ESSENTIALS OF
BIOCHEMISTRY

ESSENTIALS OF
BIOCHEMISTRY

Pankaja Naik PhD
Professor and Head
Department of Biochemistry
MVPS Dr Vasantrao Pawar Medical College
Nashik, Maharashtra
India

Foreword
PM Bulakh

JAYPEE BROTHERS MEDICAL PUBLISHERS (P) LTD
New Delhi • Panama City • London

Jaypee Brothers Medical Publishers (P) Ltd.

Headquarter

Jaypee Brothers Medical Publishers (P) Ltd
4838/24, Ansari Road, Daryaganj
New Delhi 110 002, India
Phone: +91-11-43574357
Fax: +91-11-43574314
Email: jaypee@jaypeebrothers.com

Overseas Offices

J.P. Medical Ltd.,
83 Victoria Street, London
SW1H 0HW (UK)
Phone: +44-2031708910
Fax: +02-03-0086180
Email: info@jpmedpub.com

Jaypee-Highlights Medical Publishers Inc.
City of Knowledge, Bld. 237, Clayton
Panama City, Panama
Phone: 507-317-0160
Fax: +50-73-010499
Email: cservice@jphmedical.com

Website: www.jaypeebrothers.com
Website: www.jaypeedigital.com

Publisher: Jitendar P Vij
Publishing Director: Tarun Duneja
Cover Design: Seema Dogra

Essentials of Biochemistry
First Edition: **2012**

ISBN 978-93-5025-491-2

Printed at

Dedicated to

My Parents
whose love and support
are indispensable to me
in a million ways

Foreword

It gives me a great pleasure to write foreword to *Essentials of Biochemistry* book written by Dr Pankaja Naik, Professor and Head, Department of Biochemistry, Dr Vasantrao Pawar Medical College, Nashik, Maharashtra, India. She is a dedicated teacher with more than twenty years of teaching experience. She is also a recognized postgraduate teacher of Maharashtra University of Health Sciences, Nashik, Maharashtra, India.

The study of Biochemistry is prerequisite not only for understanding the basic functions of the living body at cellular and molecular levels, but also finding remedies for a variety of ailments that afflict human beings.

The publication of this book on Biochemistry certainly meets the requirements of students, teachers, professionals and layman also. One of the salient features of this book is that it covers course contents as per the recommendations of Medical Council of India (MCI). The book has also been written in a lucid language that enables the students to understand and grasp the fundamentals and its applications. Moreover, in order to enable the reader to understand the topic in its proper perspective, additional information has been provided in the form of diagrams, tables and flow charts. Importantly, in the book, each chapter concludes with a summary of topic, multiple choice questions (MCQs) and clinical cases.

In addition to medical students, this book will also be useful for other branches and learning of health sciences.

I congratulate Dr Pankaja Naik for doing an excellent job in writing this valuable book and wish her all the very best in this endeavor.

PM Bulakh
Director
Board of College and University Development
Bharati Vidyapeeth University
Pune, Maharashtra, India
Formerly
Professor and Head (Biochemistry) and Dy Dean
BJ Medical College
Pune, Maharashtra, India

Preface

Essentials of Biochemistry, is based on the earlier well-established book *Biochemistry*. It has been streamlined to focus primarily on the essential biochemical concepts important to medical students. If further details are needed, it is advised that the students should refer to larger parent book *Biochemistry*.

The curriculum of biochemistry for medical students is vast and the time available for its study is comparatively short. All this has prompted me to write a concise textbook on biochemistry, which should be complete, comprehensive, easily understandable and exam oriented.

The entire text has been rewritten, simplified and conveniently divided into thirty-five chapters, that encompasses all the syllabus recommended by the Medical Council of India (MCI). Excessive details, redundancy and verbosity have been minimized as far as possible without compromising the clarity of expression and accuracy of meaning. Wherever possible, additional information has been provided in the form of diagrams, tables and flow charts. The book is well illustrated and each chapter begins with a chapter outline that indicates the topics covered and concludes with a "Summary" of the contents. Multiple choice questions (MCQs) and clinical cases given at the end of the chapters are intended to help the students in self assessment. I do hope the students will appreciate this book as a resource for securing knowledge and procuring good marks in the examination.

In addition to medical students, this book will also be useful for students of dental, physiotherapy, occupational therapy, nursing and other life sciences. If this book truly helps the students in understanding biochemistry, I shall consider that I have done my job in providing a secured base for students to excel further in medical sciences.

I will be highly obliged if readers can respond with their feedback in the form of comments, constructive criticism and suggestions at the following e-mail address: **pankajanaik@hotmail.com**.

Pankaja Naik

Acknowledgments

I express my profound gratitude to late Dr Vasantrao Pawar, Sarchitnis, Nashik District Maratha Vidya Prasarak Samaj, Nashik, Maharashtra, India for his keen interest in all the academic activities of the faculty members.

I thank Mrs Chetna Malhotra Vohra, Senior Manager and Mr CS Gawade, Branch Manager, Mumbai branch of M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India who provided me inspiration to write this book.

I sincerely express my gratitude to Smt Neelimatai Pawar, Sarchitnis, Nashik Maratha Vidya Prasarak Samaj, Nashik, Maharashtra, India for her continuous support and encouragement.

I would like to thank Dr (Mrs) Mrunal Patil (Dean) of my college for all the cooperation extended to me.

I thank all my fellow teachers and senior professors from various institutes and universities across the world for their suggestions which enabled me to bring out *Essentials of Biochemistry* successfully.

My colleague in the department, Mr Momin (Assistant Professor) helped me in designing the cover page. Indeed, I sincerely acknowledge his help. Without the able DTP operator, the manual would not have been completed within the due period. I profusely thank Mr Mukesh Kale in Nashik for the same.

I would also like to acknowledge and thank the help rendered by Mr Chavan, Mr Rayate, Mr Gosavi, Mr Labhade and Mr Khelukar of my department.

I am indebted to Shri Jitendar P Vij, the dynamic Chairman and Managing Director, M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi and his dedicated team of professionals: Mr Tarun Duneja (Director-Publishing), Mr KK Raman (Production Manager), Mrs Samina Khan (PA to Director-Publishing), Mr Neelambar Pant (Production Coordinator), Mrs Yashu Kapoor, Mr Varun Rana & Mr Sunil Rawat (DTP Operators), Mr Manoj Pahuja (Graphic Designer) and Mr Abhas Mukherjee (Proofreader) for their continuous cooperation, enthusiasm and their excellent work in bringing out this book.

Last but not least, I thank my husband Mr Shekhar and my son Sushrut for understanding and supporting me to complete the task successfully.

Contents

CHAPTER

1

Cell and Membrane Transport

INTRODUCTION

'Cell' means a small room or chamber, cells are the structural and functional units of all living organisms. The major parts of a cell are the **nucleus** and the **cytoplasm.**

The electron microscope allowed classification of cells into two major groups, **prokaryotes** and **eukaryotes,** based on the presence and absence of the true nucleus.

Eukaryotes

- Eukaryotes have nucleus which is covered by nuclear membrane. (Greek: Eue = true, karyon = nucleus). Animals, plants and fungi belong to the eukaryotes.
- Eukaryotic cells are much larger than prokaryotes.
- Unlike prokaryotes, eukaryotes have a variety of other membrane-bound organelles (subcellular elements) in their cytoplasm, including:
  - Mitochondria
  - Lysosomes
  - Endoplasmic reticulum and
  - Golgi complexes.

The biochemical functions of subcellular organelles of the eukaryotic cell is given in **Table 1.1**.

Prokaryotes

- Prokaryotes have no typical nucleus and subcellular components. (Greek: Pro = before). Bacteria and blue green algae belong to the prokaryotes.

STRUCTURE AND FUNCTIONS OF A CELL AND ITS SUBCELLULAR COMPONENTS (FIGURE 1.1)

A cell has three major components:

1. Cell membrane (Plasma membrane)
2. Cytoplasm with its organelles
3. Nucleus.

Cell Membrane (Plasma Membrane)

- The cell is enveloped by a thin membrane called **cell membrane** or **plasma membrane.**

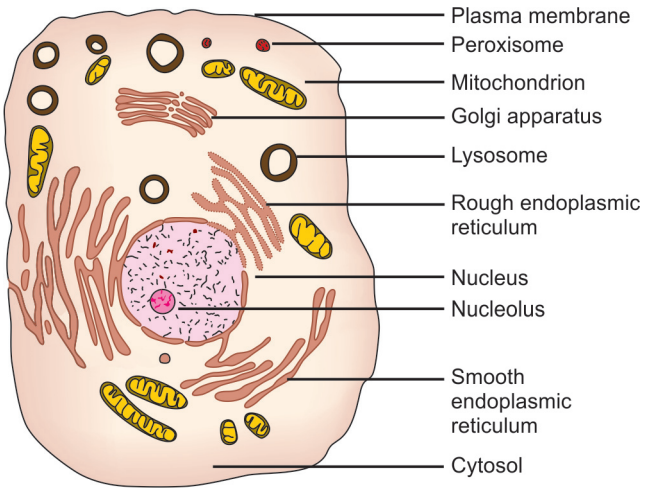


Figure 1.1: A diagrammatic representation of a typical eukaryotic cell

Table 1.1: Biochemical functions of subcellular organelles of the eukaryotic cell

Subcellular organelles	*Function*
Plasma membrane	Transport of molecules in and out of cell, receptors for hormones and neurotransmitters
Lysosome	Intracellular digestion of macromolecules and hydrolysis of nucleic acid, protein, glycosaminoglycans, glycolipids, sphingolipids
Golgi apparatus	Post-transcriptional modification and sorting of proteins and export of proteins
Rough endoplasmic reticulum	Biosynthesis of protein and secretion
Smooth endoplasmic reticulum	Biosynthesis of steroid hormones and phospholipids, metabolism of foreign compounds
Nucleus	Storage of DNA, replication and repair of DNA, transcription and post-transcriptional processing
Peroxisomes	Metabolism of hydrogen peroxide and oxidation of long-chain fatty acids
Nucleolus	Synthesis of rRNA and formation of ribosomes
Mitochondrion	ATP synthesis, site for tricarboxylic acid cycle, fatty acid oxidation, oxidative phosphorylation, part of urea cycle and part of heme synthesis
Cytosol	Site for glycolysis, pentose phosphate pathway, part of gluconeogenesis, urea cycle and heme synthesis, purine and pyrimidine nucleotide synthesis

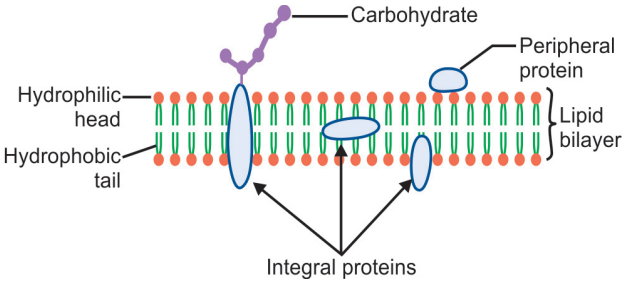


Figure 1.2: The basic organization of biological membrane

- Cell membranes mainly consist of **lipids, proteins** and smaller proportion of **carbohydrates** that are linked to lipids and proteins.
- The basic organization of biologic membranes is illustrated in **Figure 1.2.**
- The cell membrane is an organized structure consisting of a **lipid bilayer** primarily of **phospholipids** and penetrated **protein** molecules forming a **mosaic-like pattern (Figure 1.3).**

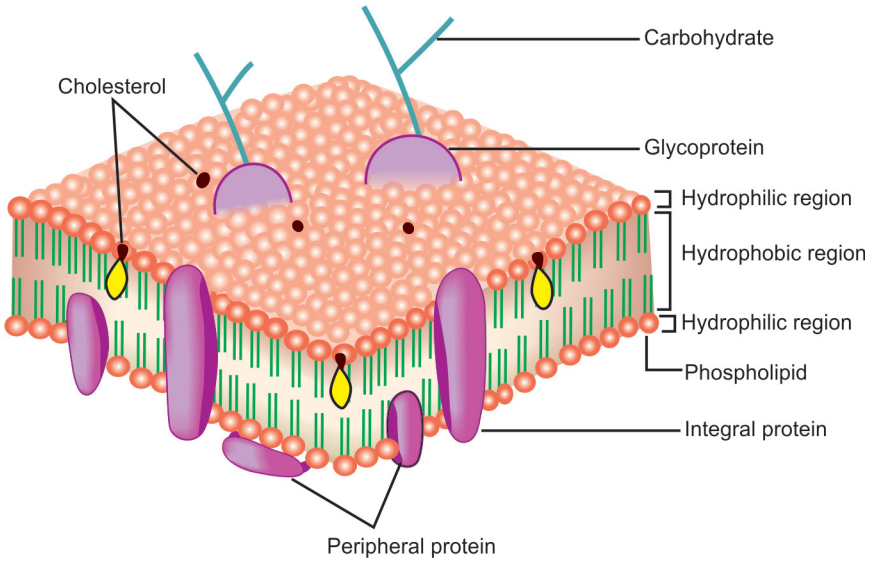


Figure 1.3: The fluid mosaic model of cell membrane

Membrane Lipids

- The major classes of membrane lipids are:
  - Phospholipids
  - Glycolipids
  - Cholesterol.

  They all are **amphipathic** molecules, i.e. they have both hydrophobic and hydrophilic ends.
- Membrane lipids spontaneously form bilayer in aqueous medium, burrying their hydrophobic tails and leaving their hydrophilic ends exposed to the water **(Figure 1.2).**

Membrane Proteins

- Proteins of the membrane are classified into two major categories:
  - Integral proteins or intrinsic proteins or transmembrane proteins and
  - Peripheral or extrinsic proteins.
- **Integral proteins** are either partially or totally immersed in the lipid bilayer. Many integral membrane proteins span the lipid bilayer from one side to the other and are called **transmembrane protein** whereas others are partly embedded in either the outer or inner leaflet of the lipid bilayer **(Figure 1.2). Transmembrane proteins** act as **enzymes** and **transport carriers** for ions as well as water soluble substances, such as glucose.
- **Peripheral proteins** are attached to the surface of the lipid bilayer by electrostatic and hydrogen bonds. They bound loosely to the polar head groups of the membrane phospholipid bilayer **(Figure 1.2)**. Peripheral proteins function almost entirely as **enzymes** and **receptors.**

Membrane Carbohydrates

- Membrane carbohydrate is not free. It occurs in combination with proteins or lipids in the form of ***glycoproteins*** or ***glycolipids.*** Most of the integral proteins are glycoproteins and about one-tenth of the membrane lipid molecules are glycolipids. The carbohydrate portion of these molecules protrude to the outside of the cell, dangling outward from the cell surface **(Figure 1.2).**
- Many of the carbohydrates act as **receptor** for hormones. Some carbohydrate moieties function in **antibody** processing.

Functions of Cell Membrane

- **Protective function:** The cell membrane protects the cytoplasm and the organelles of the cytoplasm.
- **Maintenance of shape and size of the cell.**
- **As a semipermeable membrane:** The cell membrane permits only some substances to pass in either direction, and it forms a barrier for other substances.

The Fluid Mosaic Model of Cell Membrane

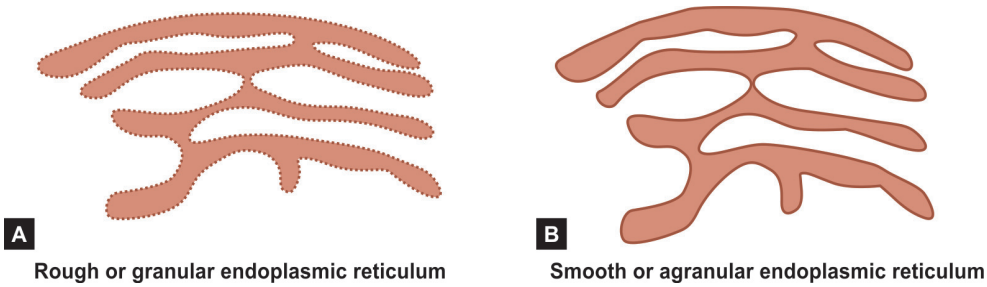
- In 1972, Singer and Nicolson postulated a theory of membrane structure called the **fluid mosaic model**, which is now widely accepted.
- A mosaic is a structure made up of many different parts. Likewise, the plasma membrane is composed of different kinds of macromolecules like **phospholipid, integral proteins, peripheral proteins, glycoproteins, glycolipids** and **cholesterol.**
- According to this model, the membrane structure is a **lipid bilayer** made of phospholipids.
- The bilayer is fluid because the hydrophobic tails of phospholipids consist of an appropriate mixture of saturated and unsaturated fatty acids that is fluid at normal temperature of the cell.
- Proteins are interspersed in the lipid bilayer, of the plasma membrane, producing a mosaic effect **(Figure 1.3).**
- The peripheral proteins literally float on the surface of 'sea' of the phospholipid molecules, whereas the integral proteins are like icebergs, almost completely submerged in the hydrophobic region.
- There are no covalent bonds between lipid molecules of the bilayer or between the protein components and the lipids.
- Thus, there is a mosaic pattern of membrane proteins in the fluid lipid bilayer.
- Fluid mosaic model allows the membrane proteins to move around laterally in two dimensions and that they are free to diffuse from place to place within the plane of the bilayer. Whereas they cannot tumble from one side of the lipid bilayer to the other.
- **The Singer-Nicolson model can explain many of the physical, chemical and biological properties of membranes and has been widely accepted as the most probable molecular arrangement of lipids and proteins of membranes.**

Cytoplasm and its Organelles

Cytoplasm is the internal volume bounded by the plasma membrane. The clear fluid portion of the cytoplasm in which the particles are suspended is called ***cytosol.***

Six important organelles that are suspended in the cytoplasm are:

1. Endoplasmic reticulum
2. Golgi apparatus
3. Lysosomes



Figures 1.4 A and B: Structure of endoplasmic reticulum

4. Peroxisomes
5. Mitochondria
6. Nucleus.

Endoplasmic Reticulum (ER)

- Endoplasmic reticulum is the interconnected network of tubular and flat vesicular structures in the cytoplasm **(Figures 1.4A and B).**
- Endoplasmic reticulum forms the link between nucleus and cell membrane by connecting the cell membrane at one end and the outer membrane of the nucleus at the other end **(see Figure 1.1).**
- A large number of minute granular particles called ***ribosomes*** are attached to the outer surface of many parts of the endoplasmic reticulum, this part of the ER is known as **rough** or **granular ER.**
- During the process of cell fractionation, rough ER is disrupted to form small vesicles known as **microsomes**. It may be noted that microsomes as such do not occur in the cell.
- Part of the ER, which has no attached ribosomes, is known as **smooth endoplasmic reticulum.**

Functions of the ER

- Rough ER functions in the biosynthesis of protein.
- The smooth endoplasmic reticulum functions in the synthesis of steroid hormones and cholesterol.
- Smooth endoplasmic reticulum is the site of the metabolism of certain drugs, toxic compounds and carcinogens (cancer producing substances).

Golgi Apparatus

Golgi apparatus is present in all cells ***except in red blood cells.*** It is situated near the nucleus and is closely related to the endoplasmic reticulum. It consists of four or more membranous sacs. This apparatus is prominent in secretory cells.

Functions of golgi apparatus

The Golgi apparatus functions in association with the endoplasmic reticulum.

- Proteins synthesized in the ER are transported to the Golgi apparatus where these are processed by addition of carbohydrate, lipid or sulfate moieties. These chemical modifications are necessary for the transport of proteins across the plasma membrane.
- Golgi apparatus are also involved in the synthesis of intracellular organelles, e.g.lysosomes and peroxisomes.

Lysosomes

- Lysosomes are vesicular organelles formed from Golgi apparatus and dispersed throughout the cytoplasm.
- ***Among the organelles of the cytoplasm, the lysosomes have the thickest covering membrane to prevent the enclosed hydrolytic enzymes from coming in contact with other substances in the cell and therefore, prevents their digestive actions.***
- Many small granules are present in the lysosome. The granules contain more than 40 different hydroxylases (hydrolytic enzymes). All the enzymes are collectively called ***lysozymes***.

Functions of lysosomes

Lysozymes present in lysosomes digest proteins, carbohydrates, lipids and nucleic acids. Apart from the digestive functions, the enzymes in the lysosomes are responsible for the following activities in the cell:

- Destruction of bacteria and other foreign bodies.
- Removal of excessive secretory products in the cells of the glands.
- Removal of unwanted cells in embryo.

Peroxisomes

- These organelles resemble the lysosomes in their appearance, but they differ both in function and in their synthesis.

- They do not arise from Golgi membranes, but rather from the division of pre-existing peroxisomes. Or perhaps through budding off from the smooth endoplasmic reticulum.

Functions of peroxisomes

- Peroxisomes contain enzymes ***peroxidases*** and ***catalase*** which are concerned with the metabolism of peroxide. Thus, the peroxisomes are involved in the detoxification of peroxide.
- Peroxisomes are also capable of carrying out β-oxidation of fatty acid.

Mitochondria (Power House of Cell)

- Mitochondria are called ***"Power Plant"*** of the cell since they convert energy to form ATP that can be used by cell.
- A mitochondrion is a double-membrane organelle **(Figure 1.5)** that are fundamentally different in composition and function.
  - — **The outer membrane** forms a smooth envelope. It is freely permeable for most metabolites.
  - — **The inner membrane** is folded to form ***cristae,*** which give it a large surface area and are the site of ***oxidative phosphorylation.*** The components of the electron transport chain are located on the inner membrane.
- The space within the inner membrane is called the ***mitochondrial matrix.*** It contains the enzymes of the:
  - — Citric acid cycle
  - — β-oxidation of fatty acid
  - — Some other degradative enzymes.
- Mitochondria contain DNA (mtDNA) which encodes a few polypeptides involved in oxidative phosphorylation.

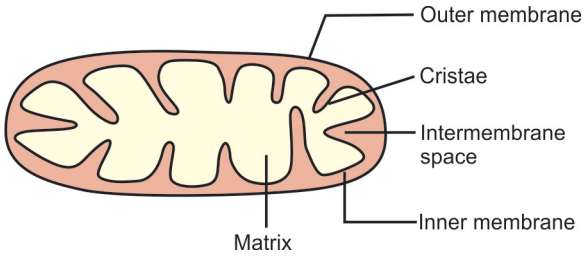


Figure 1.5: Structure of mitochondria

It is worth noting that sperms contribute no mitochondria to the fertilized egg, so that mitochondrial DNA is inherited exclusively through the female line. ***Thus, mitochondria are maternally inherited.***

Nucleus

The cells with nucleus are called ***eukaryotes*** and those without nucleus are known as ***prokaryotes.*** Most of the cells have only one nucleus but ***cells of skeletal muscles have many nuclei. The matured red blood cell contains no nucleus.***

Structure of Nucleus

- The nucleus is spherical in shape and situated near the center of the cell. The nucleus is surrounded by the nuclear envelope.
- The space enclosed by the nuclear envelope is called ***nucleoplasm,*** within this the ***nucleolus*** is present. Nucleolus is an organized structure of DNA, RNA and protein that is involved in the synthesis of ribosomal RNA. The remaining nuclear DNA is dispersed throughout the nucleoplasm in the form of ***chromatin fibers.*** At mitosis, chromatin is condensed into discrete structures called ***chromosomes.***

Functions of Nucleus

The major functional role of the nucleus is that of:

- **Replication:** Synthesis of new DNA.
- **Transcription:** The synthesis of the three major types of RNA:
  1. Ribosomal RNA (rRNA)
  2. Messenger RNA (mRNA)
  3. Transfer RNA (tRNA).

CYTOSKELETON

- The cytoplasm of most eukaryotic cells contains network of protein filaments, that interact extensively with each other and with the component of the plasma membrane. Such an extensive intracellular network of protein has been called **cytoskeleton.** The plasma membrane is anchored to the cytoskeleton. The cytoskeleton is not a rigid permanent framework of the cell but is a dynamic, changing structure.
- The cytoskeleton consists of three primary protein filaments:
  1. Microfilaments
  2. Microtubules
  3. Intermediate filaments.

1. **Microfilaments** are about 5 nm in diameter. They are made up of protein **actin.** Actin filaments form a meshwork just underlying the plasma membrane of cells and are referred to as **cell cortex,** which is labile. They disappear as cell motility increases or upon malignant transformation of cells. The function of microfilaments is:
   — To help muscle contraction

— To maintain the shape of the cell
— To help cellular movement.

2. **Microtubules** are cylindrical tubes, 20 to 25 nm in diameter. They are made up of protein **tubulin.** Microtubules are necessary for the formation and function of mitotic spindle. They provide stability to the cell. They prevent tubules of ER from collapsing. These are the major components of **axons** and **dendrites.**
3. **Intermediate filaments** are so called as their diameter (10 nm) is intermediate between that of microfilaments (5 nm) and of microtubules (25 nm).

- Intermediate filaments are formed from fibrous protein which varies with different tissue type.
- They play role in cell-to-cell attachment and help to stabilize the epithelium. They provide strength and rigidity to axons.

Functions of Cytoskeleton

- The cytoskeleton gives cells their characteristic shape and form, provides attachment points for organelles, fixing their location in cells and also makes communication between parts of the cell possible.
- It is also responsible for the separation of chromosomes during cell division.
- The internal movement of the cell organelles as well as cell locomotion and muscle fiber contraction could not take place without the cytoskeleton. It acts as **"track"** on which cells can move organelles, chromosomes and other things.

MEMBRANE TRANSPORT

- One of the functions of the plasma membrane is to regulate the passage of a variety of small molecules across it.
- Biological membranes are **semipermeable** membranes through which certain molecules freely diffuse across membranes but the movement of the others is restricted because of **size**, **charge** or **solubility.**
- The two types of transport mechanisms are **(Figure 1.6):**
  1. Passive transport or passive diffusion
  2. Active transport.

Passive Transport or Passive Diffusion

- Passive transport is the process by which molecules move across a membrane without energy (ATP).
- The direction of passive transport is always from a **region of higher concentration to one of lower concentration.**
- There are two types of passive transport as follows:
  1. Simple diffusion
  2. Facilitated diffusion.

Simple Diffusion

- Lipid soluble, i.e. lipophilic molecules can pass through cell membrane, without any interaction with carrier proteins in the membrane. Such molecules will pass through membrane along the concentration gradient, i.e. from a region of higher concentration to one of lower concentration. This process is called **simple diffusion.**

Facilitated Diffusion

- The movement of water soluble molecules and ions across the membrane requires specific transport system. They pass through specific **carrier proteins.** A carrier protein binds to a specific molecule on one side of the membrane and releases it on the other side. This type of crossing

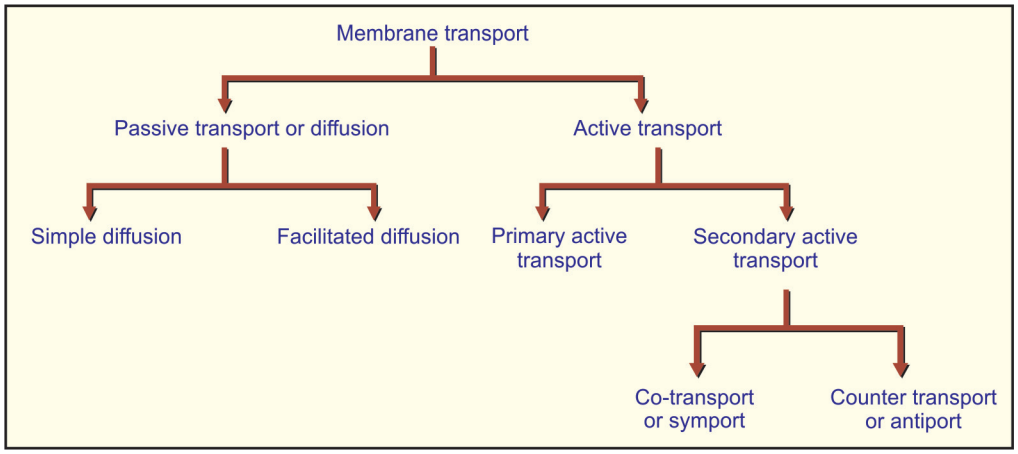


Figure 1.6: Types of membrane transport mechanism

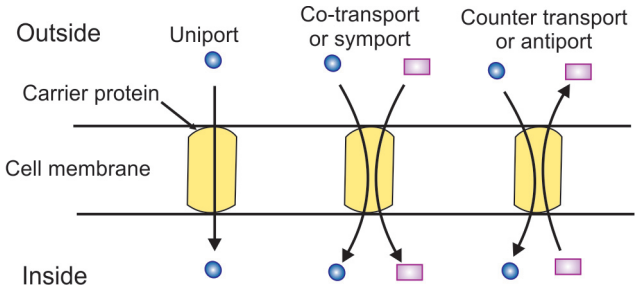


Figure 1.7: Uniport, symport and antiport transport of substance across the cell membrane

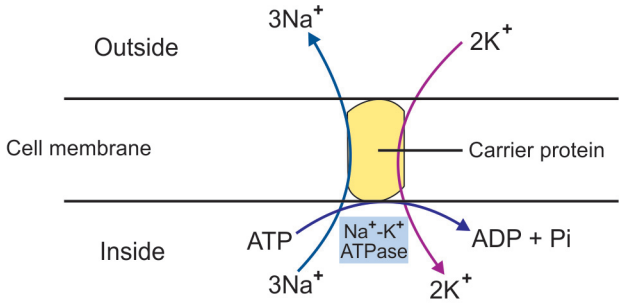


Figure 1.8: Mechanism of sodium-potassium pump (primary active transport)

the membrane is called **facilitated diffusion** or **carrier-mediated diffusion.**

- An example of facilitated diffusion is the movement of glucose and most of the amino acids across the plasma membrane.
- These diffusion processes are not coupled to the movement of other ions, they are known as **uniport transport processes (Figure 1.7).**

Active Transport

- If a molecule moves against a concentration gradient, an external energy source is required; this movement is referred to as **active transport**.
- Substances that are actively transported through cell membranes include, **Na^+, K^+, Ca^{++}, H^+, CI^-,** several different **sugars** and most of the **amino acids.**
- Active transport is classified into two types according to the source of energy used as follows :
  i. Primary active transport
  ii. Secondary active transport.
- In both instances, transport depends on the **carrier proteins;** like facilitated diffusion. However, in active transport, the carrier proteins function differently from the carrier in facilitated diffusion. **Carrier protein for active transport is capable of transporting substance against the concentration gradient.**

Primary Active Transport

- In primary active transport, the energy is derived **directly** from **hydrolysis of ATP.**
- Sodium, potassium, calcium, hydrogen and chloride ions are transported by primary active transport.

Primary active transport of Na^+ and K^+ (sodium-potassium pump)

- Na^+-K^+ Pump, a primary active transport process that pumps Na^+ ions out of the cell and at the same time pumps K^+ ions from outside to the inside generating an electrochemical gradient.
- Carrier protein of Na^+-K^+ pump has three receptor sites for binding sodium ions on the inside of the cell and two receptor sites for potassium ions on the outside. The inside portion of this protein has **ATPase activity (Figure 1.8).**
- The pump is called **Na^+-K^+ ATPase** because the hydrolysis of ATP occurs only when three Na^+ ions bind on the inside and two K^+ ions bind on the outside of the carrier proteins. The energy liberated by the hydrolysis of ATP leads to conformational change in the carrier protein molecule, extruding the three Na^+ ions to the outside and the two K^+ ions to the inside.

Physiological importance of Na^+-K^+ pump

- The active transport of Na^+ and K^+ is of great physiological significance. The Na^+-K^+ gradient created by this pump in the cells, **controls cell volume.**
- It carries the active transport of sugars and amino acids.

Secondary Active Transport

Secondary active transport uses an energy generated by an electrochemical gradient. It is not directly coupled with hydrolysis of ATP. Secondary active transport is classified into two types:

1. **Co-transport** or **symport**, in which both substances move simultaneously across the membrane in the same direction **(Figure 1.7),** e.g. transport of Na^+ and glucose to the intestinal mucosal cells from the gut.
2. **Counter transport** or **antiport**, in which both substances move simultaneously in opposite direction **(Figure 1.7),** e.g. transport of Na^+ and H^+ occurs in the renal proximal tubules and exchange of Cl^- and HCO_3^- in the erythrocytes.

Transport of Macromolecules Across the Plasma Membrane

- The process by which cells take up large molecules is called **endocytosis (Figure 1.9)** and the process by which cells release large molecules from the cells to the outside is called **exocytosis (Figure 1.10).**

Endocytosis

- There are two types of endocytosis:
  1. Pinocytosis (cellular drinking)
  2. Phagocytosis (cellular eating).

Pinocytosis

- Pinocytosis is the cellular uptake of fluid and fluid contents and is a cellular drinking process.
- Pinocytosis is the only process by which most macromolecules, such as most proteins, polysaccharides and polynucleotides can enter cells **(Figure 1.9).**
- These molecules first attach to specific receptors on the surface of the membrane.

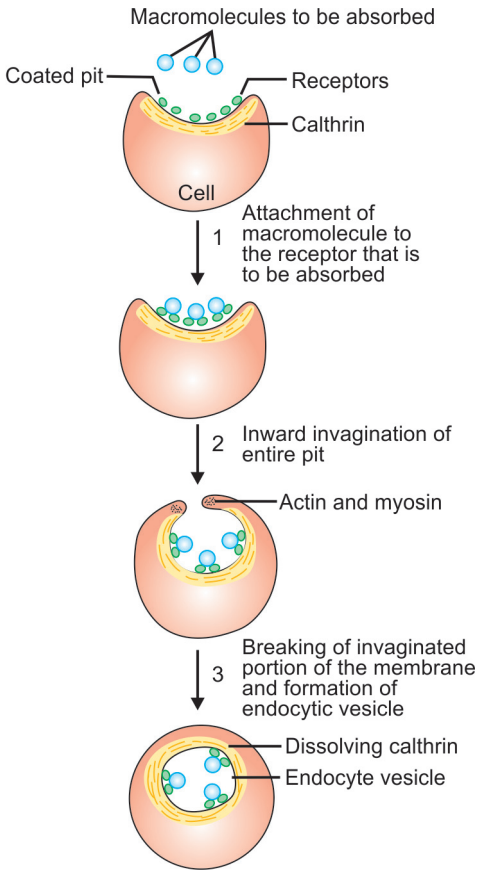


Figure 1.9: Three stages of the absorption of macromolecules by endocytosis

- The receptors are generally concentrated in small pits on the outer surface of the cell membrane. These receptors are coated on the cytoplasmic side with a fibrillar protein called **calthrin** and contractile filaments of **actin** and **myosin**.
- Once the macromolecules (which is to be absorbed) have bound with the receptors, the entire pit invaginates inward, and the fibrillar protein by surrounding the invaginating pit causes it to close over the attached macromolecule along with a small amount of extracellular fluid.
- Then immediately, the invaginated portion of the membrane breaks away from the surface of the cell forming **endocyte vesicle** inside the cytoplasm of the cell.

Phagocytosis

- Phagocytosis involves the ingestion of large particles such as viruses, bacteria, cells, tissue debris or a dead cell.
- It occurs only in specialized cells such as **macrophages** and some of the **white blood cells.**
- Phagocytosis occurs in much the same way as pinocytosis.

Exocytosis

- Most of the endocytotic vesicles formed from pinocytosis fuse with lysosomes. Lysosomes empty

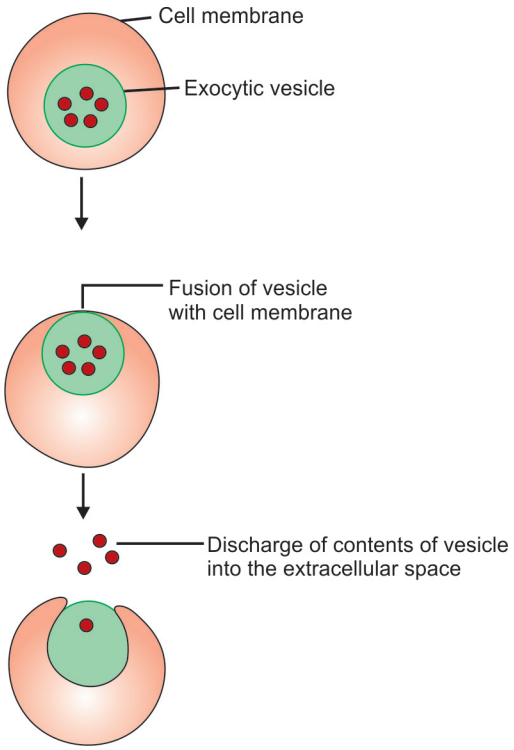


Figure 1.10: Stages in exocytosis

their acid hydrolases to the inside of the vesicle and begin hydrolyzing the proteins, carbohydrate, lipids and other substances in the vesicle.

- The macromolecular contents are digested to yield amino acids, simple sugars or nucleotides and they diffuse out of the vesicle and reused in the cytoplasm.
- Undigestible substances called residual body is finally excreted through the cell membrane by a process called **exocytosis**, opposite to endocytosis **(Figure 1.10).**
- The undigestible substances produced within the cytoplasm may be enclosed in membranes to form vesicles called **exocytic vesicles.**
- These cytoplasmic exocytic vesicles fuse with the internal surface of the plasma membrane.
- The vesicle then ruptures releasing their contents into the extracellular space and their membranes are retrieved (left behind) and reused.

CELL FRACTIONATION

- To obtain purified preparations of organelles, the tissue is first carefully broken up in a homogenizing apparatus using isotonic 0.25 M sucrose solution.
- Sucrose solution is used because it is not metabolized in most tissues and it does not pass through membranes readily and thus, does not cause inter organelles to swell.
- Then homogenate is centrifuged at a series of increasing centrifugal force. **(Figure 1.11).**
- The subcellular organelles, which differ in size and specific gravity, sediment at different rates and can be isolated from homogenate by differential centrifugation.
- The dense nuclei are sedimented first, followed by the mitochondria, and finally the microsomal fraction at the highest forces. After all the particulate matter has been removed, the soluble remnant is the cytosol.
- Organelles of similar sedimentation coefficient obviously cannot be separated by differential centrifugation. For example, mitochondria isolated in this way are contaminated with lysosome and peroxisomes. These may be separated by **isopyknic centrifugation technique.**

MARKER ENZYMES

- The purity of isolated subcellular fraction is assessed by the analysis of **marker enzymes.**
- Marker enzymes are the enzymes that are located exclusively in a particular fraction and thus become characteristic of that fraction.
- Analysis of marker enzymes confirms the identity of the isolated fraction and indicates the degree of contamination with other organelles. For example, isolated mitochondria have a high specific activity of cytochrome oxidase but low catalase and acid phosphatase, the catalase and acid phosphatase activities being due to contamination with peroxisomes and lysosomes respectively.
- Some typical subcellular markers are given in **Table 1.2.**

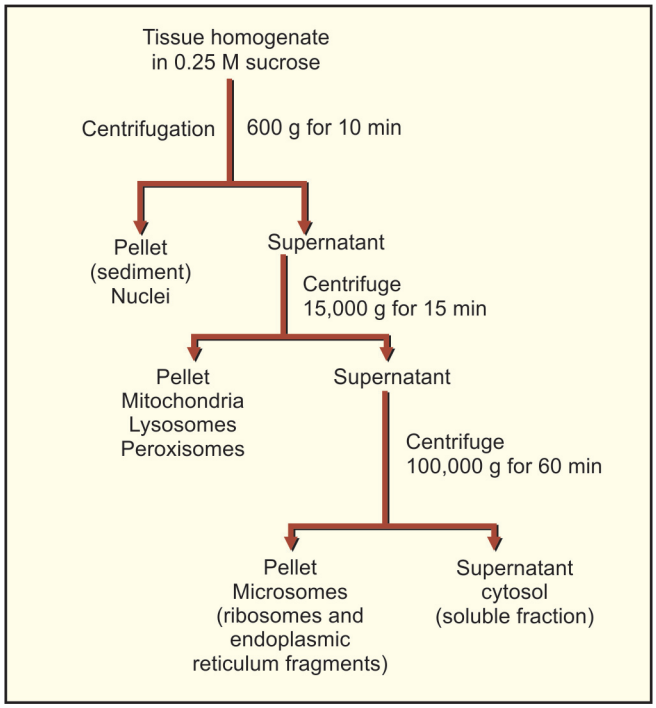


Figure 1.11: Subcellular fractionation of cell by differential centrifugation

Table 1.2: Marker enzymes of subcellular fractions

Fraction	*Enzymes*
Plasma membrane	5 Nucleotidase, Na^+-K^+-ATPase
Nucleus	DNA polymerase RNA polymerase
Endoplasmic reticulum Golgi bodies	Glucose-6-phosphatase Galactosyl transferase
Lysosomes	Acid phosphatase β-glucuronidase
Mitochondria	Succinate dehydrogenase Cytochrome C-oxidase
Peroxisomes	Catalase
Cytosol	Lactate dehydrogenase Glucose-6-phosphate dehydrogenase

SUMMARY

- Cells are the structural and functional units of living organism.
- In eukaryotes, the genetic material is surrounded by a nuclear envelop; prokaryotes have no such membrane.
- Cell membranes mainly consist of lipids, proteins and smaller proportion of carbohydrates that are linked to lipids and proteins.
- Electron microscopy has revealed the cell membrane as an organized structure consisting of a lipid bilayer primarily of phospholipids and penetrated protein molecules forming a mosaic-like pattern.
- The cytoplasm consists of the cytosol and organelles such as rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, mitochondria and nucleus.
- Lysosomes control the intracellular digestion of macromolecules.
- Peroxisome contains enzymes peroxidase and catalase, which converts hydrogen peroxide to water and oxygen.
- Mitochondria are called **power *plant*** of the cell. They are maternally inherited.
- The cytoplasm is permeated by a number of fibrillar elements that collectively form a supporting network. This network is called the **cytoskeleton.**
- Cytoskeleton includes microfilaments, microtubules and intermediate filaments.
- Various passive and active mechanisms are employed to transport molecules across membrane. Active transport occurs against concentration gradient which depends on the supply of energy (ATP).
- Facilitated diffusion permits the movement of ions and molecules from high to low concentration. Specific carrier proteins are involved in such processes. Whereas the movement of ions and molecules from low to high concentration by active transport requires energy.
- Macromolecules can enter or leave cells through mechanisms such as **endocytosis** or **exocytosis.**
- The subcellular organelles can be separated by differential centrifugation, on the basis of differences in sedimentation rates or density.
- Purity of subcellular fractions may be assessed by analyzing marker enzymes.

EXERCISE

Multiple Choice Questions (MCQs)

1. The following is the metabolic function of ER:
a) RNA processing
b) Fatty acid oxidation
c) Synthesis of plasma protein
d) ATP-synthesis

2. In biologic membranes, integral proteins and lipids interact mainly by:
a) Covalent bond
b) Both hydrophobic and covalent bond
c) Hydrogen and electrostatic bond
d) None of the above

3. Plasma membrane is made up of:
a) Lipid bilayer
b) Protein bilayer
c) Carbohydrate bilayer
d) Lipid single layer

4. Select the subcellular component involved in the formation of ATP:
a) Nucleus
b) Plasma membrane
c) Mitochondria
d) Golgi apparatus

5. Mitochondrial DNA is:
a) Maternal inherited
b) Paternal inherited
c) Maternal and paternal inherited
d) None of the above

6. All of the following statements about the nucleus are true, *except:*
a) Outer nuclear membrane is connected to ER
b) It is the site of storage of genetic material
c) Nucleolus is surrounded by a bilayer membrane
d) Outer and inner membranes of nucleus are connected at nuclear pores

7. Golgi apparatus is present in all of the following *except:*
a) RBC
b) Parenchymal cells
c) Skeletal muscle cells
d) Pancreatic cell

8. Peroxisomes arise from:
a) Golgi membrane
b) Lysosomes
c) Mitochondria
d) Pre-existing peroxisomes and budding off from the smooth ER

9. Na^+ - K^+ ATPase is the marker enzyme of:
a) Nucleus
b) Plasma membrane
c) Golgi bodies
d) Cytosol

Correct Answers for MCQs

1-c	2-c	3-a	4-c
5-a	6-c	7-a	8-d
9-b			

CHAPTER

2

Carbohydrate Chemistry

INTRODUCTION

The carbohydrates are widely distributed both in animal and plant tissues. Chemically, they contain the elements carbon, hydrogen and oxygen. The empirical formula of many simple carbohydrates is $\mathbf{[CH_2O]_n}$. Hence, the name ***"carbohydrate"***, i.e. hydrated carbon. They are also called ***"saccharides"***. In Greek, saccharon means sugar.

Although many common carbohydrates confirm the empirical formula $[CH_2O]_n$, others like deoxyribose, rhamnohexos do not. Some carbohydrates also contain ***nitrogen, phosphorus*** or ***sulfur.***

DEFINITION, CLASSIFICATION AND FUNCTIONS OF CARBOHYDRATES

Carbohydrates may be defined chemically as ***aldehyde*** or ***ketone*** derivatives of polyhydroxy (more than one hydroxy group) alcohols or as compounds that yield these derivatives on hydrolysis.

Functions of Carbohydrates

Carbohydrates have a wide range of functions. The following are few of them:

- Source of energy for living beings, e.g. ***glucose***
- Storage form of energy, e.g. ***glycogen*** in animal tissue and ***starch*** in plants
- Serve as structural component, e.g. ***glycosaminoglycans*** in humans, ***cellulose*** in plants and ***chitin*** in insects
- Non-digestable carbohydrates like cellulose, serve as dietary fibers
- Constituent of nucleic acids RNA and DNA, e.g. ***ribose*** and ***deoxyribose*** sugar
- Play a role in lubrication, cellular intercommunication and immunity
- Carbohydrates are also involved in detoxification, e.g. ***glucuronic acid***.

Classification of Carbohydrates

Carbohydrates are classified into three groups:

1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides.

Monosaccharides (Greek: Mono = one)

Monosaccharides are also called ***simple sugars.*** The term sugar is applied to carbohydrates that are soluble in water and sweet to taste. They consist of a single polyhydroxy aldehyde or ketone unit, and thus cannot

Table 2.1: Classification of monosaccharides and their examples

No.of Carbon	*Empirical formula*	*Type of sugar*	*Aldoses*	*Ketoses*
3	$C_3H_6O_3$	Trioses	Glyceraldehyde	Dihydroxyacetone
4	$C_4H_8O_4$	Tetroses	Erythrose	Erythrulose
5	$C_5H_{10}O_5$	Pentoses	Ribose, Xylose	Ribulose, Xylulose
6	$C_6H_{12}O_6$	Hexoses	Glucose, Galactose and Mannose	Fructose
7	$C_7H_{14}O_7$	Heptoses	Glucoheptose	Sedoheptulose

be hydrolyzed into a simpler form. They may be subdivided into two groups as follows:

1. Depending upon the number of carbon atoms they possess, e.g.
   - Trioses
   - Tetroses
   - Pentoses
   - Hexoses
   - Heptoses.
2. Depending upon the functional aldehyde (CHO) or ketone (C=O) group present:
   - Aldoses
   - Ketoses.

   Classification of monosaccharides based on the number of carbon and the type of functional group present with examples is given in **Table 2.1.** ***The most abundant monosaccharide in nature is six carbon sugar-D-glucose.*** Biologically important monosaccharides are listed in **Table 2.2.**

Oligosaccharides (Greek: oligo = few)

Oligosaccharides consist of a short chain of monosaccharide units (2 to 10 units), joined together by a characteristic bond called ***glycosidic bond*** which, on hydrolysis, gives two to ten molecules of simple sugar (monosaccharide) units. Oligosaccharides are subdivided into different groups based on the number of monosaccharide units present **(Table 2.3).**

Table 2.2: Biologically important monosaccharides

Type of monosaccharide	*Example*	*Importance*
Trioses	Glyceraldehyde and Dihydroxyacetone	• Intermediates in the glycolysis • Precursor of glycerol which is required for the formation of triacylglycerol and phospholipid
Tetroses	D-Erythrose	• Intermediate product of carbohydrate metabolism (Hexose monophosphate pathway)
Pentoses	D-Ribose	• Constituent of nucleic acid RNA and coenzymes, e.g. ATP, NAD, NADP and FAD • Intermediate product of pentose phosphate pathway
	D-Ribulose	• Intermediate product of pentose phosphate pathway
	D-Xylulose	• Constituent of proteoglycans and glycoproteins
	L-Xylulose	• An intermediate in uronic acid pathway
Hexoses	D-Glucose	• The main sugar of the body which is utilized by the tissue for energy purposes
	D-Fructose	• Can be converted to glucose in the liver and so used in the body for energy purpose
	D-Galactose	• Can be converted to glucose in the liver and metabolized • Synthesized in mammary gland to make the lactose of milk • A constituent of glycolipids, proteoglycans and glycoproteins
	D-Mannose	• A constituent of glycoprotein, glycolipids and blood group substances
Heptoses	Sedoheptulose	• An intermediate in the pentose phosphate pathway

Table 2.3: Classification of oligosaccharides and their examples

Type of oligosaccharide	*Number of monosaccharide*	*Example*	*Type of monosaccharide present*
Disaccharide	Two	Maltose	Glucose + Glucose
		Lactose	Glucose + Galactose
		Sucrose	Glucose + Fructose
Trisaccharide	Three	Raffinose	Glucose + Galactose + Fructose
Tetrasaccharide	Four	Stachyose	2 Molecules of Galactose + Glucose + Fructose
Pentasaccharide	Five	Verbascose	3 Molecules of Galactose + Glucose + Fructose

The disaccharides which have two monosaccharide units are the most abundant in nature. Oligosaccharides with more than three subunits are usually found in glycoproteins; such as blood group antigens.

Polysaccharides (Greek: Poly = many) or Glycans

Polysaccharides are polymers consisting of hundreds or thousands of monosaccharide units. They are also called ***glycans*** or ***complex carbohydrates.*** They may be either ***linear,*** (e.g. cellulose) or ***branched,*** (e.g. glycogen) in structure.

Polysaccharides have high molecular weight and are only sparingly soluble in water. They are not sweetish and do not exhibit any of the properties of aldehyde or ketone group. Polysaccharides are of two types **(Table 2.4).**

i. Homopolysaccharides (homoglycans)
ii. Heteropolysaccharides (heteroglycans).

Homopolysaccharides (Homoglycans)

- When a polysaccharide is made up of several units of one and the same type of monosaccharide unit, it is called homopolysaccharide.
- The most common homoglycans are:
  - Starch
  - Dextrins
  - Glycogen
  - Inulin
  - Cellulose.
- Some homopolysaccharides serve as a storage form of monosaccharides used as fuel, e.g. starch and glycogen, while others serve as structural elements in plants, e.g. cellulose.

Heteropolysaccharides (Heteroglycans)

- They contain two or more different types of monosaccharide units or their derivatives.
- Heteropolysaccharide present in human beings is glycosaminoglycans (mucopolysaccharides), e.g.
  - Heparin
  - Chondritin sulfate
  - Hyaluronic acid
  - Dermatan sulfate
  - Keratan sulfate
  - Blood group polysaccharides.

STRUCTURE OF GLUCOSE

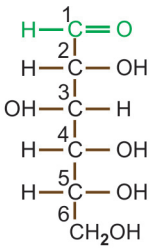
Physiologically and biomedically, glucose is the most important monosaccharide. The structure of glucose can be represented in the following ways **(Figure 2.1)**:

1. The straight chain structural formula (Fisher projection).
2. Cyclic formula (Ring structure or Haworth projection)

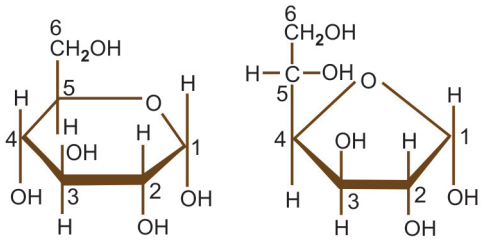
Table 2.4: Classification of polysaccharides

Polysaccharides

Homopolysaccharides or Homoglycans, e.g.	Heteropolysaccharides or Heteroglycans, e.g.
Starch	Glycosaminoglycans or Mucopolysaccharides
Dextrin	
Glycogen	
Cellulose	
Inulin	



Straight chain structure of D-glucose (Fisher projection formula)



Ring structure or Haworth projection formula of glucose

Figure 2.1: Structure of D-Glucose

- Monosaccharide in solution is mainly present in ring form. In solution, aldehyde (CHO) or ketone (C=O) group of monosaccharide react with a hydroxy (OH) group of the same molecule forming a bond ***hemiacetal*** or ***hemiketal*** respectively.
- The aldehyde group of glucose at C-1 reacts with alcohol (OH) group of C-5 or C-4 to form either six membered ring called **glucopyranose** or five membered ring called **glucofuranose**, respectively. **(Figure 2.1)**
- However, in case of glucose, the six membered **glucopyranose** is much more stable than the glucofuranose ring. In the case of fructose, the more stable form is **fructofuranose.**

ISOMERISM

The compounds possessing identical molecular formula but different structures are referred to as ***isomers.*** The phenomenon of existence of isomers is called ***isomerism.*** (Greek 'isos' means *equal,* 'meros' means parts). The five types of isomerism exhibited by sugar are as follows:

1. Ketose-aldose isomerism
2. D and L isomerism
3. Optical isomerism
4. Epimerism
5. Anomerism.

Ketose-Aldose isomerism

Glucose and fructose are isomers of each other having the same chemical (molecular) formula $C_6H_{12}O_6$, but they differ in structural formula with respect to their

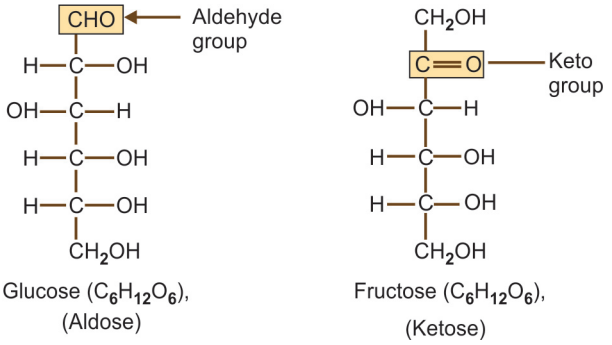


Figure 2.2: Ketose-Aldose isomerism

functional groups. There is a ***keto*** group in position two of fructose and an ***aldehyde*** group in position one of glucose **(Figure 2.2).** This type of isomerism is known as ***ketose-aldose isomerism.***

D and L isomerism

- D and L isomerism depends on the orientation of the H and OH groups around the asymmetric carbon atom adjacent to the terminal primary alcohol carbon, e.g. carbon atom number 5 in glucose determines whether the sugar belongs to D or L isomer.
- When OH group on this carbon atom is on the right, it belongs to **D-series,** when it is on the left, it is the member of the **L-series.** The structures of D and L-glucose based on the reference monosaccharide, D and L glyceraldehyde, a three carbon sugar **(Figure 2.3).**

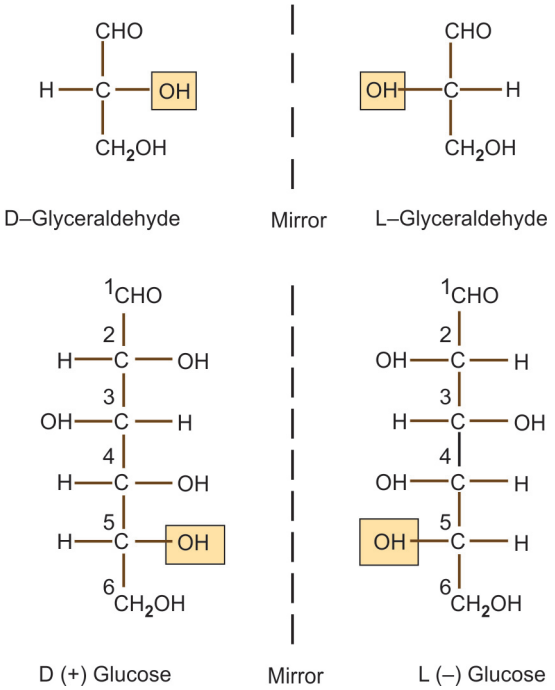


Figure 2.3: D and L isomers (enantiomeric pairs) of glyceraldehyde and glucose

- D and L isomers are mirror images of each other. These two forms are called **enantiomers.**
- Most of the monosaccharides in the living beings belong to the D-series.

Optical Isomerism

- The presence of **asymmetric carbon** atoms exhibits **optical activity** on the compound. Optical activity is the capacity of a substance to rotate the plane polarized light passing through it.
- When a beam of plane-polarized light is passed through a solution of an optical isomer, it will be rotated either to the right and is said to be **dextrorotatary (d)** or (+) or to the left and is said to be, **levorotatory (l)** or (-).
- When equal amount of D and L isomers are present, the resulting mixture has no optical activity. Since the activity of each isomer cancel one another, such a mixture is said to be a ***racemic*** or ***dl mixture.***

Epimerism

When two monosaccharides differ from each other in their configuration around a single **asymmetric carbon** (other than anomeric carbon) atom, they are referred to as **epimers** of each other.

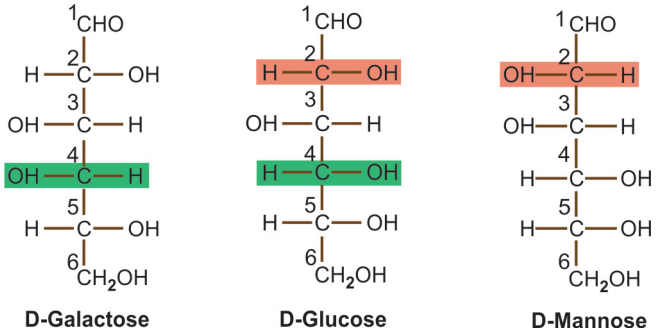


Figure 2.4: Epimers of glucose

For example, galactose and mannose are two epimers of glucose **(Figure 2.4)**. They differ from glucose in the configuration of groups (H and OH) around C-4 and C-2 respectively. Galactose and mannose are not epimers of each other as they differ in configuration at two asymmetric carbon atoms around C-2 and C-4.

Anomerism

α and β Anomerism

The predominant form of glucose and fructose in a solution are not an open chain. Rather, the open chain

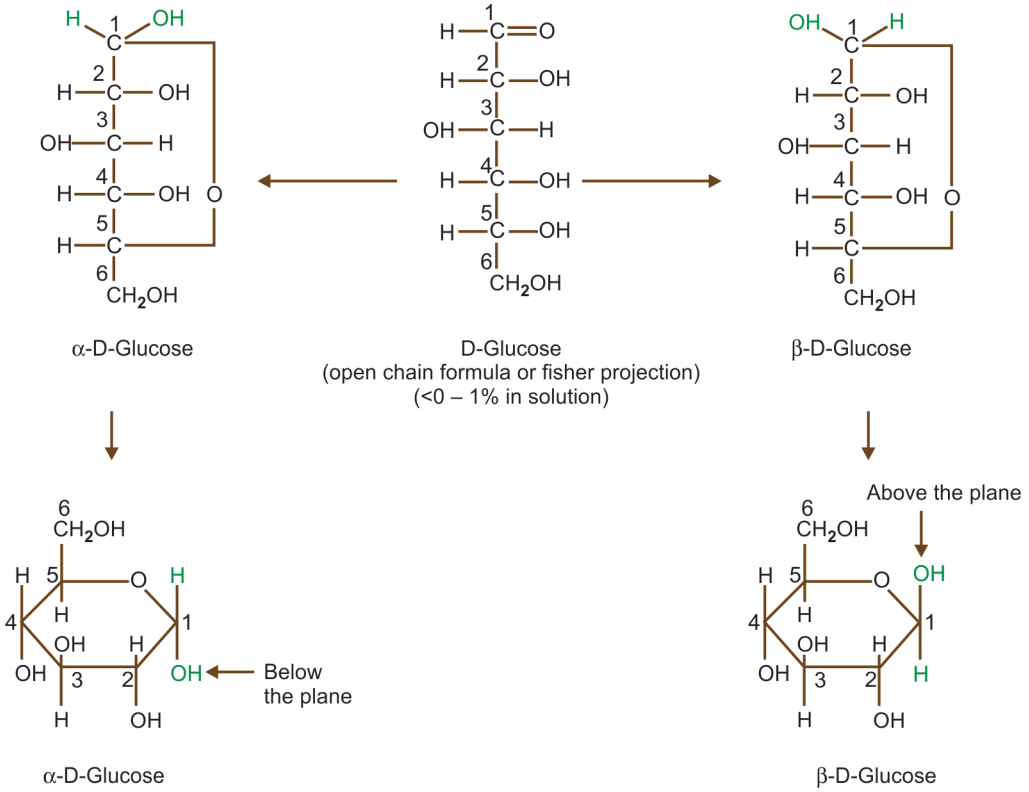


Figure 2.5: Formation of α and β anomers

form of these sugar in solution cyclize into rings. An additional asymmetric center is created when glucose cyclizes. Carbon-1 of glucose in the open chain form, becomes an asymmetric carbon in the ring form **(Figure 2.5)** and two ring structures can be formed. These are:

- α-D-glucose
- β-D-glucose.

The designation α means that the hydroxyl group attached to C-1 is below the plane of the ring, β means that it is above the plane of the ring. The C-1 carbon is called the ***anomeric carbon atom*** and so, α and β forms are **anomers.**

MUTAROTATION

- Mutarotation is defined as the change in the **specific optical rotation** by the interconversion of α and β forms of D-glucose to an equilibrium mixture.
- In water, α-D-glucopyranose and β-D-glucopyranose interconvert through the open chain form of the sugar. This interconversion was detected by optical rotation.
- The specific rotation $[\alpha]_D$, of the α and β anomers of D-glucose are +112° and +18.7°. When crystalline sample of either anomers is dissolved in water, specific rotation $[\alpha]_D$ changes with time until an equilibrium value of + 52.7° is attained **(Figure 2.6)**. This change called ***mutarotation,*** results from the formation of an equilibrium mixture containing about **one-third α-anomers** and **two-thirds β-anomers.** Very little of the open chain form of glucose is present (<1%).
- Non-reducing sugar cannot show mutarotation due to the absence of the free anomeric OH group.

CHEMICAL PROPERTIES OF MONOSACCHARIDES

Some of the important chemical properties of monosaccharides are:

1. Action of Strong Acids: Furfural formation
2. Action of Alkalies: Enolization
3. Oxidation: Sugar acid formation
4. Reduction: Sugar alcohol formation
5. Action of phenylhydrazine: Osazone formation.

Action of Strong Acids (Furfural Formation)

On heating a sugar with mineral acids (H_2SO_4 or HCI), the sugar loses water and forms ***furfural derivatives.*** These may condense with α-naphthol, thymol or resorcinol to produce colored complexes. This is the basis of the:

- Molisch's test
- Seliwanoff's test
- Bial's test
- Tollen's-phloroglucinol-HCI test.

Action of Alkalies (Enolization)

- On treatment with dilute aqueous alkalies, both aldoses and ketoses are changed to **enediols.** Enediol is the enol form of sugar because two OH groups are attached to the double bonded carbon **(Figure 2.7).**
- Enediols are good reducing agents and form basis of the **Benedict's** test and **Fehling's test**.
- Thus, alkali enolizes the sugar and thereby causes them to be strong reducing agents.
- Through the formation of a common 1, 2-enediol, glucose, fructose and mannose may isomerize into each other in a dilute alkaline solution **(Figure 2.7)**.

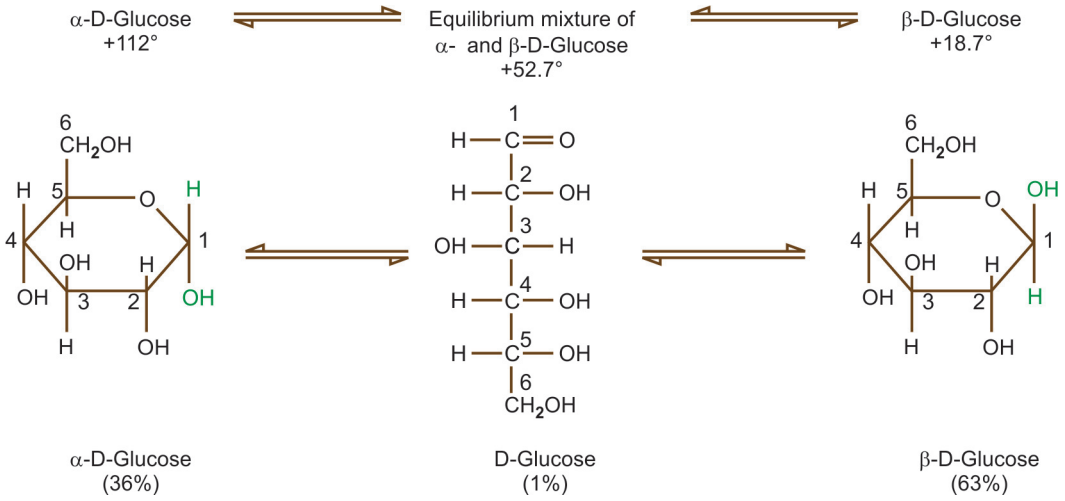


Figure 2.6: Mutarotation of glucose

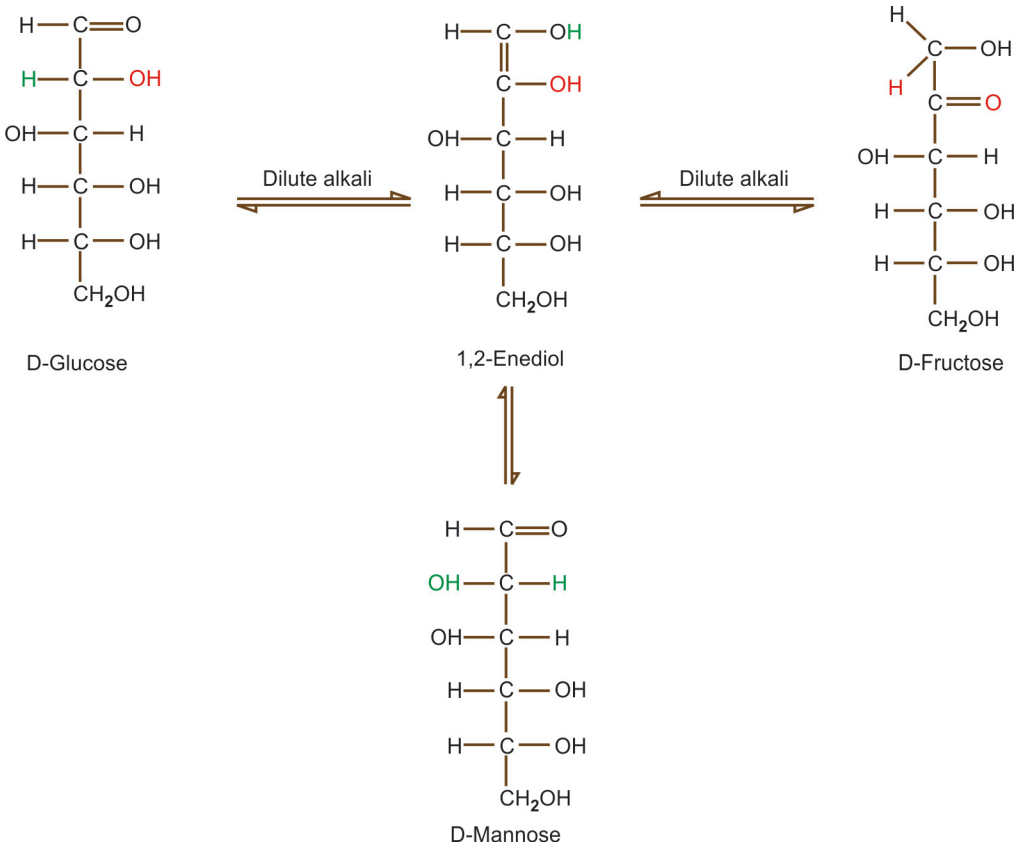


Figure 2.7: Action of alkali on reducing sugar

Oxidation (Sugar Acid Formation)

- When aldoses oxidize under proper conditions they may form:
  - Aldonic acid
  - Saccharic acids
  - Uronic acid.
- Oxidation of an aldose with hypobromous acid (HOBr), which acts as an oxidizing agent gives **aldonic acid.** Thus, glucose is oxidized to gluconic acid **(Figure 2.8)**.

