



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**

## Government of Tamil Nadu

First Edition - 2019

(Published under new syllabus)

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<b>Indian Institute of Science (IISc)</b> <i>Bangalore, Karnataka</i>	<ul style="list-style-type: none"> <li>❖ Peptides and peptidomimetics: Sugar amino acids</li> <li>❖ Delivery of therapeutic oligonucleotides</li> <li>❖ Carbohydrate-protein interactions</li> <li>❖ Functional soft and hybrid materials</li> <li>❖ Studies of dendrimers and liquid crystals</li> <li>❖ Inorganic and organic semiconductors</li> </ul>	<a href="http://www.iisc.ac.in">www.iisc.ac.in</a>
<b>Indian Institute of Technology (IITM)</b> <i>Chennai, Tamilnadu</i>	<ul style="list-style-type: none"> <li>❖ Statistical Correlations and Information Processing</li> <li>❖ Ionic liquid-templated ordered mesoporous aluminosilicates</li> <li>❖ Energy and fuel research</li> <li>❖ Molecular engineering of materials</li> <li>❖ Environmental science and engineering</li> <li>❖ Catalysis and soft matter</li> <li>❖ System engineering and data science</li> </ul>	<a href="http://www.iitm.ac.in">www.iitm.ac.in</a>
<b>National Institute of Immunology (NII)</b> <i>New Delhi</i>	<ul style="list-style-type: none"> <li>❖ Immunity and Infection,</li> <li>❖ Reproduction, Development and Cell Biology,</li> <li>❖ Genetics, Signalling and Cancer Biology, and</li> <li>❖ Chemical Biology, Biochemistry and Structural Biology.</li> </ul>	<a href="http://www.nii.res.in">/www.nii.res.in</a>
<b>National Center for Biological Sciences</b> <i>Bengaluru, Karnataka</i>	<ul style="list-style-type: none"> <li>❖ The self-packing polypeptide</li> <li>❖ Computational Approaches to Protein Science</li> <li>❖ Exploring the architecture and function of transmembrane ion channels</li> <li>❖ Computational cancer genomics</li> </ul>	<a href="http://www.ncbs.res.in">www.ncbs.res.in</a>
<b>Council of Scientific Industrial Research (CSIR)</b> <i>New Delhi</i>	<ul style="list-style-type: none"> <li>❖ Bio-actives, Cell / Stem cell / Immuno Therapies,</li> <li>❖ Regenerative / Precision / personalized Medicines</li> <li>❖ Reduction of Genetic Disease Burden.</li> </ul>	<a href="http://www.csir.res.in">www.csir.res.in</a>









 INSTITUTE	 RESEARCH AREAS	 WEBSITE
<b>CSIR - CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE(CFTRI)</b> <i>Mysuru, Karnataka</i>	<ul style="list-style-type: none"><li>❖ Ultra High Pressure System For Food Preservation</li><li>❖ Non-Aqueous Applications Of Membrane Technology</li></ul>	<a href="http://www.cftri.res.in">www.cftri.res.in</a>
<b>Central Salt &amp; Marine Chemicals Research Institute</b> <i>Bhavnagar, Gujarat</i>	<ul style="list-style-type: none"><li>❖ Salt and Marine Chemicals</li><li>❖ Inorganic Materials and Catalysis</li><li>❖ Electro Membrane Processes</li><li>❖ Reverse Osmosis</li></ul>	<a href="http://www.csmcri.org">www.csmcri.org</a>
<b>National Institute of Pharmaceutical Education and Research (NIPER)</b> <i>Mohali, Punjab.</i>	<ul style="list-style-type: none"><li>❖ Development of oral anti diabetic drugs</li><li>❖ Nano crystalline solid</li><li>❖ xanthine oxidase inhibitors</li><li>❖ microbial and marine origin for therapeutic purposes</li><li>❖ Standardization and quality control of herbal drugs and products</li><li>❖ Development of chemical process technologies for important natural products involving isolation</li></ul>	<a href="http://www.niper.gov.in">www.niper.gov.in</a>
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## LIST OF ENTRANCE EXAMINATIONS AFTER HSC

NAME OF THE EXAM	MODE OF SELECTION	COURSE	WEBSITE
<b>NEET</b> (National Eligibility cum Entrance test)	Written test	MBBS., BDS.,	<a href="http://www.cbseet.nic.in">www.cbseet.nic.in</a>
<b>AIIMS</b> (All India Institute of Medical Sciences)	Written test	MBBS.,	<a href="http://www.aiimsexams.org">www.aiimsexams.org</a>
<b>JIPMER</b> (Jawaharlal Institute Of Postgraduate Medical Education & Research)	Computer based test	MBBS.,	<a href="http://www.jipmer.edu.in">www.jipmer.edu.in</a>
<b>AFMC</b> (Armed Forces Medical College Entrance Exam)	Written test	M.B.B.S., (Should Serve 7 Years in Armed Forces)	<a href="http://www.afmc.nic.in">www.afmc.nic.in</a>
<b>IIT – JEE</b> (IIT- Joint Entrance Exam)	Written test	B.E., B.Tech., B.Arch.,	<a href="http://www.jeemain.nic.in">www.jeemain.nic.in</a>
<b>CUCET</b> (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)	<a href="http://www.cucet.co.in">www.cucet.co.in</a>
<b>NISER</b> (National Institute of Science Education and Research)	Written test	Integrated M Sc., (Biology, Chemistry, Mathematics and Physics)	<a href="http://www.niser.ac.in">www.niser.ac.in</a>
<b>IISC</b> (Indian Institute of Science Bangalore)	Written test	B.Sc., (4 years)	<a href="http://www.iisc.ernet.in">www.iisc.ernet.in</a>

 <b>NAME OF THE EXAM</b>	 <b>MODE OF SELECTION</b>	 <b>COURSE</b>	 <b>WEBSITE</b>
<b>IISER</b> (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	<a href="http://www.iiserpune.ac.in">www.iiserpune.ac.in</a>
<b>NATA</b> (National Aptitude Test in Architecture)	Computer Based Test	B.Arch.,	<a href="http://www.nata.in">www.nata.in</a>
<b>NIFT</b> (national institute of fashion technology)	Written test	B.Des., BFTech.,	<a href="http://www.nift.ac.in">www.nift.ac.in</a>
<b>IIFT</b> (indian institute of fashion technology)	Written test	B.Sc., (in fashion & apparel design)	<a href="http://www.iiftbangalore.com">www.iiftbangalore.com</a>
<b>NID NEED</b> (Nation al Entrance Exam for Design)	Written test	B.Des., (4 years)	<a href="http://www.nid.edu">www.nid.edu</a>
<b>IIST</b> (Indian Institute of Space Technology)	Written test	B.Tech., (Avionics/ Aerospace Engineering/ Physical Science)	<a href="http://www.iist.ac.in">www.iist.ac.in</a>
<b>CLAT</b> (Common Law Admission Test)	Written test	Integrated LLB ( 5 years)	<a href="http://www.cbcsneet.nic.in">www.cbcsneet.nic.in</a>
<b>NCHMCT</b> (National Council for Hotel Management Catering Technology Joint Entrance Exam)	Written test	B.Sc., (Hospitality and Hotel Administration)	<a href="http://www.nchm.nic.in">www.nchm.nic.in</a>
<b>NDA and NA</b> (National Defence Academy and Naval Academy)	Written Exam	3 Years Training for entry into ARMY/ NAVY/AIRFORCE	<a href="http://www.nda.nic.in">www.nda.nic.in</a>
<b>AIMNET</b> (All India Merchant Navy Entrance Test)	Written Exam	B.Tech., Marine Engineering B.Sc., Nautical Science B.Tech.,Navel Architecture and Ship Building	<a href="http://www.aim.net.co.in">www.aim.net.co.in</a>

## 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 titled of the structure of cell membrane in cell biology journal. In this model cell membrane consist of two phospholipid layer. 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.



## 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 system and function

## 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 Dalt in size and consist of aliphatic/aromatic hydrocarbons. There are various types of lipids that form the bilayer structure of a membrane.

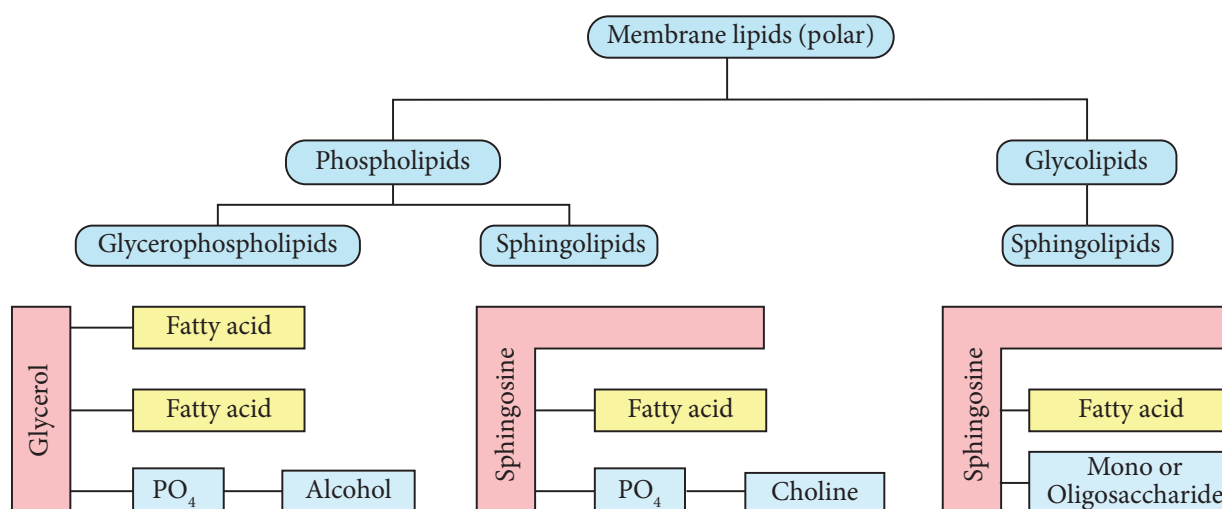


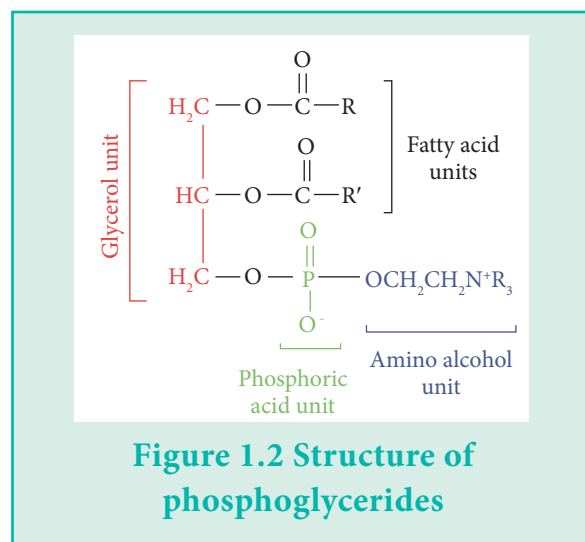
Figure 1.1 Lipid framework of a bilayered membrane



## 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

- tri-carbon (C1, C2, C3) backbone of glycerol,
- 2 long chain fatty acids esterified to C1 and C2 and
- phosphoric acid esterified to C3 of glycerol.



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 (as in C1) or can have 2 or more double bonds (as in C2).

**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**

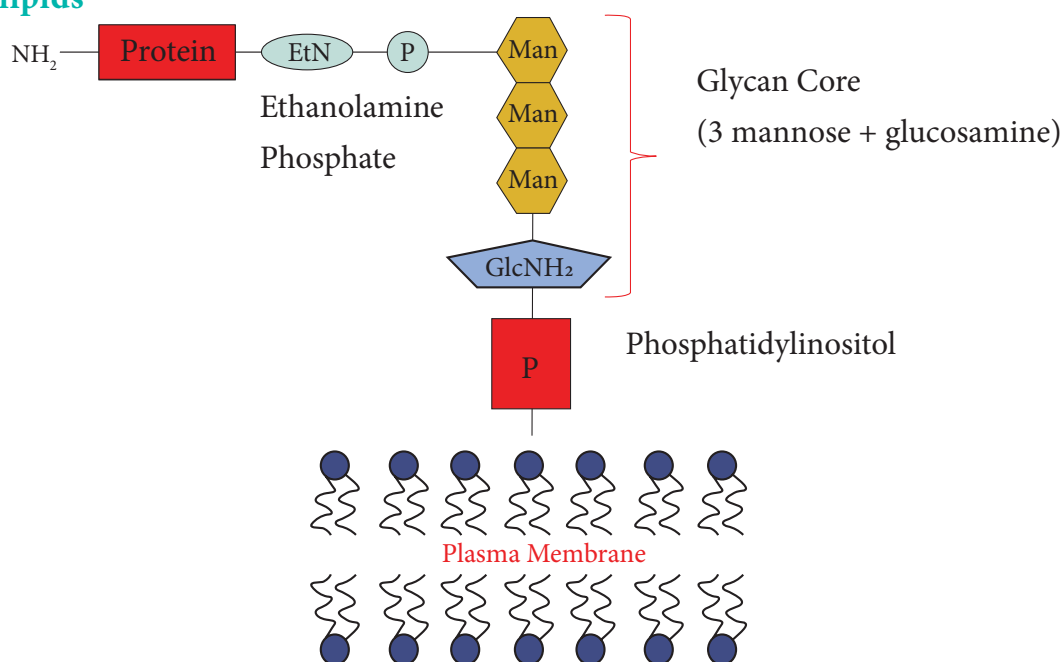
Name of glycerophospholipid	Name of X	Formula of X	Net charge (at. pH7)
Phosphatidic acid	-	-H	-1
Phosphatidylethanolamine	Ethanolamine	$-\text{CH}_2-\text{CH}_2-\text{NH}_3^+$	0
Phosphatidylcholine	Choline	$-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$	0
Phosphatidylserine	Serine	$-\text{CH}_2-\text{CH}(\text{COO}^-)-\text{NH}_3^+$	-1
Phosphatidylglycerol	Glycerol	$-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{OH}$	-1

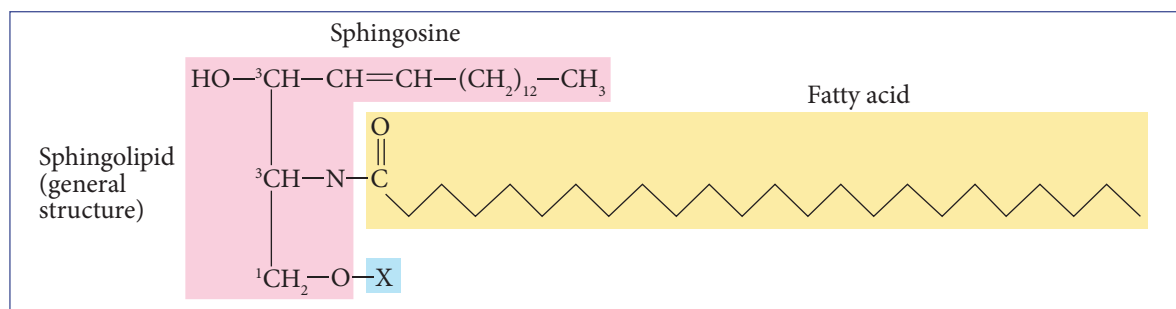
Phosphatidylinositol 4,5-bisphosphate	Myo-Inositol 4,5-bisphosphate		-4
Cardiolipin	Phosphatidylglycerol		-2

### 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 phosphatidylinositol.

### Shingolipids



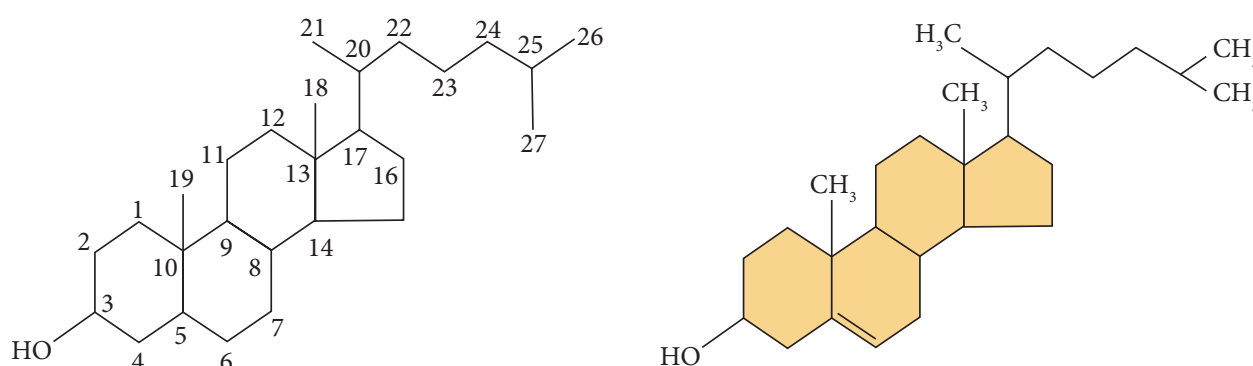


**Figure 1.3. Structure of Sphingolipids.**

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 glycosphingolipids, which may be either neutral or negatively charged. In some cases, 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.

## 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 protein present in it. You can recall that it was mentioned elsewhere that there are two classes of proteins.

Both integral and peripheral membrane proteins which form the structure of the membrane. Integral proteins both have hydrophobic and hydrophilic interaction surface with hydrocarbons 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.



## 1.2 MODELS PROPOSED FOR MEMBRANE STRUCTURE

### 1.2.1. Monolayer Model (YEAR)

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 35 Å.

### 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.

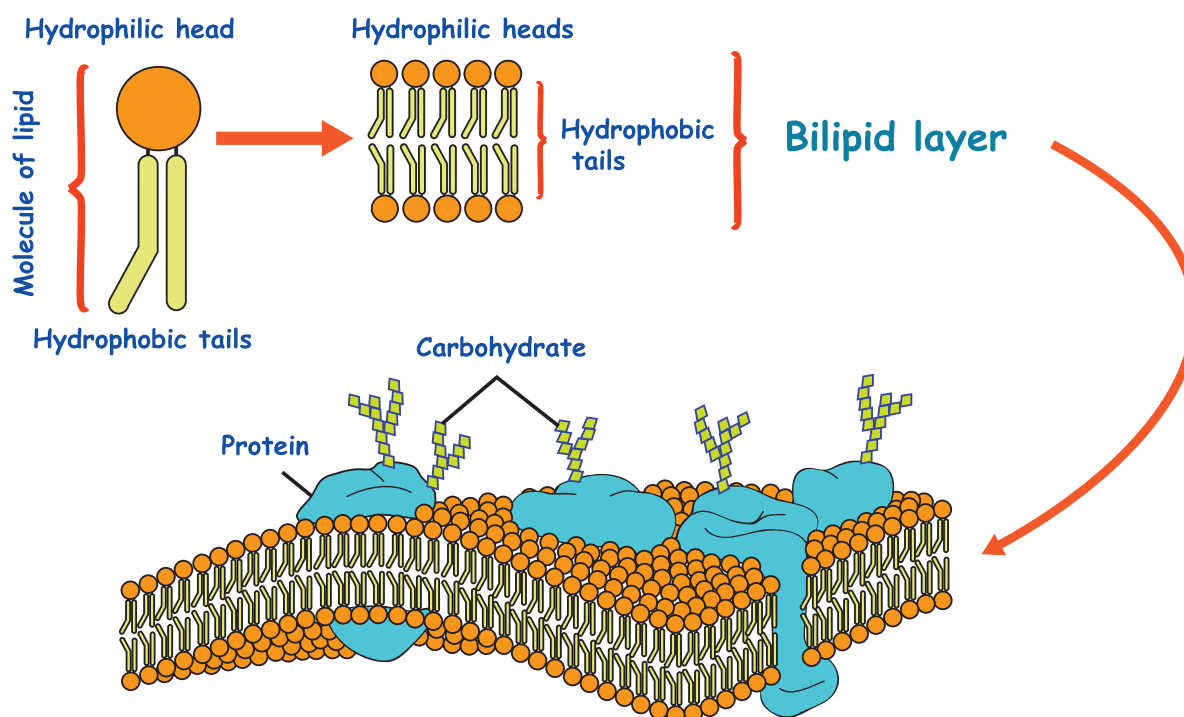
**This model is widely accepted because of the following features**

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



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.





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<sub>2</sub> and O<sub>2</sub>, do need smaller spaces between the phospholipids through which they can move quickly 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<sub>2</sub>) 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.



- 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.

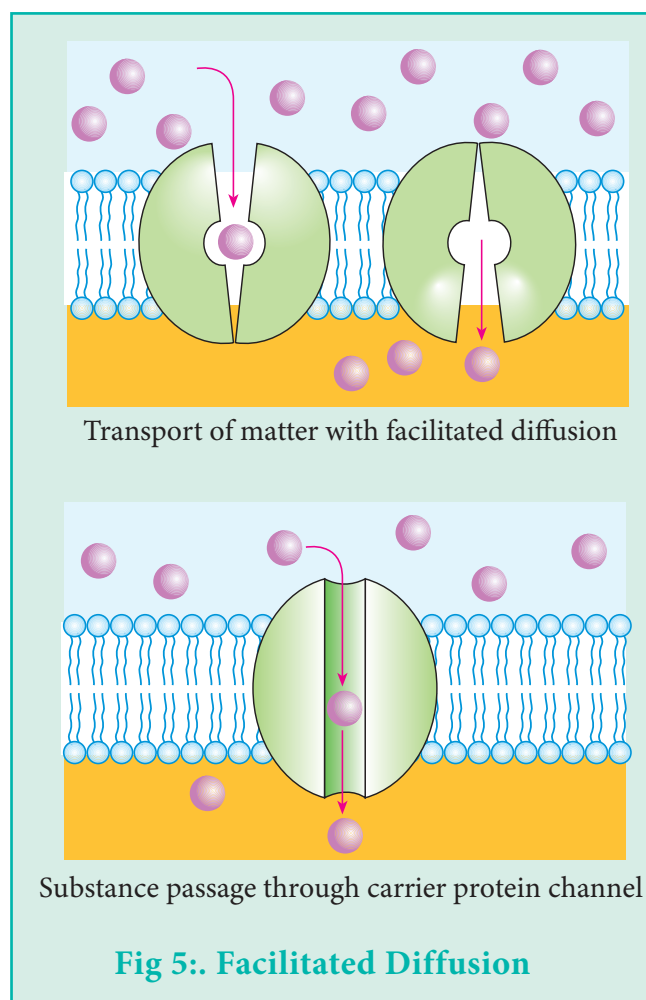
### 1.3.2. Facilitated diffusion

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 to cross.

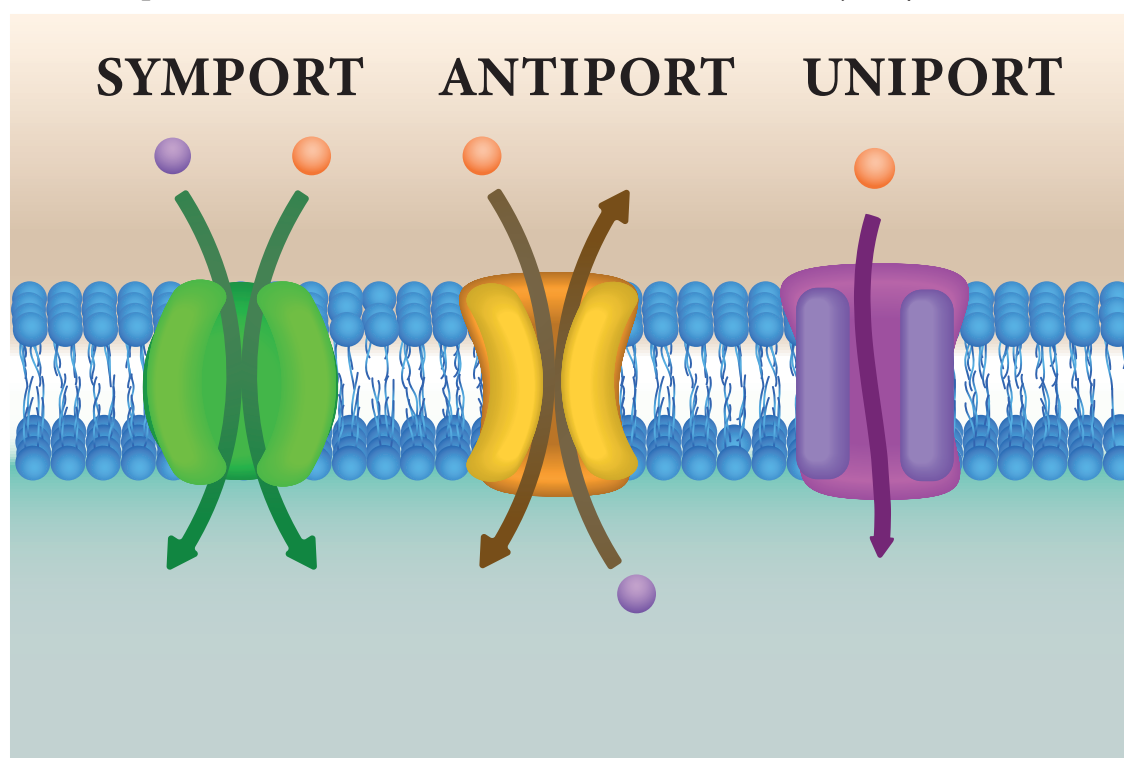
### 1.3.3. Active Transport

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).

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  $\text{Na}^+$  concentration and a high intracellular  $\text{K}^+$  concentration, along with a net negative electrical potential inside. The pump that maintains these gradients is an ATPase that is activated by  $\text{Na}^+$  and  $\text{K}^+$  ( $\text{Na}^+/\text{K}^+$  ATPase;). The ATPase is an integral membrane protein and requires phospholipids for activity. The ATPase has catalytic centers for both ATP and  $\text{Na}^+$  on the cytoplasmic side of the membrane, but the  $\text{K}^+$  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  $\text{Na}^+/\text{K}^+$  pump moves three  $\text{Na}^+$  ions out of the cell and two  $\text{K}^+$  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.

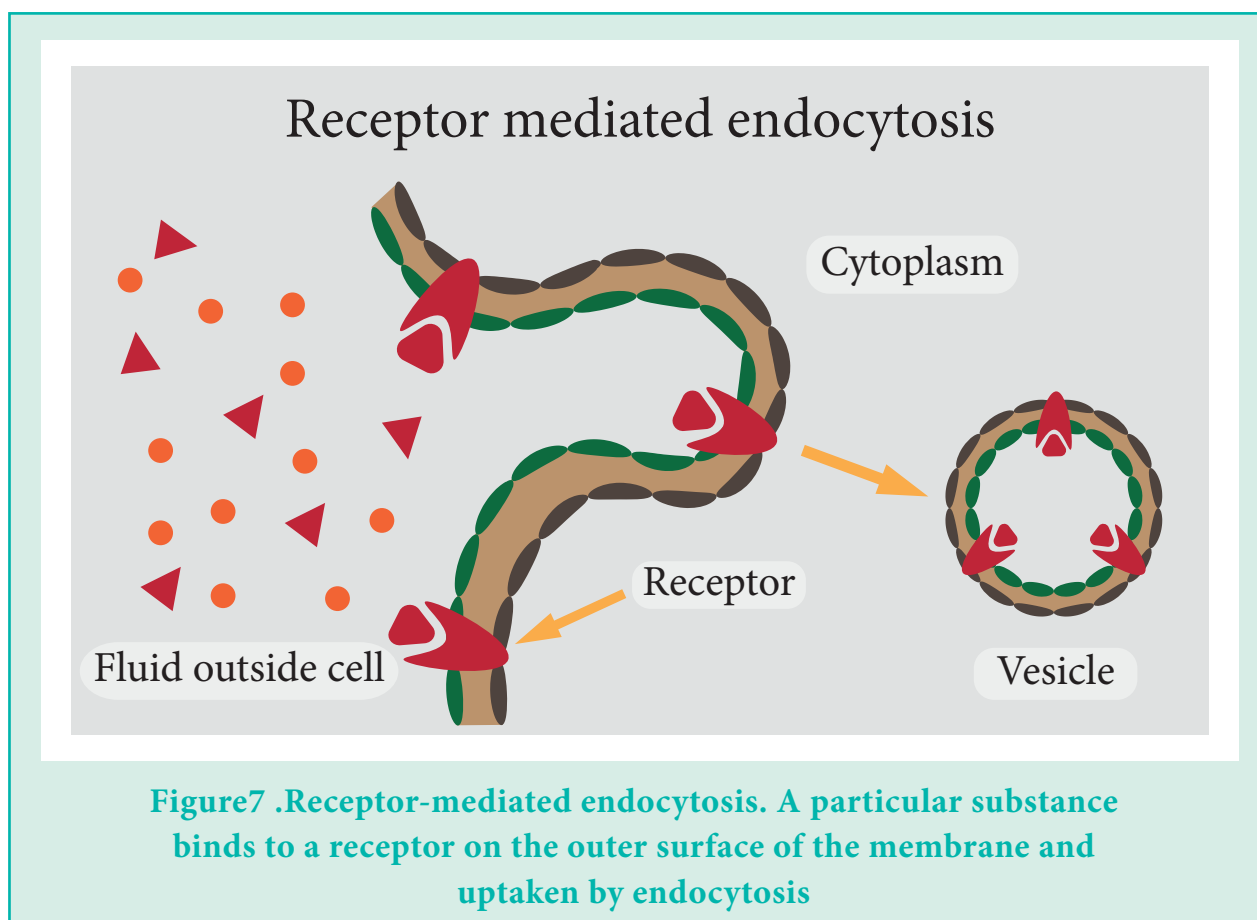
Carriers which transport a single substrate through a membrane down its concentration gradient are called uniporters. Many carriers transport substrates against or up concentration gradients also. In such cases, the carrier transport across the membrane is combined with the transport of one more substrate 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  $\text{Na}^+$ . It is called as symporter, as both glucose and  $\text{Na}^+$  are transferred in the same direction.

Another class of carriers are termed as antiporters. These are called anti as they transport substrate in the opposite direction 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.



### 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 friction 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 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.

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 pre-mature infants have low levels of this surfactant leading to acute respiratory distress.

## 1.5. OSMOSIS

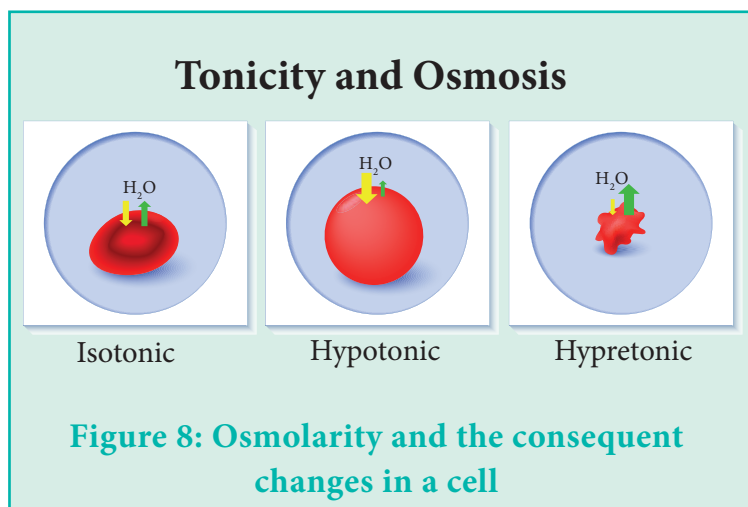
Osmosis is the net movement of water across a semi-permeable membrane from an area of lower concentration of the solute to an area of higher concentration of the solute.

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's 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's 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, concentration of solute, than the surrounding fluid, the cell interior is hypertonic to the surrounding fluid, and the surrounding fluid is hypotonic to the cell interior.





### 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, which is derived as follows.

#### Regulation

When there is a deviation of blood pH away from that of normal value, the two systems in body are actuated 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; 1. the renal system modifies the reabsorption/and or production of bicarbonate/ hydrogen ions. This balance is known as “compensation”.



### 1.7.1 Haemoglobin Buffer system

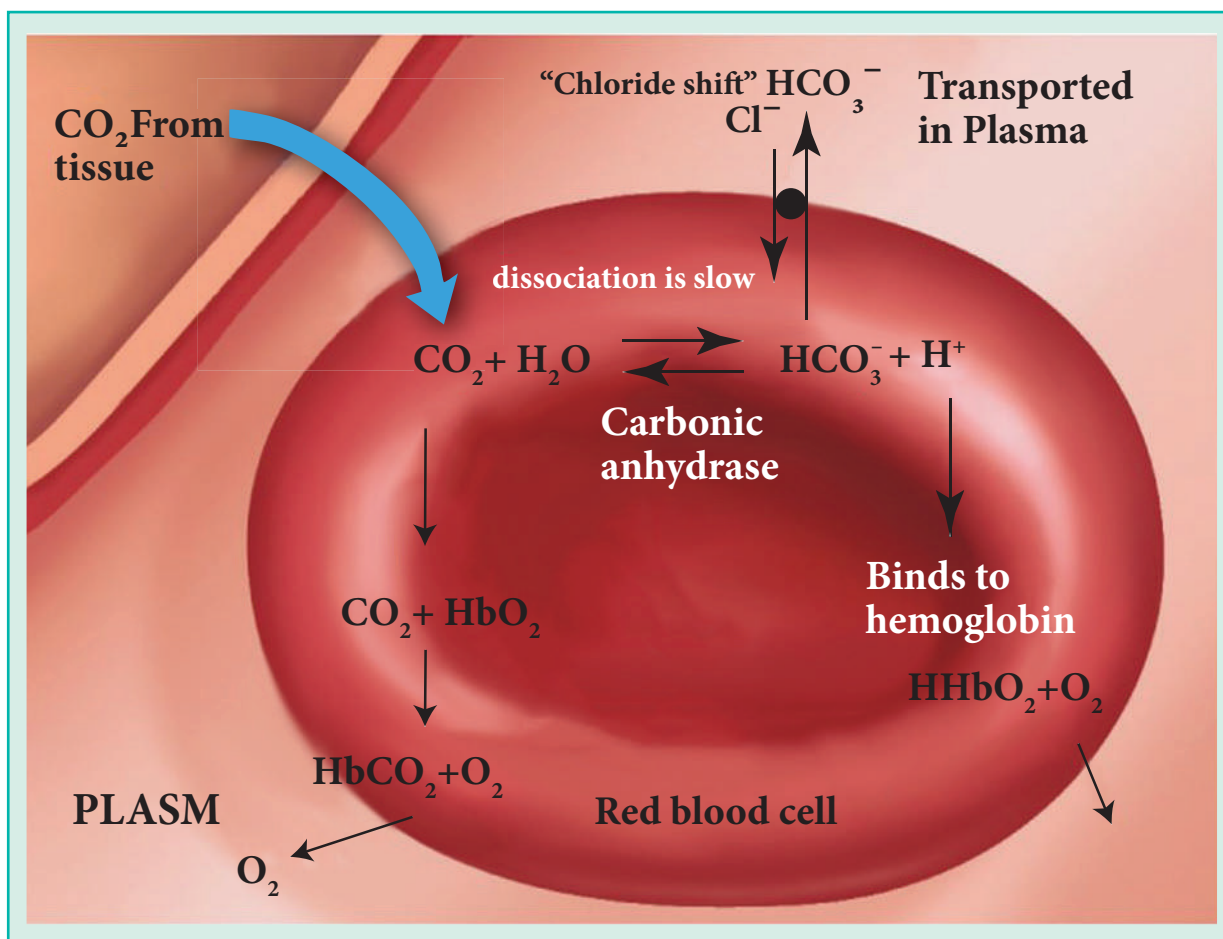
By the process of metabolism, carbon di oxide is produced in the tissues and enters the blood. This  $\text{CO}_2$  will be hydrated forming  $\text{H}_2\text{CO}_3$  and this gets ionized yielding  $\text{H}^+$  and  $\text{HCO}_3^-$ . When the oxygen tension is reduced in the tissues, oxy-hemoglobin 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  $\text{CO}_2$  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



Reduced hemoglobin will act as anion and accepts the  $\text{H}^+$  ions to produce acid-reduced hemoglobin (HHb). By the production of weak acid, the arrival of  $\text{H}^+$  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  $\text{H}^+$  ions are released . Immediately these re-released  $\text{H}^+$  ion are neutralized by  $\text{HCO}_3^-$ . This is inevitable for the lungs to release  $\text{CO}_2$ .





## Chloride Shift

1. In RBCs  $\text{CO}_2$  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 buffers.
3. Bicarbonate returns to plasma and gets exchanged with chloride ion that enters into (shifts into) the cell if the tension of  $\text{CO}_2$  increases in the blood.
4. On the contrary, if the  $\text{CO}_2$  tension is reduced, chloride will exit the cell and enters 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  $\text{CO}_2$  being carried by plasma as sodium bicarbonate.
6. The cycle continues as  $\text{CO}_2$  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.

### 1.7.2. Regulation by respiratory mechanism

We have seen that the carbonic acid will dissociate into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . If there is more of  $\text{H}^+$  within the blood more of  $\text{CO}_2$  elimination will be carried out by lungs. If there is more of  $\text{HCO}_3^-$ , the lungs will ensure low respiratory rate thus enhancing the retention of  $\text{CO}_2$  so that it can be useful for forming carbonic acid that can buffer the excess of bicarbonate.

The alveoli and bronchioles of lungs do perform such functions effectively. A phospholipoprotein molecule is secreted by a particular type of cells of 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.

### 1.7.3. 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. (Fig 9).



### Possible transport

- Simple diffusion
- Facilitated diffusion (glucose in basolateral membrane)

### Active transport

#### Active reabsorption

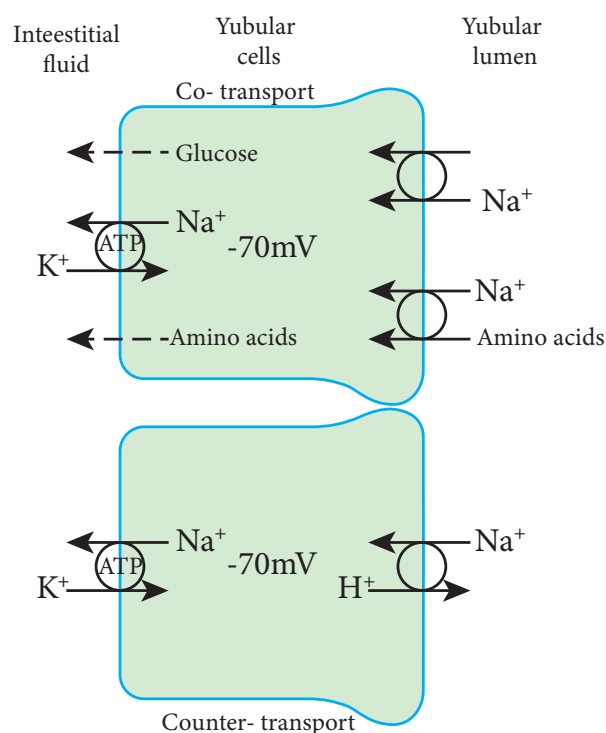
- Primary active transport (Sodium-potassium ATPase pump)
- Secondary active transport
- Secondary active reabsorption (glucose by sodium in PT)

#### Active Secretion

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

#### Osmosis

#### Pinocytosis



**Figure 9: 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.
4. Lipids and proteins (integral and peripheral) maintain the structure and function



of a 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 that govern the movement of substances across the membrane to attain the pH balance which in turn is essential for homeostasis.

## 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 bilayered 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)



4. The distribution of inherent proteins in the membrane:
  - 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.
  - 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. -----following is the major function of cell membrane.
  - A. compartmentalization
  - B. protection from extracellular components
  - C. temperature maintenance
  - D. None of the above
9. Materials are allowed to pass 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. epic
  - B. criss-cross
  - C. zigzag
  - D. mosaic



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 bring the substances into the cell
- A. Endocytosis
  - B. Exocytosis
  - C. Plasmolysis
  - D. Creantion
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

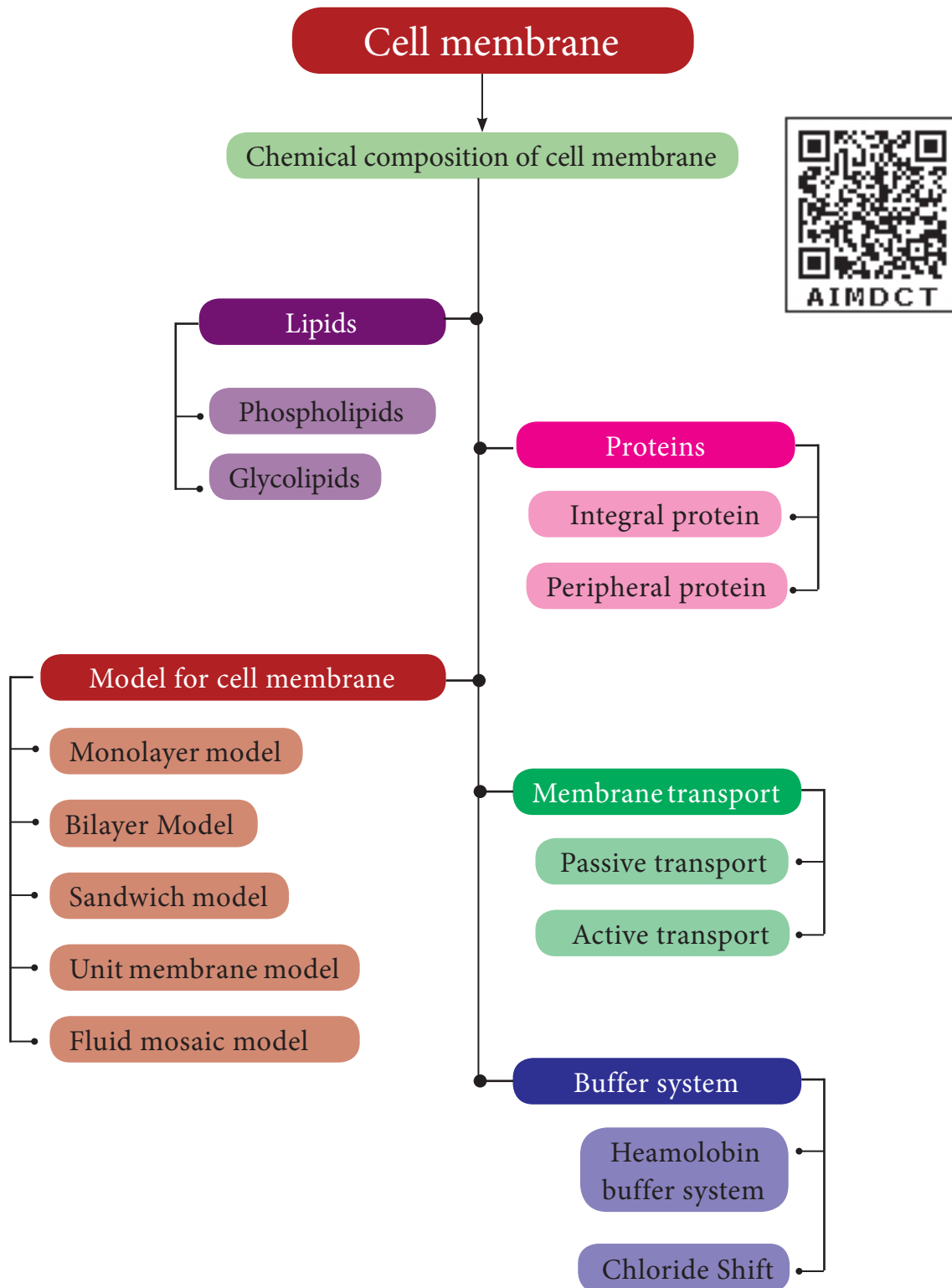
## II Answer the following

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.
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 pinoctocytosis.
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.

# CONCEPT MAP



## 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.



### 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

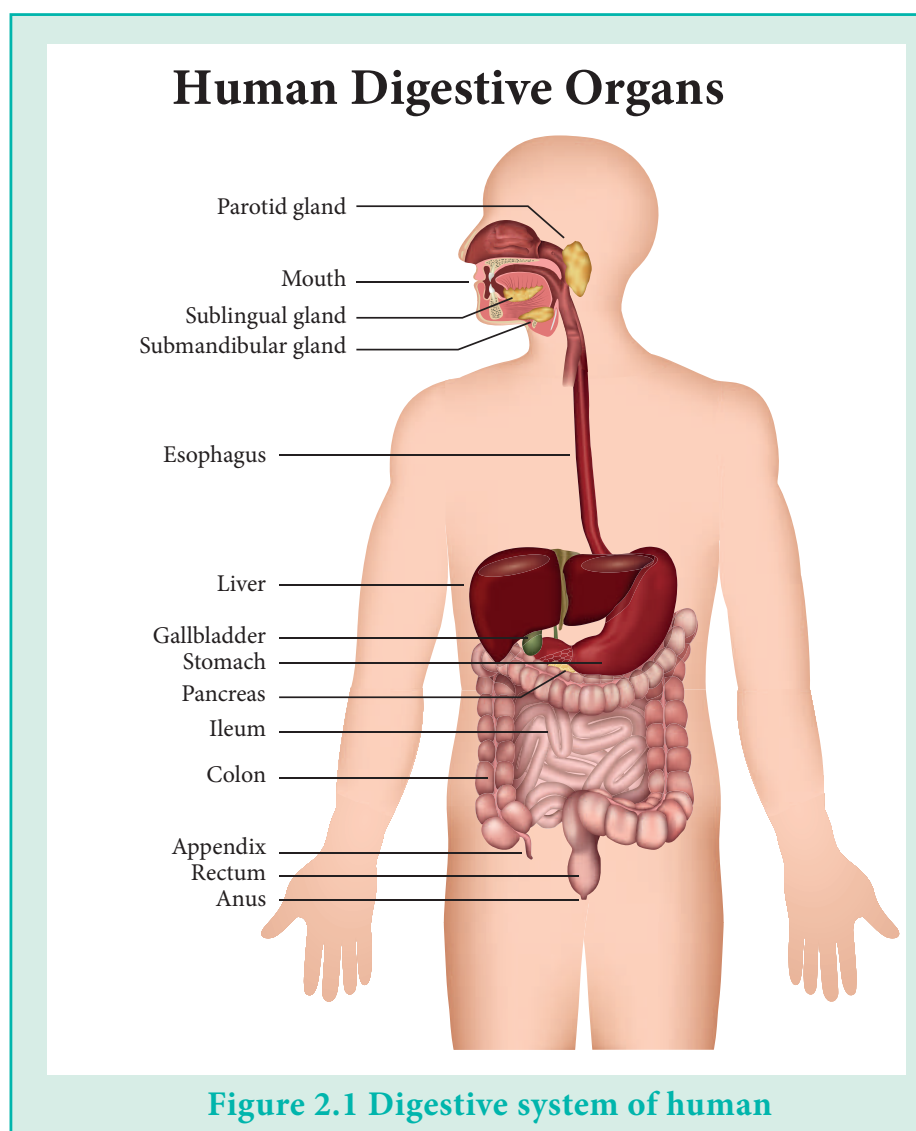
## INTRODUCTION

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:

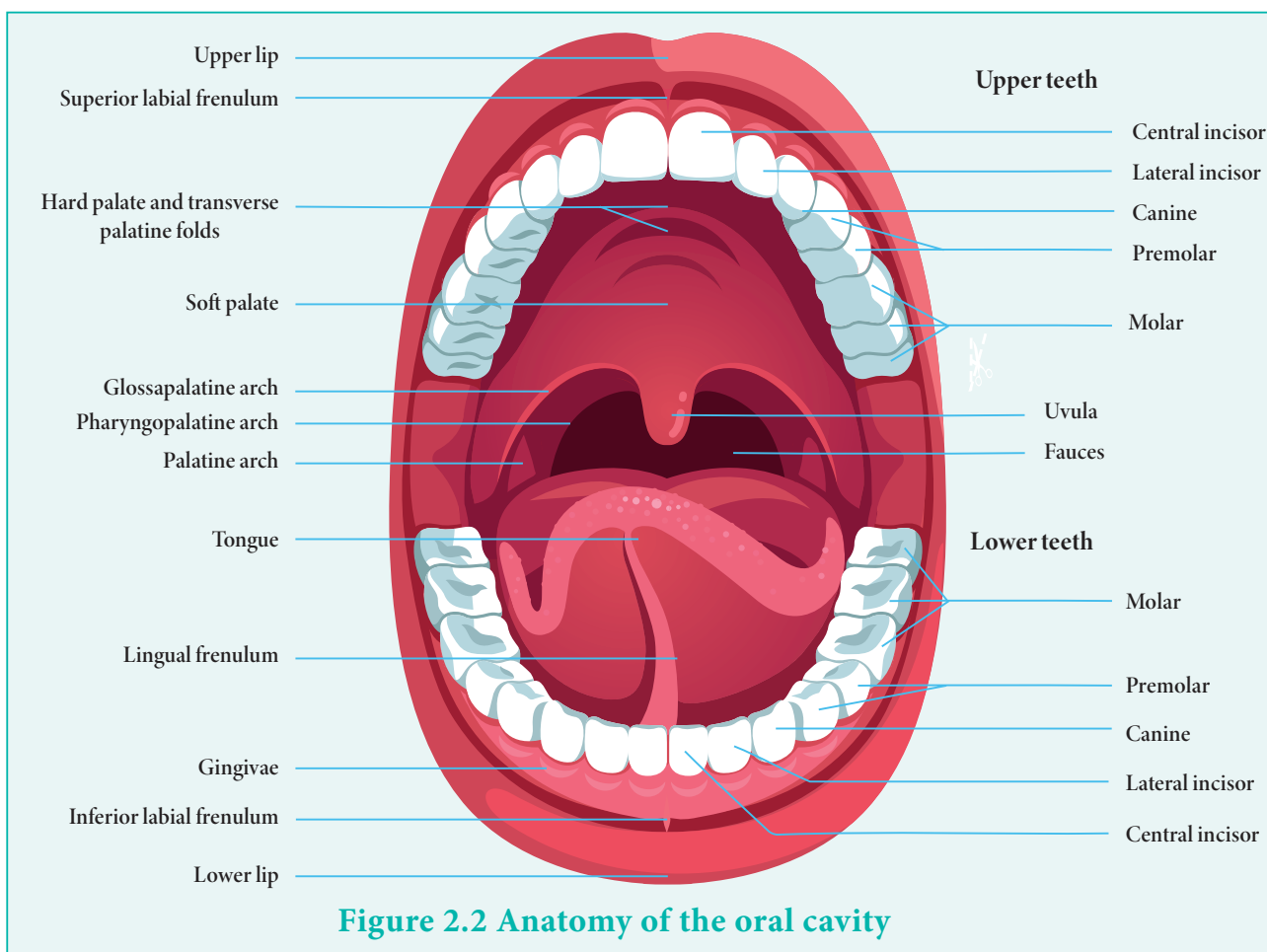
The digestive system is made up of a tubular gastro-intestinal tract (GI tract), extending from mouth till anus, and secretory organs (Figure 2.1). The organs of the GI tract include the oral cavity, pharynx, stomach, small intestine, large intestine, rectum and anus. The accessory digestive organs include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

#### 2.1.1 Gastrointestinal Tract:





### 2.1.1.1 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

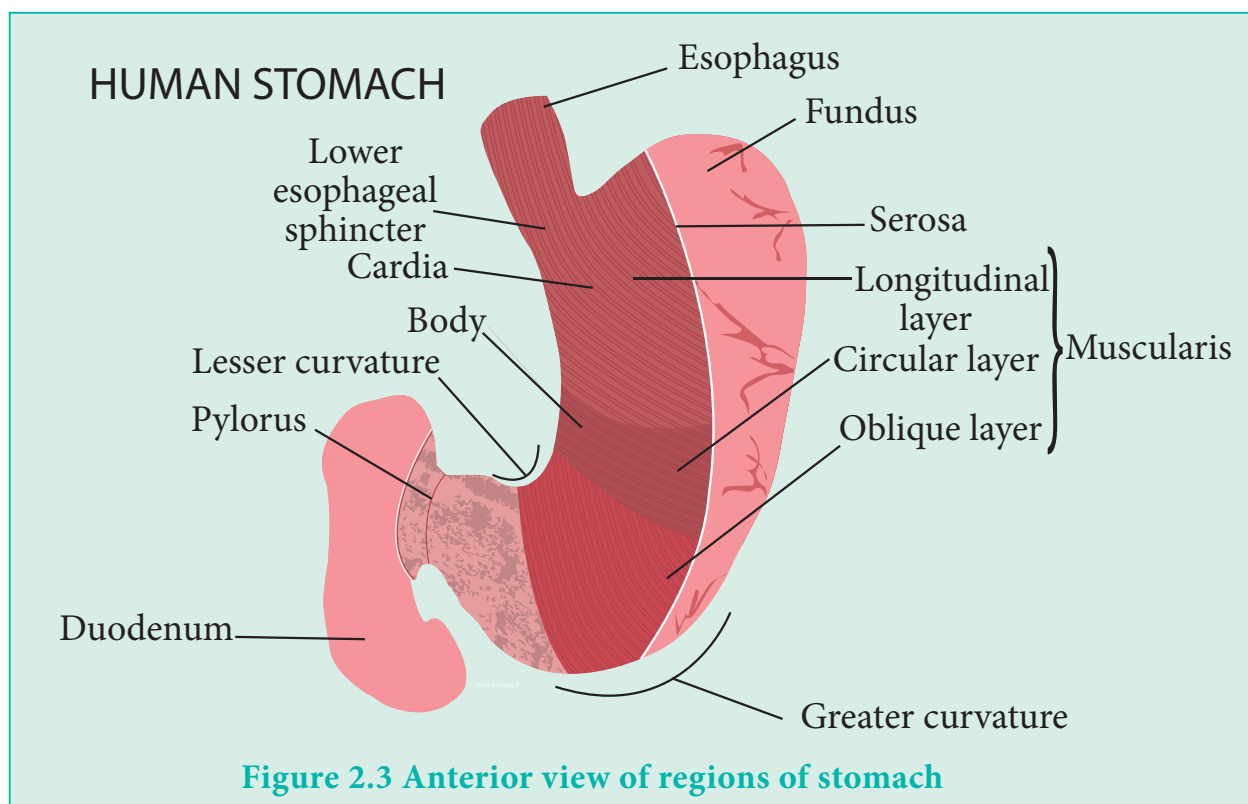
6 seconds. During this period, the swallowing center inhibits the respiratory center of the medulla, halting respiration, thereby allowing swallowing to proceed.

