 µg / ml.

### Procedure:

0.2-1.0ml of working standard ascorbic acid solution is pipetted out into five different test tubes and labelled as S1-S5 with the concentration of 20-100µg. 1.0ml of the test solution is pipetted out into a test tube and labelled as $T_1$. Final volume is made upto 1.0ml using distilled water in all the tubes. A blank is also prepared simultaneously comprising of 1.0ml of distilled water. .0.1ml of indophenol reagent is added to all the test tubes followed by the addition of 1.0ml of DNPH reagent. The contents in the test tubes are mixed well and kept in the boiling water bath for 30 minutes. The test tubes are then cooled and 3.0ml of $H_2SO_4$ is added with constant shaking. The intensity of red colour developed is measured at 540nm using a colorimeter.

A standard graph is drawn with optical density in Y- axis vs. concentration of ascorbic acid in X axis. The amount of ascorbic acid present in the given test solution is then calculated.



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## Observation

| S.No. | Particular / Reagents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | T |
|---|---|---|---|---|---|---|---|---|
| 1. | Standard Solution (ml) | - | 0.2 | 0.4 | 0.6 | 0.8 | 1 | - |
| 2. | Concentration (µg) | - | 20 | 40 | 60 | 80 | 100 | - |
| 3. | Distilled water (ml) | 1 | 0.8 | 0.6 | 0.4 | 0.2 | - | - |
| 4. | Volume of Test Solution (ml) | - | - | - | - | - | - | 1 |
| 5. | Indophenol (ml) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 6. | DNPH (ml) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep it in the boiling water bath and warm it for 30 min | | | | | | | | |
| 7. | Volume of $H_2SO_4$(ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 8. | Optical density @ 540nm | | | | | | | |

## Calculation:

1.0ml of the test solution corresponds to _____ µg of ascorbic acid

Therefore, 100ml of the test solution will contain 100 x $\frac{\text{\_\_\_\_\_ µg of ascorbic acid}}{1.0}$

= Z µg of ascorbic acid

## Result:

Vitamin C content of the given 100ml of the test solution was found to be _____µg.



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## 7. ESTIMATION OF DEOXYRIBO NUCLEIC ACID(DNA)

### (Demonstration only)

**Aim:**

To quantify the deoxyribonucleic acid content in the given test solution.

**Principle:**

The deoxyribose moiety of the DNA forms a hydroxyl levulaldehyde in TCA solution. This reacts with diphenylamine to give a blue colour which can be read at 565 nm.

**Reagents:**

1. **Dische's reagent:** Dissolve 1.5g of crystalline diphenylamine in 100 ml of glacial acetic acid and add 1.5 ml of conc. sulphuric acid.The reagent is stored in dark bottles. At the time of use add 0.1 ml of aqueous acetaldehyde (16mg/ml) for each 20 ml of the reagent.
2. 1N perchloric acid:
3. 5mMSodium hydroxide:
4. **Standard DNA solution:** A stock solution is prepared by dissolving 100mg of DNA in 5M sodium hydroxide. Working standard solution is prepared by mixing 10ml of the stock standard with 1N perchloric acid and heating at 700C for 15 minutes.

**Procedure:**

0.5-2.5ml of working standard DNA solution is pipetted out into five different test tubes and labelled as S1-S5 with the concentration of 50-250µg. 1.0ml of the test solution is pipetted out into a test tube and labelled as $T_1$. Final volume is made upto 3.0ml using distilled water in all the tubes. A blank is also prepared simultaneously comprising of 3.0ml of distilled water. 2.0ml of diphenyl amine (Dische's reagent) is added to all the test tubes. The initial turbidity formed disappears gradually. The tubes are covered with glass marbles and heated in a boiling water bath for 10-15 min. The test tubes are then cooled under tap water and the blue colour developed is measured at 565nm in a spectrophotometer.The control or blank contains only water and the Dische's reagent.