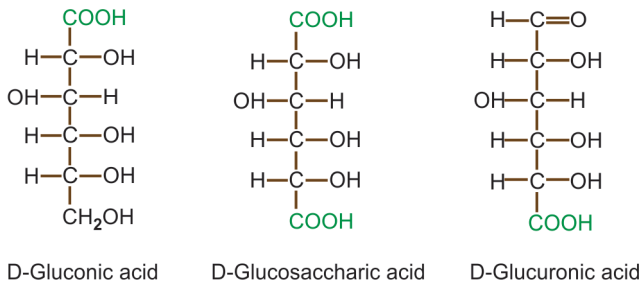


Figure 2.8: Sugar acids produced by oxidation of glucose

- Oxidation of aldoses with nitric acid under proper conditions convert both aldehyde and terminal primary alcohol groups to carboxyl groups, forming **saccharic acid.**
- When an aldose is oxidized in such a way that the terminal primary alcohol group is converted to carboxyl without oxidation of the aldehyde group (usually by specific enzymes), a uronic acid is formed **(Figure 2.8)**.

Reduction to Form Sugar Alcohol

Both aldoses and ketoses may be reduced by enzymes or non-enzymatically to the corresponding polyhydroxy alcohols. The alcohols formed from glucose, mannose, fructose and galactose are given in **Figure 2.9**.

- Manitol, the sugar alcohol derived from mannose, is frequently used medically as an osmotic diuretic to reduce cerebral edema.
- Sorbitol, the sugar alcohol derived from glucose, often accumulates in the lenses of diabetics and produces cataracts.

Figure 2.9: Reduction of sugar to form alcohol

Action of Phenylhydrazine (Osazone Formation)

Osazones are yellow or orange crystalline derivatives of reducing sugars with phenylhydrazine and have a characteristic crystal structure, which can be used for identification and characterization of different sugars having closely similar properties (like maltose and lactose).

The reactions of glucose with phenylhydrazone are shown in **Figure 2.10**.

- Osazone formed from glucose, mannose and fructose are identical because these are identical in the lower four carbon atoms.
- The osazone crystals of glucose and of the reducing disaccharides, lactose and maltose differ in forms **(Figure 2.11)**:
  - Glucosazone are needle shaped
  - Lactosazone are powder puff or tennis ball shaped
  - Maltosazone are sunflower shaped.
- Non-reducing sugars like the disaccharide sucrose cannot form osazone due to the absence of a free carbonyl (CHO or C = O) group in them.

GLYCOSIDE FORMATION

- Glycosides are formed when the hydroxyl group of anomeric carbon of a monosaccharide reacts with **OH** or **NH** group of second compound that may or may not be a carbohydrate. The bond so formed is known as **glycosidic** or **glycosyl bond.**
- The monosaccharides are joined by glycosidic bonds to form **disaccharides, oligosaccharides** and **polysaccharides.**
- In disaccharides, the glycosidic linkage may be either α or β depending on the configuration of the atom attached to the anomeric carbon of the sugar **(Figure 2.16).**

Therapeutic importance of glycosides

- Glycosides are found in many drugs, e.g. in antibiotic ***streptomycin.***

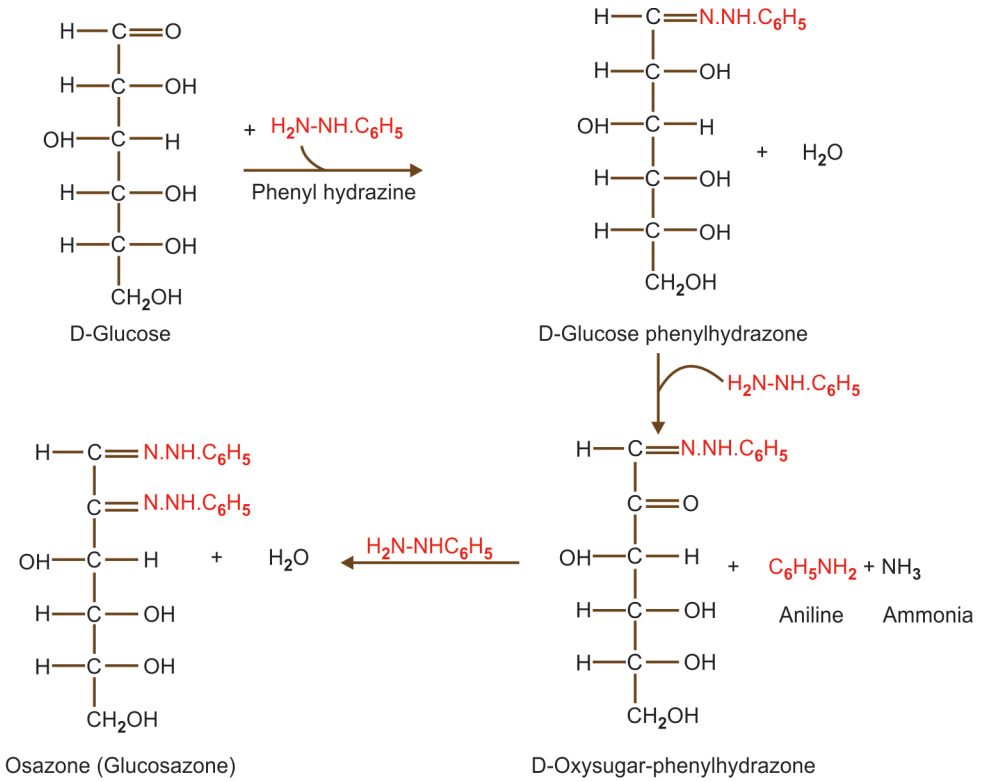


Figure 2.10: Formation of glucosazone

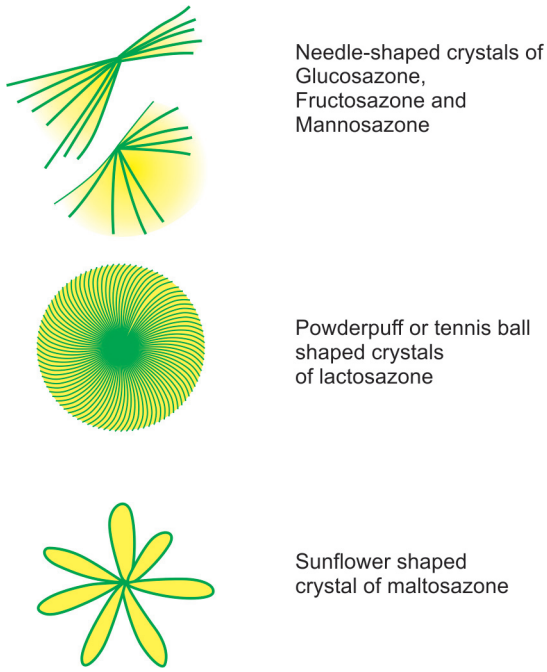


Figure 2.11: Structure of different osazones

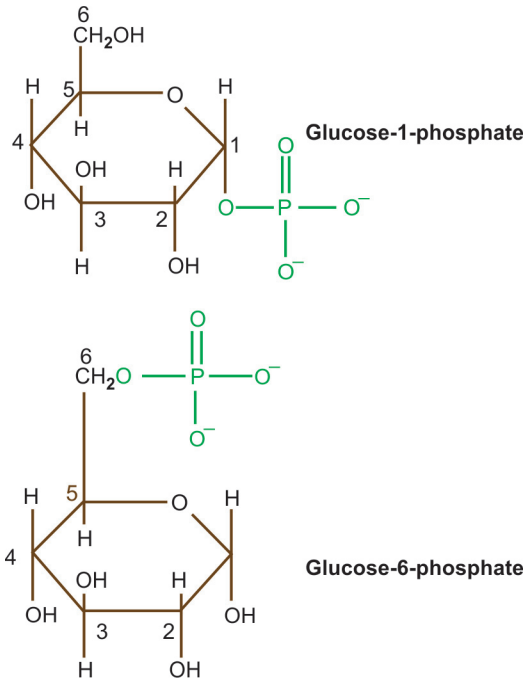


Figure 2.12: Phosphoric acid ester of glucose

- Cardiac glycosides such as ***Ouabain*** and ***digoxin*** increase heart muscle contraction and are used for treatment of congestive heart failure.
- ***Anthracycline*** glycosides (daunorubicin and doxirubicin), ***Daunorubicin*** is used to treat leukemia. ***Doxirubicin*** is used to treat a wide range of cancers.

DERIVATIVES OF MONOSACCHARIDES

Some important sugar derivatives of monosaccharides are:

- Phosphoric acid ester of monosaccharides
- Amino sugar
- Deoxy sugars
- Sugar acids
- Sugar alcohols
- Neuraminic acid
- Sialic acid.

Phosphoric Acid Ester of Monosaccharides

These are formed from the reaction of phosphoric acid with hydroxyl group of the sugar, e.g. glucose-1-phosphate or glucose-6-phosphate **(Figure 2.12).**

Importance

- Phosphorylation of sugar within cells is essential to prevent the diffusion of the sugar out of the cell.
- Nucleic acids (RNA and DNA) of cell nuclei also contain sugar phosphates of ribose and deoxyribose.

Amino Sugar

Amino sugars have a hydroxyl group replaced by an amino or an acetylated amino (acetylamino) group. For example, glucosamine, N-acetyl glucosamine **(Figure 2.13),** galactosamine and mannosamine.

Importance of amino sugar

- Amino sugars are components of glycolipid (ganglioside), glycoprotein and proteoglycans (glycosaminoglycans).
- Several antibiotics, e.g. erythromycin, carbomycin contain amino sugar.

Deoxy Sugars

Deoxy sugars possess a hydrogen atom in place of one of their hydroxy groups **(Figure 2.14)**, e.g. 2-deoxyribose found in nucleic acid DNA.

Sugar Acids

Sugar acids are produced by oxidation of the monosaccharides, for example:

- Ascorbic acid or vitamin C (not synthesized by human beings) is required for the synthesis of collagen. It acts as water soluble antioxidant.

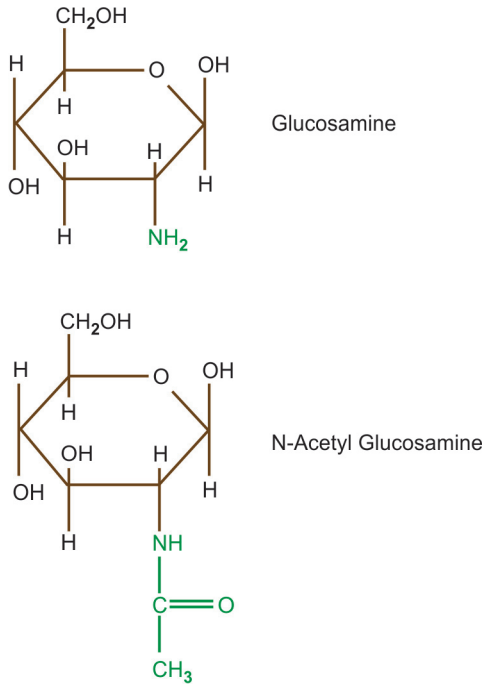


Figure 2.13: Structure of amino sugars

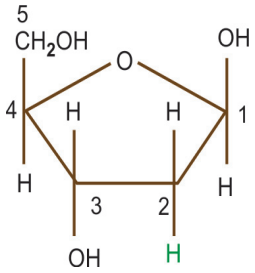


Figure 2.14: Structure of 2-deoxyribose sugar

- Glucuronic acid (uronic acid) (*See properties of monosaccharide-oxidation*).

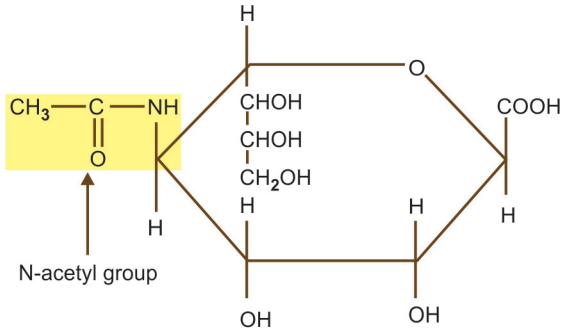
Sugar Alcohols

Discussed in properties of monosaccharide-reduction. They are not metabolically very active but have some medical importance in that they are used as non-glucose forming sweetners in food stuffs for diabetics, ***sorbitol*** and ***xylitol*** are the most commonly used.

Neuraminic Acid

Neuraminic acid is a nine carbon sugar derived from mannosamine (an epimer of glucosamine) and pyruvate.

Mannosamine + Pyruvate ————> Neuraminic acid



Sialic acid or N-Acetylneuraminic acid (NANA)

Figure 2.15: Structure of sialic acid or NANA

Sialic Acid

Sialic acids are acetylated derivatives of neuraminic acid in which amino (NH_2) or hydroxy (OH) group is acetylated **(Figure 2.15)**.

Importance

Sialic acids are constituents of both glycoproteins and glycolipids (ganglioside).

DISACCHARIDES

- Disaccharides consist of two monosaccharide units.
- They are crystalline, water soluble and sweet to taste.
- They are subclassified on the basis of the presence or absence of free reducing (aldehyde or ketone) group **(Table 2.5).**
  1. Reducing disaccharides with free aldehyde or keto group, e.g. maltose, lactose
  2. Non-reducing disaccharides with no free aldehyde or keto group, e.g. sucrose.

Maltose

- Maltose contains two glucose residues, joined by glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other, leaving one free anomeric carbon of the second glucose residue, which can act as a reducing agent. Thus, maltose is a ***reducing disaccharide***.
- The numerical description like (1 → 4) of glycosidic bond represents the number of carbon atoms that connect the two sugars as shown in **Figure 2.16**. The sugar contributing anomeric carbon is written first.
- Maltose is produced as an intermediate product in the digestion of starch and glycogen by the action of the enzyme α-amylase.

Table 2.5: Classification of disaccharides

Disaccharides

Reducing (with free aldehyde or ketone group)		*Non-reducing (absence of free aldehyde or ketone group)*	
Example	*Constituent*	*Example*	*Constituent*
Maltose	Glucose + Glucose	Sucrose	Glucose + Fructose
Lactose	Galactose + Glucose	Trehalose	Glucose + Glucose
Isomaltose	Glucose + Glucose		

Isomaltose

- It consists of two glucose molecules linked by an (α-1 → 6) glycosidic bond.
- Isomaltose is a disaccharide derived from the digestion of starch or glycogen. It is hydrolyzed to glucose in the intestinal tract by an enzyme called ***isomaltase.***

Lactose (Milk sugar)

- It is present in milk. Lactose contains one unit of **β-galactose** and one unit of **β-glucose** that are linked by a **β (1 → 4) glycosidic linkage (Figure 2.16).**
- The anomeric carbon of the glucose unit is available for oxidation and thus lactose is a reducing disaccharide.
- Lactose is hydrolyzed to glucose and galactose by ***lactase*** enzyme in human beings.

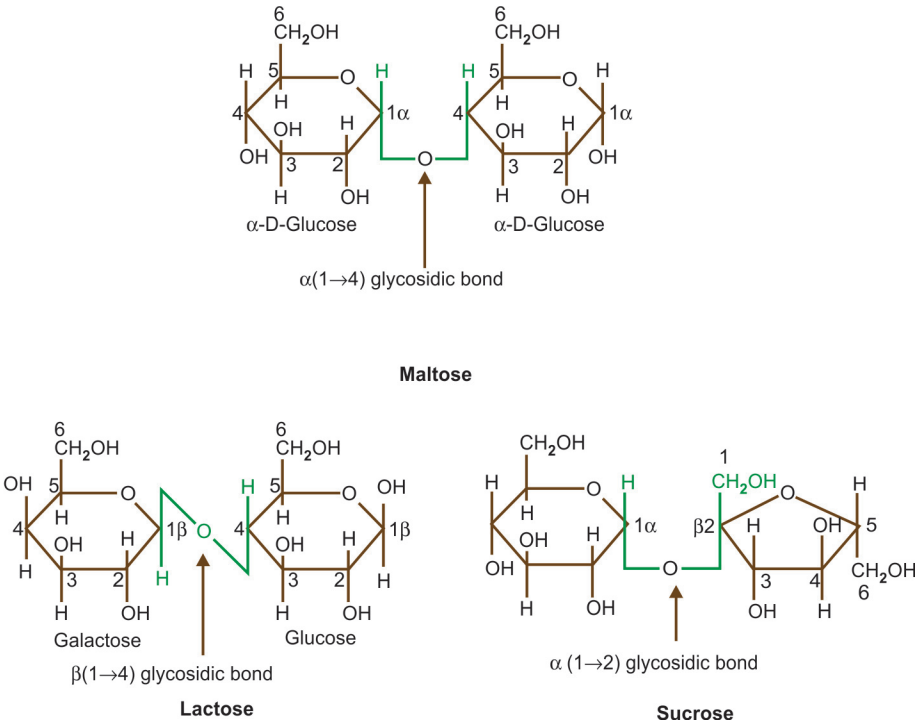


Figure 2.16: Structure of disaccharides

Sucrose (Common Table Sugar)

- Sucrose is a disaccharide of glucose and fructose. It is formed by plant but not by human beings. Sucrose is an intermediate product of photosynthesis. Sucrose is the commonly used **table sugar.**
- In contrast to maltose and lactose, sucrose contains no free anomeric carbon atom. The anomeric carbon of both glucose and fructose are involved in the glycosidic bond **(Figure 2.16).** Sucrose is therefore, a non-reducing sugar.
- Sucrose is hydrolyzed to fructose and glucose by an enzyme ***sucrase*** which is also called ***invertase.***

POLYSACCHARIDES (GLYCANS)

Carbohydrates composed of ten or more monosaccharide units or their derivatives (such as amino sugars and uronic acids) are generally classified as **polysaccharides.** Polysaccharides are colloidal in size. In polysaccharides, monosaccharide units are joined together by glycosidic linkages. Another term for polysaccharides is a ***"glycans".*** Polysaccharides are subclassified in two groups **(Table 2.4).**

1. Homopolysaccharides (Homoglycans): When a polysaccharide is made up of several units of one and the same type of monosaccharide unit only, it is called homopolysaccharide.
2. Heteropolysaccharides (Heteroglycans): They contain two or more different types of monosaccharide units or their derivatives.

Homopolysaccharides or Homoglycans

Starch

It is the storage form of glucose in plants, e.g. in potato, in grains and seeds and in many fruits. Starch is composed of two constituents viz. ***amylose*** and ***amylopectin.***

Amylose

Amylose is a linear polymer of D-glucose units joined by α-1 → 4 glycosidic linkages **(Figure 2.17).**

Amylopectin

Amylopectin is structurally identical to those of amylose (α-1→ 4 glycosidic linkages) but with side chains joining them by α-1→ 6 linkages **(Figure 2.18).**

Thus, amylopectin is a branched polymer having both α-(1 → 4) and α-(1 → 6) linkages. The branch points in amylopectin are created by α-1 → 6 bonds and occur at an interval of 20 to 30 units of glucose. **Figures 2.17** and **2.18** represent diagrammatically the difference in the amylose and amylopectin molecules.

Dextrin

Partial hydrolysis of starch by acids or α-amylase (enzyme) produces substances known as dextrins. These also occur in honey. All dextrins have few free aldehyde groups and can show mild reducing property. They are not fermented by yeast.

Glycogen (Animal Starch)

Glycogen is the major storage form of carbohydrate (glucose) in animals, found mostly in liver and muscle. It is often called ***animal starch.***

The structure of glycogen is similar to that of amylopectin, except that it is more highly branched, **(Figure 2.19)** having α-(1 → 6) linkages at intervals of about 8 to 10 glucose units.

Function

- The function of muscle glycogen is to act as a readily available source of glucose for energy within muscle itself.
- Liver glycogen is concerned with storage and maintenance of the blood glucose.

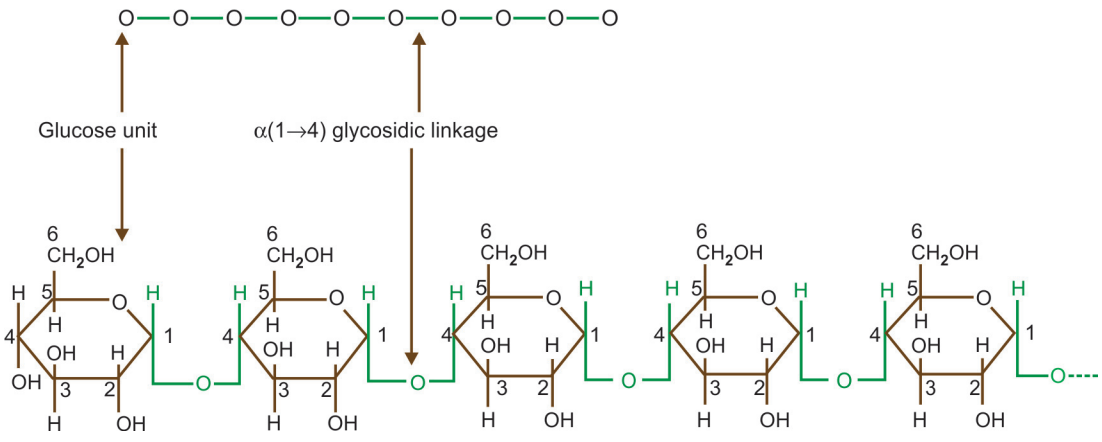


Figure 2.17: Structure of amylose

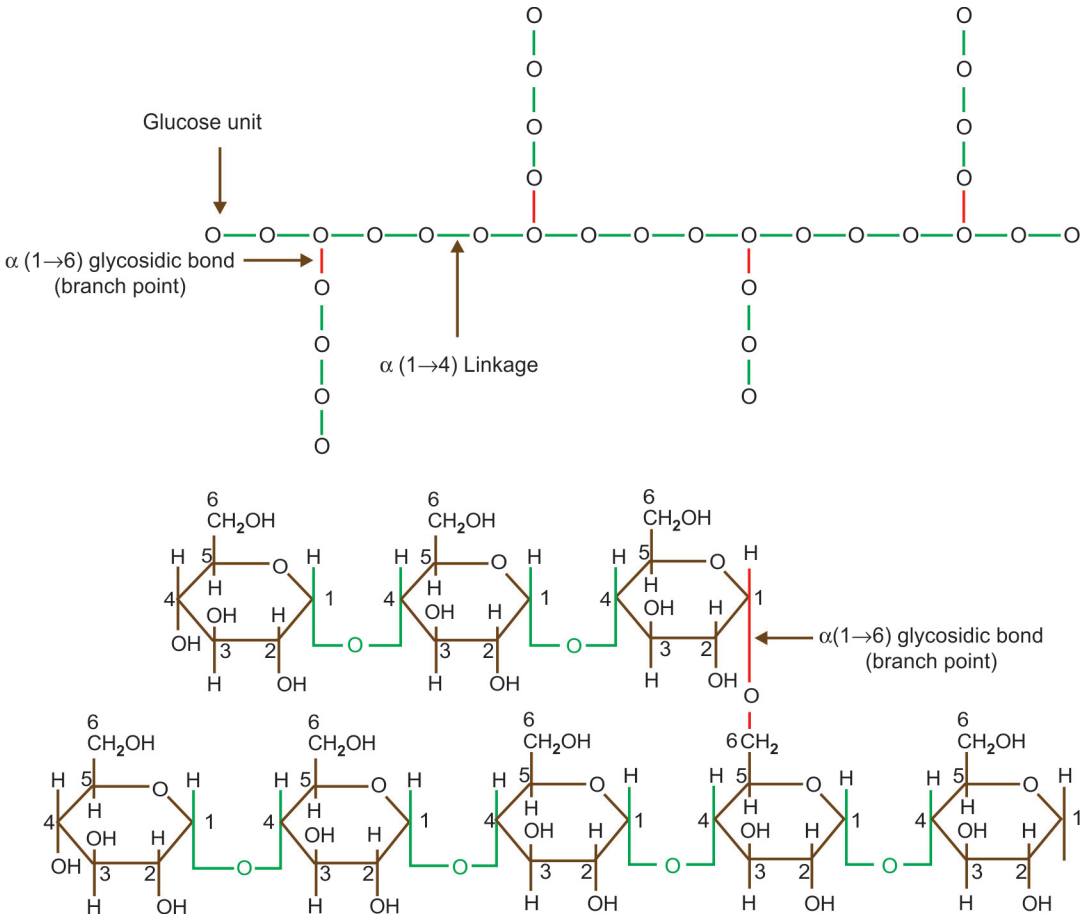


Figure 2.18: Structure of amylopectin

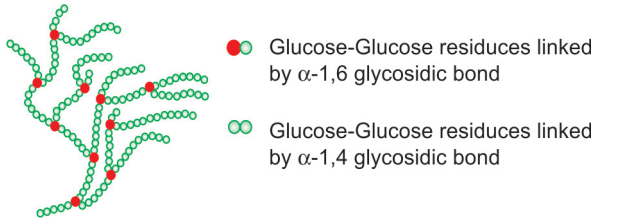


Figure 2.19: Diagrammatic representation of glycogen molecule

Cellulose

Cellulose is the chief constituent of cell wall of plants. It is an ***unbranched polymer*** of glucose and consists of long straight chains which are linked by **β-(1→4) glycosidic linkages** and not α-(1→ 4) as in amylose **(Figure 2.20)**.

Since humans lack an enzyme **cellulase** that can hydrolyze the β-(1→ 4) glycosidic linkages, ***cellulose cannot be digested and absorbed*** and has no food value unlike starch. However, the ruminants can utilize cellulose because they have in their digestive tract microorganisms whose enzymes hydrolyze cellulose.

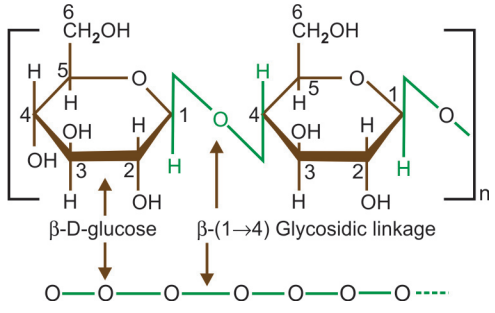


Figure 2.20: Structure of cellulose

Importance of Cellulose

For human cellulose has nutritional significance.

- Cellulose is a component of fiber in the diet.
- Although there is no known metabolic requirement for fiber, yet high fiber diet is associated with reduced incidence of a number of diseases like:
  - Cardiovascular disease
  - Colon cancer
  - Diabetes
  - Diverticulosis.
- Cellulose is present in unrefined cereals. It increases bulk of stool, aids intestinal motility, acts as a stool softener and prevents constipation.

Inulin

Inulin is a polymer of ***D-Fructose (Fructosans)*** linked together by β-(1 → 2) glycosidic linkage. It occurs in the tubers of some plants, e.g. chicory, bulb of onion and garlic. Inulin is not hydrolyzed by α-amylase but is hydrolyzed by ***inulinase,*** which is not present in the humans and so it is not utilized as food.

Clinical importance of Inulin

Inulin has clinical importance as it is used in the studies of ***glomerular filtration rates (kidney function test).***

Heteropolysaccharides or Heteroglycans

Glycosaminoglycans (GAGs) or Mucopolysaccharides (Table 2.6)

Structure of GAG

- A GAG is an unbranched heteropolysaccharide, made up of **repeating disaccharides.**
  - One component of which is always an **amino sugar** (hence the name glycosaminoglycans), either **D-glucosamine** or **D-galactosamine.**
  - The other component of the repeating disaccharide (except in the case of keratan sulfate) is a **uronic acid,** either **L-glucuronic acid** or its epimer **L-iduronic acid.**
- Thus, GAG is a polymer of **[uronic acid-amino sugar]$_n$**
- This polymer is attached covalently to extracellular proteins called ***core protein*** (except hyaluronic acid) to form ***proteoglycans.***
- A resulting structure resembles a ***"bottle brush"*** **(Figure 2.21).**
- The proteoglycan monomer associates with a molecule of hyaluronic acid to form ***proteoglycan aggregates*** **(Figure 2.22).**

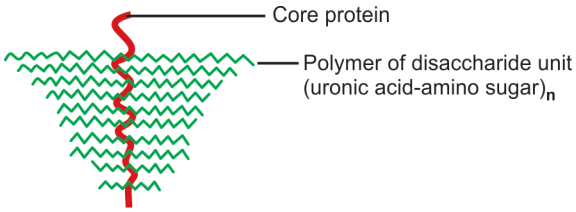


Figure 2.21: Bottle brush structure of proteoglycan monomer

Table 2.6: Structure and functions of glycosaminoglycans (GAGs)

GAG	*Disaccharide unit*	*Function*
Hyaluronic acid	N-Acetyl glucosamine -Glucuronic acid	Serves as lubricant and shock absorber, facilitates cell migration in embryogenesis, morphogenesis, wound healing
Condroitin sulfate	N-Acetyl-galactosamine-Glucuronic acid	Provides an endoskeletal structure helping to maintain their shape. Has a role in compressibility of cartilage in weight bearing
Keratan sulfate	N-Acetyl-glucosamine-Galactose (no uronic acid)	Transparency of cornea
Dermatan sulfate	N-Acetyl-galactosamine-L-Iduronic acid	Transparency of cornea and maintains the overall shape of the eye
Heparin	Glucosamine-Glucuronic acid or Iduronic acid	Serves as an anticoagulant, causes release of lipoprotein lipase from capillary walls
Heparan sulfate	Same as heparin except that some glucosamine are acetylated	Component of plasma membrane where it may act as receptor and may also participate in the mediation of cell growth, cell-to-cell communication

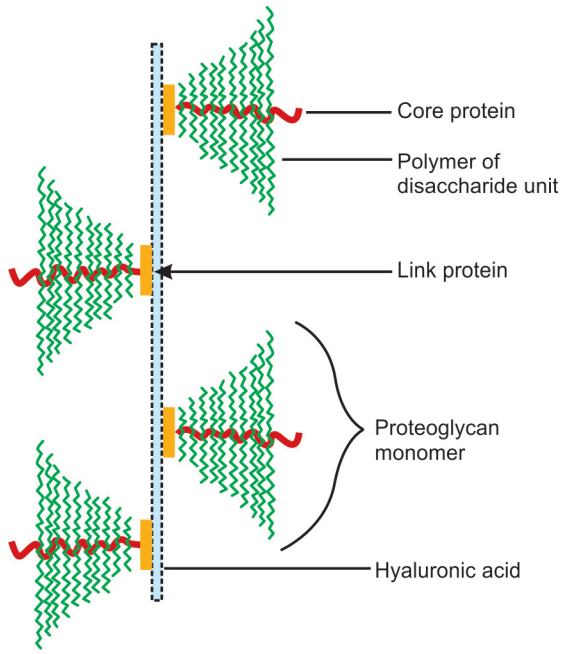


Figure 2.22: Proteoglycan aggregate

- The association is stabilized by additional small proteins called ***link proteins.***
- With the exception of hyaluronic acid, all the GAGs contain **sulfate** group.
- The amount of carbohydrates in proteoglycans is usually much greater than is found in a glycoprotein and may comprise upto 95 percent of its weight.

Occurrence of GAGs

Glycosaminoglycans are found in the:
- Synovial fluid of joints
- Vitreous humor of the eye
- Arterial walls
- Bones
- Cartilage.

Functions of GAGs

- They are major components of the extracellular matrix or ground substance.
- GAGs carry sulfate and carboxyl groups which give them a negative charge and have special ability to bind large amounts of water, thereby producing a gel-like matrix which functions as a cushion against mechanical shocks.
- They act as *"**molecular sieves**"*, determining which substances enter and leave cells.
- They also give resilience (elasticity) to cartilage, permitting compression and re-expansion.
- They lubricate joints both at the surface of cartilage and in synovial fluid.
- The viscous lubricating properties of mucous secretions are also due to the presence of glycosaminoglycans, which led to the original naming of these compounds as ***mucopolysaccharides.***

Types of GAGs

- The diferent types of GAGs are:
  - Hyaluronic acid
  - Condroitin sulfate
  - Keratan sulfate
  - Dermatan sulfate
  - Heparin
  - Heparin sulfate.
- The different types of GAGs differ from each other in the following properties:
  - Uronic acid composition
  - Amino sugar composition
  - Linkage between amino sugar and uronic acid
  - Chain length of the disaccharide polymer
  - Presence or absence of sulfate groups and their position of attachment to the sugar
  - The nature of the core protein to which they are attached
  - Their tissue and subcellular distribution and their biological function.

Table 2.6 describes structure and functions of different types of glycosaminoglycans.

GLYCOPROTEINS

- Glycoproteins are proteins to which oligosaccharides are covalently attached to their polypeptide chain.
- Glycoproteins contain much shorter carbohydrate chain than proteoglycans.
- The distinction between glycoproteins and proteoglycans may be based on the amount of carbohydrate.
  - Glycoproteins contain less than 4 percent carbohydrate in the molecule.
  - Proteoglycans contain more than 4 percent carbohydrate.

Functions of Glycoproteins

- Almost all the plasma proteins of humans are glycoproteins, except albumin.
- Many integral membrane proteins are glycoproteins.
- Most proteins that are secreted, such as antibodies, hormones and coagulation factors are glycoproteins.

- Glycoproteins serve as **lubricant** and **protective agent**, e.g. mucin of gastrointestinal and urogenital tracts.
- Glycoproteins also serve as transport molecules, such as **transferrin** and **ceruloplasmin.**

SUMMARY

- Carbohydrates are polyhydroxy aldehydes or ketones.
- Carbohydrates are classified as monosaccharides, oligosaccharides and polysaccharides.
- Sugars with free, oxidizable anomeric carbons are called reducing sugars.
- Disaccharides consists of two monosaccharides joined by the glycosidic bond, e.g. lactose, maltose and sucrose.
- Polysaccharides (glycans) contain many monosaccharide units in glycosidic linkage. Some function as storage forms of carbohydrates, e.g. starch in plants and glycogen (animal starch) in animals.
- Glycogen is branched polymer of glucose having α-(1 → 4) linkages in the main chain and α-(1 → 6) linkages at the branch points.
- Proteoglycans and glycosaminoglycans contains sugar derivatives such as amino sugar, uronic acids and sialic acids, which are associated with structural components of the tissues.
- Glycoproteins are proteins attached to oligosaccharides. Many cell surface proteins and extracellular proteins are glycoproteins.

EXERCISE

Multiple Choice Questions (MCQs)

1. All the following are composed exclusively of: glucose, *except*:
a) Glycogen b) Starch
c) Lactose d) Maltose

2. The sugar residues of glycogen are:
a) In α-(1 → 4) and α-(1 → 6) linkages
b) In β-(1 → 4) linkages
c) Fructosans
d) None of the above

3. Which of the following statements is true for fructose?
a) It is an aldose sugar
b) It usually exists in the pyranose form
c) It is involved in the formation of sucrose
d) Carbon 1 is the anomeric carbon atom

4. D-galactose and D-glucose are:
a) Epimers of each other
b) Enantiomers of each other
c) Anomers of each other
d) Mirror images of each other

5. Which of the following carbohydrate is dietary fiber?
a) Cellulose b) Starch
c) Glycogen d) Inulin

6. Glycosaminoglycans are:
a) Disaccharide
b) Homoglycans
c) Heteroglycans
d) None of the above

7. Which of the following glycosaminoglycans is unsulfated?
a) Chondroitin sulfate
b) Heparin
c) Hyaluronic acid
d) Keratan sulfate

8. Which of the following GAGs does not contain uronic acid?
a) Hyaluronic acid
b) Keratan sulfate
c) Heparin
d) Heparan sulfate

9. Inulin is:
a) Fructosans b) Glycans
c) Mannans d) Xylans

10. Which of the following is not hydrolyzed by α-amylase?
a) Starch b) Glycogen
c) Cellulose d) Dextrin

Correct Answers for MCQs

1-c	2-a	3-c	4-a
5-a	6-c	7-c	8-b
9-a	10-c		

CHAPTER

3

Chemistry of Lipids

INTRODUCTION

Lipids are a major source of energy for the body besides their various other biochemical function and their role in cellular structure. Lipids are a heterogenous group of water insoluble (hydrophobic) organic molecules. Lipids include fats, oils, steroids, waxes and related compounds.

This chapter introduces the chemistry and functions of lipids.

DEFINITION, CLASSIFICATION AND FUNCTIONS OF LIPIDS

Definition of Lipids

Lipids may be defined as organic substances insoluble in water but soluble in organic solvents like chloroform, ether and benzene. **They are esters of fatty acids with alcohol esters and are utilizable by the living organism.**

Classification of Lipids

There are many different methods of classifying lipids. The most commonly used classification of lipids is modified from Bloor as follows:

1. Simple lipids
2. Complex or compound lipids
3. Derived lipids.

Simple Lipids

These are esters of fatty acids with various alcohols. Depending on the type of alcohols, these are sub-classified as:

1. Neutral fats or triacylglycerol or triglycerides
2. Waxes.

Neutral fats or triacylglycerol or triglycerides

These are esters of fatty acids with alcohol ***glycerol,*** e.g. tripalmitin. Because they are ***uncharged,*** they are termed as ***neutral fat.*** The fat we eat are mostly triglycerides. A fat in liquid state is called an **oil**, e.g. vegetable oils like groundnut oil, mustard oil, corn oil, etc.

Waxes

True waxes

These are esters of fatty acids with higher molecular weight ***monohydric long chain alcohols.*** These compounds have no importance as far as human metabolism is concerned. For example,

- Lanolin (from lamb's wool)
- Bees-wax
- Spermacetic oil (from whales).

These are widely used in pharmaceutical, cosmetic and other industries in the manufacture of lotions, ointments and polishes.

Other waxes

These are esters of fatty acid with alcohol, e.g.

- Cholesterol forms cholesterol ester
- Retinol (vitamin A) forms vitamin A ester
- Cholecalciferol (vitamin D) forms vitamin D ester.

Complex or Compound Lipids

These are esters of fatty acids, with alcohol containing additional (prosthetic) groups. These are subclassified according to the type of prosthetic group present in the lipid as follows:

1. Phospholipids
2. Glycolipids
3. Lipoproteins.

Phospholipids

Lipids containing, in addition to fatty acids and an alcohol, a ***phosphoric acid*** residue. They also have nitrogen containing bases and other substituents. Phospholipids may be classified on the basis of the type of alcohol present in them as:

- Glycerophospholipids
- Sphingophospholipids.

Glycerophospholipids

The alcohol present is ***glycerol.*** Examples of glycerophospholipids are:

- Phosphatidyl choline (lecithin)
- Phosphatidyl ethenolamine (cephalin)
- Phosphatidyl serine
- Phosphatidyl inositol
- Lysophospholipid
- Plasmalogens
- Cardiolipins.

Sphingophospholipids

The alcohol present is **sphingosine**, e.g.

- Sphingomyelins.

Glycolipids

Lipids containing fatty acid, alcohol **sphingosine** and additional residue are ***carbohydrates*** with ***nitrogen base.*** They do not contain phosphate group. These sugar containing sphingolipids are also called ***glycosphingolipids.*** For example:

- Cerebrosides
- Gangliosides.

Lipoproteins

Lipoproteins are formed by combination of lipid with a prosthetic group protein, e.g. serum lipoproteins like:

- Chylomicrons
- Very low density lipoprotein (VLDL)
- Low density lipoprotein (LDL)
- High density lipoprotein (HDL).

Derived Lipids

Derived lipids include the products obtained after the hydrolysis of simple and compound lipids which possess the characteristics of lipids, e.g.

- Fatty acids
- Steroids
- Cholesterol
- Lipid soluble vitamins and hormones
- Ketone bodies.

Functions of Lipids

Lipids serve as:

- **Storage form of energy:** The fats and oils are used almost universally as stored forms of energy in living organisms.
- **Structural Lipids:** Lipids are major structural components of membranes, e.g. phospholipids, glycolipids and sterols.
- **Cholesterol,** a sterol, is a precursor of many **steroid hormones, vitamin D** and is also an important component of plasma membrane.
- Lipid acts as a **thermal insulator** in the subcutaneous tissues and around certain organs.
- Nonpolar lipids act as **electrical insulators** in neurons.
- Lipids are important dietary constituents because of the fat soluble vitamins and essential fatty acids which are present in the fat of natural foods.
- Lipids help in absorption of fat soluble vitamins (A,D,E and K). They act as a solvent for the transport of fat soluble vitamins.

FATTY ACIDS

Fatty acids are carboxylic acids with hydrocarbon chains ($-CH_2-CH_2-CH_2-$) and represented by a chemical formula **R-COOH,** where R stands for hydrocarbon chain. The fatty acids are ***amphipathic*** in nature, i.e. each has hydrophilic (COOH) and hydrophobic (hydrocarbon chain) groups in the structure.

Table 3.1: Some naturally occurring fatty acids

Common name	*Carbon atoms*	*Double bonds*	*Position of double bond*	*Unsaturated fatty acid class*
Saturated fatty acids				
Acetic acid	1	0		
Propionic acid	3	0		
n-butyric acid	4	0		
Valeric acid	5	0		
Louratic acid	12	0		
Myristic acid	14	0	-	-
Palmitic acid	16	0		
Stearic acid	18	0		
Arachidic acid	20	0		
Behenic acid	22	0		
Lignoceric acid	24	0		
Unsaturated fatty acid				
• **Monoenoic acid** (one double bond)				
Palmitoleic acid	16	1	9	ω-7
Oleic acid	18	1	9	ω-9
Nervonic acid	24	1	15	ω-9
• **Dienoic acids** (two double bonds)				
Linoleic acid	18	2	9, 12	ω-6
• **Trienoic acid** (three double bonds)				
Linolenic acid	18	3	9, 12, 15	ω-3
• **Tetraenoic acid** (four double bonds)				
Arachidonic acid	20	4	5,8,11,14	ω-6
• **Pentaenoic acid** (five double bonds)				
Clupandonic acid	22	5	7, 10,13, 16, 19	ω-3
• **Docosa hexaenoic acid** (DHA)				
Cervonic acid	22	6	4,7,10,13,16,19	ω-3

Some naturally occurring fatty acids are given in **Table 3.1.**

Classification of Fatty Acids

Fatty acids are classified into four major classes **(Figure 3.1).**

1. Straight chain fatty acids
2. Branched chain fatty acids
3. Substituted fatty acids
4. Cyclic fatty acids.

Straight Chain Fatty Acids

Fatty acids, in which the carbons are arranged linearly, are subclassified into two classes:

i. Saturated fatty acids
ii. Unsaturated fatty acids.

Saturated fatty acids

There is no double bond in the hydrocarbon chain of these fatty acids. Saturated fatty acids are subclassified into two classes:

a. **Even carbon acids** carry even number of carbons, e.g. palmitic acid and stearic acid.
b. **Odd carbon acids** carry odd number of carbons, e.g. propionic acid.

Unsaturated fatty acids

These contain double bonds in their hydrocarbon chains.

These are subclassified according to the number of double bonds present in the structure as follows:

a. Monoenoic or monounsaturated fatty acid
b. Polyenoic or polyunsaturated fatty acid.

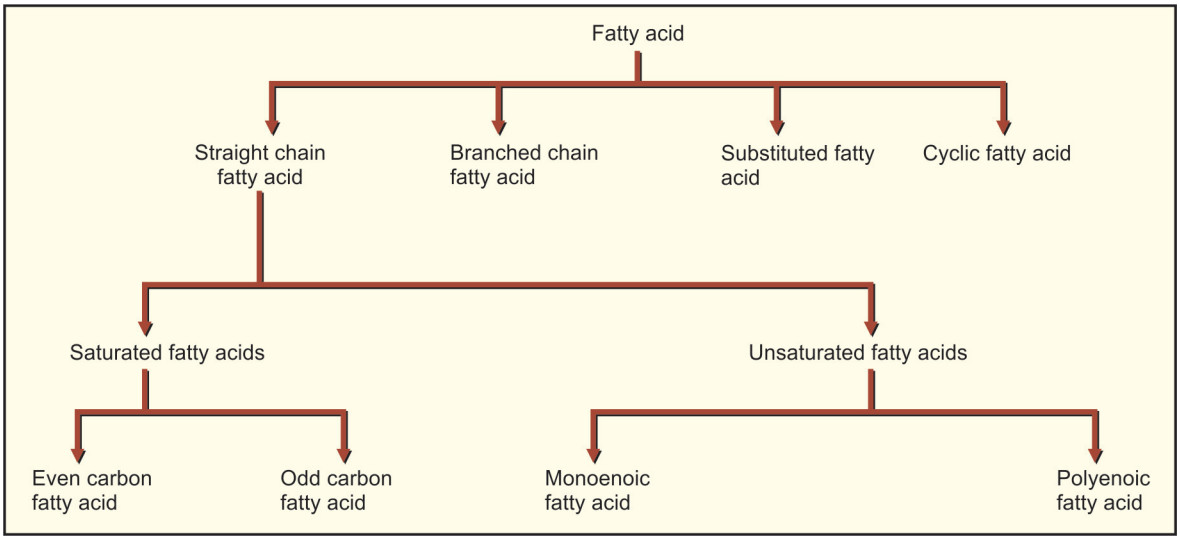


Figure 3.1: Classification of fatty acids

- **Monoenoic** or monounsaturated fatty acids carry a single double bond in the molecule, e.g. oleic acid.
- **Polyenoic** or polyunsaturated fatty acids contain two or more double bonds; for example:
  - **Dienoic acids** have two double bonds, e.g. linoleic acid present in soyabean, sunflower, saffola and groundnut oil.
  - **Trienoic acids** have three double bonds, e.g. Linolenic acid present in poppyseed oil, linseed oil.
  - **Tetraenoic acid** with four double bonds, e.g. arachidonic acid present in groundnuts.

Branched Chain Fatty Acids

These are less abundant than straight chain acids in animals and plants, e.g.

- Isovaleric acid
- Isobutyric acid.

Substituted Fatty Acids

In substituted fatty acids one or more hydrogen atoms have been replaced by another group, e.g.

- Lactic acid of blood
- Cerebronic acid and oxynervonic acids of brain glycolipids
- Ricinoleic acid of castor oil.

Cyclic Fatty Acids

Fatty acids bearing cyclic groups are present in some bacteria and seed lipids, e.g. hydnocarpic acid (Chaulmoogric acid) of chaulmoogra seed.

Functions of Fatty Acids

Fatty acids have three major physiological functions.

1. They serve as building blocks of ***phospholipids*** and ***glycolipids.*** These amphipathic molecules are important components of biological membranes.
2. Fatty acid derivatives serve as ***hormones,*** e.g. ***prostaglandins***.
3. Fatty acids serve as a major fuel for most cells.

Numbering of Fatty Acid Carbon Atoms (Figure 3.2)

- Fatty acid carbon atoms are numbered starting at the carboxyl terminus.

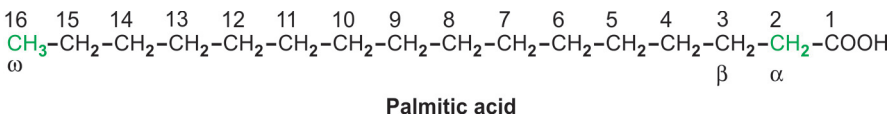


Figure 3.2: Numbering of fatty acid carbon

- Carbon atoms 2 and 3 are often referred to as α and β respectively.
- The methyl carbon atom at the distal end of the chain is called omega (ω) carbon.

Representation of Double Bonds of Fatty Acids

Two systems are used to designate the position of double bond:

- C-system
- ω- or n-system.

C-System

In C-system (i.e. C_1 being the carboxyl carbon) the position of double bond is represented by the symbol Δ (delta), followed by a superscript number.

For example, oleic acid is a C_{18} fatty acid with one double bond between carbon number 9 and 10 is represented as C: 18:1: Δ^9 **(Figure 3.3).**

ω– or n-System

In this system, '*ω*' or '*n*' refers to the carbon of their terminal methyl group in a fatty acid. In *ω*-system or n-system, the oleic acid is denoted as C: 18:1:ω-9 **(Figure 3.3)** to indicate that:

- *ω*-9 represents the double bond position which is found between 9th and 10th carbon atoms, the first carbon atom being that of the terminal methyl group. This method is widely used by nutritionists.
- Naturally occurring unsaturated fatty acids belong to ***ω-9, ω-6*** and ***ω-3*** series. For example,
  - *ω*-9 : Oleic acid (C:18:1:*ω*-9)
  - *ω*-6 : Linoleic acid (C:18:2:*ω*-6)
    Arachidonic acid (C:20:4:*ω*-6)
  - *ω*-3 : Linolenic acid (C:18:3:*ω*-3)

ESSENTIAL FATTY ACIDS

Fatty acids, that are required for optimal health and cannot be synthesized by the body and should be supplied in the diet are called ***essential fatty acids.***

They are polyunsaturated fatty acids, namely **linoleic acid** and **linolenic acid. Arachidonic acid** can be synthesized from linoleic acid. Therefore, in deficiency of linoleic acid, arachidonic acid also becomes essential fatty acids.

Humans lack the enzymes to introduce double bonds at carbon atoms beyond C_9 in the fatty acid chain. Hence, humans cannot synthesize linoleic acid and linolenic acid having double bonds beyond C_9. And thus, linoleic and linolenic are the essential fatty acids.

Functions of Essential Fatty Acids (EFA)

Synthesis of Eicosanoids

Linoleic acid and linolenic acid supplied by the diet are the precursors for the synthesis of a variety of other unsaturated fatty acids. Arachidonic acid, a fatty acid derived from linoleic acid is an essential precursor of eicosanoids, which include:

- Prostaglandins
- Thromboxanes
- Prostacyclin
- Leukotrienes.

Maintenance of Structural Integrity

EFAs are required for membrane structure and function. These fatty acids are important constituents of phospholipids in cell membrane and help to maintain the structural integrity of the membrane.

Development of Retina and Brain

Docosahexaenoic acid (DHA: ω-3), which is synthesized from linolenic acid is particularly needed for development of the brain and retina during the neonatal period.

Antiatherogenic Effect

Essential fatty acids increase esterification and excretion of cholesterol, thereby lowering the serum cholesterol level. Thus, essential fatty acids help to prevent the atherosclerosis.

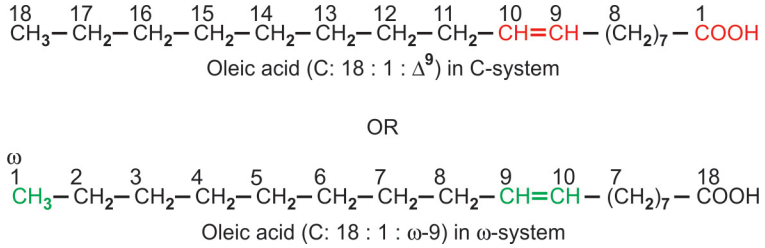


Figure 3.3: Representation of double bonds of unsaturated fatty acid

Essential Fatty Acid Deficiency

- Deficiency of EFAs is characterized by scaly skin, eczema (in children), loss of hair and poor wound healing.
- Impaired lipid transport and fatty liver may occur due to deficiency of EFAs.
- EFAs deficiency decreases efficiency of biological oxidation.

REACTIONS OF LIPIDS

Saponification

Hydrolysis of a fat by ***alkali*** is called ***saponification.*** The products are glycerol and the alkali salts of the fatty acids, which are called **soaps (Figure 3.4).** Acid hydrolysis of a fat yields the free fatty acids and glycerol.

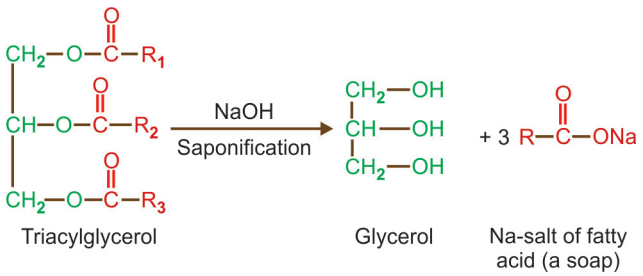


Figure 3.4: Saponification of fat

Hydrogenation

Hydrogenation of unsaturated fats in the presence of a catalyst (nickel) is known as ***"hardening".*** It is commercially valuable as a method of converting these liquid fats, usually of plant origin into solid fats as margarines, vegetable *ghee,* etc.

Peroxidation

Peroxidation (auto-oxidation) of lipids exposed to oxygen is responsible not only for deterioration of foods (rancidity) but also for damage to tissues *in vivo*, where it may be a cause of **cancer.** Lipid peroxidation is a chain reaction generating free radicals that initiate further peroxidation. To control and reduce peroxidation, humans make use of ***antioxidants.*** Naturally occurring antioxidants include vitamin E (tocopherol) and β-carotene (provitamin A), which are lipid soluble and vitamin C, which is water soluble.

Rancidity

The unpleasant odor and taste, developed by natural fats upon aging, is referred to as ***"rancidity".*** Rancidity may be due to ***hydrolysis*** or ***oxidation*** of fat.

- **Rancidity due to hydrolysis:** Naturally occurring fats, particularly those from animal sources, are contaminated with enzyme **lipase.** The action of lipase brings about partial hydrolysis of glycerides of fat.
- **Rancidity may also be caused by various oxidative processes.** For example, oxidation at the double bonds of unsaturated fatty acids of glycerides may form peroxides, which then decompose to form aldehydes of unpleasant odor and taste, this process is increased by exposure to light or heat.

Many natural vegetable fats and oils may contain antioxidants like vitamin E which prevent onset of rancidity. Therefore, vegetable fats can be preserved for a longer time than animal fats.

CHARACTERIZATION OF FAT (TESTS FOR PURITY OF FAT)

Fats are characterized and their purity assayed by the following tests:

Saponification Number

It is defined as, number of mgs of KOH required to saponify one gm of fat. It is inversely proportional to the molecular weight of fat. This value is high in fats containing a short chain fatty acids. For example, the saponification number of:

- Butter = 220
- Coconut oil = 260

Iodine Number

The number of gms of iodine required to saturate 100 gms of a given fat is known as iodine number. Since iodine is taken up by the double bonds, a high iodine number indicates a high degree of unsaturation of the fatty acids in fat, e.g.

- Butter fat = 27
- Coconut oil = 8
- Linseed oil = 200.

Iodine number is important in the identification of the fat or oil as well as is used for identification of adulteration of oils.

Acid Number

Number of mg of KOH required to neutralize the free fatty acids present in one gm of fat is known as acid number. The acid number indicates the degree of rancidity of the given fat. Acid number is directly proportional to the rancidity. The edibility of a fat is inversely proportional to the acid number.

Refined oil should not contain free fatty acids. The presence of free fatty acids in any oil indicates that it is not pure.

Reichert Meissl Number

The number of ml of 0.1 N alkali, required to neutralize the volatile fatty acids distilled from 5 gm of fat, e.g. the Reichert Meissl value for:

- Butter = 26
- Coconut oil = 7.

It is less than one for other edible oils. The admixture of certain fats may be used to prepare synthetic butter which may simulate butter in most of the constants except RM value and hence, can be detected.

TRIACYLGLYCEROLS OR TRIACYLGLYCERIDES OR NEUTRAL FAT

These are esters of fatty acids with glycerol. Triacylglycerol consists of three fatty acids, which are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of neutral fat. **(Figure 3.5).**

- Triacylglycerols containing the same kind of fatty acid in all three positions are called ***simple triacylglycerols.***
- ***Mixed triacylglycerols*** contain two or more different fatty acids. The fatty acid on carbon 1 is usually saturated. That on carbon 2 is usually unsaturated and that on carbon 3 can be either of the two. The stereospecific numbering (sn) of the glycerol carbon atom is shown in **Figure 3.5**.
- As the polar hydroxyl groups of glycerol and polar carboxyl groups of the fatty acids are bound in ester linkages, triacylglycerols are nonpolar, hydrophobic and neutral (in charges) molecules, essentially insoluble in water.
- The presence of the unsaturated fatty acid(s) in triacylglycerol decreases the melting temperature of the lipid and remains in liquid form (oil).
- Vegetable oils such as corn and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature.
- Triacylglycerols containing only saturated fatty acids, such as beef fat, are white greasy solids at room temperature.
- Triacylglycerols are highly concentrated storage form of metabolic energy.

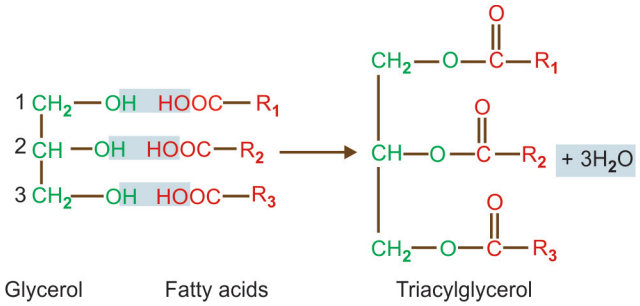


Figure 3.5: Glycerol and triacylglycerol

PHOSPHOLIPIDS

- These are made up of ***fatty acid, glycerol*** or other alcohol, ***phosphoric acid*** and ***nitrogenous base.***
- Phospholipids are the major lipid constituents of cell membranes.
- Like fatty acids, phospholipids are ***amphipathic*** in nature, i.e. each has a hydrophilic or polar head (phosphate group) and a long hydrophobic tail (containing two fatty acid chains) **(Figure 3.6).**

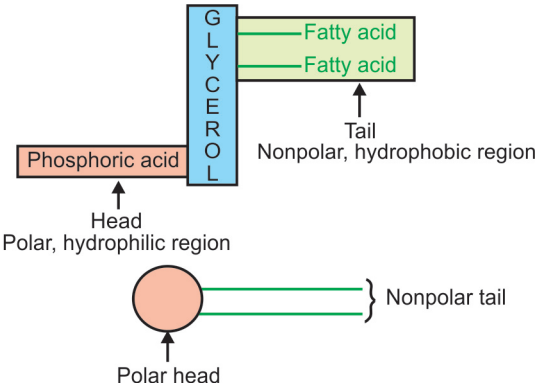


Figure 3.6: Diagrammatic representation of amphipathic phospholipid

Classification of Phospholipids

There are two classes of phospholipids **(Figure 3.7):**

1. Glycerophospholipids or phosphoglycerides, that contain glycerol as the alcohol.
2. Sphingophospholipids that contain sphingosine as the alcohol.

Glycerophospholipids or Phosphoglycerides

- Phospholipids derived from glycerol are called **phosphoglycerides** or **glycerophospholipids.**

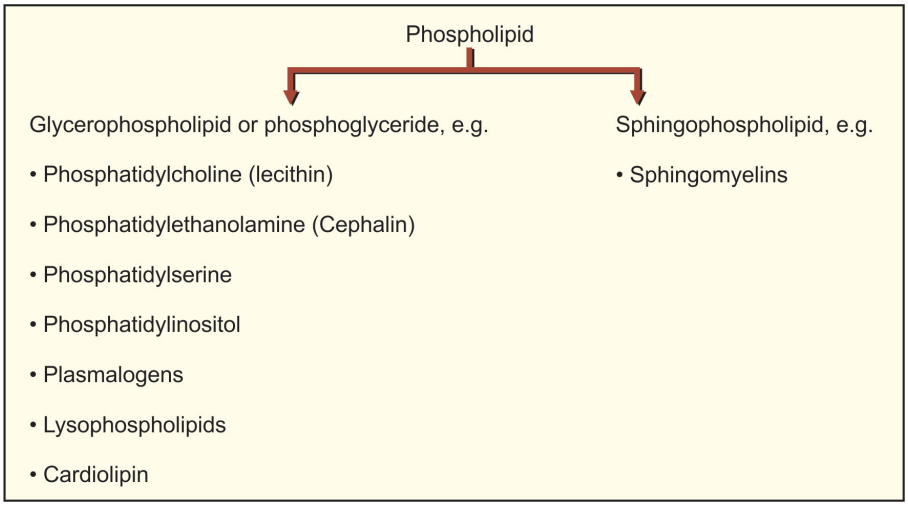


Figure 3.7: Classification of phospholipids

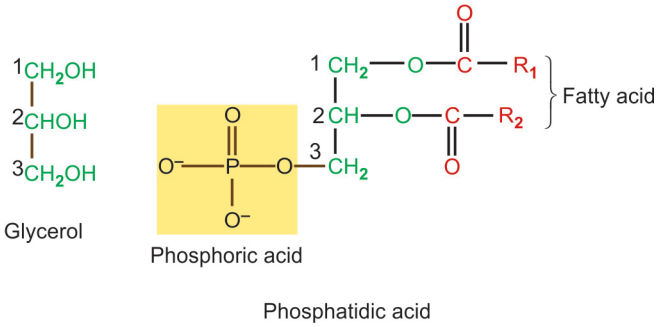


Figure 3.8: Structure of glycerol and phosphatidic acid

- In glycerophospholipid, the hydroxyl groups at C_1 and C_2 of glycerol are esterified with two fatty acids. The C_3 hydroxyl group of the glycerol is esterified to phosphoric acid and resulting compound called, ***phosphatidic acid*** **(Figure 3.8).**
- Phosphatidic acid is a key intermediate in the biosynthesis of other glycerophospholipids.
- In glycerophospholipid, phosphate group of phosphatidic acid becomes esterified with the hydroxyl group of one of the several nitrogen base or other groups. Different types of glycerophospholipids are discussed below.

Phosphatidylcholine (lecithin)

- These are glycerophospholipids containing choline **(Figure 3.9).** These are most abundant phospholipids of the cell membrane having both structural and metabolic functions.
- Dipalmitoyl lecithin is an important phosphatidylcholine found in lungs, secreted by **pulmonary type II epithelial** cell. It acts as a ***lung surfactant*** and is necessary for normal lung function. It reduces surface tension in the alveoli, thereby prevents alveolar collapse (adherence of the inner surfaces of the lungs).

> ***Acute Pulmonary Respiratory Distress Syndrome (RDS)***
> - RDS in infants is associated with insufficient ***surfactant dipalmitoyl phosphatidylcholine*** production.
> - The lungs of immature infants do not have enough type II epithelial cells to synthesize sufficient amounts of dipalmitoyl phosphatidylcholine (DPPC).
> - In its absence, the lungs tend to collapse and this condition is known as ***respiratory distress syndrome.***

Phosphatidylethanolanine (Cephalin)

- They differ from lecithin in having nitrogenous base ethanolamine in place of choline **(Figure 3.9).**
- ***Thromboplastin*** (coagulation factor III), which is needed to initiate the clotting process, is composed mainly of cephalins.

Phosphatidylserine

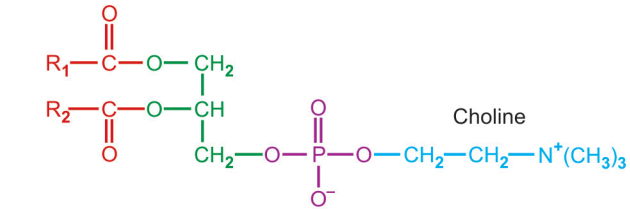
It contains the amino acid serine rather than ethanolamine and is found in most tissues **(Figure 3.9).**

Phosphatidylinositol

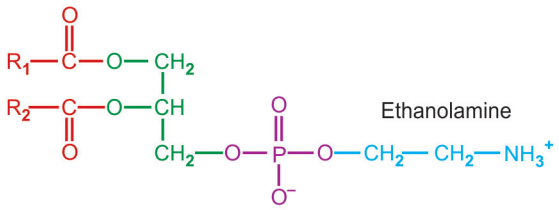
- In phosphatidylinositol, inositol is present as the stereoisomer ***myoinositol*** **(Figure 3.9).**
- Phosphatidylinositol is a **second messenger** for the action of hormones like oxytocine and vasopressin.

Plasmalogens

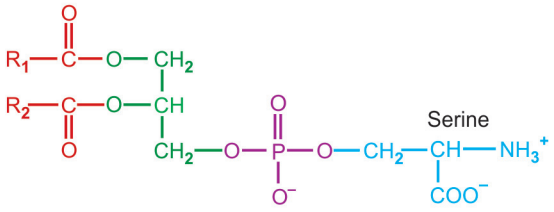
- Plasmalogens are generally similar to other phospholipids but the fatty acid at C_1 of glycerol is linked through an **ether**, rather than an ester bond **(Figure 3.9)**.



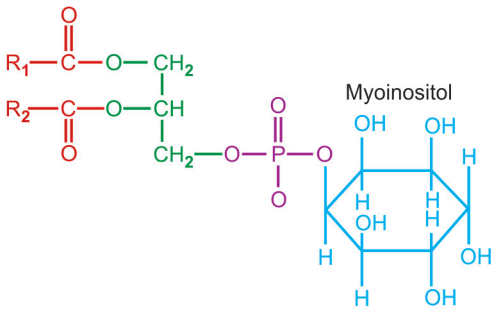
Phosphatidylcholine (Lecithin)



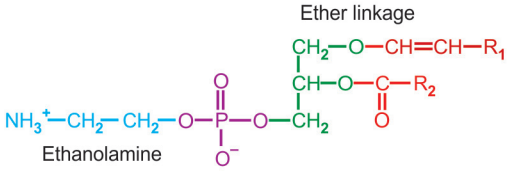
Phosphatidylethanolamine (Cephalin)



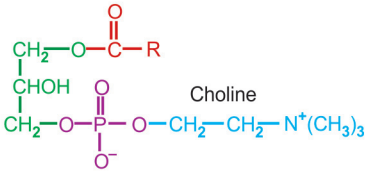
Phosphatidylserine



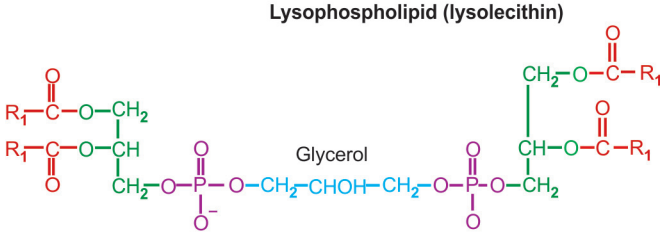
Phosphatidylinositol



Plasmalogen



Lysophospholipid (lysolecithin)



Cardiolipin or diphosphatidyl glycerol

Figure 3.9: Structure of different phospholipids

- There are three major classes of plasmalogens:
  i. Phosphatidylcholines
  ii. Phosphatidylethanolamines
  iii. Phosphatidylserines.
- These are found in myelin and in cardiac muscle.
- Plasmalogen is a platelet activating factor (PAF) and involved in platelet aggregation and degranulation.

Lysophospholipids

Lysophospholipids are produced when one of the two fatty acid is removed from glycerophospholipid. The most common of these are lysophosphatidylcholine (lysolecithin) **(Figure 3.9)** and lysophosphatidylethanolamine.

Cardiolipin (Diphosphatidylglycerol)

- Cardiolipin is composed of two molecules of phosphatidic acid connected by a molecule of glycerol.
- Two molecules of phosphatidic acid esterified through their phosphate groups with a molecule of glycerol are called cardiolipin **(Figure 3.9).**
- Cardiolipin is a major lipid of mitochondrial membrane and is necessary for optimum function of the electron transport process.

- This is only human glycerophospholipid that possess ***antigenic properties.***

Sphingophospholipids

Phospholipids derived from alcohol ***sphingosine*** instead of glycerol are called sphingophospholipids, e.g. sphingomyelin.

Sphingomyelin

- Sphingomyelin is the only phospholipid in membranes that is not derived from glycerol. Instead, the alcohol in sphingomyelin is ***sphingosine,*** an amino alcohol.
- In sphingomyelin, the amino group of the sphingosine is linked to a fatty acid to yield ***ceramide*** (sphingosine-fatty acid complex).
- In addition, the primary hydroxy group of sphingosine is esterified with phosphorylcholine **(Figure 3.10).**
- Sphingomyelin is one of the principal structural lipids of membranes of nerve tissue.