### 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).



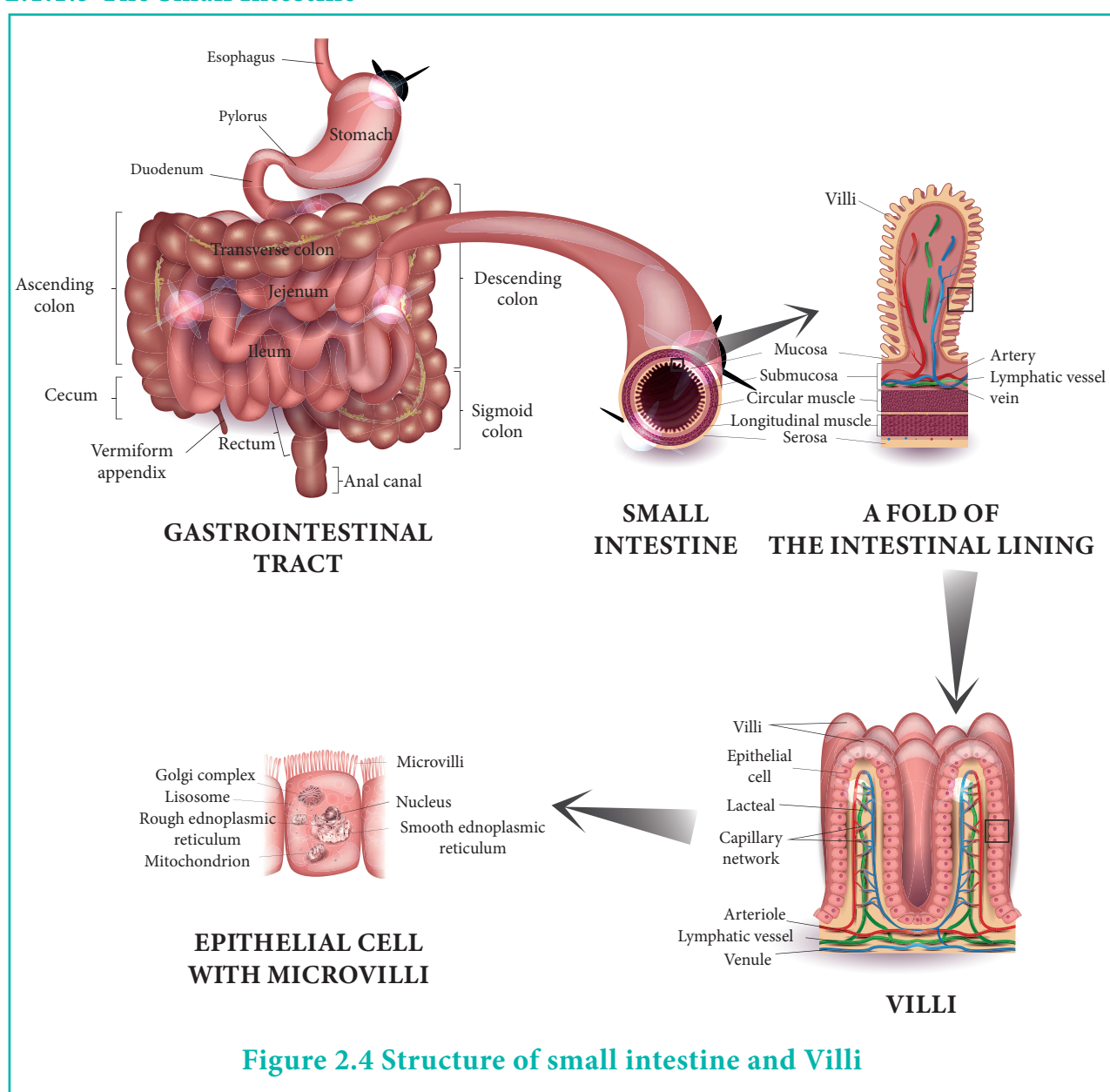
The functions of the stomach are:

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

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



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

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.

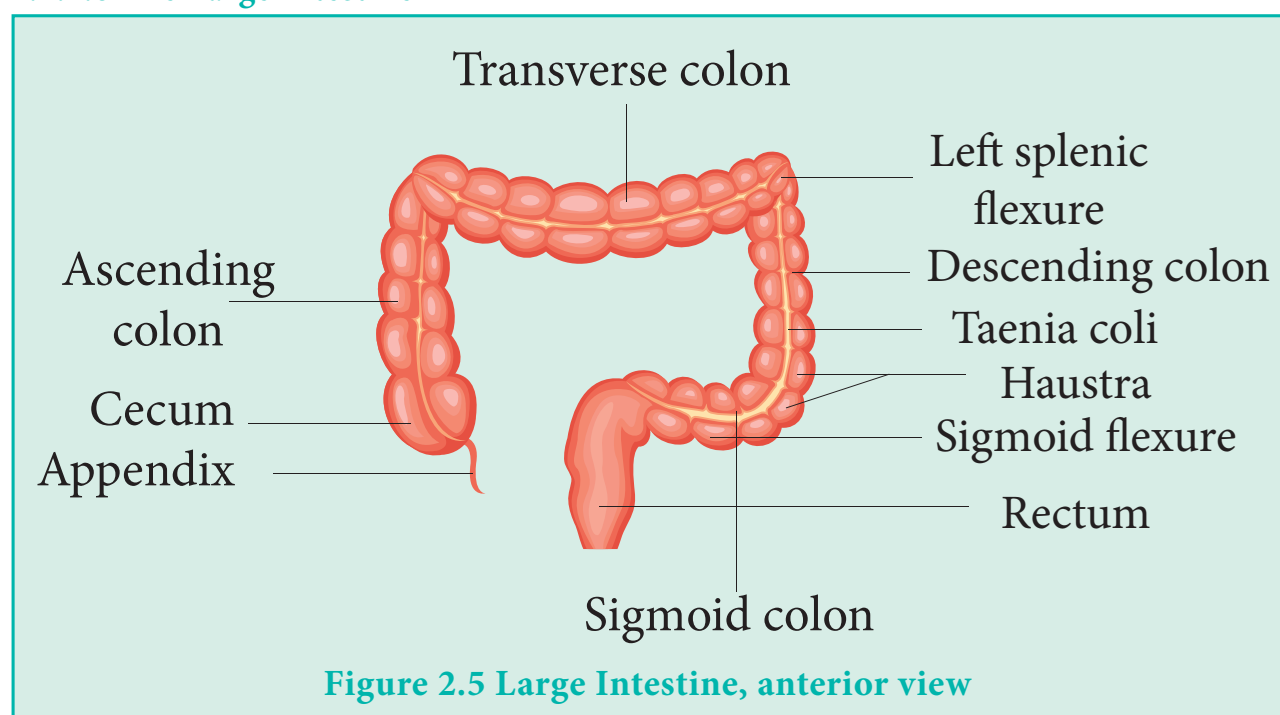
The small intestine is divided into three regions: The duodenum, a C shaped tube, which starts at the pyloric sphincter that extends about 25 cm (10 inches) and merges with the jejunum. The jejunum is about 1 m (3 ft) long and extends to the ileum, the final and longest region of the small intestine, 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.

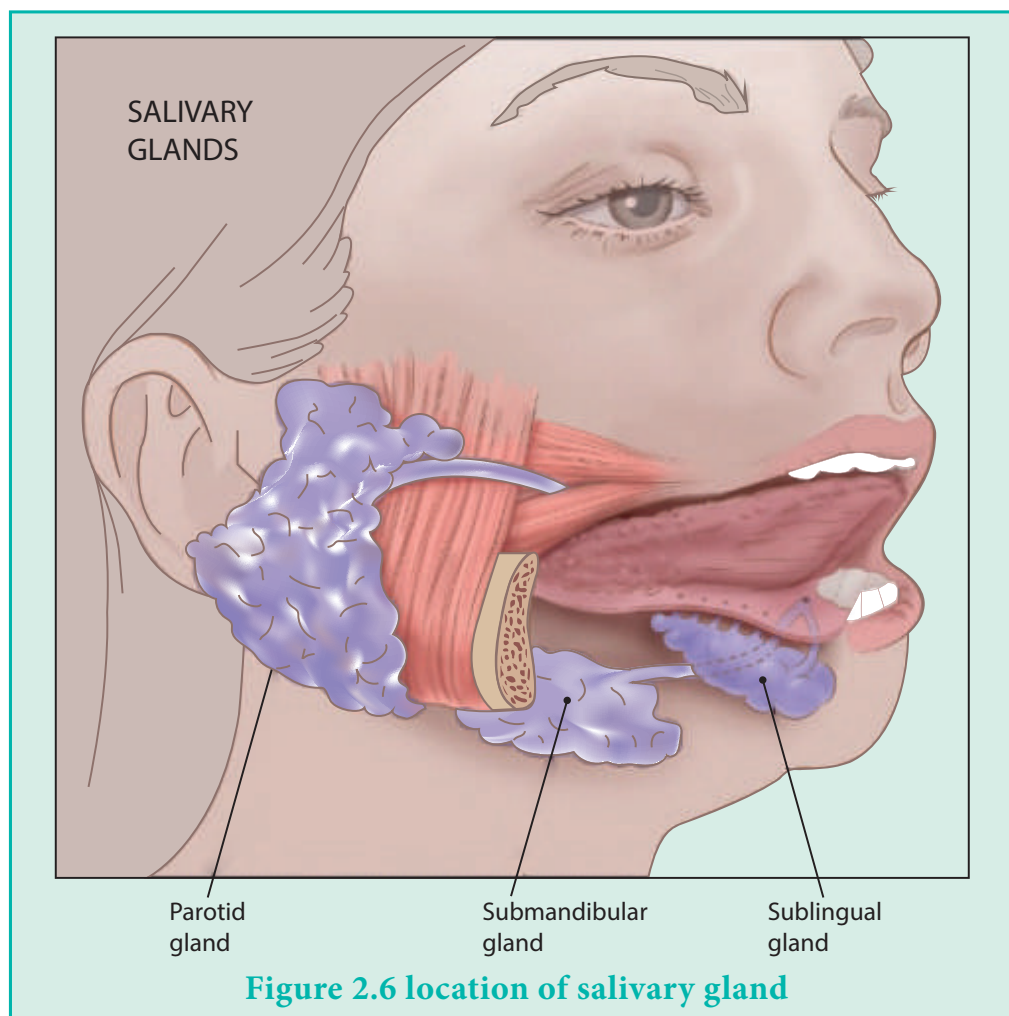
#### 2.1.1.6 The Large Intestine



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

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



#### 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



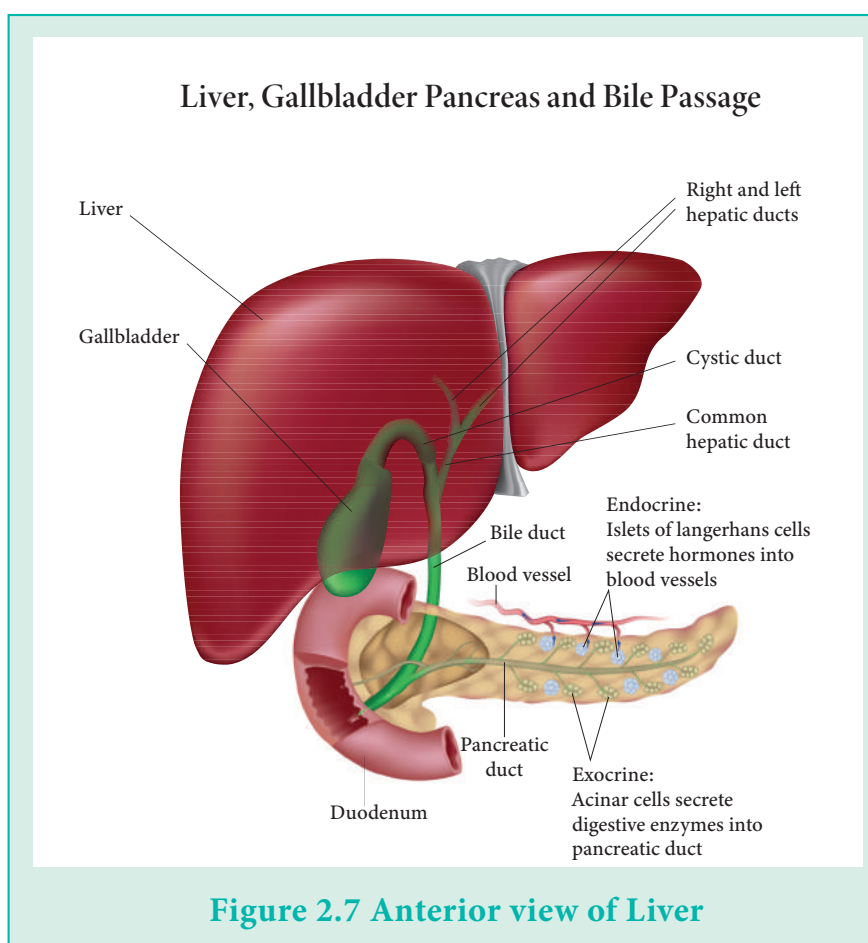
receptors (gustatory receptors), while the chloride ions activate amylase to digest starch. The IgA in the saliva acts against the microbes. The saliva helps in rolling the food into a ball so that it can be swallowed.

### 2.1.2.2 Gastric glands

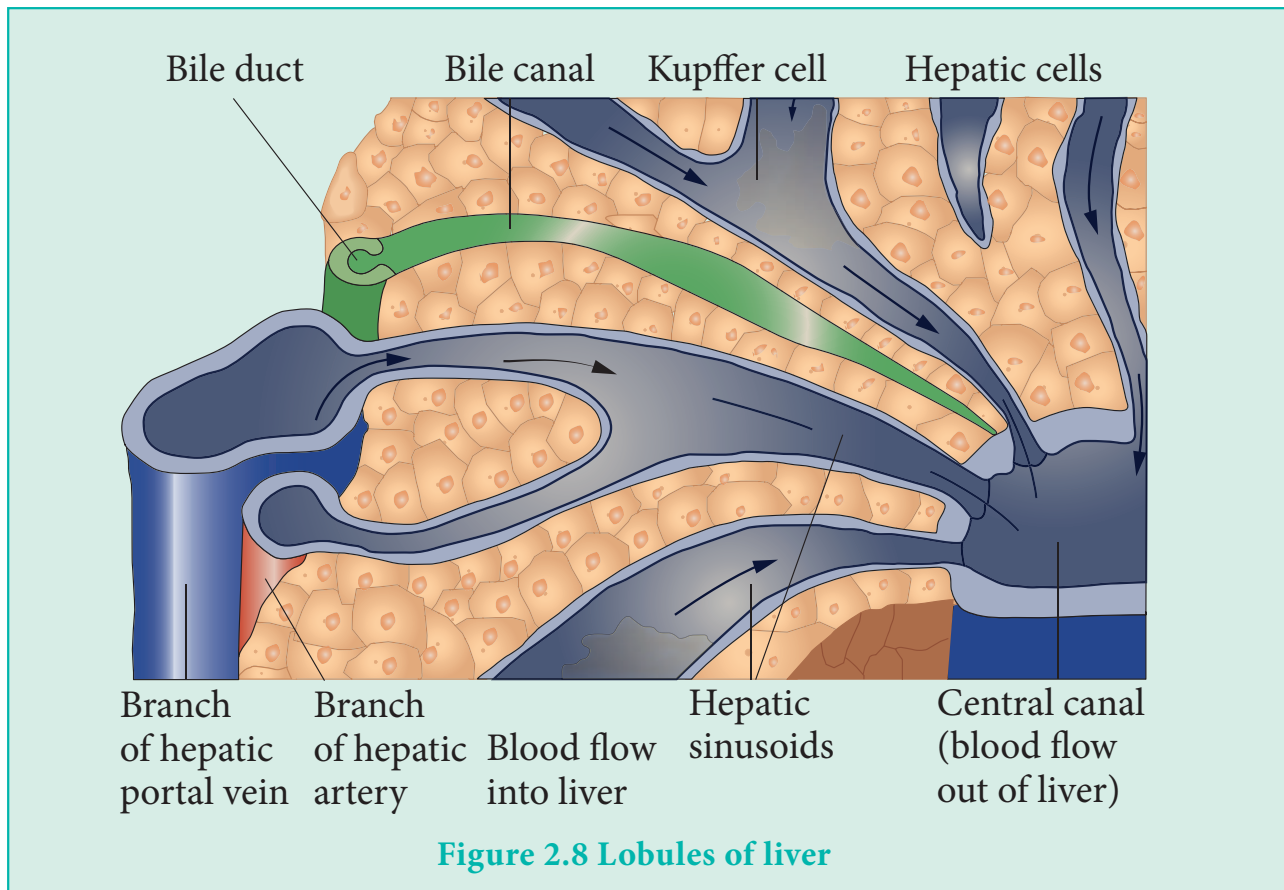
Gastric juice is secreted by the different types of exocrine cells present in the gastric glands lining the stomach. These glandular cells empty their secretions into the gastric pit. There are three types of exocrine cells: Intrinsic factor and HCl secreting parietal cells, chief cells that secrete 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).



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.



### 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

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

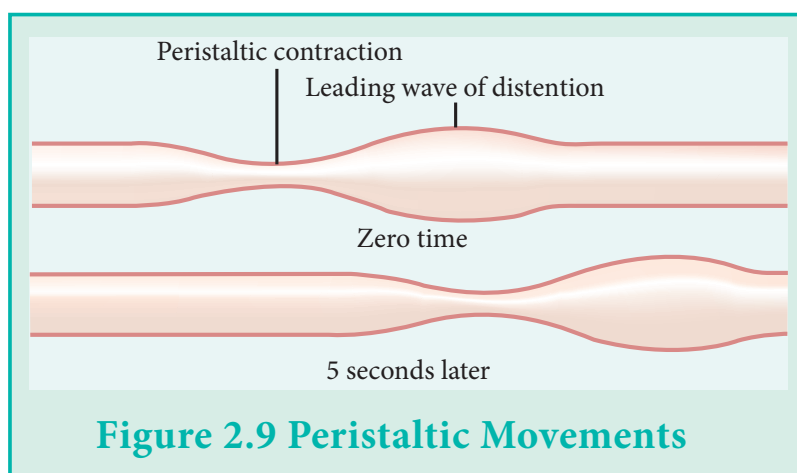
GASTROINTESTINAL TRACT	SECRETORY GLANDS
Oral cavity	Salivary glands
Pharynx	Gastric glands
Oesophagus	Liver and Gall bladder
Stomach	Pancreas
Small Intestine	Duodenal glands (Brunner's glands)
Large Intestine	
Rectum	
Anus	



### 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.
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 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).

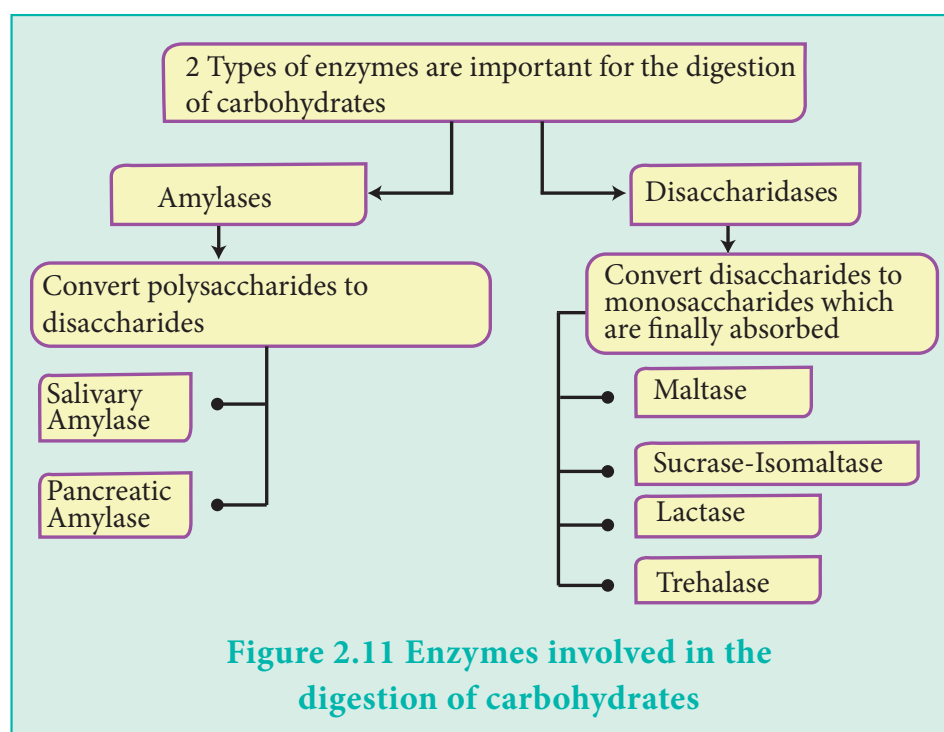
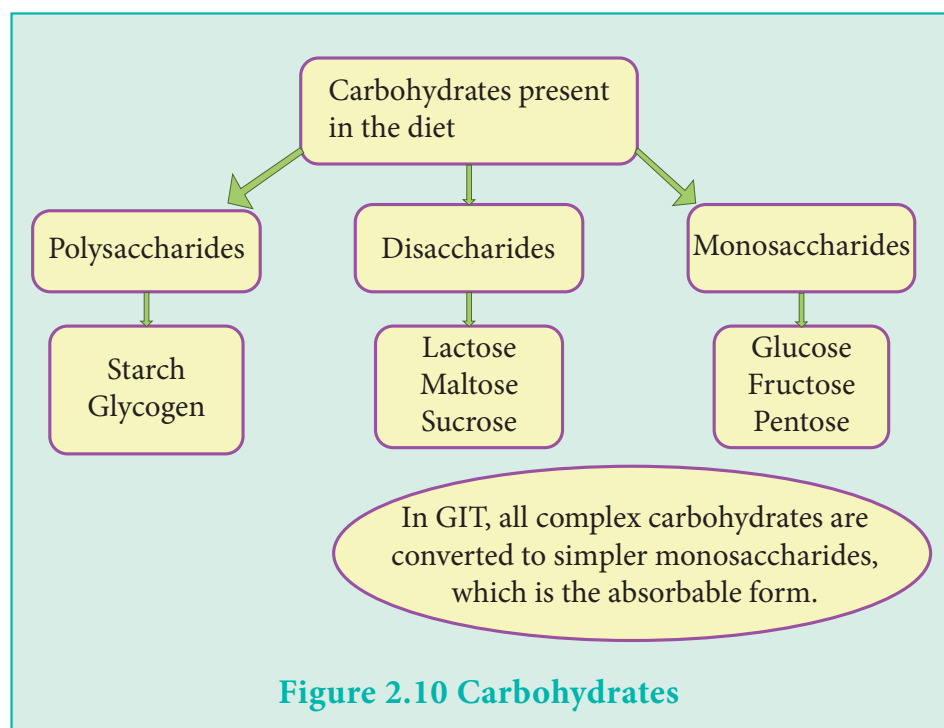
Digestion of carbohydrates takes place briefly in the mouth and largely in the intestine. The polysaccharides get hydrated during cooking, which is essential for efficient digestion.

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 2.10.2).

### 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  $\alpha$ -amylase. This enzyme with an optimum pH of 6.8, hydrolyzes the  $\alpha 1 \rightarrow 4$  glycosidic bonds to

release smaller oligosaccharide fragments and a highly branched limit dextrin (as the inner  $\alpha 1 \rightarrow 4$  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.



### 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.

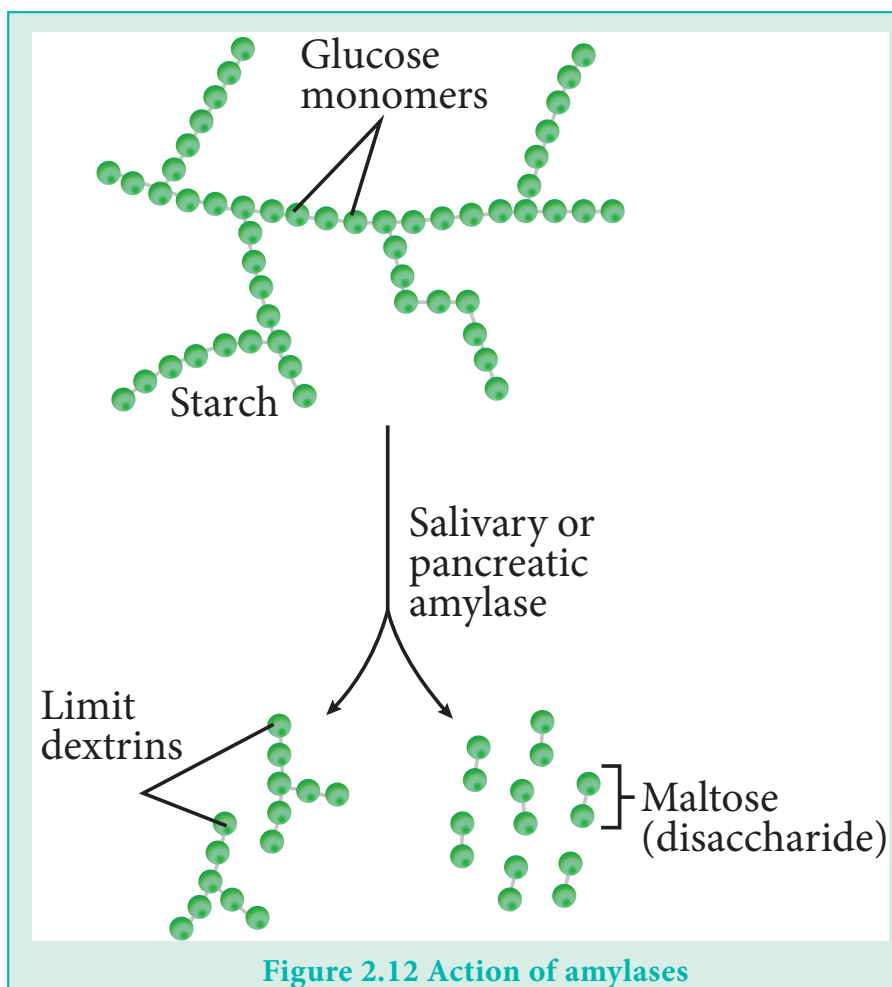


Figure 2.12 Action of amylases

### 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  $\alpha 1 \rightarrow 6$  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

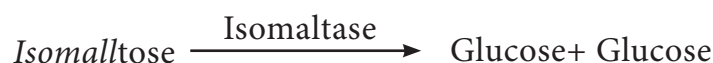
Moreover, pancreas secrete ten times more of pancreatic amylase into the intestinal lumen than that required to digest the carbohydrates in the diet.

### Disaccharidases

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

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

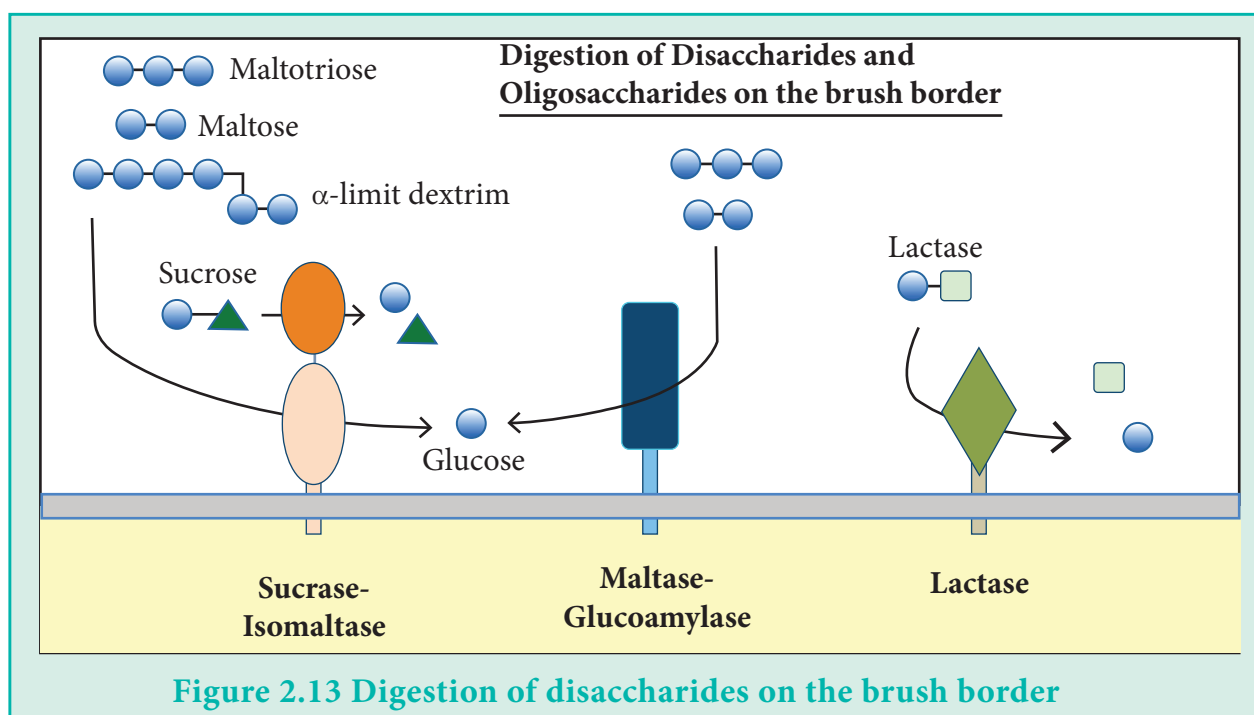
**Isomaltase:** Isomaltase co-elutes with Sucrase on purification and therefore Sucrase/isomaltase is a complex protein. Isomaltase acts on the  $\alpha 1 \rightarrow 6$  linkage and cleaves it into two molecules of glucose.



**Lactase:** The  $\beta 1 \rightarrow 4$  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, which corresponds to the typical pH of the human small intestine?.



**Maltase:** Maltose, the end product of polysaccharide digestion by amylases is cleaved into two glucose molecules by cleavage of  $\alpha 1 \rightarrow 4$  linkage by maltase to liberate glucose into the lumen. It also has an optimum pH around 6 (Figure 2.13).



Cerebrosidases – Minor enzymes are a group of enzymes that hydrolyse the gluco and galactocerebrosides present in glycolipids.

**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*	Glucosyl- and galactocerebroside
Sucrase	Sucrose; also maltose and maltotriose
Isomaltase	$\alpha(1 \rightarrow 6)$ bonds in isomaltose and $\alpha$ -limit dextrins
Trehalase	<ul style="list-style-type: none"> <li>Trehalose</li> </ul>
<p>* The lactase and cerebrosidase activities reside in two different globular domains of the same polypeptide.</p> <p># Cellobiose is a disaccharide of two glucose residues in <math>\beta(1 \rightarrow 4)</math> glycosidic linkage.</p> <ul style="list-style-type: none"> <li>Trehalose is a disaccharide with a structure of <math>\alpha</math>-D-glucopyranosyl-<math>\alpha</math>-D-glucopyranoside (in 1,1 glycosidic linkage); common only in mushrooms.</li> </ul>	

### 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.

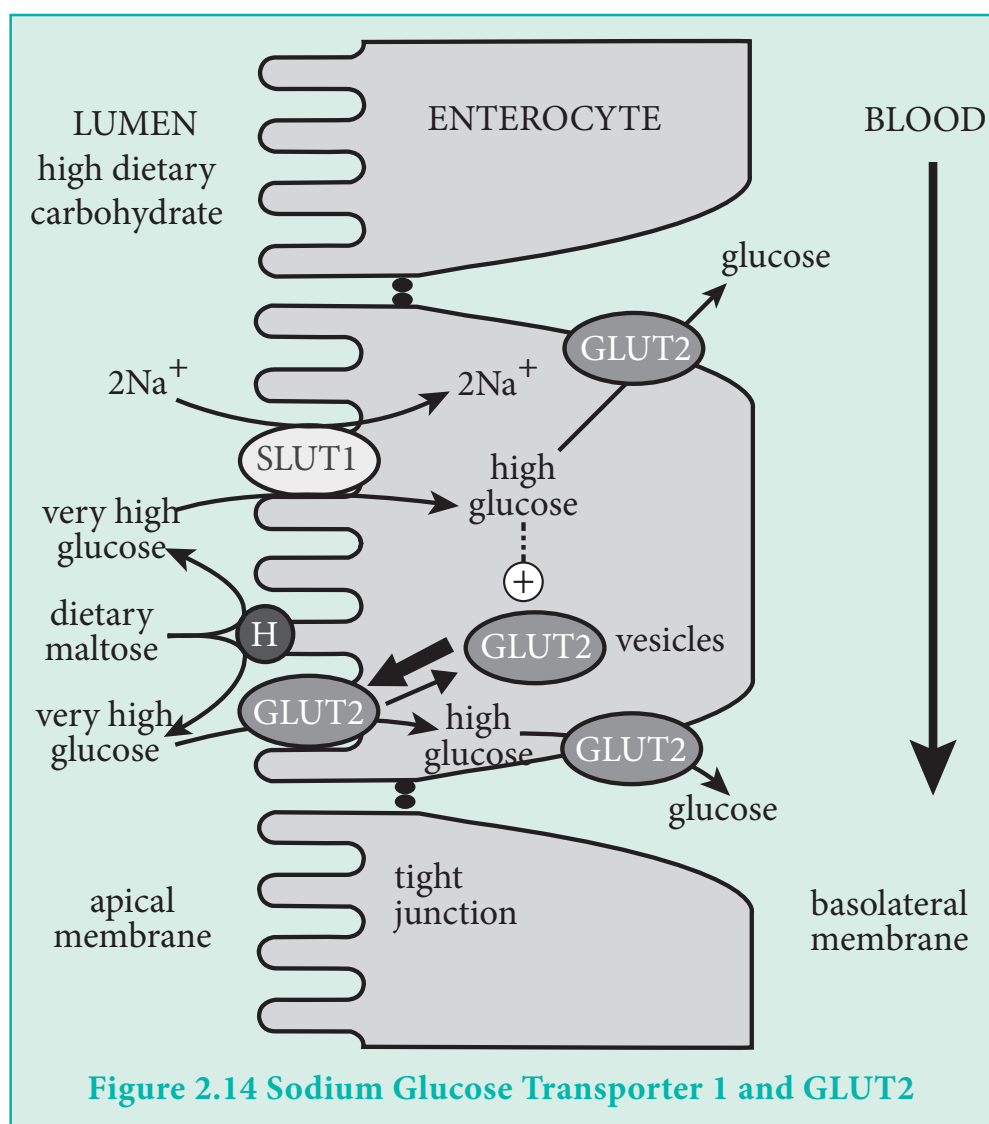
## Active Transport

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:

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, i.e. 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.



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



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 a constituents 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.

**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.

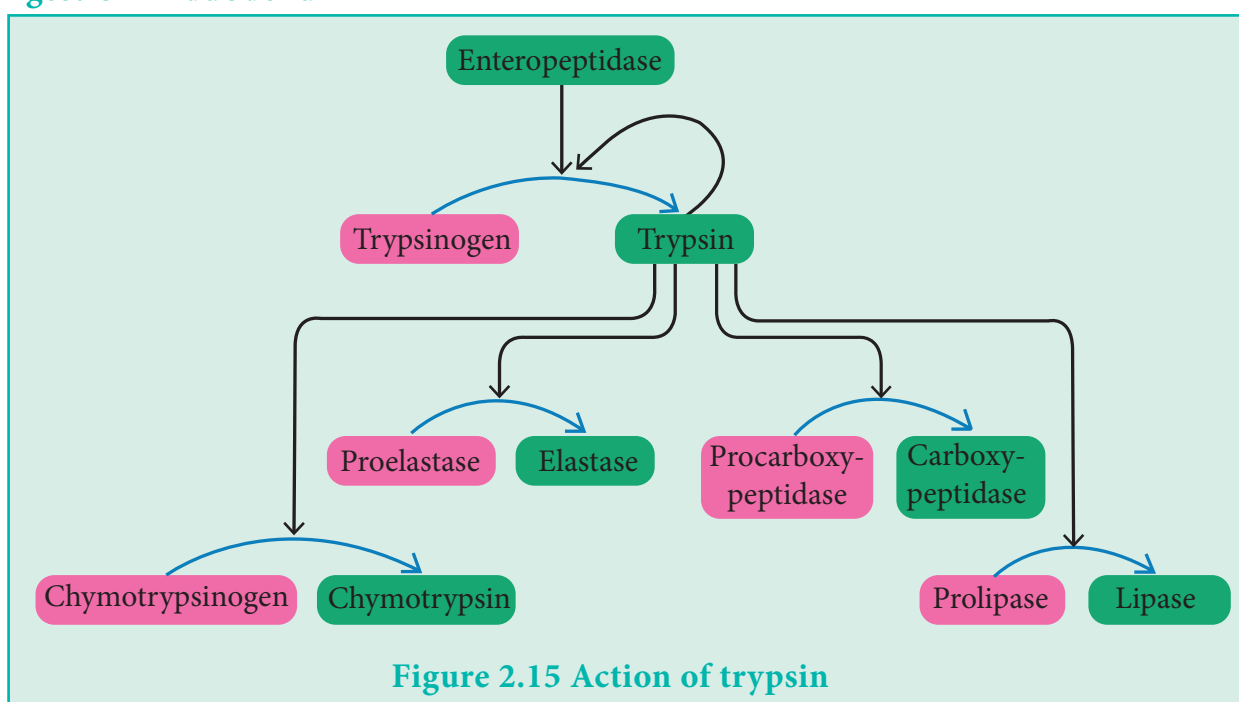
### Proteins / Peptones

- 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

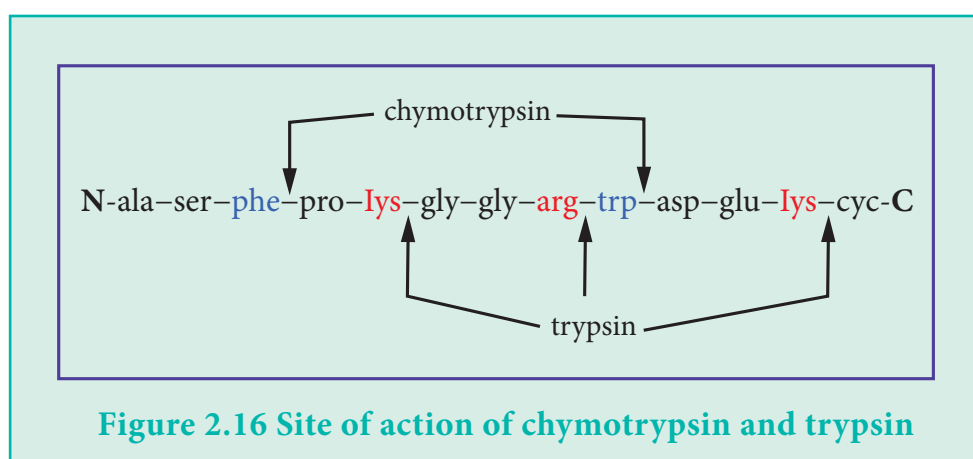




**Enteropeptidase:** Enteropeptidase or Enterokinase is a transmembrane protein expressed by the epithelial cells of the jejunum and the duodenum. It has a substrate specificity of Lys-Lys or Arg-Lys and is involved in activation of trypsin.

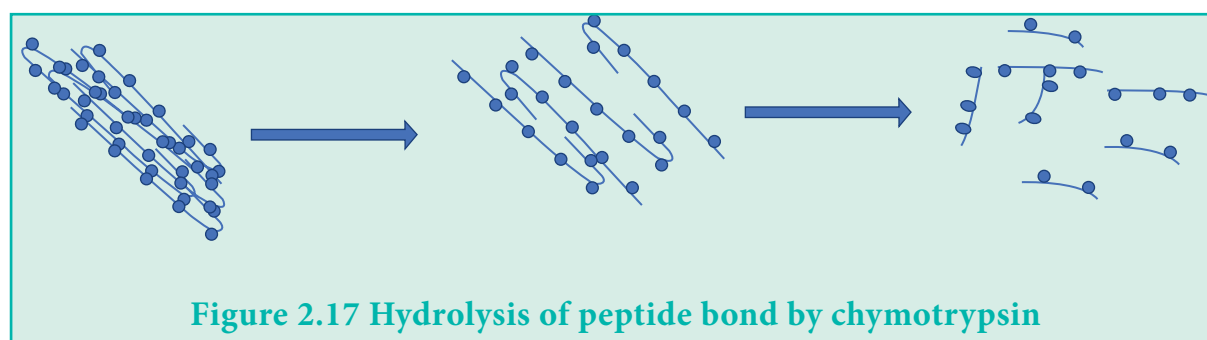
Human pancreatic juice contains zymogens of proteolytic enzymes like trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, pro-elastase along with trypsin inhibitor.

**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.



### 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).



**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.

## 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.

### 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.

### 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 2.11.4) The steps involved in the reaction are:

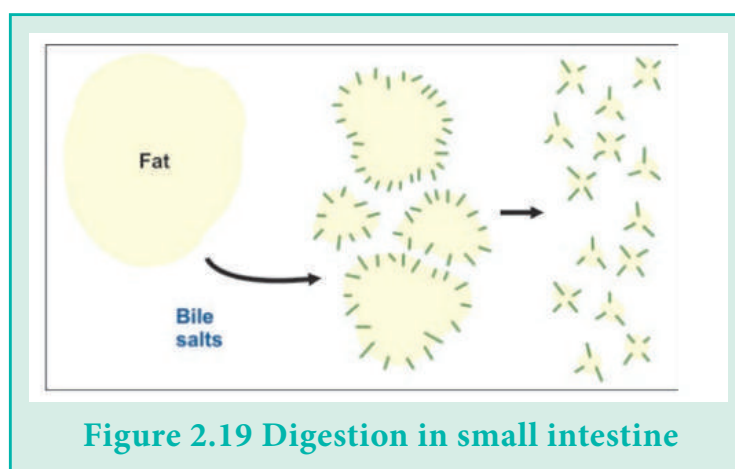


Figure 2.19 Digestion in small intestine

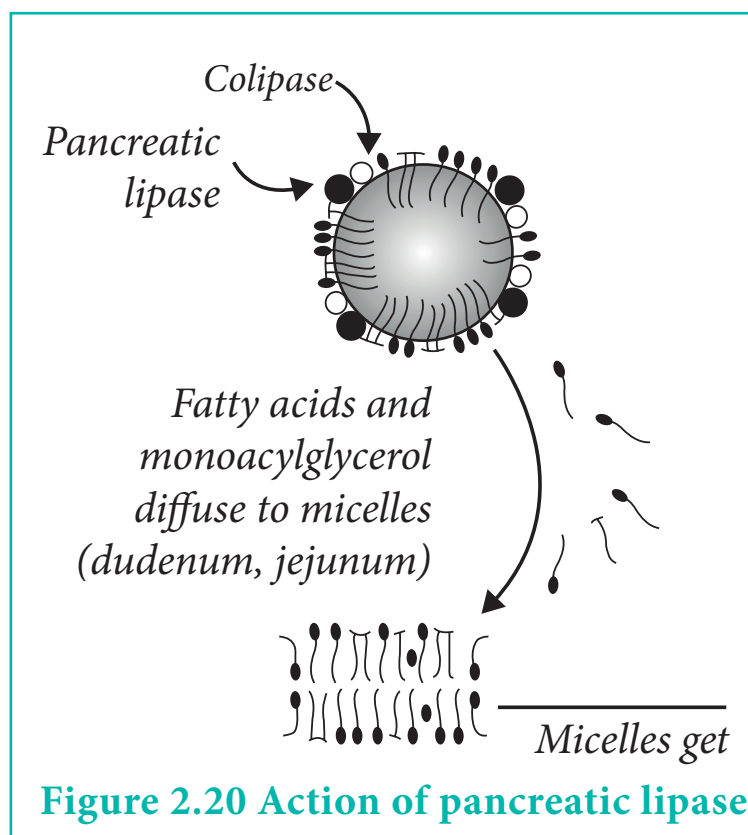


Figure 2.20 Action of pancreatic lipase

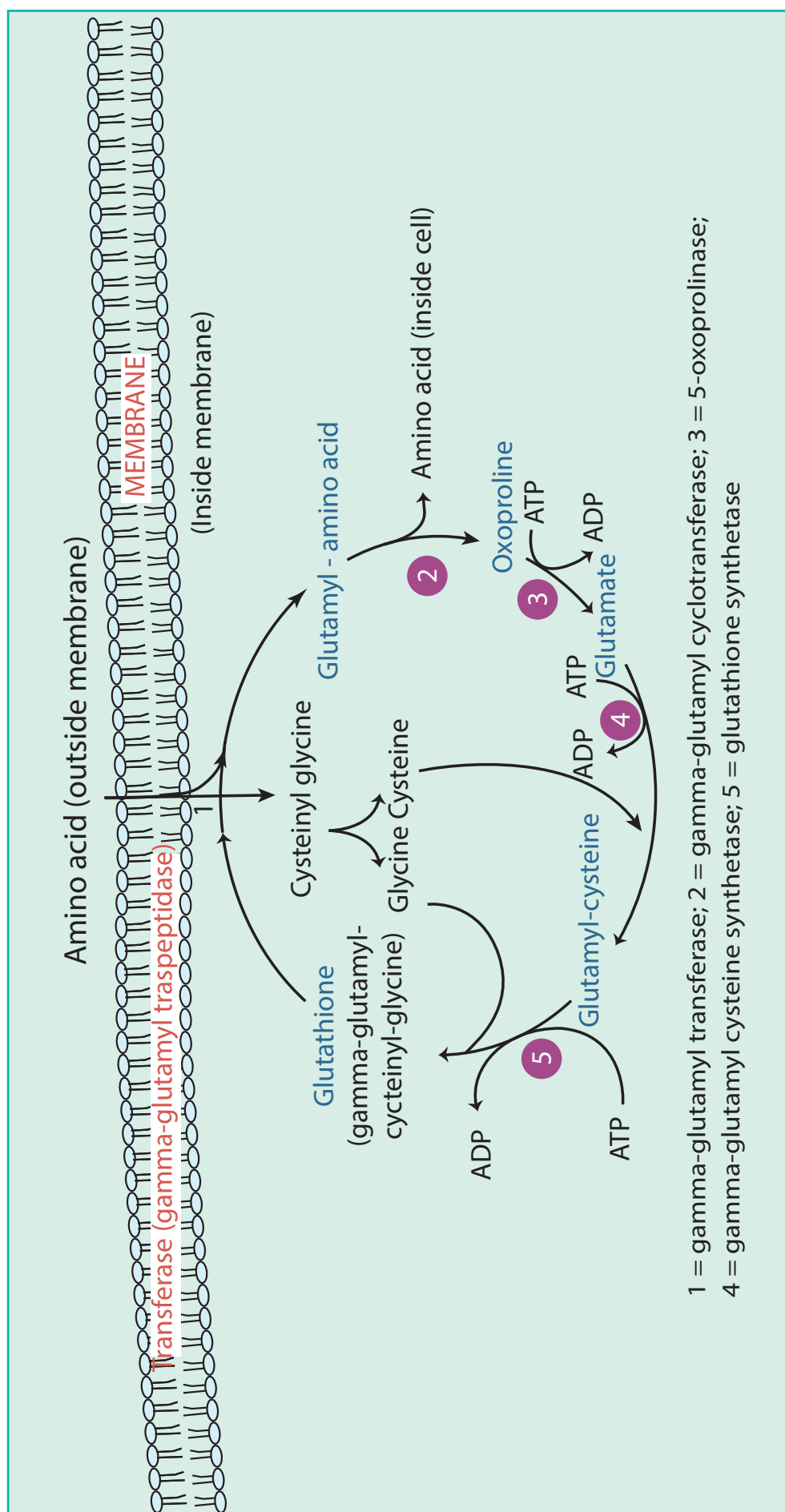


Figure 2.18 Gamma glutamyl cycle (Meister cycle)



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 immunoglobulins 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.