A standard graph is drawn with optical density in Y- axis vs. concentration of DNA in X axis. The amount of DNA present in the given test solution is then calculated.



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| S.No. | Particular/ Reagents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | T |
|---|---|---|---|---|---|---|---|---|
| 1. | Standard solution (ml) | 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | - |
| 2. | Concentration (µg) | 0 | 50 | 100 | 150 | 200 | 250 | - |
| 3. | Test solution (ml) | - | - | - | - | - | - | 1 |
| 4. | Distilled water (ml) | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 2.0 |
| 5. | Diphenyl amine (ml) | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Keep in baoiling water bath for 10 minutes, cool and take reading at 565 nm. | | | | | | | | |
| 6. | Optical density at 565 nm | | | | | | | |

## Calculation:

1.0ml of the test solution corresponds to _____ µg of DNA

Therefore, 100ml of the test solution will contain 100 x $\frac{\text{\_\_\_\_\_ µg of DNA}}{1.0}$

= Z µg of DNA

## Result:

Amount of DNA present in given 100ml of the test solution is _____ µg



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## 8. ESTIMATION OF UREA BY DIACETYL MONOXIME METHOD

### Aim:

To estimate the amount of urea present in the given test solution

### Principle:

Under acidic conditions when urea is heated with compounds containing two adjacent carbonyl groups such as diacetylmonoxime coloured products are formed. On heating, it decomposes to give hydroxylamine and diacetyl, which then condenses with urea to give rose purple diazinederivative.The colour intensity is measured colorimetrically at 520nm.

### Reagents:

1. **Acid reagent:Reagent A:** 5g of ferric chloride is dissolved in 20ml water and 100ml of orthophosphoric acid (85%) is slowly added with stirring and the volume is made up to 250ml with water;Reagent B:200ml of conc. sulphuric acid is added to 800ml water;0.5ml of reagent A is added to 1 litre of Reagent B.
2. **Colour Reagent:** Reagent C: Dissolve 20g diacetylmonoxime in 1 litre of water;Reagent D: Dissolve 5g thiosemicarbazidein l litre of water;Mixed 67ml of C and 67ml of D and made this volume upto1 litre with distilled water.
3. **Urea Stock solution:** Dissolve 100mg Urea in 100 ml of distilled water.
4. **Working standard solution:** 10ml of the stock is diluted to 100ml using distilled water.

   Concentration = 100µg / ml.

### Procedure:

0.2-1.0ml of working standard urea solution is pipetted out into five different test tubes and labelled as S1-S5 with the concentration of 20-100µg. 1.0ml of the test solution is taken in a test tube and labelled as $T_1$. The volume in all the test tubes is made up to 2.0ml with distilled water. 2.0ml of water is taken as a blank solution. 2.0ml of colour reagent and 2.0ml of acid reagent are added to all the tubes and placed in a boiling water bath for 20 min. The tubes are cooled and pink colour developed is read at 520nm.



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| S.No. | Particular – Reagents | B | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | T |
|---|---|---|---|---|---|---|---|---|
| 1. | Standard Solution (ml) | - | 0.2 | 0.4 | 0.6 | 0.8 | 1 | - |
| 2. | Concentration (µg) | 0 | 20 | 40 | 60 | 30 | 100 | - |
| 3. | Volume of test solution (ml) | - | - | - | - | - | - | 1 |
| 4. | Distilled water (ml) | 2 | 1.8 | 1.6 | 1.4 | 1.2 | 1 | 1 |
| 5. | Acid reagent (ml) | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 6. | Colouring reagent (ml) | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Boil for 15 – 20 minutes | | | | | | | | |
| 7. | Optical density at 520 nm | | | | | | | |

## Calculation:

1.0ml of the test solution corresponds to _____ µg of urea

Therefore, 100ml of the test solution will contain 100 x $\frac{\text{\_\_\_\_\_ µg of urea}}{1.0}$

= Z µg of urea

## Result:

The amount of urea present in the given test solution is ___ µg/100ml



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## 9. ESTIMATION OF CALCIUM (TITRIMETRIC METHOD)

### Aim

To estimate the amount of calcium present in the given test solution.