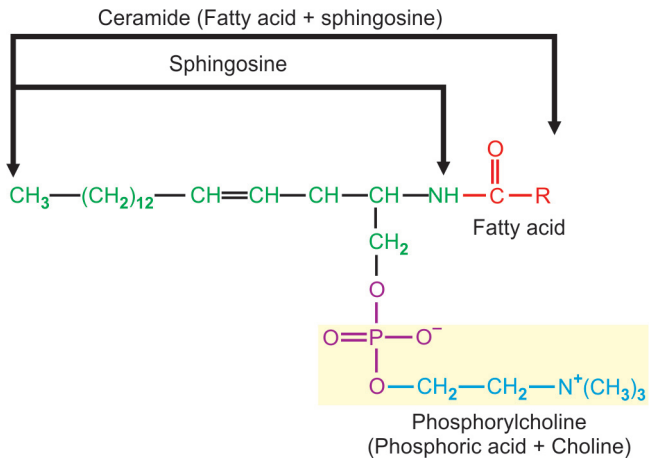


Figure 3.10: Structure of sphingomyelin

Functions of Phospholipids

- Phospholipids are the major lipid constituents of cell membranes.
- They regulate ***permeability of membranes*** as well as activation of some membrane bound enzymes.
- Phospholipids are of importance in ***insulating the nerve impulse*** (like the plastic or rubber covering around an electric wire) from the surrounding structures, e.g. sphingomyelins act as eletrical insulators in the myelin sheath around nerve fibers.
- Phospholipids are important constituents of lipoproteins.
- Phospholipids act as a ***lipotropic factor.*** Lipotropic factor is the component that prevents ***fatty liver,*** i.e. accumulation of fat in the liver.
- These are good ***emulsifying agents*** that help in intestinal absorption of lipids.
- ***Thromboplastin*** (coagulation factor III), which is needed to initiate the clotting process, is composed mainly of ***cephalins.***
- Phospholipid (lecithin) acts as ***lung surfactant,*** which prevents alveolar collapse.
- Lecithin represents a storage form of lipotropic factor **choline.**
- Phosphatidylinositol acts as a **second messenger** for the activity of certain hormones.
- In mitochondria, cardiolipin is necessary for optimum fuctions of the **electron transport process.**
- Plasmalogens (platelet activating factor) involved in **platelet aggregation** and **degranulation.**

GLYCOLIPIDS (GLYCOSPHINGOLIPIDS)

- Glycolipids as their name implies, are sugar containing lipids. Glycolipids consist of alcohol **sphingosine.**
- The amino group of sphingosine is esterified by a fatty acid and one or more sugar units are attached to the hydroxyl group of sphingosine.
- Glycolipids are widely distributed in every tissue of the body, particularly in nervous tissue such as brain.

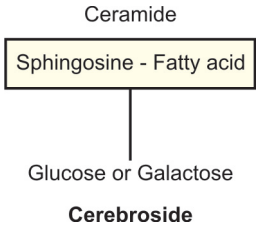
Classification of Glycolipids

Four classes of glycolipids have been distinguished:

1. Cerebrosides
2. Sulfatides
3. Globosides
4. Gangliosides.

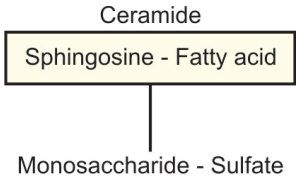
Cerebrosides (Ceramide + Monosaccharides)

- Cerebroside is the simplest glycolipid in which there is only one sugar residue, either ***glucose*** or ***galactose*** linked to ceramide and named as ***glucocerebroside*** and ***galactocerebroside*** respectively.
- Galactocerebroside is found in nerve tissue membrane. whereas glucocerebroside is the predominant glycolipid of extraneural (non-neural) tissues, where it acts as a precursor for the synthesis of more complex glycolipids, e.g. gangliosides.



Sulfatides (Ceramide + Monosaccharide + Sulfate)

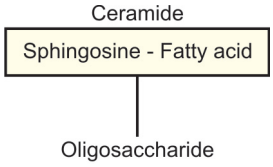
- Sulfatides are cerebrosides in which the monosaccharide contains a sulfate ester.



Sulfatide

Globosides (Ceramide + Oligosaccharide)

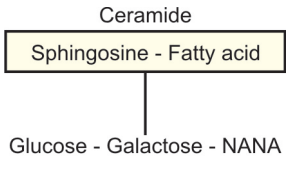
- Globosides contain two or more sugar molecules attached to ceramide.
- These glycolipids are important constituents of the RBC-membrane and are the determinants of the A,B,O blood group system



Globoside

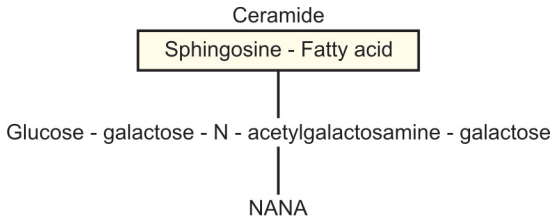
Gangliosides: (Cerebroside + Oligosaccharides + N-acetylneuraminic acid, NANA)

- Gangliosides are complex glycolipids, derived from glucocerebroside.
- Ganglioside contains oligosaccharides and one or more molecules of ***sialic acid,*** which is usually ***N-acetylneuraminic acid (NANA)*** attached to ceramide.
- Several types of gangliosides such as **GM_1, GM_2, GM_3,** etc. have been isoloted from brain and other tissues. The simplest ganglioside found in tissues is GM_3. G represents Ganglioside, M represents mono which indicate presence of one residue of NANA and subscript number assigned on the basis of chromatographic migration of ganglioside.



GM_3 - Ganglioside

- GM_1 is a more complex ganglioside derived from GM_3.



GM_1 - Ganglioside

Functions of Glycolipids

- Glycolipids are important constituents of the nervous tissue, such as brain and outer leaflet of all cell membrane.
- They play a role in the regulation of cellular interactions, growth and development.
- Glycolipids serve as cell **surface receptors** for certain hormones and a number of drugs. They also serve as receptors for cholera and tetanus toxins.
- Glycolipids are antigenic and they have been identified as a source of blood group antigens.

CHOLESTEROL (ANIMAL STEROL)

- Cholesterol is the major sterol in animal tissues. Sterols are a class of steroids containing hydroxyl group.
- It consists of steroid nucleus namely ***phenanthrene*** containing 19-carbon atoms **(Figure 3.11)**.
- It consists of methyl side chains at position C_{10} and C_{13} which are shown as single bonds.
- Cholesterol, a 27-carbon compound, has an 8-carbon side chain attached to the D ring at C_{17} and a hydroxyl group attached to C_3 of the A ring, with one double bond between carbon atoms 5 and 6 **(Figure 3.12).**

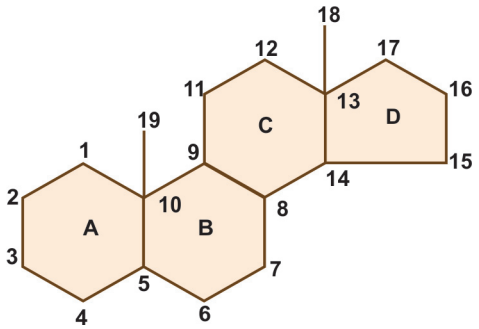


Figure 3.11: The steroid nucleus, phenanthrene (ring A, B and C), to which cyclopentane D ring is attached

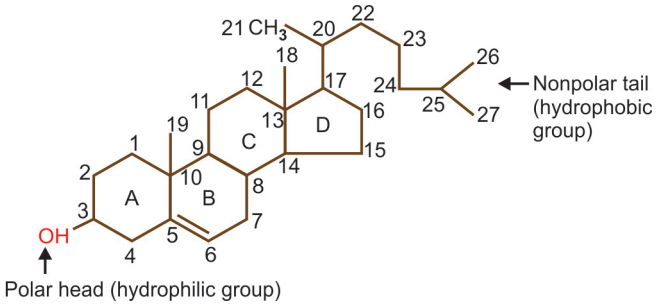


Figure 3.12: The structure of cholesterol

- Cholesterol is ***amphipathic,*** with a ***polar*** head the hydroxyl group at C_3 and a ***nonpolar,*** the steroid nucleus and hydrocarbon side chain at C_{17}.
- Most of the cholesterol in the body exists as a cholesterol ester, with a fatty acid attached to the hydroxyl group at C_3.
- Cholesterol is widely distributed in all the cells of the body but particularly in nervous tissue.
- ***It occurs in animal fats but not in the plant fats.***

Functions of Cholesterol

- It is a major structural constituent of the cell membranes and plasma lipoproteins.
- Cholesterol serves as the precursor for a variety of biologically important products, including:
  1. **Steroid hormones:** Cholesterol is the precursor of the five steroid hormones, e.g.
     i. Progesterones
     ii. Glucocorticoids
     iii. Mineralocorticoids
     iv. Androgens (male sex hormones)
     vi. Estrogen (female sex hormones).
  2. **Bile acids:** Bile acids, derived from cholesterol, act as a detergent in the intestine, emulsifying dietary fats to make them readily accessible to digestive enzyme lipase.
  3. **Vitamin D:** It is derived from cholesterol and is essential in calcium and phosphate metabolism.

LIPOPROTEINS

- Lipoproteins are large water soluble complexes formed by a combination of lipid and protein that transport insoluble lipids through the blood between different organs and tissues.
- Lipoproteins consist of a lipid core containing nonpolar ***triacylglycerol*** and ***cholesterol ester*** surrounded by a single layer of ***amphipathic phospholipids*** and ***free cholesterol*** molecules with some ***proteins***, (apoprotein) **(Figure 3.13)**.
- The protein components are referred to as an ***apoprotein*** or ***apolipoprotein.*** There are four major types of apolipoproteins designated by letters A, B, C and E with subgroups given in Roman numerals I, II, III, etc.

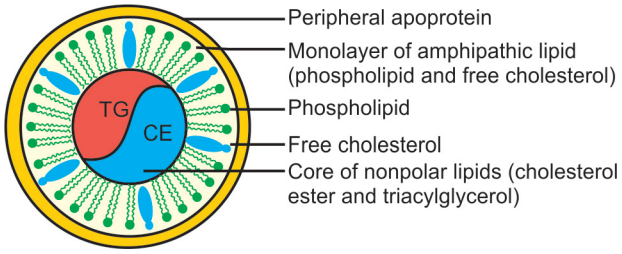


Figure 3.13: Structure of lipoprotein
where, TG: triacylglycerol, CE: cholesterol ester

Classes of Lipoproteins

Lipoproteins have been categorized into four major classes according to their physical and chemical properties **(Table 3.2).** These are :

1. Chylomicrons
2. Very low density lipoproteins (VLDL)
3. Low density lipoprotein (LDL)
4. High density lipoprotein (HDL).

- These lipoprotein complexes contain different proportions of lipids and proteins **(Table 3.2)**. The density of these lipoproteins is inversely proportional to triacylglycerol content. As the density increases, the diameter of the particle decreases as shown in **Figure 3.14.**
- Chylomicrons containing about 1 percent protein and 99 percent triacylglycerol have the lowest density.
- While HDL containing 50 percent of protein and 50 percent of lipid have the highest density.
- Triacylglycerol is the predominant lipid in chylomicrons and VLDL. Cholesterol is the predominant lipid in LDL, whereas phospholipid is the predominant lipid in HDL.
- Percentage of three major lipid classes, i.e. triacylglycerol, cholesterol and phospholipids present in lipoproteins are shown in **Table 3.2**.

Table 3.2: Characteristics of human plasma lipoproteins

Variable	*Chylomicron*	*VLDL*	*LDL*	*HDL*
Diameter (nm)	70 to 1200	25 to 70	20 to 25	5 to 10
Density (g/ml)	< 0.95	0.95 to 1.006	1.019 to 1.063	1.063 to 1.210
Lipid-Protein ratio	99:1	90:10	80:20	50:50
Major lipids	Triacylglycerol	Triacylglycerol	Cholesterol	Phospholipids
Apolipoproteins	B-48	B-100	B-100	A, C and E
Lipid components (%)				
Triacylglycerol	86	55	6	5
Cholesterol (free and ester)	5	20	50	20
Phospholipid	7	18	22	25

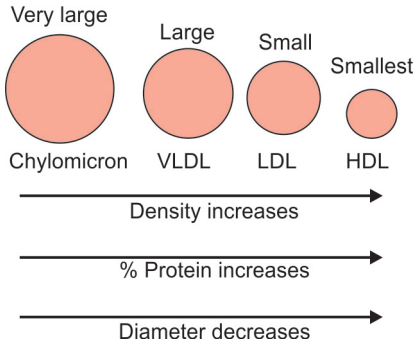


Figure 3.14: Diagrammatic representation of lipoprotein with increasing densities

Site of Synthesis and Functions of Lipoproteins

The site of synthesis of the four main lipoproteins and their functions are summarized in **Table 3.3.**

EICOSANOIDS

- **Prostaglandins** and the related compounds **thromboxanes** and **leukotriens,** are collectively known as **eicosanoids.**
- Eicosanoids are synthesized from **arachidonic acid.** A polyunsaturated fatty acid containing 20-carbon atoms from which they take their general name (Greek: eikosi means twenty).

Prostaglandins

- Prostaglandins are a group of 20-carbon compounds derived from **arachidonic acid (Figure 3.15)**.
- They derive their name from the tissue in which they were first recognized (the prostate gland) but they are now known to be present in almost all tissues.
- Chemically, the prostaglandins are derivatives of the hypothetical parent compound **prostanoic acid**, having cyclopentane (5 carbon) ring and two aliphatic side chains R_1 and R_2 **(Figure 3.15)**.
- Prostanoic acid does not occur naturally but is regarded as the parent compound of the prostaglandins and thromboxanes for the purpose of classification and carbon numbering.
- In addition to cyclopentane ring, each of the biologically active prostaglandin has a hydroxyl group at carbon 15, a double bond between carbons 13 and 14, and various substituents on the ring.

Table 3.3: The four main lipoproteins and their site of synthesis and function

Lipoprotein	*Site of synthesis*	*Function*
Chylomicrons	Intestine	Transport of dietary lipids from intestine to peripheral tissues
VLDL	Liver	Transport of triacylglycerol from liver to peripheral tissues
LDL	Plasma VLDL	Transport of cholesterol from liver to peripheral tissues
HDL	Liver and intestine	Transport of free cholesterol from peripheral tissues to the liver (Reverse cholesterol transport)

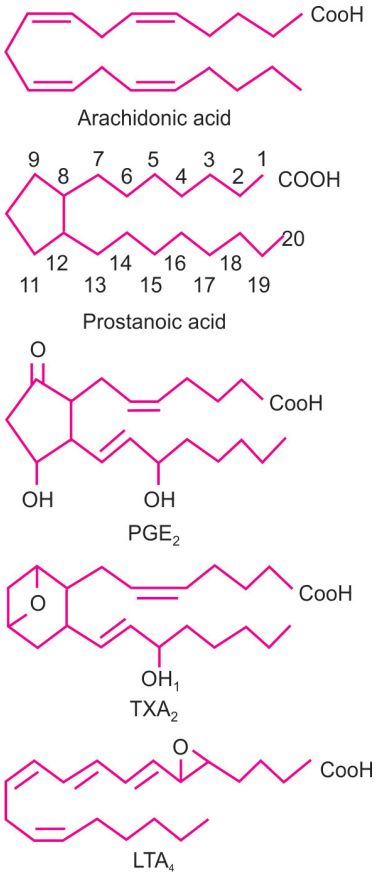


Figure 3.15: The structure of arachidonic acid, prostanoic acid, common prostaglandin (PGE_2), thromboxane (TXA_2) and leukotrienes (LTA_4)

Classification of prostaglandins

- By convention, prostaglandins are abbreviated as PG.
- They are classified into groups designated by a capital letter (A, B, C, D, E, F, G, H and I) depending on the substituents on the cyclopentane ring.
- These are subclassified by a subscript number 1, 2, or 3 corresponding to the number of double bonds in the side chains but not in the cyclopentane ring.
- Sixteen naturally occurring prostaglandins have been described but only seven are found commonly throughout the body. These are PGE_1, PGE_2, $PGF_1\alpha$, $PGF_2\alpha$, PGG_2, PGH_2, PGI_2.
- Prostaglandins are not stored, instead the precursor C_{20} arochidonic acids are stored in tissues.

Functions of prostaglandins

- Prostaglandins and other eicosanoids have hormone like actions.
- Prostaglandins in many tissues act by regulating the synthesis of cyclic AMP (cAMP). As cAMP mediates the action of many hormones, the prostaglandins affect a wide range of cellular and tissue functions. Some of these are:
  - **Smooth muscle contraction and relaxation:** For example, in pregnancy $PGF_{2\alpha}$ are produced in response to oxytocin and act to promote uterine contraction. Because of this effect, they have been used to terminate unwanted pregnancies. PGE_2 are involved in relaxation of bronchial smooth muscle.
  - **Inflammatory response:** PGs are involved in inflammatory response causing pain, edema, swelling and prolonged erythema (abnormal flushing of skin) by increasing capillary permeability.
  - **Platelet aggregation:** Prostaglandins have an effect on platelet aggregation. PGE_2 promote aggregation and are thus, involved in the blood clotting.
  - **Regulation of Blood pressure:** PGE_2 decrease blood pressure. It can lower systemic arterial pressure through their vasodilator effect.
  - **Body temperature:** Prostaglandins elevate body temperature producing fever and cause inflammation, resulting in pain.
  - **Gastric secretion:** PGE_2 suppress gastric secretion.
  - PGs are involved in **Na^+** and ***water retention*** by kidney tubules.

Thromboxanes

Thromboxanes were first isolated from blood platelets, thrombocytes—hence the name. They have six membered oxane ring **(Figure 3.15)** that includes an oxygen atom.

Nomenclature of thromboxanes

- Thromboxanes are abbreviated as TX.
  Different capital letters are used to designate different substituents of the ring (like prostaglandins).
- A subscript, if present, denotes the number of unsaturated bonds (double bonds), e.g. the most common thromboxane TXA_2 having two double bonds.

Functions of thromboxanes

- TXA_2 is produced by platelets, promotes platelets aggregation. Platelet aggregation initiates thrombus formation at sites of vascular injury.
- TXA_2 causes contractions of the smooth muscles of the arterial wall and therefore, raises blood pressure.

Leukotrienes (LT)

Leukotrienes were so named because they were initially described in leucocytes and are characterized by a conjugated **triene** system but no such ring structure that is found in prostaglandins and thromboxanes.

Nomenclature of leukotrienes

- All leukotrienes are abbreviated as LT.
- These are grouped into five classes (A to E) based on the type of substituents attached to the parent compound.
- The LTs found in humans have a subscript four to denote that they contain four double bonds **(Figure 3.15).**

Functions of leukotrienes

- The LTs facilitate chemotaxis, inflammation and allergic reactions.
- LTC_4, LTD_4 induce contraction of muscle of the lung and constrict pulmonary airways. Overproduction of LT causes asthmatic attacks.
- LTD_4 has been identified as the ***slow reacting substance of anaphylaxis (SRS-A)*** which causes smooth muscle contraction.
- LTB_4 attracts neutrophils and eosinophils to sites of inflammation.

MICELLES, LIPID BILAYER AND LIPOSOMES

Micelles

The polar lipid like phospholipid is amphipathic in nature. It has a hydrophilic or polar head (phosphate group attached to choline, ethanolamine, inositol, etc.) and a long hydrophobic tail containing two fatty acid chains **(Figure 3.16).** In aqueous systems the polar phospholipid spontaneously disperse to form micelles, in which the hydrocarbon tails of the phospholipid are hidden from the aqueous environment and electrically charged hydrophilic heads are exposed on the surface facing the aqueous medium **(Figure 3.16)**.

Lipid Bilayer

Phospholipids also readily and spontaneously form a very thin bilayer separating two aqueous compartments. In these structures, the hydrocarbon tails of the phospholipid molecules extend inward from the two surfaces to form a continuous inner hydrocarbon core and the hydrophilic heads face outwards extending into the aqueous phase **(Figure 3.16).**

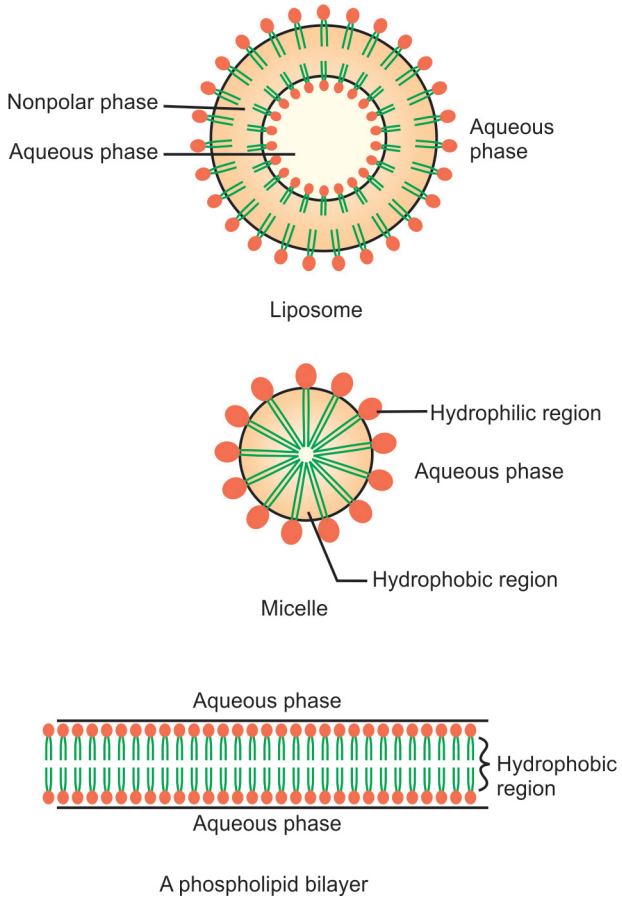


Figure 3.16: Formation of micelle, a phospholipid bilayer and liposome

Liposome (Artificially Formed Phospholipid Vesicles)

- Liposomes are artificially formed aqueous vesicles enclosed by a lipid bilayer.
- In the laboratory, liposomes (lipid bilayer) are formed by suspending phospholipid in an aqueous medium and then sonicating, i.e. agitating by high frequency sound waves to give a dispersed closed vesicles. Vesicles formed by these methods are nearly spherical in shape **(Figure 3.16)** and have a diameter of about 5 nm.
- Liposomes can be used to study membrane permeability or to deliver drugs to cells.

DETERGENTS

- Detergents are amphipathic molecules and are not natural membrane constituents. In aqueous solutions, they form micelles, with the hydrophilic

regions on the outside, interacting with the water, and the hydrophobic regions inside.
- The bile acids and bile salts are powerful naturally occurring detergents which emulsify fats in the digestive tract; they can also be used to solubilize membrane proteins.
- Lysophospholipids also have detergent like action.

SUMMARY

- Lipids are water insoluble components of cells.
- Some lipids serve as structural component of membrane and others as storage form of fuel.
- Triacylglycerols contain three fatty acid molecules esterified to the three hydroxyl groups of the glycerol and are storage fats.
- The polar lipids (phospholipids, glycolipids and cholesterol), which have polar heads and nonpolar tails, are amphipathic and major components of membranes.
- Sphingolipids, i.e. sphingomyelins, cerebrosides and gangliosides are also membrane components and contain sphingosine but no glycerol.
- Cerebrosides and gangliosides are glycolipids which contain various sugar components.
- Cholesterol, a sterol is a precursor of many steroids and is also an important constituent of plasma membranes of the cell.
- Lipoproteins are macromolecular complexes that transport insoluble lipids in the blood. The proteins of the lipoprotein are referred to as apoprotein or apolipoprotein.
- Prostaglandins, thromboxanes and leukotrienes are derived from PUFA arachidonic acid.
- Liposomes are artificially formed phospholipid vesicles.
- Detergents are amphipathic molecules, and are not natural membrane constituents.

EXERCISE

Multiple Choice Questions (MCQs)

1. The precursor for vitamin D is:
a) Cholesterol b) Arachidonic acid
c) Triacylglycerol d) Phospholipids

2. Which of the following lipids is deficient in infants with respiratory distress syndrome?
a) Sphingomyelins
b) Cardiolipins
c) Leukotrienes
d) Dipalmitoyl phosphatidylcholine

3. Which of the following carbohydrates distinguishes a ganglioside from a globoside?
a) Glucose
b) N-acetylneuraminic acid
c) N-acetylgalactosamine
d) Galactose

4. Methylated form of phosphatidyl ethanolamine is known as:
a) Phosphatidylinositol
b) Phosphatidylserine
c) Phosphatidylcholine
d) Lysophosphatidylcholine

5. Which ring of the cholesterol molecule contains a double bond?
a) A-ring b) B-ring
c) C- ring d) D-ring

6. All of the following statements are true for phosphoglycerides, *except*:
a) They are major storage of metabolic energy
b) They are found in cell membrane
c) They are amphipathic
d) They are derived from glycerol

7. All of the following are sphingolipids, *except*:
a) Sphingomyelin
b) Cerebroside
c) Phosphatidylinositol
d) Ganglioside

8. The lipoprotein particles that have the highest concentration of triacylglycerol are:
a) VLDL b) HDL
c) LDL d) Chylomicrons

9. All of the following statements about prostaglandins are true, *except*:
a) They are cyclic fatty acids
b) They are potent biologic effectors
c) They were first observed to cause uterine contraction
d) They are synthesized only in prostate gland

10. Glycerol is the backbone of:
a) Glycerophospholipid
b) Sphingophospholipid
c) Glycolipids
d) Cholesterol esters

11. All of the following statements about lipids are true, *except*:
a) They are esters of fatty acids
b) They have poor solubility in water
c) They are a source of energy
d) They are polyhydroxy aldehydes

12. Which of the following statements about cholesterol is true?

a) It is saturated
b) It contains 27-carbon atom
c) It is a major sterol of plants
d) It contains four hexane rings fused together

13. Which of the following phospholipids has an antigenic activity?

a) Lecithin b) Cardiolipin
c) Sphingomyelin d) Cephalin

14. Which of the following glycolipids is known to be the receptor in human intestine for cholera toxin?

a) GM1 b) GM3
c) Globoside d) Cerebroside

15. Which of the following is the major storage and transport form of fatty acids?

a) Cholesterol
b) Triacylglycerol
c) Albumin
d) Phospholipid

Correct Answers for MCQs

1-a	2-d	3-b	4-c
5-b	6-a	7-c	8-d
9-d	10-a	11-d	12-b
13-b	14-a	15-b	

CHAPTER

4

Chemistry of Proteins

INTRODUCTION

Proteins are the most abundant macromolecules in living cells. The term 'protein' was first used by Berzelius in 1838 and was derived from the Greek word **"protos"** which means **primary** or **holding first place.** As the name indicates, protein is the most important of cell constituents. They are responsible for almost every function that occurs in the body.

Proteins are linear chains of amino acids that are linked together by covalent, ***peptide bonds.*** Each protein has specific and unique sequence of amino acids that defines both its three-dimensional structure and its biologic function.

GENERAL NATURE OF AMINO ACIDS

- There are approximately 300 amino acids present in various animal, plant and microbial systems, but only ***20 amino acids are involved in the formation of proteins.***
- All the 20 amino acids found in proteins **(Table 4.1)** have a carboxyl group (-COOH) and an amino acid group ($-NH_2$) bound to the same carbon atom called the ***α-carbon*** **(Figure 4.1).**
- Amino acids differ from each other in their **side chains** or **R-groups,** attached to the α-carbon.
- The 20 amino acids of proteins are often referred to as the ***standard*** or ***primary*** or ***normal amino acids.***

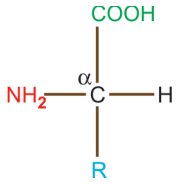


Figure 4.1: General structure of α-amino acid found in protein

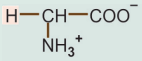
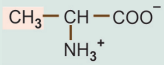
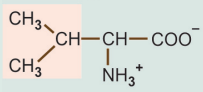
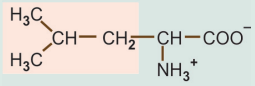
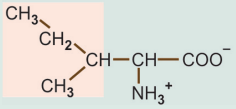
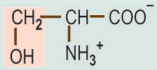
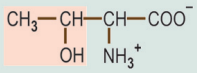
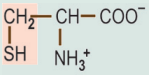
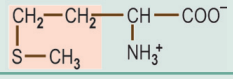
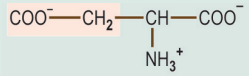
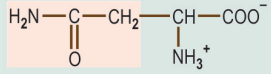
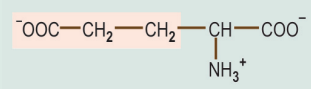
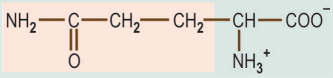
- The standard amino acids have been assigned three letters abbreviations and one letter symbol, e.g. amino acid **glycine** has abbreviated name ***Gly*** and symbol letter ***G***.
- All the amino acids found in proteins are exclusively of the ***L-configuration.***

CLASSIFICATION OF AMINO ACIDS

There are five ways of classifying amino acids depending on the:

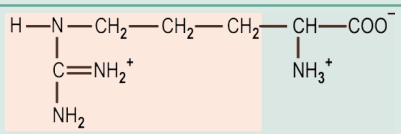
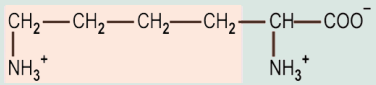
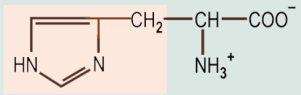
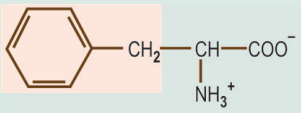
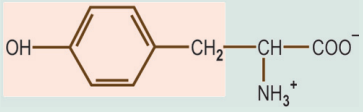
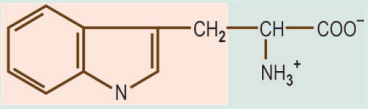
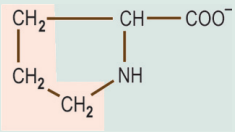
1. Chemical nature of the amino acid in the solution
2. Structure of the side chain of the amino acids
3. Nutritional requirement of amino acids
4. Metabolic product of amino acids
5. Nature or polarity of the side chain of the amino acids.

Table 4.1: The 20, L-α-amino acids (standard amino acids) found in proteins

Name	*Symbol*	*Structural formula*
Aliphatic side chain		
Glycine	Gly (G)	
Alanine	Ala (A)	
Valine	Val (V)	
Leucine	Leu (L)	
Isoleucine	Ile (I)	
Hydroxylic (OH) group containing side chains		
Serine	Ser (S)	
Threonine	Thr (T)	
Tyrosine	Tyr (Y)	See aromatic group containing side chain amino acids
Sulfur containing side chains		
Cysteine	Cys (C)	
Methionine	Met (M)	
Side chains containing acidic groups (–COOH) and their amides		
Aspartic acid	Asp (D)	
Aspargine	Asn (N)	
Glutamic acid	Glu (E)	
Glutamine	Gln (Q)	

Contd...

Table 4:1 (contd...)

Name	*Symbol*	*Structural formula*
Basic groups containing side chains		
Arginine	Arg (R)	
Lysine	Lys (K)	
Histidine	His (H)	
Aromatic group containing side chains		
Histidine	His (H)	See above
Phenylalanine	Phe (F)	
Tyrosine	Tyr (Y)	
Tryptophan	Trp (W)	
Imino acids		
Proline	Pro (P)	

Classification Based on Chemical Nature of the Amino Acid in Solution

According to this type of classification, amino acids are classified as follows:

i. Neutral amino acids
ii. Acidic amino acids
iii. Basic amino acids.

Neutral amino acids

The amino acids which are neutral in solution and are monoamino-monocarboxylic acids (i.e. having one amino group and one carboxylic group), e.g.

Glycine	Serine	Phenylalanine
Alanine	Threonine	Tyrosine
Valine	Cysteine	Tryptophan
Leucine	Methionine	Aspargine
Isoleucine	Proline	Glutamine

Acidic amino acid

These are acidic in solution and are monoamino dicarboxylic acids, e.g.

- Aspartic acid
- Glutamic acid.

Basic amino acid

These are basic in solution and are diamino-monocarboxylic acids, e.g.

- Lysine
- Arginine
- Histidine.

Classification Based on Chemical Structure of Side Chain of the Amino Acid

According to this type of classification, amino acids are classified as:

1. Aliphatic amino acids
2. Hydroxy amino acids
3. Sulfur containing amino acids
4. Dicarboxylic acid and their amides
5. Diamino acids
6. Aromatic amino acids
7. Imino acids or heterocyclic amino acids.

Aliphatic amino acids

Amino acids having aliphatic side chain, e.g.

- Glycine
- Alanine
- Valine
- Leucine
- Isoleucine.

Hydroxy amino acids

Amino acids having hydroxy group in the side chain, e.g.

- Threonine
- Serine
- Tyrosine.

Sulfur containing amino acids

Amino acids having sulfur in the side chain, e.g.

- Cysteine
- Methionine.

Dicarboxylic acid and their amides

Amino acids having carboxylic group in their side chain, e.g.

- Glutamic acid
- Glutamine (amide of glutamic acid)
- Aspartic acid
- Aspargine (amide of aspartic acid).

Diamino acids

Amino acids having amino group ($-NH_2$) in the side chain, e.g.

- Lysine
- Arginine
- Histidine.

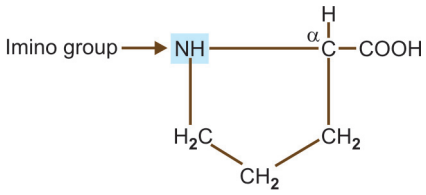
Aromatic amino acids

Amino acids containing aromatic ring in the side chain, e.g.

- Phenylalanine
- Tyrosine
- Tryptophan.

Imino acids or heterocyclic amino acids

- One of the 20 amino acids, ***proline*** is an ***imino (-NH) acid*** not an amino ($-NH_2$) acid as are other 19. The side chains of proline and its α-amino group form a ring structure and thus proline differs from other amino acids, in that it contains an imino group, rather than an amino group.



Structure of proline

Nutritional Classification of Amino Acids

On the basis of nutritional requirement, amino acids are classified into two groups:

i. Essential or indispensable amino acids
ii. Nonessential or dispensable amino acids.

Essential amino acids (also refer Chapter 11)

Essential amino acids cannot be synthesized by the body and must, therefore, be essentially supplied through the diet. Ten amino acids, essential for humans include:

- Phenylalanine
- Valine
- Threonine
- Tryptophan
- Isoleucine
- Methionine
- Histidine
- Arginine
- Lysine
- Leucine.

The mnemonics often used by students are **PVT. TIM. HALL** or **L.VITTHAL (MP)**.

Among the ten essential amino acids; **arginine** and **histidine** are known as **semi-essential** amino acids since these amino acids are synthesized partially in human body. Arginine and histidine become essential in diet during periods of rapid growth as in childhood and pregnancy.

A deficiency of an essential amino acid impairs protein synthesis and leads to a negative nitrogen balance (nitrogen excretion exceeds nitrogen intake).

Nonessential amino acids

Nonessential amino acids can be synthesized in human body and are not required in diet, e.g.

- Glycine
- Proline
- Serine
- Glutamic acid
- Glutamine
- Alanine
- Tyrosine
- Cysteine
- Aspartic acid
- Aspargine.

Metabolic Classification of Amino Acids

On the basis of their catabolic end products, the twenty standard amino acids are divided in three groups **(Table 4.2).**

i. **Glucogenic amino acids:** Those which can be converted into glucose. Fourteen out of the twenty standard amino acids are glucogenic amino acids **(Table 4.2).**
ii. **Ketogenic amino acids:** Those which can be converted to ketone bodies. Two amino acids **leucine** and **lysine** are exclusively ketogenic.
iii. **Both glucogenic and ketogenic:** Those which can be converted to both glucose and ketone bodies. Four amino acids **isoleucine, phenylalanine, tryptophan** and **tyrosine** are glucogenic and ketogenic.

Table 4.2 : Metabolic classification of amino acids

Glucogenic	*Ketogenic*	*Both ketogenic and glucogenic*
Glycine, alanine, serine, cysteine, aspartic acid, aspargine, glutamic acid, glutamine, proline, histidine, arginine, methionine, threonine, valine	Leucine Lysine	Isoleucine Phenylalanine Tyrosine Tryptophan

Classification Based on Nature or Polarity of Side Chain of Amino Acid

According to this type of classification, amino acids are classified into two major classes **(Figure 4.2):**

i. Hydrophilic or polar amino acids
ii. Hydrophobic or nonpolar amino acids.

Importance of Amino Acids

- **Formation of proteins:** Amino acids are joined to each other by peptide bonds to form proteins and peptides.
- **Formation of glucose:** Glucogenic amino acids are converted to glucose in the body.
- **Enzyme activity:** The thiol (-SH) group of cysteine has an important role in certain enzyme activity.
- **Transport and storage form of ammonia:** Amino acid glutamine plays an important role in transport and storage of amino nitrogen in the form of ammonia.
- **As a buffer:** Both free amino acids and some amino acids present in protein can potentially act as buffer, e.g. histidine can serve as the best buffer at physiological pH.
- **Detoxification reactions:** Glycine, cysteine and methionine are involved in the detoxification of toxic substances.
- **Formation of biologically important compounds:** Specific amino acids can give rise to specific biologically important compounds in the body **(Table 4.3)**.

MODIFIED OR NONSTANDARD AMINO ACIDS

In addition to the standard amino acids, a small number of modified amino acids are found in proteins. These amino acids are formed by the modification of one of the

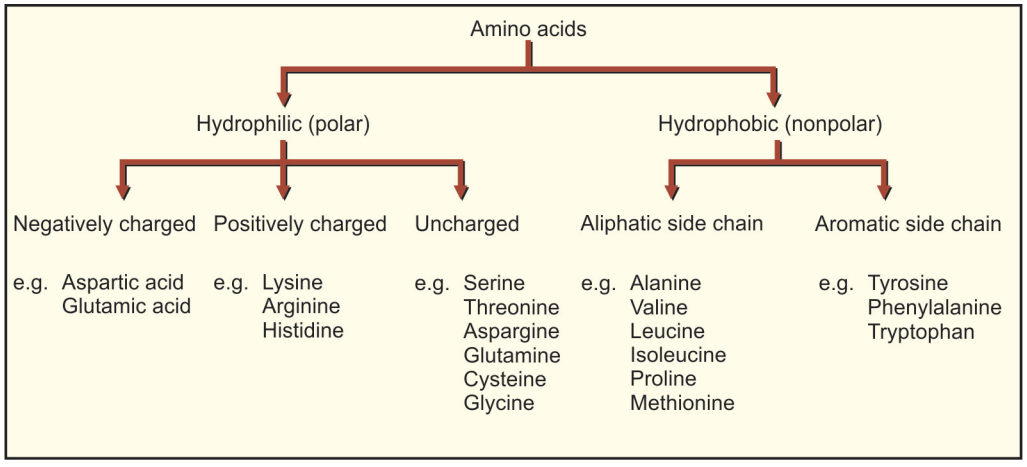


Figure 4.2: Classification of amino acids based on polarity

Table 4.3: Biologically important compounds formed by amino acids

Amino acid	*Biologically important compound*
Tyrosine	Hormone, e.g. adrenaline and thyroxine, skin pigment, e.g. melanin
Tryptophan	Vitamin, e.g. niacin
Glycine, arginine and methionine	Creatine
Glycine and cysteine	Bile salts
Glycine	Heme
Aspartic acid and glutamic acid	Pyrimidine bases
Glycine, aspartic acid and glutamine	Purine bases
β-alanine	Coenzyme-A

20 standard amino acids, after incorporation into the protein. The following modified amino acids are of particular significance.

Cystine

Cystine is formed by linkage of two cysteine side chains through a disulfide bond **(Figure 4.3)**. This modified amino acid is found in many proteins, where it provides stability to the three-dimensional structure.

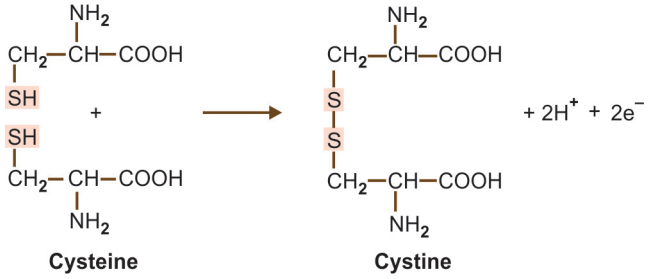


Figure 4.3: Formation of disulfide bond of cystine

Hydroxyproline and Hydroxylysine

The hydroxylation of selected lysine and proline residues is found primarily in the formation of collagen of connective tissue.

Desmosine and Isodesmosine

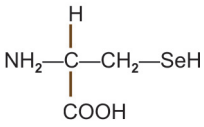
These two amino acids are formed by the oxidation and crosslinking of four lysine side chains. These are found in connective tissue protein, *elastin.*

Gamma Carboxyglutamate

Carboxylation of glutamic acid side chains occurs in many of the clotting proteins, e.g. in prothrombin.

Selenocysteine

- In addition to the 20 standard amino acids found in proteins, a 21st amino acid selenocysteine has been discovered and shown to be an essential residue in several enzyme systems.
- The amino acid **selenocysteine** is present at the active site of several human enzymes that includes :
  - Glutathione peroxidase
  - Deiodinase
  - Glycine reductase.
- Selenocysteine has a structure similar to cysteine, but containing the trace element selenium in place of sulfur atom of cysteine.



Selenocysteine

PROPERTIES OF AMINO ACIDS

Physical Properties

They are colorless, crystalline substances generally soluble in water.

Optical Properties

All naturally occurring amino acids are optically active except **glycine** which is optically inactive. At pH 7.0, all amino acids have the same L-configuration and hence L-α-amino acid **(Figure 4.4).**

Ionization of Amino Acids

Due to ionizing property of amino acids, amino acids exert:

- Acid base behavior
- Amphoteric properties (zwitter ion formation)
- Buffering activity.

Acid/base behavior of amino acids

- The acid base properties of amino acids depend on the **amino** and **carboxyl** groups attached to the α-carbon.
- The **carboxyl** (-COOH) group of an amino acid can donate proton (H^+) and behave as an acid, forming a negatively charged **anion**.
- Amino group (-NH_2) of an amino acid can accept the proton (H^+) which behave as a base, forming positively charged **cation**.

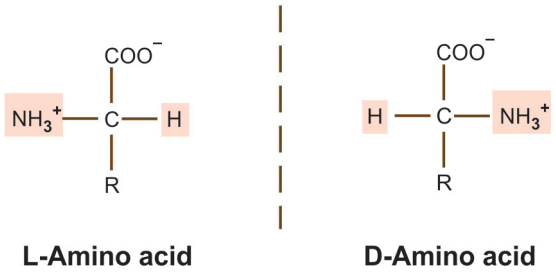


Figure 4.4: D and L forms of amino acids

Thus, amino acids in aqueous solution are ionized and act as acids and bases **(Figure 4.5)**.

Amphoteric properties of amino acids and formation of zwitter ion at isoelectric pH

Substances having a two-way property are called ***amphoteric*** or ***ampholytes*** (Greek word ***ampho*** means ***both).*** As amino acids have both acidic and basic groups, they can donate a proton or accept a proton, hence amino acids are regarded as ampholytes.

Zwitter ion (Dipolar molecule)

- Monoamino monocarboxylic acids exist in aqueous solution as ***dipolar molecule*** or ***zwitter ions,*** which means that they have both positive and negative charges on the same amino acids.
  - The ***α-COOH*** group is ionized and becomes negatively charged anion (COO^-) and
  - The ***α-NH***$_2$ group is protonated to form a positively charged cation (NH_3^+).
- Thus, the overall molecule is electrically neutral. Thus, the molecular species which contain an equal number of ionizable group of opposite charge and as a result bear no net charge, are called ***zwitter ions.***
- The net charge of an amino acid depends upon the pH of the medium.
  - At acidic pH, amino acid is positively charged because ionized COO^- group accepts a proton and becomes uncharged (COOH), so that the overall charge on the molecule is positive.
  - While at alkaline pH it is negatively charged as the NH_3^+ group loses its proton and becomes uncharged; thus the overall charge on the molecule is negative **(Figure 4.6)**.

Isoelectric pH (PI)

The pH at which amino acid bears no net charge (zwitter ion) and therefore does not move in an electric field is called ***isoelectric pH (PI).***

Buffering action of amino acid

- Amino acid can act as weak acid or weak base. In addition, each of the acidic and basic amino acids

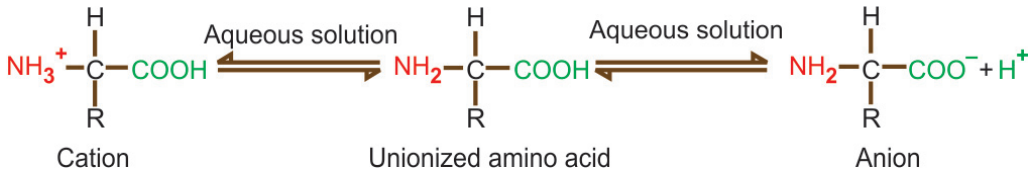


Figure 4.5: Ionization of amino acid

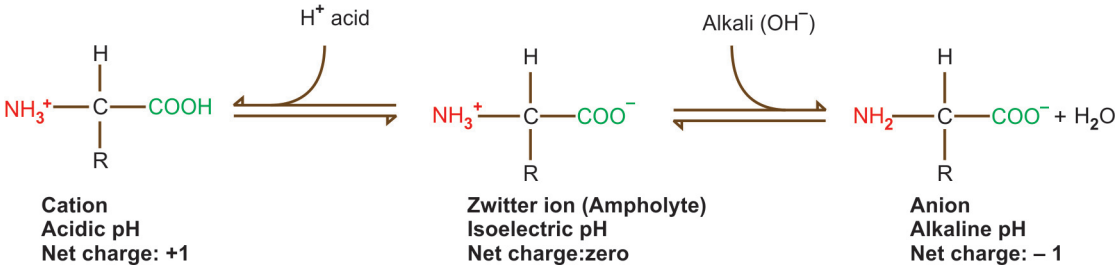


Figure 4.6: Ionic forms of amino acid in acidic, basic and isoelectric pH

contains an ionizable group in its side chain. Thus, both free amino acids and some amino acids present in proteins can potentially act as buffers.

- ***Among the 20 standard amino acids, histidine serves as the best buffer at physiological pH.*** Because the side chain of histidine has a pKa of 6.0 and can serve as the best buffer at physiological pH. All other amino acids have pKa value, too far away from pH 7 to be an effective physiological buffer. ***Maximum buffer capacity occurs at pH equal to the pKa.***

BIOLOGICALLY IMPORTANT PEPTIDES

- Peptides are chains (polymer) of amino acids. Two amino acid molecules can be covalently joined through a ***peptide bond,*** to yield ***dipeptide.*** Peptide linkage is formed by the removal of a molecule of water from the α-carboxyl group of one amino acid and the α-amino group of another **(Figure 4.7).**
- When many amino acids are joined, the product is called ***polypeptide.*** Proteins are polypeptides with thousands of amino acids.
- There are many naturally occurring small polypeptides some of which have important biological activities and are called **biologically important peptides.** A few of them are given below.

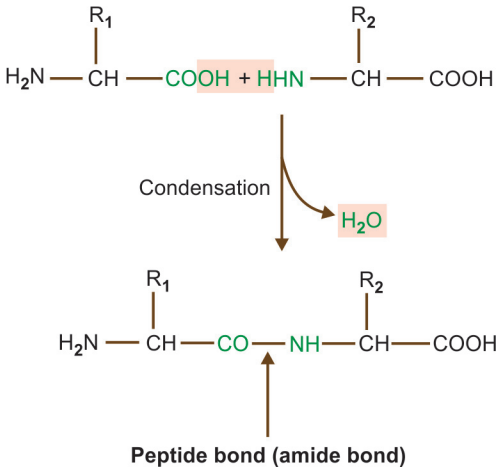


Figure 4.7: Formation of peptide bond

Glutathione (GSH)

- GSH is a tripeptide (γ glutamyl – cysteinyl – glycine) containing glutamate, cysteine and glycine.
- Glutathione is found in all mammalian cells *except* **the neurons.**
- Glutathione may exist as the reduced (GSH) or oxidized (G-S-S-G) form **(Figure 4.8)** and can thus play a role in some oxidation–reduction reactions.
- In oxidized form, two molecules of glutathione are linked by disulfide bond.
- The sulfhydryl (-SH-) is the functional group primarily responsible for the properties of glutathione.

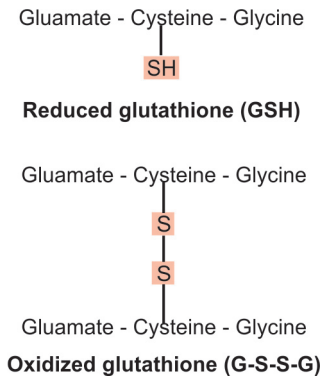


Figure 4.8: Reduced and oxidized glutathione

Functions of glutathione

- The reduced form of glutathione with a free sulfhydryl (-SH-) group serves as a **redox buffer** regulating the redox state of the cell.
- It helps in keeping the enzymes in an active state by preventing the oxidation of sulfhydryl (-SH-) group of enzyme to disulfide (-S-S-) group.
- Reduced glutathione is essential for maintaining the normal structure of red blood cells and for keeping hemoglobin in the ferrous state. Cells with lowered level of reduced glutathione are more susceptible to hemolysis.
- Glutathione plays a key role in detoxification by reducing H_2O_2—the harmful byproduct of metabolism.

$$2GSH + H_2O_2 \xrightarrow[\text{Glutathione Peroxidase}]{} G\text{-}S\text{-}S\text{-}G + 2H_2O.$$

- Glutathione is involved in transport of amino acids across the cell membrane of the kidney and intestine.

Thyrotropin Releasing Hormone (TRH)

TRH is a hypothalamic hormone of three amino acid residues. It stimulates the release of hormone thyrotropin, from the anterior pituitary gland.

Oxytocin

This is a 9-amino acid residue hormone secreted by posterior pituitary and stimulates uterine contractions.

Vasopressin

This is a 9-amino acid residue hormone secreted by posterior pituitary and it increases blood pressure and has an antidiuretic action.

Gastrin

This is a local hormone produced by stomach. It stimulates the production of gastric juice.

Angiotensin

Angiotensin II is a vasoconstrictor and elevates the arterial pressure and also promotes the synthesis of a steroid hormone called aldosterone that promotes sodium retention.

Bradykinin

It contains, 9-amino acid residue. It is a powerful vasodilator and causes contraction of smooth muscle and is mainly responsible for causing intense peripheral and visceral pain by stimulating the pain receptors.

Insulin

A pancreatic hormone contains two polypeptide chains: one having 30-amino acid residues and the other 21. Insulin regulates the glucose concentration in blood.

Glucagon

This is a pancreatic hormone of 29-residues that opposes the action of insulin.

DEFINITION, CLASSIFICATION AND FUNCTIONS OF PROTEINS

Definition

Proteins are linear chains of amino acids that are linked together by covalent, ***peptide bonds*.** Each protein has specific and unique sequence of amino acids that defines both its three-dimensional structure and its biologic function.

Classification of Proteins

Proteins have been classified in several ways. They are most conveniently classified on the basis of their:

1. Function
2. Physical and chemical properties.

Classification of Proteins Based on Functions

In a functional classification, they are grouped according to their biological role. Some functions that proteins serve and examples of specific functional proteins are as follows:

Catalytic Proteins or Enzymes

These proteins act as enzymes, e.g.

- Glucokinase
- Dehydrogenases
- Transaminases
- Hydrolytic enzymes, pepsin, trypsin, etc.

Transport Proteins

These proteins are involved in the process of transportation, e.g.

- Hemoglobin transports oxygen
- Transferrin transports iron
- Albumin carries fatty acids and bilirubin.

Storage Proteins

Many proteins serve as storage form, e.g.

- Apoferritin stores iron in the form of ferritin
- Myoglobin stores oxygen in muscles.

Contractile Proteins

Some proteins have the ability to contract and function in the contractile system of skeletal muscle, e.g.

- Actin
- Myosin.

Structural Proteins

Many proteins serve as supporting framework of cells to give biological structure, strength or protection, e.g.

- Collagen in bone
- Cartilage, elastin of ligaments
- Keratin of hair, nail.

Defence Proteins

Many proteins involved in defence mechanism against invasion of foreign substances such as viruses, bacteria and cells. Examples of defence proteins are:

- Immunoglobulins or antibodies
- Fibrinogen and thrombin are blood clotting proteins that prevent loss of blood when the vascular system is injured.

Regulatory Proteins

Some proteins regulate cellular or physiological activity, e.g.

- Many hormones, e.g. ***insulin,*** regulate sugar metabolism; ***growth hormone*** of pituitary gland regulates growth of the cells.

Classification of Proteins Based on Physical and Chemical Properties of Protein

According to the joint committee of the ***American Society of Biological Chemists*** **and** ***American Physiological Society,*** proteins are classified into three main groups as follows:

1. Simple proteins
2. Conjugated proteins
3. Derived proteins.

Simple Proteins

Simple proteins are defined as those proteins that upon hydrolysis, yield only amino acids or their derivatives. They are subclassified according to their solubility and heat coagulability as follows:

Albumins

The albumins are ***soluble in water, coagulated by heat.*** It is deficient in glycine, e.g.

- Egg albumin
- Serum albumin
- Lactalbumin of milk.

Globulins

The globulins are insoluble in water, but they are ***soluble in dilute neutral salt solution*** **and are** ***heat coagulable,*** **e.g.**

- Ovaglobulin of egg yolk
- Serum globulin
- Myosin of muscle.

Glutelins

The glutelins are ***soluble in dilute acids*** **and** ***alkalies*** but they are ***insoluble in neutral solvents.*** They are plant proteins, e.g.

- Glutelin of wheat
- Oryzenin of rice.

Prolamins or alcohol soluble proteins

The prolamins are soluble in **70 to 80%** ***alcohol,*** but they are ***insoluble in water, neutral solvent*** or ***absolute alcohol.*** The prolamins are rich in proline but are ***deficient in lysine.*** They are plant proteins, e.g.

- Zein of corn
- Gliadin of wheat.

Histones

The histones are ***soluble in water, but are not coagulated by heat.*** Histones are basic proteins as they are rich in basic amino acids. The histones, being basic, usually occur in tissues in salt combinations with acidic substances, such as nucleic acids (RNA and DNA), e.g.

- Nucleoprotein.

Protamines

They are strongly basic and rich in basic amino acid arginine. The protamines are ***soluble in water but are not heat coagulable.*** Like histones, they occur in tissues with nuleic acids, e.g. nucleoproteins.

Scleroproteins (fibrous proteins)

Fibrous proteins are also called **sclero** ***proteins.*** They are insoluble, (in all common solvents like water, neutral salt solution, organic solvents, dilute acid and alkali) high molecular weight fibers. Examples of sclero proteins are:

- Collagen found in cartilage and tendons
- Elastin found in tendon and arteries
- Keratin of hair, skin and nail.

Conjugated Proteins

Conjugated proteins are composed of simple protein combined with some non-protein substance. The non-protein group is referred to as the ***prosthetic*** (additional) group. Following are the examples of conjugated proteins:

Nucleoproteins

The nucleoproteins are composed of simple basic proteins (histones or protamines) with ***nucleic acids (RNA and DNA) as the prosthetic groups.*** They are proteins of cell nuclei, e.g.

- Nucleohistone
- Nucleoprotamine.

Glycoproteins and proteoglycans or mucoproteins

These consist of simple protein and ***carbohydrate as a prosthetic group.***

- When carbohydrate content is less than 4% of protein it is called ***glycoprotein***, e.g.
  - Mucin of saliva
  - Immunoglobulins
- When carbohydrate content is more than 4%, it is called ***mucoprotein*** or ***proteoglycans,*** e.g. glycosaminoglycans.

Chromoproteins

Chromoproteins are composed of simple proteins with a ***colored prosthetic group***, e.g.

- Hemoglobin
- Cytochromes
- Catalase
- Peroxidase.

In all these chromo proteins, prosthetic group is heme.

Phosphoproteins

The phosphoproteins are formed by a combination of protein with ***prosthetic group phosphoric acid,*** e.g.

- Casein of milk
- Vitellin of egg yolk.

Lipoproteins

The lipoproteins are formed by a combination of protein with a ***prosthetic group lipid***, e.g.

- Serum lipoproteins like:
  - Chylomicrons
  - Very low density lipoprotein (VLDL)
  - Low density lipoprotein (LDL) and
  - High density lipoproteins (HDL).

Metaloproteins

The *prosthetic group is metallic elements* such as: Fe, Co, Mn, Zn, Cu, Mg, etc., for example;

- Ceruloplasmin is a copper containing protein
- Carbonic anhydrase, carboxypeptidase and DNA polymerase are zinc containing proteins.

Derived Proteins

This class of proteins as the name implies, includes those substances formed from simple and conjugated proteins. Derived proteins are subdivided into:

- Primary derived proteins (denatured proteins)
- Secondary derived proteins.

Primary derived proteins (denatured proteins)

These protein derivatives are formed by agents, such as heat, acids, alkalies, etc. which cause only slight changes in the protein molecule and its properties without hydrolytic cleavage of peptide bond. These are synonymous with denatured proteins **(Figure 4.9)**, e.g.

- Proteans
- Metaproteins.

Proteans

These are the earliest product of protein hydrolysis by action of dilute acids or enzymes, e.g.

- Myosan from myosin
- Fibrin from fibrinogen.

Metaproteins

The metaproteins are formed by further action of acids and alkalies on proteans, e.g.

- Acid and alkali albuminates.

Secondary derived proteins

These substances are formed in the progressive hydrolytic cleavage of the peptide bonds of metaproteins (coagulated proteins) into progressive smaller molecules, e.g.

- Proteoses
- Peptones
- Peptides **(Figure 4.9)**.

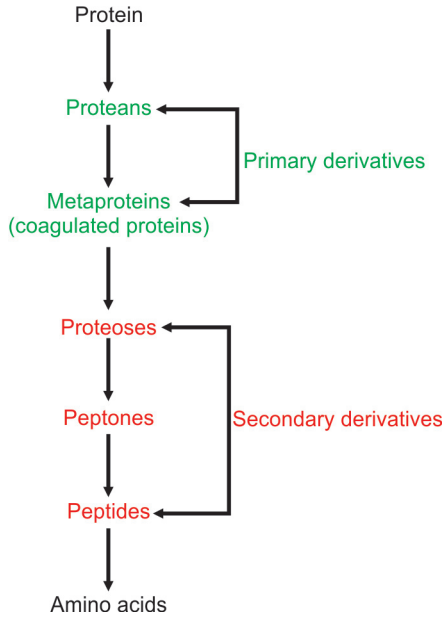


Figure 4.9: Formation of primary and secondary derived proteins

STRUCTURE OF PROTEINS

Every protein in its native state has a unique three-dimensional structure which is referred to as its ***conformation*** and made up of only 20 different amino acids. The number and sequence of these amino acids are different in different proteins. Protein structure can be classified into four levels of organization:

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure.

Primary Structure of Proteins

- The sequence of amino acids forming the backbone of proteins and location of any disulfide bond in a protein is called, the primary structure of the protein **(Figures 4.10 and 4.11)**.
- In proteins, amino acids are joined covalently by ***peptide bonds,*** which are formed between α-carboxyl group of one amino acid and α-amino group of another with the elimination of a water molecule **(Figure 4.7)**.

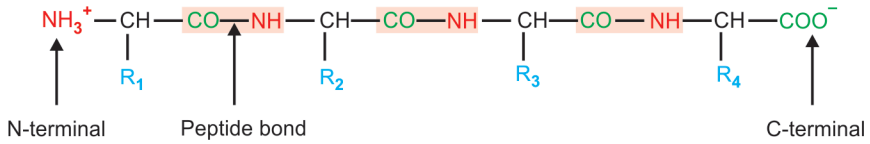


Figure 4.10: Polypeptide chain showing N-terminal and C-terminal

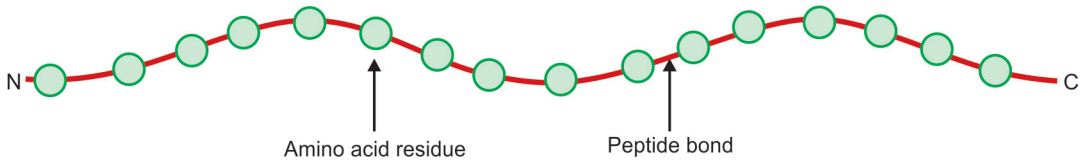


Figure 4.11: Schematic diagram for the primary structure of protein

- Linkage of many amino acids through peptide bonds results in an unbranched chain called a ***polypeptide*** **(Figure 4.10)**. Each amino acid in a polypeptide is called a **residue** or **moiety.** Each polypeptide chain is having free amino group at one end called *N-terminal* and free carboxyl group at another end, called *C-terminal*.
- Proteins have unique amino acid sequences that are specified by genes. This amino acid sequence of a protein is referred to as its primary structure.

Clinical Importance of Primary Structure

Understanding of primary structure of a protein is important because many genetic diseases result due to an abnormal amino acid sequences. If the primary structure of the normal and mutated proteins are known, this information may be used to diagnose or study the disease.

Secondary Structure of Proteins

- For stability of primary structure, **hydrogen bonding** between the hydrogen of NH and oxygen of C=O groups of the polypeptide chain occurs, which gives rise to folding or twisting of the primary structure.
- ***Thus, regular folding and twisting of the polypeptide chain brought about by hydrogen bonding is called secondary structure of protein.*** The most important kinds of secondary structure are:
  - α-Helix (helicoidal structure)
  - β-Pleated sheet (stretched structure).

α-Helix

It is called α because it was the first structure elucidated by Pauling and Corey. If a backbone of polypeptide chain is twisted by an equal amounts about each α-carbon, it forms a coil or helix. The helix is a rod-like structure.

- The helix is stabilized by hydrogen bonds beween the NH and CO groups of the same chain.
- These hydrogen bonds have an essentially optimal nitrogen to oxygen (N-O) distance of 2.8 Å. Thus, CO group of each amino acid is hydrogen bonded to the -NH of the amino acid that is situated four residues ahead in the linear sequence **(Figure 4.12)**.
- The axial distance between adjacent amino acids is 1.5 Å and gives 3.6 amino acid residues per turn of helix **(Figure 4.13).**

Helix destabilizing amino acids

Glycine and ***proline*** are the helix-destabilizing amino acids.

β-Pleated Sheet Structure or Stretched State Structure

Pauling and Corey discovered another type of structure which they named ***β-pleated sheet*** (β because it was the second structure they elucidated). The surfaces of β-sheet appear "pleated" and these structures are therefore often called ***"β-pleated sheet'.***

- A polypeptide chain in the β-pleated sheet is almost fully extended rather than being tightly coiled as in the α-helix.
- Unlike α-helix, β-pleated sheets are composed of two or more polypeptide chains.
- β-pleated sheet is stabilized by hydrogen bonds between NH and C=O groups in a different polypeptide chain whereas in α-helix, the hydrogen bonds are between NH and CO groups in the same polypeptide chain.

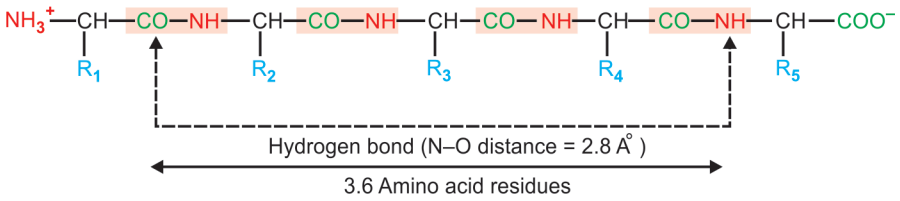


Figure 4.12: Formation of hydrogen bond in α-helix

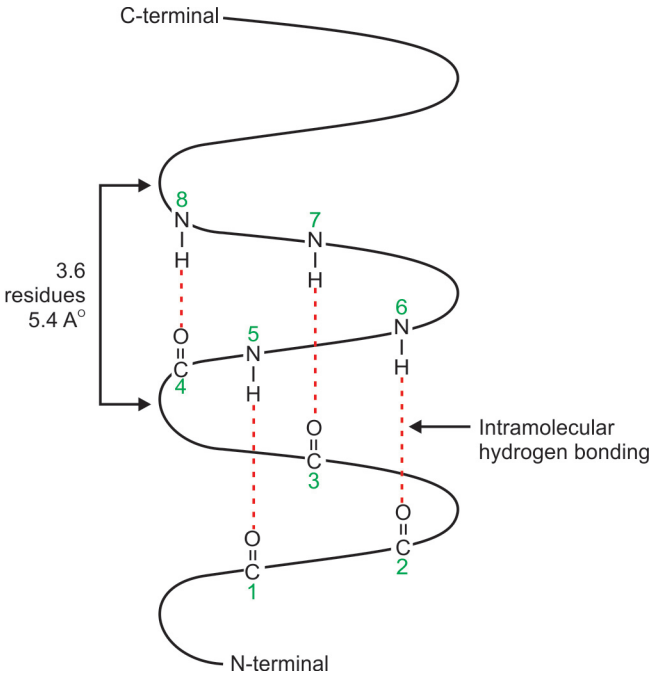


Figure 4.13: Schematic diagram of α-helical structure of protein. C=O group of each amino acid is hydrogen bonded to the NH of the amino acid that is situated four residues ahead (Number indicates the amino acid residues)

- In β-sheet, the hydrogen bonds are perpendicular to the polypeptide backbone rather than parallel as in the α-helixes.

The arrangement of polypeptide chains in β-pleated sheet conformation can occur in two ways:

1. Parallel pleated sheet
2. Anti-parallel pleated sheet.

Parallel pleated sheet

In parallel pleated sheet:

- The polypeptide chains lie side-by-side and in the same direction (with respect to N- and C-terminal), so that their N-terminal residues are at the same end (N-terminal faces to N-terminal) and stabilized by hydrogen bonding.
- Here the hydrogen bonds are (interchain) formed between NH of a polypeptide in one chain and carbonyl (C=O) of a neighboring chain **(Figure 4.14 B)**.

Anti-parallel pleated sheet

In the anti-parallel pleated sheet:

- The polypeptide chains lie in opposite directions, i.e. N-terminal end of one is next to the C-terminal of the other. (N-terminal faces to C-terminal) **(Figure 4.14A).**
- It is stabilized by interchain hydrogen bonding.

Other types of secondary structures

Besides the α- and β-structures described above, the b-bends, loop regions and disordered regions, are also found in proteins.

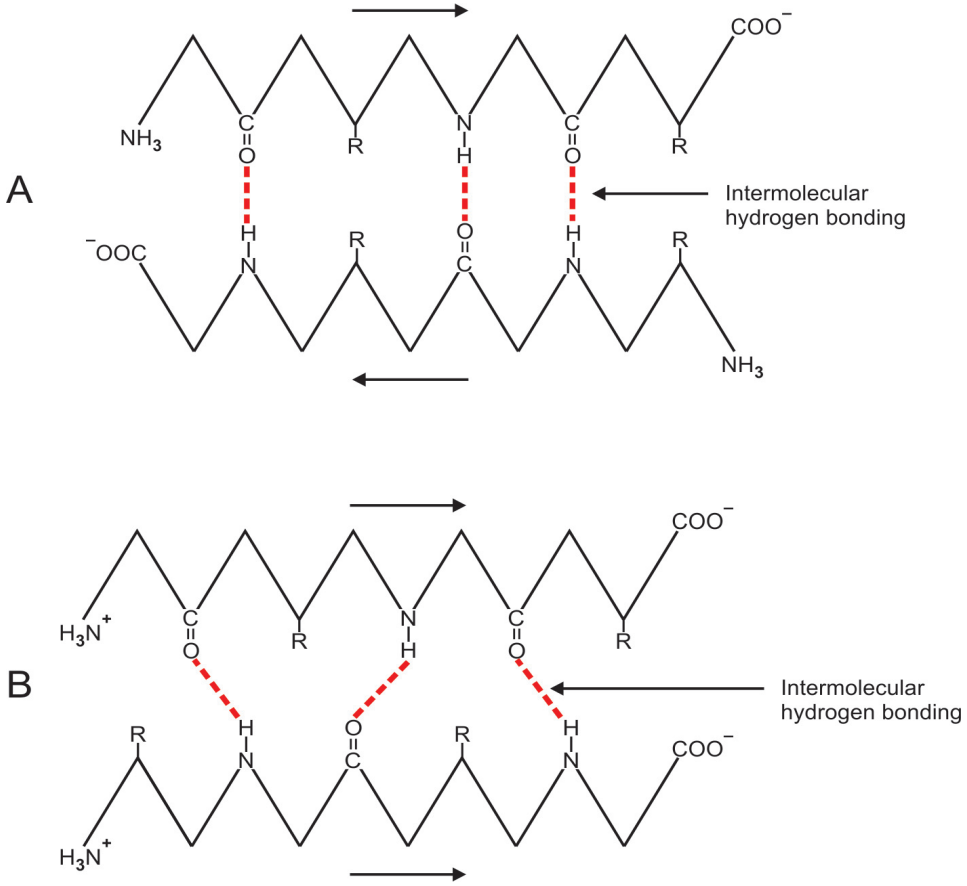
Tertiary Structure

- The peptide chain, with its secondary structure, may be further folded and twisted about itself forming three-dimensional arrangement of the polypeptide chain **(Figure 4.15)**.
- Amino acid residues which are very distant from one another in the sequence can be brought very near due to the folding and thus form regions essential for the functioning of the protein, like **active site** or catalytic site of enzymes.
- Thus, the three-dimensional folded compact and biologically active conformation of a protein is referred to as its tertiary structure, e.g. myoglobin.