## 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	FFA+2MAG
Intestinal lipase with bile acids	Small intestine	TAGs with medium chain FAs	3 FFA+ glycerol
Phospho-lipase A <sub>2</sub> + 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:

## 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.

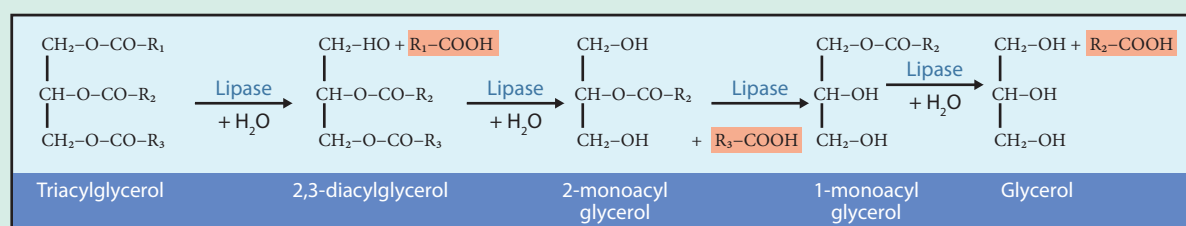


Figure 2.21 Complete hydrolysis of triglycerides

## Absorption of lipids

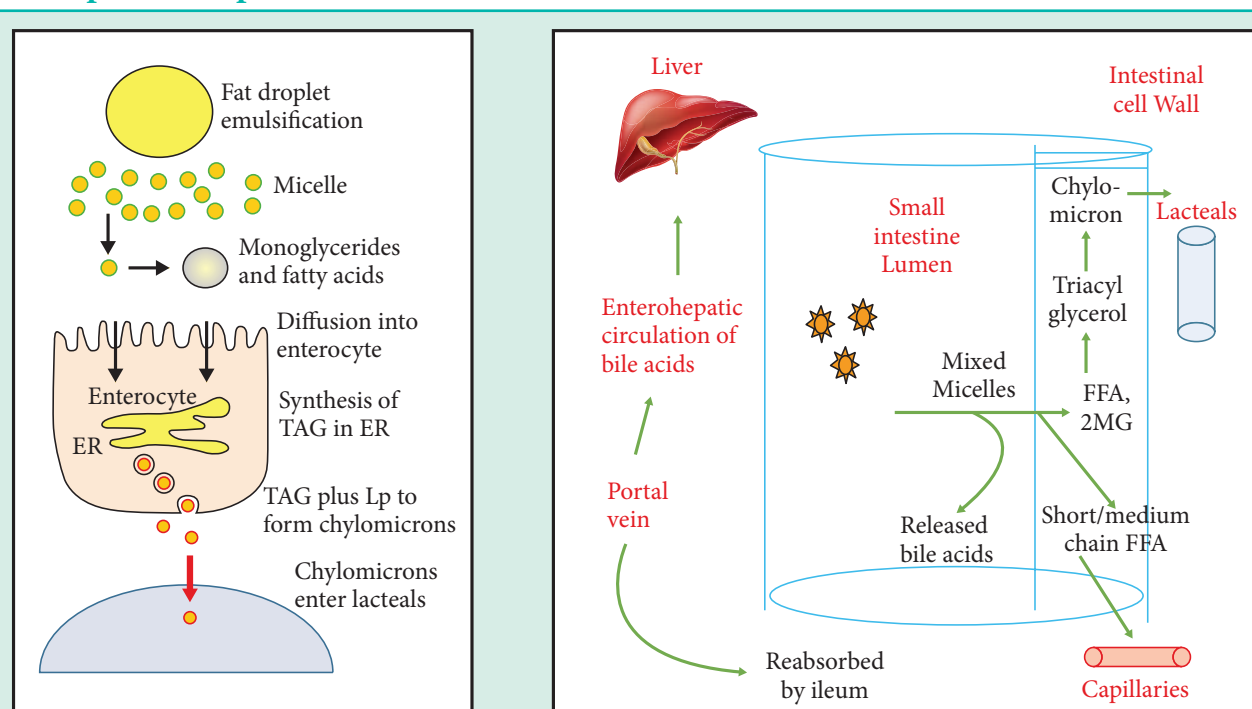


Figure 2.22 Absorption of fat as a chylomicron



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  $\mu\text{m}$  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

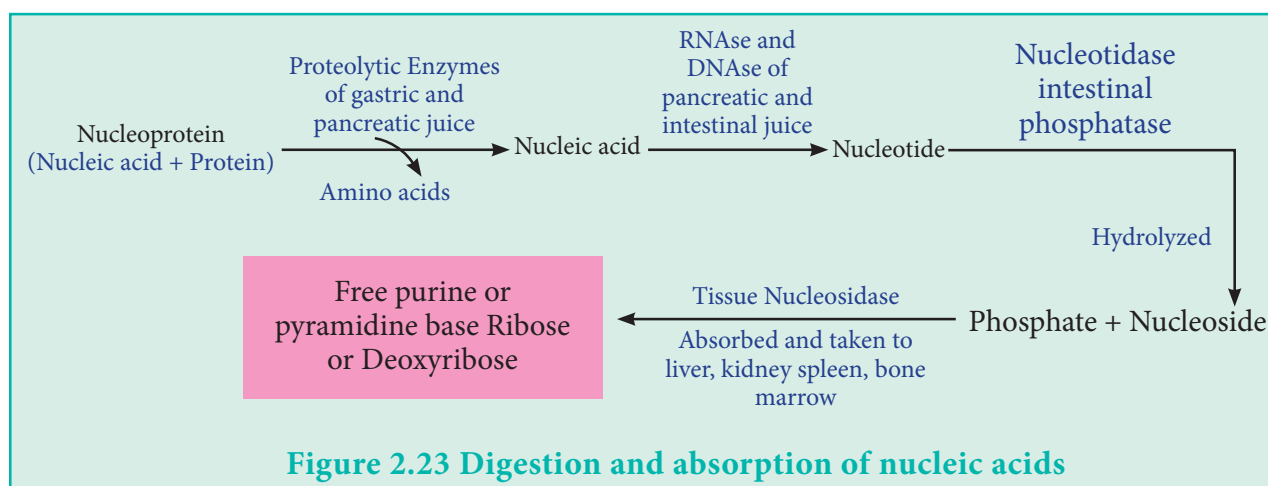
**Digestion in mouth:** No digestion of nucleic acid has been reported in the mouth.

**Digestion in stomach:** Nucleoproteins are denatured 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.



**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. 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.



### 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 Distention Nerve (Acid inhibits release)	G cells of the antrum, duodenum, and jejunum	Stimulates Gastric acid secretion Mucosal growth
Cholecystokinin	Protein Fat Acid	I cells of the duodenum, jejunum, and ileum	Stimulates Pancreatic enzyme secretion Pancreatic bicarbonate secretion Gallbladder contraction Growth of exocrine pancreas Inhibits





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

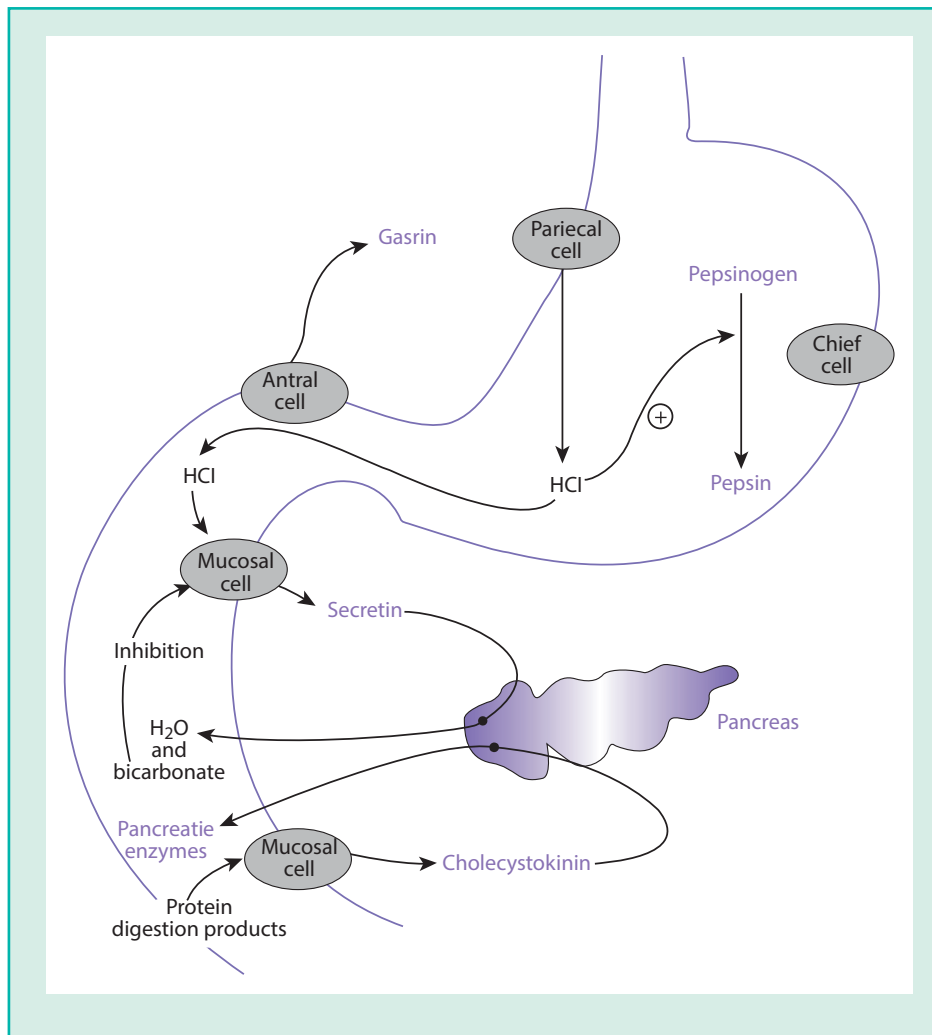
There are five major hormones that help in digestion. They are

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. Cholecystikinin (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 our appetite.





**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



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. Gglucose 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 other 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, Cholecystikin, enterogastrone, secretin and motilin are the five major hormones that help in digestion.



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.



## 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. The codont 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



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 amino acid.
- 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 has function 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. Promelain
- 17) The inner mucosal layer of stomach fold into invagination called \_\_\_\_\_.  
(a) Rugae  
(b) Frenulum  
(c) Villi  
(d) Uvula
- 18) The duodenal glands are also called as \_\_\_\_\_.  
(a) Adrenal gland  
(b) Brunner's gland  
(c) Thyroid gland  
(d) Glands of Lieberkuhn



- 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 deodenum
  - (c) I cells of deodenum
  - (d) C cells of deodenum
- 24) \_\_\_\_\_ is a hormone responsible for signaling to eat.
- (a) Ghrelin
  - (b) PYY
  - (c) Enterogastrone
  - (d) Motilin



25) Gall bladder is \_\_\_\_\_ shaped sac

- |          |              |
|----------|--------------|
| (a) Pear | (b) Globular |
| (c) Oval | (d) Tubular  |

## II Give short answer for the followings

- 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 micelle 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 of optimum pH of trypsin.

## III Give short answer for the followings

- 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 enzyme that act upon proteins.
- 7) Write any 3 factors that affects 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.



#### 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.
- 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

### 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

### 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.

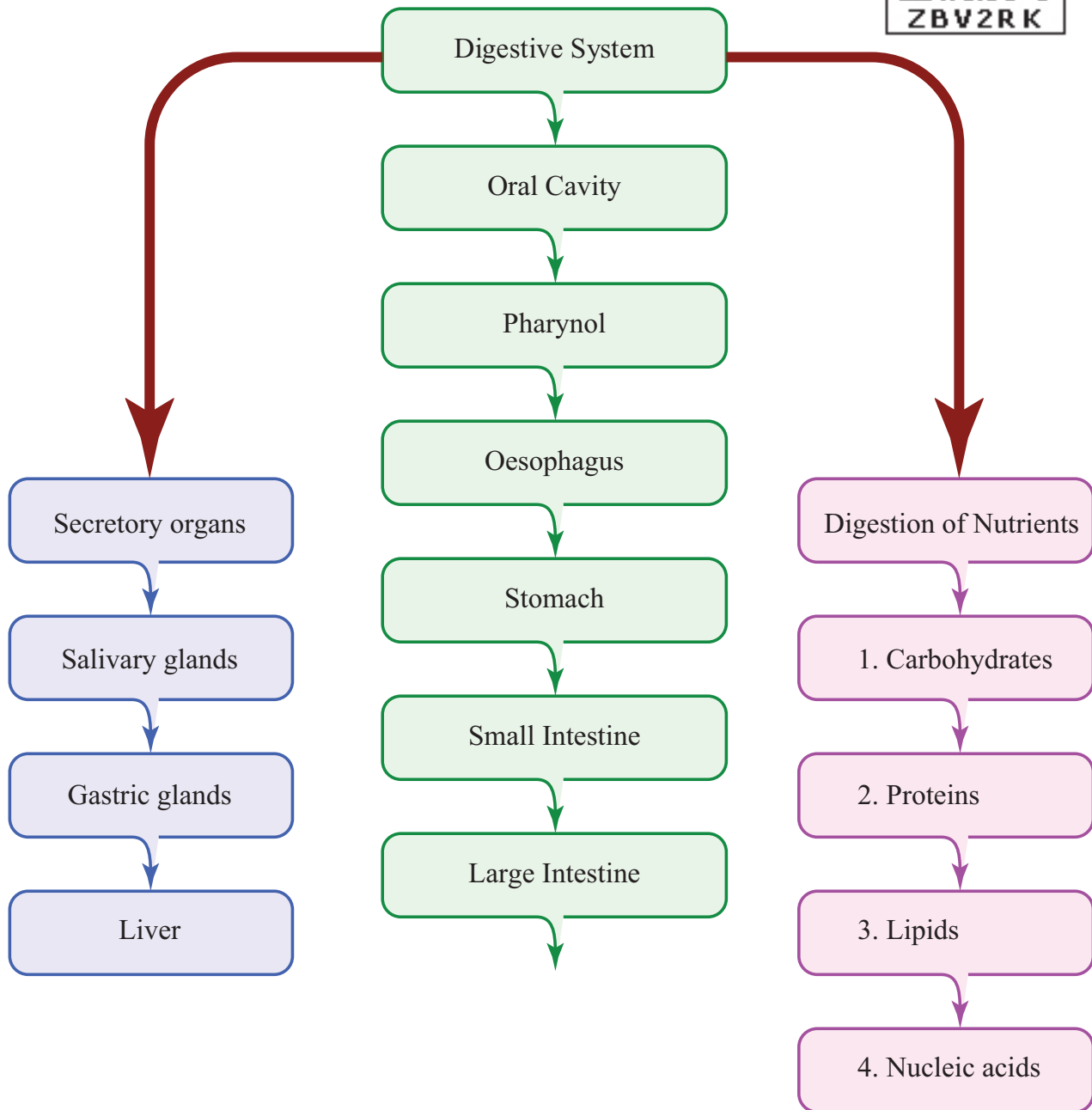
### Answer:

- 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 not the correct explanation of A
- 5. c) A is true but R is false



## CONCEPT MAP

### Digestion



## 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.



### Learning Objectives

After studying this unit the students will be able to

- Describe the metabolic processes catabolism and anabolism.
- Describe the reactions of glycolysis and gluconeogenesis.
- Describe the reactions of Hexose monophosphate 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.

## INTRODUCTION

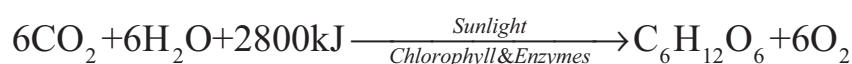
We have already learnt in XI standard about the structures and functions of various biomolecules such as proteins, amino acids, carbohydrates etc... We know that biomolecules are important for the normal functioning of any living system. These biomolecules can be obtained either directly from the diet 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, lipids etc. The 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

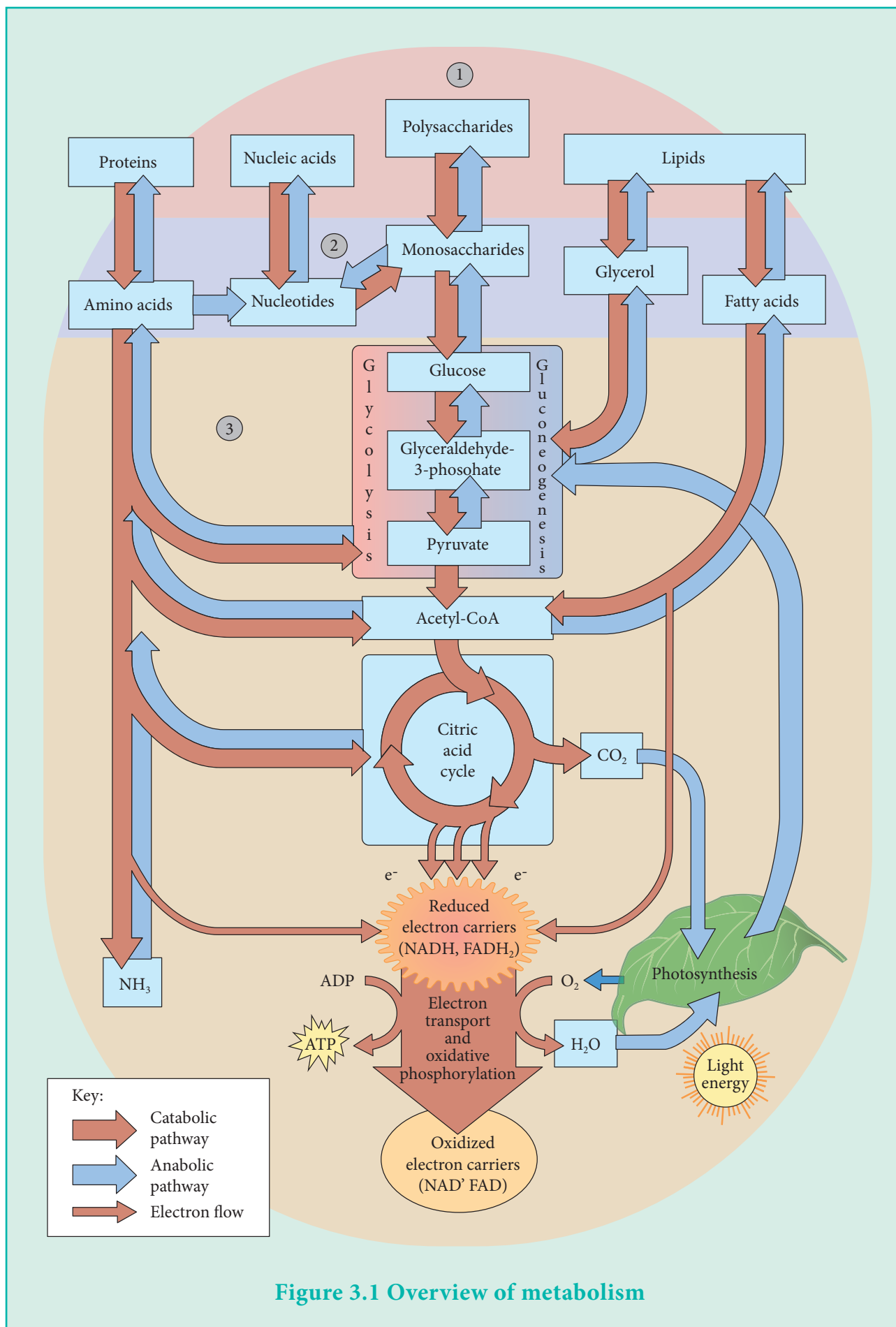
Every cell contains thousands of reactions comprising many metabolic pathways. There are two types of metabolic reactions namely anabolism and catabolism. The process of synthesising the required larger biomolecules from the small molecules is called anabolism. These reactions use up energy for example, in photosynthesis, small molecules  $\text{CO}_2$  and water are converted into a six carbon carbohydrate. In this reaction sun light provides the energy.



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

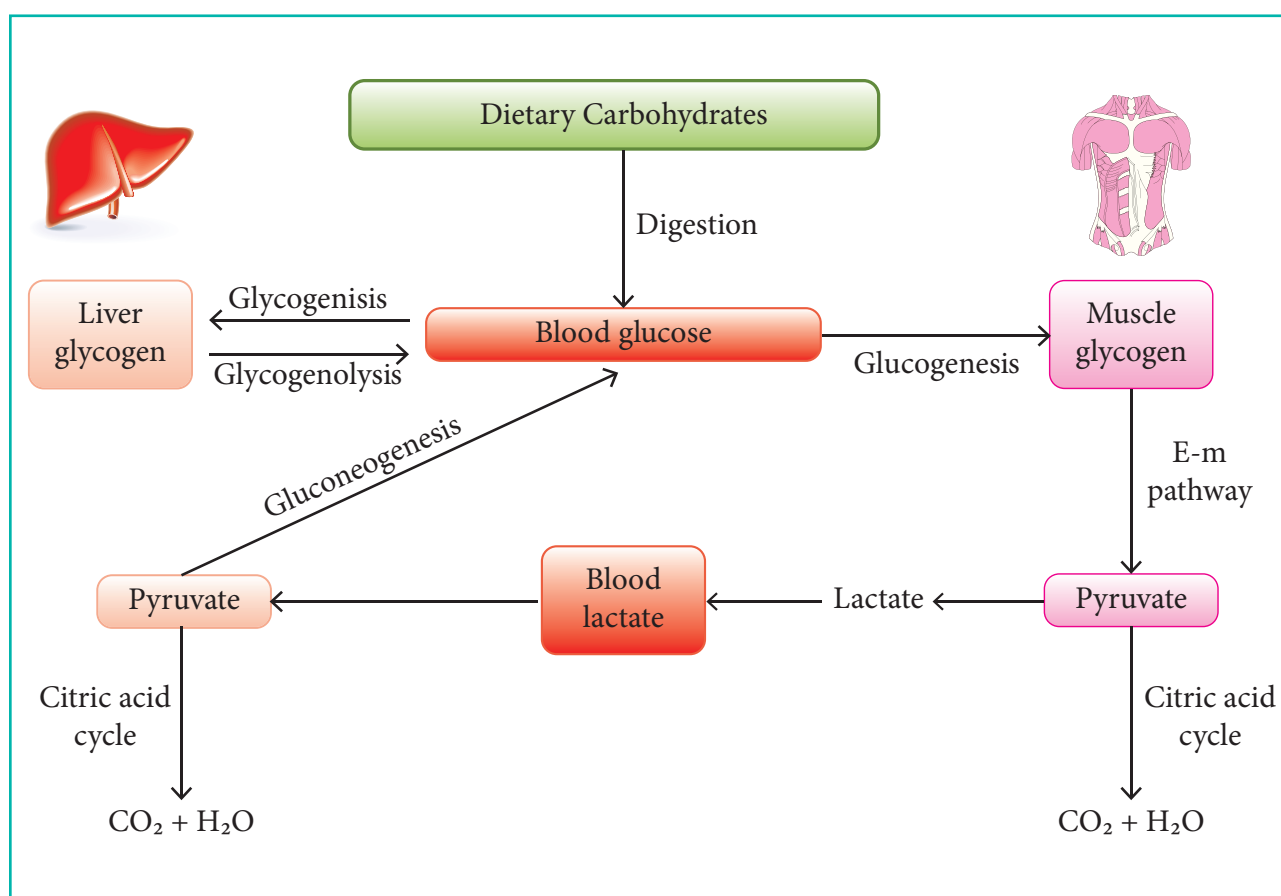


The above reaction is the reversal of photosynthesis reaction in which large (6C glucose) molecules are broken into small  $\text{CO}_2$  and  $\text{H}_2\text{O}$  molecules. If the glucose is burnt in the lab we get  $\text{CO}_2$  & water and large amount of energy is released. Whereas, in living 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 a muscle contraction, ATP is utilised.



### 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 a 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 from 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. The following figure gives an overview of carbohydrate metabolism.





### 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  $\text{NAD}^+$ . 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  $\text{CO}_2$  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-phosphate is converted into pyruvate. This is an energy producing stage in which two molecules of ATP is 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.



##### 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 hexokinase enzyme.

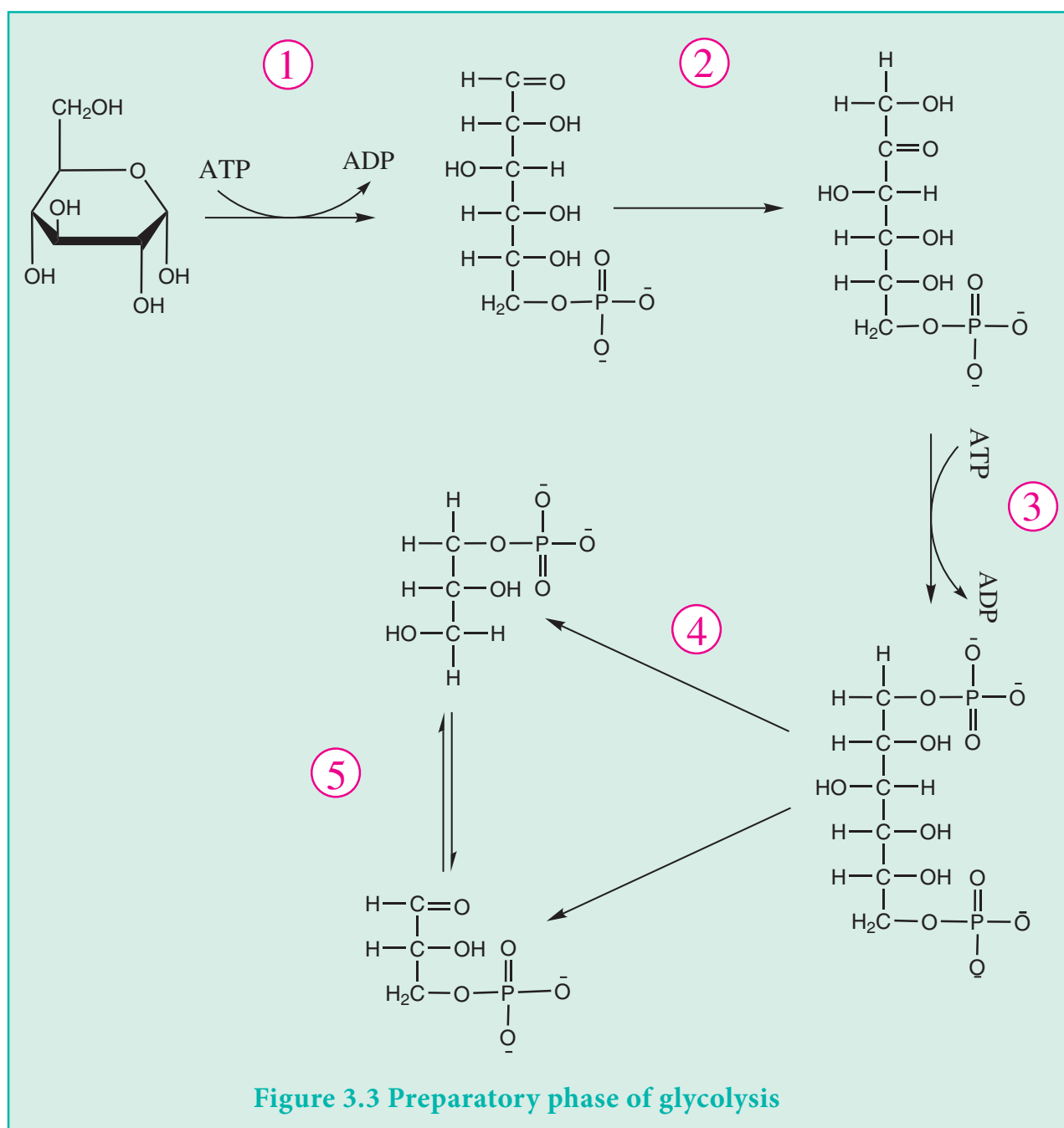
**Step 2:** Glucose-6-phosphate is isomerised to form fructose-6-phosphate. This step is catalysed by the enzyme phosphoglucose isomerase

**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.



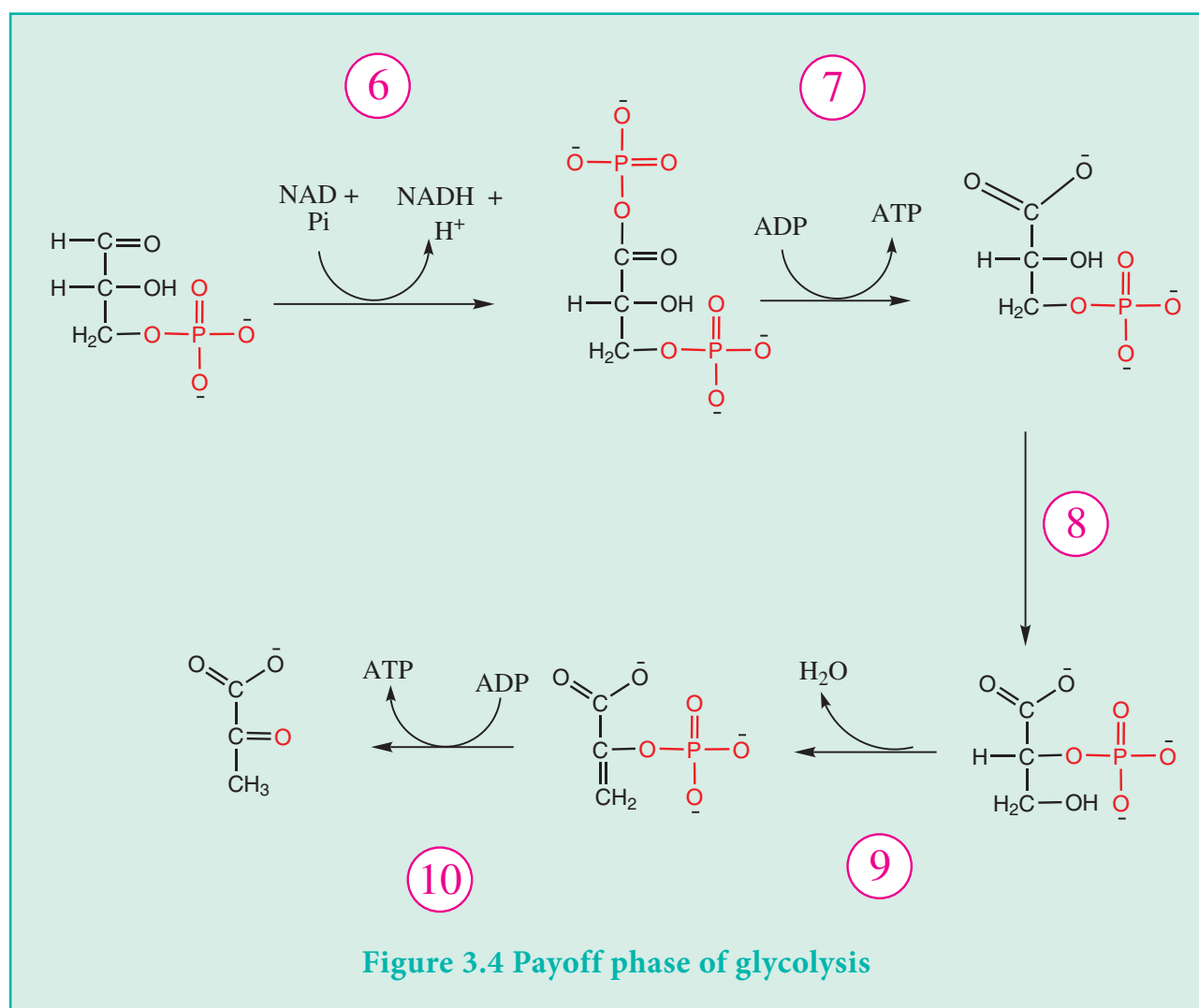
**Step 4:** In this step, the six carbon, fructose-1,6-bisphosphate splits to form two three-carbon sugars namely dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate. They are isomers of each other. This step is catalysed by the enzyme aldolase.

**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.

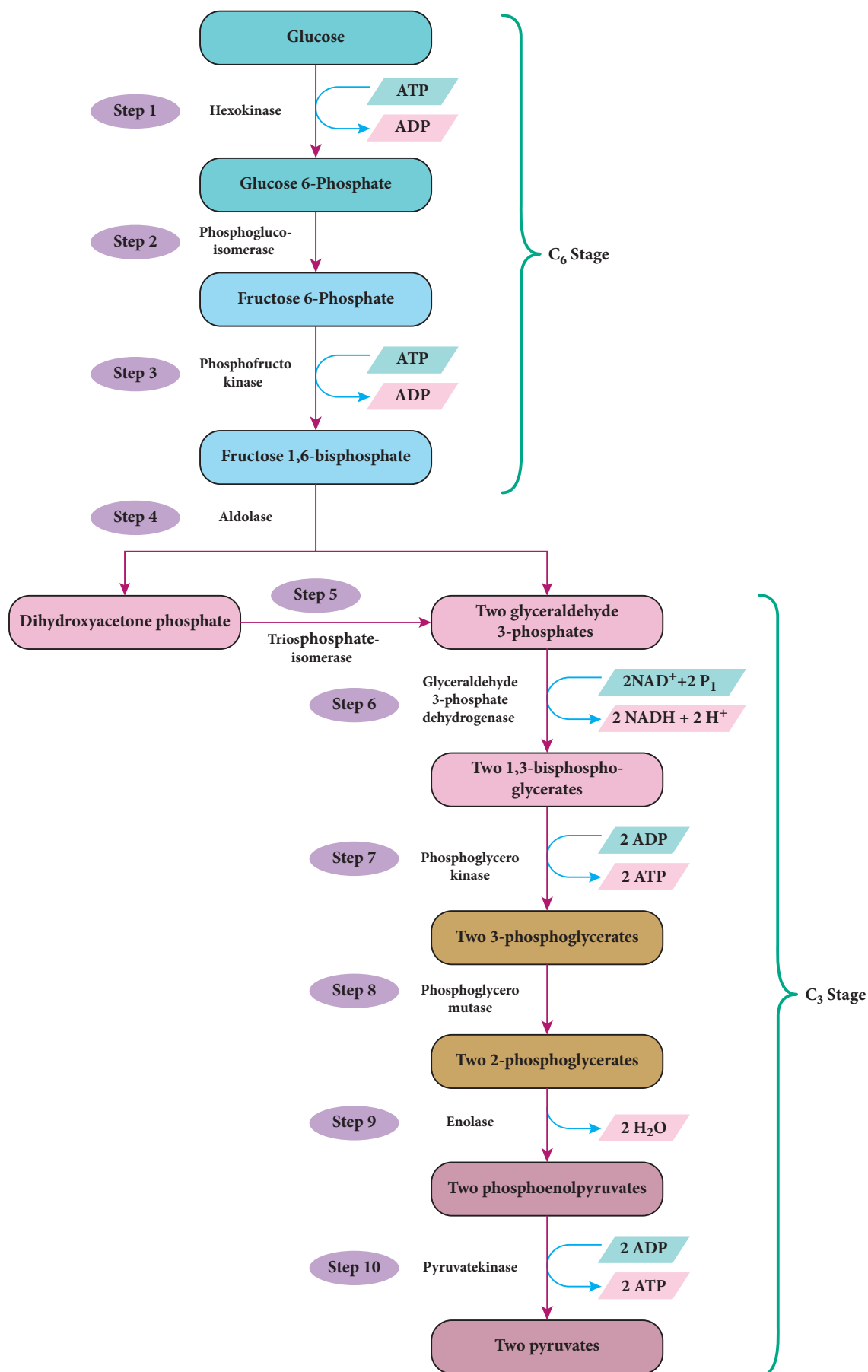


### Reactions of Payoff phase:

- Step 6:** Glyceraldehyde-3-phosphate is oxidized by  $\text{NAD}^+$  which gets reduced to  $\text{NADH}$  and  $\text{H}^+$ . 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 ( $\text{P}_i$ ) is used for phosphorylation. Glyceraldehyde-3-phosphate dehydrogenase catalyses this step.
- Step 7:** The 1,3-bisphosphoglycerate is converted into 3-phosphoglycerate transferring one of its phosphate group to ADP to form ATP. The enzyme involved in this process is phosphoglycerate 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.



# Glycolysis



### 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  $\text{NAD}^+$  molecules respectively. To regenerate the coenzyme  $\text{NAD}^+$ , NADH must be re-oxidised. If oxygen is available, the pyruvate is oxidized all the way to carbon dioxide in cellular respiration, regenerates  $\text{NAD}^+$ . During this process more ATP molecules are produced. Under anaerobic conditions it is converted to lactate by lactate dehydrogenase enzyme and regenerates  $\text{NAD}^+$ . 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 serve as energy source. The NADH molecules are oxidised in mitochondria producing more number of ATP molecules (approximately 3 ATPs per NADH molecule).

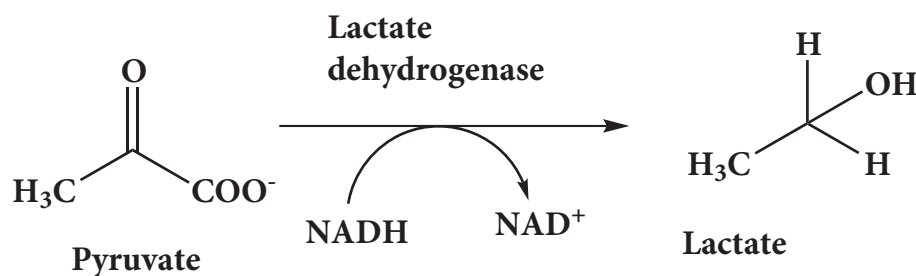
Step No.	Description	Number of ATP/ NADH formed/ consumed
6	Formation of 1,3-bisphospho glycerate from glyceraldehyde 3-phosphate	2 NADH
7	Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate	2 ATP
10	Formation of pyruvate from phosphoenol pyruvate	2 ATP
1,3	Formation of Glucose-6-phosphate & 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:

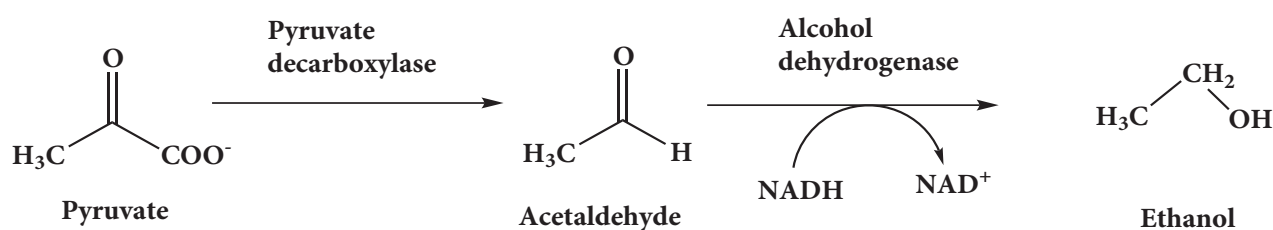
Some organisms are able to continually convert energy without the presence of oxygen. They undergo glycolysis, followed by the anaerobic process to produce ATP.

As discussed earlier anaerobic glycolysis is the transformation of glucose to lactate when limited amounts of oxygen ( $\text{O}_2$ ).

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  $\text{NAD}^+$ .



Many yeasts convert glucose to ethanol and CO<sub>2</sub> 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<sup>2+</sup>. 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<sup>+</sup>.



### 3.4. TRICARBOXYLIC ACID CYCLE:

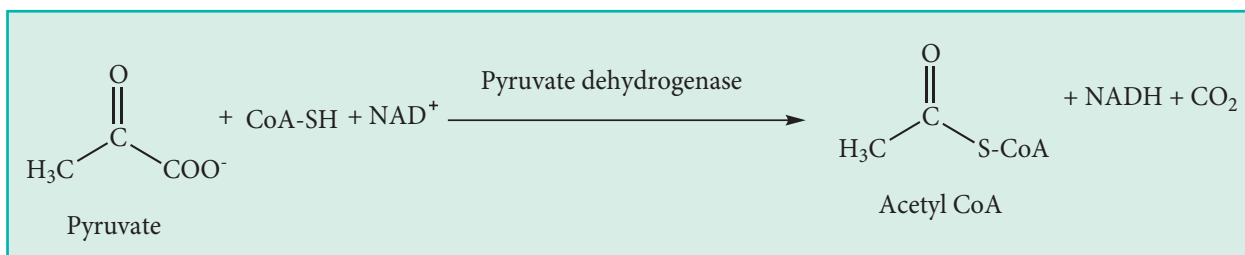
Citric acid cycle, the common mode of oxidative degradation in eukaryotes and prokaryotes. This process occurs in the mitochondria. This cycle, which is also known as the tricarboxylic acid (TCA) cycle or Krebs cycle, is the central driver of cellular respiration. It accounts for the major part of oxidation 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<sub>2</sub> and GTP molecules. The reduced electron carriers NADH and FADH<sub>2</sub> generated in the TCA cycle, will pass their electrons into the electron transport chain resulting oxidative phosphorylation, 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 provide a link between the glycolysis and the TCA Cycle which consists the following eight steps.



**Step 1:** In the first step of the cycle, the acetyl CoA (two-carbon) reacts with a 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,  $\alpha$ -ketoglutarate releasing a molecule of  $\text{CO}_2$ . In this process two electrons are also released which reduce a  $\text{NAD}^+$  molecule to NADH. This step is catalysed by isocitrate dehydrogenase.

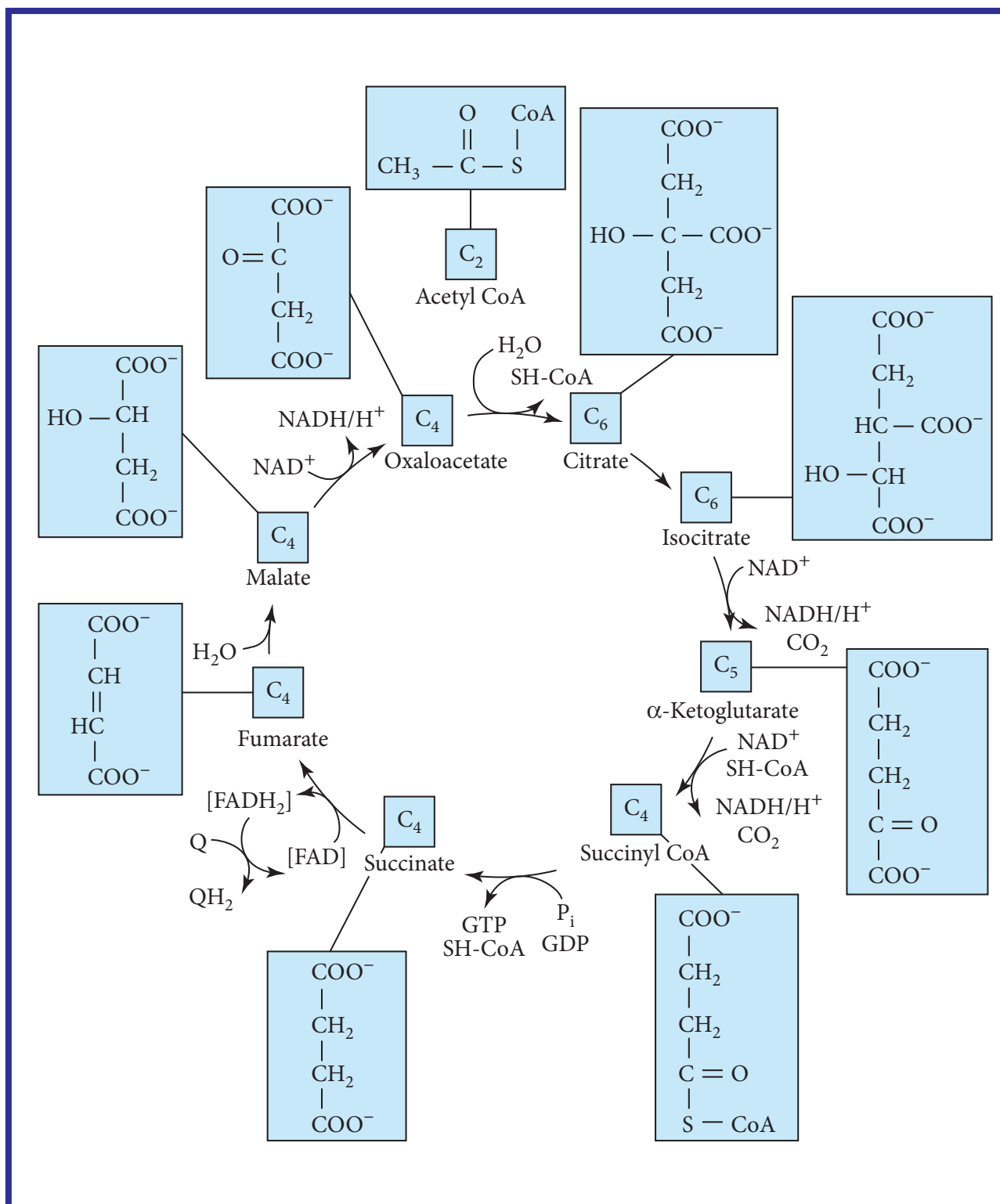
**Step 4:** In the step four,  $\alpha$ -ketoglutarate is oxidised to succinyl CoA a four carbon molecule in the prescence of Coenzyme A. In this step also a  $\text{NAD}^+$  molecule is reduced to NADH. This reaction is catalysed by  $\alpha$ -ketoglutarate dehydrogenase enzyme

**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 of a guanine diphosphate (GDP) to guanine 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.

**Step 6:** Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing  $\text{FADH}_2$ . 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.



### 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  $\text{FADH}_2$  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 <sub>2</sub>	1	2	2
GTP	1	1	1
Total no. of ATP per acetyl coA molecule			12

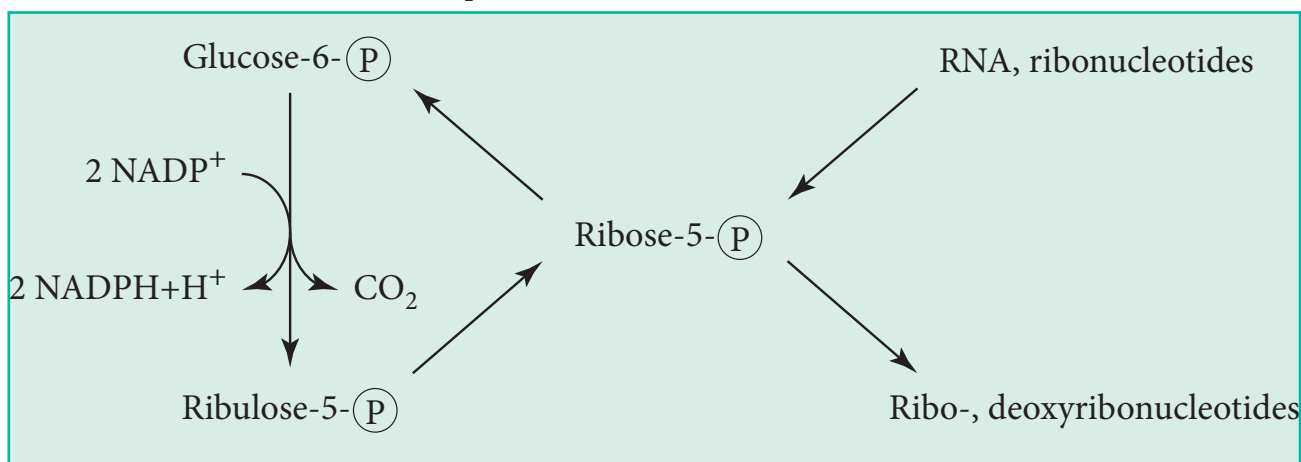
### 3.5. HEXOSE MONOPHOSPHATE SHUNT

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 molecule 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<sub>2</sub>.