### Principle

Calcium is precipitated as Calcium Oxalate with ammonium oxalate. The precipitate is washed with ammonia to remove the chloride ions. The washed precipitate is then made to react with 1N sulphuric acid. The liberated oxalic acid is now estimated by titrating against standardised potassium permanganate. The amount of oxalic acid liberated is proportional to the amount of calcium.

### Reagents required

1. **Ammonium Oxalate solution (4%)**

   4g of ammonium oxalate dissolved in 100ml of distilled water.

2. **Ammonia Solution (2%)**

   2ml of ammonia of specific gravity 0.88 is diluted to 100ml with distilled water.

3. **Potassium Permanganate(0.1N)**

   This is prepared by dissolving 3.16 g of potassium permanganate in 1 litre of distilled water.

4. **Standard oxalic acid solution (0.1N)**

   It is prepared by dissolving 630mg of oxalic acid in 100ml of distilled water.

5. **Sulphuric acid(1N)**

6. **Calcium oxalate solution 100mg of calcium oxalate dissolved in 100ml of distilled water**

### Procedure

**Standardisation of Potassium Permanganate (Titration I)**

10ml of oxalic acid is pipetted out into a clean conical flask and 10ml of dilute sulphuric acid is added and heated at 60˚C . It is titrated against potassium permanganate in the burette. The end point is the appearance of pale permanent pink colour. Titration is repeated for concordant values.

**Estimation of Calcium Oxalate (Titration II)**

10ml of calcium oxalate solution is pipetted out into a conical flask followed by the addtion of 10ml of 1N sulphuric acid and warmed for solubilisation. This solution is now titrated against potassium permanganate and the volume consumed is noted. The end point is the appearance of pale permanent pink colour. Titration is repeated for concordant values.



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10ml of 1N sulphuric acid is treated as blank and titrated against potassium permanganate. The end point is the appearance of pale permanent pink colour. Titration is repeated for concordant values and the amount of calcium present is then calculated.

**Tabular Column**

## TITRATION-I

**=Standardisation of Potassium permanganate**

**Standard Oxalic acid Vs Potassium permanganate**

| S. No. | Volume of Oxalic acid(ml) | Burette | | Volume of Potassium Permanganate (ml) | Indicator |
|---|---|---|---|---|---|
| | | Initial (ml) | Final(ml) | | |
| 1. | 10 | 0 | x | x | Self |
| 2. | 10 | 0 | x | | |

Volume of oxalic acid $V_1$ = 10ml

Normality of oxalic acid $N_1$ = 0.1N

Volume of Potassium permanganate = x ml

Normality of potassium permanganate = ?

Normality of potassium permanganate $N_2$ = $\frac{V_1N_1}{V_2}$

= $\frac{10 \times 0.1}{X} = N_2$

= $YN$



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## TITRATION-I I

**Estimation of Calcium in Serum**

| S. No. | Volume of Oxalic acid(ml) | Burette | | Volume of Potassium Permanganate (ml) | Indicator |
|---|---|---|---|---|---|
| | | Initial (ml) | Final(ml) | | |
| 1. | **Test Solution** Oxalic acid liberated from calcium oxalate +10ml of sulphuric acid | 0 | X1 | X1-X2 | Self |
| 2. | **Blank Solution** 10ml of sulphuric acid | 0 | X2 | (X3) | |

### Calculation

The amount of calcium present in the given sample can be calculated by using the equation.

1ml of 0.1N potassium permanganate is equivalent to 0.2mg of calcium. Therefore, X3 ml of 'YN' potassium permanganate is equivalent to

$$0.2 \times X3 \times Y / 1 \times 0.1 = Z \text{ mg of Calcium.}$$

10ml of the test solution contains Z mg of Calcium.