Tertiary Structure Stabilizing Forces

The three-dimensional tertiary structure of a protein is stabilized by:

- Hydrogen bonds
- Hydrophobic interactions
- Van der Waals forces
- Disulfide bond
- Ionic (electrostatic) bonds or salt bridges.



Figures 4.14 A and B: β-pleated sheet structure. (A) Anti-parallel; (B) Parallel

Figure 4.15: Tertiary structure of protein

Quaternary Structure of Protein

- Only those proteins that have **more than one polypeptide chain (polymeric)** have a quaternary structure. Not all proteins are polymeric. Many proteins consist of a **single polypeptide chain and are called monomeric** proteins, e.g. myoglobin.
- The arrangement of these polymeric polypeptide subunits in three-dimensional complexes is called the quaternary structure of the protein **(Figure 4.16)**.
- Examples of proteins having quaternary structure are:
  - Lactate dehydrogenase
  - Pyruvate dehydrogenase
  - Hemoglobin.

Quaternary Structure Stabilizing Forces

The subunits of polymeric protein are held together by noncovalent interactions or forces such as:

- Hydrophobic interactions
- Hydrogen bond
- Ionic bonds.

Bonds Responsible for Protein Structure

Protein structure is stabilized by two types of bonds

1. Covalent bond, e.g.
   - Peptide bonds
   - Disulfide bond

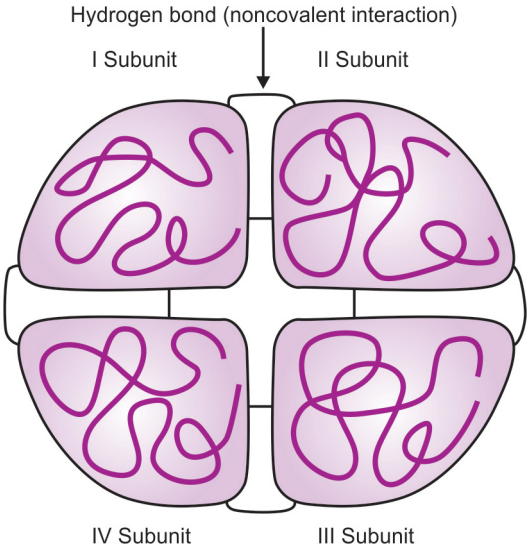


Figure 4.16: Schematic representation of quaternary structure of polymeric protein

2. Noncovalent bond, e.g.
   - Hydrogen bond
   - Hydrophobic bond or interaction
   - Electrostatic or ionic bond or salt bond or salt bridge
   - Van der Waals interactions.

Covalent Bond

Peptide bonds (–CO-NH–)

- A peptide bond is formed by the condensation of the amino group of one amino acid with the carboxyl group of another amino acid with a removal of a water molecule **(See Figure 4.7)**.

Disulfide bond (-S-S-)

- A covalent bond formed between the sulfhydryl group (-SH) of side chain of cysteine residues in the same or different peptide chains.
- These disulfide bonds help to stabilize against denaturation and confer additional stability.

Noncovalent Bonds

Hydrogen bond

- Bond formed between -NH and -CO groups of peptide bond by sharing single hydrogen.
- Hydrogen bond may occur within the same polypeptide chain (intrachain) or between different polypeptide chains (interchain).
- Side chains of 11 out of the 20 standard amino acids can also participate in hydrogen bonding.

Hydrophobic bond or interaction

These are formed by interaction between nonpolar hydrophobic R groups (side chain) of amino acids like alanine, valine, leucine, isoleucine, methionine, phenylalanine and tryptophan.

Electrostatic or ionic bond or salt bond or salt bridge

These are formed between oppositely charged groups when they are close, such as amino (NH_3^+) terminal and carboxyl (COO^-) terminal groups of the peptide and the oppositely charged R-groups of polar amino acid residues.

Van der Waals interactions

Van der Waals forces are extremely weak and act only on extremely short distances and include both an attractive and a repulsive forces (between both polar and nonpolar side chain of amino acid residues).

PROPERTIES OF PROTEINS

Colloidal Nature

Protein molecules exist in the form of colloidal particle, 5-100 mμ in dimension, are heavier than water and sink.

Colloidal Osmotic Pressure

- Colloidal protein molecules exert **osmotic pressure.** The osmotic pressure generated by plasma proteins is often called the ***colloidal osmotic pressure*** or ***oncotic pressure*** of plasma.
- The osmotic pressure of protein is proportional to its concentration, but inversely proportional to its molecular weight.
- In blood plasma, albumin contributes 75-80% of osmotic pressure (although it represents no more than half the plasma proteins), because its molecular weight is lower.
- Oncotic pressure exerted by protein is clinically important in maintaining blood volume.

Molecular Weight

Proteins are macromolecules and have very high molecular weights with wide variations, e.g. molecular weight of:

- Albumin = 69,000
- γ-Globulin = 1,60,000.

Solubility

In general, globular proteins, such as, albumin have higher solubility than elongated fibrous proteins. Moreover, smaller molecules are more soluble than larger molecules.

Shape of the Protein

There is a wide variation in the protein shape.

- Scleroproteins like keratin, collagen are in the form of fibers.
- While soluble proteins tend to be of rounded shape and are called globular proteins.

Hydration of Proteins

- Proteins, when brought into contact with water, absorb water and swell up.
- The polar groups like COOH, NH_2, OH of protein bind to the molecules of water by hydrogen bonds to hold a considerable amount of water. Thus, a relatively immobile shell-like layer of water, called the ***"solvation layer"*** or ***water envelope*** is held around each protein particle in an aqueous medium.

Amphoteric Nature and Isoelectric pH of the Proteins

The isoelectric pH of amino acid has been described previously. One end of the protein molecule has free amino group, while the other end has free COOH group.

- In acid solution, the NH_2 groups accept H^+ ion and present as NH_3^+ (cation). Therefore protein in acid solution will be positively charged.
- In alkaline pH, the COOH groups donate H^+ ion and are present as COO^- (anion). Hence, proteins in alkaline solution are negatively charged.

So, proteins are **ampholytes** acting both as donors and acceptors of H^+ ion.

Isoelectric pH of the Protein

- For every protein in solution, there is a particular pH at which the number of anions formed is exactly equal to the number of cations, and the solution is electrically neutral. That pH is called the ***isoelectric pH (PI)*** of that protein and the protein exists as ***zwitter ion.***
- At isoelectric pH:
  - Protein molecules do not migrate in an electric field.
  - Solubility, buffering capacity and viscosity will be minimum and precipitability will be maximum.
- PI of some proteins are given below:
  - Pepsin: 1.1
  - Casein: 4.6
  - Albumin: 4.7
  - Globulin: 6.4.
- At pH values above or below the isoelectric pH, they carry a net negative or positive charge and migrate to anode (+vely charged electrode) or cathode (–vely charged electrode).

Precipitation of Proteins

The stability of protein in solution depends on the **charge** and **hydration** of the protein molecule.

- The factors which neutralize the charge or remove water of hydration will cause precipitation of proteins. The factors used for precipitation of proteins are:
  - Salting out
  - Isoelectric pH
  - Heavy positive or negative ions
  - Organic solvents.

Salting out method

When neutral salts such as ammonium sulfate or sodium sulfate are added to a protein solution, the addition may precipitate a protein from its solution.

- Mineral ions attract water molecules and consequently remove the shell of hydration (solvation layer) from around protein molecules.
- Since water layer around protein particles is removed, the protein is precipitated. This is called ***salting out.***
- Higher molecular weight protein requires less salt to precipitate than low molecular weight protein. Thus, globulins are precipitated at half saturation of ammonium sulfate or 22% Na-sulfate, but albumin requires full saturation of ammonium sulfate or 28% Na-sulfate.

Application: These methods are therefore useful in separating albumin and globulin from serum proteins.

Precipitation at isoelectric pH

All proteins are least soluble at their isoelectric pH (PI) and can then be precipitated.

Precipitation by heavy positive or negative ions

- On the acidic side of its PI, a protein remains as cation (+vely charged) and may then be precipitated by neutralizing the charge on protein by adding anion (-vely charged) or alkaloidal reagents like ***tungstate, trichloroacetate*** or ***picrate.***
- On the alkaline side of its PI, a protein exists as an anion and may then be precipitated as metal proteinates by heavy metal ions like Zn^{2+}, lead, mercury, etc.

Precipitation by organic solvents

Organic solvent like alcohol, dehydrates and precipitates the proteins.

DENATURATION OF PROTEINS

The three-dimensional conformation, the ***primary, secondary, tertiary*** and even in some cases ***quaternary*** structure is characteristic of a native protein. ***Hydrogen***

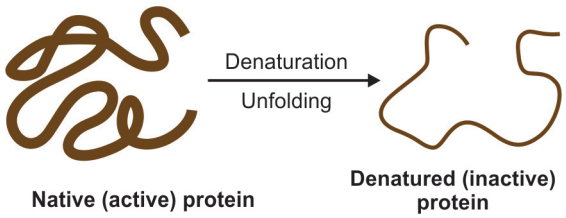


Figure 4.17: Denaturation of protein

bond, ionic bond and ***hydrophobic bond*** stabilize the structure to maintain its conformation in space. This conformation can upset and disorganized ***without breakage of any peptide linkage,*** only by the rupture of ionic bond, hydrogen bonds and hydrophobic bond which stabilize the structure. This is called ***denaturation*** **(Figure 4.17)**. Denaturation of proteins leads to:

- Unfolding of natural coils of native protein.
- Decrease in solubility and increase in precipitability.
- Loss of biological activities, (e.g. enzyme activity) and antigenic properties.
- Increased digestibility.

Denaturing Agents

- Denaturation is brought about by certain:
  - Physical agents
  - Chemical agents
  - Mechanical means.
- **Physical agents :** Heat, Ultraviolet rays and ionizing radiations can denature proteins.
- **Chemical agents :** Acids, alkalies and certain acid solutions of heavy metals, e.g. mercury, lead, detergents; organic solvents like alcohol, acetone, etc. denature proteins.
- **Mechanical means :** Vigorous shaking or grinding leads to denaturation of the protein.

Examples of Denatured Protein

Cooked meat or boiled egg, milk paneer, etc.

Significance of Denaturation

- Digestibility of native protein is increased on denaturation by gastric HCI or by heat on cooking. Denaturation causes unfolding of native polypeptide coil so that hidden peptide bonds are exposed to the action of proteolytic enzyme in the gut. It also increases reactivity of certain groups.
- Denaturation property of a protein is used in blood analysis to eliminate the proteins of the blood (deproteinization of blood).

Coagulation

Denaturation may, in rare cases be reversible, in which case the protein refolds into its original native structure, when the denaturing agent is removed. However, most proteins, once denatured, remain permanently disordered and are called ***irreversible denaturations*** or ***coagulation,*** e.g. coagulated egg white of boiled egg.

SUMMARY

- The basic structural units of proteins are amino acids.
- All proteins in all species from bacteria to humans are constructed from the same set of twenty amino acids called standard amino acids.
- Each amino acid contains an α-carboxyl group, an α-amino group and distinctive R (side chain) group attached to the α-carbon atom.
- The α-carbon atom of the amino acids except glycine is asymmetric and thus can exist in stereoisomeric forms (D and L), only L-α-amino acid (L-stereoisomer) are found in protein.
- Amino acids can be classified on the basis of nature of amino acids in the solution or structure of the side chain or nutritional requirement or metabolic products of amino acids.
- PI is the pH at which an amino acid bears no net charge and does not move in an electrical field.
- Among the 20 standard amino acids, histidine serves as the best buffer at physiological pH.
- Proteins are classified on the basis of their function, and physical and chemical properties.
- Every protein has a unique three-dimensional structure that reflects its function.
- There are four levels of protein structure: Primary, secondary, tertiary and quaternary (only for oligomeric proteins).

EXERCISE

Multiple Choice Questions (MCQs)

1. **All amino acids found in proteins are optically active, *except:***
   a) Serine b) Glycine
   c) Threonine d) Tyrosine
2. **All of the following forces may play a role in the formation of tertiary structure, *except:***
   a) Hydrogen bond
   b) Disulfide bridges
   c) Hydrophobic interaction
   d) Peptide bonds

3. The greatest buffering capacity at physiological pH would be provided by a protein, rich in which of the following amino acids:
a) Glycine b) Lysine
c) Histidine d) Valine

4. In proteins, triple helix is an example of:
a) Primary structure b) Secondary structure
c) Tertiary structure d) Quaternary structure

5. Which of the following amino acid is exclusively ketogenic?
a) Leucine b) Phenylalanine
c) Threonine d) Isoleucine

6. In humans all of the following amino acids are essential, *except:*
a) Valine b) Isoleucine
c) Glycine d) Phenylalanine

7. During denaturation of protein the following bonds are disrupted, *except:*
a) Hydrogen b) Hydrophobic
c) Peptide d) Sulfide

8. The imino acid present in protein is:
a) Phenylalanine b) Valine
c) Leucine d) Proline

9. Glycine is used for synthesis of the following, *except:*
a) Heme b) Serotonin
c) Purine d) Creatine

10. Which class of amino acids contain only non-essential amino acids?
a) Aromatic b) Acidic
c) Branched chain d) Basic

11. Which of the following is a non-protein amino acid?
a) Proline
b) Histidine
c) Ornithine
d) Aspargine

12. Which of the following is a kind of secondary structure?
a) α-helix
b) β-bend
c) Triple helix
d) All of the above

13. Glutathione is found in all mammalian cells *except:*
a) RBC b) Neurons
c) Skeletal muscle d) Argentaffin cells

14. Albumin is deficient in which of the following amino acid?
a) Glycine b) Tryptophan
c) Cysteine d) Methionine

15. Which of the following acts as a redox buffer?
a) Insulin b) Glucagon
c) Glutathione d) Angiotensin

Correct Answers for MCQs

1-b	2-d	3-c	4-b
5-a	6-c	7-c	8-d
9-b	10-b	11-c	12-d
13-b	14-a	15-c	

CHAPTER

5

Plasma Proteins and Immunoglobulins

- Introduction
- Plasma Proteins
- Immunoglobulins
- Summary
- Exercise

INTRODUCTION

Plasma contains a variety of proteins with different functions. At present time over 100 different plasma proteins have been described. There are many proteins whose function remains to be determined . In this chapter, we shall see those plasma proteins which are present in significantly high concentrations, e.g. albumin, globulin and fibrinogen.

PLASMA PROTEINS

- The plasma proteins are:
  1. Albumin
  2. Globulin
  3. Fibrinogen
- The normal value of plasma proteins are:
  - Total protein 6 to 8 gm %
  - Serum albumin 3.5 to 6 gm %
  - Serum globulin 2 to 3.5 gm %
  - Fibrinogen 200 to 400 mg %
  - A/G ratio 1.2:1 to 2.5:1

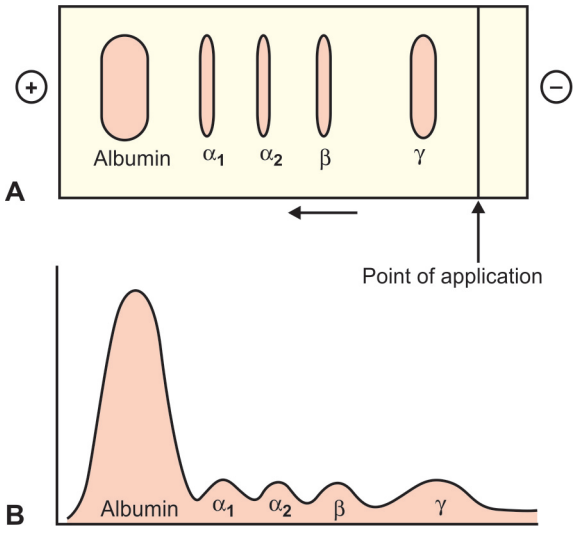
Synthesis of Plasma Proteins

- All the ***albumin*** and ***fibrinogen*** are essentially synthesized by the **liver** only.
- Similarly, 50 to 80 percent of the globulin is formed in the liver. The remainder of the globulins are formed almost entirely in the **lymphoid tissues**. They are ***gamma globulins*** that constitute the ***antibodies*** used in the immune system.
- In severe liver disease, there is thus a lowered concentration of plasma albumin but globulin fraction may not show substantial fall, as gamma globulins are not synthesized by the liver. ***The A/G ratio therefore can be altered in the liver disease.***

Separation of Plasma Proteins

- The plasma proteins can be separated by the following methods:
  1. Salting out
  2. Electrophoresis
  3. Ultracentrifugation
  4. Immunoelectrophoresis.
- ***Electrophoresis:*** This is the most commonly employed analytical technique for the separation of plasma/serum proteins. The basic principles of electrophoresis are described in **Chapter 35**.
- Serum proteins separated on cellulose acetate membrane generally have five bands **(Figures 5.1A and B):**
  1. Albumin
  2. α_1-globulin
  3. α_2-globulin
  4. β-globulin
  5. γ-globulin.

These five bands appear as five peaks on the densitometer graph.



Figures 5.1A and B: Electrophoretic separation of serum proteins (A) Electrophoretogram of normal serum on cellulose acetate strip; (B) Densitometric scanning from cellulose acetate strip converts bands to characteristic peaks of albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin

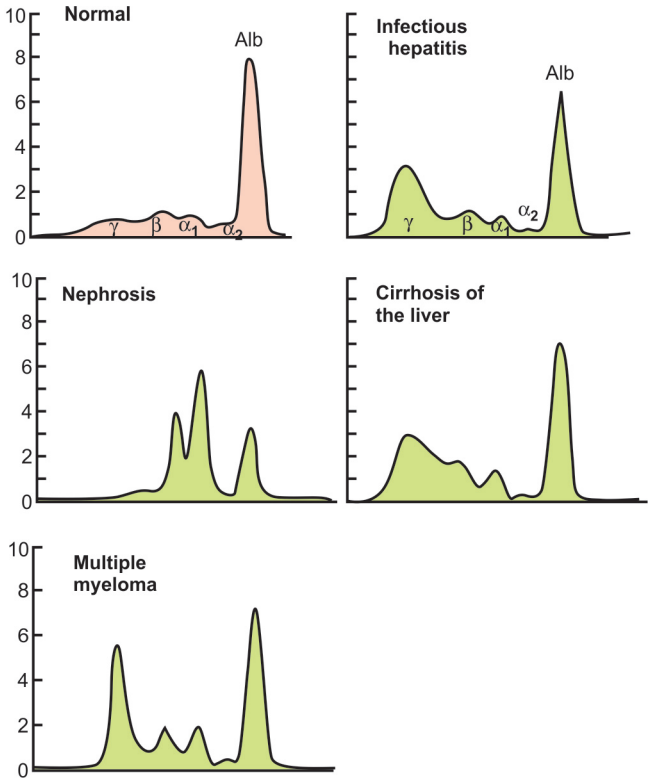


Figure 5.2: Different electrophoretic patterns of serum compared with normal serum

- Serum protein electrophoretic patterns provide useful diagnostic information.
- Different electrophoretic patterns of serum associated with various disorder, compared with normal serum are shown in **Figure 5.2**.
- Changes in serum protein fractions associated with various conditions are given below:
  - Infective hepatitis shows slight decrease in albumin and significant increased γ-globulins.
  - Liver cirrhosis shows elevations in β and γ-globulins with a decrease in albumin.
  - Nephrosis shows low level of albumin, significantly elevated α_2-globulin and elevated β-globulin.
  - Multiple myeloma shows marked increase in γ-globulin.

Major Classes of Plasma Proteins (Table 5.1)

Albumin

- Albumin is a globular protein consisting of single polypeptide chain with a molecular weight of about 69,000 in the humans. It comprises of some 585 amino acid residues.
- It is the most abundant protein found in plasma, accounting for approximately 50 percent of plasma protein mass.
- Albumin is exclusively synthesized by the liver for this reason, serum albumin levels are determined to assess liver function (synthesis decreased in liver diseases).
- The normal plasma **half-life** of albumin is **15** to **20 days.**

Functions of albumins

- Albumin's primary function is the maintenance of **colloidal osmotic pressure.** Albumin makes the biggest contribution to the plasma oncotic pressure. Thus, albumin plays a predominant role in maintaining blood volume and body fluid distribution. Very low albumin concentration develops edema.
- A second important function of albumin is to bind and transport many metabolites which are poorly soluble in water such as:
  - Fatty acids
  - Bilirubin
  - Inorganic constituent of plasma like calcium
  - Certain steroid hormones and drugs.
- **Buffering function:** Among the plasma proteins, albumin has maximum buffering capacity due to its high concentration in blood.

Table 5.1: Major classes of plasma proteins, their functions and diagnostic importance

Classes	*Examples*	*Principal functions*
Albumin		Exert colloidal osmotic pressure and transport function
Globulin	α_1-protease inhibitor (API) or α_1-antitrypsin (AAT)	Antiprotease, natural inhibitor of proteolytic enzyme elastase
	Ceruloplasmin	Transport of copper
	Haptoglobin	Conservation of iron by binding free hemoglobin
	α_2-Macroglobulin (AMG)	Natural antiprotease, inhibits thrombin, trypsin and pepsin
	Hemopexin	Binds heme and prevents loss of iron
	Transferrin	Transport of iron
	C-reactive protein (CRP)	Body's defense mechanism
	β_2-microglobulin (BMG)	Body's defense mechanism
	Immunoglobulins IgG, IgA, IgM, IgD and IgE	Body's defense mechanisms
Fibrinogen		Blood coagulation

- **Nutritive function :** Degradation of albumin provides essential amino acids during malnutrition.

Clinical Significance

Serum albumin measurements are used to assess the various clinical conditions.

Hypoalbuminemia

Decreased level of plasma albumin is seen in :

- **Malnutrition**
  In malnutrition, due to insufficient intake of proteins, the availability of amino acid is reduced and so albumin synthesis is affected.
- **Nephrotic syndrome**
  In nephrotic syndrome, large amounts of protein are lost in urine (proteinuria).
- **Cirrhosis of liver**
  In cirrhosis, albumin synthesis is decreased and so blood level is lowered.

Hyperalbuminemia

Increased levels of plasma albumin are present only in acute dehydration and have no clinical significance.

Analbuminemia

Analbuminemia is a rare hereditary abnormality in which plasma albumin concentration is usually less than 1.0 gm/L (normal level is 3.5 to 6 gm/dl). However, there may be no symptoms or signs not even edema due to compensatory increase in plasma globulin concentration.

Globulins

Globulins are bigger in size than albumins. Globulins constitute several fractions. These are:

- α_1-globulin
- α_2-globulin
- β-globulin
- γ-globulin

Biological functions of various globulins are summarized in Table 5.1, which are discussed below.

α_1-Protease inhibitor (API) or also known as α_1 antitrypsin (AAT)

- API is one of the plasma proteins that inhibits activity of **proteases** particularly **elastase,** which degrades elastin, a protein that gives elasticity to the lungs.
- In a normal individual, the activity of elastase is regulated by API.
- A genetic deficiency in API can lead to **emphysema** (lung disorder). Excessive cigarette smoking also leads to emphysema as cigarette smoke inhibits the activity of API.

Prothrombin

- It is synthesized by liver with the help of vitamin K and involved in **blood *clotting***.
- Liver damage causes lengthening of prothrombin time.

Ceruloplasmin (Ferro-oxidase)

- This is a copper containing protein.
- It has oxidase activity.

- Ceruloplasmin is the major transport protein for copper, an essential trace element.
- It is also essential for the regulation of the oxidation-reduction, transport and utilization of iron.
- Plasma ceruloplasmin level is reduced in **Wilson's disease,** in patients with malnutrition and in the nephrotic syndrome.

Haptoglobin
- It plays an important role in the conservation of iron by preventing its loss in the urine.
- Haptoglobin binds free hemoglobin to form a complex which is too large to be filtered by the kidney and thus prevents the loss of iron in the urine.

α_2-macroglobulin (AMG)
- This is major α_2-globulin, which is a natural inhibitor of ***endopeptidase*** such as trypsin, chymotrypsin, plasmin, thrombin, etc.
- Plasma AMG is decreased in myeloma, peptic ulcer and increased in nephrotic syndrome, cirrhosis and collagen disorders.

Hemopexin
- Like haptoglobin, hemopexin also plays an important role in the conservation of iron by preventing its loss in the urine.
- Hemopexin binds **free heme,** not hemoglobin
- The complex hemopexin-heme is too large to be filtered by the kidney and thus prevents the loss of iron in the urine.

Transferrin
- Transferrin is synthesized in liver.
- It transports iron (two molecules of Fe^{3+} per molecule of transferrin) through blood to the sites where iron is required.
- Increased serum levels are seen in iron deficiency anemia and pregnancy.
- Low levels occur in chronic infections and in malnutrition.

C-reactive protein (CRP)
- CRP is named because it reacts with C-polysaccharides of the cell wall of pneumococci bacteria.
- CRP is involved in the body's defense mechanism.
- It is useful in differentiating bacterial infection from viral infections because the ***level of CRP is increased in bacterial infections only.***

β_2-microglobulin
- This protein forms part of the ***human leucocyte antigen (HLA) system.***
- It is derived from myeloid and lymphoid cells and is normally synthesized at the constant rate.
- Plasma levels are increased whenever, there is malignant lymphoid or myeloid proliferation and renal failure.

Immunoglobulins
(discussed later separately)

Fibrinogen (Blood Clotting Factor I)

- It is a glycoprotein and constitutes about 4 percent of total plasma proteins. It is synthesized in liver and secreted in blood where it is involved in **blood coagulation.**
- During blood coagulation, fibrinogen is converted to fibrin which polymerizes to form fibrin clot.
- Plasma level of fibrinogen decreases in severe hepatic diseases.

IMMUNOGLOBULINS (IG)

- The immunoglobulins are **γ-globulins,** called **antibodies**. All antibodies are immunoglobulin but all immunoglobulins may not be antibodies.
- They constitute about 20 percent of all the plasma proteins
- Immunoglobulins are produced by **plasma cells** and to some extent by **lymphocytes**.

Structure of Immunoglobulins (Figure 5.3)

- Immunoglobulins are **glycoproteins** made up of **light (L)** and **heavy (H)** polypeptide chains. The term **"light"**

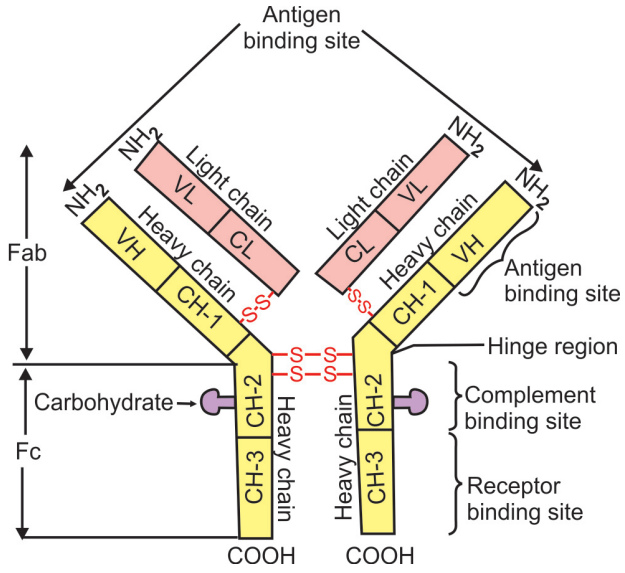


Figure 5.3: Schematic structure of IgG to show basic structure of immunoglobulin molecule
VH: Variable heavy chain VL: Variable light chain
CH: Constant heavy chain CL: Constant light chain

Table 5.2: Different classes of immunoglobulins corresponding to the type of heavy chains

Ig classes	*Types of Heavy chains*
IgG	γ (gamma)
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	ε (epsilon)

and **"heavy"** refer to **molecular weight.** Light chains have a molecular weight of 25,000 whereas heavy chains have a molecular weight of 50,000 to 70,000.

- All immunoglobulins have the same basic structure. The basic immunoglobulin is a **'Y'** shaped molecule and consists of four polypeptide chains, **two H** and **two L** chains.
- The four chains are linked by **disulfide** bonds.
- L chain may be either of two types, **Kappa (κ)** or **Lambda (λ)** but not both.
- The heavy chains may be of five types and are designated by Greek letter:
  1. Alpha (α)
  2. Gamma (γ)
  3. Delta (δ)
  4. Mu (μ)
  5. Epsilon (ε).
- Immunoglobulins are named as per their heavy chain type as **IgA, IgG, IgD, IgM** and **IgE (Table 5.2)**

The L and H chains are subdivided into **variable** and **constant** regions.

- L chain consists of one **variable (VL)** and one **constant (CL)** domain or region.
- Most H-chains consist of one **variable (VH)** and three **constant (CH-1, CH-2, and CH-3)** domains.
- Each immunoglobulin molecule has **hinge region** between CH-I and CH-2, which allows a better fit with the antigen surface.
- The variable regions of both the light and heavy chains form **antigen binding site.**
- Enzyme (papain) digestion splits the immunoglobulin molecule into two fragments named as **Fab (Fragment for antigen binding)** and **Fc (crystallizable fragment or fragment for complement binding (Figure 5.3)**

Functions of Immunoglobulins (Antibody)

- The primary function of antibodies is to protect against infectious agents.
- In addition to these functions, antibodies can act as an enzyme to catalyze the synthesis of ozone (O_3) that has microbicidal activity.

Immunoglobulin Classes

IgG (Heavy chain γ)

- IgG is a smaller **monomeric** molecule with two antigen binding sites **(Figure 5.3).**
- IgG is the major class of immunoglobulin which accounts for 70 percent of the total serum immunoglobulins.
- IgG is the only antibody that **crosses the placenta** and is the **maternal antibody** that protects the fetus.
- It is produced mainly in the **secondary response** and provides defense against bacteria and viruses.

IgA (Heavy chain α)

- IgA is the **second most abundant class** constituting about 20 percent of serum immunoglobulins. It is **monomer** or **dimer (Figure 5.4).**
- Dimeric IgA molecule formed by two monomer units, joined together at their carboxy terminals by a protein termed **J-chains** (J for joining).
- IgA is found in external secretions such as **colostrum, saliva, tears** and **respiratory, intestinal** and **genital tract secretions**.
- It prevents attachment of microorganisms like bacteria and viruses to mucous surfaces and helps to protect

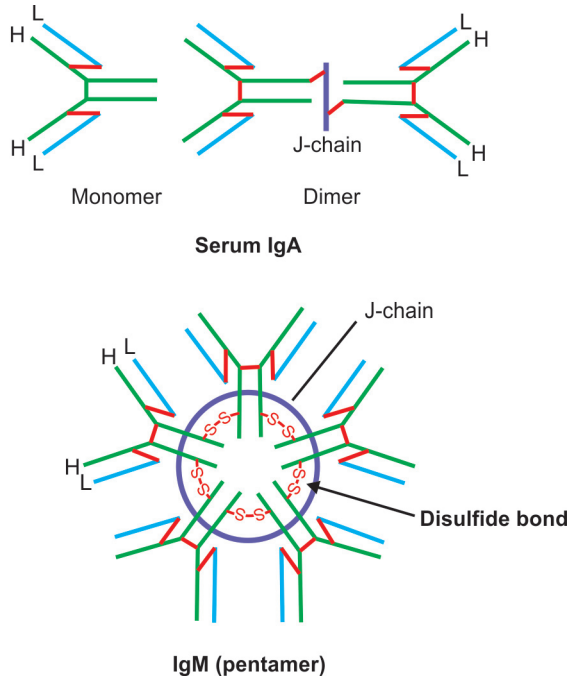


Figure 5.4: Diagrammatic representation of monomeric, dimeric and pentameric forms of immunoglobulins

mucous surfaces from antigenic attack and prevent access of foreign substances to the circulation.

IgM (Heavy chain μ)

- It is a **pentamer** consisting of five identical immunoglobulin molecules, joined together by **disulfide bridges.** This pentamer is closed in a ring structure by J-chain **(Figure 5.4).**
- IgM is the first antibody to be produced in response to an antigen and is important in defense against bacteria and viruses.
- IgM present on the surface of **B lymphocytes** are **monomer,** where it functions as an antigen binding **receptor**.

IgD (Heavy chain δ)

- It is a **monomer** and resembles IgG structurally.
- IgD has no known antibody function but may function as an **antigen receptor.**
- Like IgM, it is present on the surface of many **B lymphocytes.**
- The circulating concentration of IgD in blood is very low.

IgE (Heavy Chain ε)

- IgE is a **monomeric** molecule similar to IgG. It is sometimes called **reagin.**
- Although IgE is present in trace amounts, (approximately 0.004%) in normal persons with allergic activity have greatly increased amounts.
- IgE is responsible for **anaphylactic (immediate) type of hypersensitivity and allergy.** Its activity is mediated by histamine.
- IgE also participates in defense against certain **parasites,** e.g. **helminths** (worms).

Structure, function and characteristics of different types of immunoglobulins are summarized in **Table 5.3.**

Table 5.3: Structure, functions and characteristics of different types of immunoglobulins

Types	*Structure*	*Approximate percentage of the total immunoglobulin in serum*	*Characteristics*	*Functions*
IgG	Monomer	70%	• It is the only immunoglobulin which crosses the **placenta** and is the only maternal antibody which protects the fetus	• Neutralizes bacterial toxins and binds to microorganisms making them easier to phagocytize
IgA	Monomer or Dimer	20%	• Major component of colostrum • Also occurs in saliva, tears and respiratory, intestinal and genital tract secretions	• Prevents attachment of bacteria and viruses to mucous membranes and helps protect mucous surface from antigenic attack
IgM	Pentamer	8-10%	• Main antibody in the primary response to an antigen • Produced by fetus	• Promotes phagocytosis and causes lysis of antigenic cells (bacteria) • Antigen receptor on the surface of B lymphocytes
IgD	Monomer	Less than 1%	• Labile molecules	• May function as an antigen receptor. No known antibody function
IgE	Monomer	Trace amount 0.004%	• Activity is mediated by histamine	• Antiallergic and antiparasitic • Mediates immediate hypersensitivity by causing release of histamine

Disorders of Immunoglobulins

Abnormally large amounts of certain immunoglobulins may be found in the plasma in several diseases of humans. As well as deficiency of γ-globulins is also found in rare hereditary disease.

Multiple Myeloma

Multiple myeloma, a plasma cell cancer results due to abnormally high concentration of serum **immunoglobulins,** usually **IgG** or **IgA** (refer Bence Jones proteins also).

Amyloidosis

Amyloidosis is the accumulation of immunoglobulin **light chain fragments** in the tissues of multiple myeloma patients.

Bence Jones Proteins

- In multiple myeloma, more light chains are produced than heavy chains and enter the bloodstream, because they are of relatively low molecular weight, they pass through glomerular membrane and appear in the urine. These protein chains of low molecular weight are known as **Bence Jones Proteins.**
- Bence Jones proteins have the remarkable characteristic of precipitating on heating urine from 45° to 60°C and redissolve when the heating is continued above 80°C.
- Multiple myeloma with Bence Jones protein in the urine is called **"light chain disease."** The chain may be of either of the κ-(kappa) or the λ-(lambda) type.

SUMMARY

- Plasma proteins are classified into albumin, globulin (α_1, α_2, β and γ) and fibrinogen based on their electrophoretic mobility.
- Albumin, α and β globulin and fibrinogen are synthesized in the liver, while the γ-globulins are synthesized mostly by lymphoid system.
- Albumin is the protein with the highest concentration in plasma, responsible for the transport of hydrophobic fatty acids, bilirubin and drugs.
- Albumin is the main determinant of plasma oncotic pressure, decreased level of albumin leads to edema.
- Hypoalbuminemia may be caused by reduced synthesis in the liver or abnormal losses.
- α_1-antitrypsin plays an important role in inhibiting the action of elastase and a genetic deficiency in API can lead to emphysema.
- Ceruloplasmin is a copper containing protein. Plasma ceruloplasmin level is reduced in Wilson's disease.
- Haptoglobin binds extravascular hemoglobin, prevents its loss into kidney and urine. Hemopexin also plays an important role in the conservation of iron by preventing its loss in urine.
- C-reactive protein is involved in body's defense mechanism.
- Transferrin binds iron and transport it to sites where it is required.
- β_2-microglobulin (BMG) forms a part of human leucocyte antigen (HLA) system.
- Immunoglobulins are unique molecules with a common structure that participate in defense against antigens.
- Five classes of immunoglobulins are:
  i. IgG is the immunoglobulin that can cross the placenta.
  ii. IgM is the first antibody produced by B cell upon induction of a immune response.
  iii. IgA is found widely in secretions, protecting mucosal surfaces.
  iv. IgD is the surface receptor for antigen in B lymphocytes.
  v. IgE is responsible for anaphylactic type of hypersensitivity and allergy.

EXERCISE

Multiple Choice Questions (MCQs)

1. In multiple myeloma which of the following plasma protein level is increased?
a) Albumin b) Hemopexin
c) α_1-fetoprotein d) γ-globulin

2. Bence Jones proteins are:
a) Monoclonal light chains
b) Monoclonal heavy chains
c) Intact γ-globulins
d) All of the above

3. Cigarette smoke inhibits the activity of:
a) α_1-antitrypsin
b) Ceruloplasmin
c) α_2-macroglobulin
d) C-reactive protein

4. Emphysema is due to inhibition of:
a) α_1-antitrypsin
b) Fibrinogen
c) Ceruloplasmin
d) α_1-fetoprotein

5. Hypoalbuminemia may be due to:
a) Reduced synthesis
b) Increased catabolism
c) Excessive losses
d) All of the above

6. The antibody class which can pass through the placenta to protect fetus is:
a) IgA b) IgG
c) IgM d) IgD

7. Plasma albumin performs the following function, *except*:
a) Maintenance of osmotic pressure
b) Transport
c) Solubilization of glucose
d) Nutritive

8. Following are pentameric immunoglobulins:
a) IgG b) IgM
c) IgD d) IgE

9. IgM present on the surface of B lymphocyte is:
a) Monomer b) Dimer
c) Pentamer d) Tetramer

10. Which of the following antibody is responsible for anaphylactic type of hypersensitivity and allergy?
a) IgG b) IgM
c) IgE d) IgD

Correct Answers for MCQs

1-d	2-a	3-a	4-a
5-d	6-b	7-c	8-b
9-a	10-c		

CHAPTER 6

Enzymes

INTRODUCTION

Enzymes are biological materials with catalytic properties, i.e. they increase the rate of chemical reactions in biological and *in vitro* (in the laboratory) systems, that otherwise proceed very slowly. The study of enzymes and of the changes in the enzyme activity that occur in body fluids has become a valuable diagnostic tool for the elucidation of various diseases and for testing organ function.

DEFINITION

Enzymes are biological catalyst produced by living tissues. They are proteins (except a small group of RNA acting as ribozyme) that have the property of accelerating specific chemical reactions without being consumed in the process.

ZYMOGEN OR PROENZYME

- A number of proteolytic enzymes found in the blood or in the digestive tract are present in an inactive (precursor) form, called ***zymogen*** or **proenzymes.**
- For example, chymotrypsin is secreted by the pancreas as **chymotrypsinogen.** It is activated in the digestive tract by the proteolytic enzyme trypsin.
- Precursor proteins or inactive enzyme names have the prefix ***"pro"*** like prothrombin, proelastase, etc. or suffix ***"ogen"*** like chymotrypsinogen, trypsinogen, pepsinogen, etc.

COFACTORS (COENZYME AND ACTIVATOR)

- Some enzymes require an additional nonprotein component for its optimum activity. This additional component is called ***cofactor*** which may be either loosely or tightly bound to the protein portion of the enzyme.
- These cofactors may be:
  - Organic compounds, called ***coenzymes***
  - Inorganic ions, called ***activators***.
- Enzymes without its cofactor is referred to as an **apoenzyme;** the complete catalytically active enzyme is called **holoenzyme.**

  Apoenzyme + cofactor = holoenzyme.
- Many vitamins function as coenzymes. Coenzymes derived from vitamins will be considered for more details ***in chapter 7.***
- The lists of coenzyme and activators are given in **Tables 6.1 and 6.2** respectively.

Table 6.1: Some common coenzymes and their functions

Vitamin	*Coenzyme*	*Function as coenzyme*
Thiamine (Vit B_1)	TPP (Thiamine pyrophosphate)	Oxidative decarboxylation and transketolase reaction
Riboflavin (Vit B_2)	FAD and FMN (Flavin Adenine Dinucleotide and Flavin Mononucleotide)	Oxidation and reduction reactions
Niacin	NAD^+ (Nicotinamide Adenine Dinucleotide), $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate)	Oxidation and reduction reactions
Pyridoxine (Vit B_6)	PLP (Pyridoxal phosphate)	Transamination, deamination decarboxylation reactions of amino acids
Biotin	Biocytin	Carboxylation reactions
Folic acid	THF (Tetrahydrofolate)	Carrier of one carbon group
Pantothenic acid	Coenzyme A	Acyl carrier
Cynocobalamine	Methylcobalamine and Deoxyadenosylcobalamine	Transfer of CH_3 group and isomerizations

Table 6.2: Enzymes requiring or containing inorganic elements as cofactors (activators)

Enzyme	*Cofactor (activator)*
Ferroxidase (ceruloplasmin), Ascorbic acid oxidase	Copper
Carbonic anhydrase, DNA-polymerase, Porphobilinogen synthase, Carboxypeptidase	Zinc
Cytochrome oxidase, Catalase	Iron
Glucose-6-Phosphatase, Hexokinase	Magnesium
Glutathione peroxidase	Selenium
Arginase, Pyruvate carboxylase	Manganese
Xanthine oxidase	Molybdenum

HOW ENZYMES WORK

Energy Changes Occur During the Reaction

Virtually all chemical reactions have an energy barrier, separating the reactants and the products. This barrier, called the *free energy of activation*, is the energy difference between the energy of the reactant and high energy intermediates that occurs during the formation of a product. **Figure 6.1** shows the changes in energy during the conversion of a molecule of reactant 'S' to product 'P' through the transition state S*.

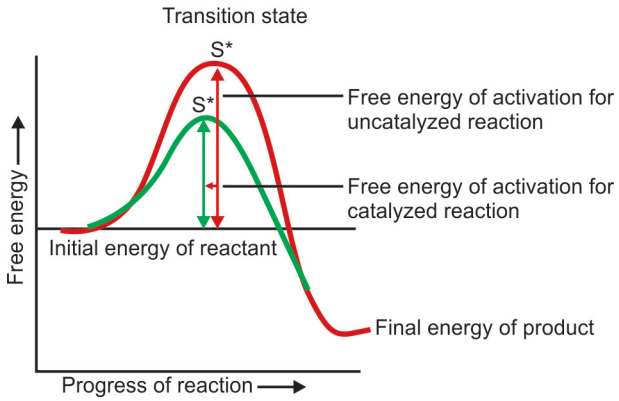


Figure 6.1: Comparison of the free energy of activation of a catalyzed and uncatalyzed reaction, S*: Transition state

The peak of free energy activation, represents the transition state, in which the high energy intermediates (S*) are formed during the conversion of a reactant to a product **(Figure 6.1).**

- An enzyme lowers the energy required for activation to the transition state.
- Without a catalyst, the reaction will occur only if enough heat energy is added to the reaction system.
- ***With an enzyme* as a *catalyst, the reaction may easily proceed at the normal physiological temperature.***

MECHANISM OF ENZYME ACTION

- Formation of an enzyme-substrate (ES) complex is the first step in enzymatic catalysis.

- Substrate is bound through multiple noncovalent interactions at the **active site** of the enzyme forming an **enzyme-substrate (ES)** complex which is subsequently converted to product and free enzyme.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

- The active site of an enzyme is the region that binds the substrate and which contains the specific amino acid residues.
- Two models for substrate binding to the active site of the enzyme, have been proposed to explain the specificity that an enzyme has for its substrate:
  1. Lock and key model or rigid template model of Emil Fisher.
  2. Induced fit model or hand-in-glove model of Daniel E Koshland.

Lock and Key Model or Rigid Template Model of Emil Fisher

- This model was proposed by Emil Fisher in 1890.
- In this model, enzyme is preshaped and the active site has a rigid structure that is complementary to that of the substrate **(Figure 6.2).**
- This model is called lock and key model, because in this model the substrate fits into the active site in much the same way that a key fits into a lock.
- This model has been useful in understanding how some enzymes can bind only a specific substrate but will not bind another compound with an almost identical structure. For example, most enzymes in carbohydrate metabolism can bind the D-isomer of hexoses but cannot bind the corresponding L-isomer, which differs only in the configuration around a single carbon atom.
- This model explains all mechanisms but do not explain the changes in the enzyme activity in the presence of allosteric modulators.

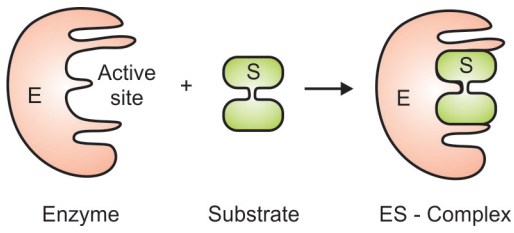


Figure 6.2: Representation of formation of an ES-complex according to the Fisher's lock and key model. The active site of the enzyme is complementary in shape to that of substrate

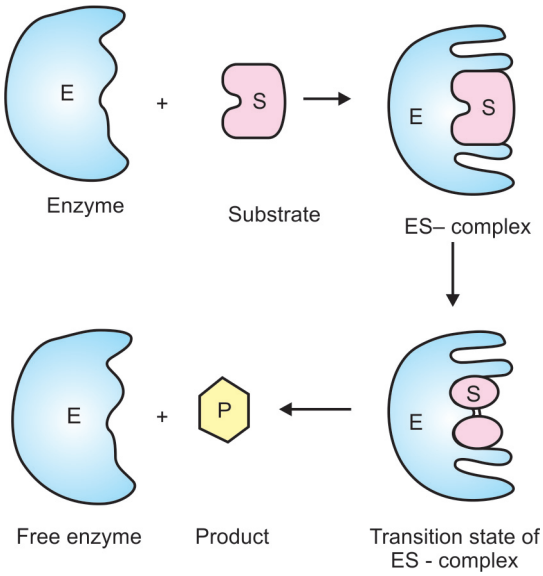


Figure 6.3: Schematic representation of induced fit model of Koshland

Induced Fit Model or Hand-in-glove Model of Daniel E Koshland (Figure 6.3)

Fisher's model explained the specificity of enzyme substrate interaction but the implied rigidity of the enzymes active site failed to explain the dynamic changes that must take place during catalysis. A model that accounts for both of these aspects of enzyme catalysis is the **induced fit model of Daniel E Koshland.**

- In the Fisher's model the catalytic site is presumed to be preshaped to fit the substrate. However, Daniel E Koshland in 1958 postulated that the **enzymes are flexible and shapes of the active site can be modified by the binding of the substrate**.
- In the induced fit model, the substrate induces a conformational change in the enzyme, in the same manner in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape. Therefore, this model is also known as **hand-in-glove model**.
- Conformational change in enzyme in turn induces reciprocal changes in its bound substrate that alters their orientation and configuration and strains the structure of the bound substrate. Due to such changes energy is librated, which is called **intrinsic binding energy (Figure 6.3).**
- This intrinsic binding energy due to the substrate-enzyme interaction is made available for the transformation of the substrate into product.

- This model is believed to describe more accurately the specificity of substrate binding than does lock and key model of E Fisher.

ENZYME CLASSIFICATION

The classification of enzyme was described in 1961 by enzyme commission of the International Union of Biochemistry (IUB). According to this classification, each enzyme is characterized by a code number called **enzyme code number** or **'EC' number,** consisting of four digits.

According to the IUB system, enzymes are classified into six major classes as follows:

1. EC-1 : Oxidoreductases
2. EC-2 : Transferases
3. EC-3 : Hydrolases
4. EC-4 : Lyases
5. EC-5 : Isomerases
6. EC-6 : Ligases.

Some common examples of different classes of enzymes are given in **Table 6.3.**

EC-1 Oxidoreductases

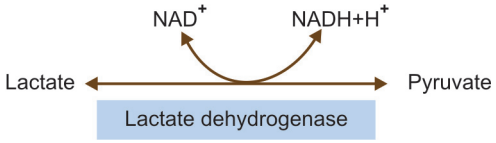
Those enzymes that catalyze oxidation-reduction reactions, are included in this class which can be illustrated schematically as follows:

$$\underset{\text{(red)}}{A} + \underset{\text{(ox)}}{B} \rightleftharpoons \underset{\text{(ox)}}{A} + \underset{\text{(red)}}{B}$$

Enzymes in this category include :

- Dehydrogenases
- Reductases
- Oxidases
- Peroxidases.

Specific Example



EC-2 Transferases

Those enzymes that catalyze the transfer of a group such as, ***amino, carboxyl, methyl*** or ***phosphoryl,*** etc. from one molecule to another are called transferases. These reactions can be illustrated as follows:

$$A\text{–}X + B \rightleftharpoons A + B\text{–}X$$

Table 6.3: Examples of enzymes with their main class

Class	*Example*
EC-1:Oxidoreductases	Lactate dehydrogenase (LDH) Glucose 6-phosphate dehydrogenase (G-6-PD) Cytochrome oxidase
EC-2: Transferases	Aspartate aminotransaminase(AST) Alanine aminotransaminase (ALT) Hexokinase
EC-3: Hydrolases	Lipase α-Amylase Trypsin Lactase Sucrase Pepsin
EC-4: Lyases	Aldolase Argininosuccinase Carbonic anhydrase
EC-5: Isomerases	Phosphoglucomutase Triphosphate isomerase Phosphohexose isomerase
EC-6: Ligases	Glutamine synthetase Pyruvate carboxylase DNA ligases

Some common enzymes in this category include :

- Amino transferase or transaminase
- Kinase
- Transcarboxylase.

Specific Example

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons[\text{PLP}]{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

where, ALT : Alanine aminotransferase
PLP : Pyridoxal phosphate (coenzyme)

EC-3 Hydrolases

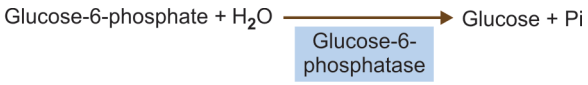
Enzymes of this class catalyze the cleavage of **C-O, C-N**, **C-C** and some other bonds with the addition of water. These can be illustrated as follows:

$$A\text{-}B + H_2O \longrightarrow A\text{-}OH + B\text{-}H$$

Some common enzymes in this category are:

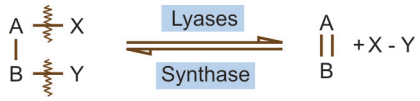
- Acid phosphatase
- All digestive enzymes like α-amylase, pepsin, trypsin, chymotrypsin, etc.

Specific Example



EC-4 Lyases

Lyases catalyze the cleavage of **C-O, C-C** and **C-N** bonds by means other than hydrolysis or oxidation, giving rise to compound with double bonds or catalyze the reverse reaction, by the addition of group to a double bond. In cases where reverse reaction is important, then synthase, (not synthetase of group EC-6) is used in the name. This type of reaction is illustrated as follows:

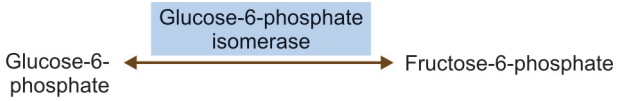


Specific Example

EC-5 Isomerases

Isomerases catalyze intramolecular structural rearrange ment in a molecule. They are called **epimerases, isomerases** or **mutases,** depending on the type of isomerism involved. This reaction can be illustrated as follows:

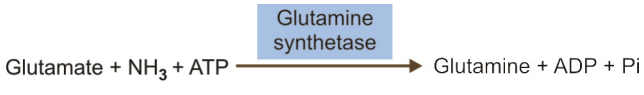
Specific Example



EC-6 Ligases (Synthetases)

Ligases catalyze the joining of two molecules coupled with the hydrolysis of ATP. The reaction is illustrated as follows:

Specific Example



SPECIFICITY OF ENZYME ACTION

Specificity refers to the ability of an enzyme to discriminate between two competing substrates. Enzymes are highly specific both in the reaction catalyzed and in their choice of substrates. Specificity makes it possible for a number of enzymes to co-exist in the cell without interfering in each other's actions.

Types of Specificity

The following types of specificity have been recognized:

1. Substrate specificity
2. Reaction specificity
3. Stereo specificity.

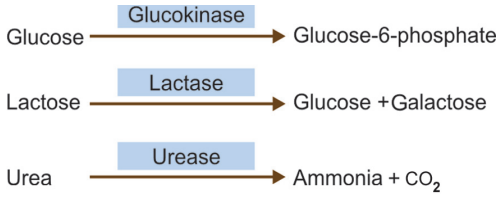
Substrate Specificity

There are following types of substrate specificity:

i. Absolute substrate specificity
ii. Relative substrate specificity
iii. Broad substrate specificity.

Absolute substrate specificity

Certain enzymes will act on only one substrate and catalyze one reaction, e.g. Glucokinase, lactase, urease, etc.



Relative substrate specificity

In this case, enzyme acts on more than one substrate. The relative substrate specificity is of two types:

a. Group specificity
b. Bond specificity.

- In **group specificity,** an enzyme acts on more than one substrate containing a particular group, e.g. chymotrypsin acts on several proteins by hydrolyzing peptide bonds attached to aromatic amino acids. Trypsin hydrolyzes peptide linkages involving arginine or lysine.
- In **bond specificity,** an enzyme acts on more than one substrate containing a particular kind of bond, e.g. salivary α-amylase cleaves α-(1→4) glycosidic bonds of carbohydrates, lipase hydrolyzes ester bonds of lipids.

Broad substrate specificity

In this case, an enzyme acts on more than one structurally related substrates, e.g. hexokinase catalyzes the phosphorylation of more than one kind of hexoses such as glucose, fructose and mannose.

Reaction Specificity

In this case, an enzyme is specific to a particular reaction but not to substrate (s) and catalyzes only one type of reaction. For example, pyruvate can undergo several reactions. Each reaction is catalyzed by a separate enzyme, which catalyzes only that reaction and none other as shown in **Figure 6.4.**

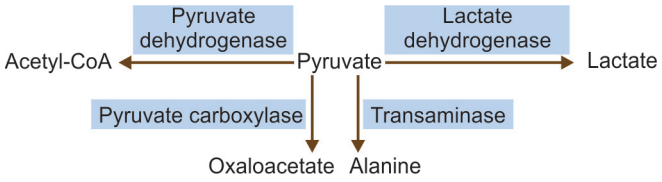


Figure 6.4: Example of reaction specificity

Stereo Specificity

Many enzymes show specificity towards stereoisomers, i.e. they act on only one type of isomer. For example,

- L-lactate dehydrogenase will act only on L-lactic acid and not D-lactic acid.
- Likewise, L-amino acid oxidase and D-amino acid oxidase are distinct enzymes which act only on L and D-amino acids respectively.
- D-glucose oxidase can similarly act only on D-glucose and not on L-glucose.
- Salivary α-amylase acts on the α-1,4 glycoside linkage and is inactive on β-1,4 glycoside linkage.
- Isomerase and epimerase do not show stereospecificity.

FACTORS AFFECTING THE VELOCITY OF ENZYME REACTION

Various factors that affect enzyme activity are:

- Substrate concentration
- Enzyme concentration
- pH i.e. H^+ ion concentration
- Temperature
- Product concentration
- Activators and coenzymes
- Time
- Physical agents
- Inhibitors

Effect of Substrate Concentration

For a given quantity of enzyme, the velocity of the reaction increases as the concentration of the substrate is increased. At first, this relationship is almost linear but later, the reaction curve becomes hyperbolic in shape **(Figure 6.5).**

- At relatively low concentrations of substrate, V_0 increases almost linearly with an increase in substrate concentration [S], a condition known as ***First order kinetics***.
- At higher substrate concentrations, V_0 increases by smaller amounts in response to increase in substrate concentration [S].
- Finally, a part is reached beyond which there are only vanishingly small increase in V_0 with increasing [S], a condition known as ***zero order kinetics*** and a plateau is called ***maximum velocity, V_{max}*** **(Figure 6.5).** Under these conditions, all the free enzymes will have been converted into ES form so that any further increase in substrate concentration has no effect on the rate and the reaction achieves a steady state.

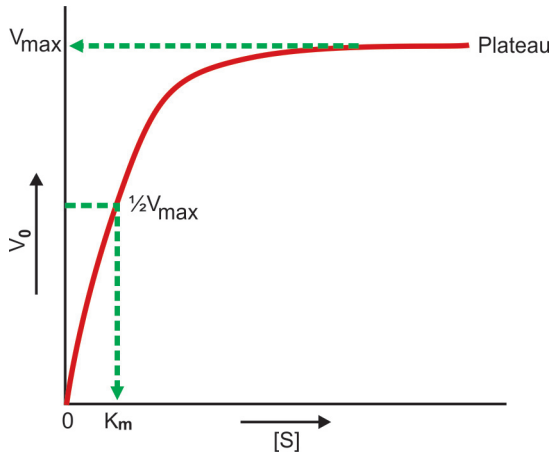


Figure 6.5: Effects of substrate concentration [S] on enzyme activity keeping enzyme concentration constant
where, V_0 : initial velocity
V_{max} : maximum velocity
K_m : 1/2 V_{max} = Michaelis Menten constant
[S] : substrate concentration

Effect of Enzyme Concentration

- The velocity of a reaction is directly proportional to the amount of enzyme present as long as the amount of substrate is not limiting.
- The substrate must be present at a concentration sufficient to ensure that all of the enzyme molecules have substrate bound to their active site **(Figure 6.6).**

Effect of Hydrogen Ion Concentration pH

- Each enzyme has an ***optimum pH***, i.e. a pH at which the enzyme activity is maximum. Below or above this pH, enzyme activity is decreased. The optimum pH

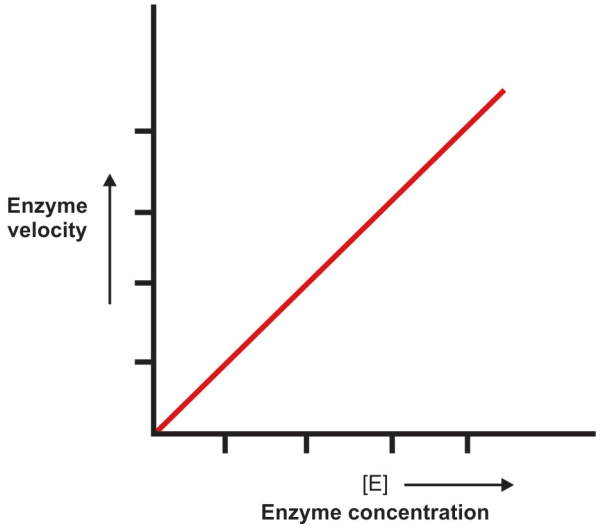


Figure 6.6: Effect of enzyme concentration on enzyme activity

differs from enzyme to enzyme, e.g. optimum pH for:
- Pepsin = 1.2
- Trypsin = 8.0

- A bell shaped curve is obtained when enzyme velocity is plotted against pH **(Figure 6.7)**.
- Changes in pH can alter the following:
  - Ionization state of the amino acid residues present in the active site of the enzyme. Enzyme activity is related to the ionic state of active site of the enzyme.
  - The ionization state of the substrate. The active site of an enzyme may require particular ionic state of the substrate for optimum activity.
  - Drastic change in pH denatures the enzyme protein.

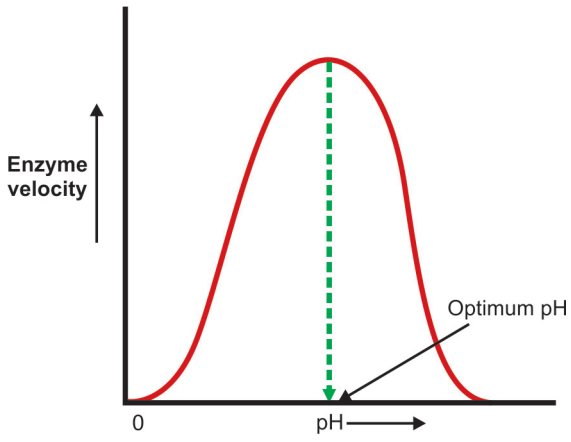


Figure 6.7: Effect of pH on enzyme activity

All these affect the enzyme substrate complex formation and decrease the rate of enzyme reaction. ***As pH affects the enzyme activity, in enzyme studies, buffers are used to keep enzyme at an optimum or at least a favorable H^+ ion concentration.***

Effect of Temperature

- Enzyme catalyzed reactions show an increase in rate with increasing temperature only within a relatively small and low temperature range.
- Each enzyme shows the highest activity at a particular temperature called ***optimum temperature***.
- The activity progressively declines both above and below this temperature.
- A bell shaped curve is obtained when we plot the enzyme velocity Vs. temperature **(Figure 6.8).**

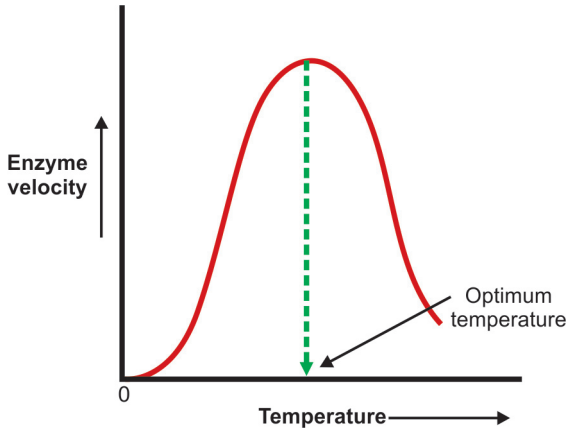


Figure 6.8: Effect of temperature on enzyme activity

- Increase in velocity is due to the increase in the kinetic energy. Further elevation of the temperature results in a decrease in reaction velocity due to denaturation of the enzyme protein.
- Low temperature also decreases enzyme activity and enzymes may be completely inactive at temperature of 0°C and below.
- The inactivity at low temperature is reversible. So, many enzymes in tissues or extracts may be preserved for months by storing at –20°C or –70°C.
- The reaction velocity of most chemical reactions increases with temperature approximately doubles for each 10°C rise called temperature coefficient Q_{10}.
- Most of the body enzymes have the optimum temperature close to 37°C to 38°C and have progressively less activity as the temperature rises.

Effect of Product

Accumulation of products of the reaction causes the inhibition of enzyme activity for some enzymatic reactions, this form of control will limit the rate of formation of the product when the product is under used. In biological systems, however, the product is usually removed as it becomes a substrate for a succeeding enzyme in a metabolic pathway.

Effect of Activators and Co-enzymes

The activity of many enzymes is dependent on the activators (metallic ions) like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, etc. and coenzymes for their optimum activity. In absence of these activators and coenzymes, enzymes become functionally inactive.

Effect of Time

Under optimum conditions of pH and temperature, time required for an enzyme reaction is less. The time required for the completion of an enzyme reaction increases with changes in temperature and pH from its optimum.

Effect of Physical Agents

Physical agent like light rays can inhibit or accelerate certain enzyme reactions. For example, the activity of salivary amylase is increased by red and blue light. On the other hand, it is inhibited by ultraviolet rays.

Effect of Inhibitors

The substances which stop the enzymatic reaction are called inhibitors. Presence of these substances in reaction medium decreases the rate of enzyme reaction. Different types of enzyme inhibitors are discussed separately later.

ENZYME KINETICS

The study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as ***kinetics.***

One of the key factors affecting the rate of a reaction catalyzed by an enzyme *in vitro* (in laboratory) is the amount of substrate present, [S]. The effect on V_0 (initial velocity) of varying substrate [S] concentration, when enzyme concentration is held constant, is shown in **Figure 6.5.**

Michaelis-Menten Equation

Enzyme catalyzed reactions occur in two stages as shown in the following equations

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \quad \text{.....1)}$$

$$ES \xrightarrow{K_3} E + P \quad \text{.....2)}$$

K_1, K_2 and K_3 are rate constant. The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration. The relationship between reaction rate and substrate concentration shown in **Figure 6.5** is described mathematically by the 'Michaelis-Menten equation' as follows:

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

where,

V_0 = initial reaction velocity (is the rate of reaction as soon as enzyme and substrates are mixed).

V_{max} = maximum velocity (is observed when all active sites on the enzyme are filled with substrate).

K_m = Michaelis-Menten constant, [is the substrate concentration, at which the reaction rate is half of its maximum velocity (V_{max})].

[S] = Substrate concentration

Every enzyme has the characteristics V_{max} and K_m which are sensitive to change in pH, temperature and ionic strength.

Significance of K_m (Michaelis Constant)

- K_m, the Michaelis constant is equal to the substrate concentration at which the reaction rate is half of its maximal value. The K_m value of an enzyme depends on the substrate and environmental conditions such as pH, temperature and ionic strength.
- The Michaelis constant, K_m has two significances:
  1. K_m provides a measure of the substrate concentration required for significant catalysis to occur.
  2. It is a measure of the **affinity of the enzyme for its substrate,** a high K_m indicates weak binding and a low K_m indicates strong binding with its substrate.

Significance of V_{max} (Maximal Velocity)

- The V_{max} of a reaction is an index of the catalytic efficiency of an enzyme. The V_{max} is useful in comparing the activity of one enzyme with that of another.

Lineweaver-Burk Plot or Double-Reciprocal Plot

Lineweaver-Burk plots are used to obtain values for V_{max} and K_m. A more accurate method of determining

values for V_{max} and K_m uses Lineweaver-Burk equation shown below. This equation is obtained by taking the reciprocal of the Michaelis-Menten equation.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

- When $1/V_0$ is plotted against $1/[S]$, a straight line is obtained **(Figure 6.9)**, with a slope equal to K_m/V_{max}.
- The point, at which line intersects the y-axis, is numerically equal to $1/V_{max}$.
- The point at which the line intersects the x-axis is numerically equal to $-1/K_m$.
- This plot is useful to determine the mechanism of action of enzyme inhibitors.

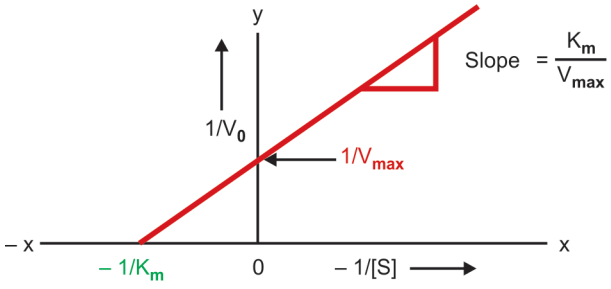


Figure 6.9: Lineweaver-Burk plot (Double reciprocal plot)

ENZYME INHIBITION

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called inhibitor. Two general classes of inhibitors are recognized according to whether the inhibitor action is **reversible** or **irreversible (Figure 6.10)**

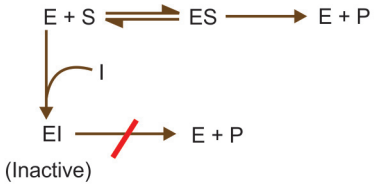
1. Reversible inhibitor
2. Irreversible inhibitor.

Reversible Inhibitor

- Reversible inhibitors bind to enzymes through **non-covalent** bonds and the activity of the enzyme is restored fully when the inhibitor is removed from the system.
- Different types of reversible inhibitors are:
  i. Competitive or substrate analogue inhibitor
  ii. Noncompetitive inhibitor
  iii. Uncompetitive inhibitor.