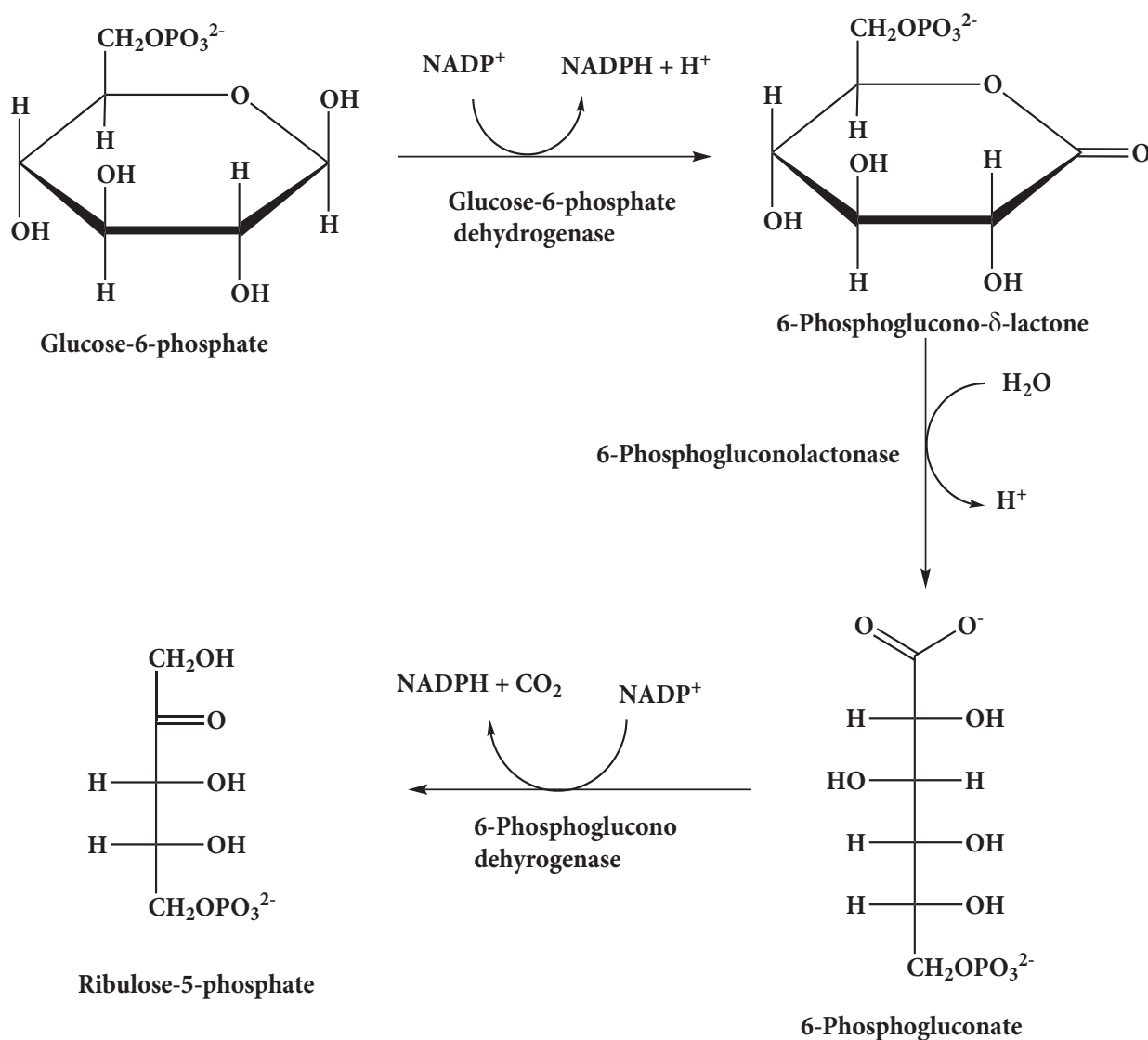
### Reactions of oxidative phase

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- $\delta$ -lactone and reduces one molecule of  $\text{NADP}^+$  to  $\text{NADPH}$ .

**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  $\text{NADP}^+$  and decarboxylates 6-phosphogluconate to the pentose, ribulose-5-phosphate.



### 3.5.2. Non-oxidative phase

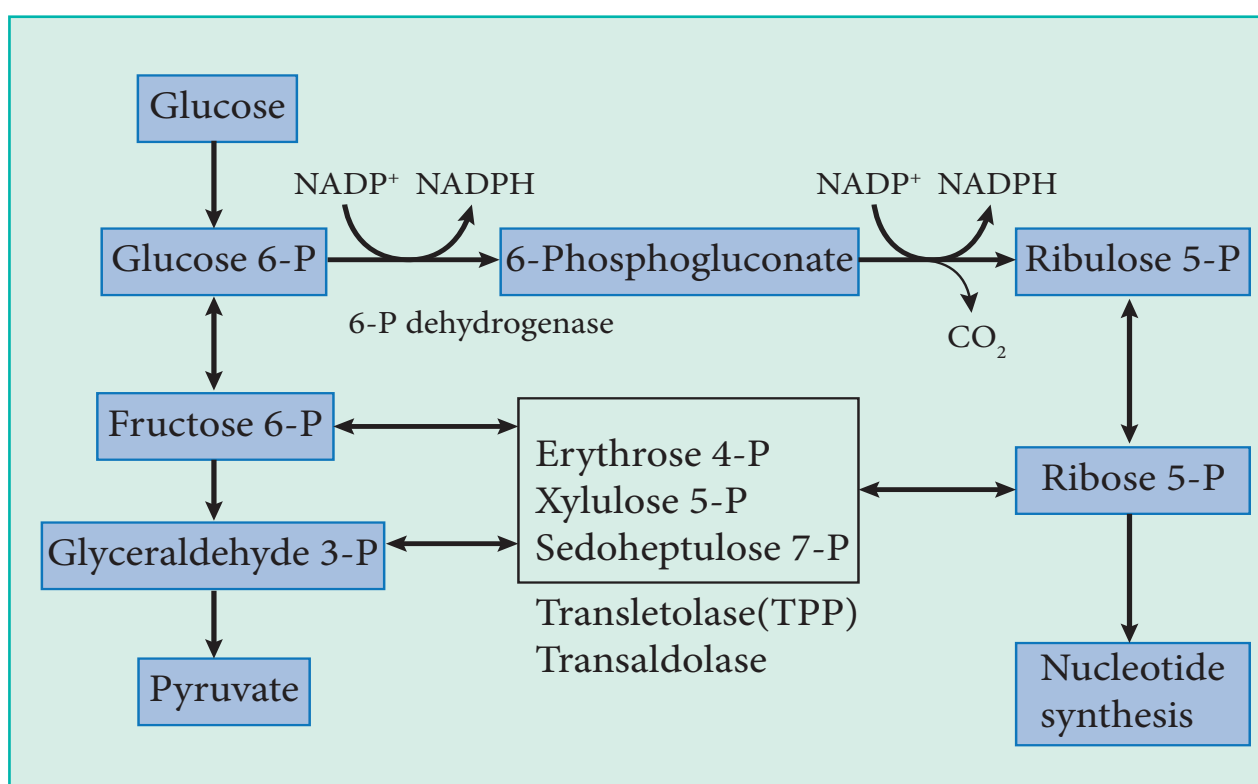
The non-oxidative phase involves the enzymes ribulose-5-phosphate epimerase (RE), ribulose-5-phosphate isomerase (RI), transketolase (TK), and transaldolase (TA). They bring about the following reactions:

**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.

**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

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,



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 to glucose-1-phosphate.

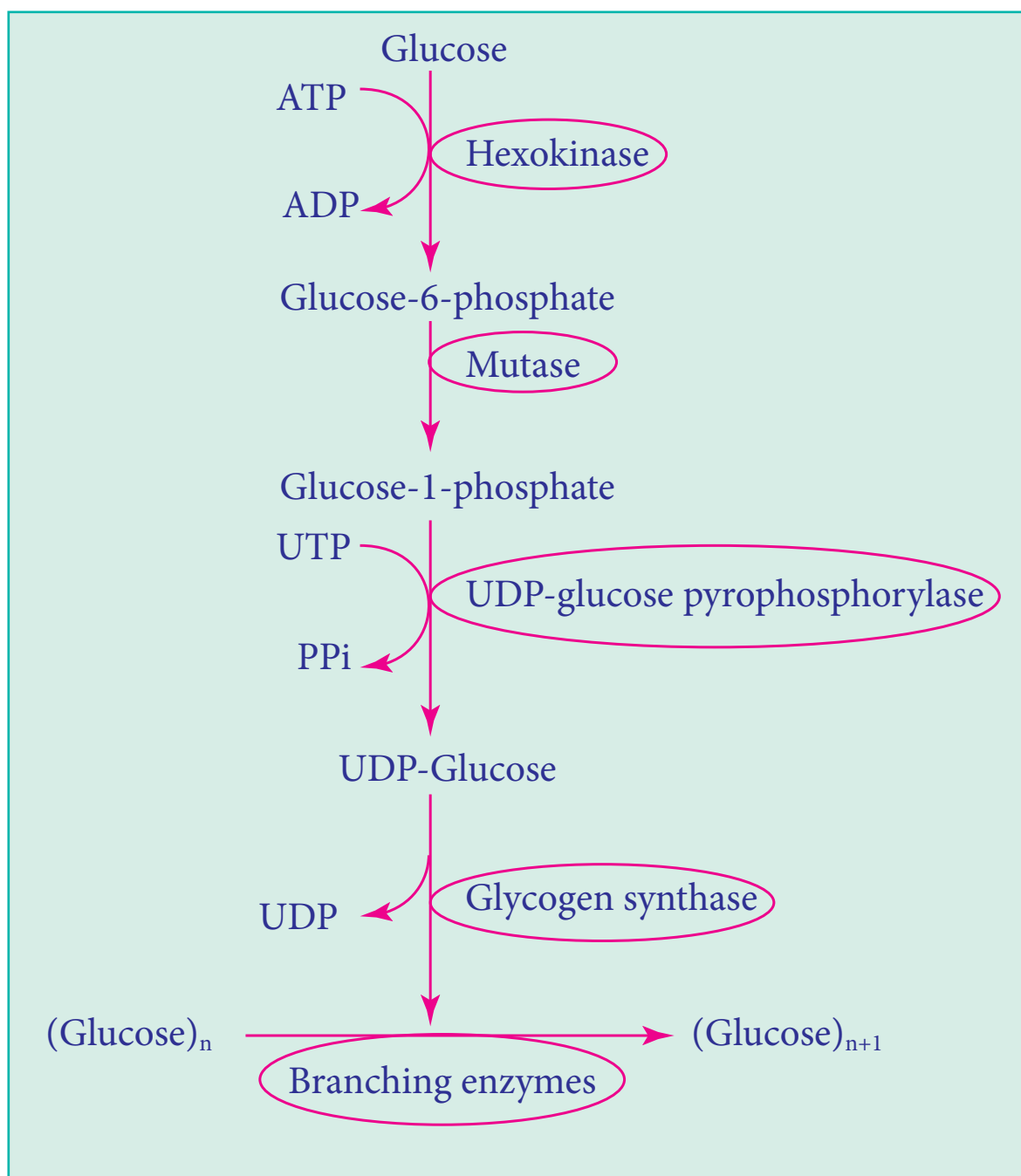
**Step 3:** UTP (uridine triphosphate) attaches to Glucose-1-phosphate in the third step. This step focuses on the reaction of glucose-1-phosphate to UTP thereby forming active nucleotide UDP-Glucose (Uridine diphosphate glucose). The one responsible for such reaction is the enzyme UDP Glucose pyrophosphorylase.

**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. 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. It will be the one to accept all glucose molecules.

**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.

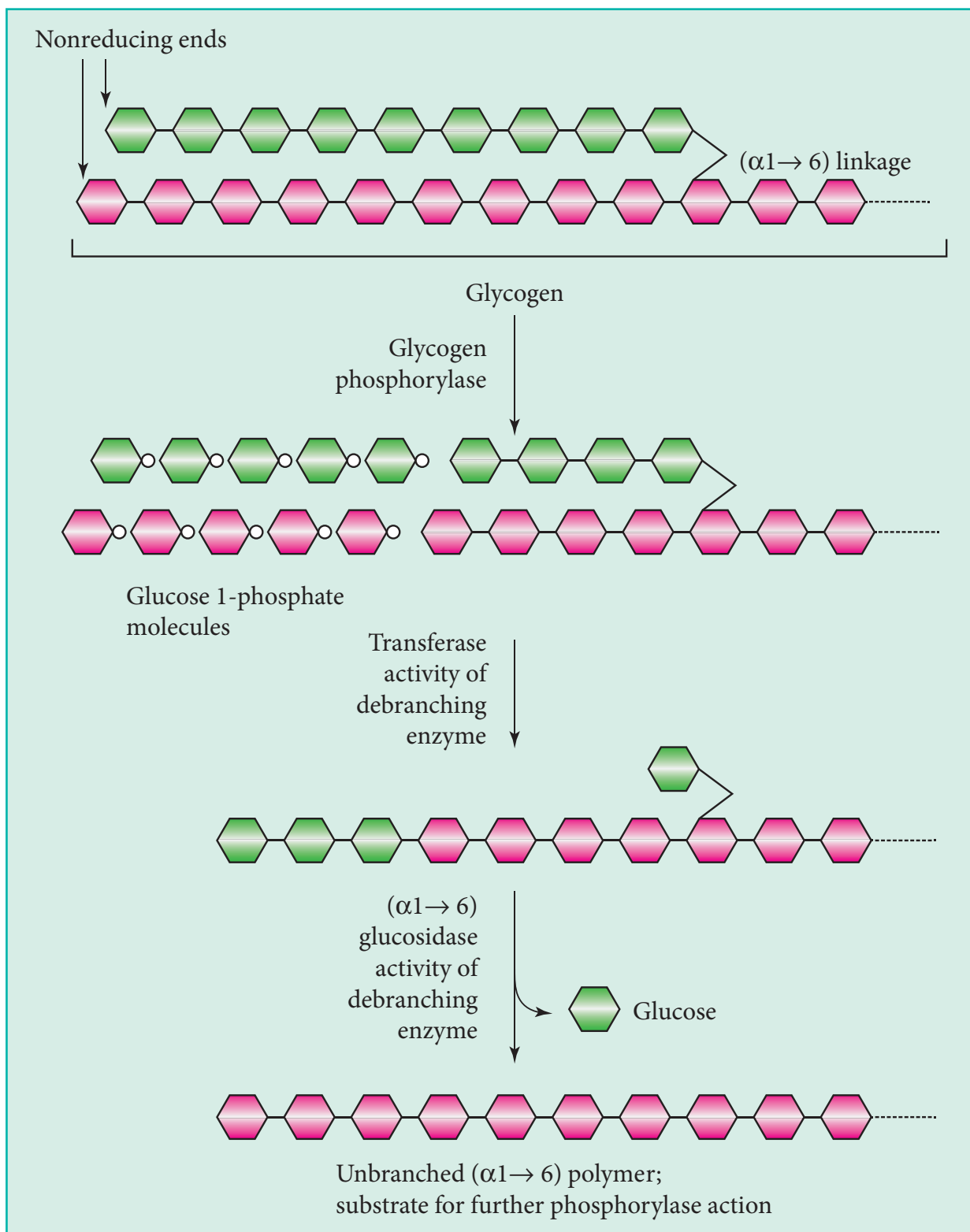


### 3.6.2. Glycogenolysis

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  $\alpha$  [1→4] linkage producing glucose-1-phosphate and glycogen that contains one less glucose molecule.

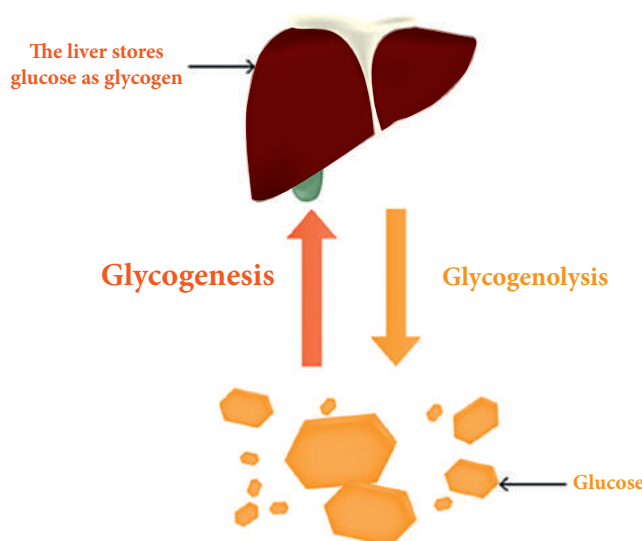
A second enzyme, phosphoglucomutase, converts the glucose-1-phosphate to glucose-6-phosphate. Glycogen involves two types of glycosidic linkage: the linear  $\alpha$  [1 $\rightarrow$ 4] linkage and the branching  $\alpha$  [1 $\rightarrow$ 6] linkages. During glycogenolysis glucose units are phosphorolysed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



A third enzyme, glycogen debranching enzyme transfers three of the remaining four glucose units to the end of another glycogen branch, exposing the  $\alpha$  [1 $\rightarrow$ 6] branching point. This glycosidic bond is hydrolysed by a fourth enzyme  $\alpha$  [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.

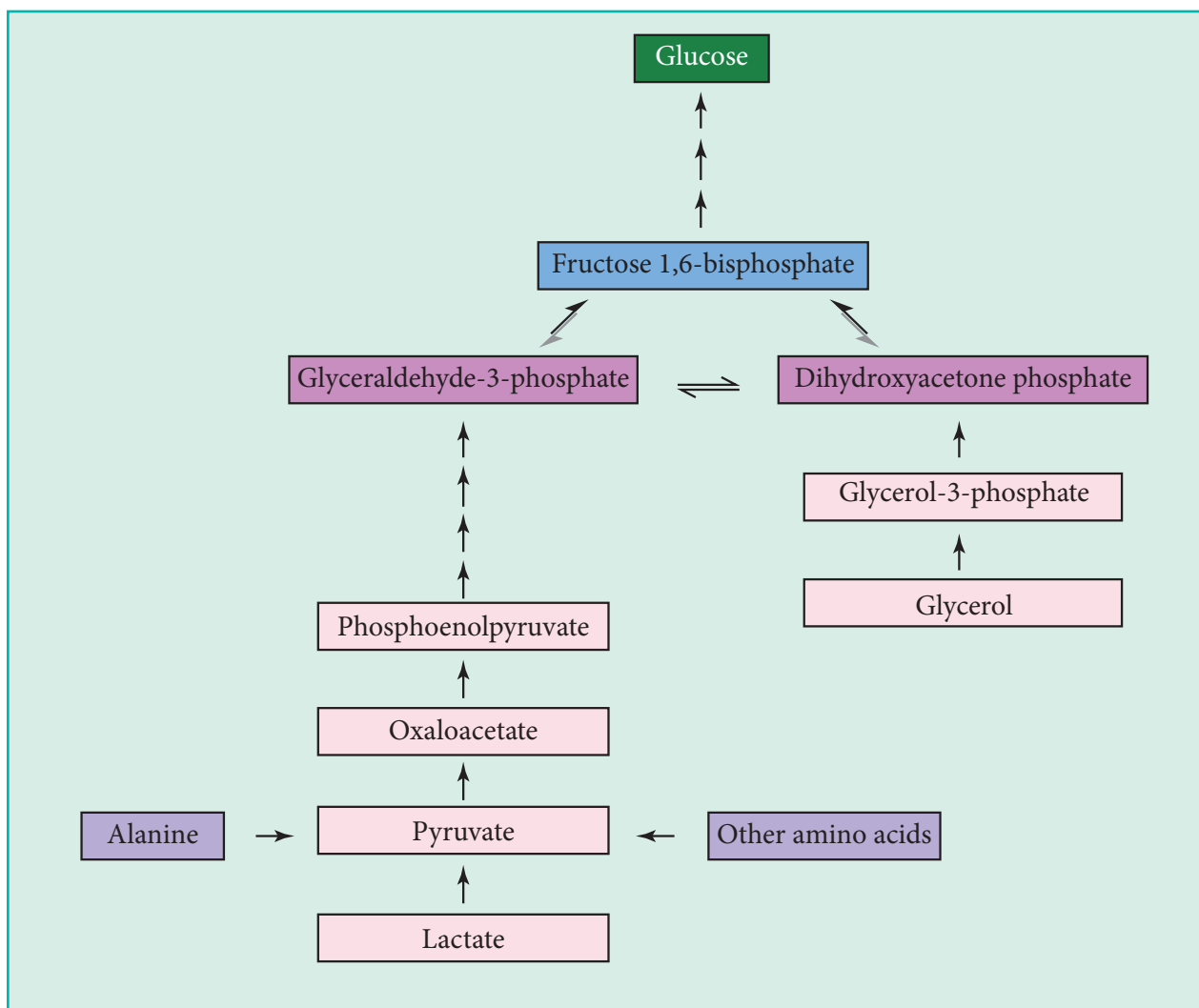
In muscle, glycogenolysis serves to provide an immediate source of glucose-6-phosphate for glycolysis to provide energy for muscle contraction but not for other body tissues. Muscle cells lack the enzyme glucose-6-phosphatase and thus cannot convert glucose-6-phosphate (which cannot be transported across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



### 3.7. GLUCONEOGENESIS

Gluconeogenesis, 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.

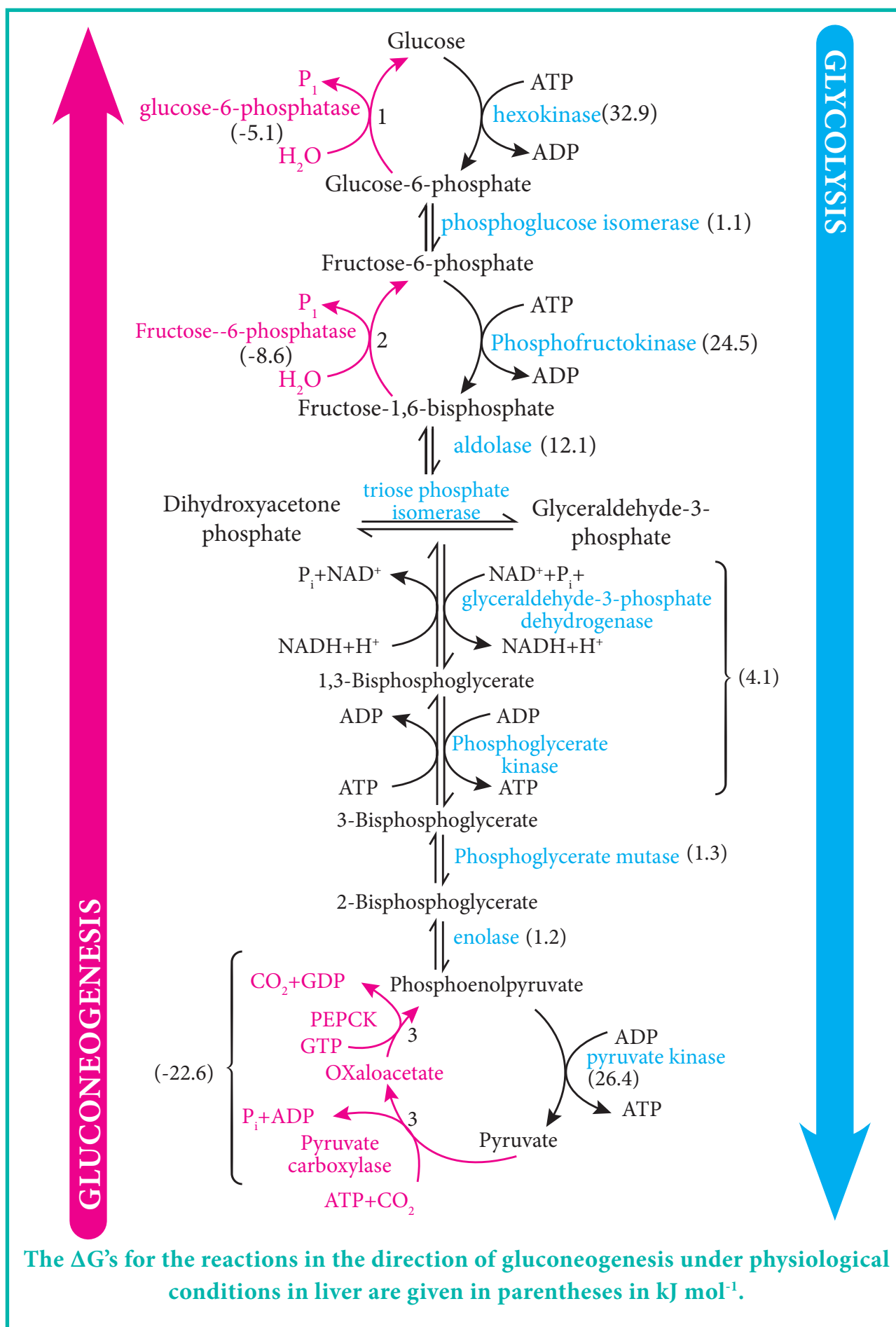


### 3.7.1 Key reactions in gluconeogenesis

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 highly exergonic 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 would require high energy. The beauty of the nature is that, the above reactions of gluconeogenesis are catalysed by different enzymes namely glucose-6-phosphatase, fructose-6-bisphosphatases and PEP carboxykinase.







**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 mitochondria 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-bisphosphate 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.

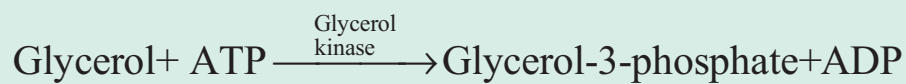
Because it is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. In glycolysis and gluconeogenesis seven of the ten steps occur at or near equilibrium. In gluconeogenesis the conversion of pyruvate to PEP, 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

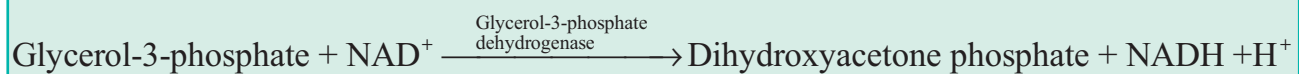
**Gluconeogenesis of amino acids:** Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination

**Gluconeogenesis of 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 of 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.



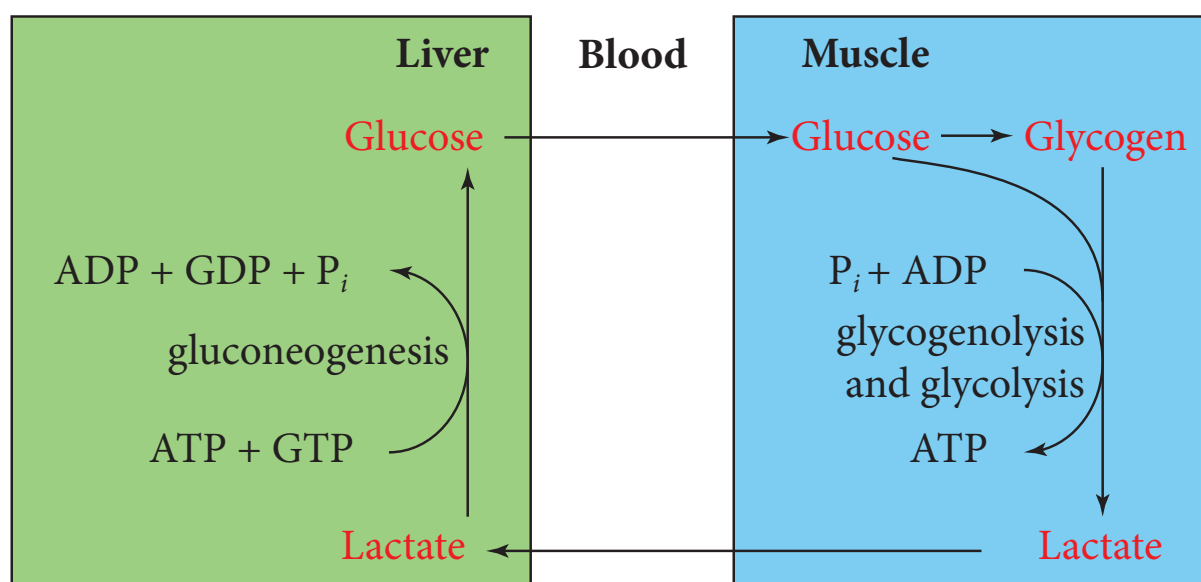
This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD<sup>+</sup> and glycerol 3-phosphate dehydrogenase.



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



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  $\beta$  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  $\beta$  cells or inadequate insulin secretion of insulin by  $\beta$  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.
- **Polydipsia:** The excessive thirst which leads to increased consumption of water. This condition is known as polydipsia. This is to replace the volume of water excreted due to polyuria.
- **Polyuria:** This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).



- **Polyphagia:** Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but in spite 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

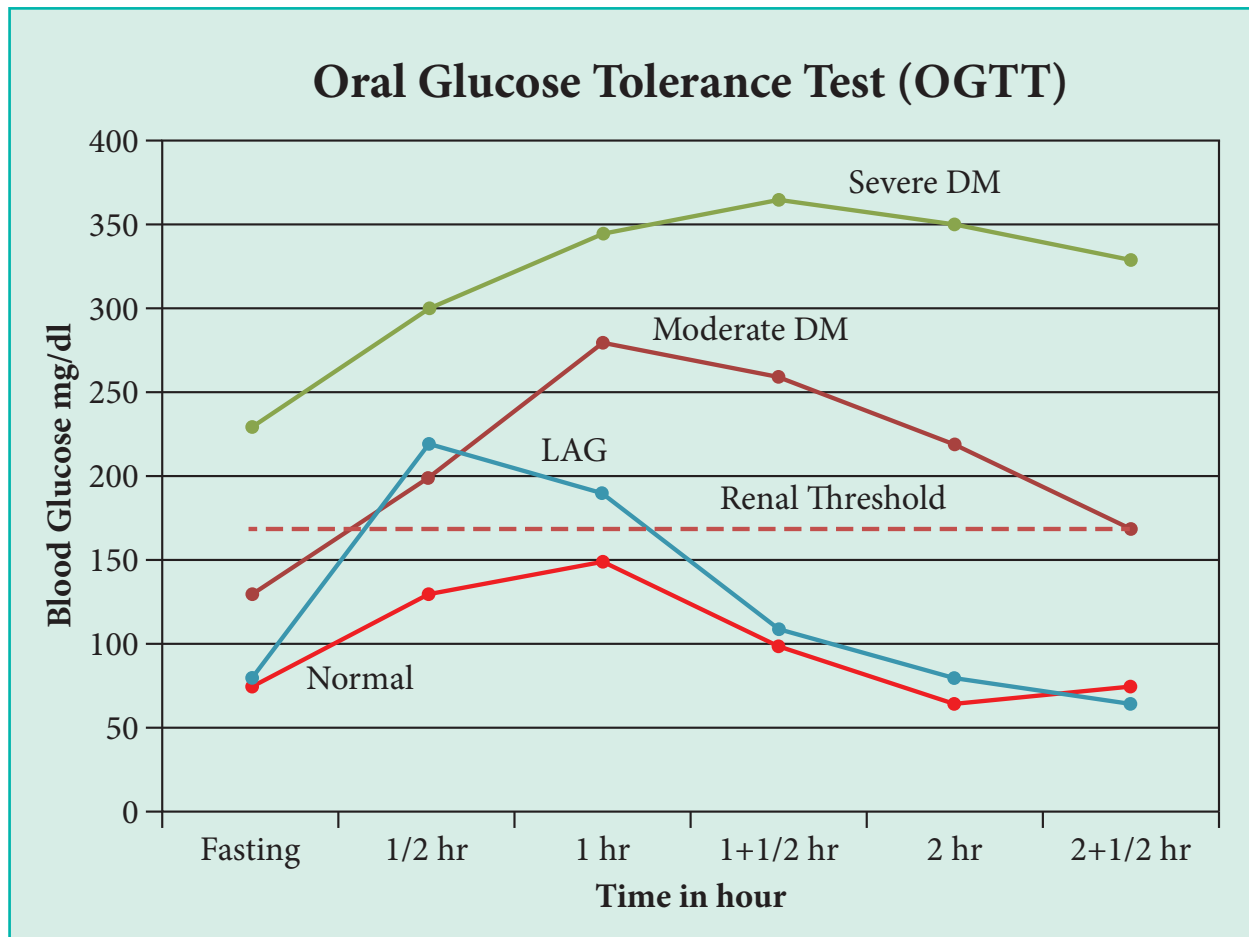
Diabetes is diagnosed with fasting sugar blood tests or with A1c blood tests, also known as **glycated hemoglobin tests**. You do not have to be fasting for an A1c blood test. A fasting blood sugar test is performed after you have had nothing to eat or drink for at least eight hours. Normal fasting blood 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.



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

- How many NADHS are produced from 7.5 mol of acetyl CoA by the citric acid cycle?
  - 15 mol
  - 7.5 mol
  - 75 mol
  - none of these
- Blood sugar is
  - Sucrose
  - Lactose
  - Glucose
  - Fructose





3. How many ATP molecules are generated during 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 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. FADH<sub>2</sub>
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
  - b. Kidney
  - c. lung
  - d. How many irreversible steps occurs in glycolysis
    - a. 2
    - b. 4
    - c. 3
    - d. 5
10. Insulin is secreted by
  - a. Liver
  - b. Kidney
  - c. Pancreas
  - d. Thyroid
11. 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



12. Glycolysis is termed anerobic because
- molecular oxygen is required
  - only small amounts of A+P are formed
  - molecular oxygen is not required
  - NADH is produced
13. In citric acid cycle the acetyl group of acetyl CoA is
- reduced to two carbondioxide
  - oxidised to two carbondioxide
  - oxidised to two NADGHs
  - reduced to two NADHs
14. Which of the following enzyme links glycolysis and TCA cycle
- Glucokinase
  - PFK
  - LDH
  - Pyruvate dehydrogenase
15. Glycolysis is termed anerobic because
- molecular oxygen is required
  - only small amounts of A+P are formed
  - molecular oxygen is not required
  - NADH is produced
16. Which of the following compounds cannot be used to synthesis glucose in gluconeogenesis
- glycerol
  - lactate
  - acetyl CoA
  - aminoacid

## II. Fill up the blanks

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

## III. Say true of false

- Phosphoglycerate kinase converts 1,3 bisphosphoglycerate to 3 - phosphoglycerate
- 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

#### 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

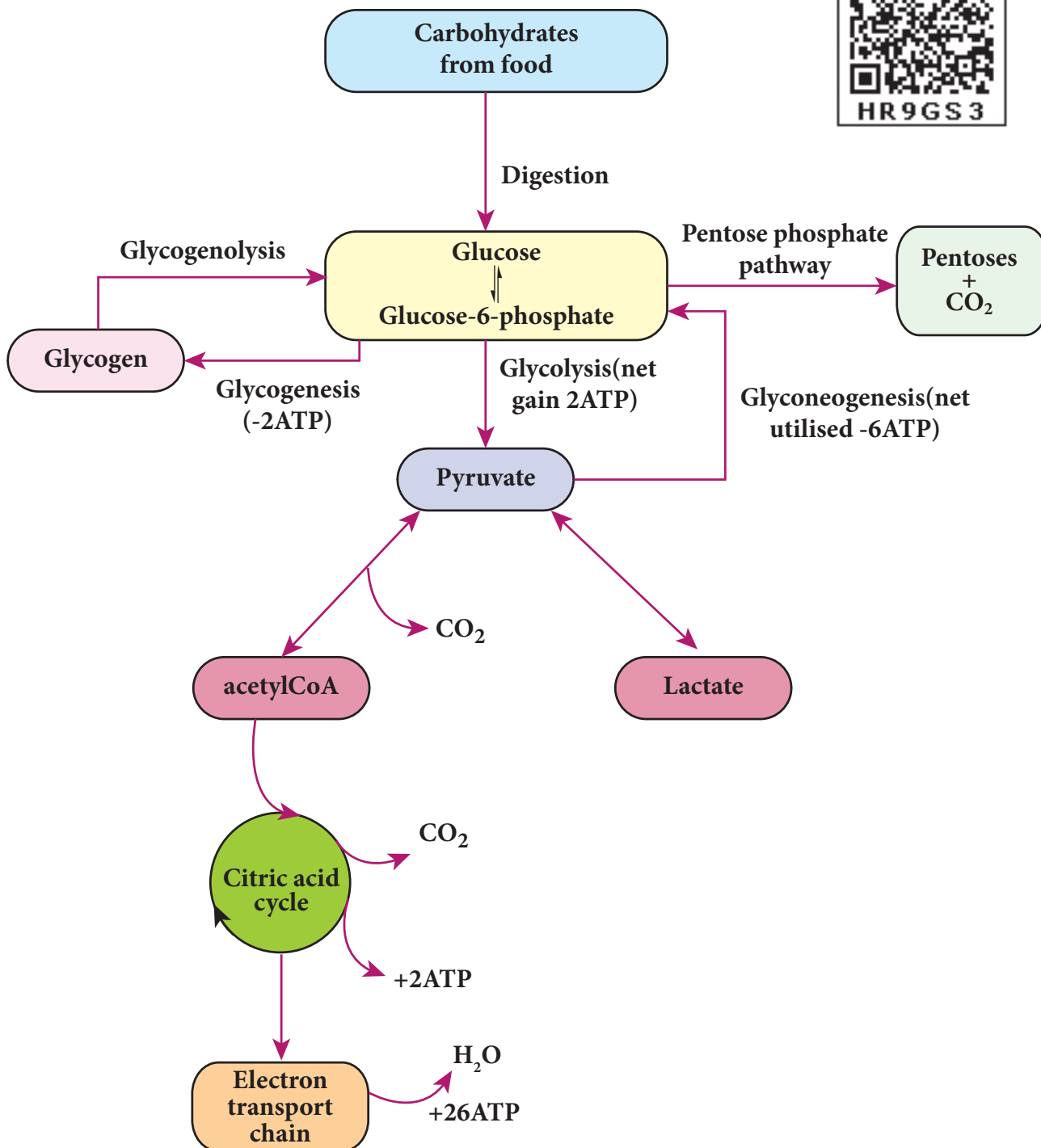
1. Give short note on cori cycle
2. Briefly discuss several common fats for glucose produced through gluconeogenesis in the liver.
3. Name the enzymes which are involved for NADH formation in TCA cycle?
4. What is the mean by aerobic and anaerobic phases?
5. What is the difference between NADH and NADPH?
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?
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 hyperglycemic (or) hypoglycaemic?
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 glycoreogenesis get around the three irreversible steps of glycolysis.

# CONCEPT MAP

## Carbohydrate metabolism



## 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.



### 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 are also serve as precursors for many biologically important compounds

## 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.

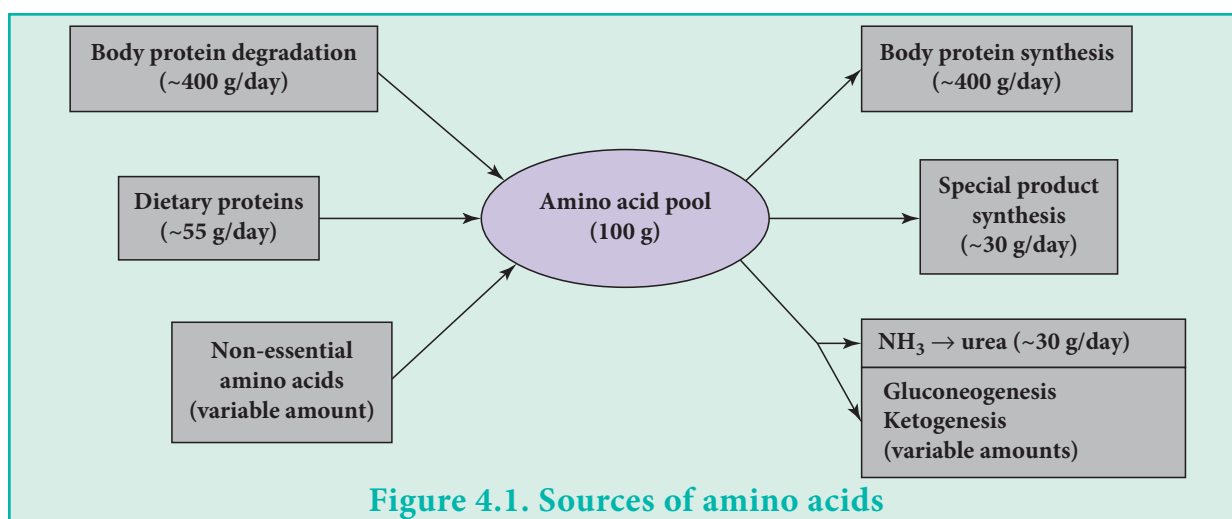


Figure 4.1. Sources of amino acids

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).

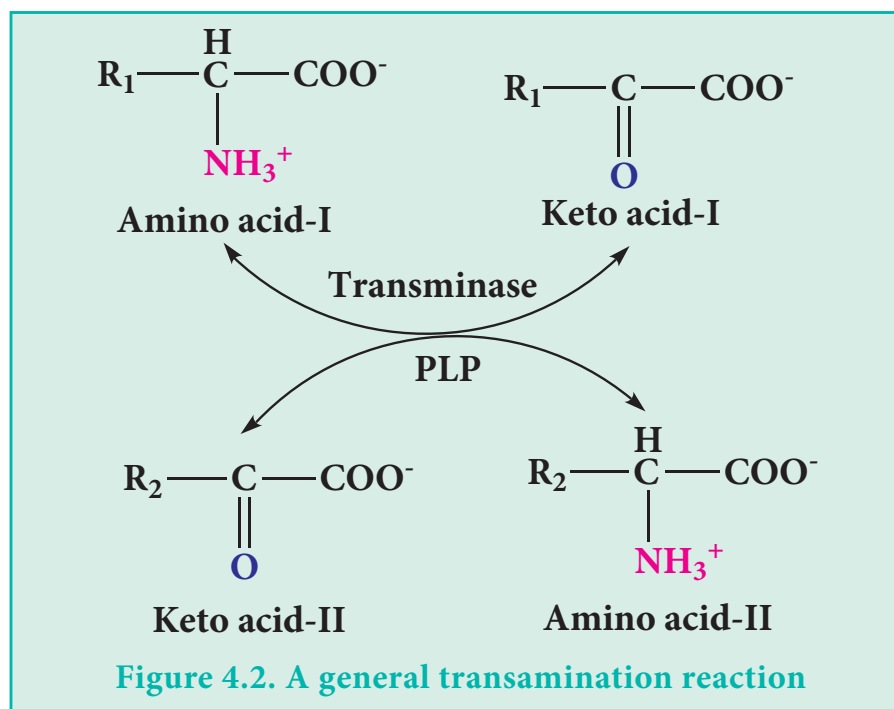
### 4.1 General reactions of amino acid

Non-essential amino acids are synthesized from  $\alpha$ -keto acids by transfer of amino groups with the help of transaminases. However, essential amino acids cannot be synthesized from  $\alpha$ -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

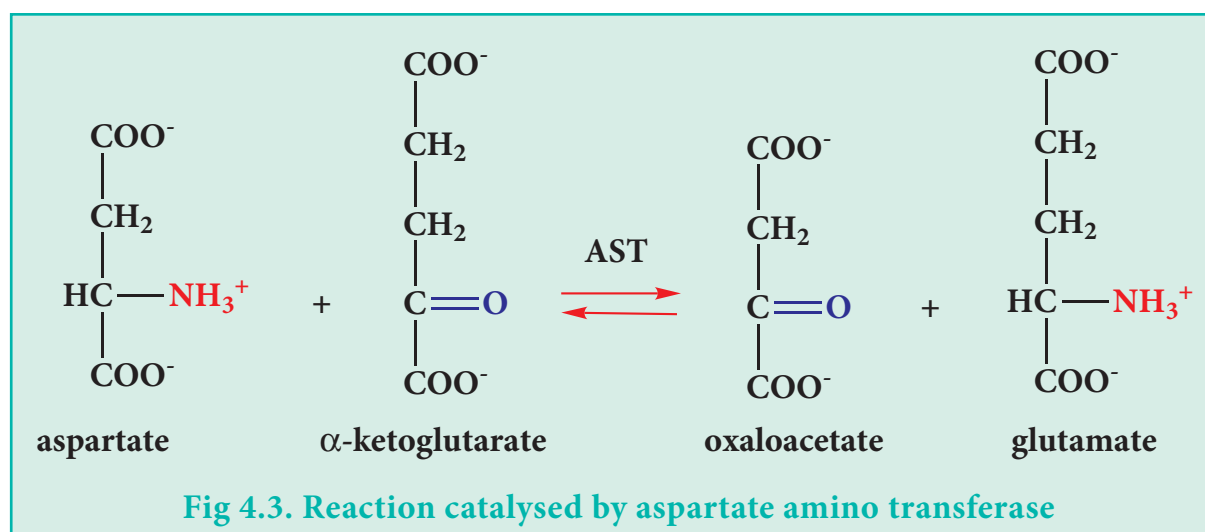
Even though, each amino acid has a specific pathway for its catabolism, the first step in the catabolism of amino acids is the removal of its amino group. This can happen in one the two different ways namely, transamination and deamination.

#### 4.1.2.2 Transamination (AST and ALT)

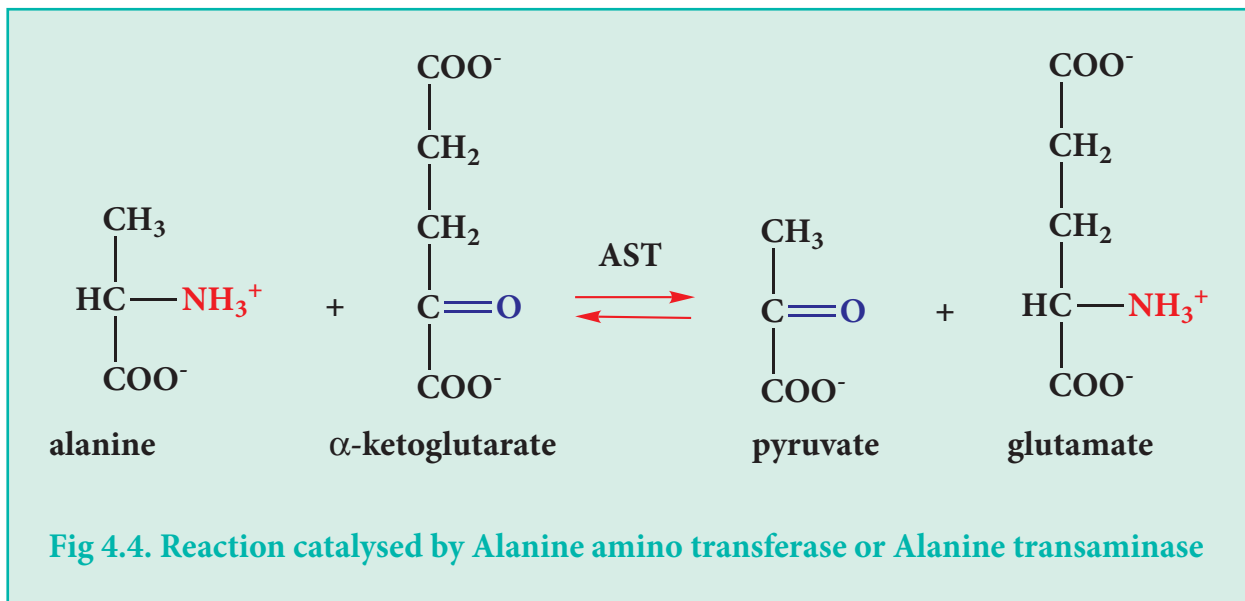


**Transamination** is a process, in which transfer of amino groups occur between a keto acid and an amino acid. It is just shuffling of amino groups and not removal of amino groups. Each amino acid has a specific transaminase (Figure 4.2). However, Alanine transaminase (ALT) and Aspartate transaminase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

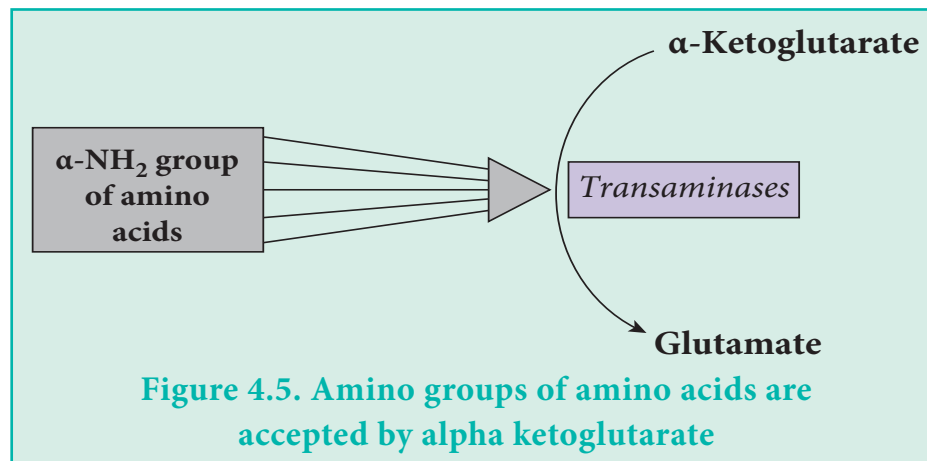
The reactions catalysed by ALT and AST are :



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



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).