Therefore, 100ml of the test solution will contain = $100 \times \frac{Z}{10}$ mg of Calcium

### Result:

The amount of calcium present in the given test solution is _____ mg/100ml.



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# 10. ESTIMATION OF AMINO ACID BY SORENSEN'S FORMOL TITRATION METHOD

## Aim:

To estimate the amount of amino acid present in100ml the given solution by formol titration

## Principle:

The carboxyl group of amino acid cannot be accurately measured by titration with alkali because it reacts with a basic amino acid group to form zwitter ions of amphoteric nature at neutral pH. They may not be decomposed at the end point. But in the presence of formaldehyde with alkali, the amino acid forms an amino acid formol complex. The complex is acidic because of the basic character of amino acid group is suppressed and hence it can be titrated with alkali using phenolphthalein as indicator.

## Reagents:

1. **Stock glycine solution:** 7g of glycine is made up to 100ml with distilled water.
2. 630mg of oxalic acid is made up to 100ml with distilled water.
3. Sodium hydroxide solution (0.1N): 4g of made up to 1000ml with distilled water.
4. 40% Formaldehyde
5. Phenolphthalein indicator.

## Procedure:

**Standardisation of sodium hydroxide:** 0.1N of oxalic acid was prepared (0.63g of oxalic acid was dissolved in 100 ml of water). 10ml of oxalic acid was pipetted out into a conical flask and titrated against sodium hydroxide using phenolphthalein as indicator. End point is the appearance of pale pink colour. The experiment was repeated for concordant values.

**Estimation of amino acid:** 10 ml of formaldehyde and 10ml of distilled water are pipetted out into a conical flask. This is taken as blank. This is titrated against standard sodium hydroxide using phenolphthalein as indicator. A permanent pink colour obtained is the end point. Burette reading is noted and the titration is repeated for concordant values.

10ml of 0.1 N glycine, 10ml of formaldehyde and 10ml of distilled water are pipette out into a conical flask. The solution is allowed to stand for ten minutes to complete the reaction. The above mixture is titrated against sodium hydroxide using phenolphthalein as indicator. A pale pink colour was obtained. End point was noted and the experiment was repeated for concordant values. Similarly the titration is repeated with the unknown glycine and the amount of glycine in the unknown sample is calculated.



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### Titration: 1 Sodium hydroxide vs oxalic acid

| S.No. | volume of solution in conical flask | Burette readings (ml) | | Volume of NaOH consumed (ml) | Concordant volume |
|---|---|---|---|---|---|
| | | Initial | Final | | |
| | 10 | 0 | | | |
| | 10 | 0 | | | |

### Titration : 2 Blank titration (sodium hydroxide vs formaldehyde)

| S.No. | volume of solution in conical flask | Burette readings (ml) | | Volume of NaOH consumed (ml) | Concordant volume |
|---|---|---|---|---|---|
| | | Initial | Final | | |
| | 20 | 0 | | | |
| | 20 | 0 | | | |

### Titration : 3 Sodium hydroxide vs unknow solution

| S.No. | volume of solution in conical flask | Burette readings (ml) | | Volume of NaOH consumed (ml) | Concordant volume |
|---|---|---|---|---|---|
| | | Initial | Final | | |
| | 30 | 0 | | | |
| | 30 | 0 | | | |

### Calculation

**Titration 1**

Volume of oxalic acid (V1) = 10ml
Normality of oxalic acid (N1) = 0.1N
Volume of NaOH (V2) =
Normality of NaOH (N2) = N1V1 / V2

**Titration 2**

Volume of NaOH (V3) =

**Titration 3**

Volume of NaOH (V4) =
Net Volume of NaOH (V5) = V4 – V3

Normality of NaOH (N2) =

Volume of amino acid (glycine) (V6) = 10ml
Normality of amino acid (glycine) = N2 V5 / V6
Normality = (Weight / L) / equivalent weight
Weight /L = Normality x equivalent weight
= Z gram of glycine/1000ml (1L)
Therefore, 100ml of the test solution (glycine) will contain 100 x $\frac{Z}{1000}$ gram of glycine

### Result:

The amount of glycine present in 100ml of the given solution was found to be ____ g.