Competitive or Substrate Analogue Inhibitor

- A competitive inhibitor is usually a structural analogue of the substrate.
- The chemical structure of the inhibitor (I) closely resembles that of the substrate (S) and binds to the enzyme at the active site, forming an EI complex rather than ES-complex **(Figure 6.11).**



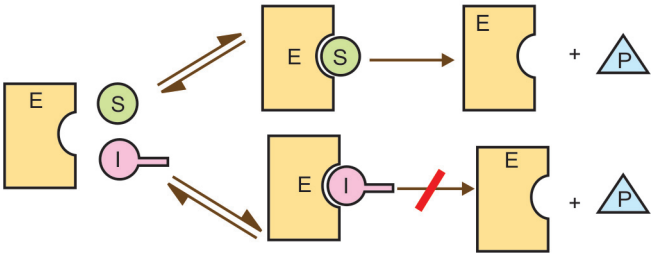


Figure 6.11: Diagrammatic representation of competitive inhibition where, E: Enzyme; S: Substrate; I: Competitive inhibitor; P: Product

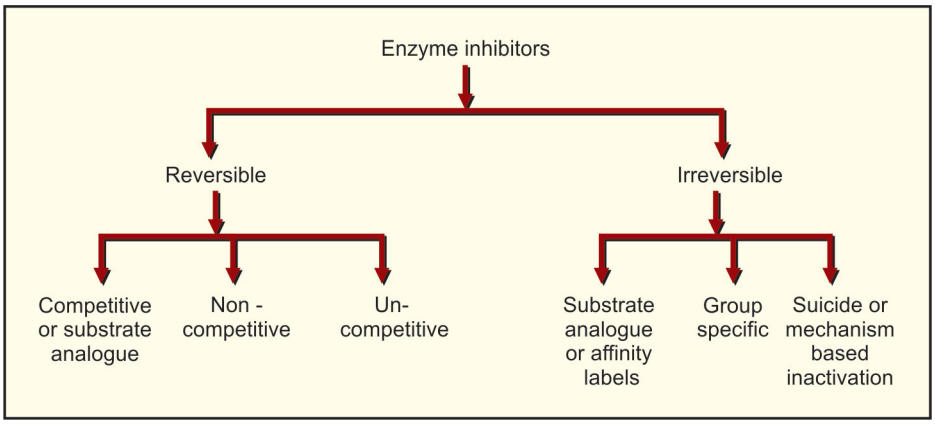


Figure 6.10: Classes of enzyme inhibitors

- But because it is not identical with the substrate, breakdown into products does not take place.
- When both the substrate and this type of inhibitor are present, they compete for the same binding site on the enzyme.
- The inhibition could be overcome by increasing substrate concentration. In competitive inhibition, the **K_m increases** whereas **V_{max} remains unchanged (Figure 6.12)**.

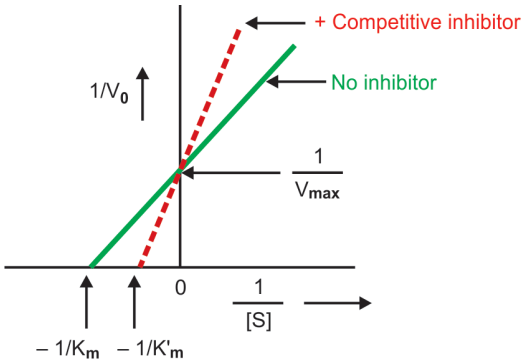


Figure 6.12: A double reciprocal plot of enzyme kinetics in presence and absence of competitive inhibitor, V_{max} is unaltered whereas K_m is increased

- The classical example is the competitive inhibition of **succinate dehydrogenase** by the **malonate.**
- Succinate dehydrogenase is one of the enzymes of the citric acid cycle. Succinate dehydrogenase is inhibited by malonate which resembles succinate.
- Many drugs which act as competitive inhibitors are given below and some more are given in **Table 6.4.**

Sulfonamide

- Sulfonamide is an analogue of P-aminobenzoic acid (PABA) and inhibits **pteroid synthetase** enzyme required for the synthesis of **folic acid** in microorganisms.
- Since folic acid is involved in the biosynthesis of purines and thymine, sulfonamides inhibit growth of the pathogenic organisms.

Isoniazide [Isonicotinic acid hydrazine (INH)]

It is an antituberculous drug, an analogue of nicotinamide.

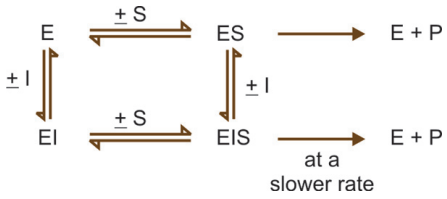
INH interferes with the biosynthesis of NAD and restrict the growth of the organisms that cause tuberculosis.

Dicumarol

It is an anticoagulant drug structurally similar to vitamin K. It inhibits the vitamin K activity and inhibits the formation of prothrombin.

Noncompetitive Inhibitors

- As the name implies, in this type of inhibition no competition occurs between substrate and inhibitor. Inhibitor is usually structurally different from the substrate.
- It binds at a site on the enzyme molecule other than the substrate-binding site and thus there is no competition between inhibitor and substrate.
- Since inhibitor and substrate may bind at different sites; formation of both EI and EIS-complexes is possible.
- Since EIS may breakdown to form product at a slower rate than does ES, the reaction is slowed but not halted. The following reactions may occur:



- For noncompetitive inhibition, the **K_m value is unchanged** while **V_{max} is lowered (Figure 6.13)**

Table 6.4: Commonly used drugs that are enzyme inhibitors

Drug	*Type of inhibition*	*Target enzyme*	*Therapeutic use*
Mevinolin Lovastatin	Competitive	HMG-CoA reductase (3-Hydroxy-3-Methyl-Glutaryl CoA-reductase)	Hypercholesterolemia
Allopurinol	Competitive	Xanthine oxidase	Gout
Methotrexate	Competitive	Dihydrofolate reductase	Cancer
5-Fluorouracil	Suicide	Thymidylate synthase	Cancer
Aspirin	Suicide	Cyclo-oxygenase	Anti-inflammatory
Penicillin	Suicide	Bacterial transpeptidase	Antibacterial

- Examples of noncompetitive inhibitors are:
  - **Ethanol** or certain **narcotic drugs** are noncompetitive inhibitor of **acid phosphatase**.
  - **Trypsin inhibitors** occur in **soybean** and **raw egg white**, inhibit activity of trypsin noncompetitively.
  - As well as ***Ascaris* parasites** (worm) contain **pepsin** and **trypsin inhibitors**, which inhibit non-competitively action of pepsin and trypsin, that is why ascaris worm is not digested in human intestine.

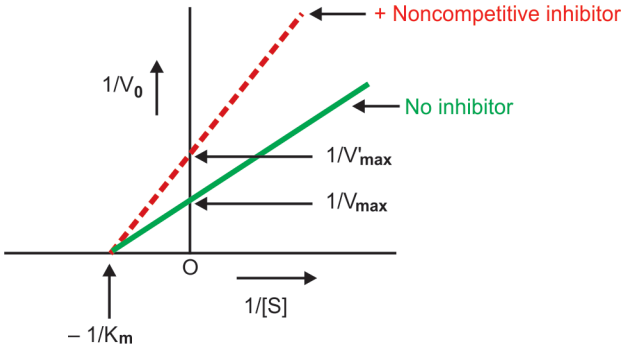


Figure 6.13: A double reciprocal plot of enzyme kinetics in presence and absence of noncompetitive inhibitor; K_m is unaltered by noncompetitive inhibitor, whereas V_{max} is decreased

Uncompetitive Inhibitor

- Uncompetitive inhibitor can bind only to the enzyme-substrate (ES) complex.
- It does not have affinity for free enzyme. Uncompetitive inhibitor **decreases both V_{max}** and **K_m (Figure 6.14).**
- This form of inhibitor is rare with single substrate but more common in multiple substrate reaction.

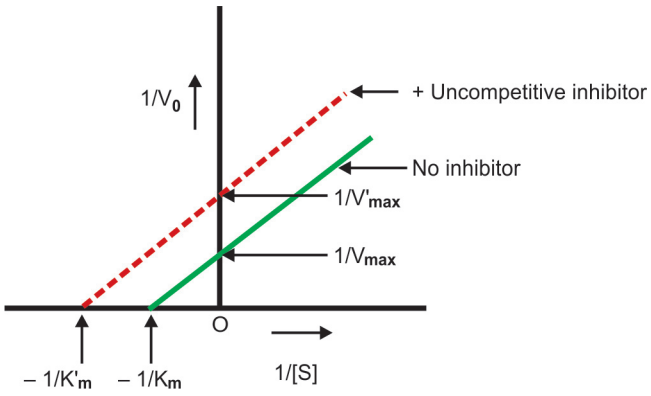


Figure 6.14: Effect of uncompetitive inhibitor on the double reciprocal plot; it shows parallel lines with decrease in both V_{max} and K_m

Irreversible Inhibitor

- An irreversible inhibitor binds with an enzyme tightly **covalently** and forms a stable complex.
- An irreversible inhibitor cannot be released by dilution or dialysis or simply by increasing the concentration of substrate.
- Irreversible inhibitors can be divided into three categories:
  i. Group specific inhibitors
  ii. Substrate analogue inhibitor or affinity labels
  iii. Suicide inhibitor or mechanism based inactivation.
- In terms of enzyme kinetics, the effect of an irreversible inhibitor is like that of the **reversible noncompetitive** inhibitors resulting in a **decreased in V_{max}** but having **no effect on the K_m (Table 6.5).**

Table 6.5: Effect of inhibitors on kinetic properties of enzymes

Type of inhibitor	*Km*	*V_{max}*
Irreversible	No effect	Decreased
Reversible competitive	Increased	No effect
Reversible noncompetitive	No effect	Decreased
Reversibe uncompetitive	Decreased	Decreased

Group Specific Irreversible Inhibitor

These inhibitors react with specific R-groups (side chain) of amino acid residues in the active site of enzyme. Examples of group specific irreversible inhibitors are:

Di-isopropylphosphofluoride (DIPF)

- DIPF can inhibit an enzyme **acetylcholine esterase** by covalently reacting with **hydroxyl group** of a **serine** residue present at the active site of the enzyme **(Figure 6.15)**

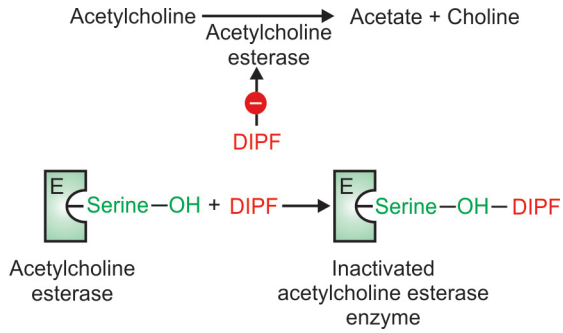


Figure 6.15: Irreversible inhibition of acetylcholine esterase by a group of specific inhibitor, diisopropylphosphofluoride (DIPF)

- DIPF has also been found to inhibit **trypsin, chymotrypsin, elastase** and **phosphoglucomutase**.

Iodoacetamide and heavy metals

- Iodoacetamide and heavy metals like, Pb^{2+}, Ag^{+}, Hg^{2+}, etc. which react with **sulfhydryl (-SH)** group of **cysteine** residues present at the active site of the enzyme and makes them inactive.

Substrate Analogue Irreversible Inhibitor or Affinity Labels

- Substrate analogues or affinity labels are molecules that are structurally similar to the substrate.
- These substrate analogues possess a highly reactive group which is not present in the natural substrate.
- The reactive group of substrate analogues covalently reacts with amino acid residues of the active site of the enzyme and permanently block the active site of the enzyme, e.g. 3-Bromoacetol phosphate (BAP).
- BAP is a substrate analogue of the normal substrate dihydroxyacetone phosphate (DHAP) for the enzyme phosphotriose isomerase of glycolysis.

Suicide Inhibitor or Mechanism Based Inactivation

- These compounds are relatively unreactive until they bind to the active site of a specific enzyme.
- On binding to the active site of the enzyme they carry out the first few catalytic activities of the normal enzyme reaction.
- Instead of being transformed into a normal product, however, the inhibitor is converted to a very reactive compound that combines irreversibly with the enzyme leading to its irreversible inhibition.
- The enzyme literally commits suicide. These are also called **mechanism based inactivation** because they utilize the normal enzyme reaction mechanism to inactivate the enzyme.
- These inhibitors act as drugs for example.

Penicillin

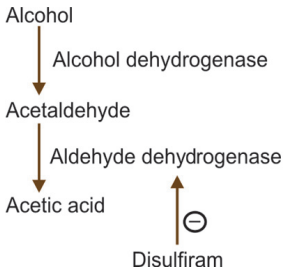
- Penicillin irreversibly inactivates an essential bacterial enzyme **glycopeptidyl transpeptidase** involved in the formation of bacterial cell wall.

Aspirin

- Aspirin inactivates an enzyme **cyclo-oxygenase** which catalyzes the first reaction in the biosynthesis of prostaglandins from arachidonic acid.

Disulfiram (antabuse)

- Disulfiram is a drug used in the treatment of alcoholism. It inhibits irreversibly **aldehyde dehydrogenase** enzyme resulting in accumulation of acetaldehyde in the body. Accumulation of acetaldehyde in the tissue leads to alcohol avoidance.



Clinical Application of Enzyme Inhibitor

Enzyme inhibitors have therapeutic applications. Most antibiotics and anticancer drugs that are used therapeutically are either competitive inhibitor or mechanism based suicide inhibitor. Some commonly used drugs that exert their effects by inhibiting enzymes are described in **Table 6.4.**

ALLOSTERIC ENZYME

Allosteric enzyme is a **regulatory enzyme.** The term allosteric derives from Greek word, **allo** means **other** and **steros** means **space** or **site.** ***Allosteric enzymes are those having other site.***

Like all enzymes, allosteric enzymes have active site for binding of the substrate but they also have one or more regulatory (or allosteric) sites for binding regulatory metabolites which is called ***modulator.***

- Allosteric enzymes may be inhibited or stimulated by their modulators **(Table 6.6)**.
- Modulators that inhibit enzyme activity are termed ***negative modulators.*** Whereas those that increase enzyme activity are called ***positive modulators.***
- Just as the active site of an enzyme is specific for its substrate, the allosteric site is specific for its modulators. Allosteric enzymes are generally larger and more complex than those of simple enzymes.

Feedback Allosteric Inhibition

In some multienzyme systems, the first enzyme of the sequence is the regulatory enzyme and has distinctive characteristics.

- It is inhibited by the end product of the multienzyme system whenever the end product of such metabolic reaction produced in excess of the cells needs. The end product of the pathway acts as a specific inhibitor of the first or regulatory enzyme in the pathway.

Table 6.6: Allosteric enzymes and its modulators			
Pathway	*Enzyme*	*Inhibitor*	*Activator*
Glycolysis	Phosphofructokinase-I	ATP	AMP
Pyruvate to acetyl-CoA	Pyruvate dehydrogenase	ATP	-
TCA cycle	Isocitrate dehydrogenase	ATP	ADP
Gluconeogenesis	Pyruvate carboxylase	-	Acetyl-CoA
Fatty acid synthesis	Acetyl-CoA carboxylase	-	Citrate

- The whole enzyme system thus slows down to bring the rate of production of its end product back into balance with the cell's needs.

This type of regulation is called ***feedback inhibition.*** For example, first enzyme **δ-aminolevulenic acid synthase** is an allosteric enzyme in heme synthesis is inhibited by its end product heme. **(Figure 6.16)**.

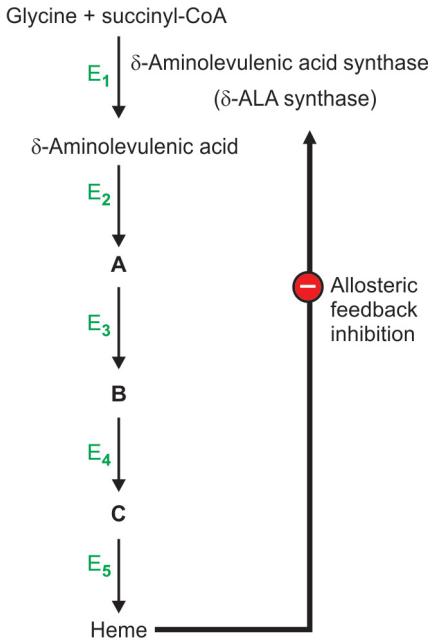


Figure 6.16: Feedback inhibition. First enzyme δ-ALA-synthase is an allosteric enzyme inhibited by its end product heme

ISOENZYME

- Isoenzymes or isozymes are multiple forms (isomers) of the same enzyme that catalyze the same biochemical reaction. Isoenzymes show different chemical and physical properties like electrophoretic mobility and kinetic properties.
- Not all enzymes have isoenzymes. In fact, it was found that only those enzymes, which are active in polymeric form demonstrate isoenzyme. For example:
  1. Lactate dehydrogenase (LDH)
  2. Creatine kinase (CK) (formerly called creatine phosphokinase (CPK).
- Some more examples of isoenzymes are given in **Table 6.7.**

Table 6.7: Examples of isoenzymes	
Enzyme	*Isoenzyme forms*
Acid phosphatase	Prostate, erythrocytes, platelets, liver, spleen, kidney and bone marrow
Alkaline phosphatase	Bone, liver, placenta, intestine and kidney
Amylase	Salivary and pancreatic
Hexokinase	Liver (glucokinase) and muscle

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme that catalyzes the oxidation of L-lactate to pyruvate.

- LDH has five isoenzymes:
  - LDH_1
  - LDH_2
  - LDH_3
  - LDH_4
  - LDH_5.
- Since LDH is a **tetramer,** made up of two types of polypeptide **M *(muscle)*** type and ***H (heart)*** type, five combinations are possible with varying ratios of two kinds of polypeptides **(Table 6.8).**
- Five isoenzymes of LDH can be detected by electrophoresis as they have different electrophoretic mobilities.
- **LDH_1** is the **fastest moving** fraction towards the anode and **LDH_5** is the **slowest moving** isoenzyme of LDH **(Figure 6.17)**.

- LDH_1 predominates in cells of cardiac muscle, and erythrocytes and LDH_5 is the most abundant form in the liver and in skeletal muscle **(Table 6.8).**

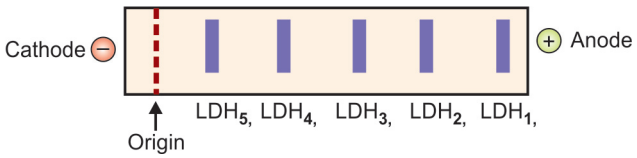


Figure 6.17: Electrophoretic separation of LDH isoenzyme

Clinical Applications of LDH

The LDH isoenzyme analysis may be useful in the following clinical situations **(Table 6.8)**:

- Significant elevation of LDH_1 and LDH_2 (LDH_1>LDH_2) occurs within 24 to 48 hours after myocardial infarction.
- Predominant elevation of LDH_2 and LDH_3 occur in leukemia. LDH_3 is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of LDH_5 occurs after damage to the liver or skeletal muscle.

Creatine Kinase (CK)

- Creatine kinase isoenzymes are **dimer** that are made up of two types of polypeptide chains, which may be either ***M (muscle)*** type or **B *(brain)*** type, generating three isoenzymes **(Table 6.8).**
  - CK_1 (BB) : It is present in the brain
  - CK_2 (MB) : Cardiac tissue is the only tissue which has the mixed MB (CK_2) isoenzyme
  - CK_3 (MM) : It is present in skeletal muscle.

Clinical Application

- CK_1 may be elevated in neonates particularly in damaged brain or very low birth weight newborn.
- Increased level of **CK_2** in blood is characteristic of **damage of heart** tissue from myocardial infarction because cardiac tissue is the only tissue which has the mixed MB (CK_2) isoenzyme.
- Elevated levels of **CK_3** in serum occur in all types of **dystrophies** and **myopathies.**

CLINICAL SIGNIFICANCE OF ENZYMES

Certain enzymes are used:

- For the diagnosis of the disease
- As therapeutic agents
- As analytical reagents.

Diagnostic Use of Enzymes

Enzymes are known as marker of **cellular damage** and their measurement in plasma is used in the investigation of diseases of **liver, heart, skeletal muscle,** the **biliary tract** and the **pancreas.** The enzymes that are found in plasma can be categorized into two major groups: The **plasma specific** enzyme and the **plasma nonspecific** enzyme.

- The **plasma specific** enzymes are present in higher concentration in plasma than in most tissues; e.g.
  - The enzymes involved in blood coagulation
  - Ferroxidase
  - Pseudocholinesterase
  - Lipoprotein lipase.
- These enzymes are clinically of interest when their concentration decreases in plasma.

Table 6.8: Type, composition, location and diagnostic importance of lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes

Type	*Composition*	*Location*	*Diagnostic importance (cause of elevated level)*
LDH_1	HHHH	Heart, RBC	Myocardial infarction
LDH_2	HHHM	Heart, RBC	Megaloblastic anemia
LDH_3	HHMM	Brain	Leukemia, malignancy
LDH_4	HMMM	Lung, spleen	Pulmonary infarction
LDH_5	MMMM	Liver, muscle	Liver diseases, Muscle damage/diseases
CK_1	BB	Brain	Neurological injury
CK_2	BM	Heart	Myocardial infarction
CK_3	MM	Skeletal muscle	Muscular dystrophies and myopathies

- The **plasma nonspecific** enzymes are present in very high concentration in tissues than in the plasma. Estimation of plasma nonspecific enzymes is very important for the diagnosis of several disease.
- The important enzymes useful for the diagnosis of specific diseases are listed in **Table 6.9**. A brief account of selected diagnostic enzymes is discussed.

Alanine transaminase (ALT)

- Alanine transaminase was known formerly as glutamate pyruvate transaminase (GPT).
- The plasma ALT normal value for adult is 10 to 40 U/L.
- ALT level is elevated in **liver diseases** (viral or toxic hepatitis), **jaundice** and **cirrhosis** of liver.

Aspartate transaminase (AST)

- It was known formerly as glutamate oxaloacetate transminase (GOT).
- The plasma AST normal value for adults is 10 to 30 U/L.
- Increased AST level occurs after **myocardial infarction.** The plasma AST level starts increasing after 6 to 8 hours after the onset of chest pain with peak values 18 to 24 hours and the values fall to normal level by the fourth or fifth day.
- It is moderately elevated in liver disease.

Alkaline phosphatase (ALP)

- ALP hydrolyzes organic phosphate at alkaline pH.
- Normal serum level for adults is 3-13 KA units/dl.
- It is elevated in certain **bone** and **liver disease.**
- Very high levels may be noticed in **obstructive jaundice, bone diseases** such as **Paget's disease,** rickets, osteomalacia, carcinoma of bone and hyperparathyroidism.

Acid phosphatase (ACP)

- It hydrolyzes phosphoric acid ester at pH 5 to 6.
- Normal serum value for ACP is 0.5 to 4 KA units/dL.
- Prostatic acid phosphatase enzyme is useful for the diagnosis and prognosis of **prostate cancer.** ACP is therefore an important **tumor marker.**

Amylase

- It catalyzes hydrolysis of starch and glycogen.
- Normal serum value is 50-120 U/L.
- The activity of serum amylase is increased in **acute pancreatitis.**
- Elevated activity of amylase is also found in urine of the patient of acute pancreatitis.
- Increase in serum levels are also seen in **chronic pancreatitis, mumps** and **obstruction of pancreatic duct.**

Creatine kinase (CK)

Refer isoenzyme.

Lactate dehydrogenase (LDH)

Refer isoenzyme.

Prostate specific antigen (PSA Seminogelase)

- It is a glycoprotein with mild protease activity and involved in the liquification of the seminal coagulum formed after ejaculation.

Table 6:9: Enzymes of diagnostic importance

Enzyme	*Clinical Application*
Acid phosphatase	Prostatic cancer
Alanine aminotransferase	Liver disease (viral or toxic hepatitis), jaundice and liver cirrhosis
Aldolase	Muscle diseases
Alkaline phosphatase	Obstructive jaundice, bone diseases such as Paget's disease, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism
Amylase	Acute pancreatitis, mumps, obstruction in pancreatic duct
Aspartate transaminase	Myocardial infarction, liver diseases
Cholinesterase	Organophosphorus insecticide poisoning, hepatic parenchymal diseases
Creatine kinase	Myocardial infarction, muscle diseases
γ-Glutamyl transferase	Hepatobiliary disease, alcoholism
Lactate dehydrogenase	Myocardial infarction, leukemia, muscular dystrophy, hepatic diseases
5'-Nucleotidase	Hepatitis, obstructive jaundice
Prostate specific antigen	Prostate cancer
Trypsin	Pancreatic disease, cystic fibrosis

- It is produced exclusively by prostate glands.
- Elevated blood levels of PSA occur in prostate cancer.

Enzyme Assay in Myocardial Infarction

The enzymes' assay, which are most helpful in the diagnosis of myocardial infarction are listed in **Table 6.10** and **Figure 6.18.**

Therapeutic Use of Enzymes

Some enzymes are used in the treatment of some diseases of human being. For example:

- **Bacterial asparginase** is used in the treatment of some types of leukemia. Asparginase which hydrolyzes aspargine to aspartic acid. Aspargine is necessary for the formation of leukemic white cell.
- **Chymotrypsin:** Used for dissolving ligaments of the lens during the extraction of cataract.
- **Collagenase:** Used for debridement (cleaning of wound by removing dead tissue) of dermal ulcers and severe burns.
- **Fibrinolysin:** It is used in the venous thrombosis, pulmonary and arterial embolism (blood clot).
- **Hyaluronidase:** It is used to promote the rapid absorption of drugs injected subcutaneously. It acts by increasing tissue permeability. It is used in the treatment of traumatic or postoperative edema.
- **Lysozyme** (an antibiotic) found in human tears and egg white, is used in the infection of eye. It has antibacterial action, it acts on cellulose of bacteria.
- **Penicillinase** (bacterial enzyme) is used for the treatment of persons who are allergic to penicillin, penicillinase destroys penicillin.
- **Rennin** is used for the treatment of gastric achylia.
- **Streptokinase:** Streptokinase and urokinase are used in myocardial infarction to dissolve blood clot or purulent (containing pus) material. It causes fibrinolysis.
- **Trypsin:** It is a proteolytic enzyme and is used to clean the wounds by dissolving purulent material and in the treatment of acute thrombophlebitis to dissolve the blood clot.

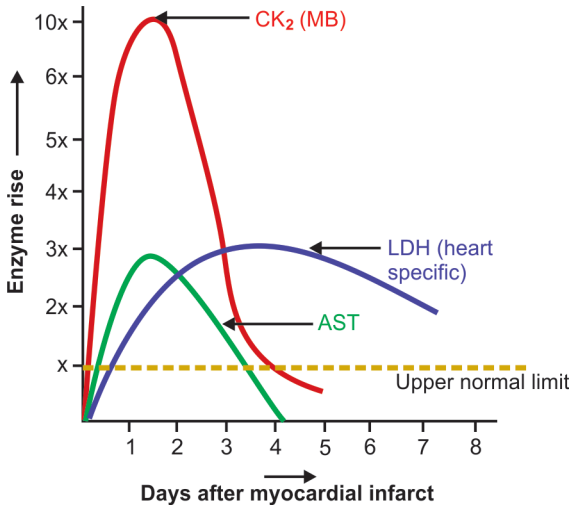


Figure 6.18: Typical rise in serum enzyme activities following a myocardial infarction

- **Pepsin, trypsin peptidase, lipase, amylase elastase** and **cellulase** are used in gastrointestinal tract (GIT) disorders and chronic pancreatitis.

Analytical Use of Enzymes

- Enzymes can be used as ***reagents*** and ***labels.***
- In addition to measurement of serum enzyme activity for the diagnosis and management of disease, enzymes are widely used in the clinical laboratory as reagents for the estimation of serum constituents. Some examples are given in **Table 6.11.**
- Many enzymes have been used as the label in various immunoassays (enzyme linked immunosorption assay, ELISA) for determining the serum concentration of drugs, hormones or other compounds of interest. Commonly used label enzymes are:
  - Glucose-6-phosphate dehydrogenase
  - Alkaline phosphatase
  - β-galactosidase
  - Peroxidase.

Table 6.10: Enzymes in diagnosis of myocardial infarction and time course of plasma enzyme activity changes after myocardial infarction

Enzyme	*Abnormal activity detectable (hours)*	*Time for maximum rise (hours)*	*Time for return to normal (Days)*
CK_2 (MB-isoenzyme)	3-10	12-24	2-3
AST	6-12	24-48	4-6
LDH (heart specific)	8-16	48-72	7-12

Table 6.11: List of enzymes used in the clinical laboratory as reagents for assays

Enzymes as reagents	*Assays*
Alcohol dehydrogenase	Ethanol
Lactate dehydrogenase	Lactate
Glucose oxidase and peroxidase	Glucose
Hexokinase and glucose-6-phosphate dehydrogenase	Creatine kinase
Uricase	Uric acid
Urease	Urea
Cholesterol oxidase and peroxidase	Cholesterol
Lipase, glycerol kinase, glycerol phosphate dehydrogenase	Triacylglycerol

SUMMARY

- Virtually every biochemical reaction is catalyzed by enzymes. With the exception of ribozymes (catalytic RNAs), all known enzymes are proteins.
- Many enzymes are synthesized as inactive precursor called zymogens or proenzymes.
- Enzymes are classified into six main classes according to the type of reaction it catalyzes.
- Temperature, pH, enzyme concentration, substrate concentration and inhibitors affect the rate of enzyme catalyzed reactions.
- Substances that reduce the activities of enzymes are called inhibitors. They may be irreversible, reacting covalently with the enzyme or reversible inhibitors bind noncovalently to enzymes.
- Kinetic analysis distinguishes competitive, non-competitive or uncompetitive inhibition. Many clinically important drugs are enzyme inhibitors.
- Isoenzymes are physically distinct forms of the same enzyme. Separation and identification of isoenzymes is of diagnostic value.
- The quantitative analysis of certain plasma enzymes have been used for diagnostic purpose.

EXERCISE

Multiple Choice Questions (MCQs)

1. Enzymes increase reaction rates by:
a) Selectively enhancing the rate of the forward reaction
b) Altering the change in free energy of the reaction
c) Changing the equilibrium constant of the reaction
d) Decreasing the rate of activation

2. The Michaelis constant (K_m) is:
a) Not changed by the presence of non-competitive inhibitor
b) Equal to 1/2 V_{max}
c) The substrate concentration at 1/2 V_{max}
d) The equilibrium constant for the dissociation of ES to E + P

3. Which of the following non-proteins can act as an enzyme?
a) DNA b) RNA
c) Phospholipid d) Glycolipid

4. The substrate concentration at which an enzyme exhibits half the maximum velocity is known as:
a) V_{max} b) [S]
c) K_m d) Keq

5. All of the following serum enzymes are elevated in myocardial infarction, *except*:
a) Alkaline phosphatase
b) Lactate dehydrogenase
c) Aspartate transaminase
d) Creatine kinase

6. Enzymes may be used as the following, *except*:
a) Therapeutic agents b) Nutrients
c) Diagnostic agents d) Laboratory reagents

7. Enzyme activity in biological systems may be affected by the following:
a) Negative modifiers
b) Change in pH
c) Change in enzyme concentration
d) All of the above

8. Isoenzymes can be characterized as:
a) Non-protein part of enzyme
b) Enzymes with same quaternary structure
c) Similar enzymes that catalyze different reactions
d) Multiple forms of given enzyme that catalyze same type of reactions

9. Transaminase enzymes belong to the class:
a) Hydrolases b) Transferases
c) Oxidoreductases d) Isomerases

10. Liver diagnostic enzyme is:
a) Alkaline phosphatase
b) Acid phosphatase
c) Alanine transaminase
d) Amylase

11. Which of the following enzyme levels increases in obstructive jaundice?
a) Lipase
b) Amylase

c) Acid phosphatase
d) Alkaline phosphatase

12. The digestive enzymes belong to:
a) Isomerases b) Transferases
c) Hydrolases d) Ligases

13. The enzyme useful in the treatment of leukemia is:
a) α-chymotrypsin b) Hyaluronidase
c) Asparginase d) Streptokinase

14. Earliest marker of myocardial infarction is:
a) CK-1 b) CK-2
c) CK-3 d) AST

Correct Answers for MCQs

1-d	2-c	3-b	4-c
5-a	6-b	7-d	8-d
9-b	10-c	11-d	12-c
13-c	14-b		

CHAPTER

7

Vitamins

INTRODUCTION

The name *'Vitamine'* was proposed in 1911 by Polish chemist Casimir Funk for the nutrient compound required to prevent the nutritional deficiency disease ***beriberi,*** because of its vital (vita = life) need and because chemically it was found to be an ***amine.*** Later, after a number of other essential organic nutrients were discovered, the ***"e"*** was dropped, when it was found that not all of them are amines. The term ***'Vitamin'*** *has* now been adopted universally and applied to a group of biologically essential compounds that include 14 compounds which cannot be synthesized by human beings. They must, therefore, be supplied through food.

Since their chemical nature was unknown letter designations were applied for their nomenclature, e.g. vitamins A, B and C. Later, vitamin B was shown to consist of several substances and subscripts were added, i.e. vitamin B_1, B_2, B_6, etc. and collectively called ***vitamin B complex.***

DEFINITION AND CLASSIFICATION OF VITAMINS

Vitamins are organic nutrients that are required in small quantities (in micrograms to milligram quantities per day) for a variety of biochemical functions and which generally cannot be synthesized by the body and must, therefore, be supplied by the diet. Some can be synthesized by intestinal microorganisms, but in quantities that are not sufficient to meet our needs. They may be water or fat soluble.

Classification

The vitamins are grouped into two categories based on their solubility:

1. Water soluble vitamins
2. Fat soluble vitamins

- **Water soluble vitamins which include**
  - i. Vitamin B complex, e.g.
    - Thiamine (vitamin B_1)
    - Riboflavin (vitamin B_2)
    - Niacin (vitamin B_3)
    - Pantothenic acid (vitamin B_5)
    - Pyridoxine (vitamin B_6)
    - Biotin
    - Folic acid
    - Cobalamin (vitamin B_{12})
  - ii. Vitamin C or ascorbic acid.
- **Fat soluble vitamins, which include**
  - Vitamin A or retinol
  - Vitamin D or cholecalciferol
  - Vitamin E or tocopherol
  - Vitamin K.

Table 7.1 summarizes the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins.

Table 7.1: Summary of the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins

Name	*Active form*	*Sources*	*Daily requirements*	*Functions*	*Deficiency manifestations*
WATER SOLUBLE VITAMINS					
Thiamine (Vitamin B_1)	TPP	Cereals, meat, nuts green vegetables, eggs	1.0–1.5 mg	Coenzyme for oxidative decarboxylation and transketolase reactions	Beriberi, Wernicke Korsakoff syndrome
Riboflavin (Vitamin B_2)	FMN, FAD	Yeast, germinating seeds, green leafy vegetables, milk, eggs, liver, meat	1.3–1.7 mg	Coenzyme for oxidation-reduction reactions	Cheilosis, glossitis, dermatitis, vascularization of cornea
Niacin (Vitamin B_3)	NAD^+ and $NADP^+$	Yeast, legumes, liver, meat	15–20 mg	Coenzyme for oxidation reduction reactions	Pellagra
Pantothenic acid (Vitamin B_5)	Coenzyme A, (CoA-SH)	Wheat germs, cereals, yeast, liver, eggs	5–10 mg	Acyl carrier	Burning feet syndrome
Pyridoxin (Vitamin B_6)	PLP	Yeast, unrefined cereals, pulses, vegetables, meat, fish, egg yolk	1.6–2 mg	Coenzyme for transamination, decarboxylation, non-oxidative deamination, trans-sulfuration reactions	Epileptic convulsions, dermatitis, hypochromic microcytic anemia
Biotin (Vitamin B_7)	Biocytin (Enzyme bound biotin)	Liver, kidney, egg yolk, vegetables	150–300 µg	Coenzyme for carboxylation reactions	Rare dermatitis
Folic acid (Vitamin B_9)	**THF** (Tetrahydrofolic acid)	Green leafy vegetables, liver, yeast	200 µg	Carrier of one carbon unit. Synthesis of methionine, purines and pyrimidines	Megaloblastic anemia, Neural tube defects
Cynocobalamin (Vitamin B_{12})	Methylcobalamin, Deoxyadeno-sylcobalamin	Only animal origin, meat, egg, liver, fish	3 µg	Coenzyme for reactions: Homocysteine to Methionine. Methylmalonyl-CoA to Succinyl-CoA	Pernicious anemia Megaloblastic anemia Neuropathy (dementia) Methylmalonic aciduria
Ascorbic acid (Vitamin C)	Ascorbic acid	Citrus fruits, Amla, leafy vegetables, tomatoes	60–70 mg	Antioxidant, involved in hydroxylation reactions in the synthesis collagen, steroid hormones, adrenaline, etc. facilitates absorption of iron from intestine	Scurvy

Contd...

Contd...

Table 7.1

Name	*Active form*	*Sources*	*Daily requirements*	*Functions*	*Deficiency manifestations*
FAT SOLUBLE VITAMINS					
Vitamin A (Retinol)	Retinol, Retinal, Retinoic acid	Fish liver oils, mik, milk products, Green leafy vegetables, carrots, yellow and red fruits	800–1000 retinol equivalents	Retinal and retinol are involved in vision. Retinoic acid regulates the expression of gene during growth and development. Antioxidant	Night blindness xerophthalmia formation of Bitot's spots, dry, rough and scaly skin. Retardation of growth in children
Vitamin D (Cholecalciferol)	1, 25-Dihydroxy-cholecalciferol	Cod liver oil, sunlight induced synthesis of vitamin D_3 in skin, egg yolk	200–400 IU	Regulation of the plasma level of calcium and phosphorus, calcification of bone	Rickets (in children) Osteomalacia (in adults)
Vitamin E (α -Tocopherol)	α-tocopherol,	Soya and corn oils, germ oil, fish oil, eggs, alfalfa	8–10 mg	Natural antioxidant and acts as a scavenger of free radicals. Protects the RBCs from hemolysis Prevents peroxidation of PUFA in cell membrane	Hemolytic anemia. Retrolental fibroplasia (RLF) in premature infants
Vitamin K	Phylloquinone (Vitamin K_1) Menaquinone (Vitamin K_2)	Green leafy vegetables, tomatoes, cheese, meat, egg yolk	70–140 μg	Required for activation of blood clotting factors. Required for γ carboxylation of glutamic acid residue in clotting and oseocalcin proteins	Hemorrhagic disorder, increased clotting time

DIFFERENCE BETWEEN FAT SOLUBLE AND WATER SOLUBLE VITAMINS

- Water soluble vitamins function as precursor for coenzymes and antioxidants while fat soluble vitamins function as coenzymes, hormones and antioxidants.
- Water soluble vitamins are usually ***non-toxic*** since excess amounts of these vitamins are excreted in the urine, while fat soluble vitamins are ***toxic*** and even lethal when taken in excessive quantities.
- Water soluble vitamins are not stored extensively **except *vitamin* B_{12},** and so their intake has to be more frequent than that of other fat soluble vitamins which are stored.

WATER SOLUBLE VITAMINS

Thiamine (Vitamin B_1)

Structure

Thiamine consists of a pyrimidine ring attached to a thiazole ring **(Figure 7.1)** by methylene bridge.

Active Form of Thiamine

Thiamine pyrophosphate (TPP) is an active coenzyme form of vitamin thiamine **(Figure 7.2).**

Sources

- It is present in all natural foods but particularly good dietary sources are unrefined cereals, meat, nuts, green vegetables, eggs, etc.

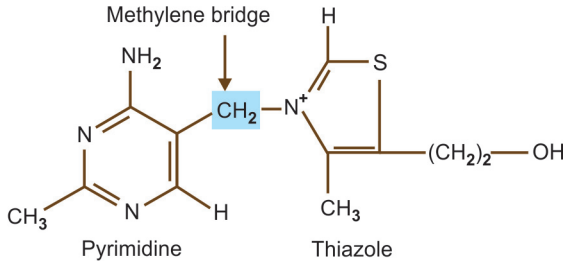


Figure 7.1: Structure of thiamine

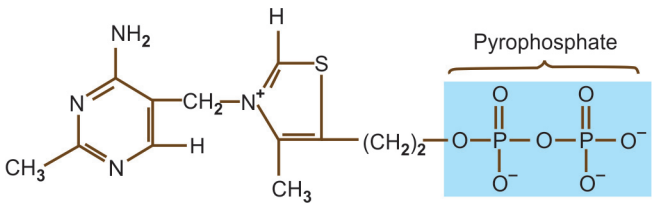


Figure 7.2: Thiamine pyrophosphate, active coenzyme form of thiamine (OH-group of thiazole is replaced by pyrophosphate)

- White bread and polished rice are very poor sources of the vitamin thiamine.

Functions

- Thiamine is required mainly for ***carbohydrate metabolism***
- Thiamine pyrophosphate (TPP) is a coenzyme involved in several enzymatic reactions mainly for ***oxidative decarboxylation*** and ***transketolase*** reactions as follows:
  1 TPP is a coenzyme for ***pyruvate dehydrogenase complex*** which catalyzes the conversion of pyruvate into acetyl CoA by oxidative decarboxylation **(Figure 12.5).** Acetyl-CoA is a precursor for the synthesis of the **neurotransmitter acetylcholine** and also for the synthesis of myelin. ***Thus, thiamine is required for the normal functioning of the nervous system.***
  2 TPP is a coenzyme for ***α-ketoglutarate dehydrogenase*** which catalyzes the conversion of α-ketoglutarate to succinyl-CoA in TCA cycle ***(See Figure 12.6).***
  3 TPP is a coenzyme for the enzyme ***transketolase,*** in the pentose phosphate pathway of glucose oxidation ***(See Figure 12.16).***

Nutritional Requirements

- Nutritional Research Council recommends daily intake of ***1.0 to 1.5 mg*** of thiamine for adults which is increased with increased muscular activity, dietary carbohydrates and in pregnancy and lactation.

Deficiency Manifestations

- The deficiency of vitamin B_1 results in a condition called **beriberi.** Deficiency of thiamine occurs in population who consume exclusively **polished rice** as staple food. Polishing of rice removes thiamine.
- The early symptoms of thiamine deficiency are anorexia, nausea, mental confusion, peripheral neuritis, muscle fatigue and irritability.
- Thiamine deficiency leads to three types of beriberi namely
  1. Dry beriberi
  2. Wet beriberi
  3. Infantile beriberi

Dry beriberi (neuritic beriberi)

- It develops when the diet chronically contains slightly less than the thiamine requirements.
- This form of beriberi is characterized primarily by **peripheral neuritis,** severe muscular weakness and fatigue. Other symptoms of dry beriberi include dry skin, mental confusion and poor appetite.

Wet beriberi (cardiac beriberi)

- It develops when the deficiency is more severe in which cardiovascular system is affected in addition to neurological symptoms.
- Wet beriberi is characterized primarily by **edema** of extremities, heart enlargement and cardiac insufficiency. Other symptoms include tachycardia or bradycardia and palpitation.
- Both forms of beriberi may overlap to a varying degree and patients of beriberi may die due to heart failure, if not treated.

Infantile beriberi

- Infantile beriberi is observed in breast fed infants born to mother suffering from thiamine deficiency. The breast milk of these mothers is deficient in thiamine.
- It is characterized by cardiac dilation (enlargement of heart), tachycardia, convulsions, edema and GI disturbances such as vomiting, abdominal colic, etc. In acute condition, the infant may die due to cardiac failure.

Wernicke-Korsakoff Syndrome

- It is also known as **cerebral beriberi** and mostly seen in alcoholics.
- In chronic alcoholics, the nutritional deficiencies result from either poor intake of food or malabsorption of nutrients from intestine.
- Wernicke-Korsakoff syndrome is characterized by anorexia, nausea, vomiting, nystagmus, depression, ataxia, loss of memory, mental confusion, peripheral paralysis, muscular weakness, etc.

Antimetabolites

Thiamine can be destroyed if the diet contains ***thiaminase.*** Thiaminase is present in raw fish and seafood.

Thiamine Assay

Whole blood or Erythrocyte ***transketolase*** *(requiring TPP as a coenzyme)* activity is used as a measure of thiamine deficiency.

Riboflavin (Vitamin B_2)

Structure

Riboflavin is a yellow compound (Flavus = ***yellow*** in Latin) consisting of a ***isoalloxazine ring*** with a ***ribitol*** (sugar alcohol) side chain **(Figure 7.3)**. Riboflavin is relatively ***heat stable*** but decomposes in the presence of visible light (photosensitive).

Active Form of Riboflavin

The active or coenzyme forms of the riboflavin are:

- Flavin mononucleotide (FMN), and
- Flavin adenine dinucleotide (FAD).

Sources

- The main dietary sources of riboflavin are yeast, germinating seeds, green leafy vegetables milk and milk products, eggs, liver and meat.
- Cereals are a poor source.

Functions

- Riboflavin is a precursor of coenzymes ***FMN*** and **FAD,** which are required by several ***oxidation-reduction*** reactions in metabolism. FMN and FAD serve as coenzymes for *oxidoreductase enzymes* involved in carbohydrate, protein, lipid, nucleic acid metabolism and electron transport chain. Some examples are given in **Table 7.2.**
- It is needed for maintenance of mucosal epithelial and the ocular tissues.
- They are also involved in protection against peroxidation in ***metabolism of xenobiotics.***

Table 7.2: Examples of enzymes requiring FMN or FAD as a coenzyme and reaction where they are involved

Flavoprotein enzyme	*Pathway/Reaction*
Amino acid oxidase	Deamination of amino acids
Xanthine oxidase	Purine degradation
Succinate dehydrogenase	Citric acid cycle
Acyl-CoA dehydrogenase	Fatty acid oxidation
NADH dehydrogenase	Respiratory chain into mitochondria
Pyruvate dehydrogenase, and α-Ketoglutarate dehydrogenase	Oxidative decarboxylation of pyruvate and α-ketoglutarate

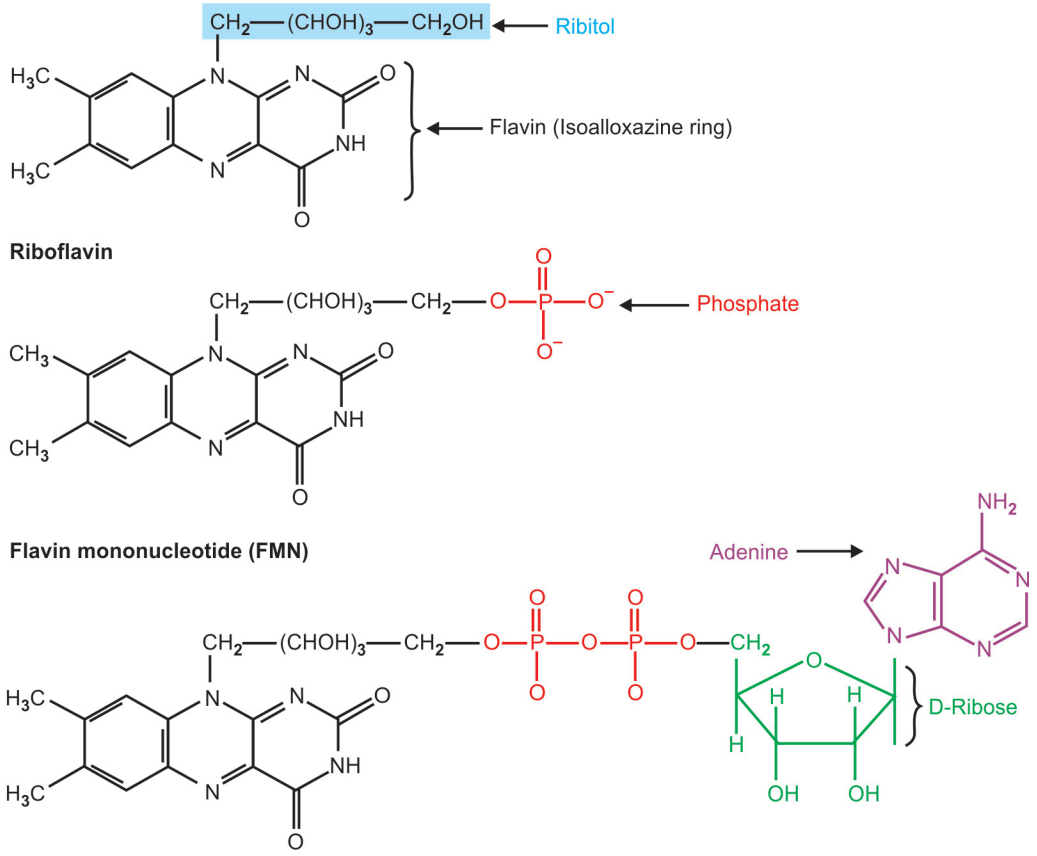


Figure 7.3: Structure of riboflavin and its active coenzyme forms FMN and FAD

Nutritional Requirements

- The RDA for vitamin B_2 is **1.3 to 1.7 mg** for adults.
- It is related to protein use and increases during growth, pregnancy, lactation and wound healing.

Deficiency Manifestations

- Riboflavin deficiency is quite rare as it has a wide distribution in food stuffs. It is usually seen along with deficiencies of other vitamins of B-complex group. It is most commonly seen in chronic alcoholics.
- The characteristic symptoms of riboflavin deficiency are:
  - Cheilosis: Fissures at the angles of the mouth,
  - Glossitis: Inflammation of the tongue that becomes swollen and magenta colored
  - Dermatitis: Rough and scaly skin
  - Vascularization (the development of blood vessels) of cornea, etc.

Riboflavin Assay

A commonly used method for assessment of riboflavin status uses the determination of FAD dependent **glutathione reductase** activity in freshly lyzed erythrocytes.

Niacin (Vitamin B_3)

Structure

Niacin is a general name for the ***nicotinic acid*** and ***nicotinamide,*** either of which may act as a source of the vitamin in the diet. Niacin is a simple derivative of ***pyridine.***

Active Form

Active forms of niacin are:

- Nicotinamide adenine dinucleotide (NAD^+)
- Nicotinamide adenine dinucleotide phosphate ($NADP^+$) **(Figure 7.4).**

Sources

- Yeast, liver, legumes and meats are major sources of niacin.
- Limited quantities of niacin can also be obtained from the metabolism of tryptophan. ***For every 60 mg of***

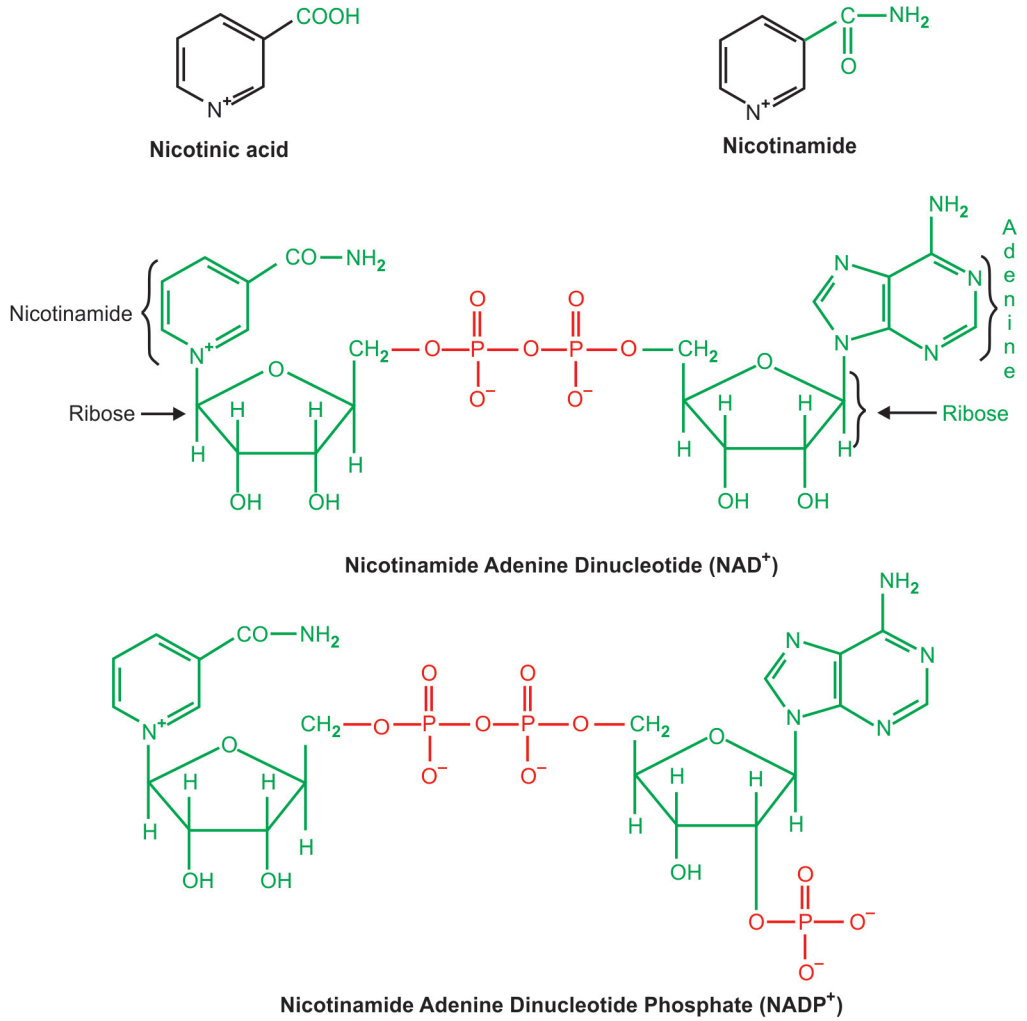


Figure 7.4: Structure and active coenzyme forms of niacin

tryptophan, 1 mg equivalent of niacin can be generated.

Functions

- Niacin is a precursor of coenzymes, ***nicotinamide adenine dinucleotide (NAD+)*** and ***nicotinamide adenine dinucleotide phosphate (NADP+).***
- NAD^+ and $NADP^+$ are involved in various ***oxidation*** and ***reduction reactions*** catalyzed by dehydrogenases in metabolism.
- They are, therefore involved in many metabolic pathways of carbohydrate, lipid and protein. Generally, ***NAD+*** linked dehydrogenases catalyze oxidation-reduction reactions in ***oxidative pathways,*** e.g. citric acid cycle and glycolysis.
- Whereas ***NADP+*** linked dehydrogenases or reductases are often found in pathways concerned with ***reductive synthesis,*** e.g. synthesis of cholesterol, fatty acid and pentose phosphate pathways.
- Selected examples of enzymes and the reactions they catalyze are given in **Table 7.3.**

Nutritional Requirement

- The RDA for niacin is ***15*** to ***20 mg.***
- ***Tryptophan*** can only provide about 10% of the total ***niacin*** requirement.

Deficiency Manifestation

Pellagra

- Deficiency of niacin in human causes pellagra, a disease involving the:

Table 7.3 : Examples of enzymes requiring NAD^+ or $NADP^+$ or NADPH and reaction where they are involved

Enzyme	*Pathway/Reaction*
NAD dependent	
Glyceraldehyde-3-phosphate dehydrogenase	Glycolysis: Glyceraldehyde-3 phosphate to 1,3-bisphosphoglycerate
Pyruvate dehydrogenase	Oxidative decarboxylation of pyruvate to acetyl-CoA
α-Ketoglutarate dehydrogenase	TCA cycle: α-ketoglutarate to succinyl-CoA
β-Hydroxy acyl-CoA dehydrogenase	β-Oxidation of fatty acid: β- Hydroxy acyl-CoA to β-Keto acyl-CoA
NADP dependent	
Glucose-6-phosphate dehydrogenase	Pentose phosphate pathway: Glucose 6-phosphate to 6-phosphogluconolactone
Malic enzyme	Transfer of acetyl-CoA from mitochondria to cytosol
NADPH dependent	
3-Ketoacyl reductase	Fatty acid synthesis: 3 Ketoacyl enzyme to 3-Hydroxyacyl enzyme
HMG CoA reductase	Cholesterol synthesis: HMG-CoA to Mevalonate

- Skin
- Gastrointestinal tract
- Central nervous system.

- The symptoms of pellagra are characterized by **three 'Ds'**:
  1. Dermatitis
  2. Diarrhea
  3. Dementia and if not treated death.

Dermatitis: Skin inflammation is seen in any area exposed to direct sunlight.

Diarrhea: Frequent diarrhea nausea, vomiting, anorexia are the disorders of GI tract.

Dementia: Dementia (loss of memory) is associated with degeneration of nervous tissues.

- To produce niacin deficiency, diet must be poor in both available niacin and tryptophan. Niacin deficiency occurs in:
  - Population dependent on maize (corn) or sorghum *(jowar)* as the staple food.
  - ***Deficiency of vitamin B_6*** (pyridoxal phosphate) leads to ***niacin deficiency*** as it is involved as a coenzyme in the pathway of synthesis of niacin from tryptophan.
  - **Malignant carcinoid syndrome** in which tryptophan metabolism is diverted to formation of serotonin.
  - In **Hartnup disease,** a genetic disorder in which tryptophan absorption and transportation is impaired.

Therapeutic Uses of Niacin

Nicotinic acid (not nicotinamide), used at high doses (1–2 gm/day), has been shown to lower total cholesterol, LDL cholesterol and VLDL triglyceride in patients with hyperlipoproteinemias.

Toxicity

High intake of the vitamin has undesirable side effects, mainly vasodilation and flushing and liver damage.

Pantothenic Acid (Vitamin B_5)

The name pantothenic acid is derived from the Greek word ***'pantothene,'*** meaning from ***"everywhere"*** and gives an indication of the wide distribution of the vitamin in foods.

Structure

Pantothenic acid is formed by a combination of ***pantoic acid*** and ***β-alanine*** **(Figure 7.5)**.

Active form

Active forms of pantothenic acid are:
- Coenzyme-A (CoA-SH)
- Acyl carrier protein (ACP).

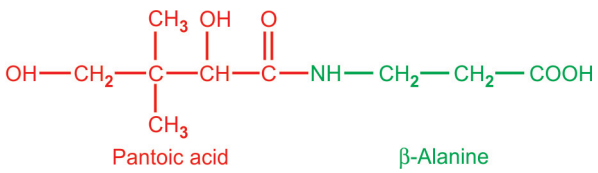


Figure 7.5: Structure of pantothenic acid

Source

Eggs, liver, yeast, wheat germs, cereals, etc. are important sources of pantothenic acid, although the vitamin is widely distributed.

Functions

- Pantothenic acid is a component of ***coenzyme-A (CoA-SH)*** and ***acyl carrier protein (ACP).*** The thiol (-SH) group of CoA-SH and ACP acts as a carrier of acyl groups.
- Coenzyme-A participates in reactions concerned with:
  - Reactions of citric acid cycle
  - Fatty acid synthesis and oxidation
  - Synthesis of cholesterol
  - Utilization of ketone bodies.
- ACP participate in reactions concerned with fatty acid synthesis.

Nutritional Requirement

The RDA of pantothenic acid is not well established. A daily intake of about 5–10 mg is advised for adults.

Deficiency Manifestations

No clearcut case of pantothenic acid deficiency has been reported (becuase the substance is widely distributed in foods) except in malnourished prisoners of war in the far East in 1940s, where *neurological condition,* known as the ***burning feet syndrome,*** was reported and ascribed to pantothenic acid deficiency. As these people were severely malnourished and were deficient in other vitamins as well, it is not possible to attribute this specific effect to pantothenic acid deficiency.

Clinical signs observed in experimentally induced deficiencies are:

- Paresthesia (abnormal tingling sensation)
- Headache
- Dizziness
- Gastrointestinal malfunction.

Pyridoxine (Vitamin B_6)

Structure

Vitamin B_6 consists of a mixture of three different closely related pyridine derivatives **(Figure 7.6)** namely:

1. Pyridoxine
2. Pyridoxal
3. Pyridoxamine.

All the three have equal vitamin activity, as they can be interconverted in the body.

Active Form of Vitamin B_6

Pyridoxal phosphate (PLP) is the active form of vitamin B_6 **(Figure 7.7).** PLP is formed from phosphorylation of all three forms of vitamin B_6.

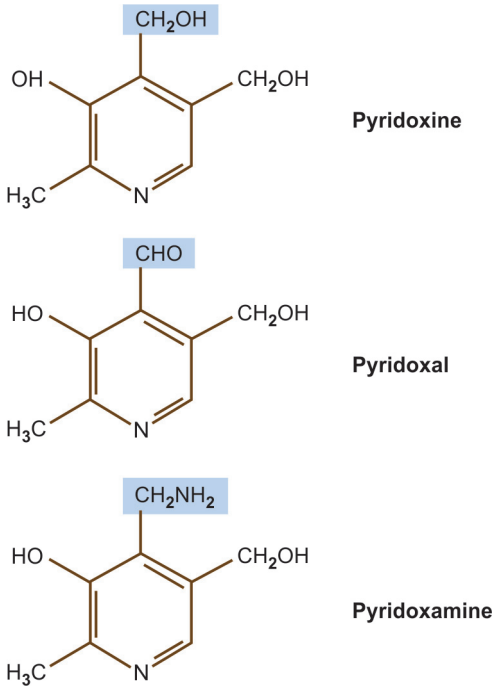


Figure 7.6: Structure of three different forms of vitamin B_6

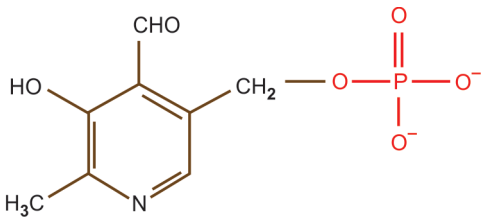


Figure 7.7: Structure of pyridoxal phosphate: an active form of vitamin B_6

Sources

- Pyridoxine occurs mainly in plants, whereas pyridoxal and pyridoxamine are present mainly in animal products.
- Major dietary sources of vitamin B_6 are yeast, unrefined cereals, pulses, meat, poultry fish, potatoes and vegetables.
- Dairy products and grains contribute lesser amounts.

Functions

- Active form of vitamin B_6, pyridoxal phosphate (PLP) acts as coenzyme in large number of reactions of amino acid metabolism. For example:
  - Transamination
  - Decarboxylation
  - Nonoxidative deamination
  - Trans-sulfuration
  - Condensation reactions of amino acids.

Transamination reactions: Transamination reactions are catalyzed by transaminases and PLP acts as coenzyme converting amino acid to keto acid, e.g. aspartate transaminase (AST) and alanine transaminase (ALT) **(Figure 14.6).**

Decarboxylation reaction: PLP acts as coenzyme in decarboxylation of some amino acids. The amino acids are decarboxylated to corresponding amines. The important biogenic amines synthesized by PLP decarboxylation are given below. **(Figure 14.45).**

- **γ-Amino butyric acid (GABA):** It is an inhibitory neurotransmitter derived from glutamate on decarboxylation hence in vitamin B_6 deficiency underproduction of GABA leads to convulsions (epileptic seizures) in infants and children.
- **Serotonin and melatonin:** These are produced from tryptophan. Serotonin is a neurotransmitter and stimulates the cerebral activity. Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body.
- **Histamine:** Histamine produced by decarboxylation of histidine. It is a vasodilator and lowers blood pressure. It is involved in allergic reactions.
- **Catecholamines** (dopamine, norepinephrine and epinephrine) Synthesis of catecholamines from tyrosine requires PLP-dependent DOPA decarboxylase. Catecholamines are neurotransmitters and involved in metabolic and nervous regulation.

Non-oxidative deamination: Hydroxyl group containing amino acids (serine, threonine) are non-oxidatively deaminated to α-keto acids and ammonia, which requires PLP. **(Figure 14.9).**

Trans-sulfuration reaction: PLP is a coenzyme for **cystathionine synthase** involved in synthesis of cysteine from methionine **(Figure 14.25).** In these reactions transfer of sulfur from methionine to serine occurs to produce cysteine.

Condensation reactions: Pyridoxal phosphate is required for the condensation reaction of L-glycine and succinyl CoA to form δ-aminolevulinic acid, a precursor of heme **(Figure 18.2).**

Other PLP-dependent reactions:

- Pyridoxal phosphate is required for niacin coenzyme ($NAD^+/NADP^+$) synthesis from tryptophan **(Figure 14.22).**
- PLP is required for synthesis of serine from glycine **(Figure 14.12).**
- The enzyme glycogen phosphorylase contains covalently bound PLP.
- PLP is required for the synthesis of sphingosine, a component of sphingomyelin.

Nutritional Requirement

The RDA for vitamin B_6 is ***1.6*** to ***2.0 mg***. Requirements increase during pregnancy and lactation.

Deficiency Manifestations

As pyridoxine occurs in most foods, the dietary deficiency of vitamin B_6 is rare. The main clinical symptoms of deficiency are given below:

- Vitamin B_6 deficiency causes neurological disorders such as **depression, nervousness** and **irritability.** These symptoms are due to decreased production of neurotransmitters, catecholamines, GABA and serotonine.
- Severe deficiency of pyridoxine causes epileptic seizures (convulsions) in infants due to reduced production of GABA.
- Demyelination of nerves causes peripheral neuropathy. Since vitamin B_6 is required for synthesis of sphingolipids needed for myelin formation.
- Vitamin B_6 deficiency causes **hypochromic microcytic anemia** due to decreased heme synthesis. Since PLP is required for the synthesis of heme.
- The commonest cause of pyridoxine deficiency is:
  - ***Drug antagonism,* e.g. *isoniazide (INH),*** used in the treatment of tuberculosis and ***penicillamide*** used in the treatment of Wilson's disease and rheumatoid arthritis can combine with pyridoxal phosphate forming an inactive derivative with pyridoxal phosphate.
  - ***Alcoholism:*** Alcoholics may be deficient owing to metabolism of ethanol to acetaldehyde, which stimulates hydrolysis of the phosphate of the pyridoxal phosphate.

Vitamin B_6 Assay

Activities of blood **transaminases** have been used frequently as indirect measurements of vitamin B_6 status. Erythrocyte levels of aspartate and alanine aminotransferase provide a better information of vitamin B_6 status.

Therapeutic Uses

Pyridoxine is used for the treatment of:

- **Seizures.**
- **Down's syndrome,** a state of mental subnormality (incomplete development of mind) due to chromosome defect.
- **Autism,** psychiatric disorder of childhood.
- **Premenstrual tension syndrome** (PMS).

Toxicity

Pyridoxine seems to be safe at levels of 100–150 mg but taking 500–5000 mg per day, has shown peripheral neuropathy within 1–3 years.

Biotin

Biotin was known formerly as vitamin H.

Structure

Biotin is an imidazole derivative **(Figure 7.8).** It consists of a **tetrahydrothiophene** ring bound to an imidazole ring and a **valeric acid** side chain.

Sources

- It is widely distributed in foods.
- Liver, kidneys, vegetables and egg yolk are the important sources of biotin.
- Biotin is also synthesized by intestinal bacteria.

Active Form of Biotin

Enzyme-bound biotin, **biocytin** is an active form of biotin. Biotin is covalently bound to ε-amino group of lysine of an enzyme to form **biocytin.**

Functions

Biotin is a coenzyme of carboxylase reactions, where it is a carrier of CO_2. Some of the carboxylation reactions requiring biotin are given below.

- Conversion of acetyl-CoA into malonyl-CoA catalyzed by **acetyl-CoA carboxylase** in fatty acid synthesis **(Figure 13.17).**
- Conversion of pyruvate into oxaloacetate, catalyzed by **pyruvate carboxylase** in gluconeogenesis **(Figure 12.18).**
- Conversion of propionyl-CoA to D-methyl malonyl-CoA catalyzed by ***propionyl-CoA carboxylase*** in the pathway of conversion of propionate to succinate **(Figure 13.8).**
- It is also involved in the catabolism of branched chain amino acid catalyzed by **β-methyl-crotonyl-CoA carboxylase (Figure 14.32).**

Biotin Independent Carboxylation Reaction

There are few carboxylation reactions which do not require biotin. For example:

- Formation of carbamoyl phosphate by **carbamoyl phosphate synthetase** in urea cycle.
- Addition of CO_2 to form C_6 in purine ring.
- Conversion of pyruvate to malate by malic enzyme.

Nutritional Requirements

A daily intake of about 150–300 µg is recommended for adults. Biotin is synthesized by intestinal micro-organisms in such a large quantities that a dietary source is probably not necessary.

Deficiency Manifestation

- Since biotin is widely distributed in plant and animal foods and intestinal bacterial flora supply adequate amounts of biotin, the natural deficiency of biotin is not well characterized in humans.
- The experimentally induced symptoms of biotin deficiency are nausea, anorexia, glossitis, dermatitis, alopecia (loss of hair), depression and muscular pain.
- Deficiency of biotin occurs in:
  - The people with the unusual dietary habit of consuming large amounts of uncooked eggs. Egg white contains the glycoprotein ***avidin***, which binds the imidazole group of biotin and prevents biotin absorption.
  - Use of antibiotics, that inhibit the growth of intestinal bacteria, eliminates this source of biotin and leads to deficiency of biotin.

Folic Acid

Structure

Folic acid consists of three components, **pteridine ring, p-amino benzoic acid (PABA)** and **L-glutamic acid (Figure 7.9).** In a folic acid molecule, the number of glutamic acid residues varies from one to seven. Folic acid usually has one glutamic acid residue.