### 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.

#### 4.1.2.1 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.

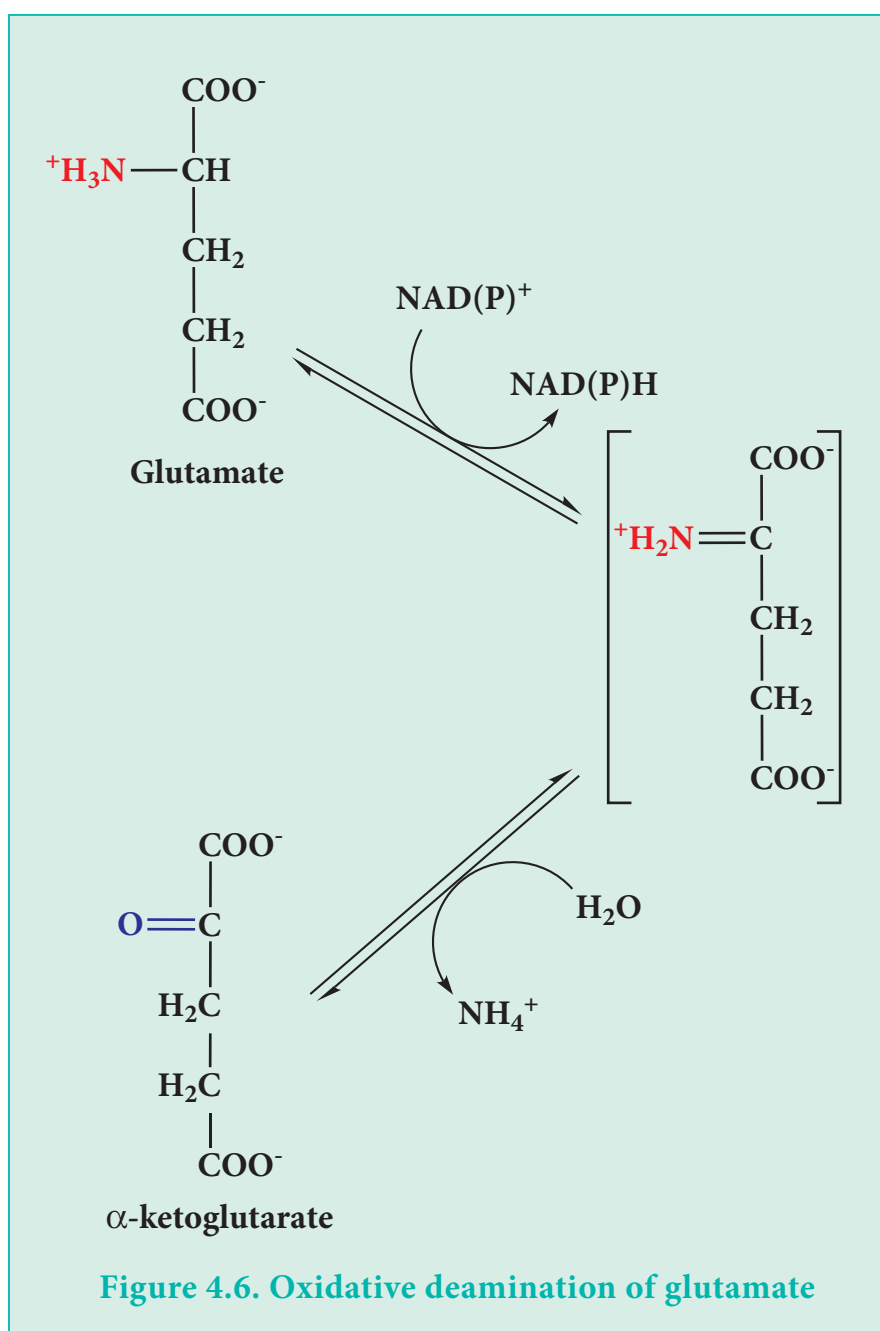




## Oxidative Deamination

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.

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  $\text{NAD}^+/\text{NADP}^+$  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).



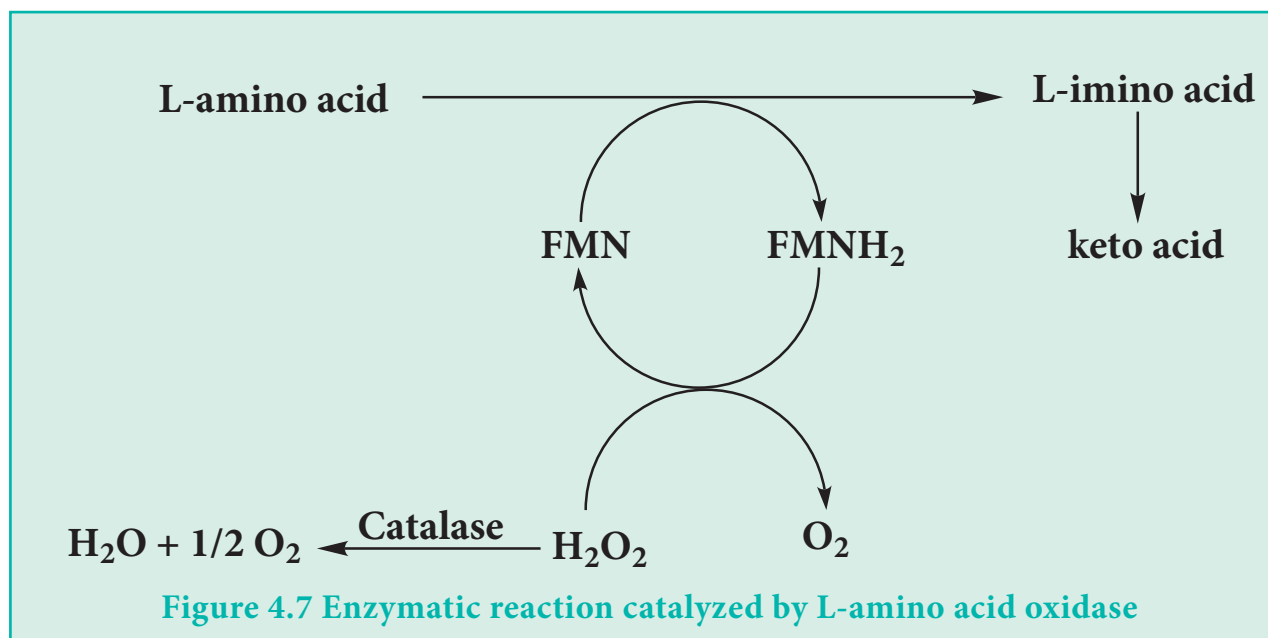
As glutamate acts as a collection centre for all alpha amino groups by transamination and subsequently glutamate liberates ammonia, the process is collectively called as trans-deamination.

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



### 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).



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 sulfhydryl group of cysteine to form pyruvate.

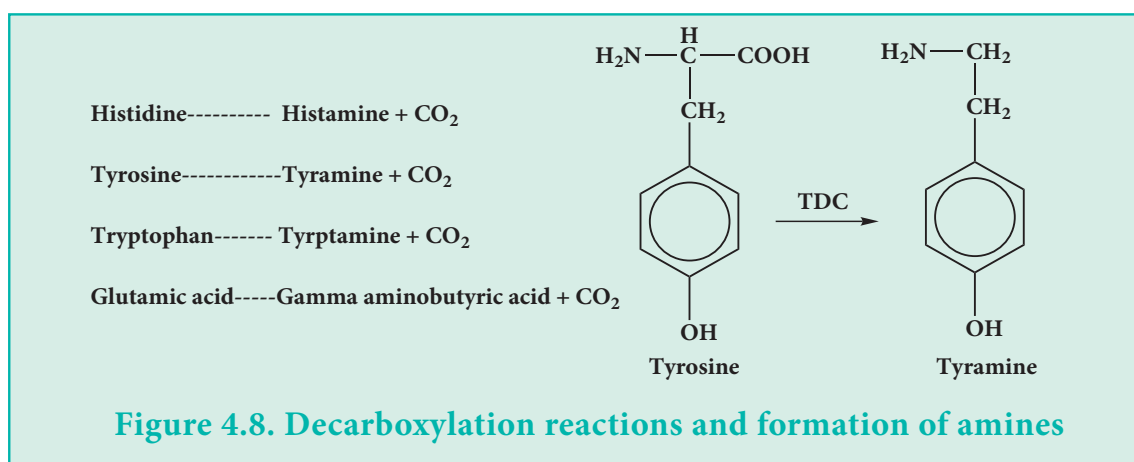
The amide groups of glutamine and asparagine are removed by glutaminase and asparaginase, respectively.

#### 4.1.2.3 Decarboxylation

The removal of carboxyl group from the amino acid in the form of CO<sub>2</sub> 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.

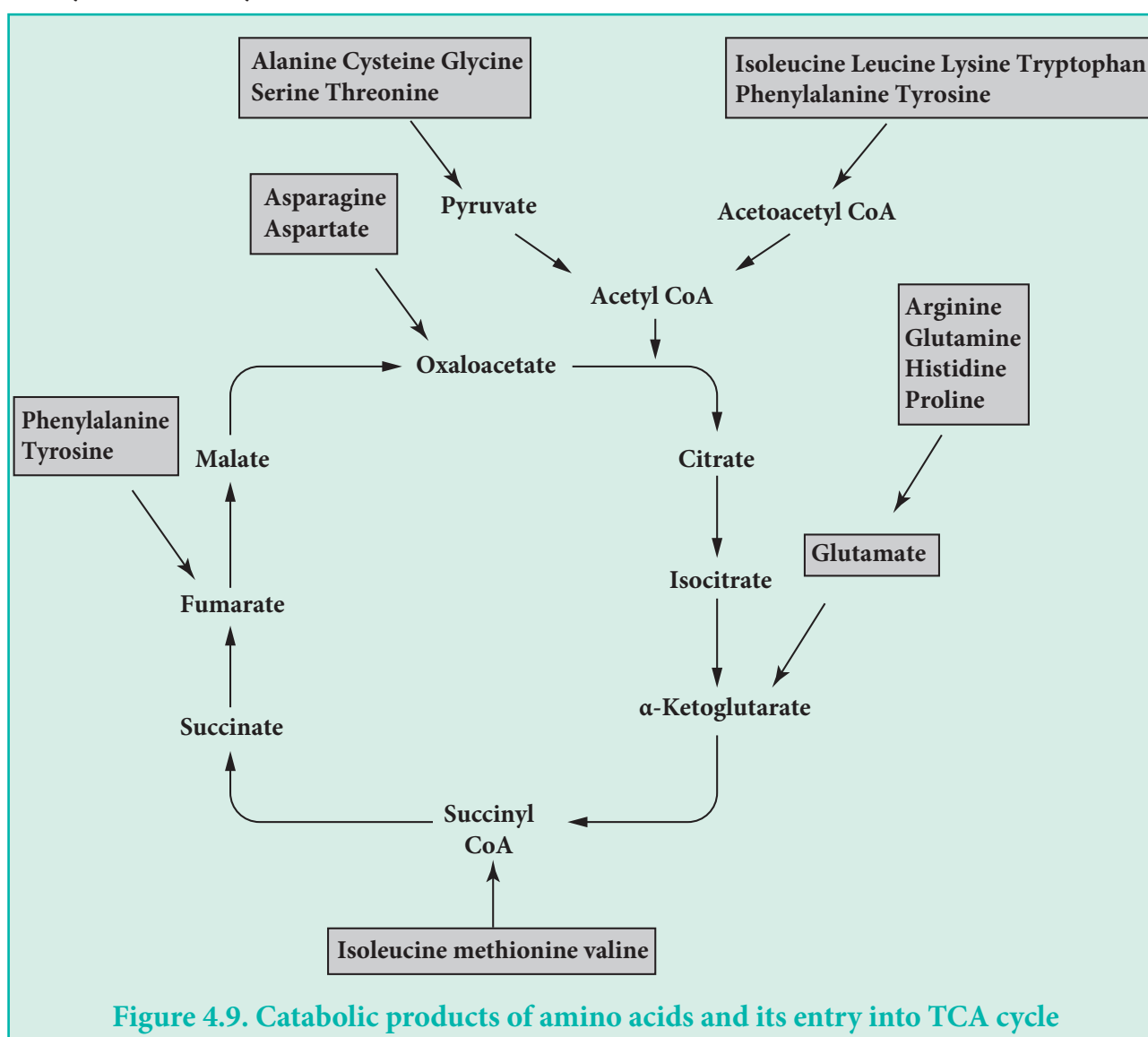


For example,



#### 4.1.2.4 Fate of carbon skeleton of amino acids

##### 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.

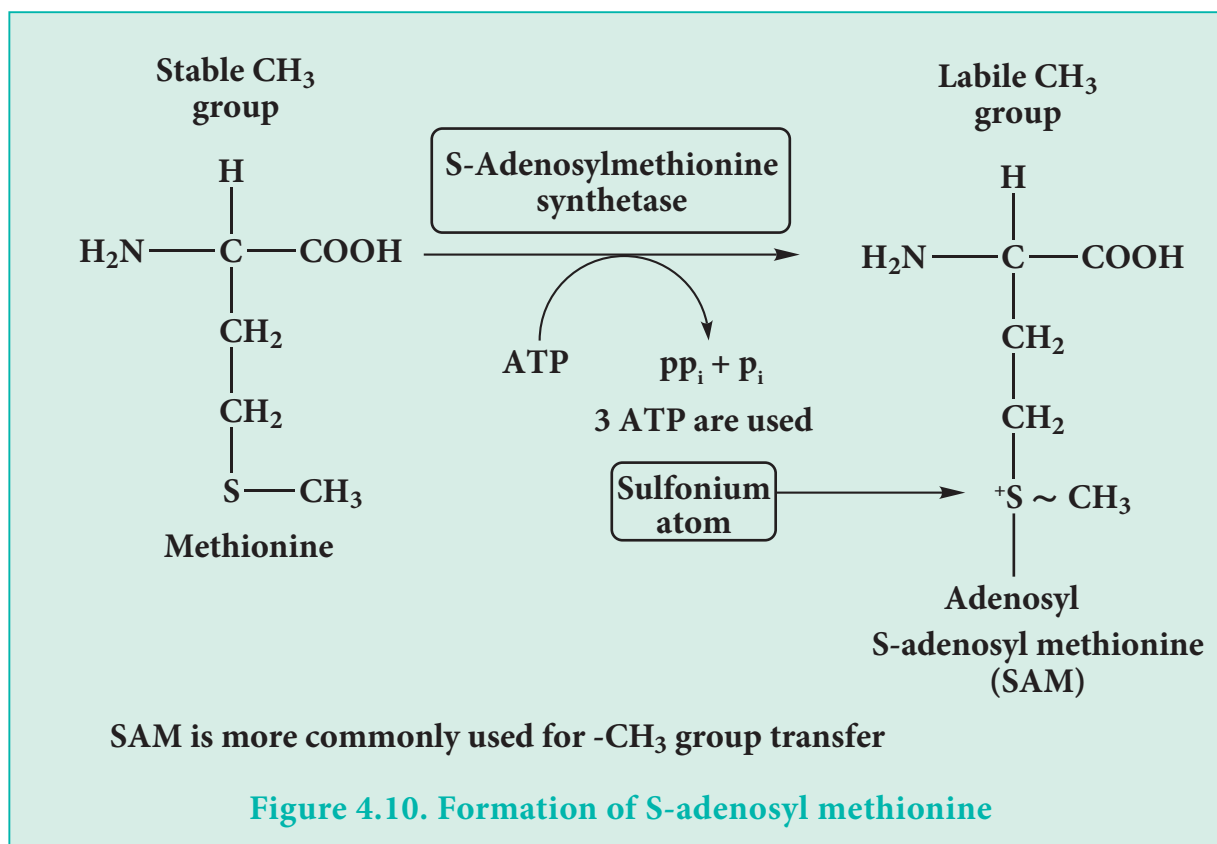
Catabolism of phenylalanine and tyrosine yields fumarate that also enters TCA cycle for further catabolism. These intermediates that enter into TCA cycle can act as precursors of gluconeogenesis (Figure 4.9). Hence, the amino acids that can contribute their carbon skeletons for the synthesis of glucose 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.

#### 4.1.2.5 Transmethylation

##### One carbon metabolism



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

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  $\alpha$ -ketoglutarate to form glutamate, thus depleting the levels of  $\alpha$ -ketoglutarate and impairing the function of TCA cycle in neurons.

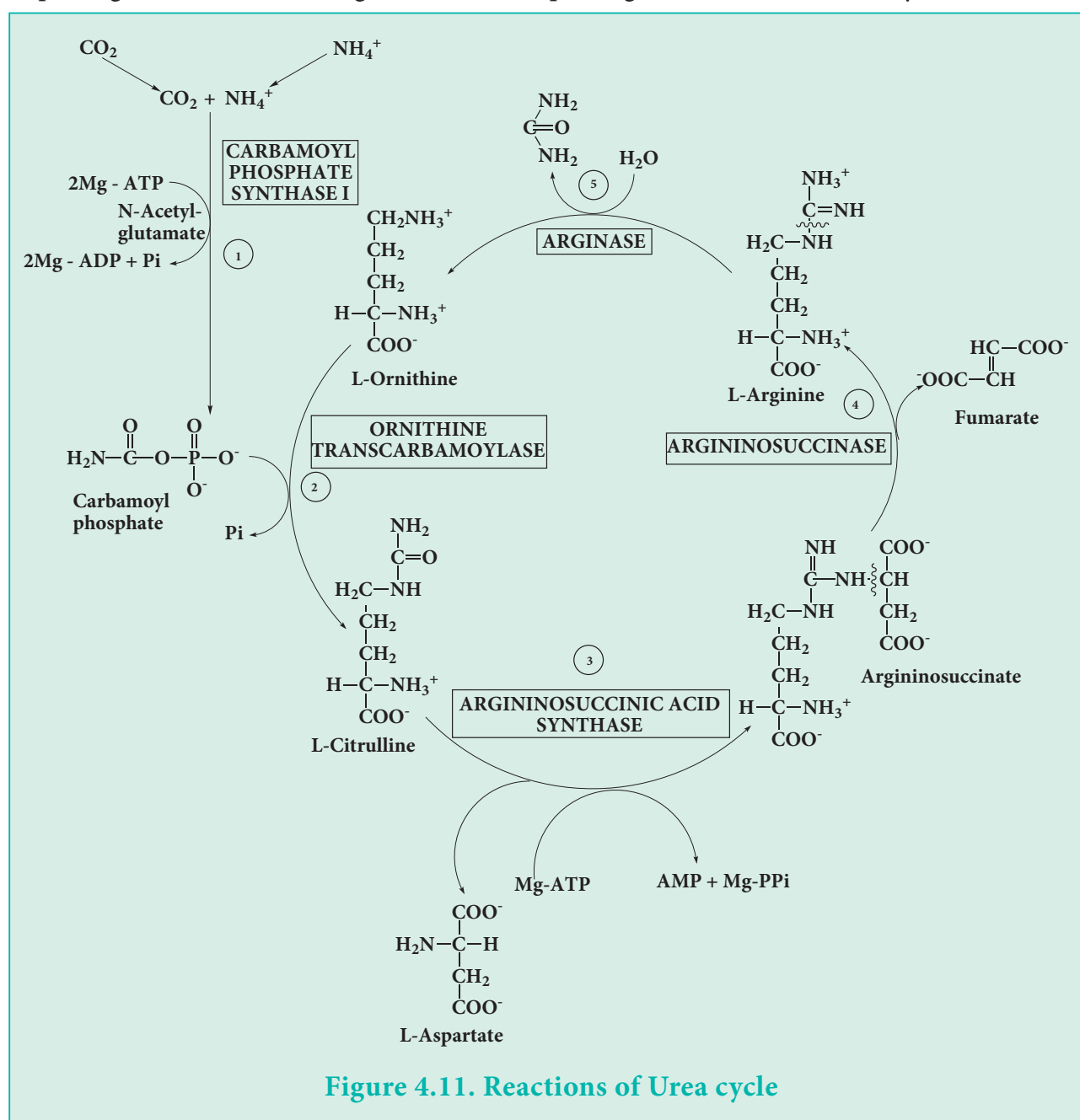


Figure 4.11. Reactions of Urea cycle



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.

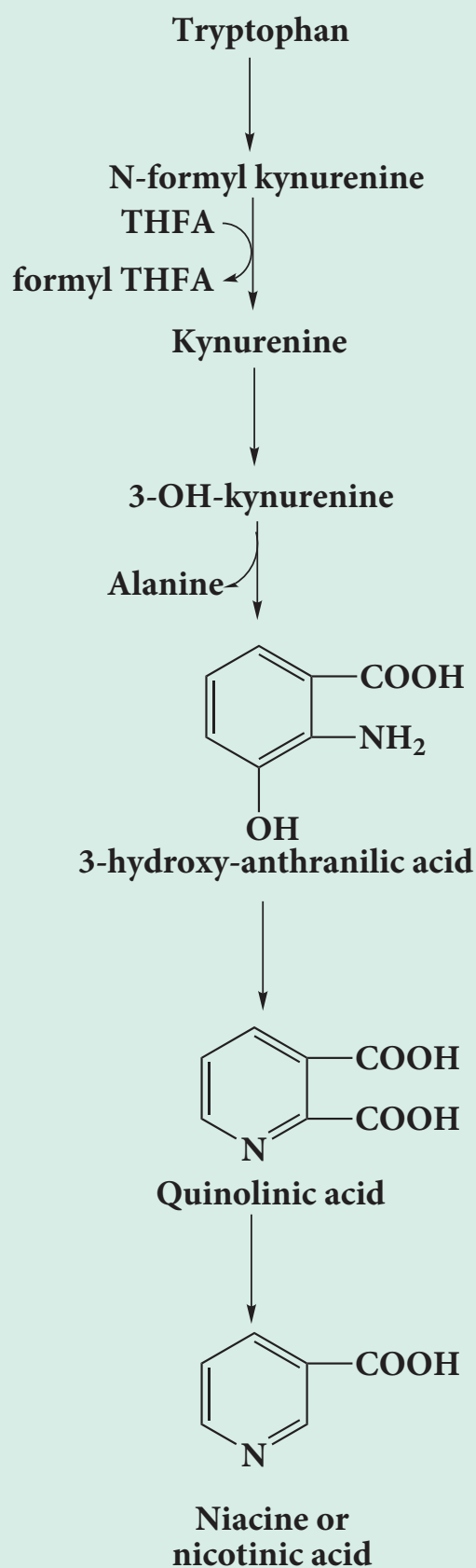
One molecule of Urea is synthesized from one molecule of carbondioxide, one molecule of ammonia and the amino group of aspartate. The reactions consume four high energy bonds equivalent to four ATPs.

### 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:

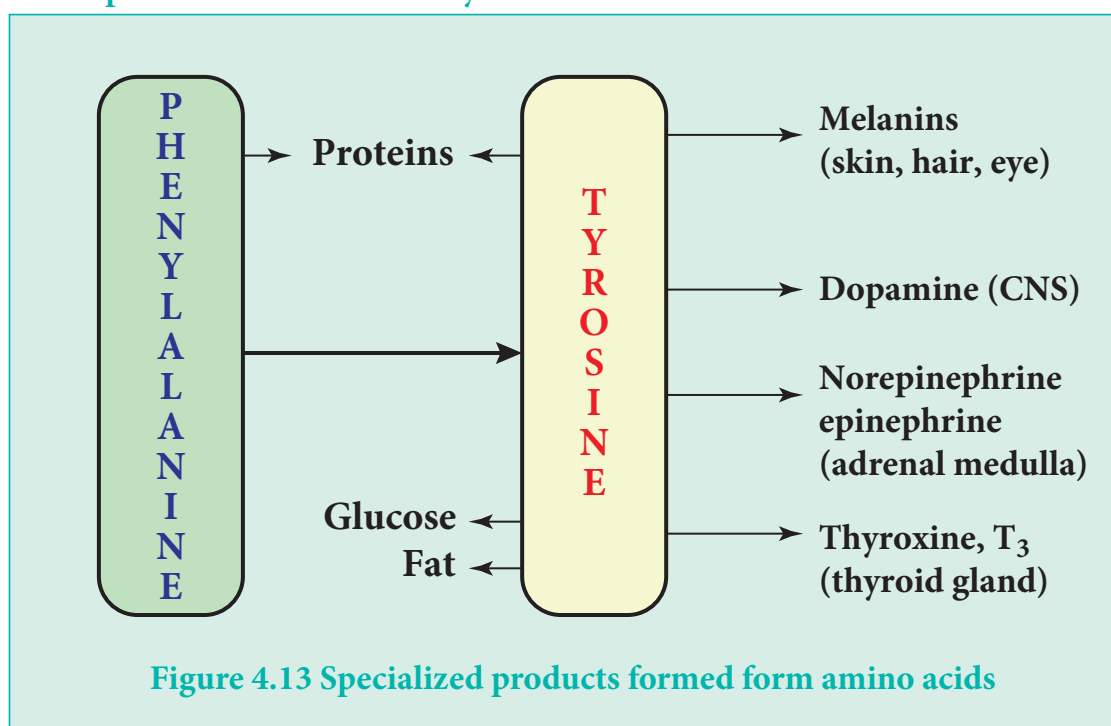
1. Tryptophan is oxidized to N-formyl kynurenine.
2. N-formyl kynurenine transfers its formyl group to Tetrahydrofolate and results in the formation of Kynurenine
3. Kynurenine is hydroxylated at the 3<sup>rd</sup> position to form 3-hydroxy kynurenine.
4. Alanine is removed from 3-hydroxy kynurenine 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**



### Specialized products formed from tyrosine



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 (T<sub>3</sub>) and tetraiodothyronine (T<sub>4</sub>-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.

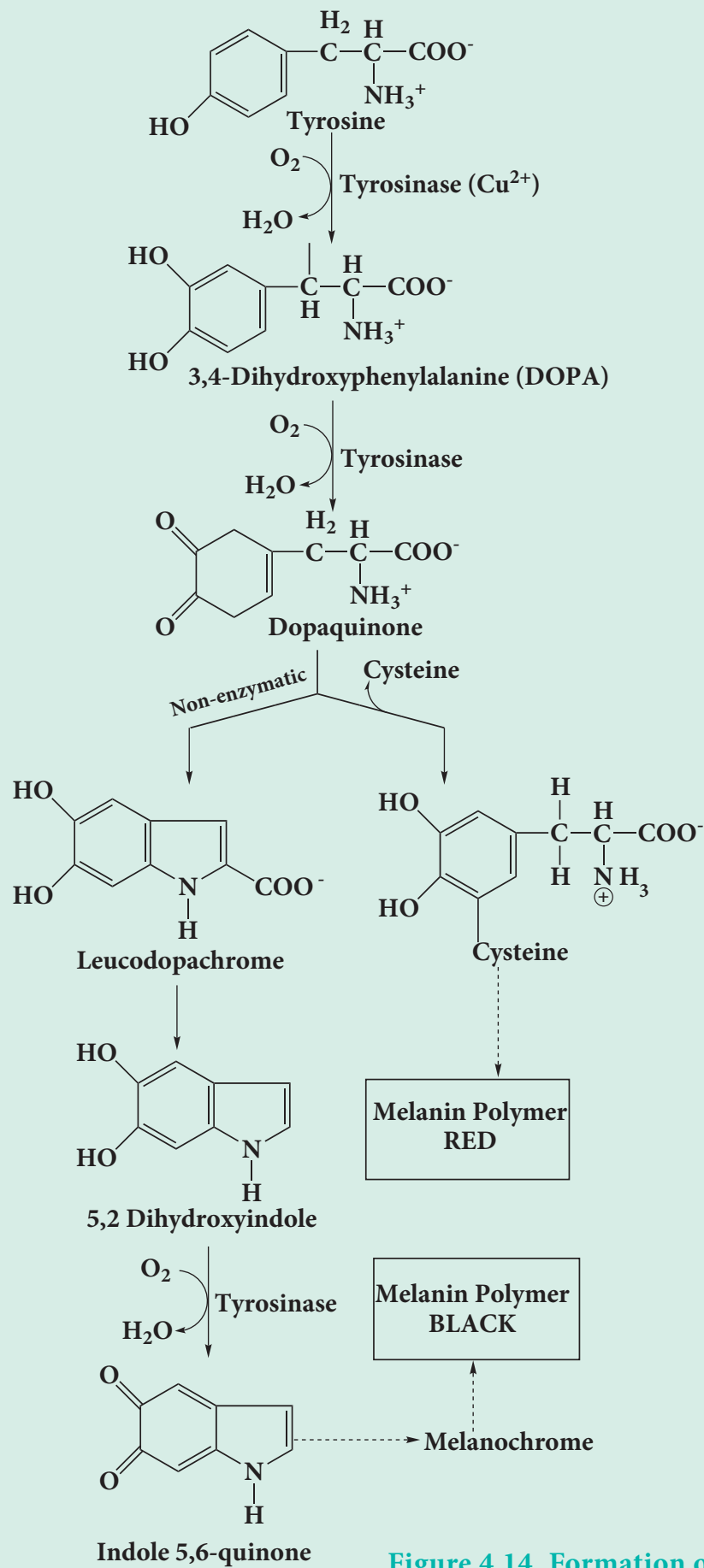


Figure 4.14. Formation of melanin

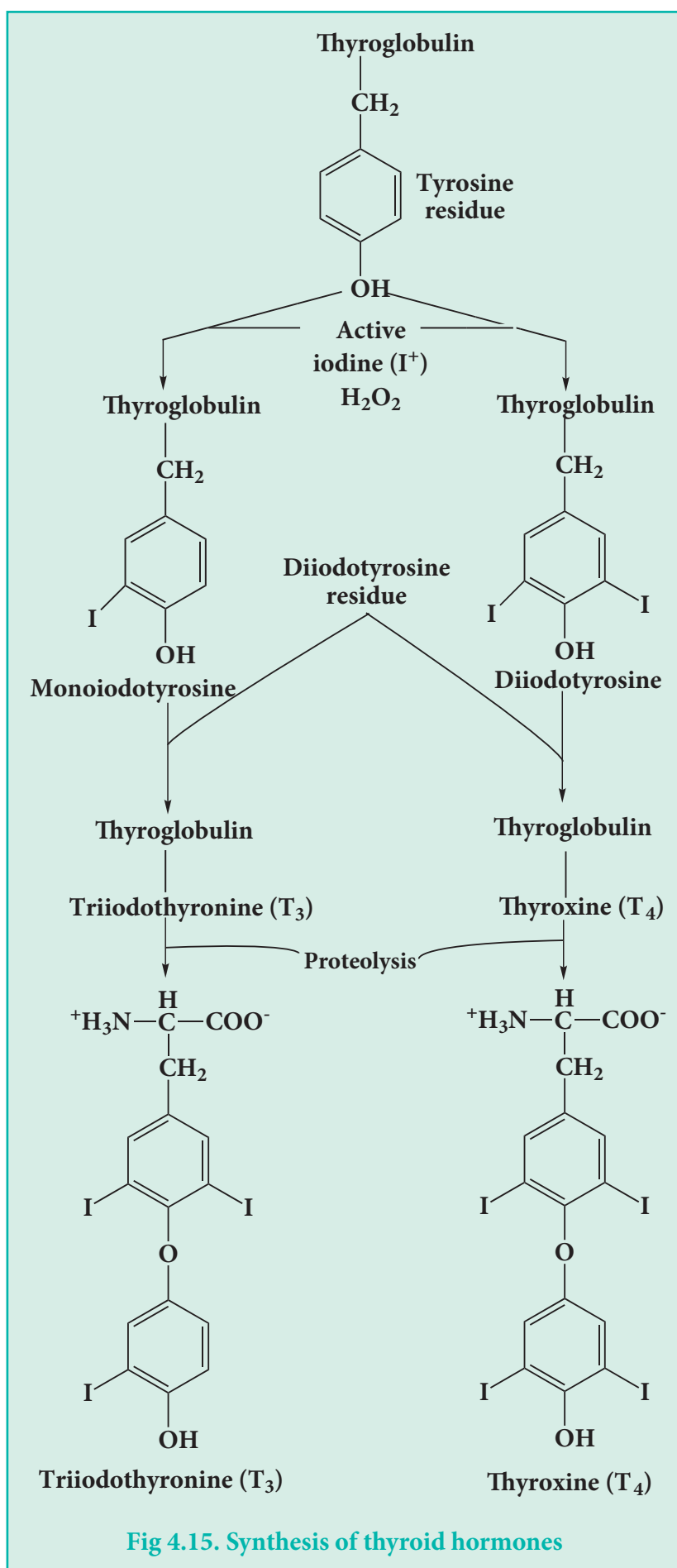


## 4.5 Formation of thyroid hormones

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 diiodotyrosine
3. Formation of diiodothyronine 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 T<sub>3</sub> and T<sub>4</sub>.



## 4.6 Formation of Catecholamines

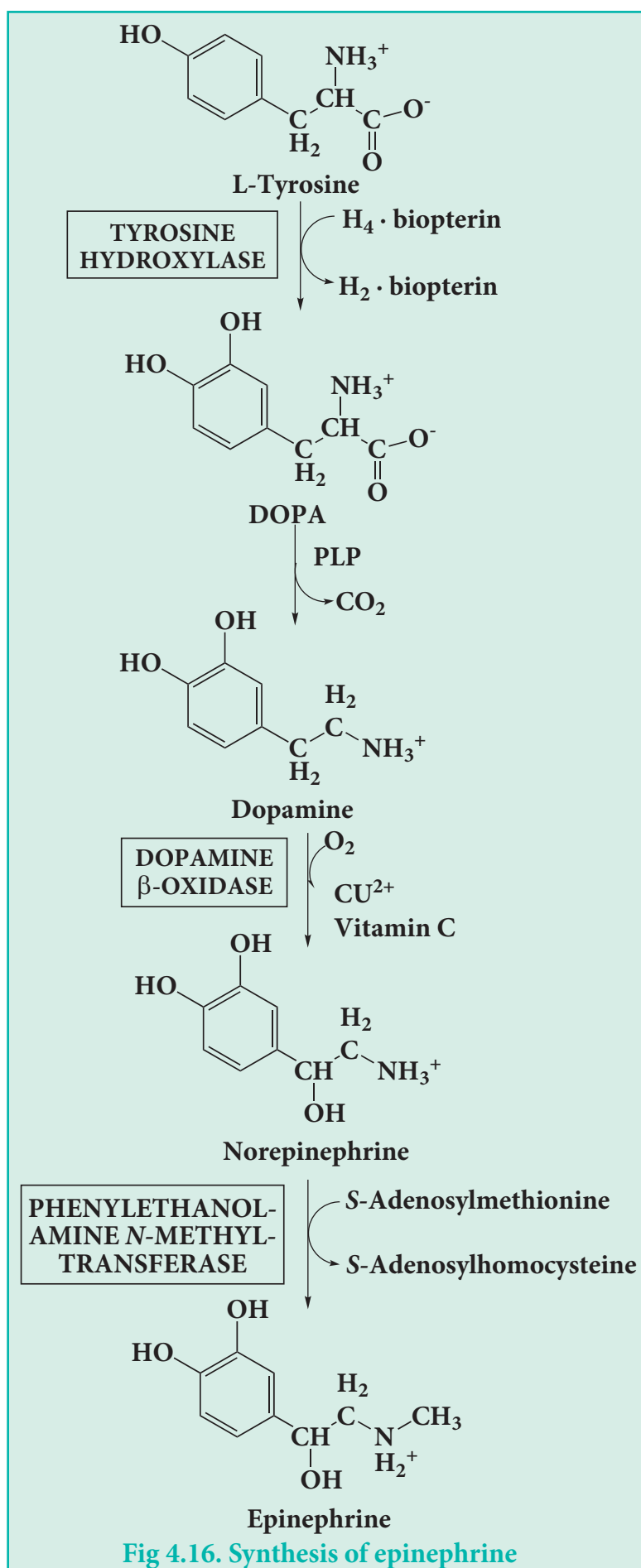
Adrenal glands present in the top of the kidneys are responsible for the secretion of hormones that regulate mineral metabolism (secreted from the adrenal cortex) along with hormones that are secreted in response to stress (secreted by the adrenal medulla), such as epinephrine and nor-epinephrine, that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





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  $\text{CCl}_4$  and  $\text{CHCl}_3$ .
- 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.



- 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.



## 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





- 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  $\text{NADH}^+/\text{NADPH}^+$   
b) Irreversible and  $\text{NAD}^+/\text{NADP}^+$   
c) **Reversible and  $\text{NAD}^+/\text{NADP}^+$**   
d) Reversible and  $\text{NADH}^+/\text{NADPH}^+$
12. \_\_\_\_\_ amino acids are both glucogenic and ketogenic in nature.  
a) **Isoleucine and tyrosine**  
b) Glycine and alanine  
c) Leucine and lysine



- 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)  $\alpha$ -ketoglutarate
  - c) Malate
  - d) **Fumarate**
15. Co-enzyme required for the conversion of L-Amino acid into L-Imino acid.
- a)  $\text{FAD}^+$
  - b) **FMN**
  - c)  $\text{NAD}^+$
  - d)  $\text{NADP}^+$

## 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.

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

### III Answer the following

- Write in detail about oxidative and Non-oxidative deamination.
- Elaborate on Urea Cycle and its significance.
- Write a note on specialized products formed from tyrosine.
- Briefly discuss on the synthesis and functions of thyroid hormones.
- 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.

- If both A and R are true and R is correct explanation of A
- If both A and R are true but R is not the correct explanation of A
- If R is true but A is false
- If both A and R are false.



**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  $\alpha$ -ketoglutarate occurs due to its conversion to glutamate.

**3. Assertion:**  $\alpha$ -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  $\alpha$ -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

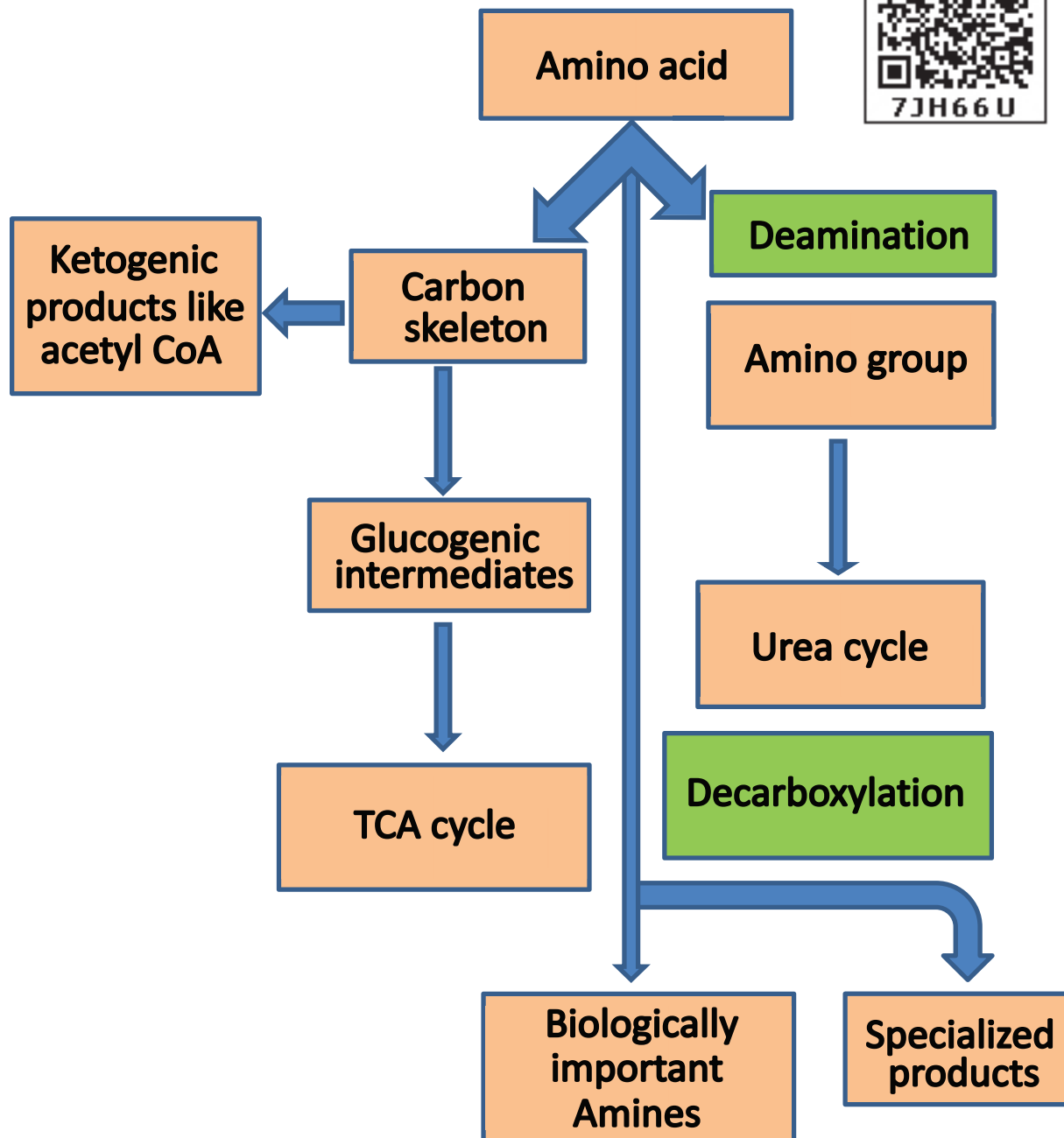
3. c) R is true but A is false

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



## Concept Map



# LIPID METABOLISM

## UNIT 5



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.



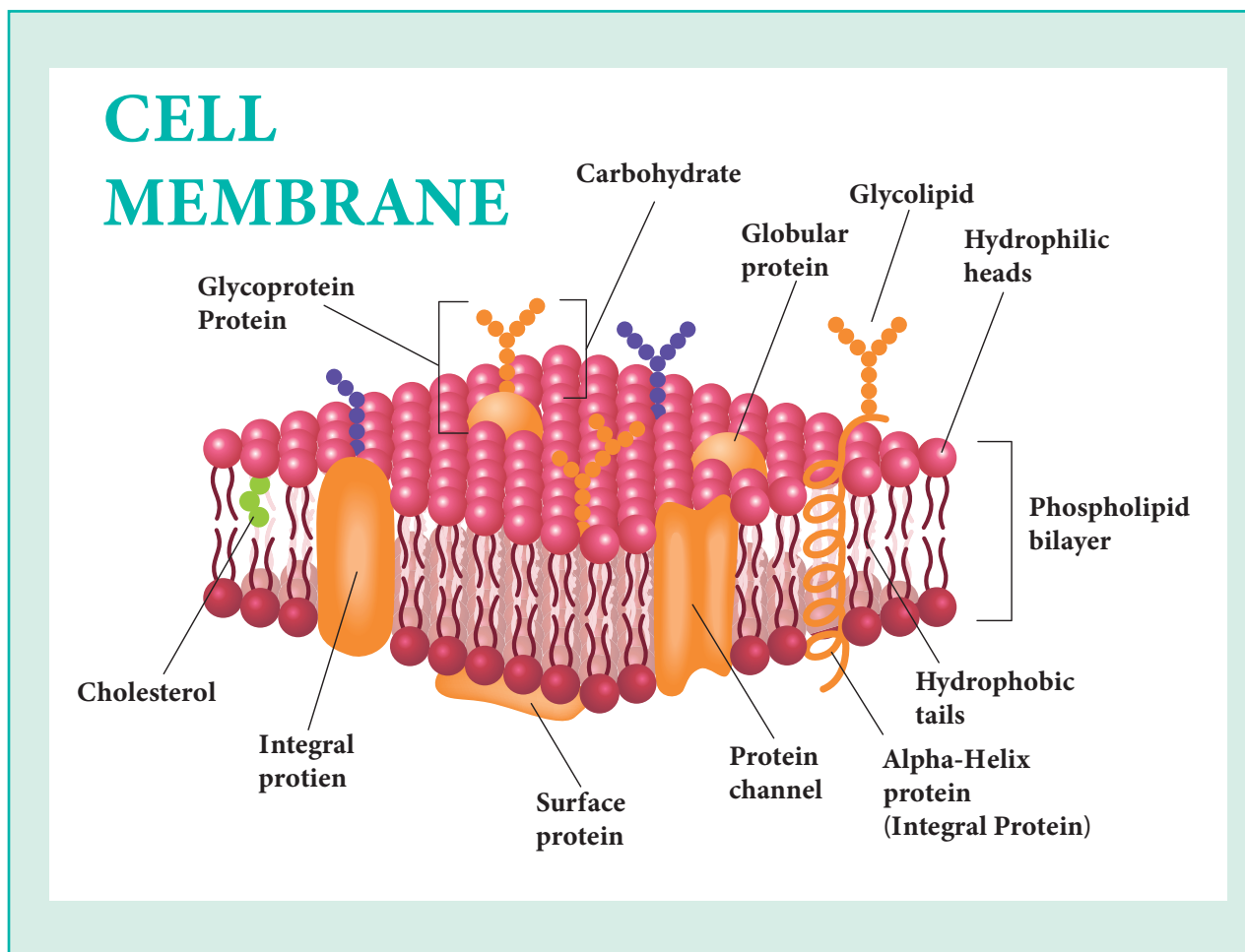
### Learning Objectives

After studying this unit the students will be able to

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

## 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 function of Lipids

**Lipids perform several important functions:**

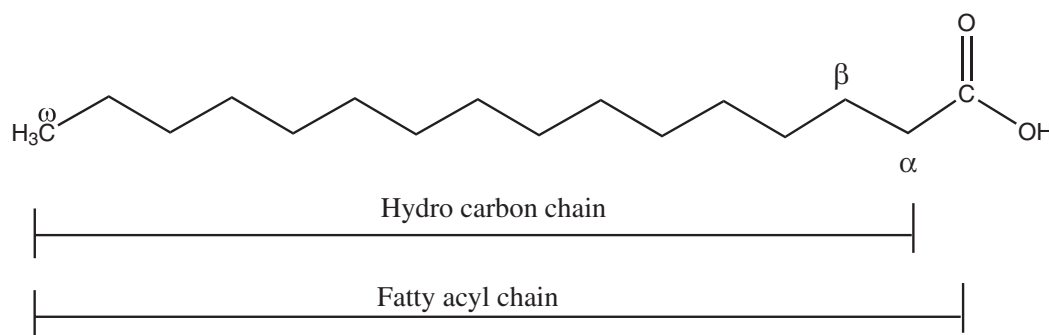
- They are the concentrated fuel reserve of the body (triacylglycerols)
- Lipids are the constituents of biological membranes and regulate membrane permeability (phospholipids and cholesterol)
- They serve as a source of fat-soluble vitamins (A, D, E and K)
- Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins)
- Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body



- 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

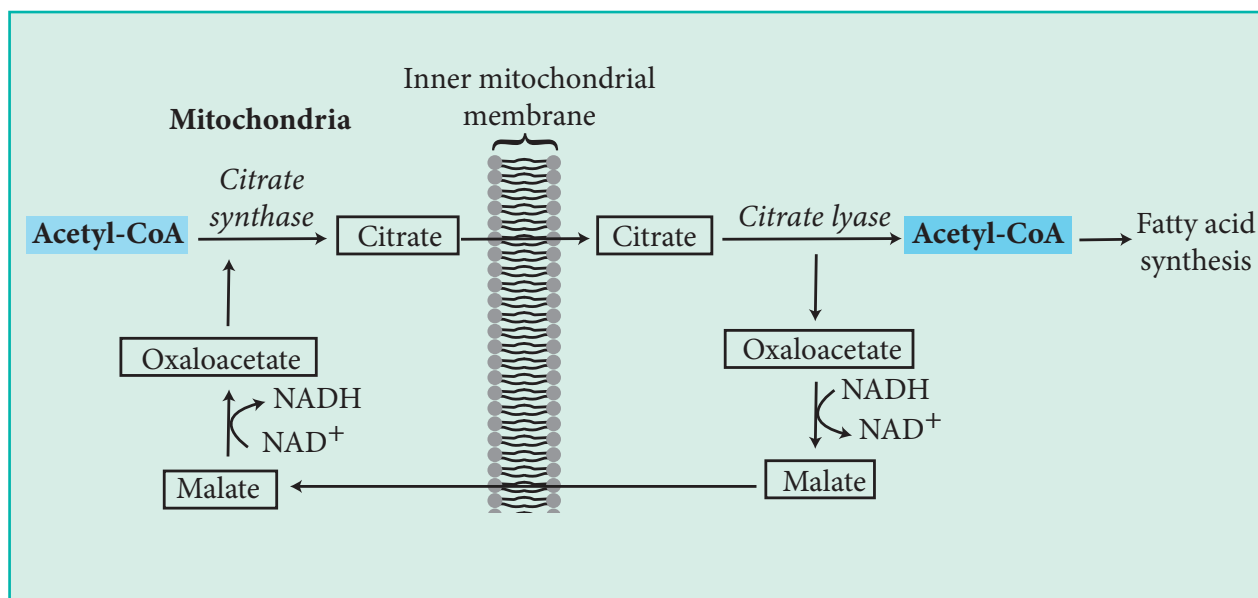
Fatty acids are the simplest form of lipids and they serve as the constituent in a large number of complex form of lipids. They are long chain hydrocarbons (4 to 36 carbons long) with one carboxyl group. Fatty acids are amphipathic in nature; that is, they have both polar and non-polar ends.



In a fatty acid the second carbon is referred as  $\alpha$  carbon, third carbon as  $\beta$  carbon and the methyl carbon is called as  $\omega$  carbon. The alkyl chain in fatty acids maybe 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.





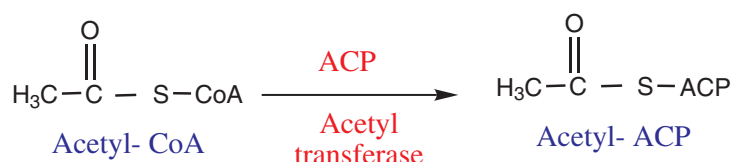
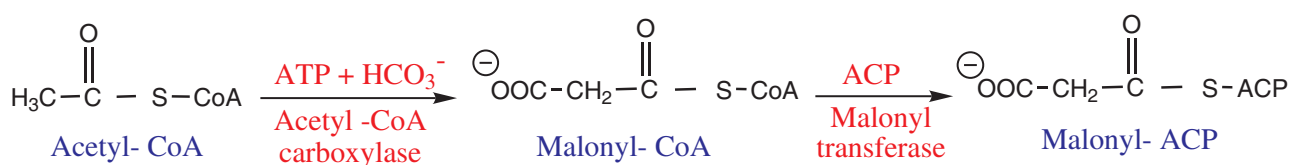
Within the mitochondria, the acetyl CoA reacts with oxaloacetate in a reaction catalyzed by citrate synthase to form citrate. The acetyl group passes out of the mitochondria as citrate. In the cytosol, the citrate reacts with cytosolic CoA to form acetyl CoA and oxaloacetate. Oxaloacetate is reduced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

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.

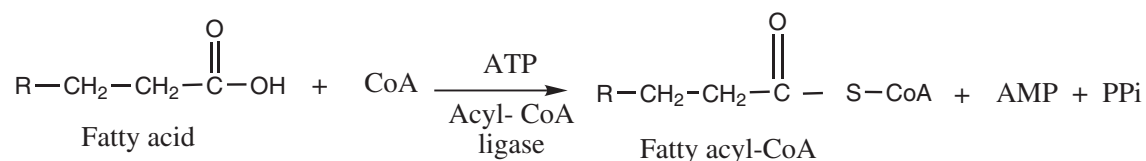
The fatty acids in the body are mostly oxidized by  $\beta$ -oxidation in the mitochondria.  $\beta$ -oxidation may be defined as the oxidation of fatty acids on the  $\beta$ -carbon atom. This results in the sequential removal of a two-carbon fragment, acetyl CoA as described below.