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## Glossary - கலைச்சொற்கள்

| | |
|---|---|
| Acidosis | அமிலத் தேக்கநோய் |
| Activator | இயக்குவிப்பான் |
| Active site | கிளர்வு மையம் |
| Active transport | செயல்மிகு கடத்தல் |
| Agglutination | திரட்சி வினை |
| Albinism | வெளிறுதல்(தோல், கண்) |
| Amphipathic | நீர்வெறுக்கும் |
| Antibody | எதிர்காப்பு மூலங்கள் |
| Antigen | உடற்காப்பு ஊக்கி |
| Antiporters | எதிர் திசைக் கடத்திகள் |
| Atherosclerosis | தமனி தடிப்பு |
| Avidity | இனையும் திறன் |
| Bacillus | உருளைவடிவ பாக்டீரியா |
| Bee-sting | தேனீ கொடுக்கு |
| Bile salt | பித்த உப்புகள் |
| Biocatalyst | உயிரூக்கிகள் |
| Biogenic | உயிரிவழித்தோற்ற |
| Biosynthesis | உயிர்த் தொகுப்பு |
| Blood vessel | இரத்த குழல்கள் |
| Cartilage | குருத்தெலும்பு |
| Catabolism | சிதைமாற்றம் |
| Cataract | கண்புரை |
| Cereals | தானியங்கள் |
| Chemotaxis | வேதித் தூண்டியக்கம் |



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|---|---|
| Choroids | விழியடிகரும்படலம் |
| Coccus | கோளவடிவ பாக்டீரியா |
| Competitive inhibition | போட்டித்தன்மையுள்ள தடுத்தல் |
| Convulsion | வலிப்பு |
| Cortex | புறணி |
| Crenation | செல் சுருங்குதல் |
| Cytosol | உயிரணுக்கணிகம் |
| Debranching enzyme | கிளை நீக்கும் நொதி |
| Devoid | காலியான |
| Diarrhoea | வயிற்றுப் போக்கு |
| Duodenum | சிறுகுடலின் முன்பகுதி |
| Duodenum | முன்சிறுகுடல் |
| Electron transport chain | எலக்ட்ரான் நகர்வுச் சங்கிலி |
| Electrophiles | எலக்ட்ரான் கவர் காரணிகள் |
| Elongation factors | நீளமாக்கும் காரணிகள் |
| Emaciated | உடல் இளைத்தல் |
| Endoplasmic reticulum | எண்டோபிளாச வலைப் பின்னல் |
| Erythrocyte | இரத்த சிவப்பு அணுக்கள் |
| Essential fatty acids | இன்றியமையாத கொழுப்பு அமிலங்கள் |
| Facilitated diffusion | புரதவழிக் கடத்தல் |
| Fatty acids | கொழுப்பு அமிலங்கள் |
| Fibril | நுண்ணிழை |
| Foreign bodies | நோய்க்கிருமிகள் |
| Gastro intestinal tract | இரைப்பைக் குடல் |