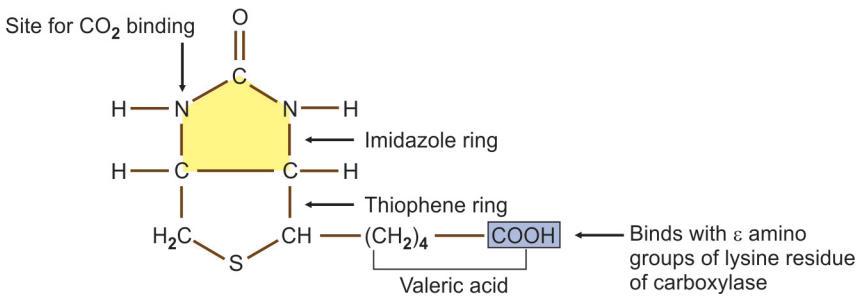


Figure 7.8: Structure of biotin

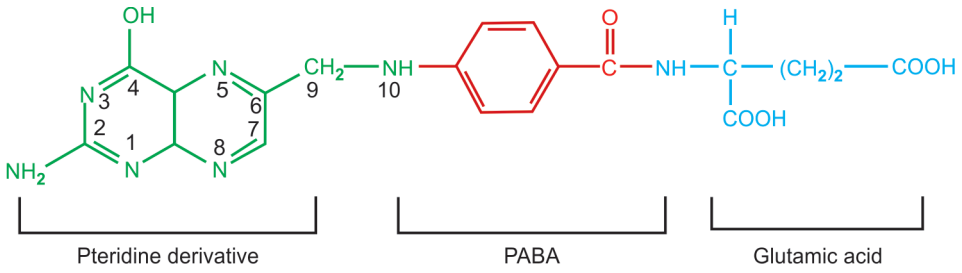


Figure 7.9: The structure of folic acid

Active Form of Folic Acid

Tetrahydrofolate (THF) is the active form of folic acid. Folate is enzymatically reduced in a two-stage process in tissues to yield the dihydro and then tetrahydrofolate, which requires vitamin C **(Figure 7.10).**

Source

Folic acid is found in green leafy vegetables, liver, yeast. The word folate is related to folium which means *leaf* in Latin.

Functions

- THF acts as a carrier of one carbon units. The one carbon units are:

Methyl	CH_3
Methylene	CH_2
Methenyl	CH
Formyl	CHO
Formimino	CH=NH

- One carbon unit binds to THF through N^5 or N^{10} or both N^5, N^{10} position. The THF coenzymes serve as acceptors or donors of one carbon units in a variety of reactions involved in amino acid and nucleic acid metabolism. Five of the major reactions in which THF is involved are given below **(Figure 7.11).**
  1. **Conversion of serine to glycine:** The conversion of serine to glycine is accompanied by the formation of N^5,N^{10}- methylene THF.
  2. **Synthesis of thymidylate (pyrimidine nucleotide):** The enzyme ***thymidylate synthase*** that converts deoxyuridylate (dUMP) into thymidylate (TMP) uses N^5, N^{10}-methylene THF as the methyl donor for this reaction. Thus, folate coenzyme plays a central role in the biosynthesis of **nucleic acids.**
  3. **Catabolism of histidine :** Histidine in the course of its catabolism is converted into ***formiminoglutamate (FIGLU).*** This molecule can donate

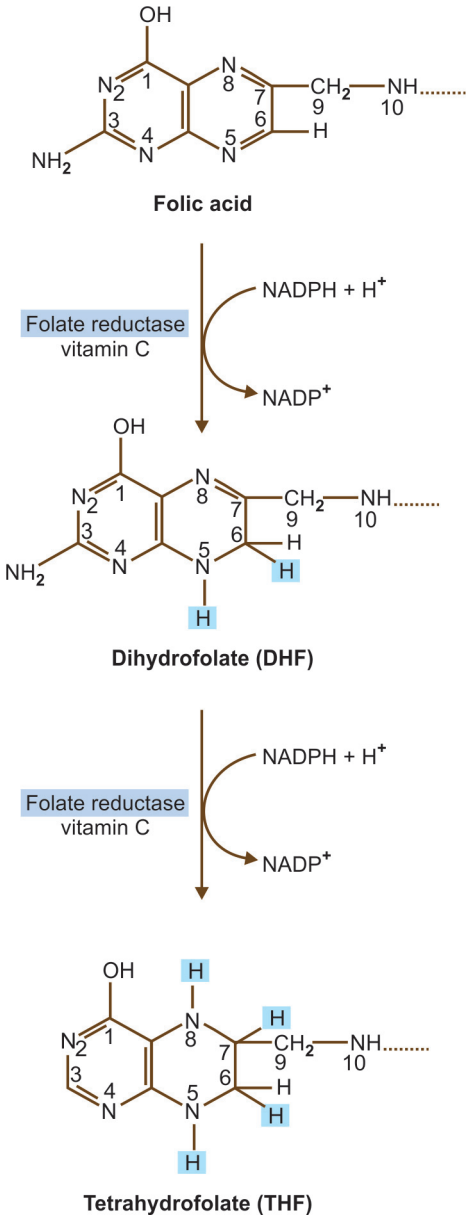


Figure 7.10: Formation of tetrahydrofolate from folic acid

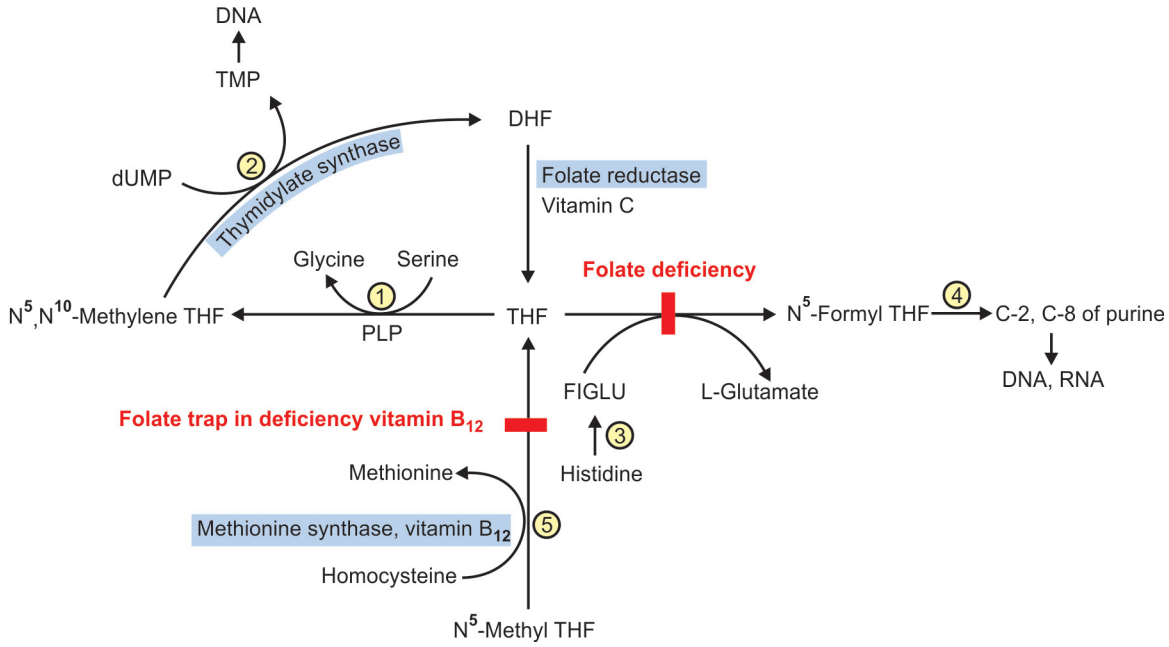


Figure 7.11 : Role of folic acid in one carbon metabolism
DHF: Dihydrofolate; FIGLU: Formiminoglutamate; THF: Tetrahydrofolate; dUMP: Deoxyuridine monophosphate; TMP: Thymidine monophosphate; PLP: Pyridoxal phosphate

the formimino group to THF to produce N-^{5} formimino THF. In case of folic acid deficiency, FIGLU accumulates and is excreted in urine.

4. **Synthesis of purine:** N^5-Formyl THF intermediate formed in histidine catabolism is used in the biosynthesis of purine and therefore in the formation of both DNA and RNA.
5. **Synthesis of methionine from homocysteine:** Homocysteine is converted to methionine in presence of N^5-methyl THF, and vitamin B_{12}.

   In this reaction the methyl group bound to cobalamin (Vitamin B_{12}) is transferred to homocysteine to form methionine and the cobalamin then removes the methyl group from N^5-methyl THF to form THF **(Figure 7.12)**.

   This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In B_{12} deficiency, conversion of N^5-methyl THF to free THF is blocked.

Nutritional Requirements

- The RDA of folate is **200 µg.**
- Requirements increase during pregnancy and lactation.

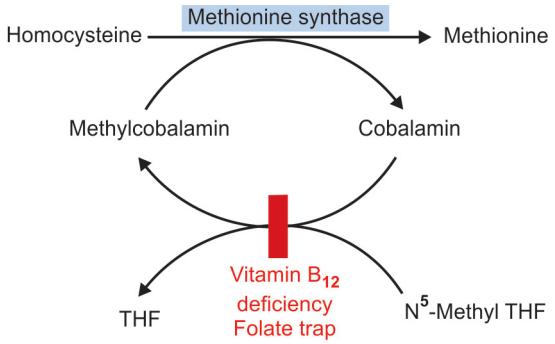


Figure 7.12: The combined roles of vitamin B_{12} and folate in the synthesis of methionine

Deficiency Manifestations

Folate deficiency frequently occurs particularly in pregnant women and in alcoholics. Clinical symptoms of folic acid deficiency include:

- **Megaloblastic or macrocytic anemia:** The deficiency of folic acid leads to impairement of synthesis of DNA. Impaired DNA synthesis, impairs the maturation of erythrocytes. Consequently,

megaloblasts are formed instead of normoblast. These megaloblasts are accumulated in the bone marrow and leads to megaloblastic anemia.

- **Accumulation and excretion of FIGLU in the urine:** Folate deficiency blocks the last step of histidine catabolism, due to lack of THF. This results in accumulation of FIGLU in body, which leads to increased excretion of FIGLU in urine **(Figure 7.13)**.

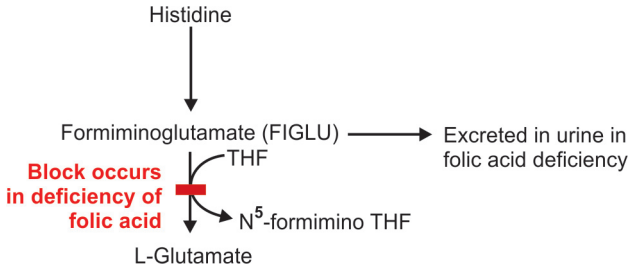


Figure 7.13: Excretion of FIGLU in folic acid deficiency

- **Hyperhomocysteinemia:** Due to folic acid deficiency the methylation of homocysteine to methionine is impaired which leads to hyperhomocysteinemia. Increased level of homocystein is a risk factor for cardiovascular disease.
- **Neural tube defect in fetus:** Since, folate is required for the formation of neural tube in early stage of gestation, the folate deficiency during early stage of pregnancy increases the risk of neural tube defect.

Therapeutic Uses

N^5-methyl THF called **folinic acid** or **citrovorum factor** is used as therapeutic drug to overcome the folate deficiency.

Cobalamin (Vitamin B_{12})

Structure

Vitamin B_{12} bears a complex ***corrin ring*** (containing pyrrols similar to porphyrin), linked to a ***cobalt atom*** held in the center of the corrin ring, by four coordination bonds with the nitrogen of the pyrrole groups. The remaining coordination bonds of the cobalt are linked with the nitrogen of ***dimethylbenzimidazole nucleotide*** and sixth bond is linked to either ***methyl*** or ***5′-deoxyadenosyl*** or ***hydroxy*** group to form ***methyl-cobalamin, adenosylcobalamin*** or ***hydroxycobalamin*** respectively **(Figure 7.14).**

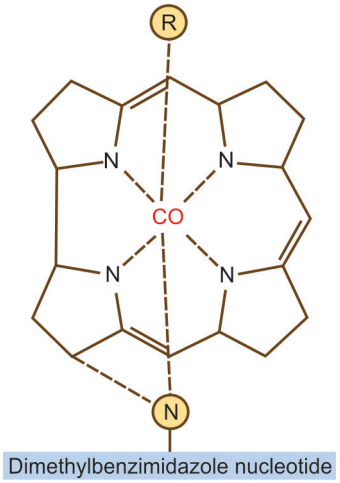


Figure 7.14: Structure of cobalamin (vitamin B_{12})
R: Either methyl or deoxyadenosyl or hydroxy group

Thus, cobalamin exists in three forms that differ in the nature of the chemical group attached to cobalt. ***Cynocobalamin is the commercial available form of vitamin B_{12}.***

Active form of Vitamin B_{12}

The active coenzyme forms of vitamin B_{12} are:

- Methylcobalamin
- Deoxyadenosylcobalamin.

Sources

- ***Dietary sources of vitamin B_{12} are of animal origin*** and include meat, eggs, milk, dairy products, fish, poultry, etc.
- ***Vitamin B_{12} is absent in plant foods.***
- Humans obtain small amounts of vitamin B_{12} from their intestinal flora.

Absorption, Transport and Storage

- The intestinal absorption of vitamin B_{12} requires an **intrinsic factor (IF),** a glycoprotein secreted by parietal cells of the stomach.
- In stomach IF binds the dietary vitamin B_{12} to form **vitamin B_{12}-IF complex**. This complex binds to specific receptors on the surface of the mucosal cells of the ileum.
- After binding to the receptor, the bound vitamin B_{12} is released from the complex and enters the illeal mucosal cells through a Ca^{2+} dependent process.
- The vitamin in mucosal cell is converted into its main plasma transport form to **methylcobalamin**. It is then transported by a vitamin B_{12} binding protein known as **transcobalamin (TC-I and TC-II)**.

- Methylcobalamin which is in excess is taken up by the liver, **stored in deoxyadenosyl B_{12}** form.
- Liver can store about 4-5 mg of vitamin B_{12} in adults, an amount sufficient to meet the body requirements of vitamin B_{12} for 3–6 years.
- Vitamin B_{12} is the only water soluble vitamin that is stored in significant amounts in the liver.

Functions

There are only two human enzyme systems that are known to require vitamin B_{12} coenzyme.

1. **Isomerization of methylmalonyl-CoA to succinyl-CoA**
   - Propionyl-CoA-is produced as catabolic end product of some aliphatic amino acids and in β-oxidation of odd chain fatty acids. The propionyl-CoA is then converted to succinyl-CoA.
   - During conversion of propionyl-CoA to succinyl-CoA vitamin B_{12} coenzyme, deoxyadenosyl cobalamine is required for the isomerization of L-methylmalonyl- CoA to succinyl-CoA.
   - In vitamin B_{12} deficiency methylmalonyl CoA accumulates and is excreted in urine as methyl-malonic acid **(Figure 7.15).**
2. **Conversion of homocysteine to methionine**
   - Methylcobalamin is a coenzyme in the conversion of homocysteine to methionine, which joins the metabolic roles of vitamin B_{12} and those of folic acid (for explanation see functions of folic acid and **Figure 7.12**). This is the only mammalian reaction known to require both vitamins.

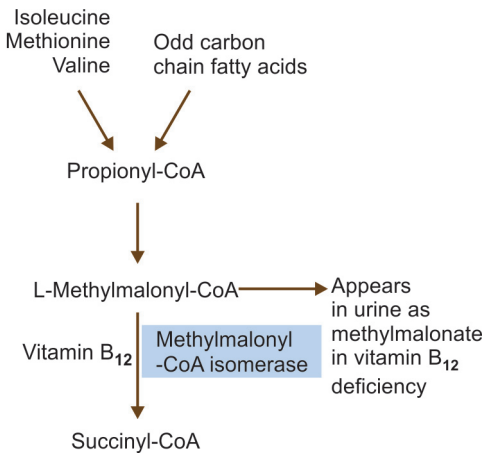


Figure 7.15: Role of vitamin B_{12} in the metabolism of propionyl-CoA

Nutritional Requirements

RDA for adult is **3 µg** with higher allowances for pregnancy and lactating women.

Deficiency Manifestations

Deficiency may arise due to decreased absorption or decreased dietary intake. Dietary deficiency is seen in strict vegetarians, since the vitamin found only in foods of animal origin or in microorganisms. Deficiency of vitamin B_{12} leads to:

- Pernicious anemia
- Megaloblastic anemia
- Methylmalonic aciduria
- Neuropathy.

1. **Pernicious anemia:** It is caused by a deficiency of **intrinsic factor** in the stomach, which leads to impaired absorption of vitamin B_{12}. It is characterized by megaloblastic anemia and **low hemoglobin** level with **neurological** disorders.
2. **Megaloblastic anemia:** It occurs due to **functional folate deficiency.** The functional folate deficiency is seen in vitamin B_{12} deficiency due to folate trap **(Figure 7.12).**
3. **Methylmalonic aciduria:** Because vitamin B_{12} is necessary for the conversion of methylmalonic acid to succinic acid, individuals deficient in vitamin B_{12} excrete excess amounts of methylmalonic acid in the urine **(Figure 7.15).**
4. **Neuropathy:** In vitamin B_{12} deficiency, many neurological symptoms appear due to progressive degeneration of myelinated nerves. Degeneration of myelinated nerves is due to accumulation of L-methylmalonyl-CoA, which impaires the myelin sheath formation. The neurological symptoms include numbness and tingling of fingers and toes, mental confusion, poor muscular coordination and dementia.

FOLATE TRAP

- Methylation of homocysteine to methionine depends on vitamine B_{12} and N^5-methyl THF. When vitamine B_{12} is deficient N^5-methyl THF cannot be converted to free THF. ***Thus, most of folic acid of the body is irreversibly "trapped" as its methyl derivative (N^5-methyl THF). This is called folate trap*** **(Figure 7.12)**.

Folate trap creates folate deficiency and an adequate supply of free THF is not available for the synthesis of purine and pyrimidine bases. Thus, a B_{12} deficiency can lead to a folate deficiency. Although the tissue folate levels are adequate or high, there is a functional folate deficiency due to the lack of THF.

Vitamin C (Ascorbic Acid)

Structure

Vitamin C is also known as ***ascorbic acid***. It is a six-carbon sugar derivative **(Figure 7.16).** Most animals can synthesize ascorbic acid. But humans cannot synthesize ascorbic acid, due to lack of the enzyme ***gluconolactone oxidase*** which is required for the synthesis of ascorbic acid. Thus, humans have a dietary requirement of ascorbic acid.

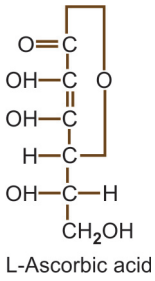


Figure 7.16: Structure of ascorbic acid

Active Form of Ascorbic Acid

Ascorbic acid itself is an active form.

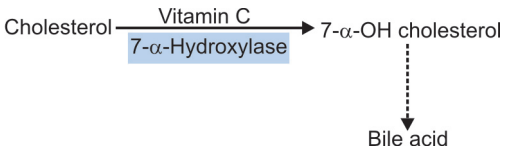
Sources

- The main dietary sources of vitamin C are leafy vegetables and fruits, especially citrus fruits, strawberries, tomatoes, spinach and potatoes.
- Cereals contain no vitamin C.
- Animal tissues and dairy products are very poor sources.

Functions

Ascorbic acid functions as a reducing agent in many metabolic processes as follows:

- **Collagen biosynthesis:** Vitamin C is required for formation of collagen, where it is needed for the hydroxylation of ***proline*** and ***lysine*** residues, of protocollagen. Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and collagen strength and stability. Since vitamin C is required for normal collagen formation, vitamin C is also involved in bone and dentin formation as well as wound healing process.
- **Steroid synthesis:** In adrenal cortex, vitamin C is involved in the hydroxylation reactions of steroids.
- **Adrenaline synthesis:** In adrenal medulla it serves as a reducing agent in hydroxylation reactions in the synthesis of adrenaline and noradrenaline from tyrosine.
- **Carnitine synthesis:** Vitamin C functions in the hydroxylation of γ-butyrobetaine to carnitine.
- **Bile acid formation:** Vitamin C is required for the hydroxylation of cholesterol in bile acid synthesis.



- **Degradation of tyrosine:** The oxidation of P-hydroxyphenylpyruvate to homogentisate requires vitamin C. The subsequent step is catalyzed by homogentisate oxidase, which is a ferrous ion containing enzyme that also requires vitamin C.
- **Folate metabolism:** Folic acid is converted to its active form tetrahydrofolate (THF) with the help of vitamin C **(See Figure 7.10).**
- **Absorption of iron:** Ascorbic acid facilitates the absorption of iron from intestine by reducing it to the Fe^{++} (ferrous) state.
- ***Ascorbic acid is a water soluble antioxidant*:** (Ascorbic acid is a strong reducing agent and acts as an antioxidant).
  - It reduces oxidized vitamin E (tocopherol) to regenerate functional vitamin E.
  - Vitamin C, thought to be involved in the prevention of atherosclerosis and coronary heart disease by preventing oxidation of LDL.
  - Antioxidant property of vitamin C is also associated with prevention of cancer by inhibiting nitrosamine formation from naturally occurring nitrates during digestion.

Nutritional Requirements

The recommended daily allowance is about **60–70 mg.** Additional intakes are recommended for women during pregnancy and lactation.

Deficiency Manifestation

Deficiency of ascorbic acid causes ***scurvy.*** Symptoms of scurvy are related to ***deficient collagen formation*** (Refer functions of vitamin C). These include:

- Spongy, swollen, bleeding gums, loosening of teeth
- Abnormal bone development and osteoporosis
- Poor wound healing
- Anemia due to impaired erythropoiesis
- Easy bruising and bleeding due to fragile capillaries.

Therapeutic Uses

Use of vitamin C in preventing cold and cancers has not been scientifically supported. Although the incidence of common cold is not reduced by vitamin C, the duration of cold episodes and severity of symptoms can be decreased.

Toxicity

Vitamin C can be taken in doses up to 2–3 g/day without undesirable effects. Above these levels, however, it cannot be absorbed from the intestine and can cause severe diarrhea and deposition of oxalate stones in kidneys.

FAT SOLUBLE VITAMINS

Vitamin A

Structure

Vitamin A contains a single 6-membered ring to which is attached an 11-carbon side chain **(Figure 7.17).** Vitamin A is an ***alcohol (retinol),*** but can be converted into an ***aldehyde (retinal),*** or an ***acid (retinoic acid).***

Active Form

Vitamin A consists of three biologically active molecules which are collectively known as **retinoids.**

1. Retinol: Primary alcohol (CH_2OH) containing form
2. Retinal: Aldehyde (CHO) containing form
3. Retinoic acid: Carboxyl (COOH) containing form

- Each of these compounds are derived from the plant precursor molecule, **β-*carotene*** (a member of a family of molecules known as carotenoids).

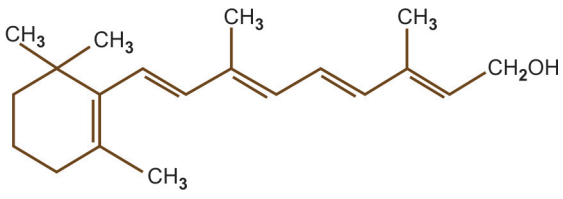


Figure 7.17: Structure of vitamin A, retinol

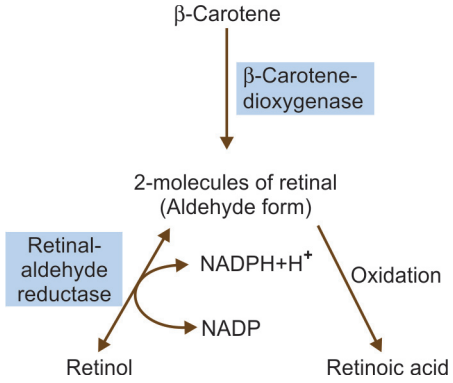


Figure 7.18: Conversion of β-carotene (provitamin) to biologically active forms of vitamin A

- β-carotene which consists of two molecules of retinal linked at their aldehyde ends is also referred to as the ***provitamin*** form of vitamin A.
- The retinol and retinal are interconverted by enzyme **retinal aldehyde reductase.** The retinoic acid is formed by oxidation of retinal. The retinoic acid connot be reduced to either retinol or retinal **(Figure 7.18).**

Absorption, Transport and Storage

- Ingested β-carotene is cleaved in the intestine by **β-carotene** ***dioxygenase*** to yield retinal. Retinal is reduced to retinol by ***retinaldehyde reductase,*** an NADPH requiring enzyme within the intestine **(Figure 7.18).**
- Retinol is esterified with palmitic acid incorporated into chylomicrons together with dietary lipid and delivered to the liver for storage.
- Transport of retinol from the liver to extrahepatic tissues, occurs by binding of retinol to ***retinol binding protein (RBP).***
- Transport of retinoic acid is accomplished by binding to albumin.

Sources

- The richest dietary sources of vitamin A are fish liver oils (cod liver oil). Animal livers are also rich sources but meat is rather low in vitamin A.
- Other good sources are milk and dairy products, dark-green leaves, such as spinach and yellow and red fruits and vegetables, such as carrots, tomatoes, and peaches.

Functions of Vitamin A

- Vitamin A is required for a variety of functions such as vision, cell differentiation and growth, mucus secretion, maintenance of epithelial cells, etc.
- The role of vitamin A in vision has been known through the studies of **G *Wald,*** who received the Nobel prize in 1943 for this work.
- Different forms of the vitamin have different functions.
  - Retinal and retinol are involved in vision.
  - Retinoic acid is involved in cellular differentiation and metabolic processes.
  - β-carotene is involved in antioxidant function.

Role of Vitamin A in Vision

The cyclic events occur in the process of vision, known as ***rhodopsin cycle*** or ***Wald's visual cycle*** **(Figure 7.19).** Both rod and cone cells of retina contain a photoreceptor pigment in their membrane and vitamin A is a component of these pigments. ***Rhodopsin*** or ***visual purple,*** the visual pigment of rod cells in the retina consists of ***11-cis-retinal*** bound to protein ***opsin.***

- When rhodopsin absorbs light, the 11-cis-retinal is converted to all-trans retinal.
- The isomerization is associated with a conformational change in the protein opsin.
- Conformational changes in opsin generates a nerve impulse that is transmitted by the optic nerve to the brain.
- This is followed by dissociation of the all-trans retinal from opsin.
- The all-trans retinal is immediately isomerized by retinal isomerase to 11-cis-retinal.
- This combines with opsin to regenerate rhodopsin and complete the visual cycle.

The conversion of all-trans retinal to 11-cis-retinal is incomplete and therefore remaining all-trans retinal which is not converted to 11-cis-retinal is converted to all-trans retinol by ***alcohol dehydrogenase*** and is stored in the liver. When needed, retinol re-enters the circulation and is taken up by the retina, where it is converted back to 11-cis-retinal which combines with opsin again to form rhodopsin **(Figure 7.19).**

Dark adaptation time: When a person enters from bright light to dark there is difficulty in seeing due to depletion of rhodopsin, but after few minutes the vision improves. During these few minutes, rhodopsin is resynthesized and vision is improved. The time taken for regeneration of rhodopsin is known as **dark adaptation time.** Dark adaptation time is increased in vitamin A deficient individuals.

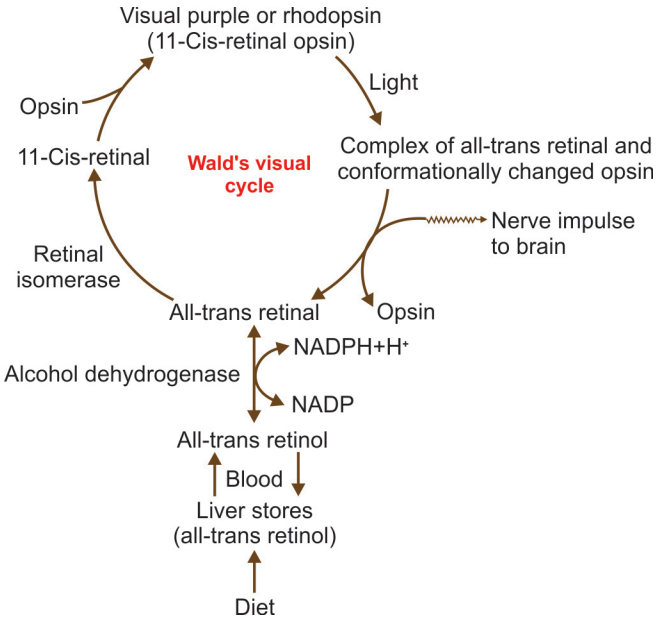


Figure 7.19: Wald's visual cycle

Color Vision

- While vision in dim light is mediated by rhodopsin of the rod cells, color vision is mediated by three different retinal containing pigments in the cone cells, the three pigments are called ***porphyropsin, iodopsin*** and ***cyanopsin*** and are sensitive to the three essential colors: **red, green** and ***blue*** respectively. All these pigments consist of **11-cis-retinal** bound to protein **opsin.**
- Thus, when light strikes the retina, it bleaches one or more of these pigments, depending on the color quality of the light. The pigments are converted to all-trans retinal, and the protein moiety opsin is released as in the case of rhodopsin. This reaction gives rise to the nerve impulse that is read out in the brain as color:
  - Red if porphyropsin is split
  - Green if iodopsin is split
  - Blue if cyanopsin is split.
- If mixtures of the three are converted, the color read out in the brain depends on the proportions of the three split.

Cellular Differentiation and Metabolic Effect

- **Retinoic acid** is an important regulator of **gene expression** especially during growth and development. Retinoic acid is essential for normal gene expression during embryonic development such as

cell differentiation in spermatogenesis and in the differentiation of epithelial cells.
- Retinoic acids exert a number of metabolic effects on tissues. These include:
  - Control of biosynthesis of membrane ***glycoproteins*** and ***glycosaminoglycans*** (mucopolysaccharide) necessary for mucus secretion. The normal mucus secretion maintains the epithelial surface moist and prevents keratinization of epithelial cell.
  - Control of biosynthesis of ***cholesterol.***

Antioxidant Function

- β-carotene is an antioxidant and may play a role in trapping peroxy free radicals in tissues.
- The antioxidant property of lipid soluble vitamin A may account for its possible anticancer activity.
- High levels of dietary carotenoids have been associated with a decreased risk of cardiovascular disease.

Nutritional Requirements

The RDA of vitamin A for adults is **800–1000 retinol equivalents.** (1 retinol equivalent = 1 μg retinol = 6 μg β-carotene).

Deficiency Manifestation

Clinically, degenerative changes in eyes and skin are observed with vitamin A deficiency.

Effect on Vision

Night blindness (nyctalopia)
- Night blindness is one of the earliest symptoms of vitamin A deficiency. This is characterized by loss of vision in night (in dim or poor light) since **dark adaptation time** is increased. Prolonged deficiency of vitamin A leads to an irreversible loss of visual cells.
- Severe vitamin A deficiency causes dryness of cornea and conjuctiva, a clinical condition termed as **xerophthalmia** (dry eyes).
- If this situation prolongs, **keratinization** and **ulceration** of cornea takes place.This results in destruction of cornea. The cornea becomes totally opaque resulting in permanent loss of vision (blindness), a clinical condition termed as **keratomalacia.** Xerophthalmia and keratomalacia are commonly observed in children.
- White opaque spots develop on either side of cornea in vitamin A deficiency are known as **Bitot's spot.**

Effect on Skin and Epithelial Cells

- Vitamin A deficiency causes keratinization of epithelial cells of skin which leads to keratosis of hair follicles, and dry, rough and scaly skin.
- Keratinization of epithelial cells of respiratory, urinary tract makes them susceptible to infections.

Other Symptoms of Vitamin A Deficiency

- Failure of growth in children.
- Faulty bone modelling producing thick cancellous (spongy) bones instead of thinner and more compact ones.
- Abnormalities of reproduction, including degeneration of the testes, abortion or the production of malformed offspring.

Therapeutic Use of Vitamin A

- The use of retinoic acid preparations, in the treatment of psoriasis, acne and several other skin diseases, is related to its involvement with epithelial cell differentiation and integrity
- Some precancerous lesions seem to respond to treatment with carotenoids.

Hypervitaminosis A

- The symptoms of hypervitaminosis A include nausea, vomiting, diarrhea, loss of hair (alopecia), scaly and rough skin, bone and joint pain, enlargement of liver, loss of weight, etc.
- In pregnant women, the hypervitaminosis A may cause congenital malformation in growing fetus (teratogenic effect).
- The excess intake of carotenoids is not toxic like vitamin A.

Why Vitamin A is Considered as a Hormone?

- Within cells both retinol and retinoic acid function by binding to specific receptor proteins present in the nucleus of target tissues.
- Following binding, the receptor-vitamin complex interacts with several genes involved in growth and differentiation and affects expression of these genes. In this capacity, retinol and retinoic acid are considered as hormones.

Vitamin D (Cholecalciferol)

Vitamin D is also known as **calciferol** because of its role in calcium metabolism and **antirachitic factor** because it prevents rickets.

Vitamin D could be thought of as a hormone rather than a vitamin because:
- As it can be synthesized in the body
- It is released in the circulation
- Has distinct target organs
- Action of vitamin D is similar to steroid hormones. It binds to a receptor in the cytosol. Following binding, the receptor vitamin complex interacts with DNA to stimulate the synthesis of calcium binding protein.

Structure

Vitamin D is a steroid compound. There are two forms of vitamin D.

1. The naturally produced D_3 or ***cholecalciferol*** **(Figure 7.20)**, is the form obtained from animal sources in the diet, or made in the skin by the action of ultraviolet light from sunlight on ***7-dehydro-cholesterol*** **(Figure 7.21)**.
2. Artificially produced form D_2 or **ergocalciferol,** is the form made in the laboratory by irradiating the plant sterol, ***ergosterol***.

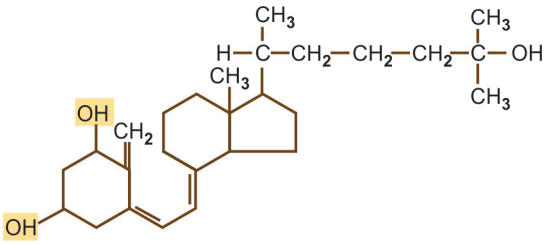


Figure 7.20: Structure of 1,25-dihydroxycholecalciferol: an active form of vitamin D_3

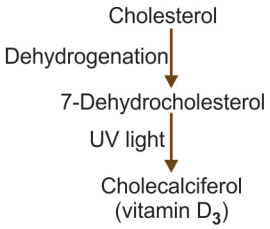


Figure 7.21: The formation of vitamin D_3 in the body

Absorption, Transport and Activation of Vitamin D

Exogenous or dietary vitamin D is absorbed in the duodenum along with lipids. It is transported to the liver through chylomicron.

Active Form of Vitamin

Cholecalciferol is an inactive form of vitamin D. It needs further metabolism to produce the active form of the vitamin. ***1,25 dihydroxycholecalciferol*** also known as **calcitriol** is the active form of vitamin D. The steps involved in activation are:

1. The first step is the conversion of cholecalciferol to 25-hydroxycholecalciferol. The ***25-hydroxylation occurs in liver*** and is catalyzed by **hydroxylase**.
2. The 25-hydroxycholecalciferol formed transported to the kidney, where it is further hydroxylated by, **1,α-hydroxylase enzyme** in the 1-position to 1,25 dihydroxycholecalciferol. **(Figure 7.22).**

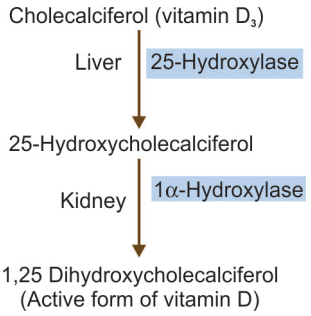


Figure 7.22: Activation of vitamin D

Sources

- Best sources are cod liver oil and often fish oils and sunlight induced synthesis of vitamin D_3 in skin.
- Egg yolk and liver are good sources.

Functions

Vitamin D (Calcitriol) plays an essential role as a hormone in the regulation of **calcium** and **phosphorus** metabolism.

It maintains the normal plasma level of calcium and phosphorus by acting on **intestine**, **kidneys** and **bones** **(Figure 7.23).**

Action of calcitriol on intestine

It increases the plasma calcium and phosphorus concentration by stimulating the absorption of calcium and phosphorus from the intestine by enhancing the synthesis of calcium binding proteins **calbindins.** This protein increases the calcium uptake by the intestine.

Action of calcitriol on kidney

It stimulates the reabsorption of calcium and phosphorus from the kidney and decreases their excretion.

Action of calcitriol on bone

It is believed that calcitriol has both **anabolic** and **catabolic** role on bone.

- Calcitriol promotes the mineralization of bones by deposition of calcium and phosphorus.
- Calcitriol along with PTH stimulates the mobilization of calcium and phosphorus from bone by stimulating the synthesis of **osteocalcin** (a calcium binding protein in bone). This causes elevation of plasma calcium and phosphorus levels.

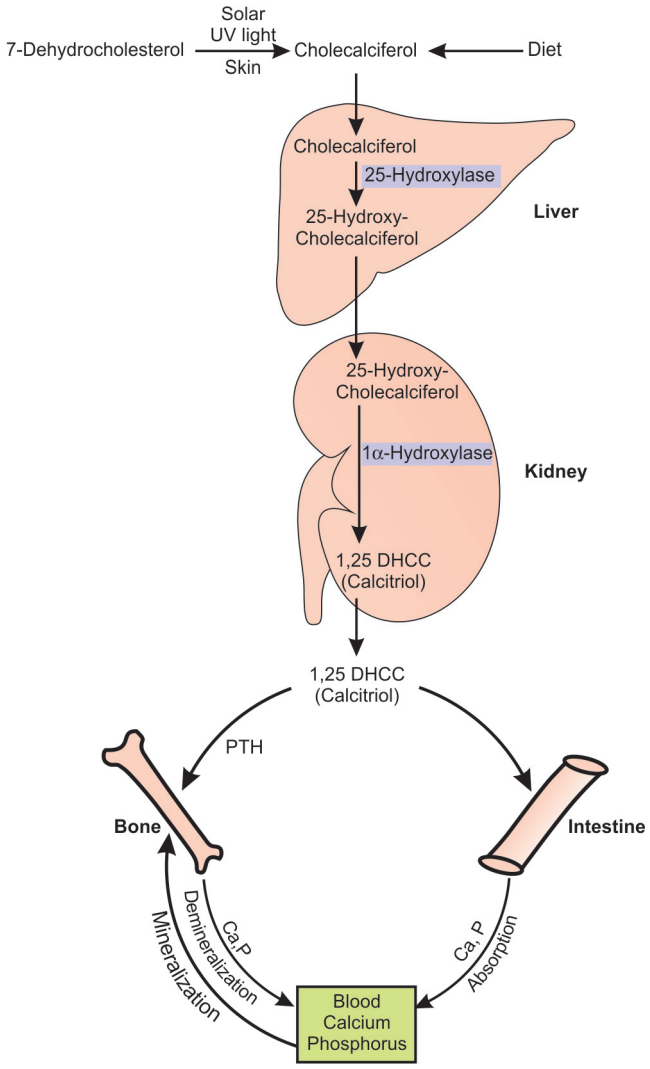


Figure 7.23: Metabolism and functions of Vitamin D where 1,25 DHCC: 1,25 dihydroxycholecalciferol; PTH: Parathyroid hormone

Nutritional Requirement

The daily requirements of vitamin D is **200–400 IU.**

Deficiency Manifestation

Deficiency of vitamin D causes **rickets (rachitis)** in growing children and **osteomalacia** in adults.

Rickets

- Rickets is characterized by formation of soft and pliable bones due to poor mineralization and calcium deficiency. Due to softness, the weight bearing bones are bent and deformed.

Figure 7.24: Bowing of legs in rickets

- The main features of the rickets are, a large head with protruding forehead, pigeon chest, bow legs, (curved legs), knock knees **(Figure 7.24)** and abnormal curvature of the spine (kyphosis).
- Rachitic children are usually anemic or prone to infections. Rickets can be fatal when severe.
- Rickets is characterized by low plasma levels of calcium and phosphorus and high alkaline phosphatase activity.

Osteomalacias (Adult Rickets)

- The deficiency of vitamin D in adults causes **osteomalacia.** This is a condition similar to that of rickets.
- Osteomalacia characterized by demineralization of previously formed bones, Demineralization of bones makes them soft and susceptible to fractures.

Renal Rickets (Renal Osteodystrophy)

In chronic renal failure synthesis of calcitriol in kidney is impaired. As a result, the deficiency of calcitriol occurs which leads to hypocalcemia and hyperphosphatemia. It can be treated by oral or intravenous administration of calcitriol (active form of vitamin D).

Vitamin D Resistant Rickets

As the name implies, this is a disease which does not respond to treatment with vitamin D. There are various possible causes of this condition and all involve a defect in the metabolism or mechanism of action of 1,25 dihydroxycholecalciferol as follows:

- Due to **defective vitamin D receptor**

- Due to a *defective 1, α-hydroxylase activity in kidney*
- Due to liver disease and kidney failure as the production of 25-hydroxycholecalciferol and 1,25 dihydroxycholecalciferol respectively will be inefficient in the damaged tissue.

Hypervitaminosis D

High doses of vitamin D over a long period are toxic.

- The early symptoms of hypervitaminosis D include nausea, vomiting, anorexia, increased thirst, loss of weight, etc.
- Hypercalcemia is seen due to increased bone resorption and intestinal absorption of calcium.
- The prolonged hypercalcemia causes calcification of soft tissues and organs such as kidney and may lead to formation of stones in the kidneys.

Therapeutic Use

Vitamin D analogues have been used in the treatment of psoriasis.

Vitamin E (Tocopherol)

Structure

Vitamin E consists of eight naturally occurring tocopherols, of which α-tocopherol is the most active form **(Figure 7.25)**.

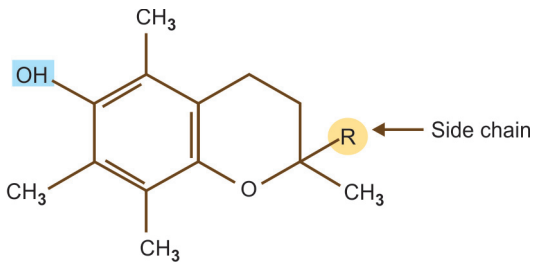


Figure 7.25: Structure of α-tocopherol

Sources

The major dietary sources of vitamin E are fats and oils. The richest sources are germ oil, corn oil, fish oil, eggs, lettuce and alfalfa.

Absorption, Transport and Storage

Vitamin E is absorbed from intestine together with dietary lipid. It is incorporated in chylomicrons. It is delivered to the liver via chylomicron. The liver can export vitamin E into very low density lipoprotein (VLDL) to target cells. In cells, tocopherols are distributed where antioxidant activity is required. ***The major site of vitamin E storage is in the adipose tissue.***

Functions

- Vitamin E acts as a natural antioxidant by scavenging free radicals and molecular oxygen.
- Vitamin E is important for preventing peroxidation of polyunsaturated fatty acids in cell membranes.
- Protection of erythrocyte membrane from oxidant is the major role of vitamin E in humans. It protects the RBCs from hemolysis.
- Vitamin E also helps to prevent oxidation of LDL. Oxidized LDL may be more atherogenic than native LDL and thus vitamin E may protect against athreomatous coronary heart disease.
- Whether vitamin E affects human fertility is unknown.
- In animals, vitamin E is required for normal reproduction and prevents sterility.

Nutritional Requirements

A daily consumption of about:

- 10 mg (15 IU) of α-tocopherol for a man
- 8 mg (12 IU) for a woman is recommended. One mg of α-tocopherol is equal to 1.5 IU.

Deficiency Manifestation

Vitamin E deficiency in humans is rare.

- The major symptom of vitamin E deficiency in human is ***hemolytic anemia*** due to an increased red blood cell fragility.
- Another symptom of vitamin E deficiency is ***retrolental fibroplasia (RLF)*** observed in some premature infants of low birth weight. Children with this defect show ***neuropathy.***

Hypervitaminosis E

Unlike other fat soluble vitamins such as A and D, vitamin E does not seem to have toxic effects.

Vitamin K

This vitamin is called an ***anti-hemorrhagic factor*** as its deficiency produced uncontrolled hemorrhages due to defect in blood coagulation.

In 1929, H Dam gave the name koagulation vitamin from the Danish word ***koagulation.*** It is now called vitamin K.

Structure (Figure 7.26)

- There are two naturally occurring forms of vitamin K:

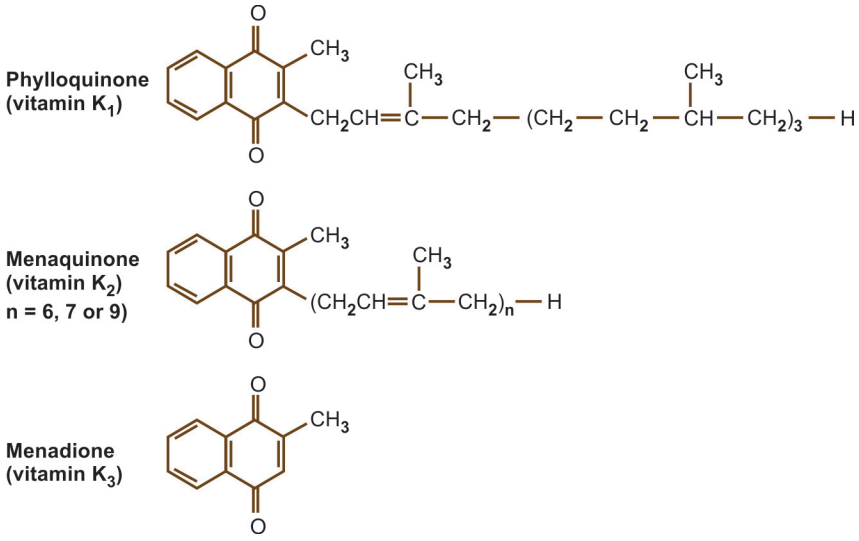


Figure 7.26: Structure of vitamin K

i. **Vitamin K_1** or **phylloquinone** derived from plant.
ii. **Vitamin K_2** or **menaquinones,** produced by microorganisms.

Both these natural types have the same general activity.

- **Vitamin K_3 or menadione** is a synthetic product, which is an alkylated form of vitamin K_2.

Sources

- Excellent sources are cabbage, cauliflower, spinach and other green vegetables.
- Good sources include tomatoes, cheese, dairy products, meat, egg yolk, etc.
- The vitamin is also synthesized by microorganisms in the intestinal tract.

Absorption, Transport and Storage

The naturally occurring vitamin K derivatives are absorbed only in the **presence *of bile salts,*** like other lipids. It is transported to the liver in the form of chylomicrons, where it is stored.

Menadione (synthetic vitamin K), being water soluble, is absorbed even in the absence of bile salt, passing directly into the hepatic portal vein.

Functions of Vitamin K

- Vitamin K plays an important role in **blood coagulation.** Vitamin K is required for the activation of blood clotting factors, prothrombin (II), factor VII, IX and X. These blood clotting proteins are synthesized in liver in inactive form, and are converted to active form by **vitamin K dependent carboxylation** reaction. In this, vitamin K dependent carboxylase enzyme adds the extra carboxy group at χ-carbon of glutamic acid residues of inactive blood clotting factors.
- Vitamin K is also required for the carboxylation of glutamic acid residues of **osteocalcin,** a Ca^{2+} binding protein present in bone.

Anticoagulants, **dicumarol** and **warfarin** are structurally similar to vitamin K and inhibit the action of vitamin K.

Nutritional Requirements

The suggested intake for adults is **70–140 µg/day**.

Deficiency Manifestation

- Vitamin K deficiency is associated with **hemorrhagic disease.** In vitamin K deficiency, clotting time of blood is increased. Uncontrolled hemorrhages occur on minor injuries as a result of reduction in prothrombin and other clotting factors.
- Vitamin K is widely distributed in nature and its production by the intestinal microflora ensures that dietary deficiency does not occur. Vitamin K deficiency, however, is found in:
  - **Patients with liver disease** and ***biliary obstruction.*** Biliary obstruction inhibits the entry of bile salts to the intestine.
  - **In newborn infants,** because the placenta does not pass the vitamin to the fetus efficiently, and the gut is sterile immediately after birth.

- **Following antibiotic therapy** that sterilizes the gut.
- **In fat malabsorption,** that impairs absorption of vitamin K.

Hypervitaminosis K

Excessive doses of vitamin K produce a **hemolytic anemia** (due to increased breakdown of RBCs) and **jaundice** (in infants).

Therapeutic Use

An important therapeutic use of vitamin K is an antidote (drug that counteracts the effects of a poison) to poisoning by dicumarol type drugs.

SUMMARY

- Vitamins are organic nutrients, essential for growth and development. They must be taken in the diet, because the body either cannot synthesize them at all or not in sufficient amounts for its needs.
- Most of the water soluble vitamins function as coenzymes.
- Thiamine (vitamin B_1) is a component of thiamine pyrophosphate, a coenzyme in oxidative decarboxylation of keto acid and of an enzyme of the pentose phosphate pathway, transketolase.
- Riboflavin and niacin are each important coenzymes in oxidation reduction reactions. Riboflavin is a component of FMN and FAD, whereas niacin is present in NAD and NADP.
- Pantothenic acid is present in coenzyme-A and acyl carrier protein which acts as a carrier for acyl groups in many reactions.
- Pyridoxine is an essential precursor of pyridoxal phosphate and is the coenzyme for several enzymes of amino acid metabolism including the trans aminases.
- Biotin is the coenzyme for several carboxylase enzymes.
- Folic acid in the form of tetrahydrofolate and vitamin B_{12}, takes part in providing one carbon residues for nucleic acid synthesis.
- Ascorbic acid is a water soluble antioxidant.
- In vegetables, vitamin A exists as provitamin, β-carotene, vitamin A is required in vision, cell differentiation, growth, mucus secretion, etc.
- Vitamin D is steroid prohormone, whose activity is carried out by its active form 1,25-dihydroxycholecalciferol (calcitriol). It is involved in the regulation of calcium and phosphate metabolism,
- Vitamin E (tocopherol) is the most important antioxidant in the body. It protects against the effect of toxic free radicals.
- Vitamin K is needed for the synthesis of several blood clotting factors, (e.g. II, VIII, IX and X). It functions as a cofactor to a carboxylase enzyme.
- In contrast to water soluble vitamins only one of the fat soluble vitamins (vitamin K) has a coenzyme function.

EXERCISE

Solve

Case History 1

A 15-year-old male has polished rice as a major component of his diet. He is hospitalized with symptoms of poor appetite, peripheral neuropathy and muscular weakness.

Questions

a. Name the probable disorder and its different types.
b. Which factor is deficient in the diet?
c. Give the active form of the deficient factor.
d. Give any reaction where this factor is required.

Case History 2

A male infant, 6 months of age, was admitted to the hospital in a coma. Blood investigation indicated that the child was anemic and that his vitamin B_{12} level was very low, A urine sample contained increased amount of methylmalonate and homocysteine.

Questions

a. Why was the infant anemic?
b. What are the sources of vitamin B_{12} in the diet?
c. Explain the high level of methylmalonate and homocysteine in the infant's urine.
d. Give active form of vitamin B_{12}.

Case History 3

A 42-year-old woman with a chronic inflammatory bowel disease was on intravenous feeding containing fat free and carbohydrate rich diet. After 3 months she began to complain of being unable to see appropriately in dim light.

Questions

a. Name the probable disorder.
b. Which factor is deficient in the diet?
c. What is the daily requirement of this factor?
d. Name the rich sources of this factor.

Case History 4

A 6-year-old girl is hospitalized with symptoms of digestive disorders, dermatitis, depression and dementia.

Questions

a. Name the disorder.
b. Disorder is due to deficiency of which biomolecule?
c. Give active form of this biomolecule.
d. Give any reaction requiring the active form of this biomolecule.

Case History 5

A ten-year-old boy presented with spongy bleeding gums with loose teeth.

Questions

a. What is the disease he is suffering from?
b. What is the cause?
c. What is the biochemical basis of the disease?
d. Give RDA for the concerned biomolecule.

Case History 6

A small 3-year-old child was brought with bow legs, protruding forehead, pigeon chest, depressed ribs and kyphosis.

Questions

a. Name the disease.
b. Which biomolecule is deficient?
c. What are the functions of the concerned biomolecule?
d. RDA of the concerned biomolecule.

Multiple Choice Questions (MCQs)

1. Which of the following coenzymes is not derived from vitamins?
a) CoASH
b) TPP
c) Pyrodoxal phosphate (PLP)
d) Coenzyme Q

2. A deficiency of vitamin B_{12} causes:
a) Scurvy b) Rickets
c) Pernicious anemia d) Beriberi

3. Rickets is due to deficiency of:
a) Vitamin D b) Vitamin A
c) Vitamin C d) Vitamin B_1

4. Which of the following vitamins would most likely become deficient in a person who developed a completely vegetarian lifestyle?
a) Vitamin C b) Niacin
c) Cobalamin d) Vitamin E

5. Pyridoxal phosphate is a coenzyme for the reactions, *except:*
a) Transamination
b) Deamination
c) Decarboxylation
d) Oxidation-reduction

6. Both folic acid and methylcobalamin are required in:
a) Phosphorylation
b) Deamination
c) Methylation of homocysteine to methionine
d) Conversion of pyruvate to acetyl-CoA

7. Beriberi is caused by a deficiency of:
a) Thiamine b) Thymine
c) Threonine d) Tyrosine

8. Precursor of CoA is:
a) Folic acid b) Thiamine
c) Riboflavin d) Pantothenic acid

9. Biotin is involved in:
a) Oxidation-reduction
b) Carboxylation
c) Decarboxylation
d) Dehydration

10. Antihemorrhagic vitamin is:
a) Vitamin A
b) Vitamin E
c) Vitamin K
d) Vitamin D

11. Both Wernicke's disease and beriberi can be treated by administering vitamin:
a) Thiamine b) Niacin
c) Riboflavin d) Ascorbic acid

12. Pellagra occurs due to deficiency of:
a) Biotin b) Niacin
c) Pantothenic acid d) Folic acid

13. All of the following vitamins have antioxidant property, *except*:
a) β-carotene
b) Ascorbic acid
c) Tocopherol
d) Cholecalciferol

14. Increased prothrombin time is observed in the deficiency of:
a) Vitamin K b) Vitamin B
c) Vitamin A d) Vitamin B_{12}

15. Thiamine pyrophosphate is required for the following enzymatic activity:
a) Hexokinase
b) Transketolase

c) Transaldolase
d) Glucose-6-phosphatase

16. Excretion of FIGLU in urine occurs in deficiency of:
a) Thiamin b) Folic acid
c) Ascorbic acid d) Nicotinic acid

17. Which of the following vitamin is required for collagen synthesis?
a) Ascorbic acid b) Nicotinic acid
c) Pantothenic acid d) Folic acid

18. Functionally active form of vitamin D is:
a) 1,25-dihydroxycholecalciferol
b) 24,25-dihydroxycholecalciferol
c) 25-hydroxycholecalciferol
d) 1,24-dihydroxycholecalciferol

19. Which of the following enzyme is used as a measure of thiamin deficiency?
a) Pyruvate dehydrogenase
b) Erythrocyte transketolase
c) α-ketoglutarate dehydrogenase
d) Decarboxylase

20. Which of the following is used as an assay of riboflavin status?
a) Transketolase
b) Glutathione reductase
c) FIGLU
d) Pyruvate dehydrogenase

Correct Answers for MCQs

1-d	2-c	3-a	4-c
5-d	6-c	7-a	8-d
9-b	10-c	11-a	12-b
13-d	14-a	15-b	16-b
17-a	18-a	19-b	20-b

CHAPTER

8

Chemistry of Hemoglobin

INTRODUCTION

Hemoglobin are conjugated proteins, with a prosthetic group **heme.** Hemoglobin found in red blood cell, carries oxygen from lungs to the tissues and carbon dioxide from tissues to the lungs. The red color of blood is due to the hemoglobin content of the erythrocytes.

STRUCTURE AND FUNCTION OF HEMOGLOBIN

Hemoglobin is a globular, oligomeric protein made up of ***heme*** and ***globin.***

Heme + globin → Hemoglobin

Heme

- The heme is iron containing compound belonging to the class of compounds called ***protoporphyrin.***
- Protoporphyrin is composed of four **pyrrole rings** which are linked by methene (=CH) bridge to form **porphyrin ring (Figure 8.1).**
- Four **methyl,** two **vinyl** and two **propionate** side chain groups are attached to the porphyrin ring. These substituents can be arranged in 15 different ways. But only one of these isomer called ***protoporphyrin IX*** is biologically active.
- The iron (Fe^{2+}) is held in the center of the proto-porphyrin molecule by coordination bonds with the four nitrogen of the protoporphyrin ring **(Figure 8.1)**

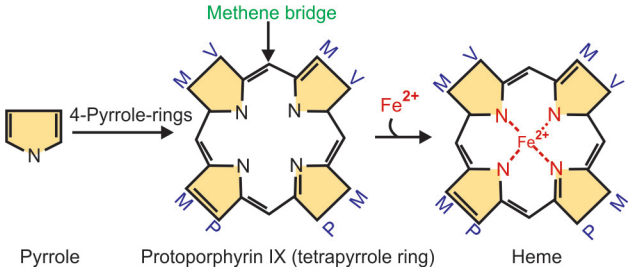


Figure 8.1: Structure of heme. Side chains of the pyrrole rings are designated, M: methyl, V: Vinyl, P: propionic acid

- The iron atom has six coordination bonds.
  - Four bonds are formed between the **iron** and **nitrogen** atoms of the porphyrin ring system.
  - Fifth bond is formed between nitrogen atom of histidine residue of the globin polypeptide chain, known as ***proximal histidine (F-8)***.
  - The sixth bond is formed with **oxygen.**
- The oxygenated form of hemoglobin is stabilized by the H-bond between oxygen and side chain of another histidine residue of the globin chain, known as ***distal histidine (F-7)*** **(Figure 8.2)**

Globin

- Globin molecule contains four polypeptide chains, ***two alpha (α)*** chains (141 amino acid residues each) and ***two beta (β)*** or ***two gamma (γ)*** or ***two delta (δ)*** as per the type of hemoglobin **(Table 8.1)**. The

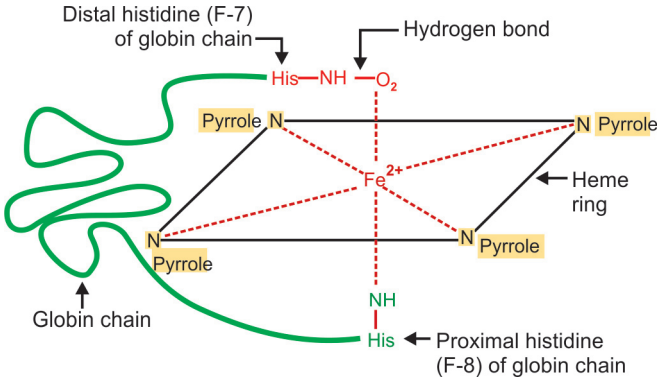


Figure 8.2: The coordination bonds of iron

Table 8.1 : Normal Human Hemoglobins

Type	*Composition*
HbA_1 (adult Hb)	$\alpha_2\beta_2$
HbA_2 (minor adult Hb)	$\alpha_2\delta_2$
HbF (Fetal Hb)	$\alpha_2\gamma_2$
HbA_{1c} (Glycosylated Hb)	$\alpha_2\beta_2$-glucose

β, γ, and δ chains have 146 amino acid residuces each.

- With each polypeptide chain, one molecule of heme is attached. A hemoglobin molecule, therefore has four heme molecules.
- Four globin polypeptide chains with four heme are held together in a definite arrangement or conformation to constitute a characteristic quaternary structure of hemoglobin, which is stabilized by:
  - Hydrogen bonds
  - Salt bridges
  - Van der Waals forces
- There is little contact between the two alpha or two beta chains. But there are many contact points between alpha and beta chains of dissimilar chain pairs $\alpha_1\beta_1$ and $\alpha_2\beta_2$.
- There is a central open channel or cavity in hemoglobin molecule **(Figure 8.3).**

Function of Globin

The function of globin chain of hemoglobin is to form a protective hydrophobic pocket for binding of heme **(Figure 8.3)** These pockets protect the reduced form of iron (Fe^{2+}) of heme from oxidizing to the ferric (Fe^{3+}) form from the aqueous environment and permits binding of oxygen with Fe^{2+} ion of heme. Exposure of

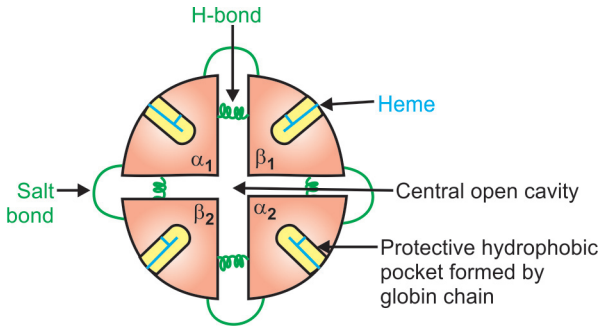


Figure 8.3: Schematic representation of quaternary structure of hemoglobin

heme iron to water results in oxidation of Fe^{2+} to Fe^{3+} form and loss of oxygen binding capacity.

Functions of Hemoglobin

- Transport of O_2 from lungs to tissues
- Transport of CO_2 and H^+ from tissues to lungs and kidney
- Acts as an intracellular buffer and is thus involved in acid-base balance.

BINDING SITES FOR OXYGEN, HYDROGEN (H^+) AND CARBON DIOXIDE (CO_2) WITH HEMOGLOBIN

- **Oxygen** is bound to the ferrous (Fe^{2+}) atom of the heme **(Figure 8.2)** to form oxyhemoglobin.
- **Hydrogen** is bound to R-groups (side chain) of histidine residues of α and β chains.
- **Carbon dioxide** is bound to N-terminal end of each of the polypeptide chains of hemoglobin to form carbamino hemoglobin **(Figure 8.4).**

TENSE (T) AND RELAXED (R) FORMS OF HEMOGLOBIN

The Tense (T) Form

The deoxyhemoglobin is called the tense (T) or taut or a stressed form, in which the four subunits of Hb are

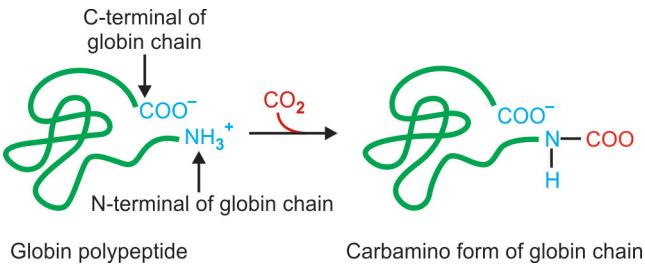


Figure 8.4 : Binding site for carbon dioxide with a globin chain of Hb

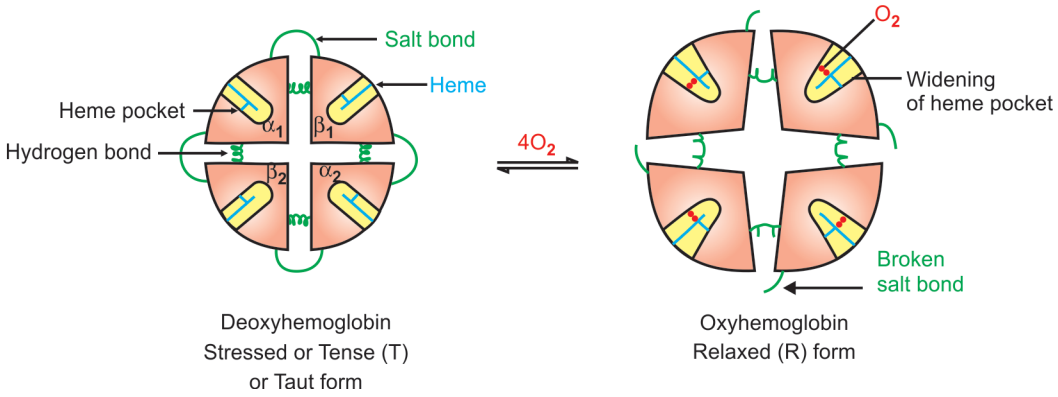


Figure 8.5: Schematic representation of tense (T) and relaxed (R) forms of hemoglobin

held together by salt bonds, hydrogen bonds and Van der Waals forces **(Figure 8.5).**

The Relaxed (R) Form

- The oxyhemoglobin is called the **relaxed (R)** form of hemoglobin.
- A molecule of O_2 bound first by the α-chain whose heme pockets are more readily accessible than those of the β-chains. Heme pockets of the ***β-chains are blocked by valine residue.***
- Binding of oxygen is accompanied by the rupture of salt bonds of all four subunits and protons are generated.
- These changes alter hemoglobin's secondary, tertiary and quaternary structures **(Figure 8.5)** and lead to widening of heme pockets of the remaining subunits and facilitates the binding of O_2 to these subunits.
- As a result, a **stressed** or ***tense (T)*** or ***taut*** structure of the deoxyhemoglobin changes to ***relaxed (R)*** form in oxyhemoglobin **(Figure 8.5).**

Cooperative Oxygen Binding of Hemoglobin

- The binding of the first oxygen to heme of the hemoglobin enhances the binding of oxygen to the remaining heme of the same molecule of hemoglobin. This is called **cooperative oxygen binding** of hemoglobin. The cooperative binding of O_2 by hemoglobin enhances oxygen transport.
- The shape of O_2 binding curve of hemoglobin is ***sigmoidal (S-shaped)*** because oxygen binding is cooperative **(Figure 8.6).** This shape indicates that the affinity of hemoglobin for binding the first molecule of oxygen is relatively very low but subsequent oxygen molecules are bound with a very much higher affinity (almost 500-fold) **(Figure 8.7)** accounting for the **steeply rising portion of the S-shaped curve.**
- Because of cooperativity between O_2 binding sites, hemoglobin delivers more O_2 to tissues than would a noncooperative protein **myoglobin (Figure 8.6)**

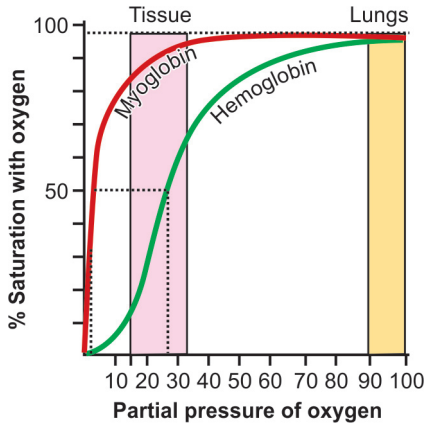


Figure 8.6: Oxygen binding curves for hemoglobin and myoglobin. Note that the curve for hemoglobin is sigmoidal while that for myoglobin is hyperbolic

Bohr Effect

- Hemoglobin also transports a significant amount (about 20%) of the total H^+ and CO_2 from tissues to the lungs and the kidney.
- The formation of CO_2 in peripheral tissues causes an increase in H^+ ion concentration (i.e. decrease in pH) in tissues as follows:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

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

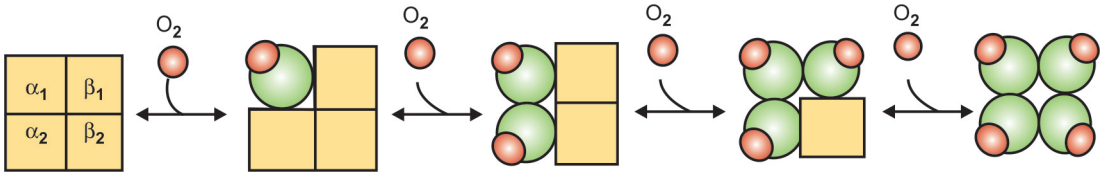


Figure 8.7: Cooperative binding of O_2 to hemoglobin

- Binding of H^+ and CO_2 to hemoglobin decreases the affinity for O_2. Thus, at a relatively low pH and high CO_2 concentration in the peripheral tissues, the affinity of hemoglobin for O_2 is decreased as H^+ and CO_2 are bound.
- Conversely, in the lungs, as CO_2 is excreted and the blood pH consequently rises, the affinity of hemoglobin for O_2 is increased.
- This effect of pH and CO_2 concentration on the binding and release of O_2 by hemoglobin is called ***Bohr* effect,** after the Danish physiologist who discovered it. **(Figure 8.8)**

Effect of 2-3 Bisphosphoglycerate (BPG) on Binding of Oxygen to Hemoglobin

2-3 BPG is found in red blood cells at nearly the same concentration as hemoglobin. It is formed as an intermediate in glycolysis in RBC **(see Figure 12.4).**

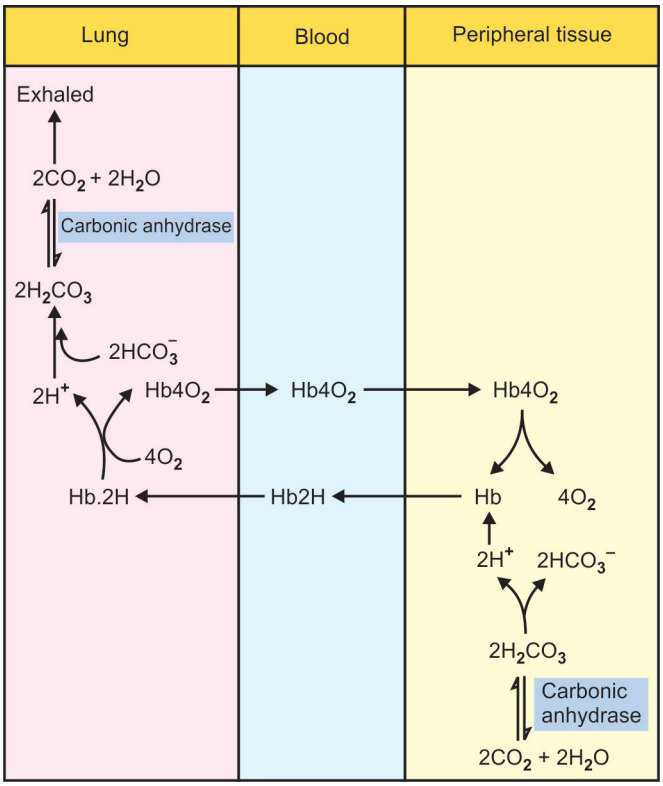
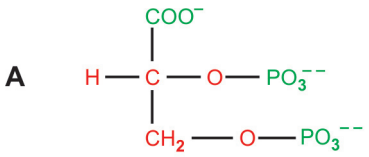


Figure 8.8: Bohr effect

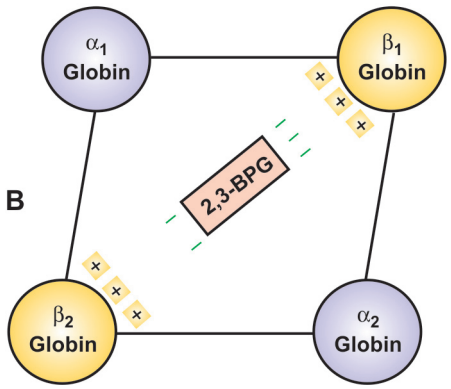
- 2-3 BPG regulates the binding of O_2 to hemoglobin.
- The presence of BPG significantly reduces the affinity of hemoglobin for oxygen.
- This reduced affinity releases oxygen efficiently in peripheral tissues.
- One molecule of 2-3 BPG binds in the central cavity of deoxyhemoglobin. It binds with β-chains through ionic bonds.
- These ionic bonds are formed between positively charged amino acids of β-chain with negatively charged phosphate groups of 2-3 BPG.
- In HbA, the binding site is made up of six +ve charges of amino acids of β-globin chains and five -ve charges of phosphate groups of 2-3 BPG **(Figures 8.9A and B).**

Importance of 2-3 BPG

- Without 2-3 BPG, hemoglobin would be an extremely inefficient oxygen transporter, releasing



2, 3-Bisphosphoglycerate



Figures 8.9A and B: (A) Structure of 2,3-BPG; (B) Schematic representation of binding of 2,3-BPG to the hemoglobin

only 8 percent of its oxygen in the tissue and the oxygen saturation curve of hemoglobin would approach that of myoglobin.