### 5.3.1 $\beta$ -Oxidation

The  $\beta$ -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:

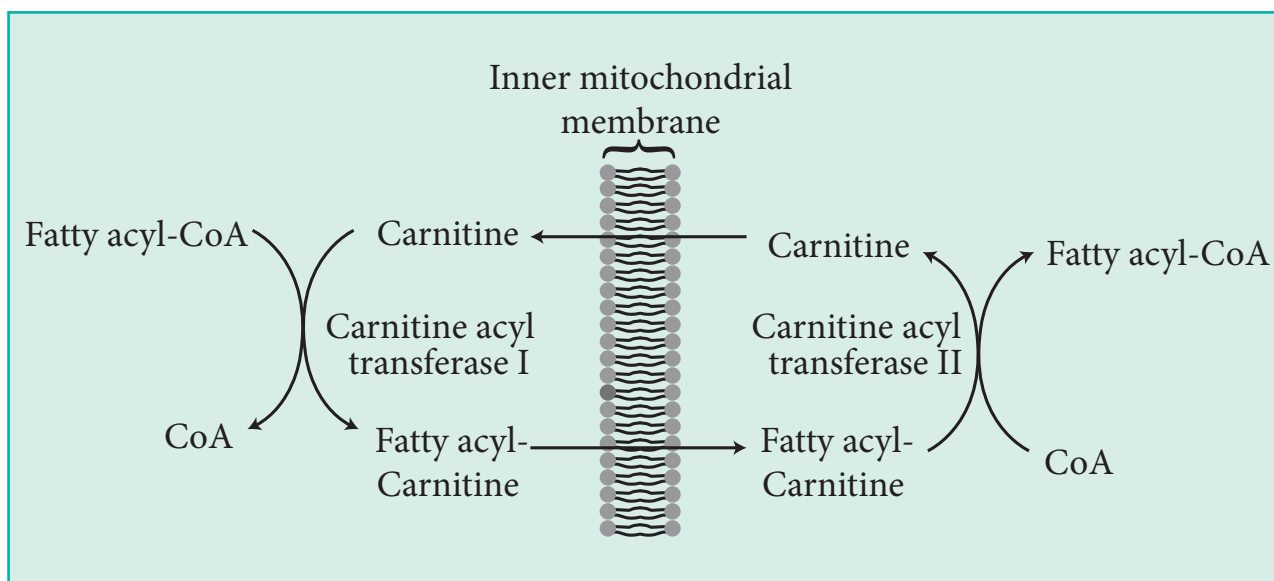
#### i. Activation of fatty acids occurring in the cytosol

Before the  $\beta$ -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

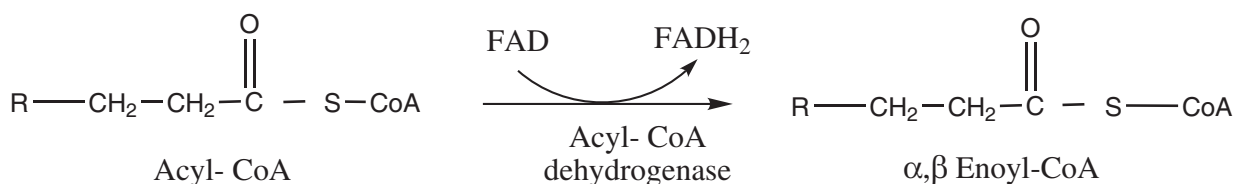
Though fatty acids are activated for oxidation in cytosol, they are oxidized in the mitochondria. Since the mitochondrial membrane is impermeable for long chain fatty acids, they are transported by a special carrier molecule called carnitine.



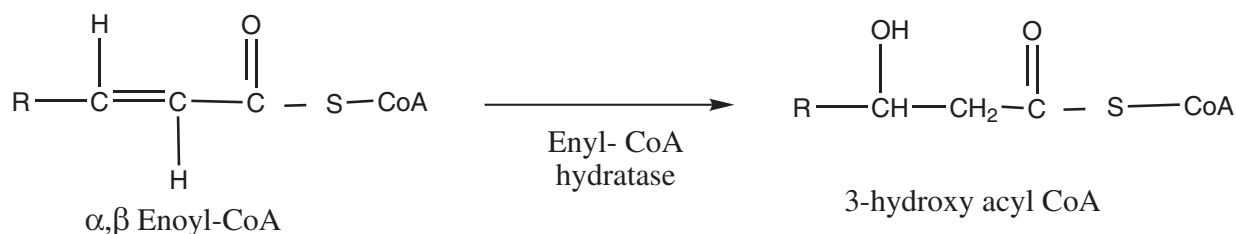
#### iii. $\beta$ -Oxidation in the mitochondrial matrix

Once inside the mitochondrial matrix, fatty acyl CoA is oxidized with  $\beta$ -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.

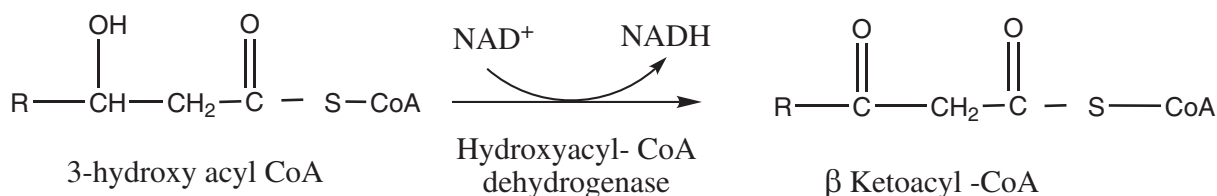
#### Oxidation



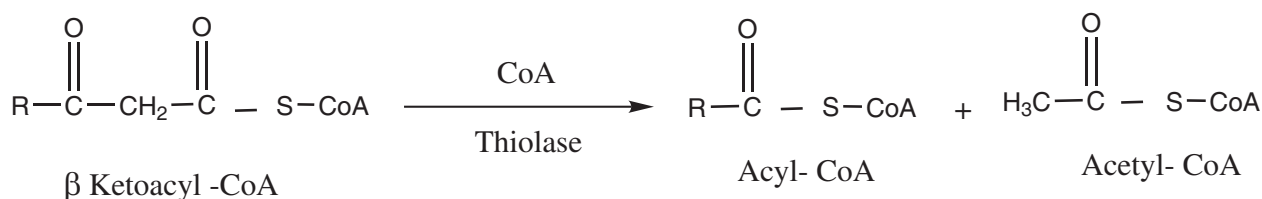
#### Hydrolysis



#### Oxidation



#### Thiolysis



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  $\text{C}_{27}$  alcohol with molecular formula  $\text{C}_{27}\text{H}_{46}\text{O}$ . It has a structure consisting of four fused hydrocarbon rings forming the bulky steroid structure. There is a

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:

- Cholesterol exists as crystals that are white, shiny and rhombic in nature
- It is tasteless and odorless
- It has a high melting point of 150°C
- It is insoluble in water and soluble in fat solvents
- 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
- Cholesterol, when oxidized under suitable conditions, undergoes rapid oxidation to form a ketone called cholestenone
- 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

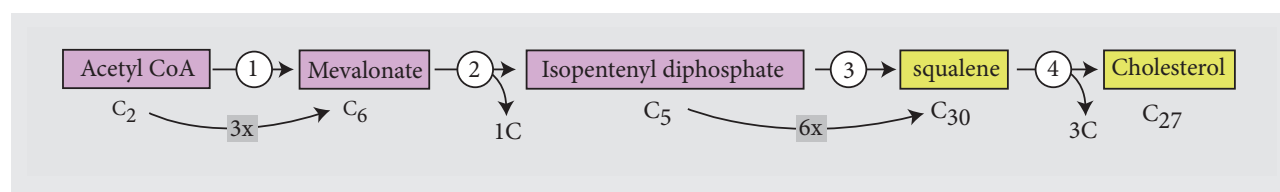
- It is a structural component of the cell membrane
- Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids
- It is an essential ingredient in the structure of lipoproteins which transport lipids in the body. Fatty acids 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, reproductive tissue etc.

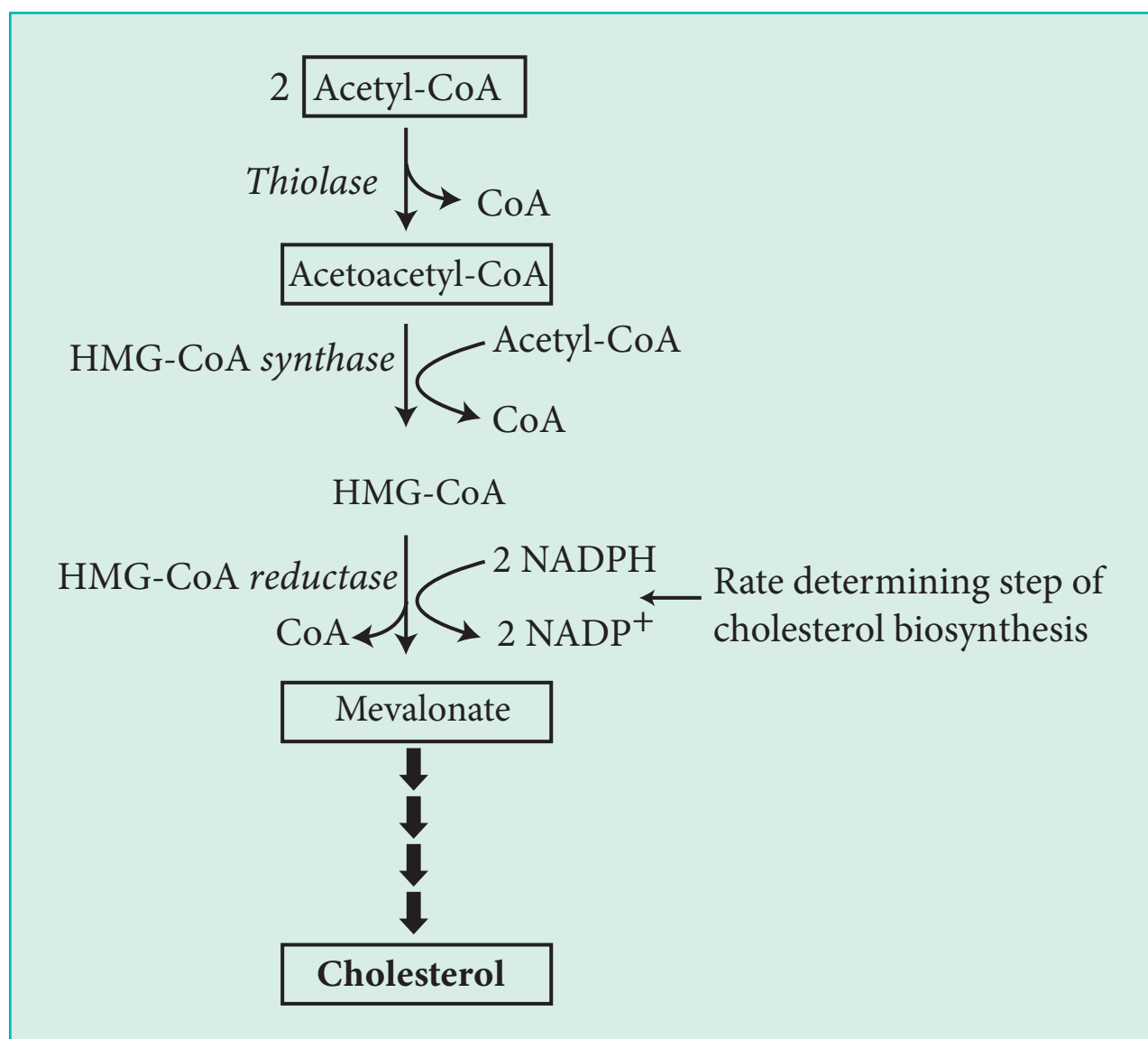
The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by NADPH while ATP provides energy.

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

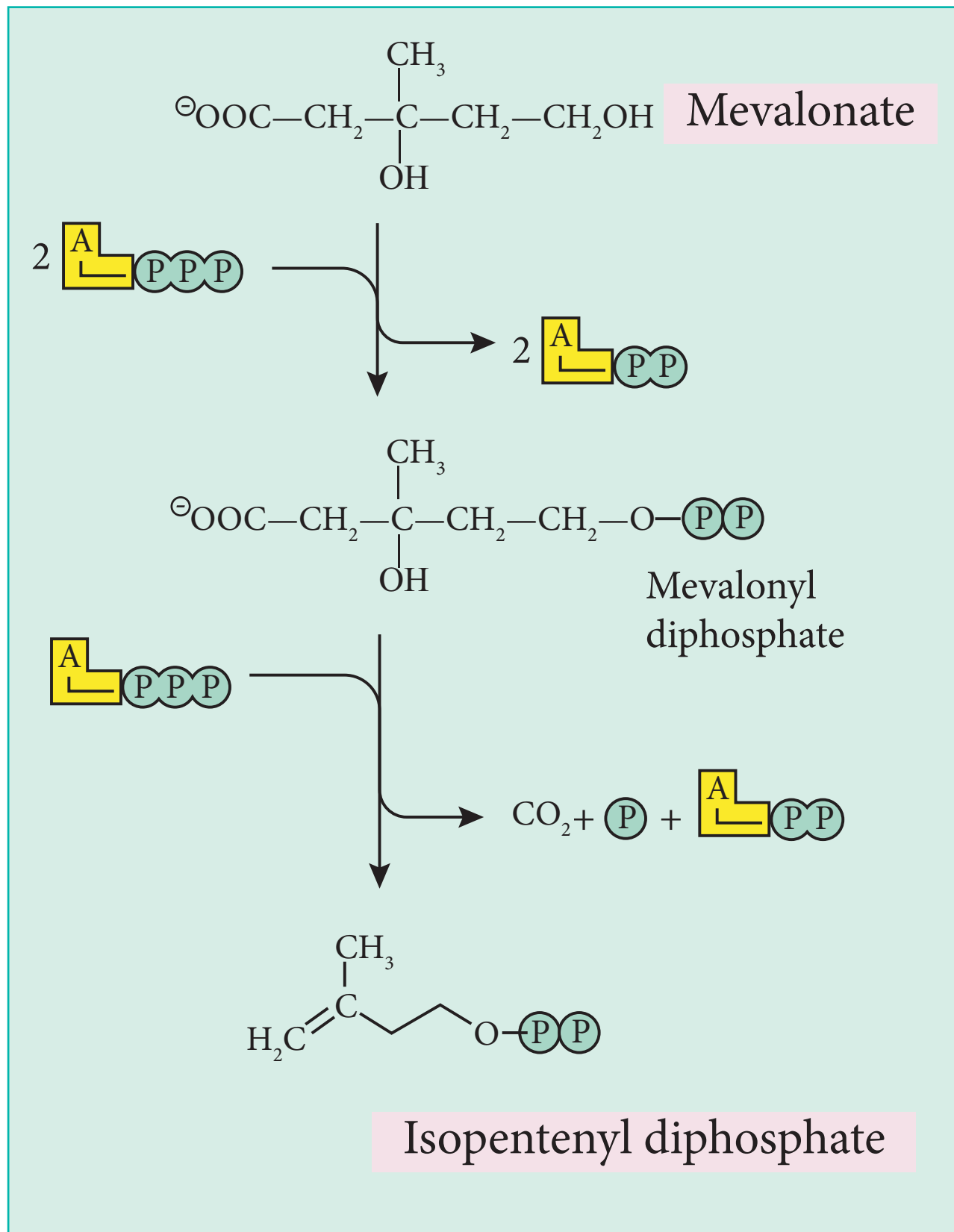


The four basic steps in cholesterol synthesis are,

- 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.

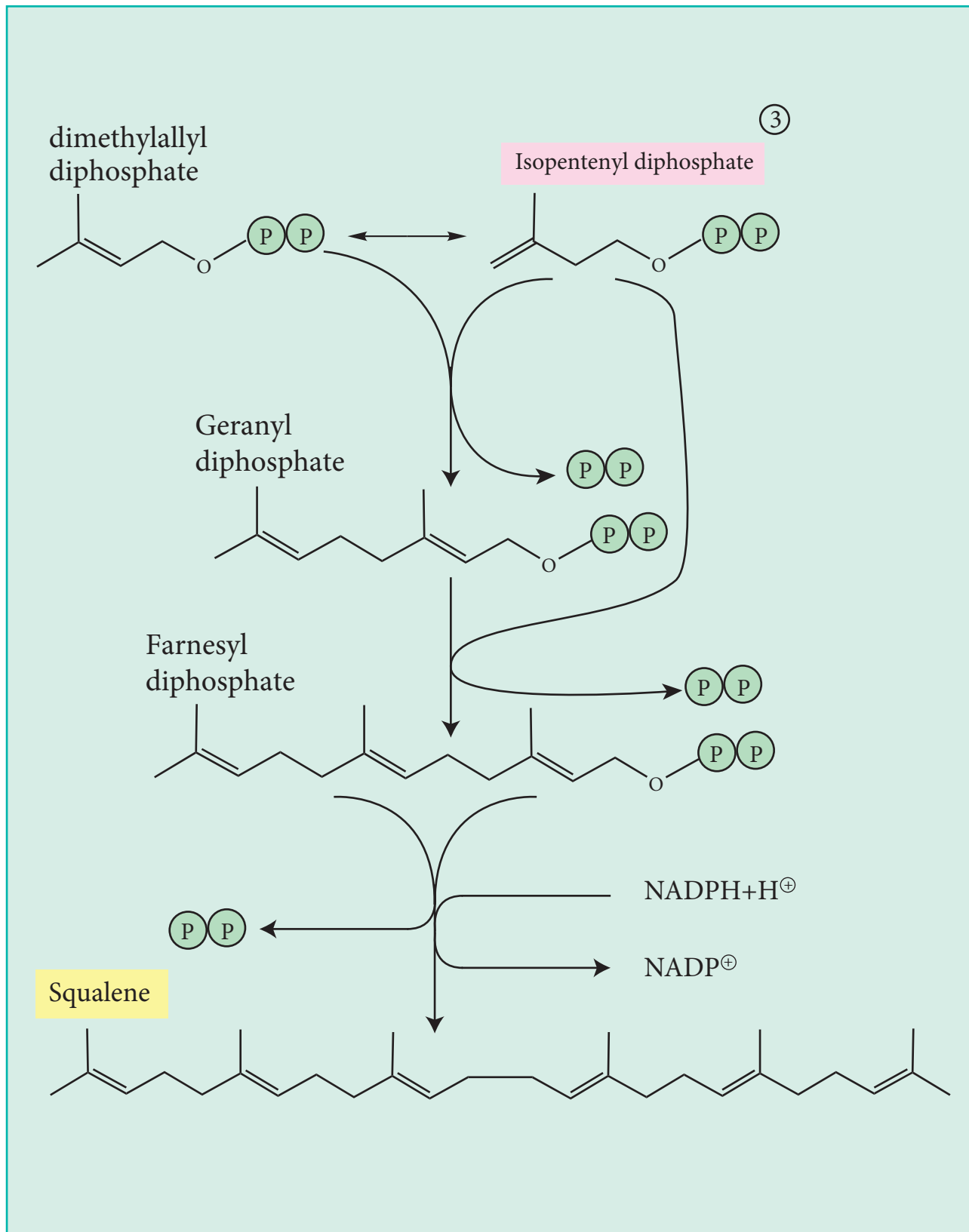


Mevalonate thus formed is then converted to squalene through various steps. Squalene, with the formation of various intermediates finally gives rise to the end-product cholesterol.

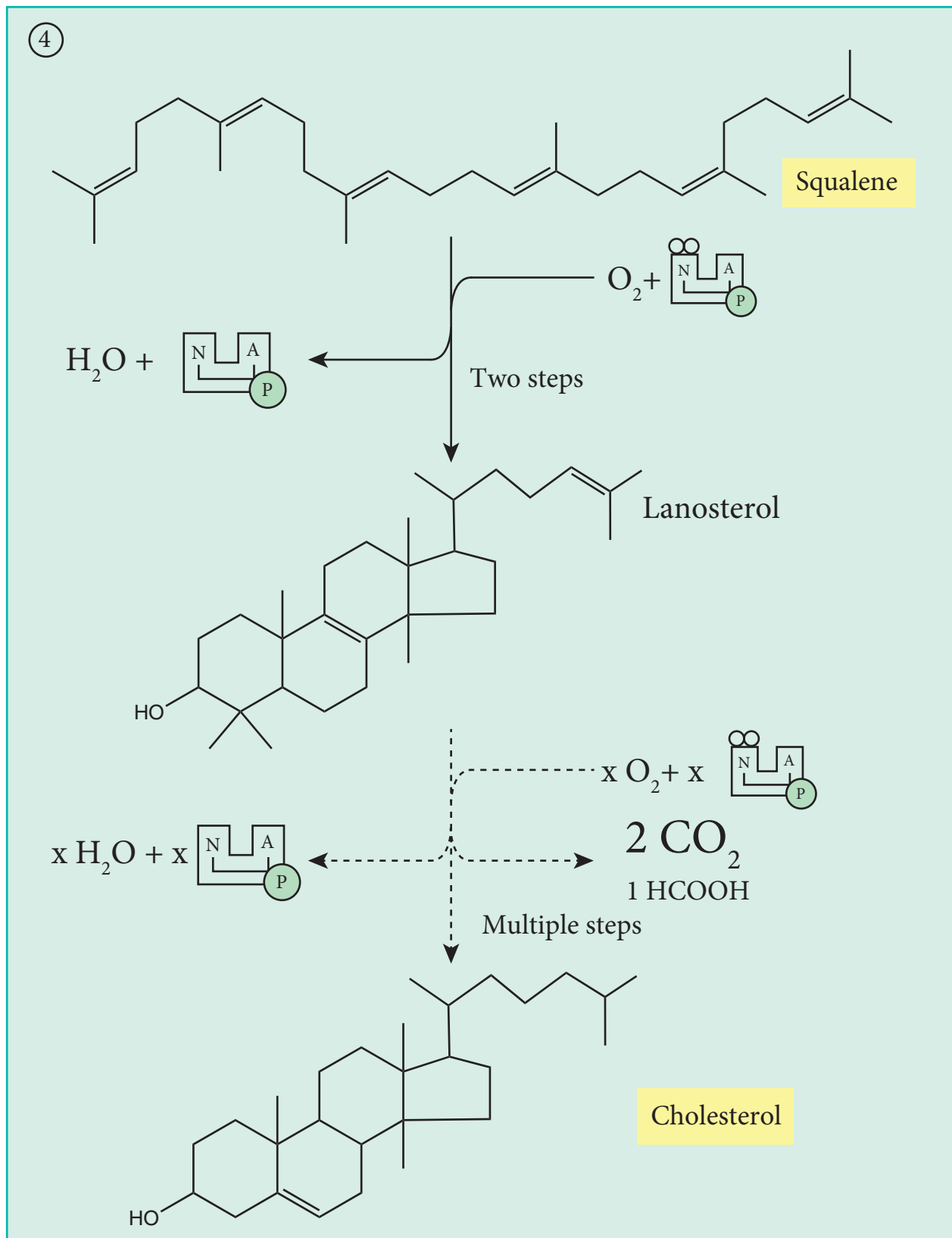


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### 5.4.2 Important derivatives of cholesterol

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.

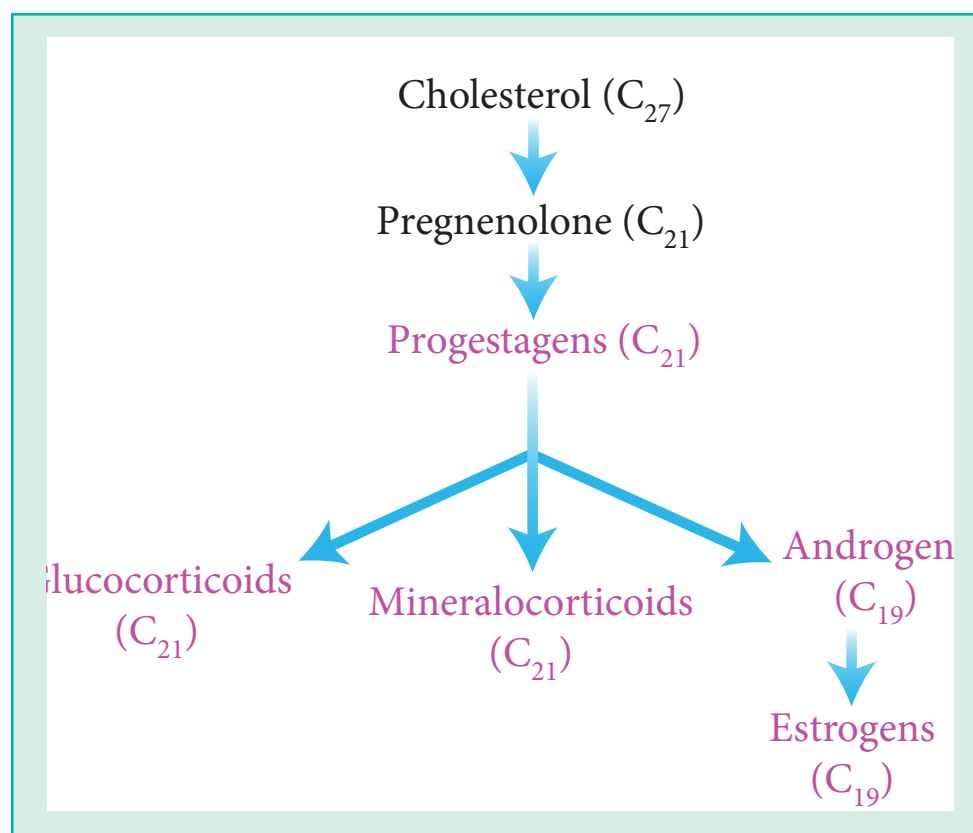
An efficient recycling system allows the bile salts to re-enter the bloodstream and return to the liver for reuse and only very small amount of bile salts escape this recycle system and this is the body's only route for cholesterol excretion.

#### 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.





## 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

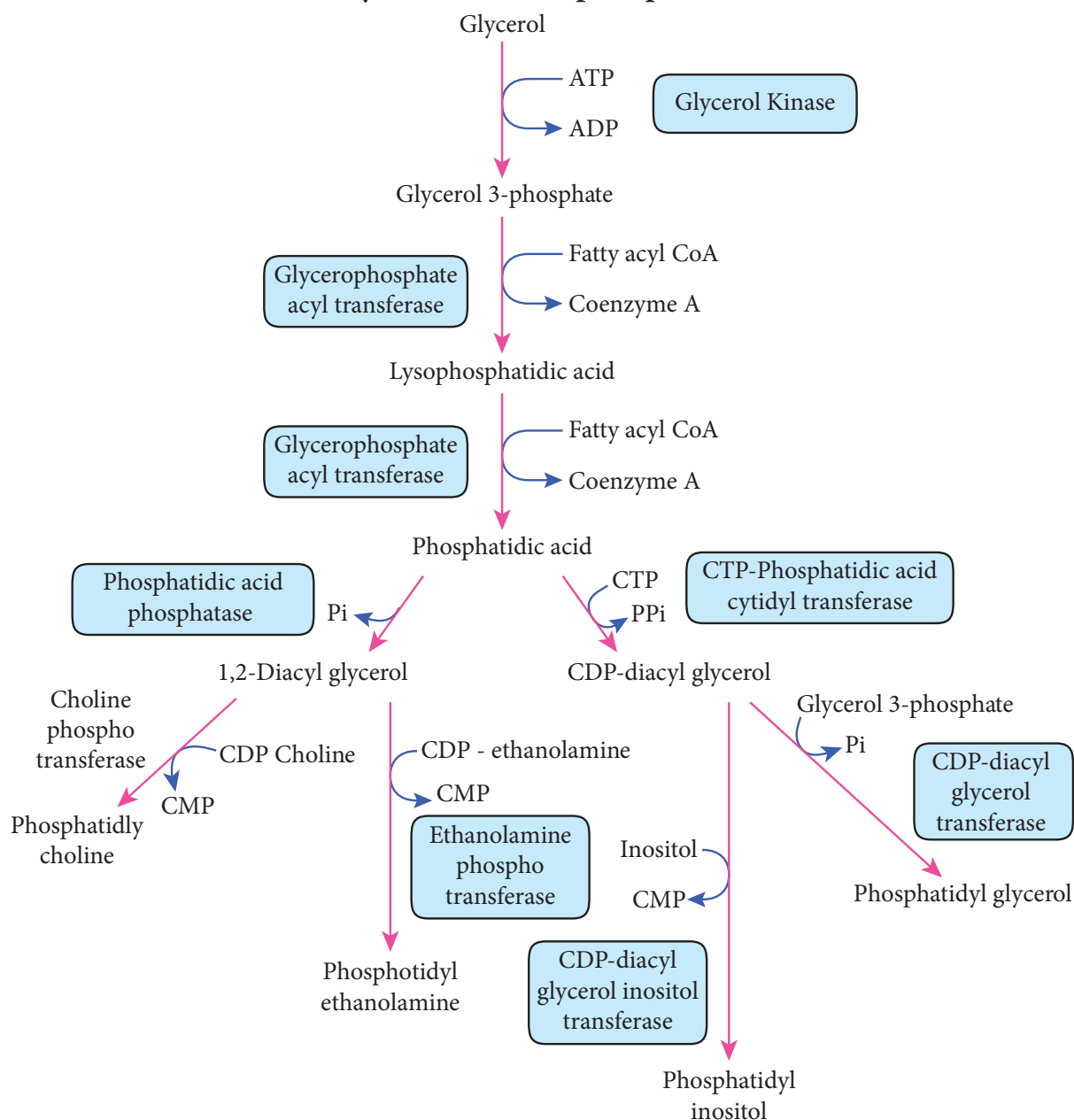
There are five general classes of phospholipids based on the type of molecule that is attached to the phosphate group. They are,

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.

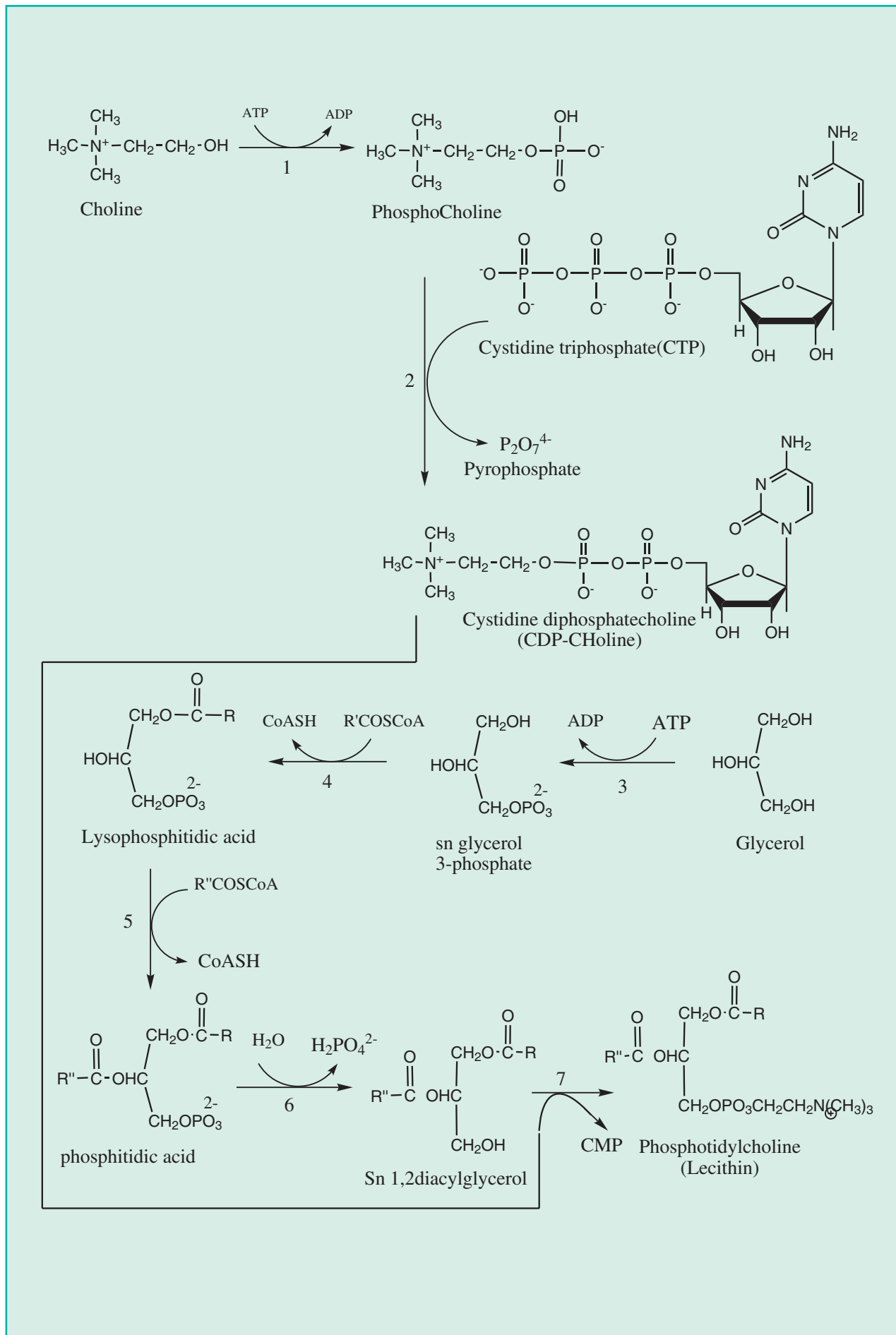
### Biosynthesis of Phospholipids



#### 5.5.3 Biosynthesis of lecithin

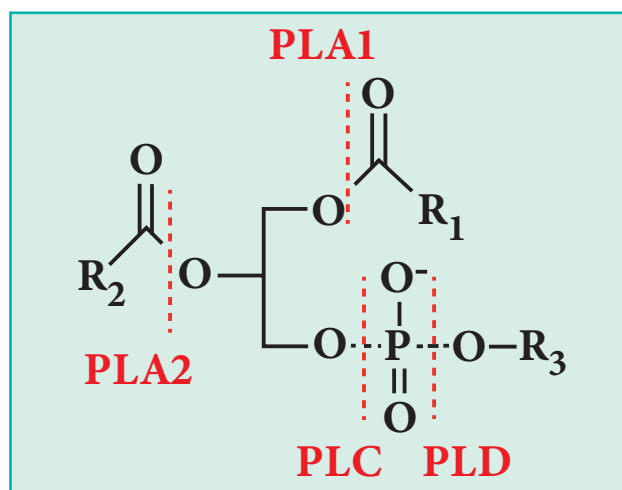
Lecithin (phosphatidylcholine) is a precursor for choline. It is involved in the synthesis of the neurotransmitter acetylcholine. Lecithin is also involved in complex intracellular processes, including the regulation of cellular membrane permeability.

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



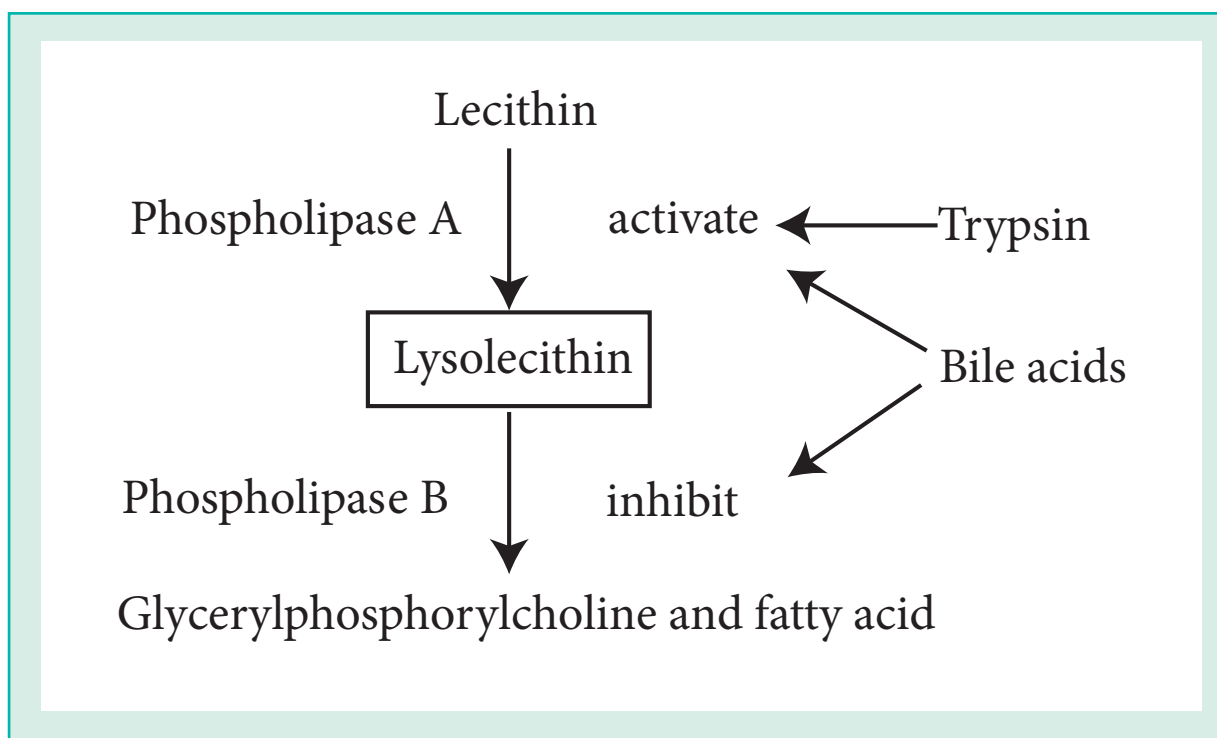
#### 5.5.4 Degradation of Phospholipids

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 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 B, but this reaction is inhibited by bile acids.



#### 5.5.6 Effects of lysolecithin

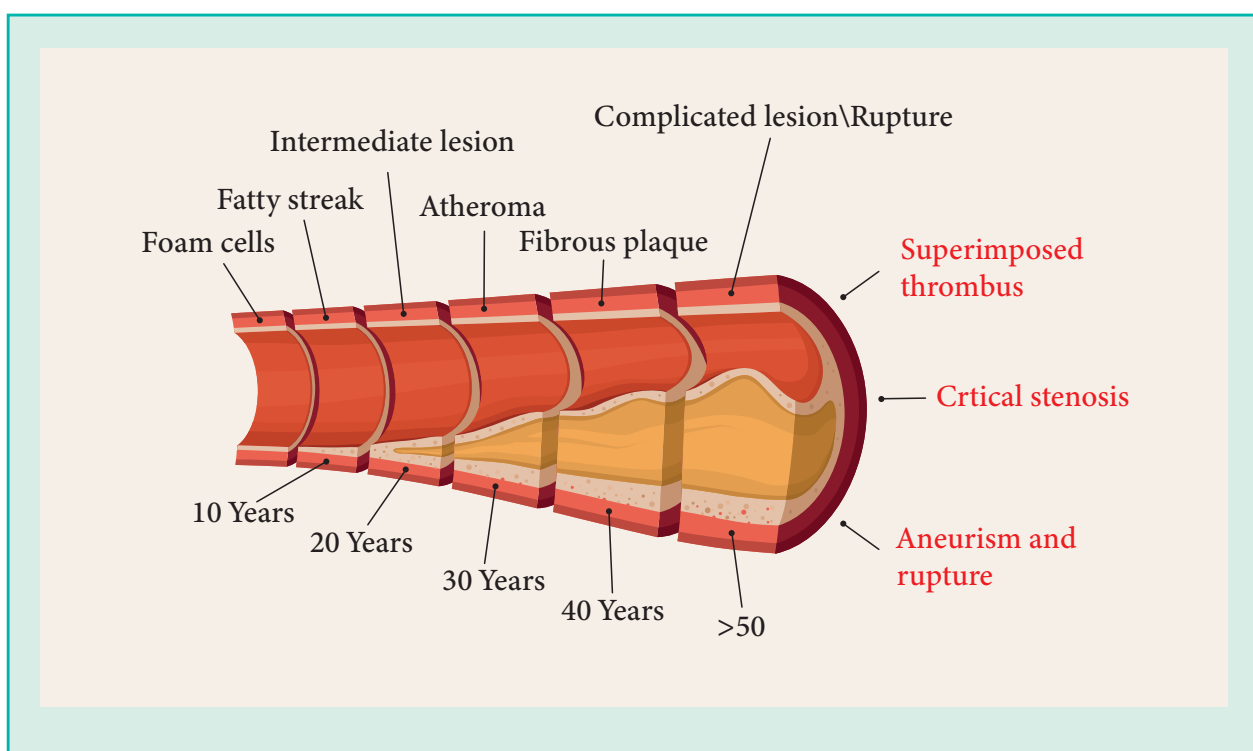
Lysolecithin is highly toxic to cell membranes and it is a strong detergent similar to bile salts. It can demyelinate nerves and destroy red blood cells. Due to its emulsification properties, it helps make the consistency of products smooth and easy to spread because of this LPC is widely used in food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

## 5.6 CEPHALIN

Cephalin ( $C_{40}H_{80}NO_8P$ ) 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 flow 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)

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  $\beta$ -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

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.

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.

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 ( $C_{40}H_{80}NO_8P$ ) 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

- \_\_\_\_\_ is not an essential fatty acid.
  - Linoleic acid
  - Linolenic acid
  - Arachidonic acid
  - Oleic acid
- How many ATP's are produced in betaoxidation of one stearic acid molecule
  - 2
  - 98
  - 0
  - none of these
- Which one is a saturated acid?
  - Oleic acid
  - Cerebronic acid
  - Nervonic acid
  - Stearic acid
- Biosynthesis of fatty acids from acetyl CoA is called
  - glucoreogenesis
  - lipogenesis
  - betaoxidation
  - both (b) and (c)
- \_\_\_\_\_ vitamin is involved for acetyl CoA carboxylation reaction
  - TPP
  - FAD
  - Biotin
  - Vitamin C
- \_\_\_\_\_ is a derivative of cholesterol
  - Vitamin A
  - Vitamin C
  - Vitamin E
  - Vitamin D
- Which of the following is an intermediate in the process of lipogenesis?
  - isopentyl pynophosphate
  - Molonyl ACP
  - Oxaloacetate
  - Acetoacetate



8. Which of the following is a ketone body
- Carnitine
  - Acetoacetate
  - Oxaloacetate
  - Acetyl CoA
9. Lysolecithin is formed by the action of \_\_\_\_\_ on lecithin
- Lecithinase A
  - Lecithinase A2
  - Lecithinase C
  - Lecithinase D
10. How many terms of the  $\beta$ - oxidation pathway are needed to process a  $C_{18}$  fatty acid molecule?
- Seven
  - eighteen
  - nine
  - none of these
11. What is the first functional group change that occurs in the  $\beta$  - oxidation pathway?
- alkane to alkene
  - $2^\circ$  alcohol to ketone
  - Alkene to  $2^\circ$  alcohol
  - Alkane to  $2^\circ$  alcohol

## II. Fill up the blanks

- Deficiency of essential fatty acids causes \_\_\_\_\_
- The active intermediate form of fatty acids to undergo  $\beta$  oxidation are \_\_\_\_\_
- \_\_\_\_\_ is the amino alcohol present in sphingolipids.
- Number of double bonds present in Arachidonic acid \_\_\_\_\_
- Atherosclerotic individual have increased levels of \_\_\_\_\_ and \_\_\_\_\_ in plasma.

## III. Say true or false

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

## IV. Match the following

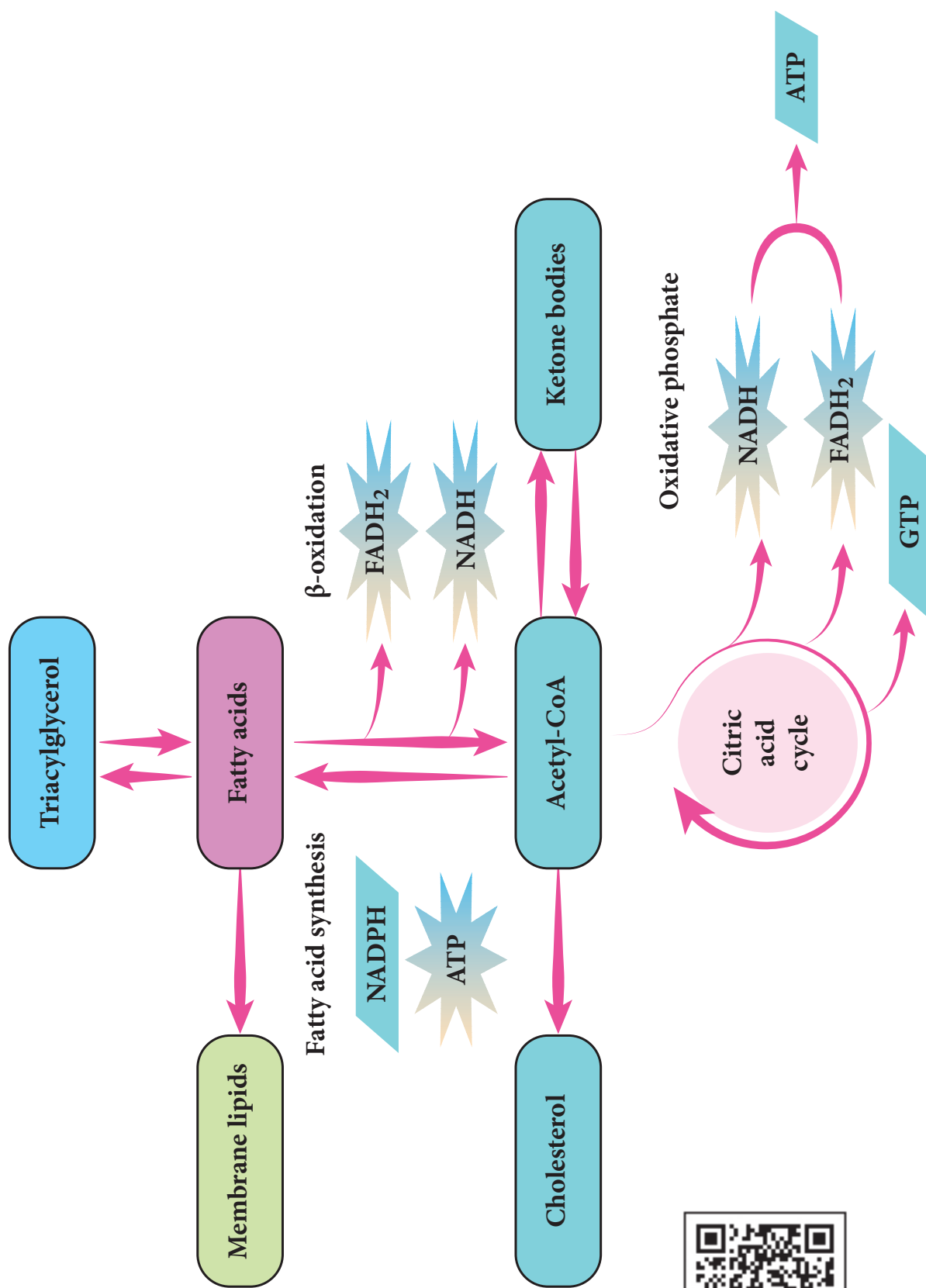
- |                           |   |                           |
|---------------------------|---|---------------------------|
| 1. Lecithin               | - | Cholesterol biosynthesis  |
| 2. Cephalin               | - | Bile salt                 |
| 3. Acetyl CoA carboxylase | - | Phosphatidyl choline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



#### V. Give short answer for the following

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

## CONCEPT MAP



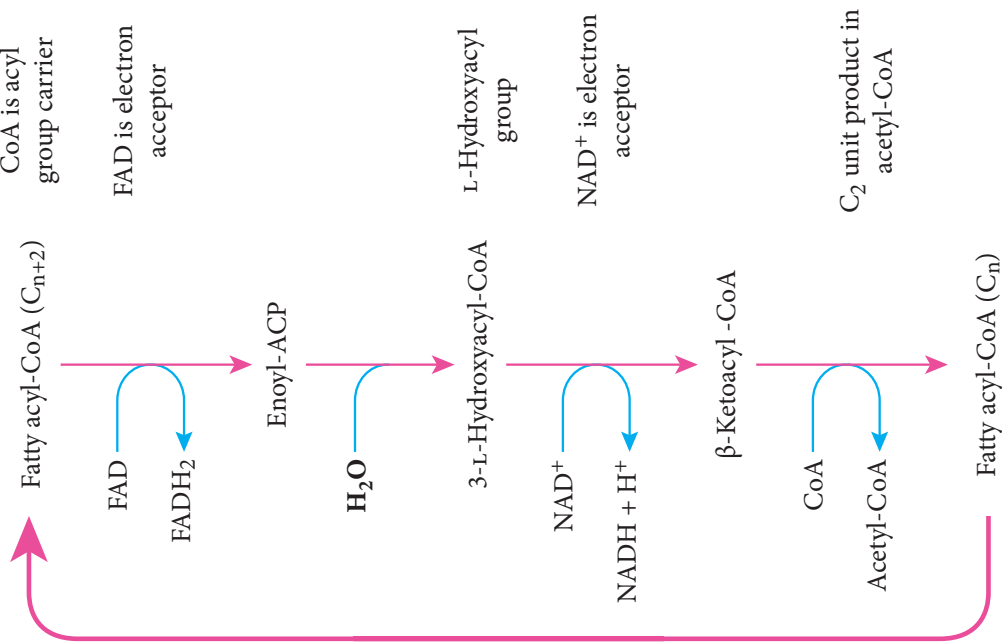
## $\beta$ -Oxidation

Occurs in mitochondria

CoA is acyl group carrier

FAD is electron acceptor

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## Biosynthesis

Occurs in cytoplasm

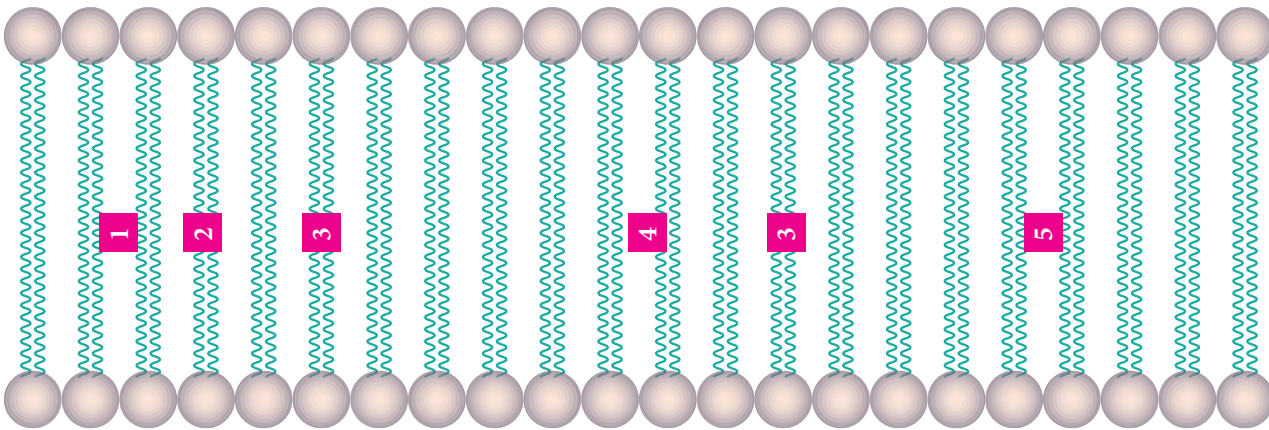
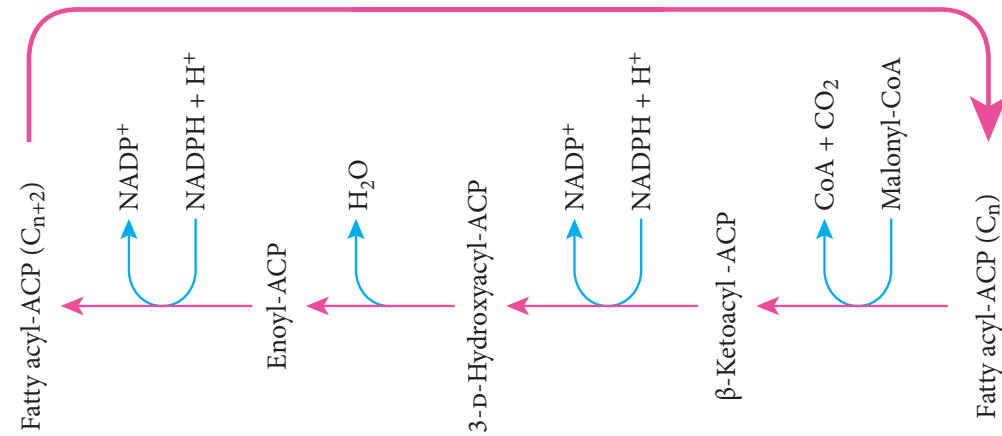
ACP is acyl group carrier

NADPH is electron donor

D- $\beta$ -Hydroxyacyl group

NADPH is electron donor

$C_2$  unit donor is malonyl-CoA



## UNIT 6

# MOLECULAR BIOLOGY



**Kary 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. Kary Mullis is an American biochemist who was responsible for this invention and was dully awarded Nobel prize in chemistry in 1993.