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| Ghosts | கூடுகள் |
| Goitre | முன்கழுத்துக் கழலை |
| Gun cotton | வெடிபஞ்சு |
| Hemolysis | இரத்தச்சிதைவு |
| Haemorrhage | இரத்தப்போக்கு |
| Hepatitis | மஞ்சள் காமாலை |
| Host | ஓம்புயிரி |
| Hydrophilic | நீர் விரும்பும் |
| Hydrophobic | நீர் வெறுக்கும் |
| Hyperglycaemia | மிகை இரத்த சர்க்கரை |
| Hypoventilation | மந்த சுவாசம் |
| Immunity | நோய் எதிர்ப்பாற்றல் |
| Infections | நோய்த் தொற்று |
| Inflammation | அழற்சி |
| Inhibitor | தடுப்பான் |
| Initiation factors | துவக்கக் காரணிகள் |
| Jaundice | மஞ்சள் காமாலை |
| Kidney | சிறுநீரகம் |
| Leukaemia | இரத்தப் புற்றுநோய் |
| Lipolysis | கொழுப்புச் சிதைவு |
| Liver | கல்லீரல் |
| Lung alveoli, | நுரையீரல் சிற்றறைகள் |
| Lymphoma | நிணநீர் நாளப்புற்று |
| Macrophage | பெருவிழுங்கணுக்கள் |
| Membrane | செல் சவ்வு |



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| Membrane transport | செல்சவ்வுக் கடத்தல் |
| Meningitis | மூளைக்காய்ச்சல் |
| Metabolism | வளர்சிதை மாற்றம் |
| Mutation | திடீர்மாற்றம் |
| Neoplasm | உயிரணுப் புற்று |
| Neurotransmission | நரம்புத் தூண்டல் |
| Non-competitive Inhibition | போட்டித் திறனற்ற தடுத்தல் |
| Nucleophiles | கருகவர்க் காரணிகள் |
| Nuts | கொட்டைகள் |
| Obesity | உடல் பெருத்தல் |
| Organelle | உள்ளுறுப்புகள் |
| Passive transport | செயலற்ற கட்த்தல் |
| Pathogen | நோய்க்கிருமி |
| Peripheral proteins | புற அமை புரதங்கள் |
| Phagocytosis | செல் விழுங்குதல் |
| Pinocytosis | செல்குடித்தல் |
| Polymerisation | பலபடியாக்கல் |
| Precursor | முதல்நிலைப் பொருட்கள் |
| Proteolytic enzymes | புரதச் சிதைவு நொதி |
| Pulses | பருப்பு வகைகள் |
| Reducing power | ஒடுக்கும் திறன் |
| Satiety value | நிறைவுத் தன்மை |
| Sclera | விழிவெண்படலம் |
| Sequential process | படிநிலைகள் |
| Serum | இரத்த திரவம் |



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| Sheath | உறை |
| Sickle cell anaemia | அரிவாளணுச்சோகை |
| Signal | சமிக்ஞை |
| Skeletal muscles | எலும்பு தசை |
| Spiders | சிலந்திகள் |
| Starvation | பட்டினி |
| Stem cell | ஆதாரச் செல் |
| Strand | இழை |
| Stress | மன உளைச்சல் |
| Symporters | ஒரு திசைக் கடத்திகள் |
| Symptoms | அறிகுறிகள் |
| Syphilis | பால்வினை நோய் |
| Ternary complex | முக்கூட்டுப் பொருள் |
| Thoracic duct | வயிற்றுக் குழல் |
| Toxic | நச்சுத்தன்மை கொண்ட |
| Transamination | அமினோ மாற்றம் |
| Transcription | படியெடுத்தல் |
| Translation | மரபுக்குறியீட்டு பெயர்ப்பு |
| Trans membrane proteins | செல்சவ்வு குறுக்கு புரதங்கள் |
| Uncompetitive inhibition | போட்டித் தன்மையற்ற தடுத்தல் |
| Uniporters | ஒரு பொருள் கடத்திகள் |
| Vascular bundles | கடத்துதிசுக் கற்றை |
| Vesicle | சிறு கொப்புளம் |
| Villi | குடலுறிஞ்சி |
| Whole grains | முழுதானியங்கள் |



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**This book has been printed on 80 G.S.M. Elegant Maplitho paper.**

**Printed by offset at:**



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