- When there is a chronic deprivation of oxygen in tissue, the level of 2-3 BPG increases, such compensatory increase occurs in:
  - Individuals who live at high altitudes
  - Patients with chronic obstructive pulmonary disease (COPD) like emphysema
  - Anemias
  - Cardiac failure.

TYPES OF NORMAL AND ABNORMAL HEMOGLOBIN

Normal Hemoglobin

Several different forms of normal hemoglobin can be found in adult humans and during human development, each containing four polypeptide subunits. Most forms of hemoglobin contain **two α-chains** plus two other chains usually **β, γ** and **δ (Table 8.1)**

Adult Hemoglobin (HbA_1)

It consists of **two alpha** and **two beta** chains and designated as **$\alpha_2\beta_2$.** Approximately 98 percent of the total hemoglobin of a normal adult is of this type.

Minor Component of Normal Adult Hemoglobin (HbA_2)

It consists of **two alpha** and **two delta** chains and is designated as $\alpha_2\delta_2$. It is present usually to the extent of 2.5 percent of the total.

Fetal Hemoglobin (HbF)

HbF is the major hemoglobin found in a fetus and a new born. It consists of **two alpha** and **two gamma** chains. After birth, the gamma (γ) subunits are replaced by beta (β) chains and becomes $\alpha_2\beta_2$ (HbA_1).

Glycosylated Hemoglobin (HbA_{1C})

HbA_1 (adult Hb) reacts spontaneously with glucose to form a derivative, known as ***glycosylated hemoglobin (HbA_{1C}).***

- The aldehyde group of glucose reacts nonenzymatically with N-terminal residue (valine) of β-chains **(Figure 8.10)**, when blood glucose enters the erythrocytes.
- Normally, the concentration of HbA_{1C} in blood is very low to the extent of about 5 percent of the total hemoglobin.

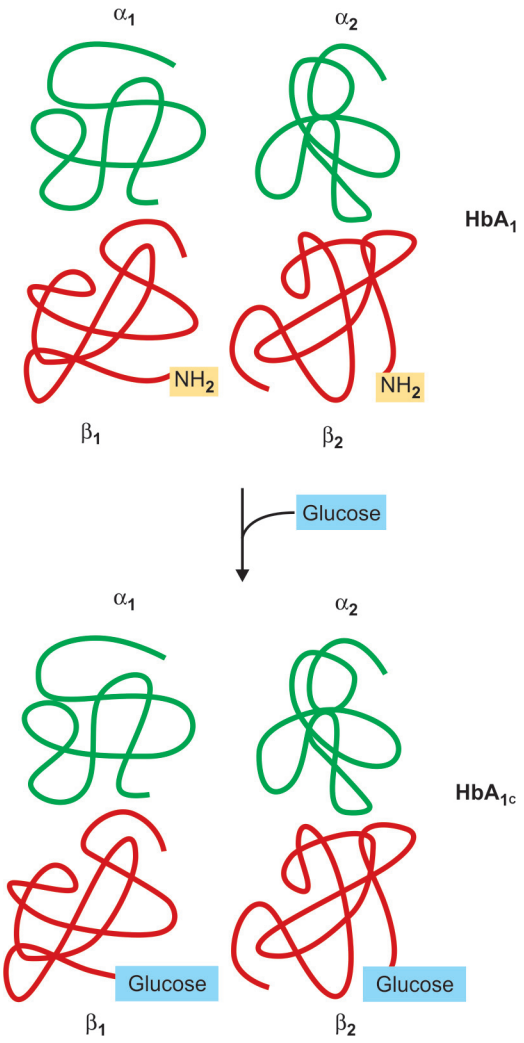


Figure 8.10: Formation of HbA_{1C}

- Formation of HbA_{1C} is proportional to blood glucose concentration.
- Increased amounts of HbA_{1C} are found in patients with diabetes mellitus, where the blood sugar level is high.
- Measurement of HbA_{1C} provides useful information for the management of diabetes mellitus, since RBCs have a life span of about 120 days, the content of HbA_{1C} is an indicator of how effectively blood glucose levels have been regulated over the previous 2 to 3 months.

Abnormal Hemoglobin

Since α, β, γ and δ chains of the globin of hemoglobin are synthesized from amino acids under genetic

control, mutations in the genes that code for globin chains can affect their formation and biological function of hemoglobin. Such hemoglobins are called ***abnormal hemoglobin.*** When biological function is altered due to a mutation in hemoglobin, the condition is called **hemoglobinopathy.** Some of the abnormal forms of hemoglobin are discussed below.

Thalassemia

- Thalassemia is a group of genetically transmitted disorder of hemoglobin synthesis, due to **lack or decreased synthesis of α or β** globin chains.
- Because the synthesis of one globin chain is reduced, there is a relative excess synthesis of the other globin chains. These globin chains may precipitate in the cell causing hemolysis, resulting in a ***hypochromic anemia***.
- The name of this group of diseases comes from the Greek word ***"thalasa"***, meaning **"sea",** because this disorder occurs more commonly among people living near the Mediterranean sea.

Types of thalassemia

Depending upon whether the genetic defect lies in synthesis of α or β globin chains, thalassemia are classified into **α-thalassemia** and **β-thalassemia** respectively.

α-thalassemia

In this condition, synthesis of α-globin chain is defective. α-globin chains are coded by **four copies of α-globin gene.** The α-thalassemia results from genetic defect in one or more copies of α-globin genes. (located on chromosome 16) and is characterized by either decreased or total absence of synthesis of α-globin chains. The α-thelassemia is of four types. These are :

a. **Silent carrier type of α-thalassemia:** In this type of α-thalassemia, only one of the four copies of α-globin gene is mutated. Since the patients of this disorder can synthesize sufficient α-globin chains, they do not show any clinical symptoms of thalassemia. They are only carriers of α-thalassemia.
b. **α-thalassemia trait:** It this type of α-thalassemia, two of the four copies of α-globin genes are mutated. They usually have only mild anemia and is not fatal.
c. **Hemoglobin H disease:** In this type of α-thalassemia three of the four copies of α-globin genes are mutated. They have moderately severe hemolytic anemia.
d. **Hydrops fetalis:** In hydrops fetalis, all four copies of α-globin genes are mutated. This is a lethal condition. Most of the affected are stillborn (death before birth) or die soon after birth.

β-thalassemia

In β-thalassemia, synthesis of **β-globin chain** is impaired due to genetic defect in β-globin genes. β-globin chains are coded by **two copies of β-globin genes** and is characterized by decreased or total absence of synthesis of β-globin chains. The β-thalassemia is of two types. These are:

a. **β-thalassemia minor (also known as β-thalassemia trait):** In this condition, one of the two copies of β-globin genes is mutated. It is a heterozygous state. The presence of one normal gene in the heterozygous allows enough normal globin chain synthesis, so that affected individuals are usually asymptomatic. The individual may be completely normal or has a mild anemia.
b. **β-thalassemia major:** β-thalassemia major is homozygous state, carrying two mutated β-globin genes. Thalassemia major leads to **severe anemia.** They regularly need blood transfusion. Bone marrow transplant has recently been introduced as a remedy.

Sickle Anemia and Sickle Hemoglobin (Hbs)

- Sickle cell anemia is a genetic disorder caused by production of an abnormal hemoglobin, known as **sickle hemoglobin (HbS).**
- Production of HbS is due to **mutation in the β-globin gene** which codes for β-globin chain. The mutant β-globin chain of HbS has an altered amino acid sequence.
- **Glutamic acid** residue normally present in the sixth position of β-chain of HbA is replaced by a **valine** residue as a result of mutation in the β-globin chain.

```
 1    2    3    4    5    6    7    8
Val—His—leu—Thr—Pro—Glu—Glu—lys—(HbA)
Val—His—leu—Thr—Pro—Val—Glu—lys—(HbS)
```

- As polar (hydrophilic) glutamic acid residue is replaced by nonpolar (hydrophobic) valine, it generates hydrophobic contact point called, **"Sticky patch",** on the outer surface of the β-globin chain. This alteration markedly reduces the solubility of deoxygenated HbS.
- When HbS is deoxygenated, the sticky patch can bind to the another deoxygenated HbS molecule. ***This binding causes polymerization of deoxy-HbS forming insoluble long tubular fiber*** **(Figure 8.11).**
- The insoluble fibers of deoxygenated HbS are responsible for deforming the red blood cells, which

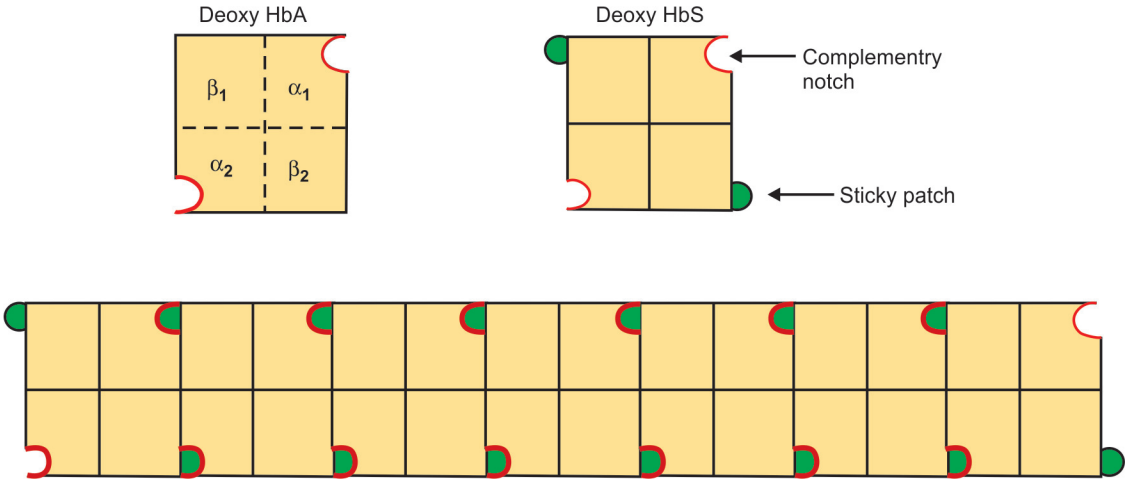


Figure 8.11: Schematic representation of polymerization of deoxy Hb-S molecules and formation of tubular fibrous structure

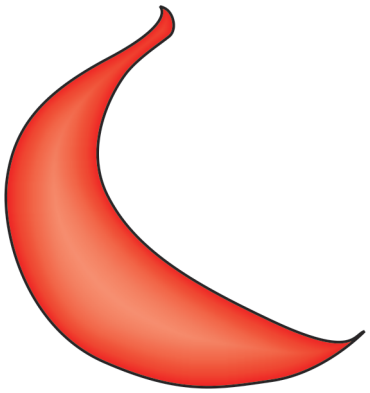


Figure 8.12: Sickle red blood cells

look like the ***blade of a sickle*** **(Figure 8.12)**. Hence, the name of the disease.

- The sickled red blood cells lose water, become fragile and have a much shorter lifespan than normal cells (17 days compared with 120 days), leading to lysis of the red blood cells and results in **hemolytic *anemia,*** called ***sickel cell anemia.***
- The more serious consequence is that, small blood capillaries in different organs become blocked by the long abnormally shaped red cell. This interrupts the supply of oxygen and leads to ***anoxia*** (oxygen deprivation) which causes pain and eventually death of the cell.

Sickle sell anemia and sickle cell trait

- **Sickle cell anemia** is a homozygous disorder in which the individual has inherited two mutant globin genes one from each parent. It is characterized by chronic hemolytic anemia, tissue damage and pain and increased susceptibility to infections. Such patients usually die in their adult age.
- **Sickle cell trait** is a heterozygous state, in which individuals have received the abnormal mutated β-globin gene from only one parent and have one normal gene. They do not show any clinical symptoms and have normal lifespan.
- Persons with sickle cell trait are resistant to malaria caused by ***plasmodium falciparum.*** This parasite spends an obligatory part of its life-cycle in the red blood cell. Since the sickle red blood cell have a shorter lifespan than normal red blood cell, the parasite cannot complete its life cycle.

HbC or Cooley's Hemoglobin

- In HbC, the glutamic acid at position 6 in the β-chain is replaced by a lysine residue.
- The red blood cells of people with HbC do not sickle, however, crystals of HbC may form within the cell.
- Both homozygous and heterozygous individuals of the disease are known. This disease is characterized by a **mild hemolytic anemia.** Clinically, heterozygous individuals are asymptomatic.

HbM

- These arise due to mutation in histidine residue of either α-or β-chains, which bound with the iron in the heme molecule.
- In HbM, ***histidine*** is replaced by ***tyrosine*** and iron is stabilized in the ferric (Fe^{3+}) intstead of ferrous (Fe^{2+})form which cannot bind oxygen and leads to cynosis.

- The letter **'M'** of HbM, signifies that the affected chains are in the ***methemoglobin*** (ferric hemoglobin) form.

DERIVATIVES OF HEMOGLOBIN

- Hemoglobin readily combines with any gas or other substances to form some products which are called the ***derivatives of hemoglobin.*** These can be grouped into:
  - Normal derivatives
  - Abnormal derivatives.
- The derivatives of hemoglobin give characteristic absorption bands in the solar spectrum by which they may be identified.
- Abnormal hemoglobin derivatives are compounds of clinical importance. Measurement of these abnormal hemoglobin derivatives can be helpful in the diagnosing and monitoring exposure to the toxic compounds.
- Abnormal hemoglobin derivatives reduce the oxygen carrying capacity of the blood.

Normal Hemoglobin Derivatives

The normal hemoglobin derivatives are :

- Oxyhemoglobin
- Reduced hemoglobin
- Carbaminohemoglobin or carbhemoglobin.

Oxyhemoglobin

This is formed by the combination of hemoglobin with O_2 by the process of oxygenations.

$$\underset{\text{Reduced Hb}}{Hb} + 4O_2 \rightleftharpoons \underset{\text{oxy Hb}}{Hb\,4O_2}$$

Oxyhemoglobin is an unstable compound and the combination is reversible, i.e. oxygen can be released from this compound. The iron remains in ferrous (Fe^{2+}) state in this compound.

Reduced Hemoglobin

When oxygen is released from oxyhemoglobin, it is called reduced hemoglobin.

Carbaminohemoglobin or Carbhemoglobin

It is a derivative of hemoglobin with carbon dioxide. Carbon dioxide can be released easily from this. The affinity of hemoglobin with CO_2 is 20 times more than for oxygen.

Abnormal Hemoglobin Derivatives

Abnormal hemoglobin derivatives are:

- Methemoglobin
- Carboxyhemoglobin (COHb).

Methemoglobin

- Methemoglobin is an oxidized hemoglobin. The iron normally present in heme in ferrous (Fe^{2+}) state being replaced by ferric (Fe^{3+}) state in methemoglobin.
- The ferrous of hemoglobin is oxidized to ferric state by **superoxide** by certain drugs and other oxidizing agents, forming methemoglobin, which cannot transport oxygen.
- Only a very small amount of methemoglobin is present in normal blood (less than 1% of the total hemoglobin), formed by spontaneous oxidation of hemoglobin.
- The erythrocyte has enzyme mechanisms for keeping methemoglobin level below 1 percent.

Carboxyhemoglobin (COHb)

Carbon monoxide combines with the heme moiety in hemoglobin. It combines at the same position in the hemoglobin molecule as oxygen but with an affinity about 210 times greater than oxygen. As a result, even small quantities of CO in the inspired air cause the formation of relatively large amounts of COHb, with a corresponding reduction in the O_2 carrying capacity of the blood. Even as little as 1% CO in inspired air can be fatal in minutes.

SUMMARY

- Hemoglobin is a globular protein, functions in transport of oxygen, carbon dioxide and protons, as well as it acts as an intracellular buffer and thus involved in acid-base balance.
- Adult hemoglobin is a tetramer of $\alpha_2\beta_2$.
- In heme Fe^{2+} is linked to all four nitrogen atoms of the porphyrin ring and to two additional coordination bonds (ligands) located above and below the plane of the heme.
- Oxygen binds cooperatively to hemoglobin which enhances oxygen transport.
- Binding of oxygen by hemoglobin is enhanced by increased pH and low CO_2 concentration, whereas release of O_2 is enhanced by decreased pH and high CO_2 concentration (Bohr's effect).
- 2-3-bisphosphoglycerate lowers the O_2 affinity of hemoglobin.

- Hemoglobinopathy is the mutation in genes that code for the α-or β-chains and can affect the biologic function of hemoglobin, e.g. sickle hemoglobin (HbS), HbM, hemoglobin C and thalassemia.
- Derivatives of hemoglobin can be grouped into two normal hemoglobin derivatives, e.g., oxyhemoglobin, reduced hemoglobin and carbhemoglobin, abnormal hemoglobin derivatives, e.g. methemoglobin, carboxyhemoglobins, etc.

EXERCISE

Solve

Case History

A 20-year-old male complaining of severe back pain was hospitalized and sickle cell anemia was diagnosed.

Questions

1. What is sickle cell anemia?
2. Give its biochemical cause.
3. Give cause for the sickling of RBC.
4. Why does a person with sickle cell trait show an increased resistance to malaria?

Multiple Choice Questions (MCQs)

1. 2, 3-BPG binds Hb by salt bonds by cross linking:
a) β_1, β_2 b) α_1, α_2
c) β_1, α_1 d) β_2, α_2

2. HbF has high affinity for O_2 than HbA due to presence of:
a) β-chain b) γ-chain
c) δ-chain d) ε-chain

3. The following are the normal forms of hemoglobin, *except*:
a) HbA_1C b) HbF
c) HbA_1 d) HbS

4. Which of the following is not true for hydrops fetalis?
a) It is a β-thalassemia
b) It is an α-thalassemia
c) Neither HbF nor HbA can be synthesized
d) Death occurs before birth

5. Persons with sickle cell trait show an increased resistance to:
a) Typhoid b) Cancer
c) Malaria d) Diabetes

6. Hb Bart consists of:
a) α_4 b) β_4
c) γ_4 d) δ_4

7. Iron in heme is linked to globin through:
a) Histidine b) Arginine
c) Glycine d) Cysteine

8. Which of the following has a protective effect against malaria?
a) HbF b) HbM
c) HbS d) Hb Bart

9. Porphyrin is formed by joining pyrrole rings by:
a) Methene bridge
b) Hydrogen bond
c) Phosphate bond
d) Glycosidic bond

10. Normal adult hemoglobin (HA_1) has the following chains:
a) $\alpha_2\beta_2$ b) $\alpha_2\delta_2$
c) $\alpha_2\gamma_2$ d) $\alpha_2\varepsilon_2$

11. Hemoglobin is a:
a) Monomeric protein
b) Trimeric protein
c) Tetrameric protein
d) Dimeric protein

Correct Answers for MCQs

1-a	2-b	3-d	4-a
5-c	6-c	7-a	8-c
9-a	10-a	11-c	

CHAPTER

9

Chemistry of Nucleic Acids

INTRODUCTION

Nucleic acids are macromolecules present in all living cells in combination with proteins to form ***nucleoproteins.*** The protein is usually **protamines** and **histones.** Genetic information is encoded in a nucleic acid molecule.

NUCLEIC ACIDS

- Nucleic acids are polymers of ***nucleotides,*** linked by **phosphodiester** bond, they are therefore called **polynucleotides.**
- The nucleic acids are of two main types:
  - Deoxyribonucleic acid or DNA
  - Ribonucleic acid or RNA.
- DNA is present in nuclei and small amounts are also present in **mitochondria,** whereas 90% of the RNA is present in cell **cytoplasm** and 10% in the **nucleolus.**

NUCLEOTIDE

Each nucleotide consists of three components:

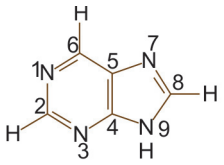
1. A nitrogenous base
2. A pentose sugar
3. A phosphate group.

Nitrogenous Bases of RNA and DNA

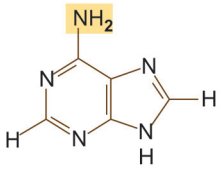
Two classes of nitrogenous bases namely **purines** and **pyrimidines** are present in RNA and DNA.

Purine Bases

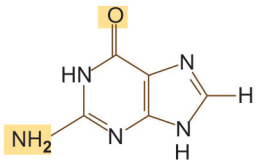
- Two principal purine bases found in DNAs, as well as RNAs **(Figure 9.1)** are:
  i. Adenine (A)
  ii. Guanine (G).



Purine ring



Adenine (6-aminopurine)



Guanine (2-amino-6-oxypurine)

Figure 9.1: Structure of purine ring and purine bases

Pyrimidine Bases

- Three major pyrimidine bases **(Figure 9.2)** are:
  i. Cytosine (C)
  ii. Uracil (U)
  iii. Thymine (T).
- Cytosine and uracil are found in RNAs and cytosine and thymine in DNA.
- Both DNA and RNA contain the pyrimidine cytosine but they differ in their second pyrimidine base. DNA contains thymine whereas RNA contains uracil.

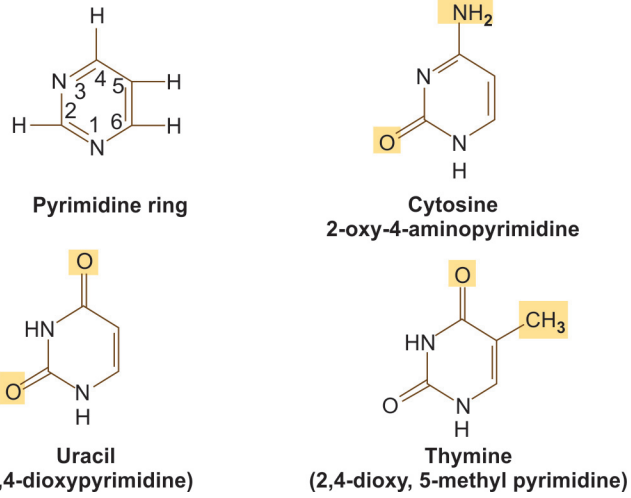


Figure 9.2: Structure of pyrimidine ring and pyrimidine bases

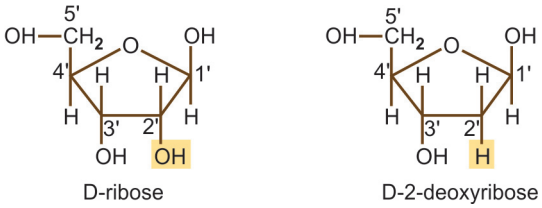


Figure 9.3: Structure of sugars present in nucleic acid

Pentose Sugars Present in RNA and DNA

- The pentose sugar is either ***D-ribose*** or ***D-2-deoxy-ribose*** **(Figure 9.3).** DNA and RNA are distinguished on the basis of the pentose sugar present. DNA contains D-2-deoxyribose and RNA contains D-ribose.
- A pentose sugar (D-ribose or D-2-deoxyribose) is linked to a base (purine or pyrimidine) via covalent **N-glycosidic bond (Figure 9.4).** The term **nucleoside** is used for structures containing only sugar and nitrogen base.
- This linkage joins nitrogen-9 of the purine base or nitrogen 1 of the pyrimidine base with carbon 1 of pentose sugar **(Figure 9.4).**
- The atoms of the base in nucleosides are given *cardinal* numbers, whereas the carbon atoms of the sugars are given **primed** numbers as shown in **Figure 9.4** to distinguish sugar atoms from those of the nitrogen base.

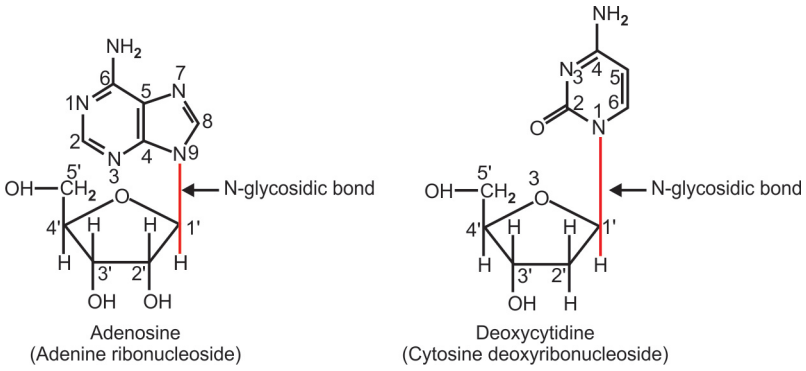


Figure 9.4: Structures of nucleoside

Table 9.1: Different major bases with their corresponding nucleosides and nucleotides

Base	*Ribonucleoside*	*Ribonucleotide*
Adenine (A)	Adenosine	Adenosine monophosphate (AMP)
Guanine (G)	Guanosine	Guanosine monophosphate (GMP)
Uracil (U)	Uridine	Uridine monophosphate (UMP)
Cytosine (C)	Cytidine	Cytidine monophosphate (CMP)
Base	*Deoxyribonucleoside*	*Deoxyribonucleotide*
Adenine	Deoxyadenosine	Deoxyadenosine monophosphate (dAMP)
Guanine	Deoxyguanosine	Deoxyguanosine monophosphate (dGMP)
Uracil	Deoxyuridine	Deoxyuridine monophosphate (dUMP)
Cytosine	Deoxycytidine	Deoxycytidine monophosphate (dCMP)
Thymine	Deoxythymidine	Deoxythymidine monophosphate (dTMP)

- The nucleosides of A, G, C, T and U are named adenosine, guanosine, cytidine, thymidine and uridine respectively.
- If the sugar is ribose, ribonucleoside is produced; if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced. The structures of these two types of nucleoside are illustrated by the examples in the **Figure 9.4. Table 9.1** indicates the bases and their corresponding nucleosides.

Structure of nucleotides

Nucleotides are phosphorylated nucleosides. Nucleosides are nitrogen bases containing pentose sugar. The phosphate group is attached to the nucleoside by an ester linkage to the hydroxyl group of the pentose sugar. The nucleotides are of two types depending on the kind of pentose sugar present.

1. **Deoxyribonucleotides:** These nucleotides contain pentose sugar, **deoxyribose** and are monomeric units of DNA **(Figure 9.5)**.
2. **Ribonucleotides:** These nucleotides contain pentose sugar, D-ribose and are monomeric units of RNA **(Figure 9.5)**.

- Mononuleotides are nucleosides in which single phosphate group is attached to hydroxyl group of the pentose sugar. For example, AMP (adenosine monophosphate) is adenine + ribose + phosphate.
- If an additional phosphate group is attached to the pre-existing phosphate of mononucleotide
  - A nucleoside diphosphate, e.g. ADP
  - A nucleoside triphosphate, e.g. ATP results **(Figure 9.6)**.
- The principle bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in **Table 9.1.**

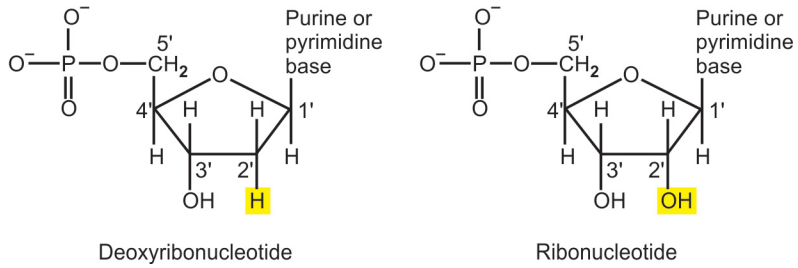


Figure 9.5: Structure of nucleotide

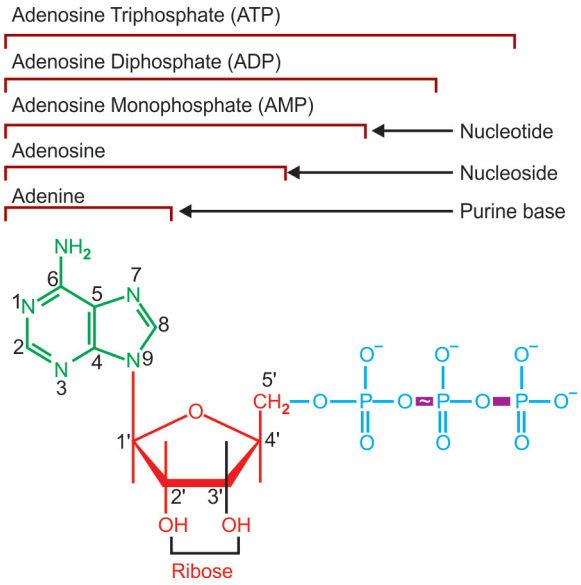


Figure 9.6: Structure of ATP and its components

BIOLOGICALLY IMPORTANT NUCLEOTIDES

Besides being the structural components of nucleic acids, several nucleotides such as ATP, ADP, c-AMP, GTP, GDP, c-GMP, UDP, CTP, CDP, etc. participate in several biochemical and physiological functions. Such nucleotides are called **biologically important nucleotides.** Nucleotides involved in a various biochemical proceses are discussed below.

ATP

ATP serves as the main biological source of energy in the cell. ATP is required as a source of energy in several metabolic pathways, e.g. fatty acid synthesis, glycolysis, cholesterol synthesis, protein synthesis, gluconeogenesis, etc. and in physiologic functions such as muscle contraction, nerve impulse transmission, etc.

AMP

AMP is the component of many coenzymes such as NAD^+, $NADP^+$, FAD, coenzyme A, etc. These coenzymes are essential for the metabolism of carbohydrate, lipid and protein.

C-AMP (Cyclic adenosine 3', 5'-monophosphate)

- c-AMP is formed from ATP by the action of ***adenylate*** **cyclase.**
- c-AMP acts as a second messenger for many hormones, e.g. epinephrine, glucagon, etc. c-AMP affects a wide range of cellular processes by acting as a second messenger.
- It enhances the degradation of storage fuels like fat and glycogen by stimulating lipolysis, glycogenolysis.
- It inhibits the aggregation of blood platelets.
- c-AMP increases the secretion of acid by the gastric mucosa.

GDP and GTP

- These guanosine nucleotides participate in the conversion of **succinyl-CoA to succinate,** a reaction which is coupled to the substrate level phosphorylation of GDP to GTP in citric acid cycle.
- GTP is required for activation of adenylate cyclase by some hormones.
- GTP serves as an energy source for protein synthesis.

C-GMP (Cyclic guanosine 3', 5'-monophosphate)

- c-GMP is formed from GTP by ***guanylyl cyclase.***
- c-GMP is an intracellular signal or second messenger that can act antagonistically to c-AMP.
- c-GMP is involved in relaxation of smooth muscle and vasodilation.

UDP (Uridine diphosphate)

- UDP participates in glycogenesis.
- UDP-glucose and UDP-galactose take part in galactose metabolism and required for synthesis of lactose and cerebrosides.
- UDP-glucuronic acid is required in detoxification processes and for biosynthesis of mucopolysaccharides such as heparin, hyaluronic acid, etc.

CTP (Cytidine triphosphate) and CDP (Cytidine diphosphate)

- CTP and CDP are required for the biosynthesis of some phospholipids. CDP-choline is involved in the synthesis of sphingomyelin.

SYNTHETIC ANALOGUES OF NUCLEOTIDES OR ANTIMETABOLITES

Chemically, synthesized analogues of purines and pyrimidines, their nucleosides and their nucleotides have therapeutic applications in medicine. An analogue is prepared either by altering the heterocyclic ring or sugar moiety. These are used **chemotherapeutically** (treatment of disease by the use of chemical substances) to control **cancer** or **infections**.

Analogues of purines and pyrimidines used in the treatment of infections or in cancer chemotherapy are:

- The nucleoside ***cytarabine (arabinosyl cytosine; ara-c)*** in which arabinose replaces ribose, is used in the chemotherapy of cancer and viral infections.

- The purine analogue ***allopurinol*** used in treatment of hyperuricemia and gout.
- ***Azidothymidine (AZT)*** is a structural analogue of thymidine used in the treatment of acquired immunodeficiency syndrome (AIDS), a disease caused by the human immunodeficiency virus (HIV).
- ***Azathioprine,*** which is used during organ transplantation to suppress events involved in immunologic rejection.
- ***5-iododeoxyuridine,*** a nucleoside analogue has antiviral activity and is used in the treatment of herpetic keratitis, an infection of the cornea by *Herpes* virus.

DNA STRUCTURE AND FUNCTION

DNA serves as the genetic material for cells both prokaryotes and eukaryotes. In eukaryotes, DNA is located in the nucleus separated from cytoplasm by the nuclear membrane. Because prokaryotes lack internal membrane systems, their DNA is not separated from the rest of the cellular contents. Eukaryotic DNA is bound to proteins, forming a complex known as chromatin.

DNA is also present in mitochondria (less than 0.1% of the total DNA) and in chloroplast of plants. Many viruses also contain DNA as their genetic material.

Structure of DNA

- DNA is a very long, thread like macromolecule made up of a large number of **deoxyribonucleotides.** Deoxyribonucleotide is composed of a ***nitrogenous base, a sugar*** and ***phosphate group.***
- The bases of DNA molecule carry genetic information, whereas their sugar and phosphate groups perform a structural role.
- The sugar in a deoxyribonucleotide is ***deoxyribose.***
- The purine bases in DNA are **adenine (A),** and **guanine (G)**.
- Pyrimidine bases are **thymine (T)** and **cytosine (C).**
- DNA is a polymer of many deoxyribonucleotides linked covalently by **3', 5' phosphodiester bonds**. The 3'-hydroxyl group of the sugar moiety of one deoxyribonucleotide is joined to the 5'-hydroxyl group of the adjacent sugar moiety of deoxyribonucleotide by a phosphodiester linkage **(Figure 9.7).**

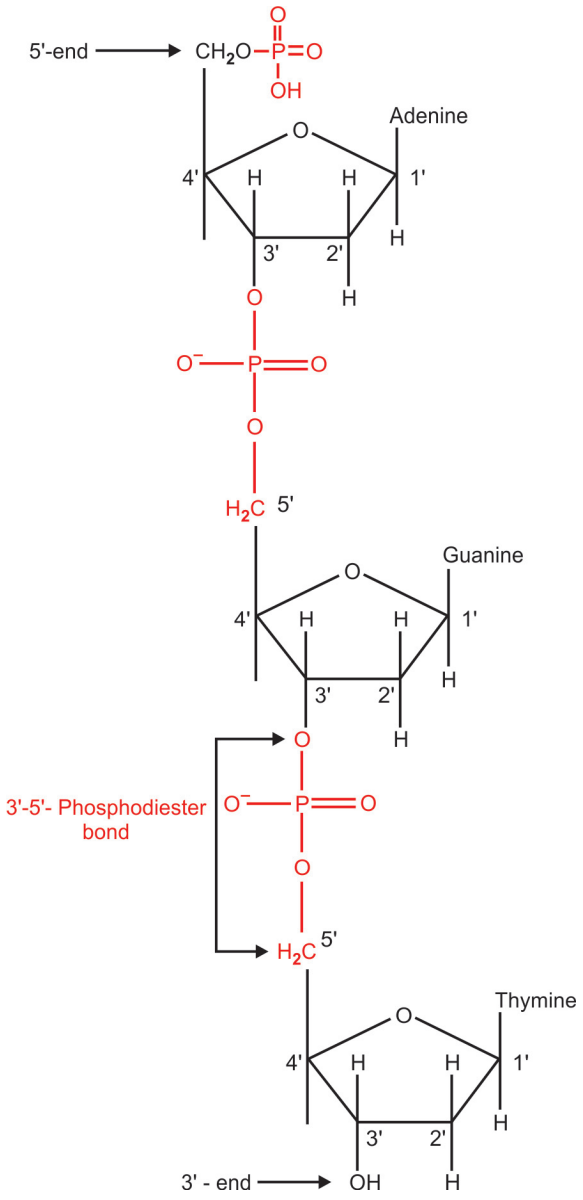


Figure 9.7: Structure of polynucleotide chain of DNA

The Watson-Crick DNA Double Helical Structure

In 1953, James Watson and Francis Crick deduced the three-dimensional structure of DNA. The important features of their model of DNA are as follows:

- Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite direction (anti parallel) **(Figure 9.8)**.
- The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and deoxyribose units are on the outside.
- The diameter of the helix is 20 Å. Adjacent bases are separated by 3.4 Å along the helix axis and the helical structure repeates after ten residues on each chain, i.e. at intervals of 34 Å **(Figure 9.9)**.

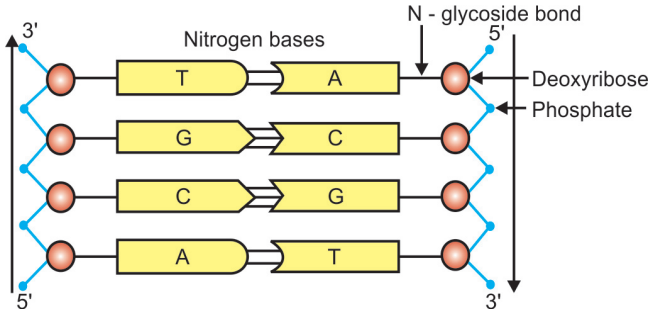


Figure 9.8: Antiparallel strands of DNA

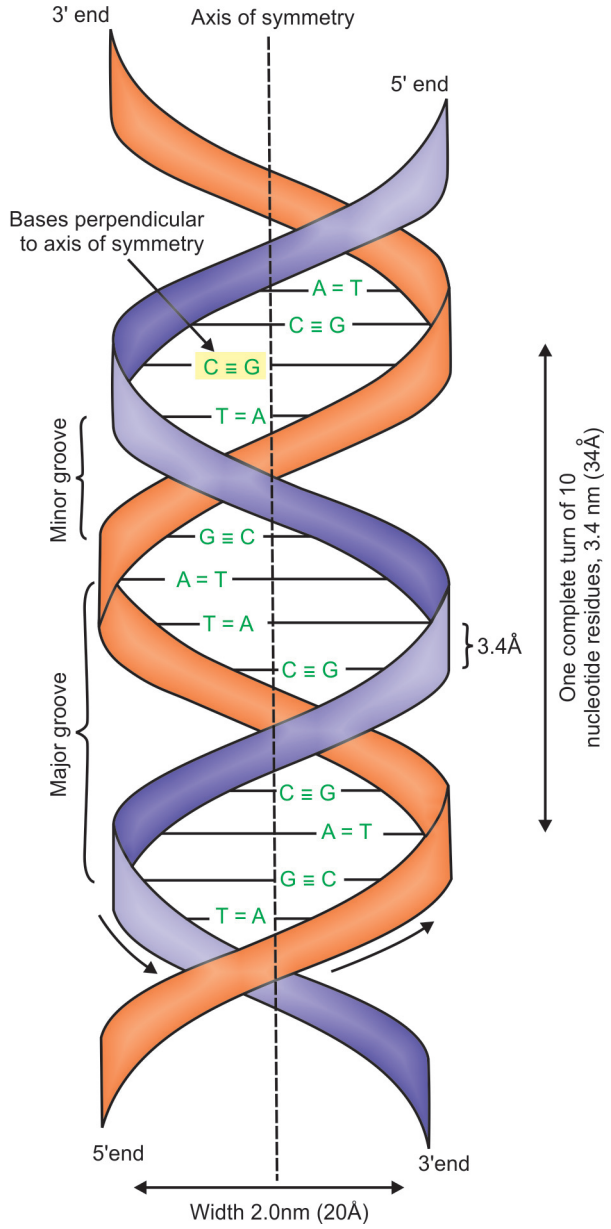


Figure 9.9: A diagrammatic representation of the Watson and Crick model of the double-helical structure of the B form of DNA

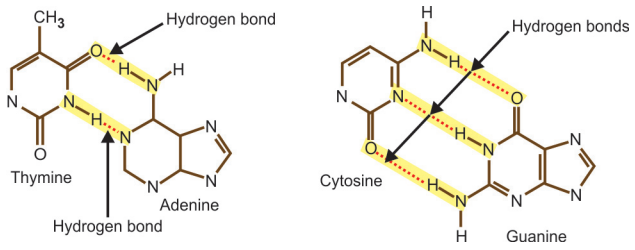


Figure 9.10: Base pairing between adenine and thymine involves the formation of two hydrogen bonds and base pairing between cytosine and guanine involves the formation of three hydrogen bonds

- The two chains are held together by hydrogen bonds between complementary pairs of bases:
  - ***Adenine is always paired with thymine by formation of two hydrogen bonds. Guanine is always paired with cytosine by formation of three hydrogen bonds*** **(Figure 9.10).**
- The two strands are always complementary to each other. In a double stranded DNA molecule, the content of adenine equals to that of thymine and the contents of guanine equals to that of cytosine. The complementary base pairing proves the **Chargaff's rule** (discussed later).
- The model proposed by Watson and Crick **(Figure 9.9)** is a **B form of DNA *(B-DNA)*** which is a right handed helix of 10 base pairs per turn, containing grooves of alternate size, known as major and minor grooves.
- Other forms of DNA may also occur, such as ***A-DNA*** and ***Z-DNA***. Under physiologic conditions, DNA is almost entirely in Watson-Crick B form.

Chargaff's Rule

- Ervin Chargaff discovered that in DNA of all species, quantity of purines is the same as that of pyrimidines (A+G=T+C). He observed that in DNA, content of adenine equals that of thymin (A=T) and content of guanine equals that of cytosine (G=C).
- Watson and Crick deduced that adenine must pair with thymine and guanine with cytosine, because of stearic and hydrogen bonding factors. Adenine cannot pair with cytosine; guanine cannot pair with thymine.
- Thus, one member of a base pair in a DNA must always be a purine and the other a pyrimidine.
- This base pairing restriction explains that in a double stranded DNA molecule, the content of A equals that of T and the content of G equals that of C.

- The ratio of purine to pyrimidine bases in the DNA is always one, i.e. G+A : T+C = 1. This is that of ***Chargaff's rule.***

Functions of DNA

DNA is the store of genetic information. The genetic information stored in the DNA serves two functions.

1. It is the source of information for the synthesis of all protein molecules of the cell and
2. It provides the information inherited by daughter cells or offspring.

ORGANIZATION OF DNA

Prokaryotic DNA

A prokaryotic cell generally contains a single chromosome composed of double stranded circular DNA, which contains over 4 x 10^6 base pairs. Because DNA molecules are so large, they require special organization/packaging to enable them to reside within cells. In *E. coli*, the circular DNA is supercoiled and attached to an RNA-protein core.

Eukaryotic DNA

- Eukaryotes contain over 1,000 times the amount of DNA found in prokaryotes. Consequently, their method of organizing or packing DNA is much more complex.
- A typical human cell contains 46 chromosomes, whose total DNA is approximately two meter in length.
- The packing of DNA in a chromosome represents a 10,000 fold shortening of its length from primary B-form DNA.
- In resting nondividing eukaryotic cells, the chromosomal material is called **chromatin**. Chromatin is made up of nucleosomes.

Different Levels of Organization of Eukaryotic DNA

Nucleosomes

- Nucleosomes are primary structural units of chromatin; which consists of **DNA bound to protein histones**.
- There are five types of histones, designated **H1, H2A, H2B, H3** and **H4.** Two molecules of each H2A, H2B, H3 and H4 associate with one another to form a structural core called **histone octomer (Figure 9.11)**.
- Around histone octomer a segment of the DNA double helix is wound nearly twice forming **nucleosome core** particles (a "bead").

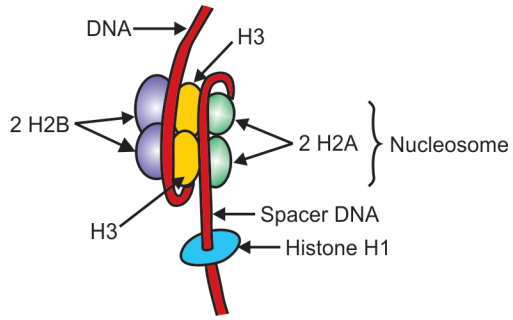


Figure 9.11: Diagrammatic representation of nucleosome

Chromatin fiber

- Chromatin is made up of repeating units of nucleosomes in which one nucleosome core joins to the next to form chromatin fiber.
- Nucleosomes are separated by spacer DNA to which histone H1 is attached to stabilize the complex **(Figure 9.12).** This continuous string of nucleosomes representing beads on a string form of chromatin is termed as **10 nm fiber.**

Solenoid structure

In addition to the net shortening of a DNA strand produced by winding of it around the histones, additional shortening packaging of eukaryotic DNA is brought about by further coiling of 10 nm chromatin fiber to produce 30 nm fiber which has a **solenoid structure** with six nucleosomes per turn. The solenoids are further folded into supercoiled loops to produce 300 nm form **chromosomes.**

A schematic view of the different levels of organization of DNA in the chromosome is shown in **Figure 9.13.**

RNA STRUCTURE AND FUNCTION

Unlike double stranded helical structure of DNA, the RNAs are single stranded. RNA is an unbranched linear polymer of ribonucleotides joined by 3′, 5′ phosphodiester bonds. The phosphodiester bonds join the 3′-OH group of ribose of one nucleotide unit to the 5′-OH group of ribose sugar of the next nucleotide.

Differences Between RNA and DNA

- In RNA, the sugar is **ribose** rather than **2'-deoxyribose** of DNA.
- RNA does not possess **thymine** except in the rare case. Instead of thymine, RNA contains **uracil.**

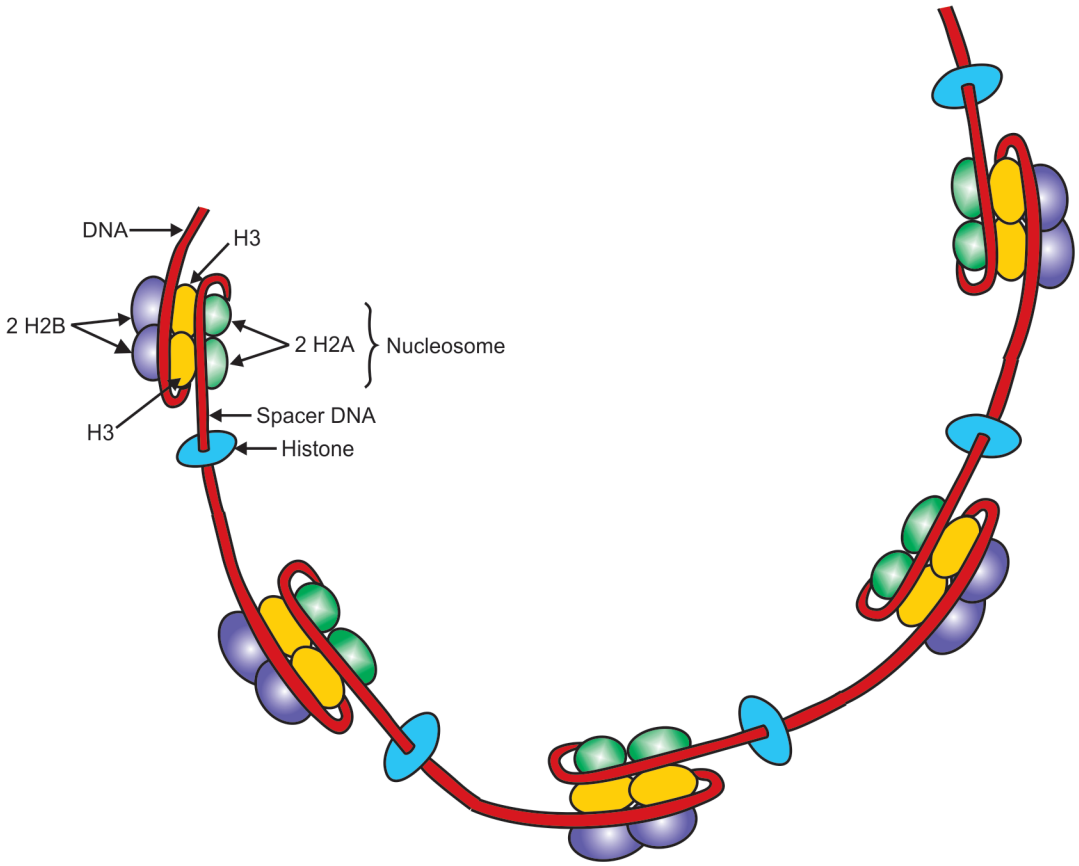


Figure 9.12: Schematic representation of chromatin fiber (Beads on a string structure)

- In RNA, adenine pairs with uracil rather than thymine.
- Unlike DNA, RNA is a single stranded and does not exhibit the equivalence of adenine with uracil and cytosine with guanine.

Types of RNA

Cell contains three major types of RNA:
1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA).

All of these are involved in the process of protein biosynthesis. Each differs from the others by size and function.

Messenger RNA (mRNA)

Structure of mRNA (Figure 9.14)

- The mRNA comprises only about 5-10% of total cellular RNA.
- mRNA is synthesized in the nucleus as heterogenous RNA (hnRNA), which are processed into functional mRNA.
- The mRNA carries the genetic information in the form of codons. Codons are a group of three adjacent nucleotides that code for the amino acids of protein.
- In eukaryotes mRNAs have some unique characteristics, e.g. the 5' end of mRNA is ***"capped"*** by a ***7-methyl-guanosine triphosphate.***
- The cap is involved in the recognition of mRNA in protein biosynthesis and it helps to stabilize the mRNA by preventing attack of 5'-exonucleases.
- A poly (A**)** ***"tail"*** is attached to the other 3'-end of mRNA. This tail consists of series of **adenylate residues**, 20-250 nucleotides in length joined by 3' to 5' phosphodiester bonds.
- The function of poly A tail is not fully understood, but it seems that it helps to stabilize mRNA by preventing the attack of 3'-exonuclease.

Function of mRNA

mRNAs serve as template for protein biosynthesis and transfer genetic information from DNA to protein synthesizing machinery.

If the mRNA codes for only one peptide, the mRNA is **monocistronic.** If it codes for two or more different polypeptides, the mRNA is **polycistronic.** In eukaryotes most mRNA are monocistronic.

Transfer RNA (tRNA)

tRNA molecules vary in length from 74 to 95 nucleotides. In eukaryotic cells, 10-20% of the nucleotides of tRNA may be modified and known as unusual nucleotides **(Figure 9.15)**, e.g.

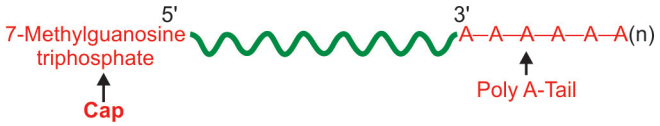


Figure 9.14: Structure of mRNA

- ***Dihydrouridine (D)***, in which one of the double bonds of the base is reduced.
- ***Ribothymidine (T),*** in which methyl group is added to uracil to form thymine.
- ***Pseudouridine (ψ)***, in which uracil is attached to ribose by a carbon-carbon bond rather than a nitrogen bond.

Structure of tRNA

- All single stranded transfer RNA molecules get folded into a structure that appears like a **clover leaf.**

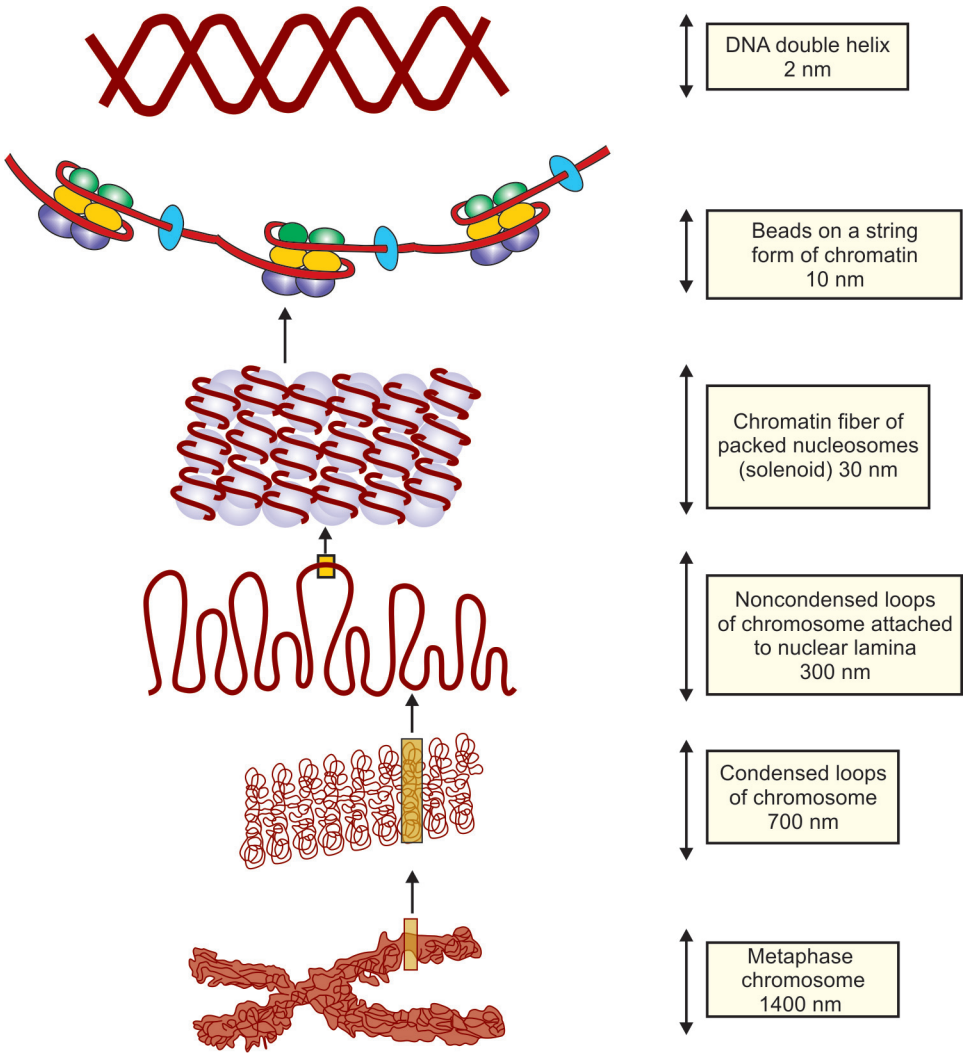


Figure 9.13: Packaging of DNA, arranged in increasing order of organization from top to bottom

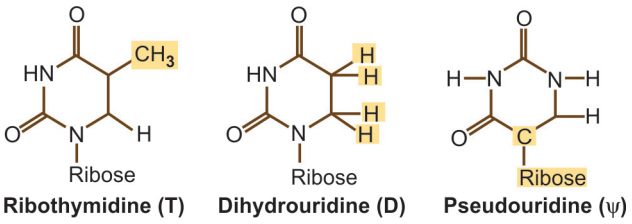


Figure 9.15: Three unusual (modified) nucleosides present in tRNA

All t-RNAs contain four main arms:

1. The acceptor arm
2. The D arm
3. The anticodon arm
4. The TψC arm.

The arms have base paired stems and unpaired loops as shown in **Figure 9.16.** The structure of tRNA molecules is maintained by the base pairing in these arms or stem regions.

- The **acceptor arm** consists of a base paired stem that terminates in the sequence **CCA** at the 3' end. This is the attachment site for the amino acid.
- The **D arm** is named for the presence of the base dihydrouridine (D).
- **The anticodon arm** contains the **anticodon** that base pairs with the codon on mRNA. Anticodon has nucleotide sequence complementary to the codon of mRNA and is responsible for the specificity of the tRNA.
- The TψC arm contains both **ribothymidine (T)** and **pseudouridine (ψ, psi)**

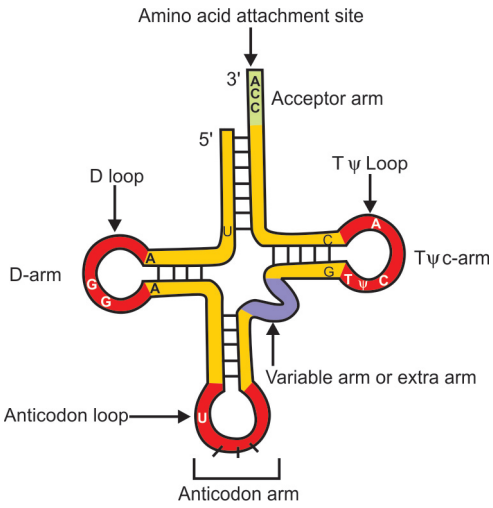


Figure 9.16: Structure of clover leaf transfer RNA

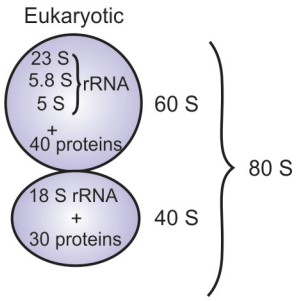


Figure 9.17: The components of eukaryotic ribosomal subunits

- The extra arm is also known as variable arm because it varies in size, is found between the anticodon and TψC arms.

Function of tRNA

tRNA carries amino acids in an activated form to the ribosome for the protein synthesis.

Ribosomal RNA (rRNA)

The RNA of the ribosomes is called the rRNA.

- A ribosome is a cytoplasmic nucleoprotein that acts as a machinery for the synthesis of proteins.
- The ribosome is a spheroidal particle and is composed of a large and a small nucleoprotein subunit.
- The eukaryotic ribosomes are composed of 60S and 40S subunits **(Figure 9.17)**.
- Each subunit is composed of one or more strand of rRNA and numerous protein molecules **(Figure 9.17).**
- The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA, while the 40S subunit contains 18S rRNA.

Functions of ribosomal RNA

The function of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are:

- Necessary to maintain ribosomal structure and also participate in protein synthesis by binding of mRNA to ribosome.
- Recent studies suggest that ribosomal RNAs may also provide some of the catalytic activities and thus is an enzyme "a ***ribozyme".***

Other Nuclear and Cytoplasmic RNAs

Besides mRNA, tRNA and rRNA, eukaryotes have some other RNAs. These are:

- Heterogenous RNAs (hnRNAs)
- Small cytoplasmic RNAs (scRNAs)
- Small nuclear RNAs (snRNAs).

Table 9.2: Different types of cellular RNAs and their functions

Types of RNA	*Functions*
mRNA (messenger RNA)	Carries the genetic information from DNA to the cytosol, where it is used for protein synthesis
tRNA (transfer RNA)	Serves as an "adaptor" molecule that carries specific amino acid to the site of protein synthesis
rRNA (ribosomal RNA)	In association with protein serves as the sites for protein synthesis. Provides catalytic activities (peptidyltransferase activity)
hnRNA (heterogeneous nuclear RNA)	Serves as precursor for mRNA
scRNA (small nuclear cytoplasmic RNA)	Involved in recognition of signal sequence in protein synthesis on membrane bound ribosomes
snRNA (small nuclear RNA)	Involved in excising introns and splicing exons

Various RNAs and their functions are given in **Table 9.2.**

SUMMARY

- The nucleic acids DNA and RNA are poly-nucleotides.
- In DNA and RNA, nucleotides are linked by 3'-5' phosphodiester bonds.
- Each nucleotide contains a nitrogenous base, a sugar and a phosphate.
- Nucleosides contain, D-ribose or D-2-deoxyribose, linked to N-1 of pyrimidines or N-9 of purines, by a N-glycosidic bond.
- DNA contains the purine bases: adenine (A) and guanine (G) and pyrimidine bases: cytosine (C) and thymine (T).
- RNA contains uracil (U) instead of thymine.
- Several synthetic analogues of purine and pyrimidine bases and their derivatives are used chemotherapeutically as anticancer drugs.
- DNA is organized into two strands by the pairing of bases A to T and G to C, on complementary strands. In contrast to DNA, RNA is single stranded structure.
- The three major types of RNA are mRNA, tRNA and rRNA, all are involved in some aspects of protein synthesis.

EXERCISE

Multiple Choice Questions (MCQs)

1. Which of the following holds DNA strands together?
a) Phosphodiester bond b) Hydrogen bond
c) Glycosidic bond d) Phosphate ester

2. Which carbon of the pentose is in ester linkage with the phosphate in a nucleotide structure?
a) C_1 b) C_2
c) C_3 d) C_4

3. Thymine is present in which of the following:
a) Ribosomal RNA
b) Messenger RNA
c) Transfer RNA
d) None of the above

4. Unusual nucleotide pseudouridylic acid is present in:
a) mRNA b) tRNA
c) rRNA d) hnRNA

5. A nucleoside can be composed of, *except*:
a) Purine base
b) Pentose sugar
c) Phosphate group
d) Pyrimidine base

6. Nucleotides perform all of the following functions *except:*
a) Structural units of DNA and RNA
b) Catalytic in nature
c) Regulators of metabolic reactions
d) Components of certain coenzymes

7. Which of the following is not a feature of Watson-Crick model of DNA?
a) Helical
b) Two strands are held by hydrogen bond
c) A + T = C+G
d) Two strands are right handed

8. The number of hydrogen bonds between guanosine and cytosine in DNA are:
a) One b) Two
c) Three d) Four

9. DNA is present in:
a) Only nucleus
b) Only mitochondria
c) Both nucleus and mitochondria
d) Cytoplasm

10. RNA is present in:
a) Nucleus
b) Only cytoplasm
c) Mitochondria
d) Cytoplasm and nucleolus

Correct answers for MCQs

1-b	2-a	3-c	4-b
5-c	6-b	7-c	8-c
9-c	10-d		

CHAPTER

10

Biological Oxidation

INTRODUCTION

Oxidation, which occurs in living systems is called ***biological oxidation.*** Biological oxidations are exergonic. During biological oxidations, the reacting chemical systems move from a higher energy level to a lower one and therefore there is liberation of energy. The energy released as heat is converted to chemical energy by formation of energy rich compound ATP.

The formation of ATP from ADP and Pi is termed **phosphorylation,** as phosphorylation is coupled with biological oxidation, the process is called ***biological oxidative phosphorylation.***

ENZYMES AND COENZYMES OF BIOLOGICAL OXIDATION

Biological oxidation is brought about with different **enzymes and *coenzymes.***

- The enzymes required for biological oxidation belong to the class of ***oxidoreductases,*** which includes:
  - Oxidases
  - Dehydrogenases
  - Hydroperoxidases
  - Oxygenases.
- Coenzymes involved in biological oxidation include:
  - Nicotinamide adenine dinucleotide (NAD^+)
  - Nicotinamide adenine dinucleotide phosphate ($NADP^+$)
  - Flavin mononucleotide (FMN)
  - Flavin adenine dinucleotide (FAD).

Oxidases

Oxidases catalyze the removal of hydrogen from a substrate in the form of H_2O or H_2O_2 (hydrogen peroxide), using oxygen as a hydrogen acceptor, e.g.

- Cytochrome oxidase
- L-amino acid oxidases
- Xanthine oxidase.

Dehydrogenases

This group constitutes several enzymes. They all catalyze the removal of hydrogen from a substrate but are not able to use oxygen as a hydrogen acceptor. These enzymes, therefore, require specific coenzymes as acceptors of hydrogen atoms. The coenzymes of dehydrogenases may be either:

- Nicotinamide coenzymes (NAD^+ or $NADP^+$)
- Flavin coenzymes (FMN or FAD).

Nicotinamide coenzymes (NAD^+ or $NADP^+$) linked dehydrogenases

- Some dehydrogenases can use either nicotinamide adenine dinucleotide (NAD^+) or nicotinamide

adenine dinucleotide phosphate ($NADP^+$) co-enzymes. These coenzymes are derived from vitamin **niacin**.

- NAD^+ or $NADP^+$ linked dehydrogenases remove two hydrogen atoms from their substrate. One of these is transferred to the NAD^+ or $NADP^+$. The other appears as hydrogen ion (H^+) in the medium. The general reaction can be written as:

$$MH_2 + NAD^+ / NADP^+ \rightarrow M + NADH + H^+ / NADPH + H^+$$

- NAD linked dehydrogenases are involved in the oxidative pathways of metabolism like in glycolysis, TCA cycle and in the mitochondrial respiratory chain.
- NADP linked dehydrogenases, on the other hand, are involved in reductive biosynthetic reactions like fatty acid synthesis and cholesterol synthesis.
- Unlike NADH, NADPH cannot be oxidized with concomitant production of energy.

Flavin-coenzyme (FMN or FAD) linked dehydrogenases

- Flavin mononucleotide (FMN) and flavin adenide dinucleotide (FAD) are derived from vitamin **riboflavin**.
- Unlike NAD^+, both hydrogen atoms from substrate are accepted by FMN or FAD. The general reaction can be written as:

$$MH_2 + FAD/FMN \rightarrow M + FADH_2/FMNH_2$$

- Most of the FMN linked dehydrogenases are concerned with mitochondrial electron transport chain, e.g. NADH-dehydrogenase.
- The examples of FAD linked dehydrogenases are **succinate dehydrogenase** in TCA cycle, **acyl-CoA dehydrogenase** in β-oxidation of fatty acid, etc.

Hydroperoxidases

Hydroperoxidases catalyze the reduction of H_2O_2 to H_2O. There are two types of hydroperoxidases:

1. Peroxidases
2. Catalases.

Oxygenases

Oxygenases are a group of enzymes that catalyze the addition of one or both of the atoms of the O_2 molecule into the substrate. **Oxygenases** ***are not concerned with energy production in the body.*** These are:

Monooxygenase

- These enzymes incorporate one oxygen atom of O_2 into the substrates in the form of hydroxyl group, while the other oxygen atom of O_2 is reduced to H_2O.
- Examples of monooxygenase is phenylalanine hydroxylase for the formation of tyrosine from phenylalanine.