### Learning Objectives

After studying this unit the students will be able to

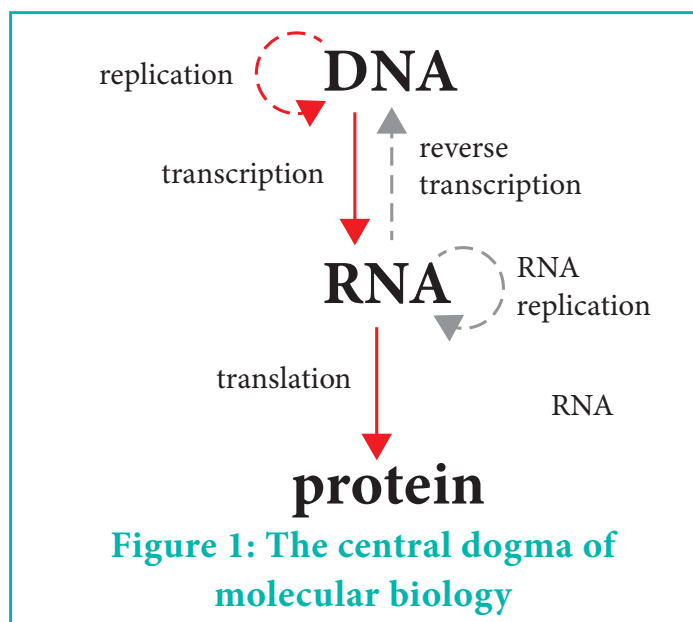
- Explain the central dogma of molecule Biology
- Explain the replication process
- Describe the mechanism of RNA synthesis.
- Explain the structure of activated tRNA.
- Describe the mechanism of protein synthesis.

## 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.

### 6.1.1 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.



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 mengo viruses.



## 6.2 DNA replication

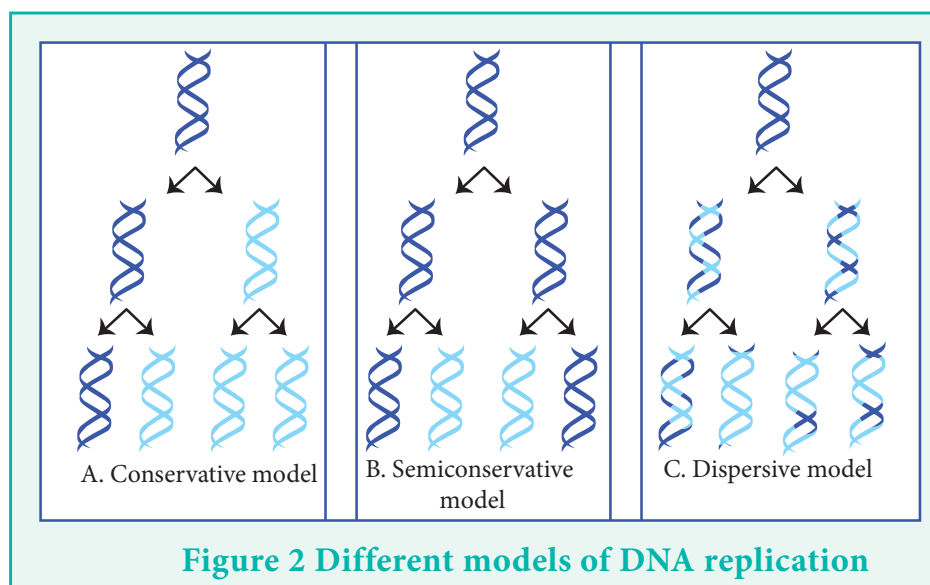
Genetic information has to flow from the parental cells or organisms to progeny by a very faithful mechanism. This copy of DNA from DNA known as replication is the first and fundamental step in the central dogma. Any DNA replication mechanisms should consider and overcome the following complexities

1. As we have studied already the strands of DNA run in opposite directions ( $5' \rightarrow 3'$ ) 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.



### 6.2.2 The conservative model

In this model the two parental strands base pair once again after forming a new copy. The newly synthesised 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



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

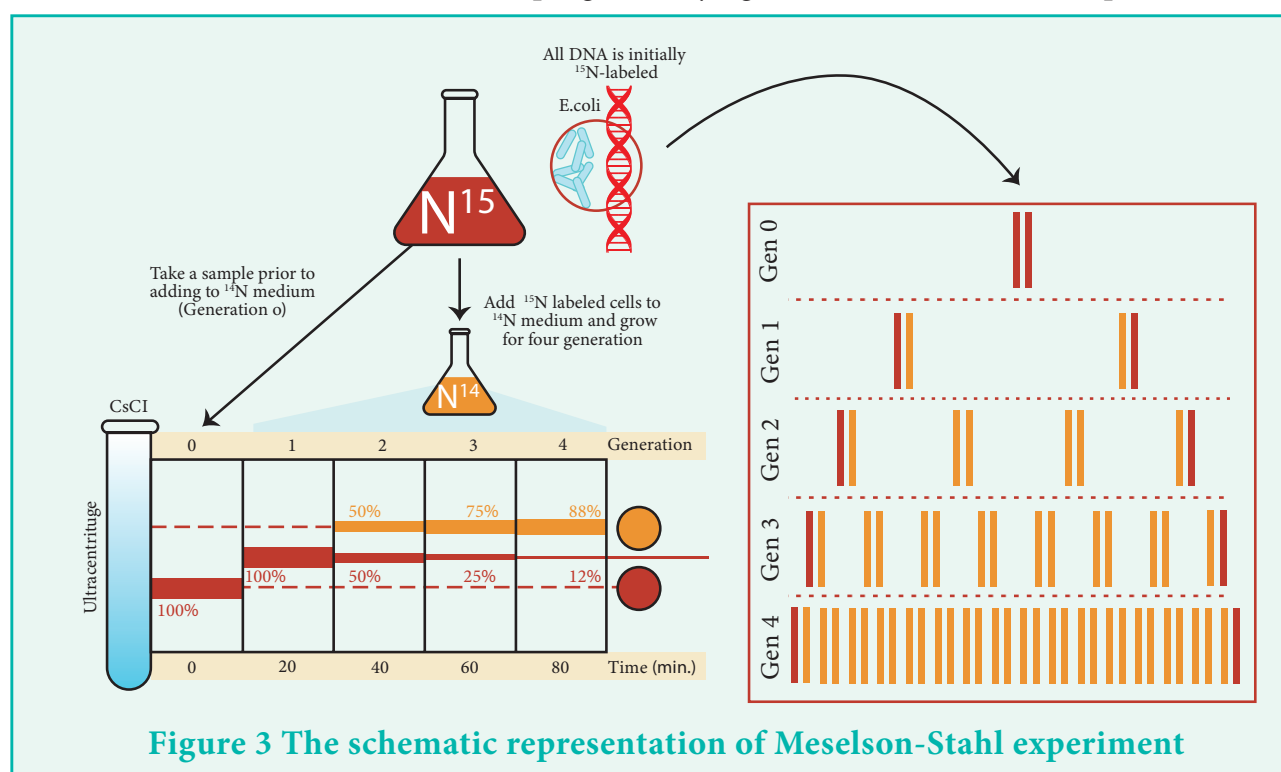
#### 6.2.4 The dispersive model

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  $^{14}\text{N}$  isotope of nitrogen is normally present in DNA, the presence of the heavier  $^{15}\text{N}$  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  $^{15}\text{N}$  as the only source of nitrogen for several generations. The bacteria took up this  $^{15}\text{N}$  for growth and eventually all the nitrogen in the DNA were  $^{15}\text{N}$  isotope. The *E. coli* were then switched suddenly to the medium containing the lighter  $^{14}\text{N}$  as the only source of nitrogen, so as the bacterium divides these lighter nitrogen gets incorporated into the DNA. After switching to the light  $^{14}\text{N}$  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 ( $\text{CsCl}$ ) 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.





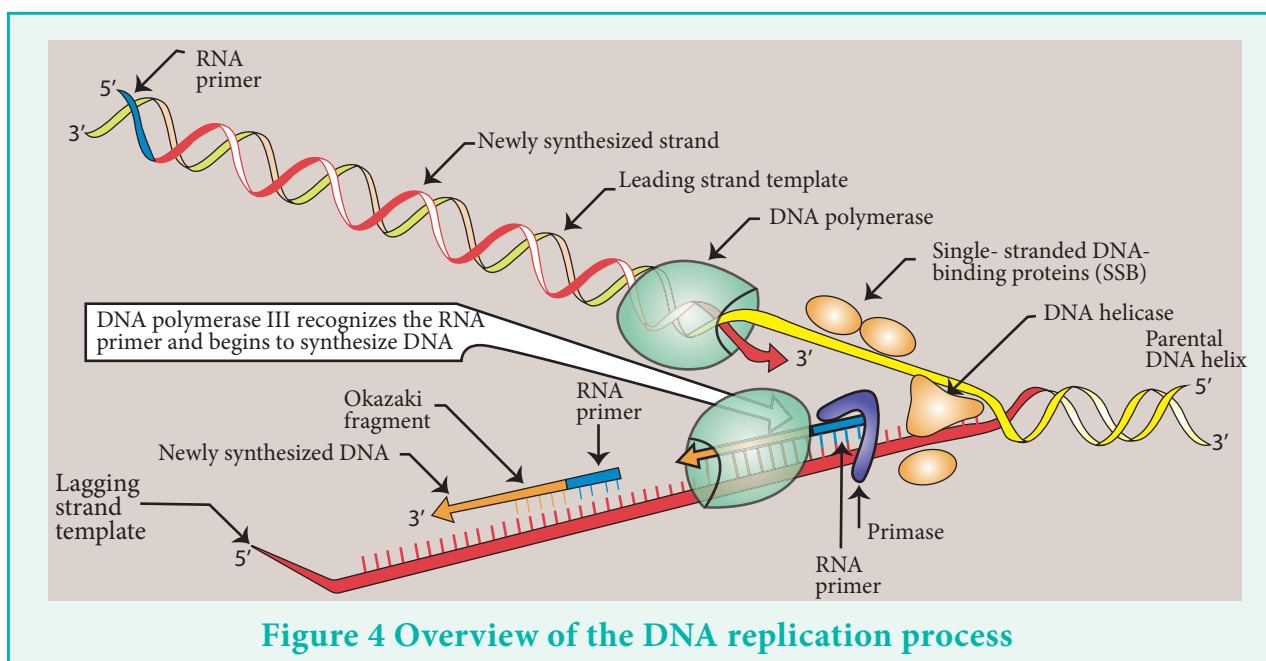
The experiment and the results are shown in fig 3. It could be seen that at generation zero, a single band at the bottom of the tube was produced. This is due to the fact that all the nitrogen in the DNA were the heavy  $^{15}\text{N}$  isotope. At generation one, a single band was produced but in the intermediary position between where  $^{15}\text{N}$  and  $^{14}\text{N}$  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  $^{14}\text{N}$  region. Subsequent generations of DNA showed these two bands, however the intermediary band was shown to become thinner and the  $^{14}\text{N}$  band becoming broader. This is due to the fact that the strands containing  $^{14}\text{N}$  isotope increases as the DNA replicates in  $^{14}\text{N}$  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 separated.
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.

### 6.2.7 The DNA polymerase.

The DNA polymerase or the DNA dependent DNA synthetase is a large multi subunit enzyme which adds deoxribonucleotides 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.,



### 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.



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.

### 6.2.8 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

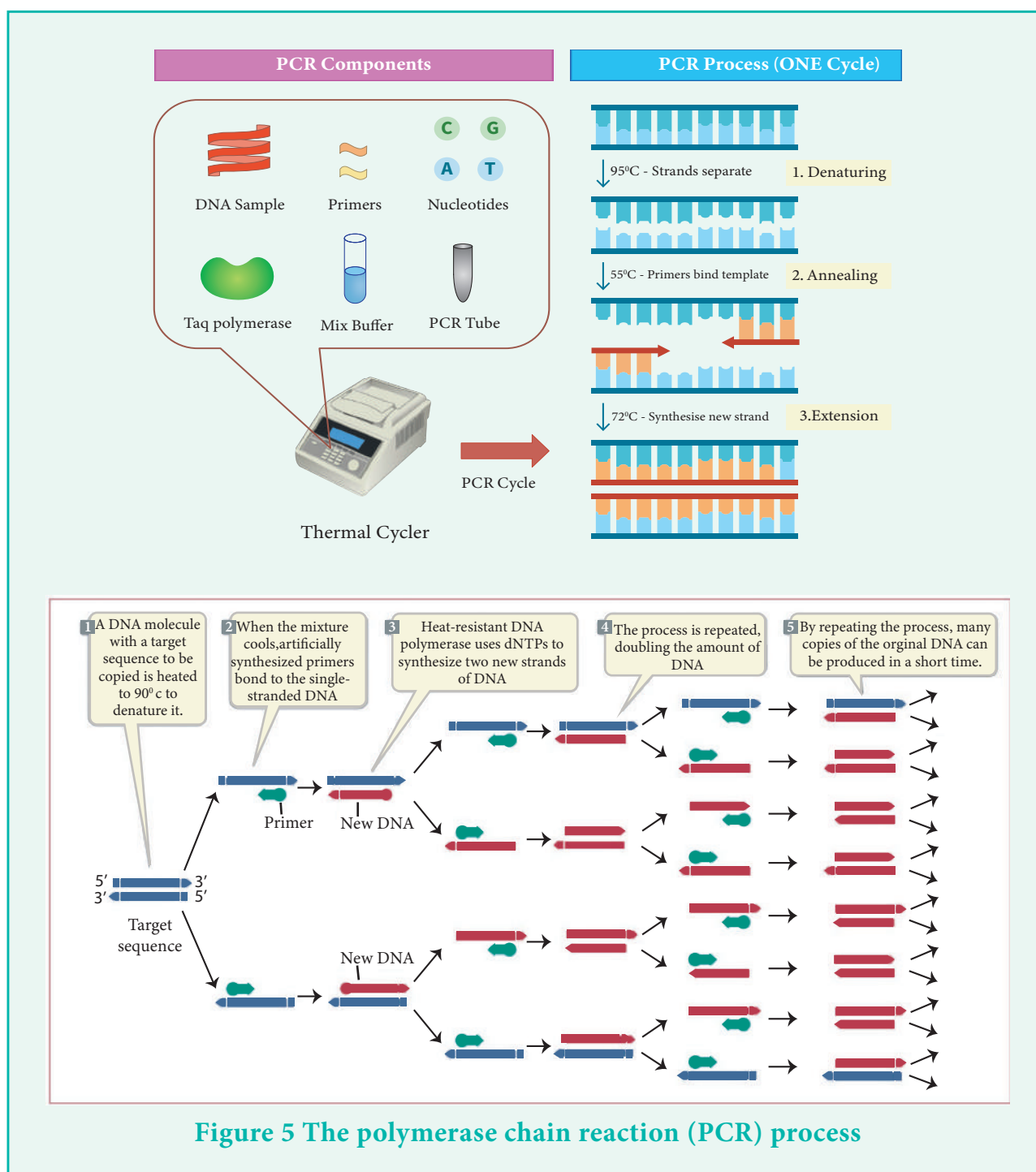
1. A gene whose function a researcher wants to understand
2. A genetic marker used by forensic scientists to match crime scene DNA with suspects.
3. A genetic marker region in DNA which could be used for diagnosis of a disease like cancer

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.,

#### 6.2.8.1 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.



## 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

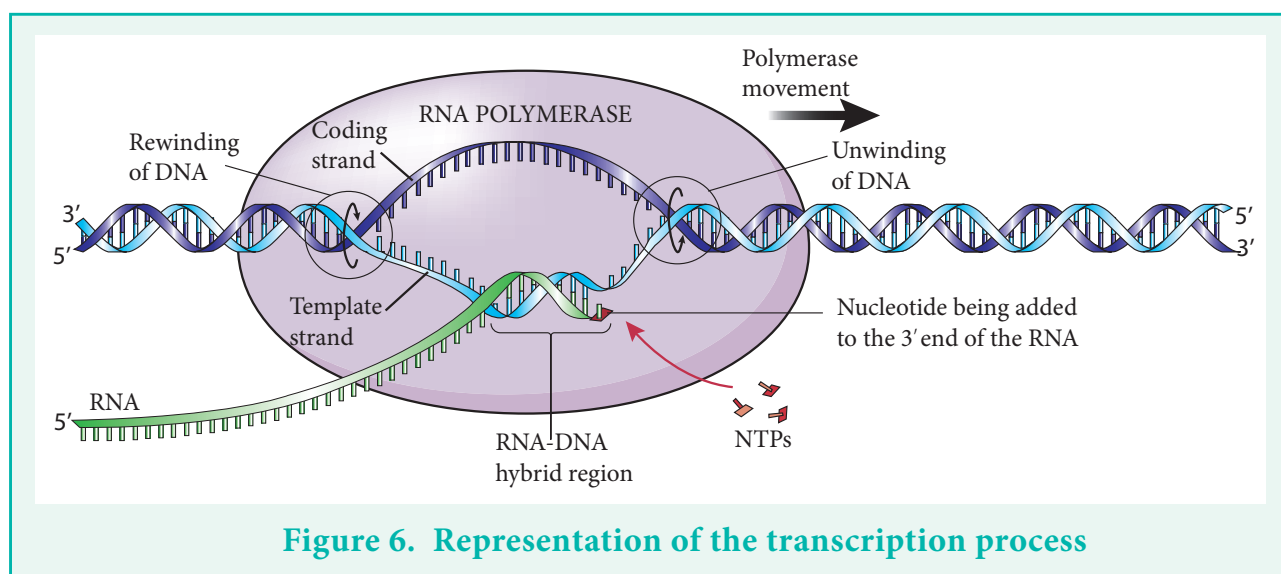
Genes are described as units of heredity in classical genetics. However in molecular biology terms, genes can be considered as a region in DNA which acts as an information

template for a protein or RNA molecule (in case of tRNA or rRNA genes). Each gene contains a distinct sequence of nucleotides which determines the amino acid sequence or nucleotide sequence a cell might synthesize. If a gene is a segment of DNA that 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 sequences.
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.

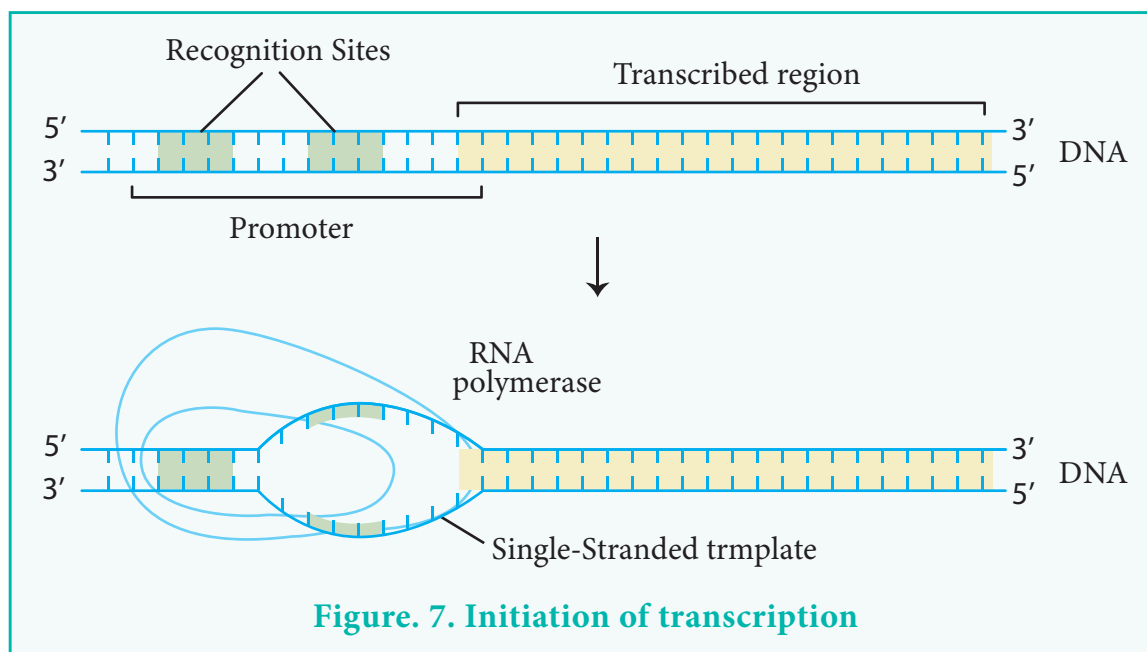
**There are three stages in transcription which are initiation, elongation and termination.**



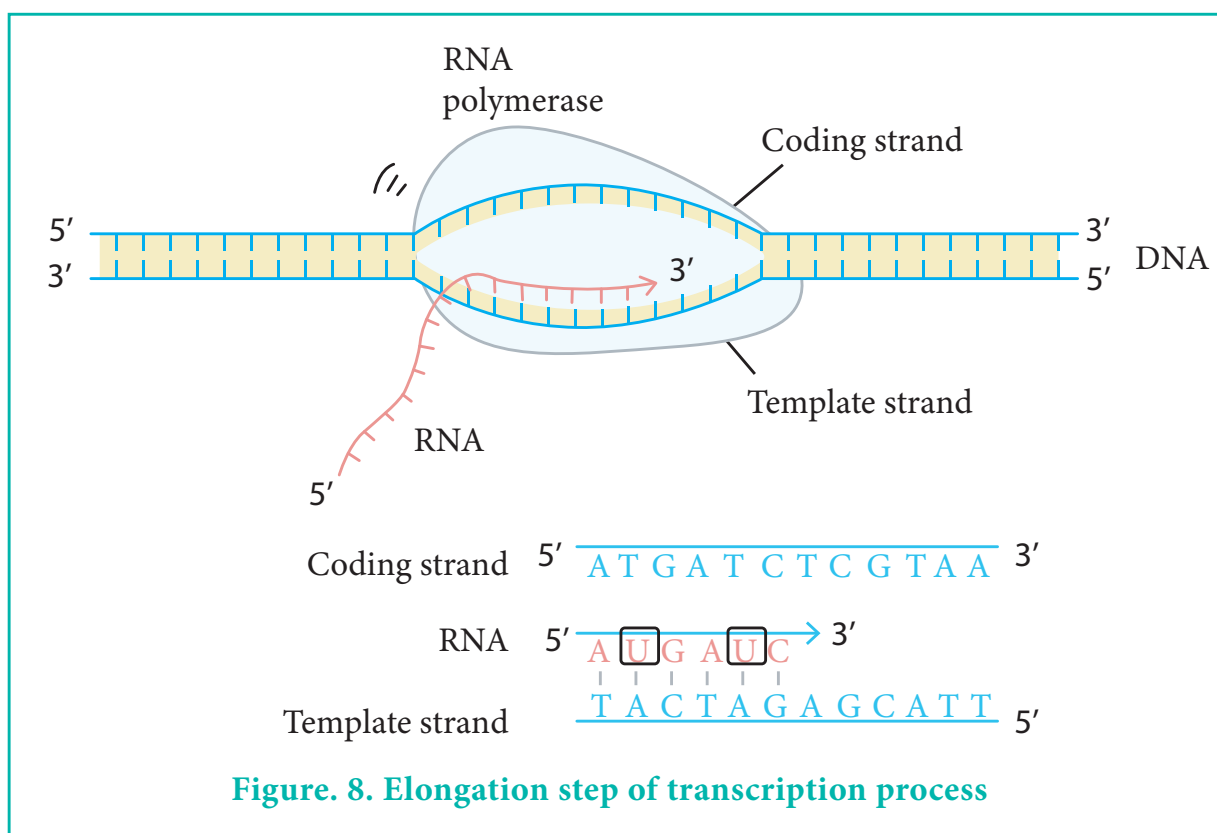
### 6.3.3 Initiation of transcription

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.

- Each gene (or group of co-transcribed genes, in bacteria) has its own promoter.
- Once bound, RNA polymerase separates the DNA strands, providing the single-stranded template needed for transcription.
- 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.



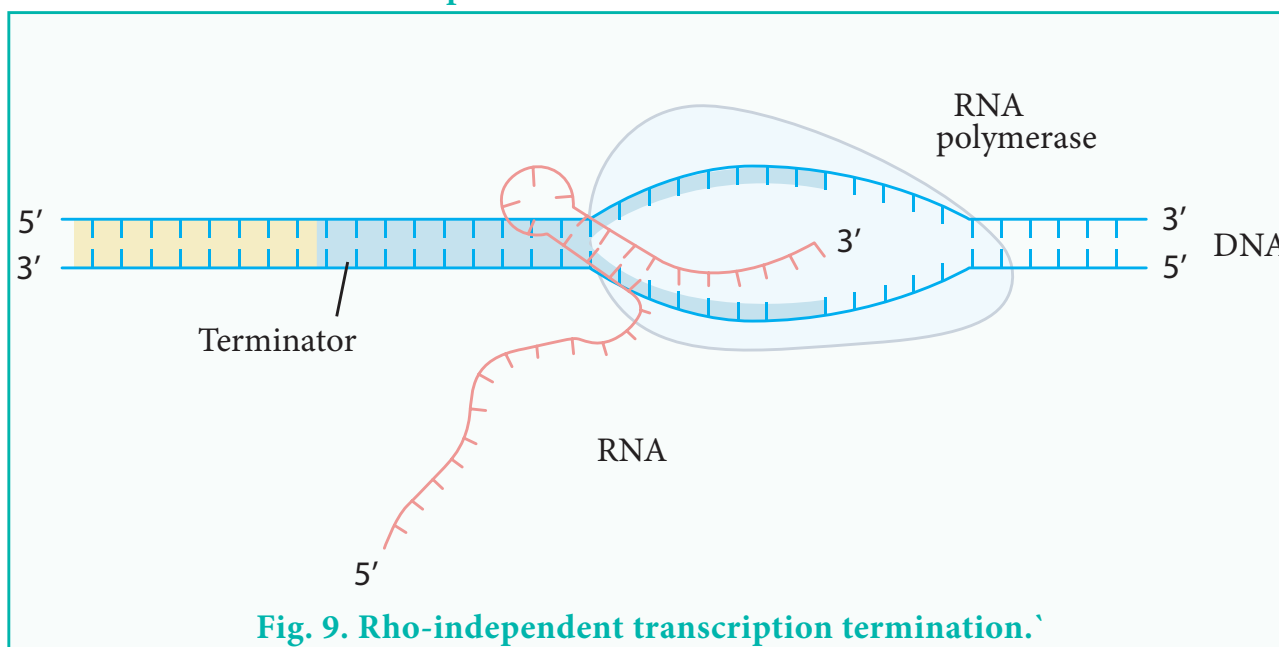
#### 6.3.4 Elongation of transcription





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).

### 6.3.5 Termination of transcription



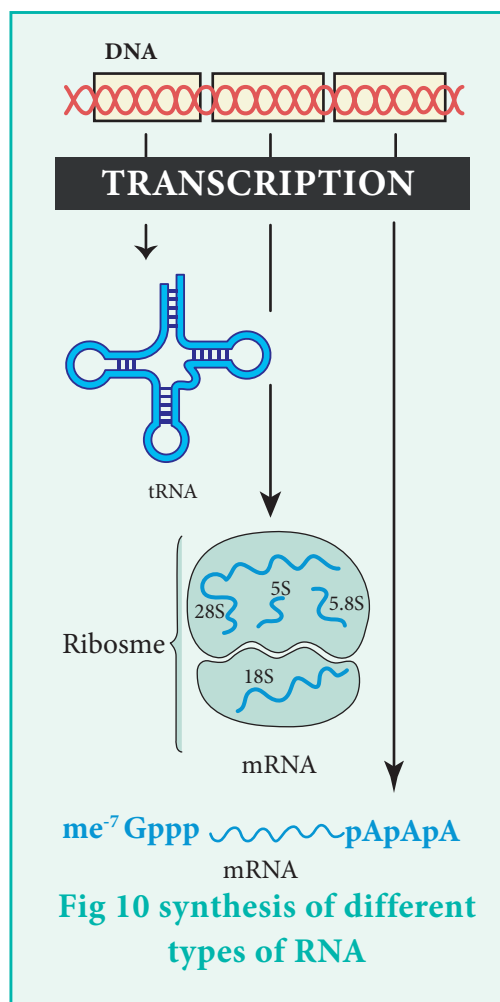
**Fig. 9. Rho-independent transcription termination.**

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.

### 6.3.6 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 or tRNA and the messenger RNA or mRNA





### 6.3.6.1 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.

### 6.3.6.2 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

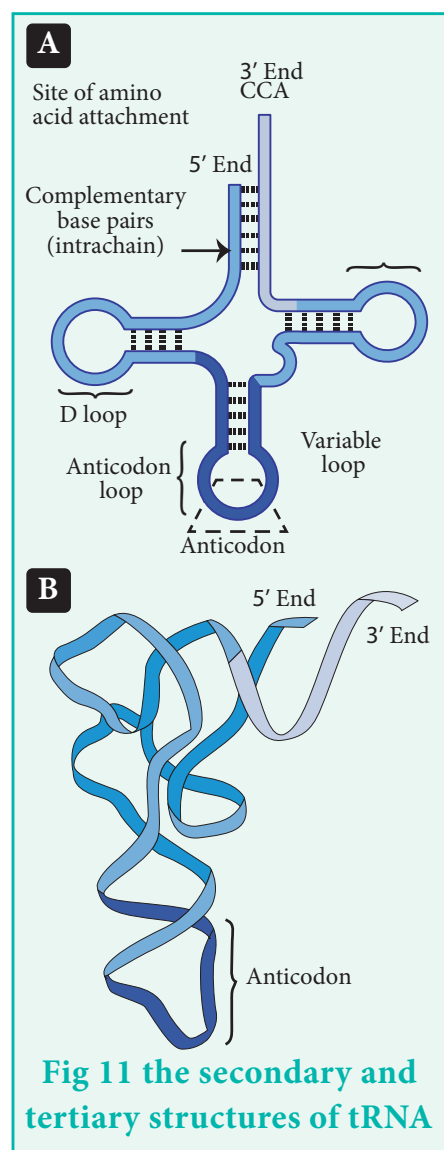
tRNA like dihydrouracil. They also form extensive intra chain base pairing forming different loops in the RNA structure like the T $\psi$ C loop, anticodon loop and D loop.

### 6.3.6.3 The messenger RNA

This carries the genetic information from the DNA to the cytoplasm 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.

### 6.3.7 Post transcriptional modification of RNA

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



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.

### 6.3.8 Difference between Prokaryotes and Eukaryotes 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 modeification(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.

### 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 amino acid. 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 amino acid methionine and it also acts as a start codon. The genetic code is universal i.e. barring a few rare instances it is followed by all organisms. Similarly the genetic code is degenerate, meaning that one codon can only represent one amino acid but one amino acid 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				
		U	C	A	G	
First Letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA STOP UAG STOP	UGU } Cys UGC } UGA STOP UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAU } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

Fig 12 The universal genetic code

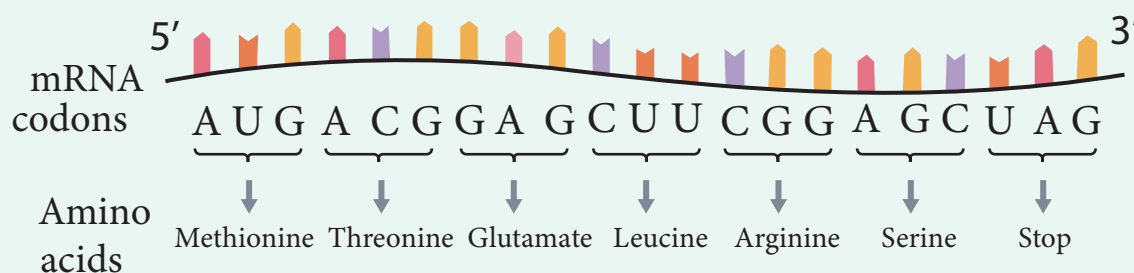


Fig 13 Representational decoding of codons in a sample mRNA to corresponding amino acid sequence.

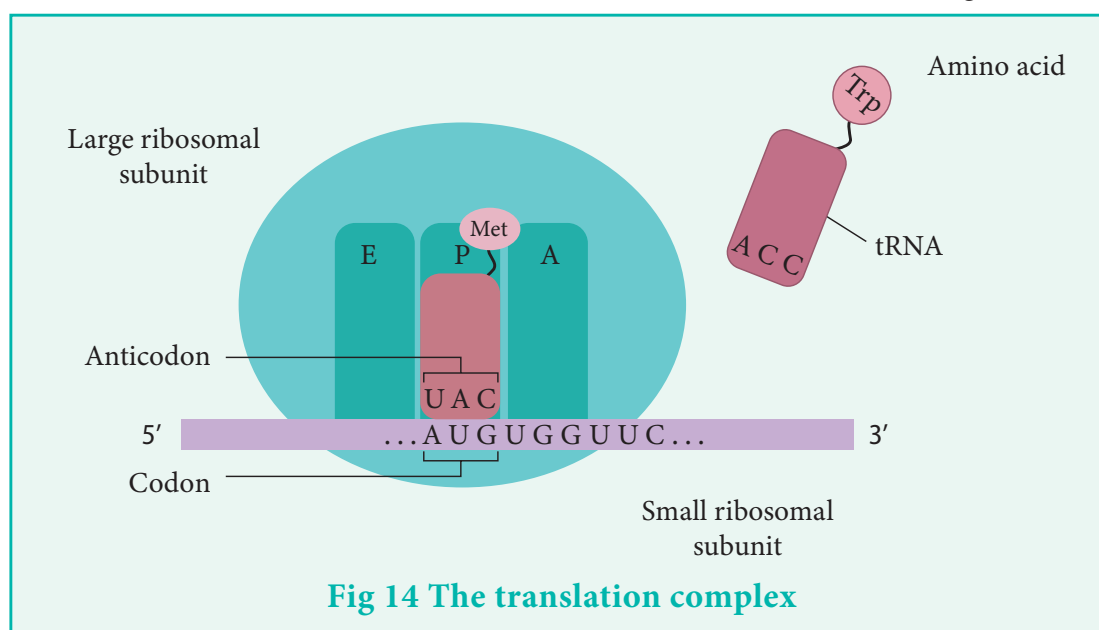
### 6.4.2 Overview of translation

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

the tRNAs play 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



The ribosome provides three different structural packets where tRNA can enter and transfer its amino acid 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

Initiation requires 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 methionine 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

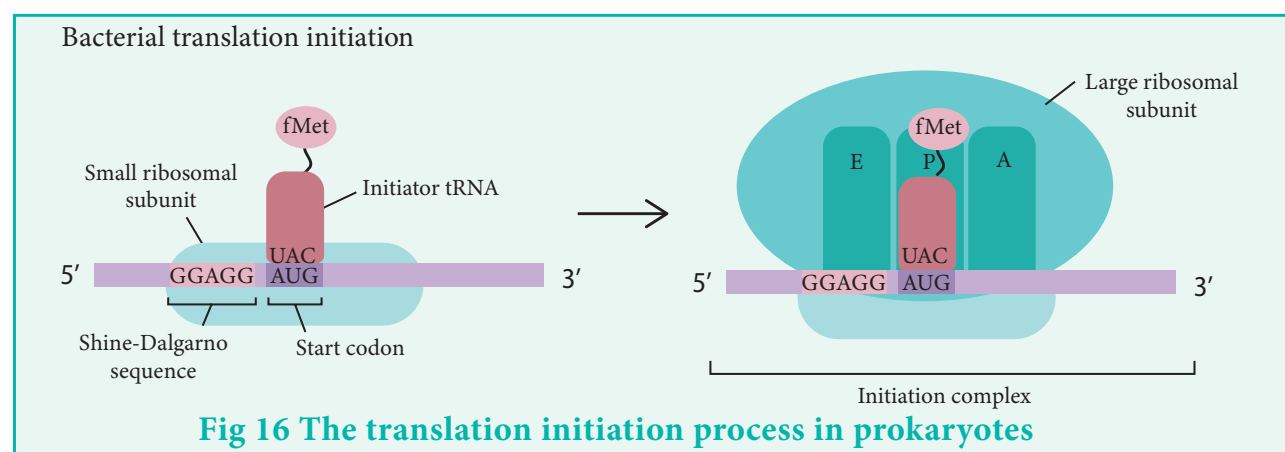
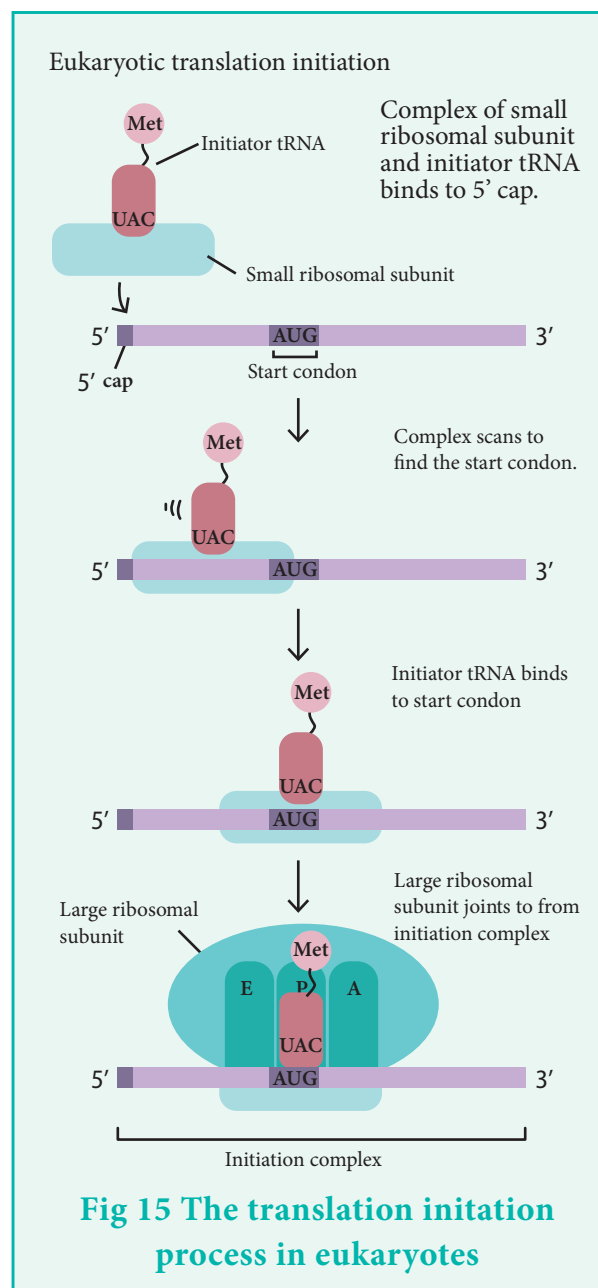
joined by the large subunit of the ribosome and this complex is known as the translation initiation complex.

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.

#### 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.



- 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.
- 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 starts growing in the ribosome.

#### 6.4.4.3 Termination of translation.

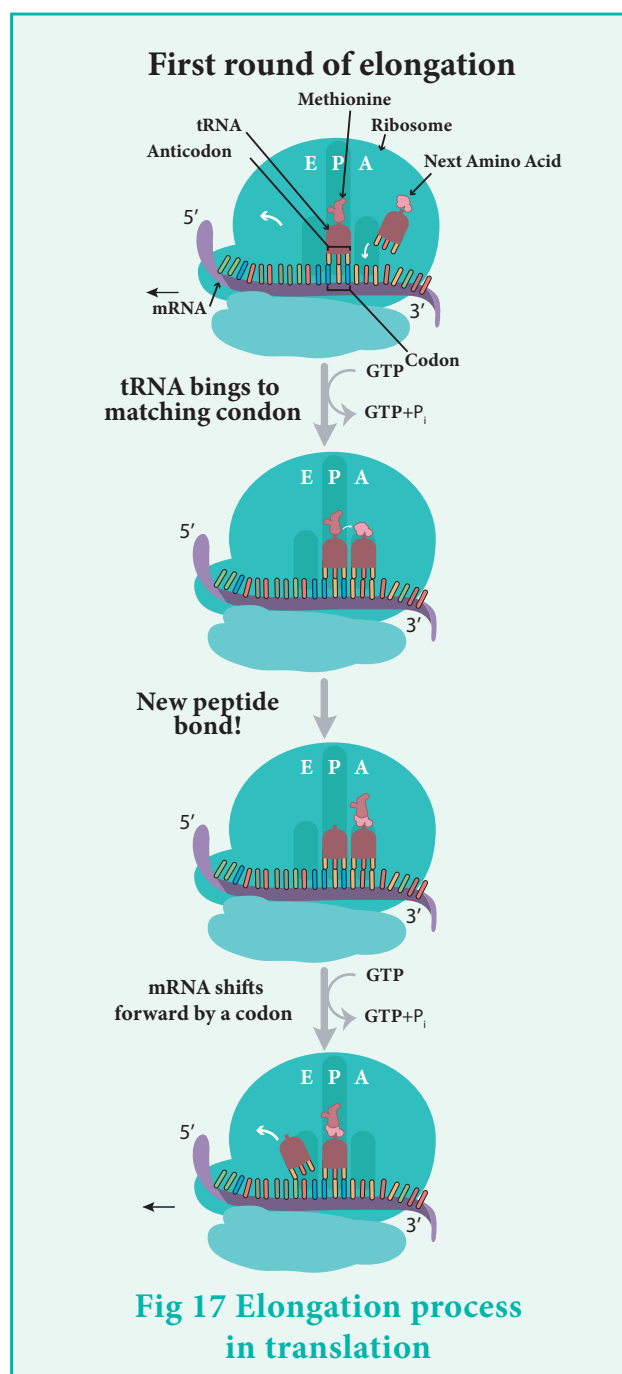
The growing polypeptide is stopped when the A site of the ribosome encounters one of the stop codons in the mRNA which are UAA, UAG, or UGA.

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.

#### 6.4.5 Post-Translational Modifications

- After translation, amino acids may be chemically altered or removed.
- The chemical modifications include phosphorylation, glycosylation, glycation, nitrosylation, methylation, acetylation, lipidation etc
- The new polypeptide will also fold into a distinct 3D structure and may join with other polypeptides to make a multi-subunit protein.



#### 6.4.6 Difference between prokaryotic and eukaryotic translation

S. No	Prokaryotes	Eukaryotes
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 cytoplasm for translation.
3	70S ribosome is involved, with a 50S larger subunit and a 30S smaller subunit	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 monocistronic

### 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.





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

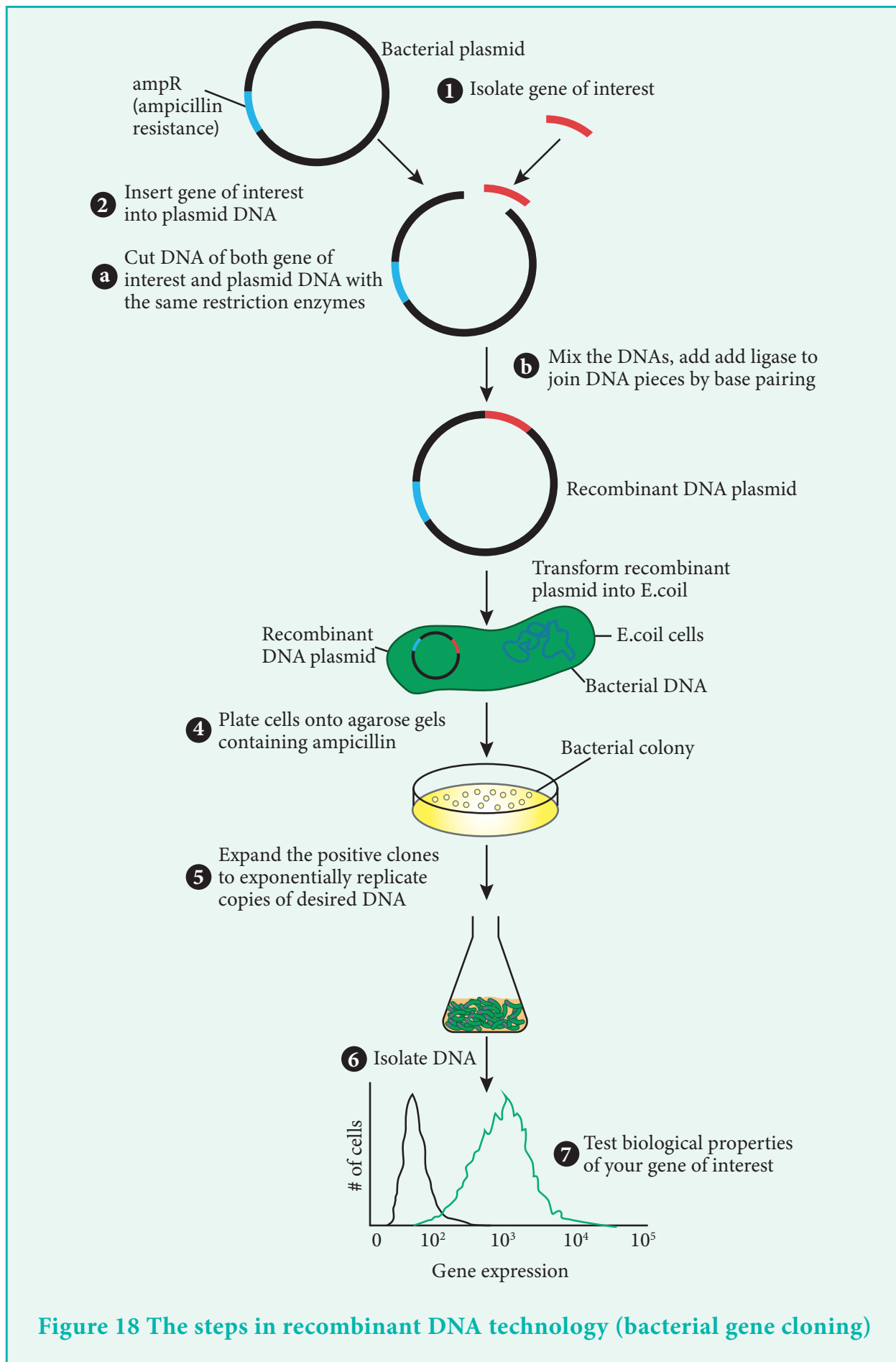
The birth of the 21<sup>st</sup> century has seen many revolutions in the field of molecular biology. Today molecular biologists are more interested in studying the collective behaviour of the genome rather than studying one individual gene. The entire information present in the DNA of an organism can 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 <sup>32</sup>P) or fluorescently labelled so the chins can be visualized in a gel electrophoresis procedure.

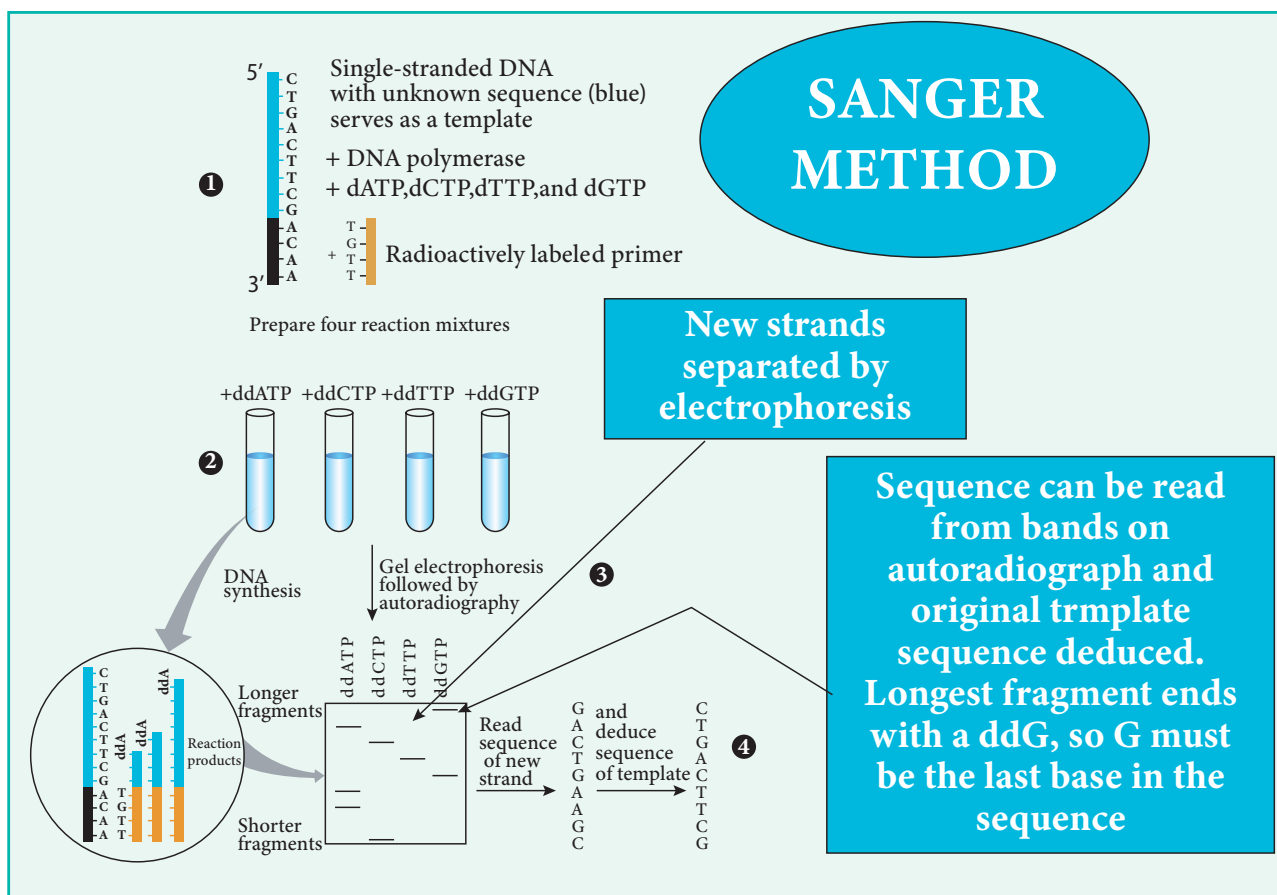
The details of Sanger sequencing procedure is depicted in figure 19 This sequencing procedure was performed in a massively parallel fashion and the human genome was sequenced in the year 2000.





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





**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 permanently 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 collaborative 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



and act as a potential resource for combating cancer.

### 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)

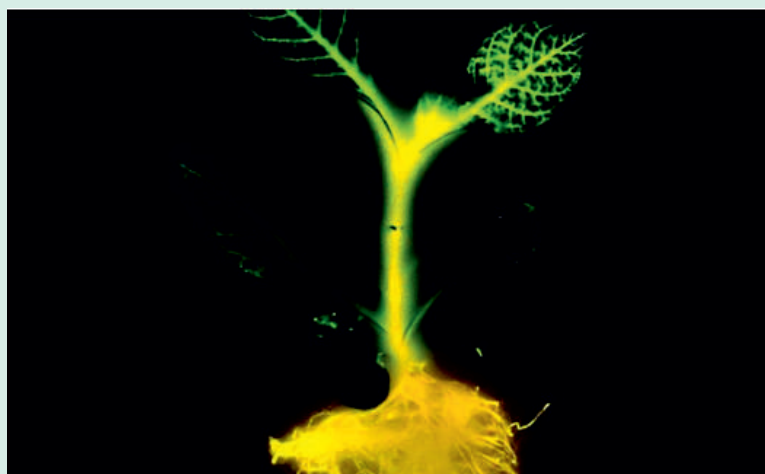
This method is also known as Chromatin Immuno Precipitation (ChIP), which precipitates the protein (histone) DNA complexes alone, by means of specific antibodies and then that region of DNA alone is sequenced. This method helps us to identify the genome regions which can interact with proteins like histones.

#### 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.



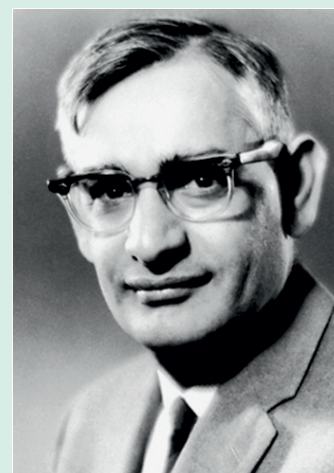
Scientist Steve Howell in 1986 created a tobacco plant that can glow in dark like a firefly. He isolated a gene called luciferase which are present in fireflies and are the reason for the fireflies to glow in dark. He introduced this gene into tobacco plants and



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 Bachelar'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.





## 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 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 synthesised, 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. 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, 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 ribosome complex.

Post-translational modifications like removal of amino acids, phosphorylation, glycosylation and hydroxylation, 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.



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 |



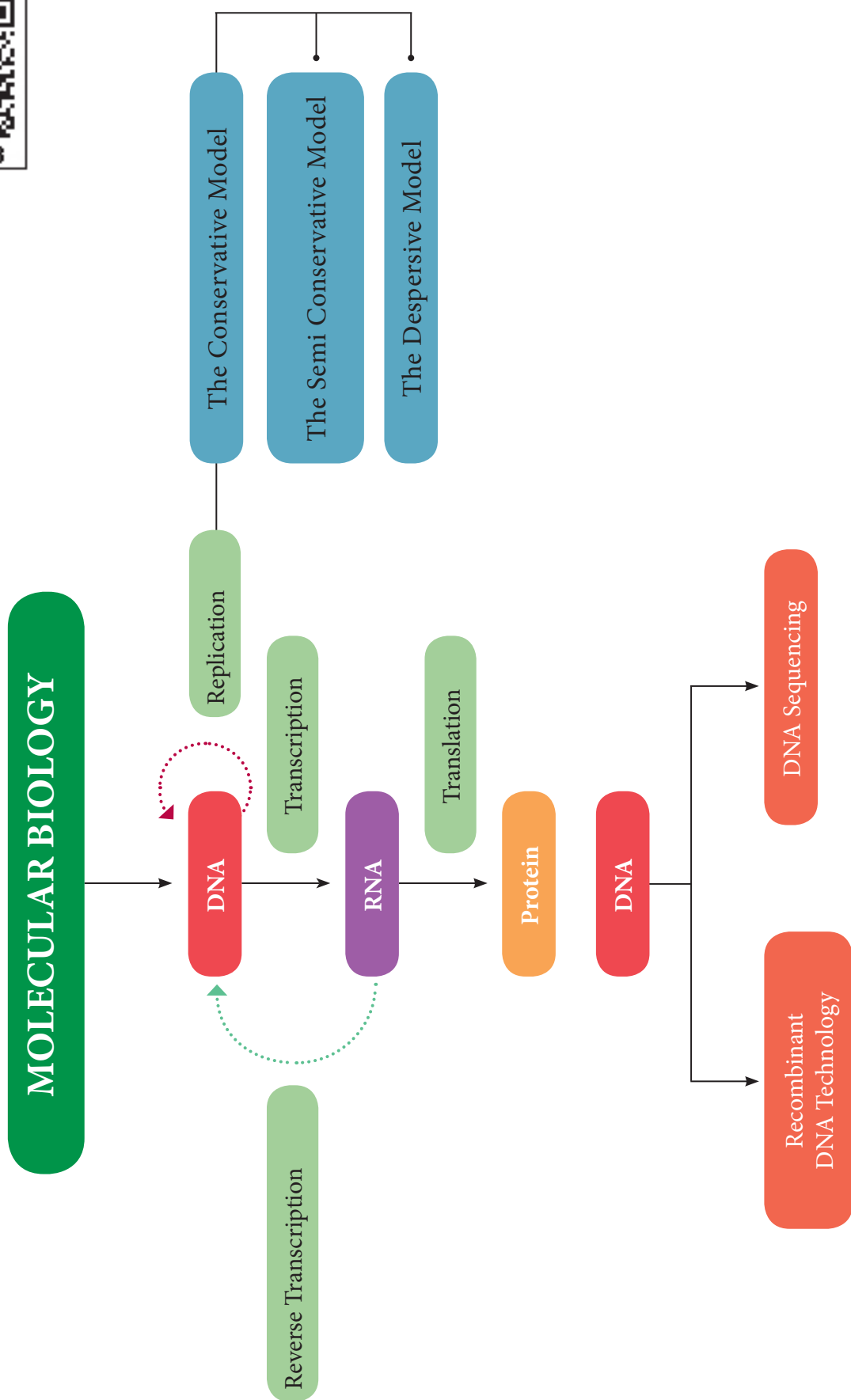


#### IV Answer the following questions:

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 Posttranscriptional 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.



# CONCEPT MAP



## 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 enzyme 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 mold *Neurospora*, confirmed Garrod’s hypothesis and proposed the ‘One gene one enzyme’ theory. This hypothesis was further modified in 1957 by Vernon Ingram, as ‘One gene one polypeptide’ since many proteins are made up of more than one polypeptide chain.



### 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.

## 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 birth defects in young infants which are inherited. IEM like galactosemia, phenylketonuria, alkaptonuria, albinism, Tay-Sachs disease and von Gierkes disease, can appear at birth 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 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 which may be essential biologically 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 amino acid 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 academia
Disorders of purine or pyrimidine metabolism	Lesch–Nyhan syndrome
Transport disorders	Cystinuria 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

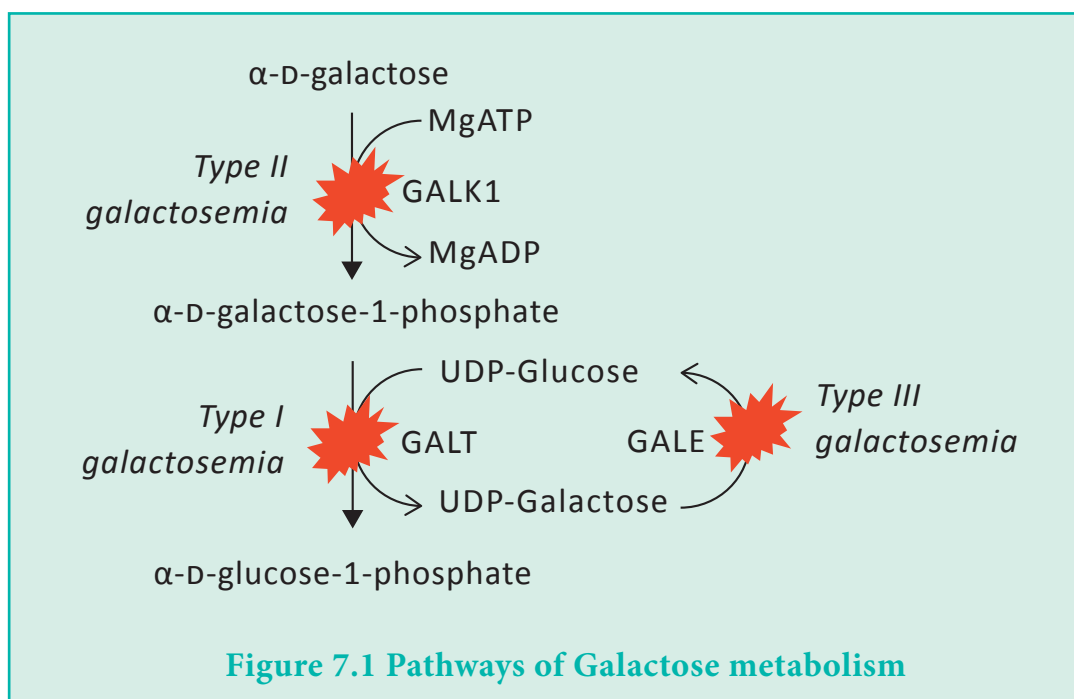


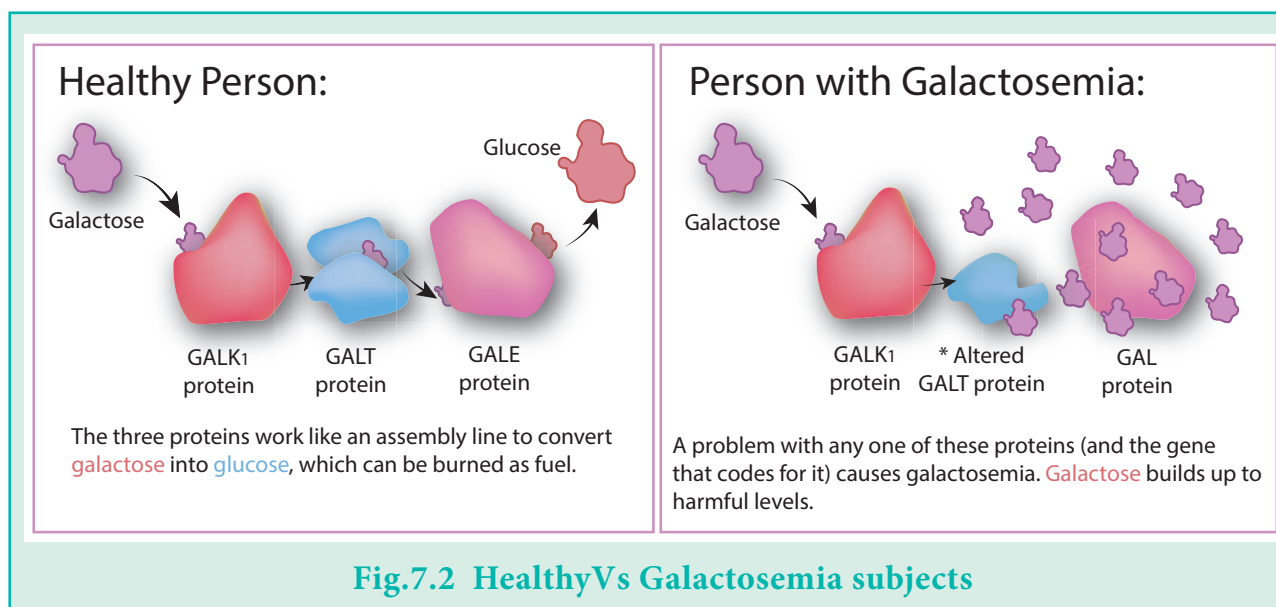
Figure 7.1 Pathways of Galactose metabolism

Galactosemia occurs when a specific enzyme known as galactose-1-phosphate uridylyltransferase (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 galactosemia, due to the absence of GALT, galactose-1-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 (Fig.7.2).



### 7.1.2 Symptoms

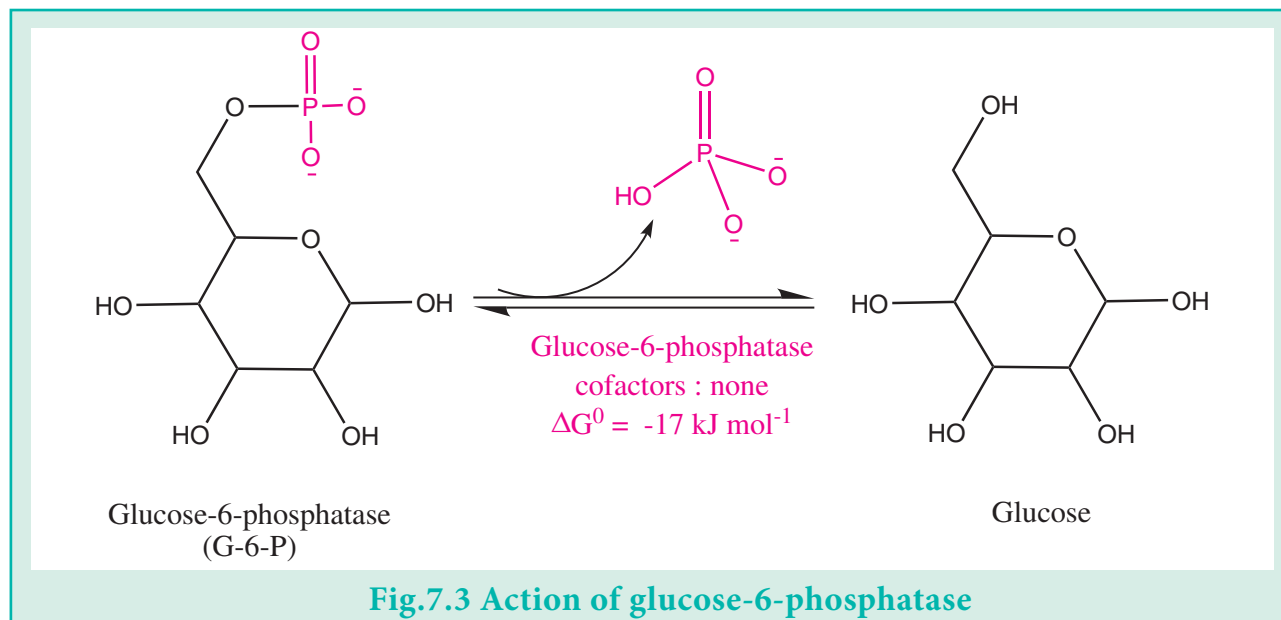
The deficiency of GALT is clinically important. Due to the enzyme defect, galactose accumulates in blood and is reduced by aldose reductase in the eye to the corresponding galactitol which causes cataract. The general condition is more severe, since, due to a deficiency in GALT, galactose 1-phosphate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

If an infant is breast feeding, but still experiences poor weight gain, it may be 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 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 by Carl 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.



Glucose -6-phosphatase catalyzes the final step leading to the release of glucose in blood stream by the liver(**Fig.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 glucose 6-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 Fig.7.4, include hypoglycemia with lactic acidosis during fasting, enlarged liver, distended abdomen, cherubic or doll like face, seizures secondary to low glucose.



### 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



**Fig.7.4 Von Gierk affected child**

## 7.3 HEMOPHILIA

Clotting factors are a set of proteins that control bleeding. When a blood vessel is affected, the walls lining 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 are chemicals that attract other cells and make



**Hemophilia**

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 form a coagulation cascade leading to the formation of fibrin clot. The fibrin clot acts as a mesh and stops bleeding.

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



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	Antihemophilic factor	Liver, lung capillaries
IX	Christmas factor Plasma thromboplastin component	Liver
X	Stuart-prower factor	Liver
XI	Plasma thromboplastin anticedant	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 haemophilia namely haemophilia A, B, and C are important.

- (i) *Haemophilia A*: Classic haemophilia 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) *Haemophilia 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) *Haemophilia C*: It is mild and is caused by a deficiency of factor XI.

### 7.3.2 Symptoms:

People with haemophilia 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 haemophilia 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



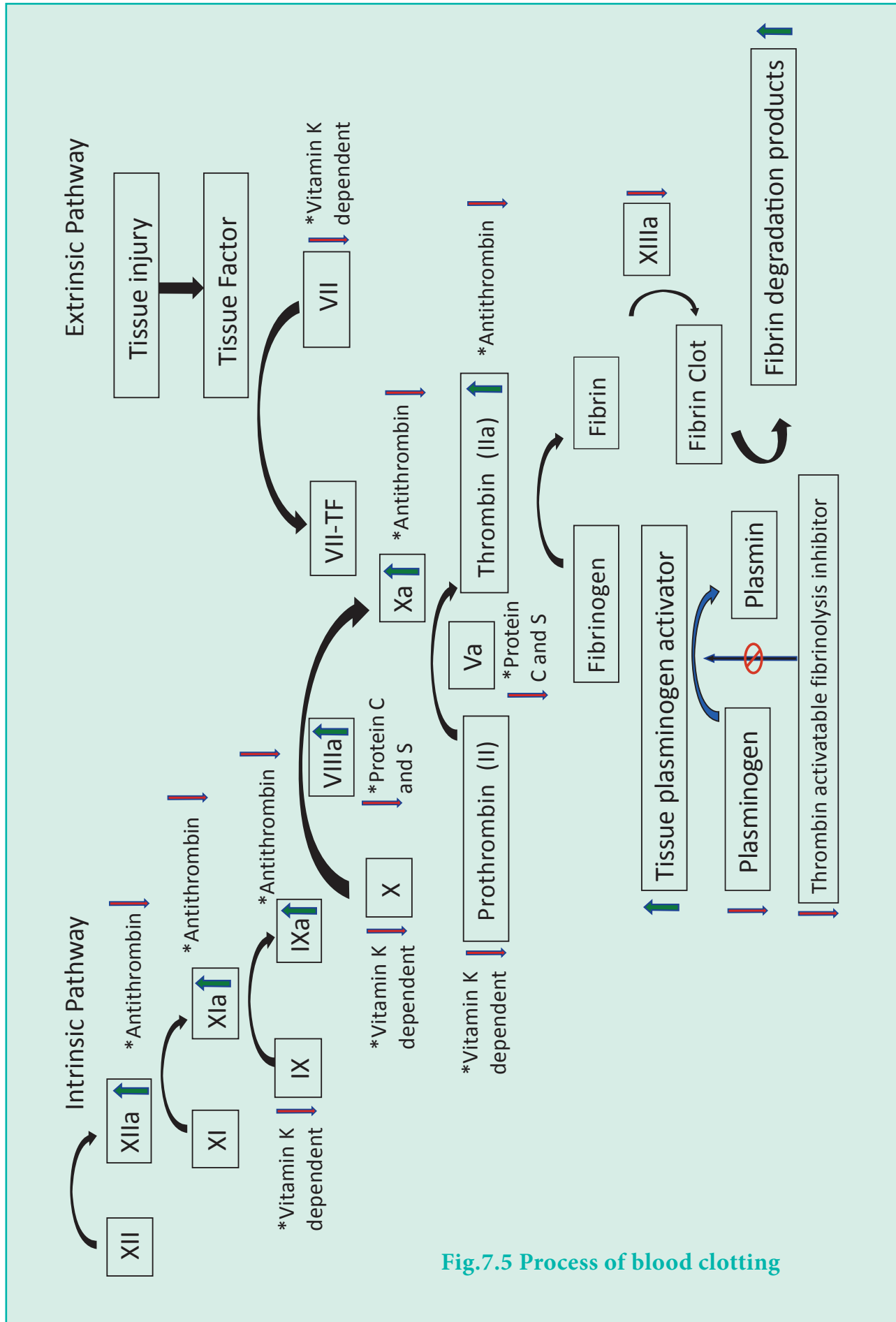


Fig.7.5 Process of blood clotting



bleeding. In certain cases, the immune system gives a negative reaction, in response to the clotting factors used to treat 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 susceptible 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 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. Melanins 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 albinism.

The synthesis of melanin from tyrosine is as shown in the figure 7.6. 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

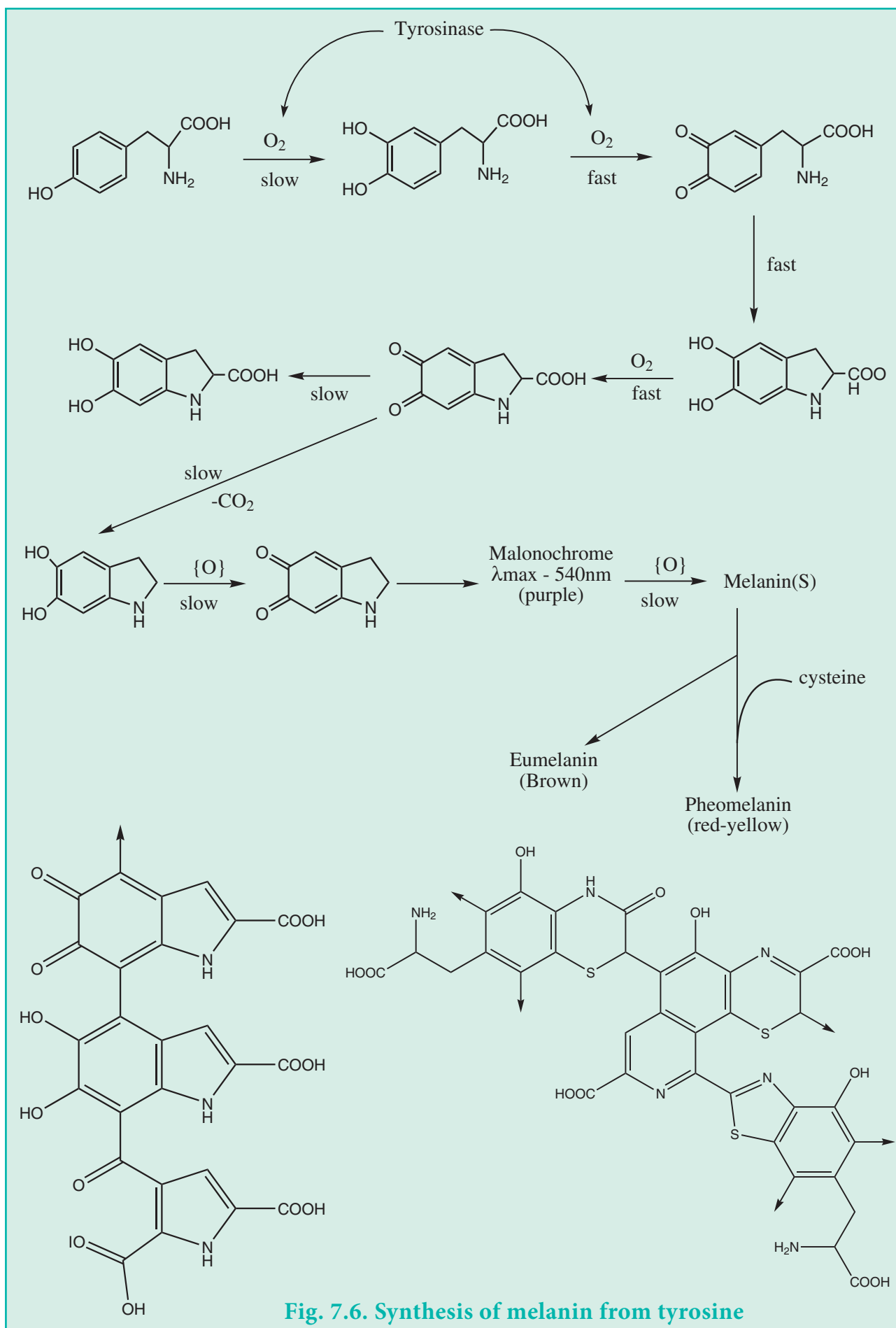


Fig. 7.6. Synthesis of melanin from tyrosine

oxygen directly to form DOPA. The synthesis of melanin occurs in the melanocyte, and the reactions starting with tyrosine are shown in **Fig.7.6**.

DOPA is converted to DOPAquinone. 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 (Fig.7.7).

### 7.4.2 Symptoms

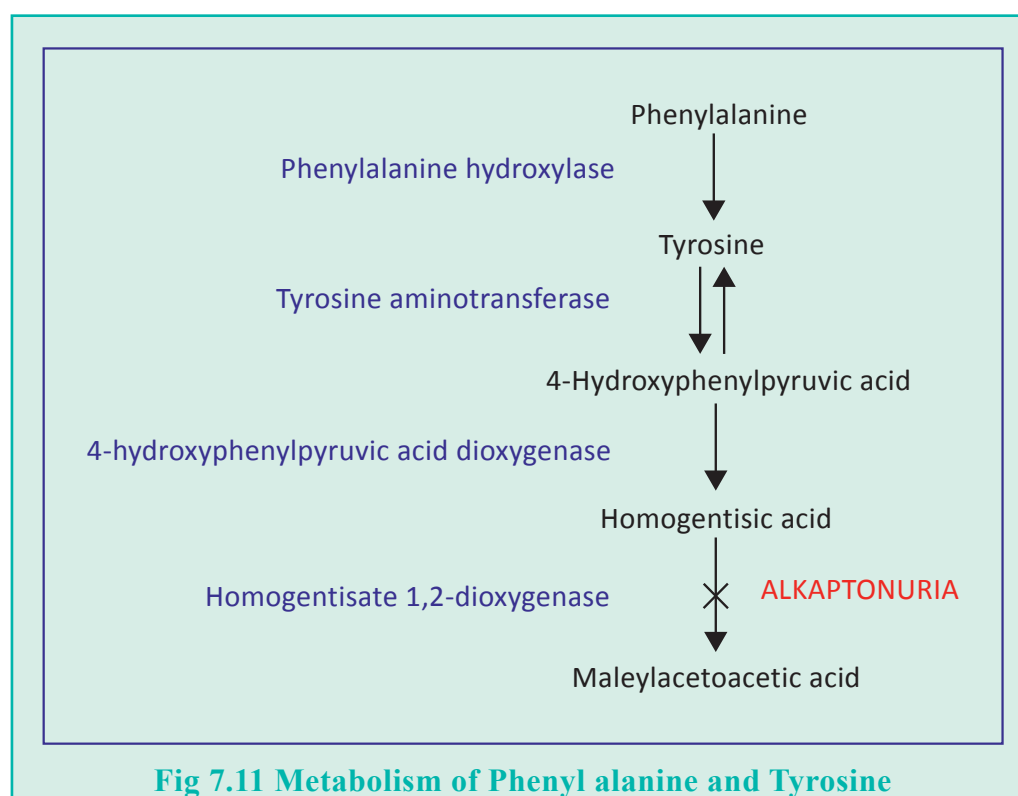
In Oculo-cutaneous albinism, there is decreased pigmentation of skin and eyes, whereas in Ocular albinism, there is decreased pigmentation of only eyes and not the skin. The common symptoms include the following

- 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)



**Fig 7.7. A woman affected with albinism**

## 7.5 ALKAPTONURIA





In 1584, Scribonius was the first to describe dark coloured urine, looking at the case of a child, which excreted urine which turned "black as ink" on exposure to air. In 1859, Boedeker used, 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 phenylalanine 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).

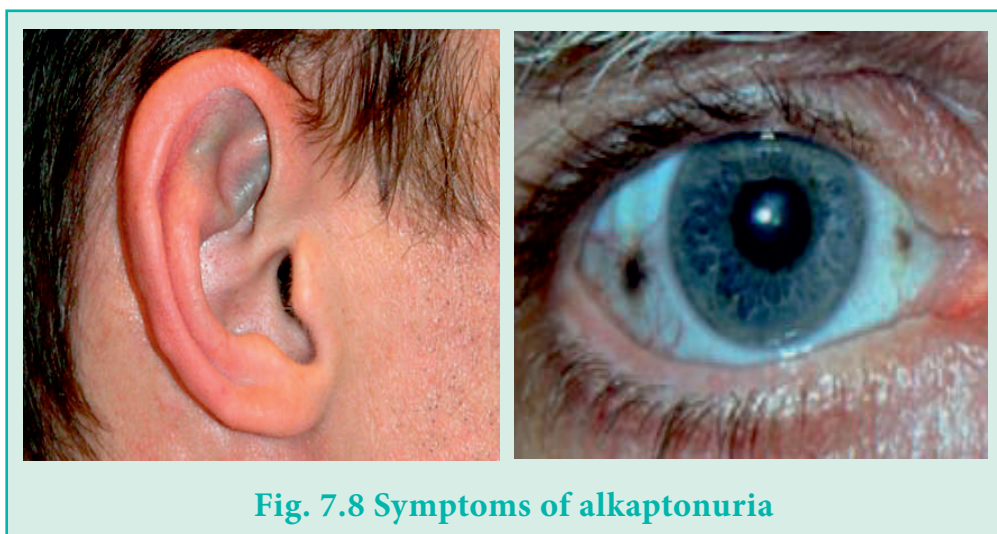


Fig. 7.8 Symptoms of alkaptonuria

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, a condition called ochronosis.

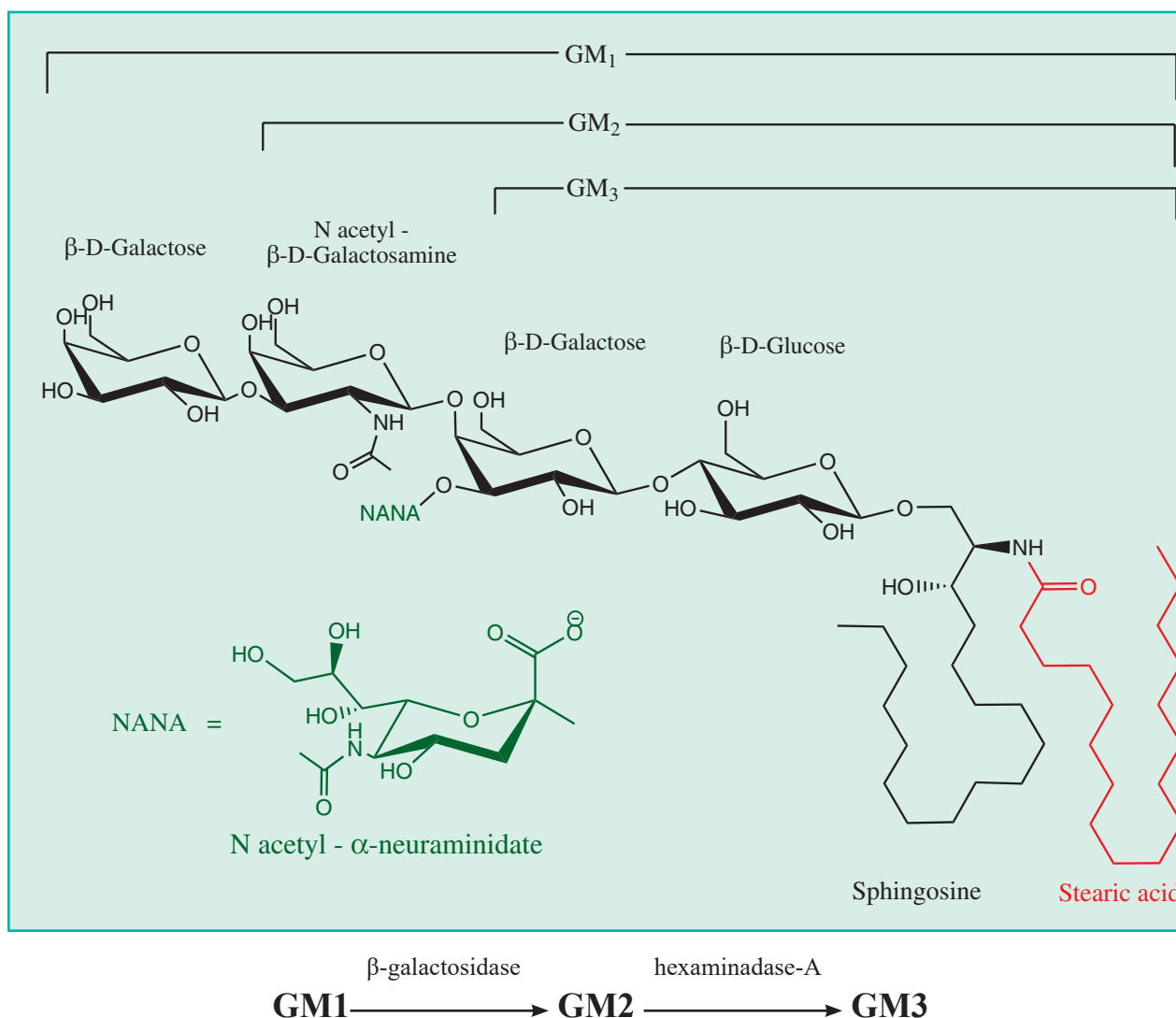
### 7.5.2 Symptoms

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 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 by lysosomal 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.



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. 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, identifying its familial characteristics, like mental disturbances, a deficiency in normal reflexes, progressive blindness and mortality, in Ashkenazi Jews (A Jewish ethnic group).

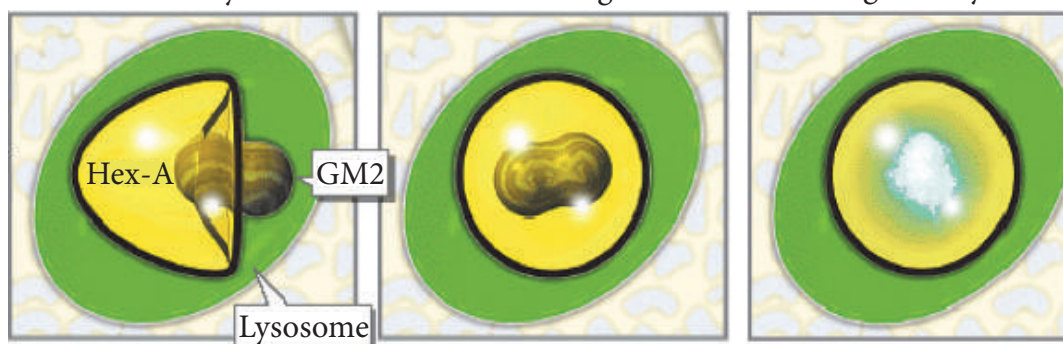
### 7.6.1 Causes

Tay-Sachs disease is classified as a lysosomal storage disease (Fig.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.

#### Cells in healthy children

In a healthy child, a lipid, or fat, called GM2 ganglioside enters the nerve cell as a source of food. Among the components of the cell are lysosomes, which might be thought of as the “stomachs” of the cell. They contain an enzyme called Hoxosaminidase A, or Hex-A, that digests the GM2.

GM2 enters the lysosome... ... where it is engulfed ... ... and digested by Hex-A.



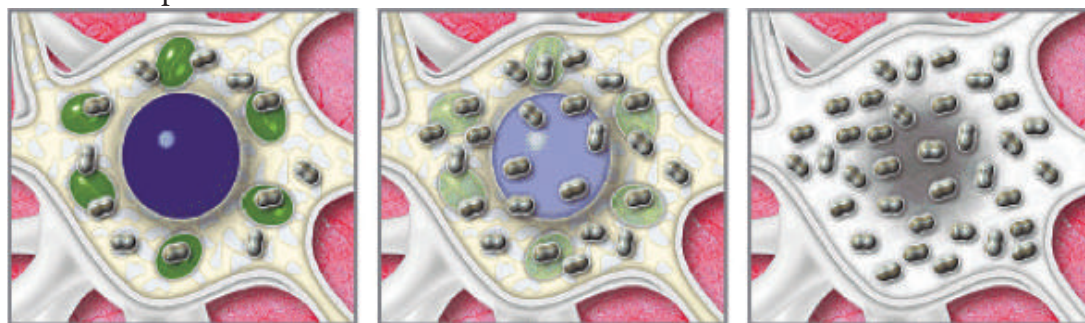
#### Cells in children with Tay-Sachs disease

Children with Tay-Sachs lack Hex-A, so the GM2 proliferates to such a degree that it eventually kills the cell, gradually shutting down the central nervous system.

If Hex-A enzyme  
is not present...

... GM2 accumulates...

... and in time chokes  
off the cells.



REID BROWN | THE PLAIN DEALER

**Fig.7.9 Tay-Sachs disease**

### 7.6.2 Symptoms

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 mother and 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.



Figure 7.10 Cherry red spot in the retina

Till now, there is no cure for Tay-Sachs disease. Treatments include gene therapy or enzyme replacement therapy. They are not promising cures 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's hypothesis followed by Beadle and Tatum's famous One gene one enzyme theory and subsequently the 'One gene one polypeptide' theory
2. Common defects in biochemical pathways include, defect in 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 chemical to the other.

4. The major classification of Inborn errors of metabolism includes protein, carbohydrate, lipid disorders.
5. The major diseases associated with IEM are described. Galactosemia is an inherited disorder characterized by inability of the body to metabolise 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 in 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 inherited disorder, characterized by deficiency in homogentisate 1,2 dioxygenase, which takes part in the degradation of tyrosine.
9. Albinism is characterized by pigmentation of skin due to deficient melanin metabolism.
10. Tay-Sachs disease, a lysosomal disorder, is characterized by the inability of hexosaminidase A to degrade the GM2 gangliosides in the 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. phosphogulcomutase
2. Liver failure and mental retardation are symptoms of
  - a. von-gierkes diseases
  - b. galactosemia
  - c. albinism
  - d. all of these
3. glucose 6-phosphatase deficiency causes
  - a. galactosemia



- b. albinism
  - c. von-gierkes diseases
  - d. haemophilia
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-Gierkes 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
  - d. All of these
10. The decreased pigmentation in eyes are called as
- a. Ocular albinism
  - b. Oculo-cutaneous albinism
  - c. Alkaptonuria
  - d. Hemophilia
11. \_\_\_\_\_ amino acids are involved in alkaptonuria
- a. Glycine & alanine
  - b. Cysteine & methionine
  - c. Tryptophane & lysine
  - d. Phenyl alanine & tyrosine
12. Deficiency of hexaminadase-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. Haemophilia
14. Most IEMs have autosomal recessive modes of inheritance. It means \_\_\_\_\_?
- a. Two copies of the bad gene must be passed on to the child for the disease to develop
  - b. The bad gene is passed down to the child from the mother.
  - c. The bad gene is passed down to the child from the father.
  - d. The bad 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 homogentistic acid oxidase causes
- a. Phenylketonuria



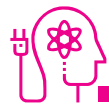
- b. Alkaptonuria
  - c. Tyrosinemia
  - d. Galactosemia
17. A child's diapers on exposure to air became dark coloured. What is your probable diagnosis?
- a. Tay-Sachs disease
  - b. Glycogen storage disease
  - c. Lysosomal storage disease
  - d. Alkaptonuria
18. ----- clot acts as a mesh and stops bleeding
- a. fibrogen
  - b. prothrombin
  - c. christmas factor
  - d. fibrin

## II. Answer the following:

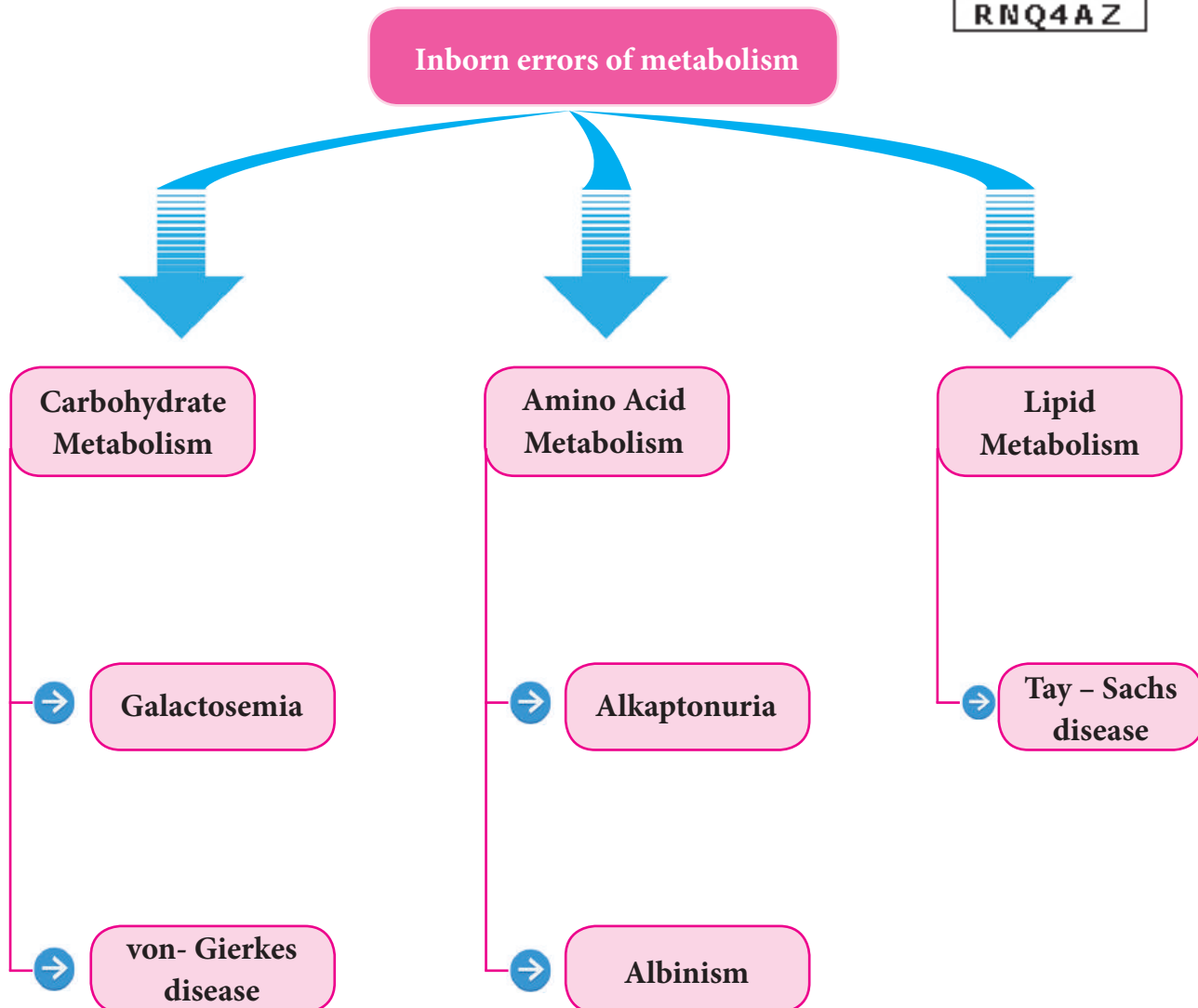
1. Classify inborn errors of metabolism.
2. Give the metabolism of galactose
3. Comment on the symptoms of Von Gierke disease
4. Describe lysosomal storage disease
5. Write a note on haemophilia
6. Give an account on the deficient enzyme involved in alkaptonuria.
7. What are symptoms of galactosemia?
8. Describe the types of Albinism.
9. Give an account on alkaptonuria.
10. How GM1 is converted into GM2?
11. Write the reaction of conversion of glucose to glucose-6-phosphate.
12. Describe the causes and symptoms of Tay-Sachs disease.

## References

1. Gardner, E.J., Simmons, M.J., Snustad, D.P. Principles of Genetics. VIII Edition. Wiley India 2008.
2. Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5<sup>th</sup> edition. 2012.



## CONCEPT MAP



## 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.



### 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



## 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).

## 8.2 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.

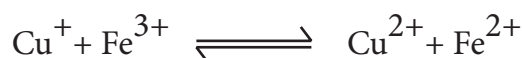


A complete redox reaction must have one reactant, an electron acceptor, which gets reduced by gaining electrons. The electron donor is called as reductant which becomes oxidized while transferring electrons to the other substrate which is called as the oxidant.





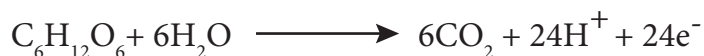
Example : oxidation of copper by iron



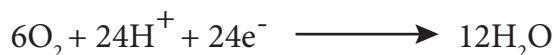
Electrons cannot be created or destroyed in a chemical reaction and therefore if one molecule is oxidized the other one must be reduced, known as a redox reaction. The complete oxidation of glucose by oxygen is described in the following reaction.



The above reaction can be divided into two half reactions. In the first reaction, the carbon atoms of glucose are oxidized as follows:



In the second half reaction, molecular oxygen is reduced



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  $\text{NAD}^+$  and  $\text{FAD}$  to form  $10\text{NADH}$  and  $2\text{FADH}_2$  through several glycolytic enzymes such as, glyceraldehyde 3 phosphate dehydrogenase, pyruvate dehydrogenase and citric acid cycle enzymes – isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. The stepwise passage of electrons from  $\text{NADH}$  and  $\text{FADH}_2$  to  $\text{O}_2$  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  $\text{P}_i$  through oxidative phosphorylation.

### 8.2.1 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  $\text{H}^+$ , 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 ( $\Delta E$ ), greater the electrons flow spontaneously from the less positive to the more positive side.  $\Delta E$  is expressed in volts.



The free energy change at the standard state of pH7 is represented as  $\Delta G^\circ$ . The standard free energy change can be calculated from the change in redox potential  $\Delta E^\circ$  of the substrate and products as represented below, where  $n$  denotes the number of electrons transferred per mole of reactants,  $\Delta E^\circ$  in Volts (V) and  $\Delta G^\circ$  in kilocalories per mole ( $\text{kcal mol}^{-1}$ ),  $F$  is Faraday's constant ( $96,494^\circ\text{C mol}^{-1}$ ). The standard free energy for the reaction is:  $\Delta G^\circ = -nF\Delta E^\circ$

### 8.3 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,  $\beta$  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.

#### 8.3.1 Structure of mitochondria

The size of a mitochondrion is close to the size of a bacterium (ranging between 1 to  $2\mu\text{m}$ ). 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 (See Box).

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.

The mitochondrial membrane is rich in proteins. Porins present in the outer membrane of the mitochondria allow small molecules to be exchanged between the cytoplasm and the inter membrane space. However the inner

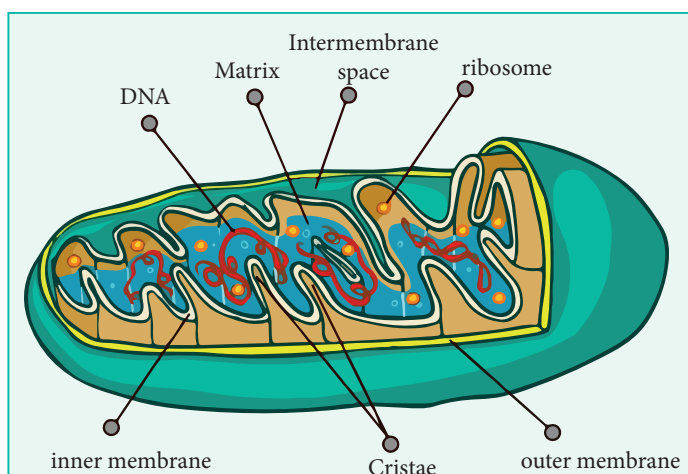


Figure 8.1. Structure of Mitochondria