Dioxygenases

- These catalyze the incorporation of both the atoms of O_2 into the substrates. For example, the enzymes such as:
  - Homogentisate oxidase of tyrosine metabolism.
  - 3-Hydroxy anthranilate oxidase and L-tryptophan dioxygenase of tryptophan metabolism.
  - Cycloxygenase involved in prostaglandin synthesis.

ELECTRON TRANSPORT CHAIN (ETC) OR RESPIRATORY CHAIN

The final steps in the overall oxidation of food stuffs (carbohydrate, fat and amino acids) result in formation of NADH and $FADH_2$.

The electron transport chain (ETC) oxidizes NADH and $FADH_2$ by transferring electrons (reducing equivalents) by a series of oxidation reduction reactions to O_2, the terminal electron acceptor. In the presence of O_2, the ETC converts reducing equivalents into energy, (ATP) by oxidative phosphorylation.

Localization of the Electron Transport Chain

The electron transport chain is present in the ***inner mitochondrial membrane (Figure 10.1).*** The enzymes of the electron transport chain are embedded in the inner membrane.

Components of the Electron Transport Chain

The major components of the electron transport chain include:

- Nicotinamide adenine dinucleotide (NAD^+).
- Flavin mononucleotide (FMN) and Flavin adenine dinucleotide (FAD).
- Ubiquinone or coenzyme Q.
- The ***iron-sulfur (Fe-S) protein*** associated with FMN and cytochrome b.
- Cytochromes (hemeproteins): ***b, c_1, c, a*** and ***a_3.*** Of these, only cytochrome c is water soluble and easily diffusible, whereas cytochromes b, c_1, a and a_3 are lipid soluble and therefore, are fixed components of

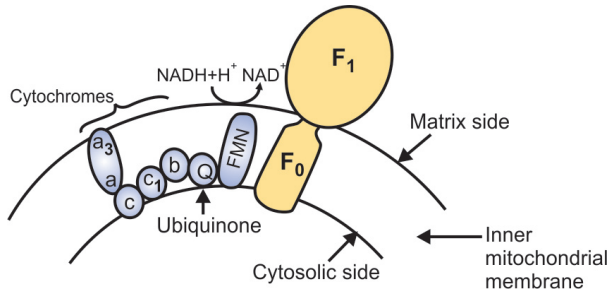


Figure 10.1: Structural organization of components of respiratory chain and F_oF_1 ATPase in the mitochondrial membrane

the membrane. Cytochrome aa_3 are also called **cytochrome oxidase;** and are **copper containing hemeproteins.**

Except coenzyme Q, all members of this chain are proteins. Coenzyme Q (CoQ) is a fat soluble quinone (ubiquinone) and is a constituent of mitochondrial lipids.

Structural Organization of Components of Electron Transport Chain

The mitochondrial electron carriers are organized into four complexes (complex I to IV) that catalyze oxidation-reduction reactions of the electron transport chain **(Figure 10.2).**

- **Complex I, NADH- CoQ reductase,** catalyzes the transfer of electrons from NADH to coenzyme Q (CoQ).
- **Complex II, Succinate-CoQ reductase,** transfers electrons from succinate to coenzyme Q.
- **Complex III, CoQ- Cytochrome c reductase,** transfers electrons from CoQ to cytochrome c.
- **Complex IV, Cyctochrome oxidase,** transfers electrons from cytochrome c to O_2.

Components of the respiratory chain are arranged in order of increasing redox potential **(Table 10.1).** Reducing equivalents flow through the chain from the components of **more negative redox potential to the components of more positive redox potential.**

Reactions of Electron Transport Chain

The following sequence of reactions occurs in the transfer of electrons from substrate to the ultimate acceptor oxygen **(Figure 10.3).**

1. NAD^+ is reduced to NADH by various dehydrogenases which remove two hydrogen atoms from their metabolite (MH_2) and get oxidized to M. In this oxidation reduction reaction, one hydrogen atom is accepted by NAD^+ to form NADH, while the second proton (H^+) is released into the aqueous medium.
2. The reduced NADH is oxidized by an enzyme **NADH dehydrogenase.** This enzyme contains **coenzyme FMN.** The coenzyme FMN accepts two electrons ($2e^-$) and a proton (H^+) from NADH and a free H^+ from the aqueous medium to form $FMNH_2$.
3. In addition to FMN, NADH dehydrogenase also consists of **Fe-S proteins,** which accepts only electron from $FMNH_2$. Thus two Fe-S protein molecules accept two electrons from one $FMNH_2$ molecule with release of two protons ($2H^+$) into the medium and $FMNH_2$ gets oxidized to FMN.
4. CoQ accepts two electrons from two Fe-S protein molecules and two protons ($2H^+$) from the medium

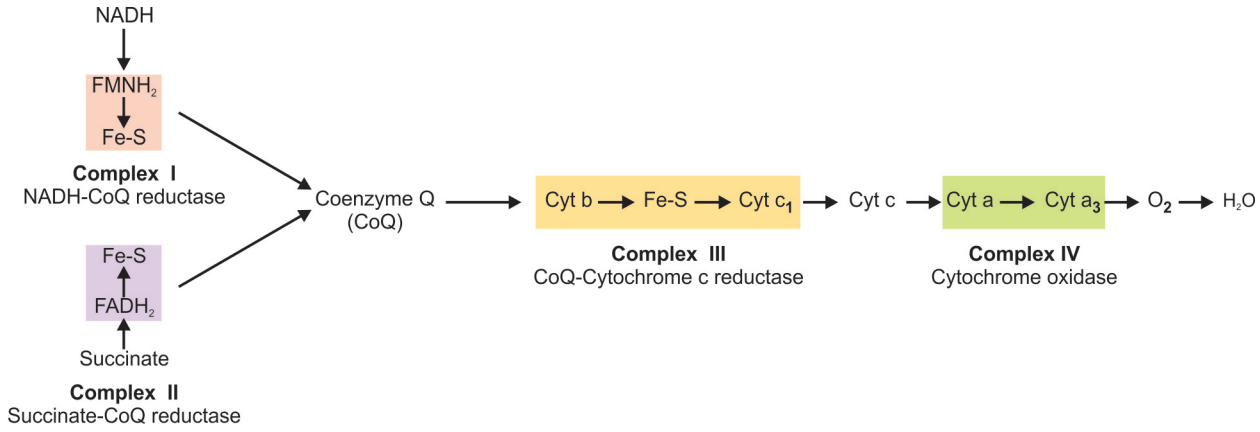


Figure 10.2: The electron transport complexes

Table 10.1: The redox potential Eo' of redox couples of components of electron transport chain

Redox couple components of electron transport chain			*Redox potential Eo' in volt*
$2H^+ + 2e^-$	→	H_2	– 0.41
$NAD + H^+ + 2e^-$	→	NADH	– 0.32
$FMN + 2H^+ + 2e^-$	→	$FMNH_2$	– 0.32
Ubiquinone $+2H^+ + 2e^-$	→	Ubiquinol	+ 0.04
Cytochrome b (ox) + e^-	→	Cytochrome b (red)	+ 0.07
Cytochrome c_1 (ox) + e^-	→	Cytochrome c_1 (red)	+ 0.23
Cytochrome c (ox) + e^-	→	Cytochrome c (red)	+ 0.25
Cytochrome a (ox) + e^-	→	Cytochrome a (red)	+ 0.29
Cytochrome a_3 (ox) + e^-	→	Cytochrome a_3 (red)	+ 0.55
$1/2O_2 + 2H^+ + 2e^-$	→	H_2O	+ 0.82

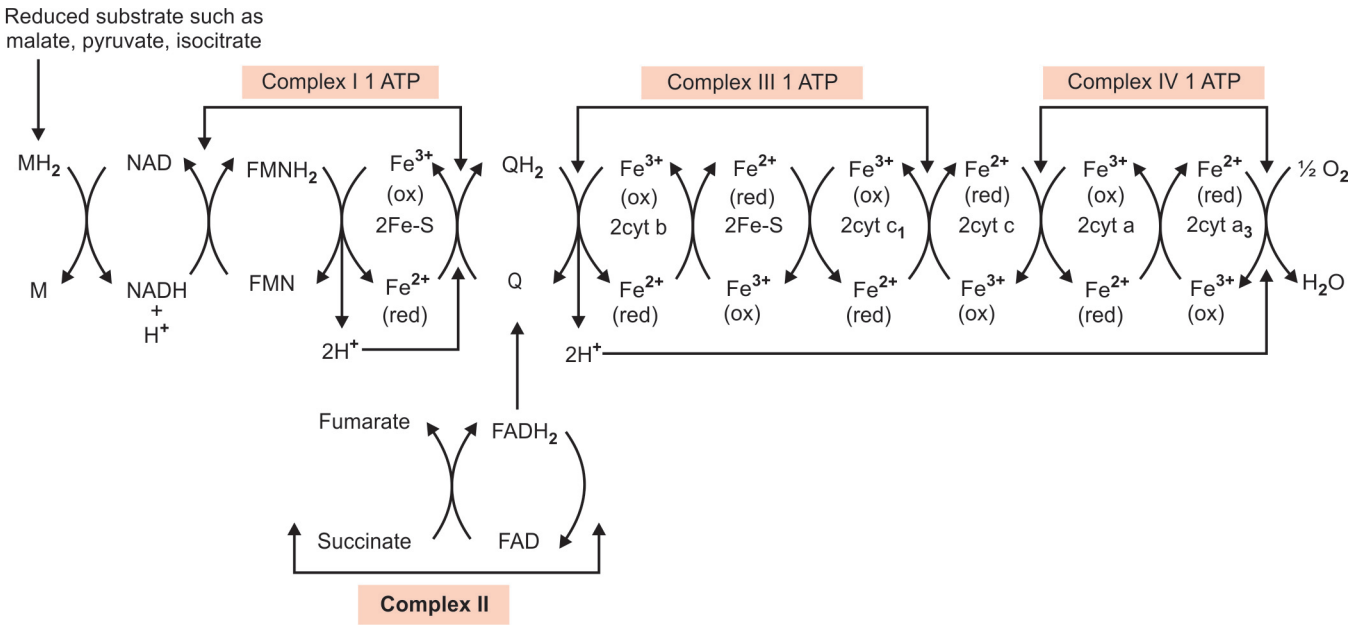


Figure 10.3: Electron transport chain

to get reduced to $CoQH_2$. CoQ also collects reducing equivalents from $FADH_2$ formed by FAD-linked dehydrogenases.

5. Beyond CoQ, oxidation reduction process occurs by removal of electrons with the help of **cytochromes.** Cytochromes accept only electrons from coenzyme QH_2 with the release of $2H^+$ in the medium. As a cytochrome can accept only one electron, $CoQH_2$ transfers its two electrons to two molecules of cytochrome b, c_1 c, a and a_3 sequentially.
6. The last cytochrome complex is cytochrome oxidase (cyt aa_3) which passes electrons from cytochrome c to molecular oxygen. Each oxygen atom accepts two electrons from cytochrome a_3 and two protons from the medium and a molecule of water results.

The reduction of O_2 by cytochrome oxidase reaction accounts for the production of about **300 ml of water/day.** This water is called ***metabolic water.***

Formation of ATP

During the transfer of electrons through the electron transport chain, energy is produced. This energy is coupled to the formation of ATP molecules by phosphorylation of ADP by an enzyme **F_0 F_1 ATPase.** The formation of ATP from ADP and Pi is termed **phosphorylation,** as phosphorylation is coupled with biological oxidation, the process is called ***biological oxidative phosphorylation.***

Sites of ATP Synthesis

- There are three ATP synthesizing sites of the electron transport chain, these are **(Figure 10.4)**:
  1 Oxidation of $FMNH_2$ by CoQ
  2 Oxidation of cytochrome b by cytochrome c_1
  3 Cytochrome oxidase reaction (oxidation of cytochrome a by cytochrome a_3.
- These sites provide the energy required to make ATP from ADP and Pi by an enzyme F_0 F_1 ATPase.
- Electrons that enter the chain through NADH pass through all three ATP synthesizing sites and thus yield three ATPs.
- However, electrons that enter the chain through $FADH_2$ pass through only two ATP synthesizing sites, as they bypass site 1, they yield two ATPs.

INHIBITORS OF ELECTRON TRANSPORT CHAIN

Inhibitors of respiratory chain may be divided into three groups.

1. Inhibitors of the electron transport chain proper
2. Inhibitors of oxidative phosphorylation (F_0F_1 ATPase)
3. Uncouplers of oxidative phosphorylation.

Inhibitors of Electron Transport Chain Proper (Figure 10.5)

Inhibitors of electron transport chain proper include, inhibitors that inhibit the flow of electrons through the respiratory chain. These inhibitors block the respiratory chain at three sites:

1. **Complex l (NADH to CoQ),** inhibited by:
   – Barbiturates such as amobarbital
   – An antibiotic piericidin A
   – The insecticide rotenone.

These inhibitors prevent the oxidation of substrates by blocking the transfer of reducing equivalents from Fe-S protein to CoQ.

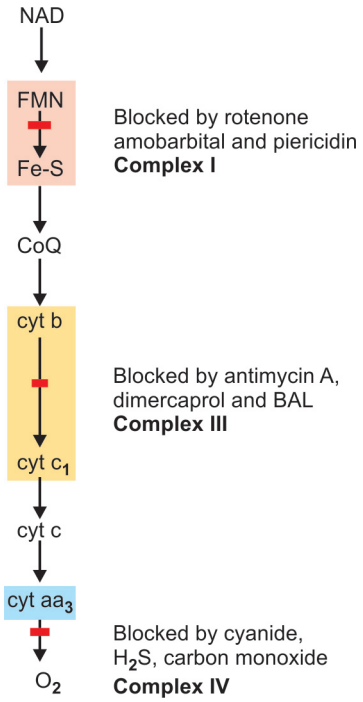


Figure 10.5: Sites of action of various inhibitors of electron transport chain

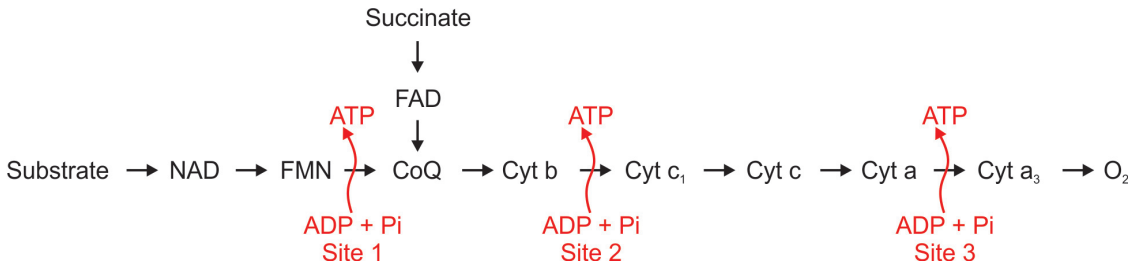


Figure 10.4: ATP synthesizing sites of electron transport chain

2. **Complex III (cytochrome b to cytochrome c_1),** inhibited by:
   - Dimercaprol
   - Antimycin A (antibiotics)
   - British antilewisite (BAL), an antidote used against war gas.

These inhibitors prevent the transfer of electrons from cytochrome b to cytochrome c_1.

3. **Complex IV (cytochrome oxidase),** inhibited by:
   - Cyanide
   - Carbon monoxide
   - H_2S.

These inhibitors prevent transfer of electrons from cyt aa_3 to molecular oxygen by inhibiting cytochrome oxidase and can therefore totally arrest respiration.

Inhibitors of Oxidative Phosphorylation (F_0F_1 ATPase)

Another set of inhibitors do not inhibit special complexes. Instead, these compounds block phosphorylation directly by inhibiting ***F_0F_1 ATPase*** enzyme. For example, antibiotic ***oligomycin*** completely blocks oxidation and phosphorylation by inhibiting an enzyme F_0F_1 ATPase required for phosphorylation.

Uncouplers of Oxidative Phosphorylation

- Uncouplers are chemical substances that allow electron transport chain in mitochondria but prevent the phosphorylation of ADP to ATP by uncoupling the essential linkage between electron transport and phosphorylation for the synthesis of ATP.
- Uncoupling agents are lipophilic (lipid soluble) compounds, which readily diffuse through the mitochondrial membrane and are capable of binding H^+ ion.
- Uncouplers allow transport of H^+ ion across the membrane towards the side with the lower H^+ ion concentration, thus preventing the formation of proton gradient which is required for the formation of ATP.
- Thus, these compounds make the inner mitochondrial membrane abnormally permeable to protons. The energy produced by the transport of electrons is released as heat rather than being used for synthesis of ATP. Examples of uncouplers include:
  - *2,4-Dinitrophenol (DNP)*
  - Dicuomarol (an anticoagulant).
  - Salicylate, a metabolite of aspirin.

Physiological uncouplers

Certain physiological substances act as uncouplers, e.g. thermogenin, thyroxine, bilirubin and free fatty acids. However, these compounds normally are not present in mitochondria in concentrations high enough to act as uncouplers.

Ionophores

Ionophores means ion carrier molecules. They are lipid soluble substances, capable of binding and carrying specific cations (other than H^+) through the mitochondrial membrane. Oxidative phosphorylation can be prevented by certain ionophores. They differ from uncoupling agents in that they promote the transport of cations other than H^+ through the membrane and abolish the membrane potential and/or pH gradient across the membrane and phosphorylation is therefore completely inhibited. For example, antibiotic **valinomycin** and **gramicidin.**

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Chemiosmotic Theory

The chemiosmotic coupling hypothesis is the most accepted theory. The chemiosmotic theory which was proposed by the ***British biochemist Peter Mitchell*** explains the mechanism of oxidative phosphorylation. The theory states that the energy released from oxidation generates the ***electrochemical potential*** by the pumping of protons across the inner mitochondrial membrane and the energy in this electrochemical potential can be converted into ATP. There are three basic principles of the theory.

1. The major electron carriers are organized into three complexes, complex I, III and IV **(Figure 10.6)**, which span the inner mitochondrial membrane. The energy released during transport of electrons from one carrier to another allows proton to be pumped across the inner mitochondrial membrane from the matrix to the outside (intermembrane space).
2. The inner mitochondrial membrane is impermeable to protons, so that their pumping results in the generation of the ***electrochemical potential.***
3. Due to this electrochemical potential or proton-motive force, the H^+ ions ejected out by electron transport flow back into the mitochondrial matrix down its electrochemical gradient through F_0F_1ATPase molecule **(Figure 10.6).** The free energy is released as H^+ ion flows back through the F_0F_1 ATPase into the zone of lower H^+ concentration. The free energy

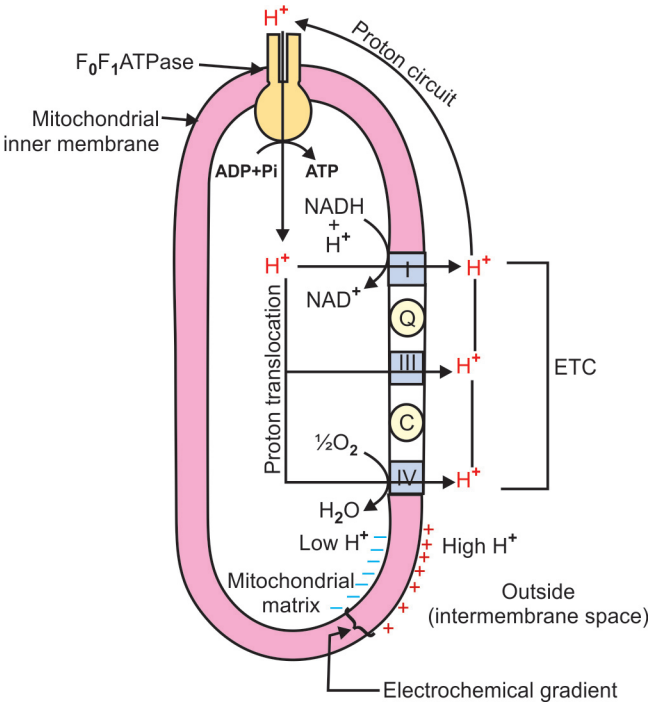


Figure 10.6: Schematic diagram of chemiosmotic theory in which respiratory complexes, I, III and IV act as a proton pump and generate electrochemical gradient

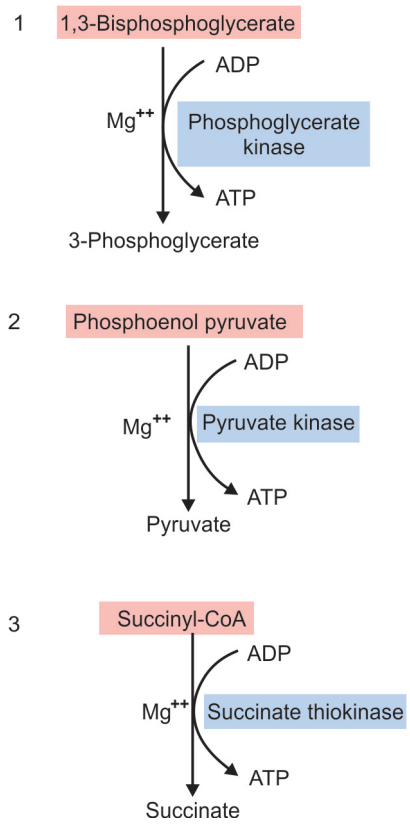


Figure 10.7: Substrate level phosphorylation processes. Reaction 1 and 2 are of glycolysis and 3 occurs in TCA cycle

released is coupled with the phosphorylation of ADP to ATP. The F_0F_1 ATPase catalyzes the addition of inorganic phosphate to ADP to form ATP.

P:O RATIO

- The P:O ratio is a measure of the number of high energy phosphates (i.e. number of ATP molecules) synthesized per atom of oxygen consumed or per molecule of water produced.
- The P: O ratio for oxidation of metabolites that yield NADH is 3 and the ratio for those that yield $FADH_2$ is 2.

SUBSTRATE LEVEL PHOSPHORYLATION

The formation of ATP, directly coupled to metabolic process without involvement of electron transport chain and molecular O_2, is called the production of ATP at the substrate level.

- Examples of substrate level phosphorylation processes are the conversion of:
  - Phosphoenolpyruvate to pyruvate
  - 1,3 bisphosphoglycerate to 3-phosphoglycerate
  - Succinyl-CoA to succinate **(Figure 10.7)**.

SHUTTLE SYSTEMS FOR OXIDATION OF EXTRAMITOCHONDRIAL NADH

- Most of the NADH and $FADH_2$ entering the mitochondrial electron transport chain arises from Kreb's cycle and β-oxidation of fatty acids, located in the mitochondria itself.
- Since, the inner mitochondrial membrane is not permeable to cytoplasmic NADH, how can the NADH generated by glycolysis, which take place outside of the mitochondria, be oxidized to NAD by respiratory chain located in mitochondria.
- Special shuttle systems carry reducing equivalents from cytosolic NADH (rather than NADH itself) into the mitochondria by an indirect route.
- Two such shuttle systems that can lead to the transport of reducing equivalent from the cytoplasm into mitochondria are:

1. The malate-aspartate shuttle
2. The glycerol phosphate shuttle.

The Malate-aspartate Shuttle System (Figure 10.8)

- The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate by cytosolic malate dehydrogenase.
- Malate, which carries the reducing equivalents, is transported across the inner membrane by a **dicarboxylate transport** system.
- The reducing equivalents carried by malate are then transferred to mitochondrial NAD^+ by **mitochondrial malate dehydrogenase** and malate itself gets reoxidized to oxaloacetate.
- The resulting mitochondrial NADH is oxidized by the mitochondrial electron transport chain, leading to formation of 3 molecules of ATP.
- The oxaloacetate so formed cannot pass through the membrane from the mitochondrion back into cytosol, so it is converted to **aspartate by transamination reaction** which is transported to the cytosolic side via amino acid transport system.
- In the cytosol, a reversal of the aspartate aminotransferase reaction gives rise to oxaloacetate and glutamate, thereby completing the "shuttle like" process.

Glycerol Phosphate Shuttle (Figure 10.9)

- The first step in this shuttle is the transfer of a pair of electrons from cytosolic NADH to dihydroxyacetone phosphate to from glycerol-3-phosphate catalyzed by cytosolic glycerol-3-phosphate dehydrogenase enzyme.
- Glycerol-3-phosphate in turn diffuses through the outer mitochondrial membrane into the intermembrane space of the mitochondria.
- Here glycerol-3-phosphate is reoxidized to dihydroxyacetone phosphate on the outer surface of

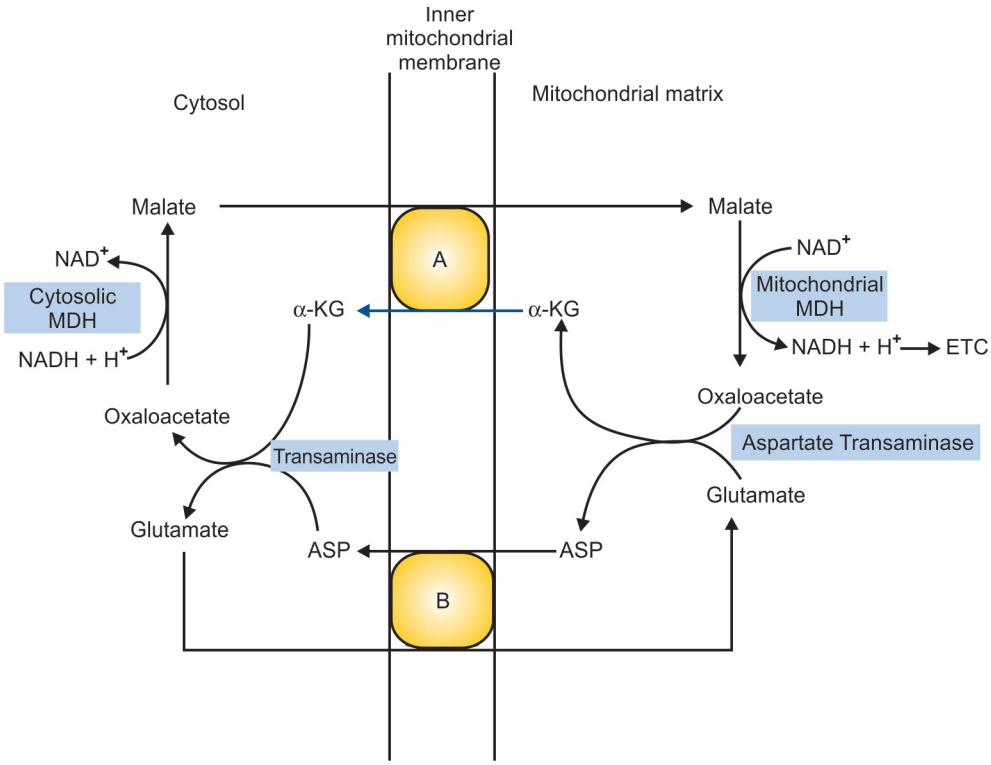


Figure 10.8: Malate-aspartate shuttle

Where	A	:	Malate/α-KG transporter
	B	:	Aspartate/glutamate transporter
	α-KG	:	α-ketoglutarate
	ASP	:	Aspartate
	MDH	:	Malate dehydrogenase
	ETC	:	Electron transport chain

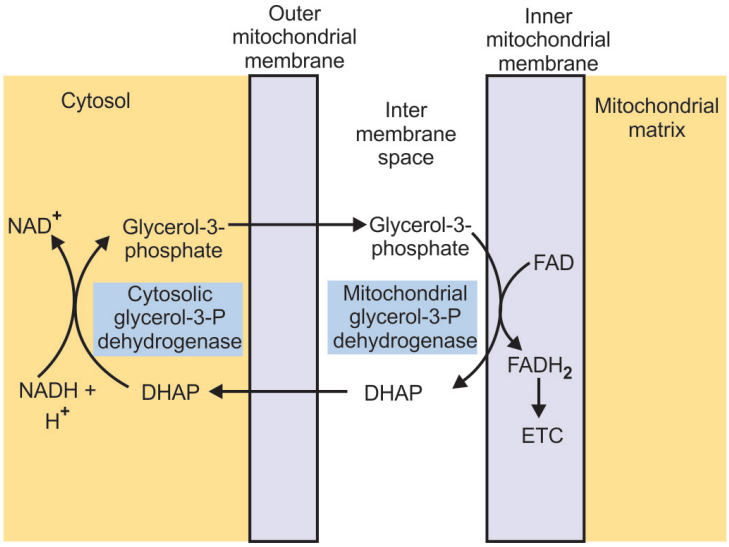


Figure 10.9: Glycerol phosphate shuttle
where, DHAP: Dihydroxyacetone phosphate

the inner mitochondrial membrane by a membrane bound FAD containing isoenzyme of glycerol-3-phosphate dehydrogenase.

- An electron pair from glycerol-3-phosphate is transferred to an FAD of the enzyme to form $FADH_2$. $FADH_2$ gets oxidized via ETC to generate 2 ATP.
- The dihydroxyacetone phosphate returns to the cytosol and can be reused for reduction of glycerol-3-phosphate.

SUMMARY

- Enzymes involved in biological oxidation and reduction are of the class oxidoreductases, which are classified into four groups: Oxidases, dehydrogenases, hydroperoxidases and oxygenases.
- Electron transport or respiratory chain is a series of highly organized oxidation-reduction enzymes, coenzymes and electron carrier proteins; cytochromes in the inner mitochondrial membrane. These are arranged into four complexes in order of increasing redox potential.
- Energy released from the oxidation of carbohydrates, fats and proteins is made available in mitochondria as reducing equivalents (H^+ or e^-), which are donated to the respiratory chain, which transfers them to molecular oxygen, reducing it to H_2O. The energy released in electron transport is used for the oxidative phosphorylation of ADP to ATP.
- According to the chemiosmotic hypothesis, the flow of electrons through complexes I, III and IV results in the pumping of protons across the inner mitochondrial membrane, making the matrix alkaline relative to extramitochondrial space. This proton gradient provides the energy (proton-motive force) for ATP synthesis from ADP and Pi with the help of F_0F_1ATPase.
- Formation of ATP can be inhibited by inhibiting electron transport or by inhibiting oxidative phosphorylation or by uncoupling oxidative phosphorylation by various compounds.
- The electrons of cytoplasmic NADH are transferred into the mitochondria by glycerol phosphate shuttle and malate-aspartate shuttle systems.

EXERCISE

Multiple Choice Questions (MCQs)

1. **All of the following are true regarding mitochondrial cytochromes, *except*:**
   a) They all contain heme groups
   b) All are bound to protein components
   c) Iron must remain in the ferrous state to function in electron transport
   d) They accept or donate one electron at a time

2. Which one of the following respiratory chain components reacts directly with molecular oxygen?
a) Coenzyme Q b) Cytochrome b
c) Cytochrome aa_3 d) Cytochrome C_1

3. In oxidative phosphorylation, the oxidation of one molecule of $FADH_2$ produces:
a) 3 ATP molecules b) 2 ATP molecules
c) 1 ATP molecule d) No ATP at all

4. All of the following statements are true regarding ETC, *except*:
a) Located in inner mitochondrial membrane
b) Components are organized in decreasing order of redox potential
c) Involved with ATP synthesis
d) Cyanide inhibits electron flow

5. The oxidation and phosphorylation of intact mitochondria is blocked by:
a) Puromycin b) Oligomycin
c) Streptomycin d) Erythromycin

6. Respiratory chain is found in:
a) Mitochondria
b) Cytoplasm
c) Nucleus
d) Endoplasmic reticulum

7. In oxidative phosphorylation, the oxidation of one molecule of NADH produces:
a) 2 ATP molecules b) 3 ATP molecules
c) 4 ATP molecules d) 1 ATP molecule

8. Which of the following vitamin is involved in ETC?
a) Thiamine b) Folic acid
c) Riboflavin d) Cobalamin

9. Which of the following trace elements has role in ETC?
a) Iron b) Iodine
c) Zinc d) Fluoride

10. An uncoupler of oxidative phosphorylation:
a) Inhibits electron transport and ATP synthesis
b) Allows electron transport to occur without ATP formation
c) Inhibits electron transport without impairment of ATP synthesis
d) Inhibits transfer of electrons from cytochrome aa_3 to molecular oxygen

11. Energy currency of the cell is:
a) High energy phosphate (~P)
b) S-adenosyl methionine (SAM)
c) Creatine phosphate
d) Glucose-6-phosphate

12. The enzyme that synthesizes ATP in oxidative phosphorylation is:
a) Hexokinase
b) NADH dehydrogenase
c) Cytochrome oxidase
d) F_0F_1ATPase

13. A naturally occurring uncoupler is:
a) Dicumarol b) 2,4-DNP
c) Thermogenin d) Salicylate

14. A biochemical substance that can act as an uncoupler is:
a) Bilirubin
b) Free fatty acid
c) Thyroxine
d) All of the above

15. Which of the following is an ionophore?
a) 2,4-dinitrophenol
b) Valinomycin
c) Thermogenin
d) Oligomycin

Correct Answers for MCQs

1-c	2-c	3-b	4-b
5-b	6-a	7-b	8-c
9-a	10-b	11-a	12-d
13-c	14-d	15-b	

CHAPTER

11

Nutrition

- Introduction
- Nutrients and their Role in Humans
- Nitrogen Balance
- Nutritional Quality of Proteins
- Basal Metabolic rate
- Thermogenic Effect (Specific Dynamic Action, SDA) of Food
- Balanced Diet
- Nutritional Disorders
- Summary
- Exercise

INTRODUCTION

Nutrition is the science of food and the nutrients and other substances contained in food. It is the study of their actions, interactions and balance in relation to health and disease. Thus, nutrition is concerned with the digestion, absorption, transport, metabolism and functions performed by the essential nutrients.

NUTRIENTS AND THEIR ROLE IN HUMANS

Nutrients are the necessary constituents of food required by organisms for growth and the maintenance of life. There are five classes of nutrients that contribute to an adequate diet **(Table 11.1)**. Each plays a special role. These may be divided into ***macronutrients*** and ***micronutrients.***

Macronutrients

These are ***proteins, fats*** and ***carbohydrates.*** They form the main bulk of food. In the Indian dietary pattern, they contribute to the total energy intake in the following proportions:

Proteins	7 to 15%
Fats	35 to 45%
Carbohydrates	50 to 70%

Table 11.1: Essential nutrients required by human beings

	Macronutrients	*Micronutrients*
1	**Carbohydrates:**	1 **Vitamins:**
	Glucose and Fiber	*Fat soluble vitamins*
		Retinol (A)
		Cholecalciferol (D)
2	**Fats:**	α-Tocopherol (E)
	Essential fatty acids	Phylloquinone (K)
	Linoleic acid	
	Linolenic acid	*Water soluble vitamins*
		Thiamine (B_1)
3	**Proteins:**	Riboflavin (B_2)
	Essential amino acids	Niacin
	Phenylalanine	Pyridoxine (B_6)
	Valine	Pantothenic acid
	Threonine	Biotin
	Tryptophan	Folic acid
	Isoleucine	Cobalamine (B_{12})
	Methionine	
	Histidine	2 **Mineral elements:**
	Arginine	Macrominerals
	Leucine	Na^+, K^+, Ca^+, Mg^{++}, P, S and Cl^-
	Lysine	Microminerals
		Cr, Co, Cu, F, I, Fe, Mn, Mo, Se, Zn

Micronutrients

These are vitamins and minerals **(Table 11.1).** They are called micronutrients because they are required in small amounts which may vary from a microgram to several grams.

Vitamins and minerals do not supply energy but they play an important role in the regulation of the metabolic activity in the body and help in the utilization of **proteins, fats** and **carbohydrates.** Minerals are also used for the formation of body structure and skeleton.

Role of Individual Nutrient

Carbohydrate

- Dietary carbohydrate is of two types:
  1. Available or digestible carbohydrate
  2. Unavailable or undigestible carbohydrate.
- The digestible carbohydrates are a major source of food energy, yielding 4 kcal/gm and provides about 50 to 70% of the energy requirement. In addition, these carbohydrates have ***protein sparing effect.***
- Unavailable or undigestible carbohydrates provide ***dietary fiber.*** Fiber is not digested by the digestive enzymes and does not serve as a source of energy. It is however a significant component of the diet.

Carbohydrate requirement

- The recommended intake of carbohydrate in balanced diet is placed so as to contribute between 50 to 70% of total energy intake.
- Most Indian diets contain amounts more than this, providing as much as 90% of total energy intake in some cases, which make the diet **imbalanced.**

Dietary Fiber

- Dietary fiber is the name given collectively to indigestible carbohydrates present in foods. These carbohydrates consist of:
  - Cellulose
  - Pectin
  - Gums
  - Mucilages.
- The dietary fiber is not digested by the enzyme of the human gastrointestinal tract, where most of the other carbohydrates like starch, sugars are digested and absorbed.
- Plant foods are the only sources of dietary fiber. It is found in vegetables, fruits, and grains.

Importance of fiber

Water holding capacity: The dietary fibers have a property of holding water and swell like sponge with a concomitant increase in viscosity. Thus, fiber adds bulk to the diet and increases transit time in the gut (gastric emptying time) due to high viscosity.

Adsorption of organic molecules: The organic molecules like bile acids, neutral sterols, carcinogens, and toxic compounds can be adsorbed on dietary fiber and facilitates its excretion.

It increases stool bulk: The fiber absorbs water and increases the bulk of the stool and helps to reduce the tendency towards constipation by increasing bowel movements.

Hypoglycemic effect of fiber: Recent studies have shown that gum present in fenugreek seeds (it contains 40% gum) is most effective in reducing blood sugar and cholesterol levels.

Hypocholesterolemic effects of fiber: Fiber has cholesterol lowering effect. Fiber binds **bile acids** and **cholesterol,** increasing their fecal exertion and thus decreasing plasma and tissue cholesterol level.

Significance of dietary fiber in medicine

High fiber diet is associated with reduced incidence of a number of diseases like:

- Coronary heart disease (CHD)
- Colon cancer
- Diabetes
- Diverticulosis
- Hemorrhoids (piles).

Adverse effect of dietary fiber

Dietary fiber also have some adverse effects on nutrition by binding some mineral elements and preventing their proper absorption. Thus, high dietary fiber intake may lead to deficiency of mineral elements.

Fats

Dietary fats are high energy yielding nutrients that provide 35 to 45% of the caloric intake. Fat yields 9 kcal/gm. Besides satisfying metabolic energy needs, there are two essential functions of dietary fat, namely, to provide:

1. A vehicle for the absorption of the fat soluble vitamins (A, D, E and K).
2. To supply essential fatty acids, **linoleic acid** and **linolenic acid** to the body.

- Dietary lipid also increases the **palatability** of food and produces a feeling of **satiety.**

Fat requirement

- The daily requirement of fat is not known with certainty. During infancy, fats contribute to a little over 50% of the total energy intake. This scales down to about 20% in adulthood.
- The ICMR Expert Group (1989) has recommended an intake of 20% of the total energy intake as fat of which at least 50% of fat intake should consist of vegetable oils rich in essential fatty acids.

Protein and Amino Acids

- Proteins are vital to any living organism.
- Proteins are important constituent of tissues and cells of the body. They form the important component of muscle and other tissues and vital body fluids like blood.
- The proteins in the form of enzymes and hormones are concerned with wide range of vital metabolic processes in the body.
- Protein as antibodies helps the body to defend against infections.
- Proteins supply **essential** and **nonessential amino acids** for the synthesis of protein and nitrogen for the synthesis of several key compounds such as neurotransmitter and heme.
- Thus, proteins are one of the most important nutrient required by the body and should be supplied in adequate amounts in the diet.
- The amino acids, which are not used for protein synthesis, are broken down to provide energy, which is a **wasteful** way of using proteins (this is not their primary function). **Hence, diet should contain adequate carbohydrate and fat to provide energy so that the proteins in the diet are most economically used for the formation of body proteins to fulfill other functions essential to life.**

Essential amino acids

- Any amino acid that humans either cannot synthesize or are unable to synthesize in adequate quantity is termed **"essential"** and rest of the amino acids are called **"nonessential"** as they can be formed in the body.
- An essential amino acid must be provided in the diet. An absence of an essential amino acid from the diet impairs protein synthesis and generally causes **negative nitrogen balance**, i.e. the total nitrogen losses in the urine, feces and sweat exceed the dietary nitrogen intake.
- Ten of the twenty amino acids found in proteins are essential for humans **(Table 11.1)** ***(Refer Chapter 4 also).***
- Of the 10 essential amino acids, 8 are essential at all times during life. The other two namely **histidine** and **arginine** are required in the diet during periods of rapid growth as in childhood and pregnancy and called **semiessential.**

Requirement of essential amino acid

Each amino acid is required in differing amounts. Minimum requirements of essential amino acids are shown in **Table 11.2**

Table 11.2 : Minimum requirement of essential amino acids

Essential amino acid	*Requirement (mg / kg body weight / per day)*
Phenylalanine	14
Leucine	11
Lysine	9
Valine	14
Isoleucine	10
Threonine	6
Methionine	14
Tryptophan	3

NITROGEN BALANCE

Catabolism of amino acids leads to a net loss of nitrogen from the body. This loss must be compensated by the diet in order to maintain a constant amount of body protein. ***Nitrogen balance studies evaluate the relationship between the nitrogen intake (in the form of protein) and nitrogen excretion.***

Three situations of nitrogen balance are possible as follows:

1. Nitrogen equilibrium
2. Positive nitrogen balance
3. Negative nitrogen balance.

Nitrogen equilibrium

- In normal adults, nitrogen intake = nitrogen excretion. The subject is said to be in nitrogen equilibrium or balance.
- In this situation, the rate of body protein synthesis is equal to the rate of degradation.

Positive nitrogen balance

- In this, nitrogen intake > nitrogen excretion, i.e. intake of nitrogen is more than excretion.

- It shows that nitrogen is retained in the body, which means that protein is laid down.
- This occurs in growing ***infants*** and ***pregnant women.***

Negative nitrogen balance

- In this, nitrogen intake < nitrogen excretion, i.e. nitrogen output exceeds input, this occurs during ***serious illness*** and **major *injury*** and ***trauma,*** in ***advanced cancer*** and following failure to ingest adequate or sufficient high quality protein, e.g. in ***kwashiorkor*** and ***marasmus.***
- If the situation is prolonged, it will ultimately lead to death.

NUTRITIONAL QUALITY OF PROTEINS

- In judging the adequacy of dietary proteins to meet the human needs, not only the quantity but the nutritional quality of the dietary proteins also matters.
- Proteins present in different foods vary in their nutritional quality because of the differences in their amino acid composition. **The quality of protein depends on the pattern of essential amino acids it supplies.**
- The best quality protein is the one which provides essential amino acid pattern very close to the pattern of the tissue proteins. Egg proteins, human milk protein, satisfy these criteria and are classified as high quality proteins and serve as **reference protein** for defining the quality of other proteins.

Assessment of Protein Quality

The quality of a protein is assessed by comparison to the **"reference protein",** which is usually egg protein. Four methods of assessment of protein quality are:

1. Chemical score or amino acid score
2. Net protein utilization (NPU)
3. Protein efficiency ratio (PER)
4. Biological value (BV).

Chemical score or amino acid score

It is a measure of the concentration of each essential amino acid in the test protein which is then compared with reference protein (usually egg protein). It is calculated by following formula:

$$\text{Amino acid score} = \frac{\text{Number of mg of one amino acid per gm of test protein}}{\text{Number of mg of the same amino acid per gm of egg protein}} \times 100$$

This mode of chemical assessment does not take into account the digestibility of dietary proteins. Hence, biological methods based on growth or nitrogen (N) retention are used to determine the overall quality of a protein.

Net protein utilization (NPU)

- It is a product of digestibility coefficient and biological value divided by 100.
- Biological measures of NPU gives a more complete expression (both absorption and retention) of protein quality than the amino acid score as said above. It is calculated by the following formula:

$$\text{NPU} = \frac{\text{Nitrogen retained by the body}}{\text{Nitrogen intake}} \times 100$$

- The protein requirement varies with the NPU of dietary protein. If the NPU is low, the protein requirement is high and vice versa.
- The NPU of the protein of Indian diets varies between 50 and 60.

Protein efficiency ratio (PER)

The overall quality, i.e. nutritive value of a food protein can be determined with laboratory animal like rat as follows.

The gain in weight of young animals per gm of protein consumed is measured and the value obtained is used to determine the protein efficiency ratio (PER) as follows:

$$\text{PER} = \frac{\text{Gain in body weight in gm}}{\text{Protein ingested in gm}}$$

Biological value

Biological value of protein is defined as the percentage of absorbed nitrogen retained by the body and is calculated by:

$$\text{Biological value (BV)} = \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

- The amount of nitrogen in the diet eaten and in excreta of adult animals are measured and the percentage of nitrogen retained by animals from out of nitrogen absorbed from the diet is calculated. The value thus obtained is the **"biological value" (BV)** of the protein.
- This test also gives an estimate of digestibility of the protein. But it cannot take into account the nitrogen that might be lost during the digestion process. The chemical score, BV, NPU, PER and deficient amino acids of some important food proteins is given in **Table 11.3.**

Table 11.3: Nutritive value of proteins of some foodstuffs

Protein source	*Protein type*	*BV*	*Chemical score*	*NPU*	*PER*	*Limiting (deficient) amino acid*
Animal protein	Egg	96	100	96	3.8	Nil
	Milk	90	65	85	2.8	S-containing amino acid
	Meat	74	70	76	3.2	S-containing amino acid
	Fish	80	60	74	3.5	Tryptophan
Vegetable proteins	Rice	80	60	77	1.7	Lysine, Threonine
Cereals	Wheat	66	42	61	1.3	Lysine, Threonine
Pulses	Bengal gram	74	45	61	1.1	S-containing amino acid
	Red gram	72	45	54	1.7	S-containing amino acid
Oil seeds	Groundnut	55	44	45	1.7	Lysine, Threonine S-containing amino acid
	Soya bean	62	55	55	2.1	S-containing amino acid

BV = Biological value; NPU = Net Protein Utilization; PER = Protein energy ratio; S-containing = Sulfur containing

Mutual supplementation of proteins

- It is seen that generally animal proteins are of higher biological value than proteins from plant foods. Plant proteins are of poorer quality since essential amino acids (EAAs) composition is not well balanced and a few EAAs deviate much from the optimal level present in egg, e.g.
  - In comparison with egg protein, cereal proteins are poor in amino acid lysine.
  - Pulses and oil seed proteins are rich in lysine but they are poor in sulfur containing amino acids.
- Such proteins individually are therefore **incomplete proteins.**
- However, relative insufficiency of a particular amino acid of any vegetable food can be overcome by judicious combination with other vegetable foods, which may have adequate level of that limiting (deficient) amino acid.
- The amino acid composition of these proteins **complement** each other and the resulting mixture will have an amino acid pattern better than either of the constituent proteins of the mixture.
- This is the procedure normally used to improve quality of vegetable proteins. This phenomenon is called **mutual supplementation effect of amino acid.**
- Thus, a protein of cereals, deficient in lysine and pulses with adequate lysine content have a mutually supplementary effect, a deficiency of an amino acid in one can be made good by an adequate level in another, if both are consumed together.
- Thus, the habitual diets of vegetarians in India based on cereal and pulse helps to overcome the deficiency of certain essential amino acids in one food.

Protein requirement

- The requirement is dependent on the quality of dietary protein.
- The ICMR Expert Group, suggested an intake of one gram of protein per kg of body weight for adult males and females, assuming, an NPU of 65 for dietary protein. The requirement should be nearly double for growing children, pregnant and lactating women.

Vitamins and Minerals

The nutritional aspects including metabolism, biochemical functions dietary sources, requirements and associated pathological conditions for vitamins (**chapter 7**) and for minerals (**chapter 17**) have already been discussed in much detail.

RECOMMENDED DAILY ALLOWANCE (RDA)

The term ***'recommended daily allowance'*** is defined as the amount of nutrient sufficient for the maintenance of health in nearly all individuals. Estimates of allowances are based on the defined minimum requirement plus a safety margin for most individuals.

RDAs for selected nutrients for adults are shown in **Table 11.4.**

Table 11.4: Recommended daily dietary allowances for Indians

Group	Particulars	Net energy kcal/d	Protein g/d	Fat g/d	Calcium mg/d	Iron mg/d	Vit A Retinol µg/d	Thiamine mg/d	Riboflavin mg/d	Nicotinic acid mg/d	Pyridoxin mg/d	Ascorbic acid mg/d	Folic acid µg/d	Vit B12 µg/d
Man	Sedentary work	2425						1.2	1.4	16				
	Moderate work	2875	60	20	400	28	600	1.4	1.6	18	2.0	40	100	1
	Heavy work	3800						1.6	1.9	21				
Woman	Sedentary work	1875						0.9	1.1	12				
	Moderate work	2225	50	20	400	30	600	1.1	1.3	14	2.0	40	100	1
	Heavy work	2925						1.2	1.5	16				
	Pregnant woman	+ 300	+ 15	30	1000	38	600	+0.2	+0.2	+2	2.5	40	400	1
	Lactation	+550	+25	45	1000	30	950	+0.3	+4	+3	2.5	80	150	1.5

ENERGY REQUIREMENTS

Energy is the principle requisite for body function and growth. Total energy required by an individual depends on the three energy requiring body processes. These are:

1. The basal metabolic rate (BMR)
2. The thermogenic effect (specific dynamic actions, SDA) of food
3. Physical activity.

Besides the above three processes, extra provision of energy has to be made for growth, pregnancy and lactation.

BASAL METABOLIC RATE (BMR)

The BMR is the energy expenditure necessary to maintain basic physiologic functions, such as:

- The activity of the heart
- Respiration
- Conduction of nerve impulses
- Ion transport across membranes
- Reabsorption in the kidney
- Metabolic activity such as synthesis of macromolecules.

Definition of BMR

It is defined as the energy expenditure at rest, awake (but not during sleep), in a thermoneutral (warm) environment 8 to 12 hours after the last meal and 8 to 12 hours after any significant physical activity.

Measurement of basal metabolism

Basal metabolism can be measured by:

- Calorimeter directly by measuring the heat dissipated under basal condition
- Indirectly by measuring oxygen consumption.

Factors affecting BMR

BMR differs among different individuals. It depends on many factors as follows:

Gender or sex: The BMR of the males is slightly higher than that of females, partly due to:

- Women's lower percentage of muscle mass (lean body mass) and higher percentage of adipose tissue (that has lower rate of metabolism), when compared to men of the same body weight.
- The difference in sex hormone profile of the two genders.

Age: Decline in BMR with increasing age is probably related to loss of muscle mass (lean body mass) and replacement of muscle with adipose tissue that has lower rate of metabolism.

Nutritional state: BMR is low in starvation and undernourishment as compared to well fed state. Starvation leads to an adaptive decrease in BMR, which results from a decrease in lean body mass.

Body size or surface area: The BMR is directly proportional to the surface area of the subject. Larger the surface area, greater will be the heat loss and equally higher will be the heat production and BMR.

Body composition: The BMR is proportionate to lean body mass, (LBM). LBM is the body weight minus non-essential (storage triacylglycerol) weight. Adipose tissue is not as metabolically active as lean body mass. BMR is often expressed as per kilogram of lean body mass or fat-free mass. Therefore, higher the percentage of adipose tissue in the body, lower the BMR/kg body weight.

Endocrinological or hormonal state: In hyperthyroidism, the BMR is increased and in hypothyroidism it may be decreased by up to 40%, leading to weight gain.

Environmental temperature or climate: In colder climate, the BMR is higher and in tropical climates the BMR is proportionally low. Stress, anxiety and disease states, especially infections, fever, burns and cancer also increase the BMR.

Drugs: Smoking (nicotine), coffee (caffeine) and tea (theophylline) increase the BMR, whereas β-blockers tend to decrease energy expenditure.

Normal values of BMR

- BMR values are expressed as **kcal** per square meter of body surface per hour. In adults, BMR for:
  - Healthy males is 40 kcal/sqm/hour
  - Healthy females, it is 37 kcal/sqm/hour.
- This means that the total caloric expenditure in 24 hours to complete basal state is 1800 kcal for adult males and 1400 kcal for adult females, assuming that the total body surface areas are 1.8 sqm and 1.6 sqm respectively.

Clinical application of BMR

- Determination of BMR is useful for the diagnosis of disorders of thyroid. In hypothyroidism, BMR is low while in hyperthyroidism it is elevated.
- BMR is used in calculating caloric requirements of an individual and planning of diets.

THE THERMOGENIC EFFECT (SPECIFIC DYNAMIC ACTION, SDA) OF FOOD

Another component of energy expenditure in man is diet induced thermogenesis, also known as ***postprandial thermogenesis.***

- This is the energy expended in the digestion, absorption, storage and subsequent processing of food. This is called ***thermogenic effect of food*** because these processes require energy and generate heat. The thermogenic effect of food is equivalent to about 5 to 10% of total energy expenditure.
- This effect was originally attributed solely to the metabolic processing of protein and was termed ***'specific dynamic action' (SDA),*** but it is now recognized as an effect produced by the consumption of all dietary fuels.
- The consumption of protein produces the greatest increase in energy loss compared to fat or carbohydrate. The thermogenic effect of food is given below:
  - Protein 20 to 30% of intake
  - Fat 2.5 to 4% of intake
  - Carbohydrate 5 to 6% of intake.
- It varies considerably from individual to individual. It has been suggested that in some people, a high degree of diet induced thermogenesis may be a factor which allows them to maintain their normal body weight even after overeating.

Physical Activity

Physical activity is the largest variable affecting energy expenditure. For convenience, the activity level may be divided into three groups - **sedentary, moderate** and **heavy** with regard to their physical activity and requirement of energy. Additional calories are to be added for each group.

Sedentary work — +30 to 40% of BMR
Moderate work — +40 to 50% of BMR
Heavy work — +50 to 60% of BMR

Table 11.5 shows energy expenditure during different types of physical activity for a 70 kg man.

Table 11.5: Energy expenditure during different types of activity for a 70 kg man

Kind of activity	*Calories/h*
Sleeping	65
Awake lying still	77
Sitting at rest	100
Standing relaxed	105
Walking slowly (2.6 miles/h)	200
Swimming	500
Running (5.3 miles/h)	570
Walking upstairs rapidly	1100

BALANCED DIET

Definition of balanced diet

A balanced diet is defined as one which contains a variety of foods in such quantities and proportions that the need for energy, amino acids, vitamins, minerals, fats, carbohydrate and other nutrients is adequately met for maintaining health, vitality and general well-being

and also makes a small provision for extra nutrients to withstand short duration of illness.

Balanced diet suggested by ICMR

The dietary pattern varies widely in different parts of the world. It is generally developed according to the:

- Kinds of food produced (which depends upon the climatic conditions of the region)
- Economic capacity
- Religion
- Customs
- Tastes and habits of the people.

ICMR has suggested balanced diet for different age groups, sex, and under various occupations for physical activity. These are given in **Table 11.6**. During pregnancy and lactation, additional food is required. This is shown in **Table 11.7**. For nonvegetarians, ICMR has recommended substitution of a part of pulses by animal food **(Table 11.8)**.

NUTRITIONAL DISORDERS

When balanced diet is not consumed by a person for a sufficient length of time, it leads to nutritional deficiencies or disorders. This nutritional status is called ***malnutrition.*** The most common nutritional disorders are discussed hereunder:

Protein Caloric Malnutrition (PCM) or Protein Energy Malnutrition (PEM)

PEM is caused by protein and energy deficiency and has been identified as a major health and nutrition problem in India.

Classification of PEM

PEM can be classified as:

- Marasmus (Greek: "to waste") or nonedematous PEM
- Kwashiorkor or edematous PEM.

Table 11.6: Balanced diet suggested by ICMR

	Adult man			*Adult woman*		
Food item	*Sedentary work*	*Moderate work*	*Heavy work*	*Sedentary work*	*Moderate work*	*Heavy work*
	Quantity gram per day			*Quantity gram per day*		
Cereals	460	520	670	410	440	575
Pulses	40	50	60	40	45	50
Leafy Vegetables	40	40	40	100	100	100
Other Vegetables	60	70	80	40	40	50
Roots and tubers	50	60	80	50	50	60
Milk	150	200	250	100	150	200
Oil and Fat	40	45	65	20	25	40
Sugar or Jaggery	30	35	55	20	20	40

Table 11.7: Additional allowances during pregnancy and lactation

Food items	*During pregnancy gm/day*	*Calories (kcal)*	*During lactation gm/day*	*Calories (kcal)*
Cereals	35	118	60	203
Pulses	15	118	60	203
Milk	15	83	100	83
Fat	-	-	10	90
Sugar	10	40	10	90
Total	-	293	-	521

Table 11.8: Suggested substitution for nonvegetarians

Food item which can be deleted in nonvegetarian diets	*Substitution that can be suggested for deleted item or items*
50% of pulses (20-30 g)	One egg or 30 g of meat or fish Additional 5 g of fat or oil
100% of pulses (40-60 g)	Two eggs or 50 g of meat or one egg + 30 g meat or fish 10 g of fat or oil

Marasmus or nonedematous PEM

- Marasmus is a chronic condition resulting from a deficiency of both protein and energy. Marasmus occurs in famine (extreme scarcity of food) areas when infants are weaned from breast milk and given inadequate bottle feedings of thin watery gruels (liquid food) of native cereals or other plant foods. These watery gruels are usually deficient in both calories and proteins.
- Marasmus is characterized by:
  - Growth retardation
  - Anemia
  - Fat and muscle wasting.
- Severe loss of body fat and muscle results in an ***emaciated*** appearance. Starvation adaptations cause serum protein and electrolyte concentrations to remain within their normal range and **do *not show edema.***

Kwashiorkor or edematous PEM

- Kwashiorkor refers to conditions caused by severe protein deficiency in individuals with an adequate energy intake. Kwashiorkor is an African word that means ***"weaning disease".*** When children are weaned from protein rich breast milk, they receive insufficient protein.
- The clinical symptoms of kwashiorkor include:
  - Anorexia
  - Severe edema associated with hypoalbuminemia
  - Moon face
  - Depigmented hair and skin
  - Fatty liver
  - Distended abdomen (due to enlarged liver).
- The edema is due to low oncotic pressure in the plasma due to ***hypoalbuminemia.*** Synthesis of plasma proteins by the liver is also decreased, this in turn impairs the export of triglycerides and other lipids from the liver, resulting in a **fatty liver.**

The major differences between kwashiorkor and marasmus are given in **Table 11.9.**

Table 11.9: Differences between kwashiorkor and marasmus

Features	*Kwashiorkor*	*Marasmus*
Age of onset	1-5 year	below 1 year
Edema	Present	Absent
Serum albumin	Hypoalbuminemia	Normal or slightly decreased
Fatty liver	Present	Absent
Muscle wasting	Absent or mild	Severe
Fat reserves	Normal to mildly diminished	Absent

Obesity

This is the pathological state resulting from the consumption of excessive quantity of food over an extended period of time. Obesity is defined as an accumulation of excess fat in the body.

The problem of obesity arises due to an ***imbalance of energy intake in relation to energy expenditure.*** The degree of obesity is commonly assessed by means of the ***body mass index (BMI)*** **(Table 11.10).**

$$\text{BMI} = \frac{\text{Body weight (kg)}}{\text{Height (m}^2\text{)}}$$

Table 11.10: Relationship between BMI and degree of obesity

Value of BMI	*Degree of obesity*
20-25	Normal
25-30	Overweight or obesity grade I
30-35	Over obesity or grade II
above 35	Gross obesity or grade III

The causes of obesity

Factors which influence the development of obesity are as follows:

- Metabolic
- Hormonal
- Genetic.

Metabolic: Obesity may result due to accumulation of triacylglycerol. The triacylglycerol accumulates when:

- Caloric intake exceeds the amount needed for body function and the amount of work being done.
- Deficiency of the enzyme ATPase which impairs normal energy metabolism and gain more weight.

Hormonal: Obesity may result from endocrine disorders like:

- Hypothyroidism
- Hypogonadism
- Hypopituitarism
- Cushing's syndrome.

Genetic: Several genes have the potential to cause obesity in humans, e.g. mutation in **leptin gene (ob gene)** results in obesity. A peptide hormone **leptin** released from adipocytes, is a product of gene **"ob gene".** Leptin leads to supression of food intake.

Grossly obese humans have a failure in production of leptin.

Obesity as a health risk

An obese person has the risk of:

- Hypertension
- Coronary heart disease and stroke
- Insulin resistant diabetes mellitus
- Atherosclerosis
- Cancer.

SUMMARY

- Nutrition is the science of food and the nutrients and other substances contained in food. Nutrients are the necessary constituents of food required by an organism.
- Nutrients are divided into two: i) macronutrients, include carbohydrates, proteins and lipids; ii) micronutrients, which include minerals and vitamins.
- Energy is the principal requisite for body function and growth. It is expressed in kilocalorie (kcal) or abbreviated with capital "C".
- The energy expenditure by an individual depends on three main factors: i) the basal metabolic rate (BMR); ii) the thermogenic effect (specific dynamic action, SDA) of food; iii) physical activity.
- A balanced diet is defined as one which contains a variety of foods in such quantities and proportions that the need for energy, amino acids, vitamins, minerals, fats, carbohydrates and other nutrients is adequately met for maintaining health, vitality, and general well-being and also makes a small provision for extra nutrients to withstand short duration of illness.
- The most common nutritional disorders due to undernutrition are protein energy malnutrition (PEM) caused by protein and energy deficiency.
- PEM can be classified as marasmus and kwashiorkor.
- Marasmus is a chronic condition resulting from a deficiency of both protein and energy and kwashiorkor is a condition caused by severe protein deficiency with an adequate energy intake.

EXERCISE

Solve

Case History 1

A 4-year-old child comes with retarded growth and pedal edema. She also has discoloration of skin and hair. On enquiring by the physician, mother told the physician that child was on breast milk up to one and a half years of age and for the past two and a half years she was being given rice and dal. The laboratory data of the child showed hypoalbuminemia.

Questions

1. Name the probable condition.
2. What is the cause of edema?
3. What are the preventive measures?

Case History 2

One-year-old female child comes to OPD with retarded growth and emaciated appearance (wasting of muscles). No edema is present. The condition is diagnosed by physician as marasmus.

Questions

1. What is marasmus?
2. What is the difference between marasmus and kwashiorkor?
3. What is the cause of emaciated appearance?

Multiple Choice Questions (MCQs)

1. **Dietary fiber has following effects, *except:***
   a) Increases stool bulk
   b) Lowers cholesterol
   c) Decreases risk of cardiovascular disease
   d) Increases risk of diabetes mellitus.

2. Which of the following dietary sources has the greatest thermogenic effect?
a) Fat
b) Protein
c) Carbohydrate
d) Vitamins

3. Following features are seen in marasmus, *except:*
a) Edema
b) Muscle wasting
c) Growth retardation
d) Anemia

4. The quality of a protein is assessed by:
a) Protein efficiency ratio (PER)
b) Biological value (BV)
c) Net protein utilization (NPU)
d) All of the above

5. An obese person has the health risk of:
a) Atherosclerosis
b) Hypertension
c) Coronary heart disease and stroke
d) All of the above

Correct Answers for MCQs

1-d 2-b 3-a 4-d 5-d

CHAPTER

12

Carbohydrate Metabolism

INTRODUCTION

The major source of carbohydrates is found in plants. Glucose is the universal fuel for human cells. The glucose concentrations in the body are maintained within limits by various metabolic processes.

DIGESTION, ABSORPTION AND TRANSPORT OF CARBOHYDRATES

Digestion of Carbohydrates

The principal sites of carbohydrate digestion are the **mouth** and **small intestine.** The dietary carbohydrate consists of:

- **Polysaccharides:** Starch, glycogen and cellulose
- **Disaccharides:** Sucrose, maltose and lactose
- **Monosaccharides:** Mainly glucose and fructose.

Monosaccharides need no digestion prior to absorption, whereas disaccharides and polysaccharides must be hydrolyzed to simple sugars before their absorption **(Figure 12.1).**

Digestion in Mouth

Digestion of carbohydrates begins in the mouth. Salivary glands secrete **α-*amylase (ptylin),*** which initiates the hydrolysis of a starch. During mastication, salivary α-amylase acts briefly on dietary starch in random manner breaking some α-(1 → 4) bonds, α-amylase hydrolyzes starch into dextrins.

Digestion in Stomach

Carbohydrate digestion halts temporarily in the stomach because the high acidity inactivates the salivary α-amylase.

Digestion in Intestine

Further digestion of carbohydrates occurs in the small intestine by **pancreatic enzymes.** There are two phases of intestinal digestion.

1. Digestion due to pancreatic **α-amylase**
2. Digestion due to intestinal enzymes : **sucrase, maltase, lactase, isomaltase.**

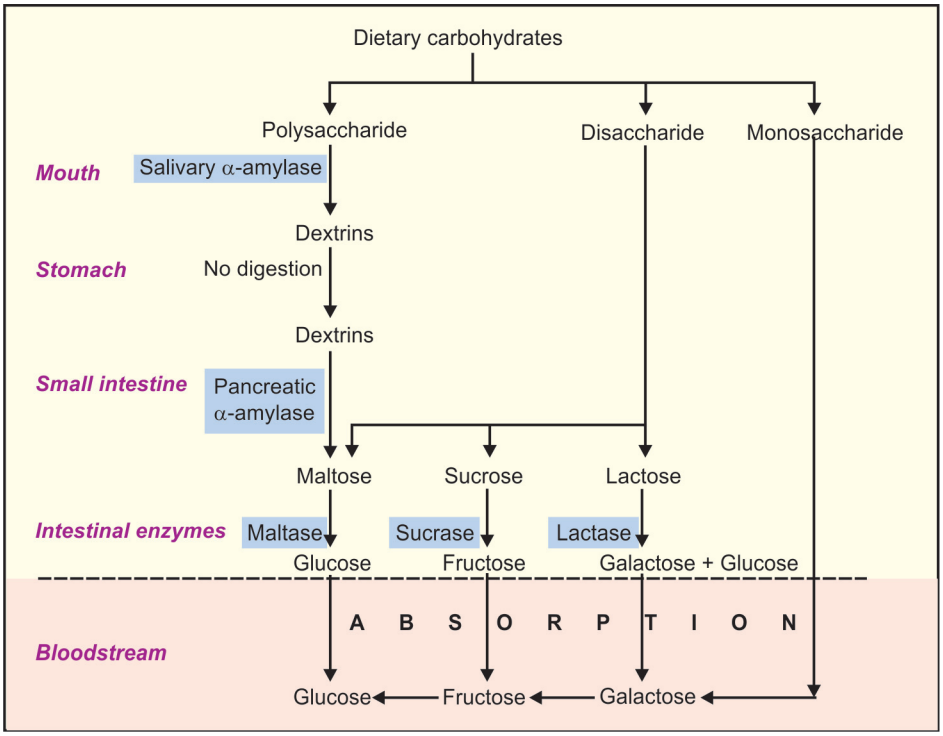


Figure 12.1: Flow sheet of digestion of carbohydrates

Digestion due to pancreatic α-amylase

- The function of pancreatic α-amylase is to degrade dextrins further into a mixture of maltose, isomaltose and α-limit dextrin.
- The α-limit dextrins are smaller oligosaccharides containing 3 to 5 glucose units.

Digestion due to intestinal enzymes

Enzymes responsible for the final phase of carbohydrate digestion are located in the brush-border membrane. The enzymes and the reactions they catalyze are as follows:

- Maltose —(Maltase)→ Glucose + Glucose
- Isomaltose —(Isomaltase)→ Glucose + Glucose
- Sucrose —(Sucrase)→ Glucose + Fructose
- Lactose —(Lactase)→ Glucose + Galactose
- α-Limit dextrin —(Dextrinase)→ Glucose + Maltose

The end products of carbohydrate digestion are ***glucose, fructose*** and ***galactose*** which are readily absorbed through the intestinal mucosal cells into the bloodstream.

Absorption of Carbohydrates

Carbohydrates are absorbed as monosaccharides from the intestinal lumen. Two mechanisms are responsible for the absorption of monosaccharides:

1. Active transport against a concentration gradient, i.e. from a low glucose concentration to a higher concentration.
2. Facilitative transport, with concentration gradient, i.e. from a higher concentration to a lower one.

Active Transport

The transport of glucose and galactose across the brush-border membrane of mucosal cells occurs by an ***active transport.*** Active transport is an energy requiring process that requires a specific transport protein and the presence of sodium ions **(Figure 12.2).**

- A sodium dependent glucose transporter (SGLT-1) binds both glucose and Na^+ at separate sites and

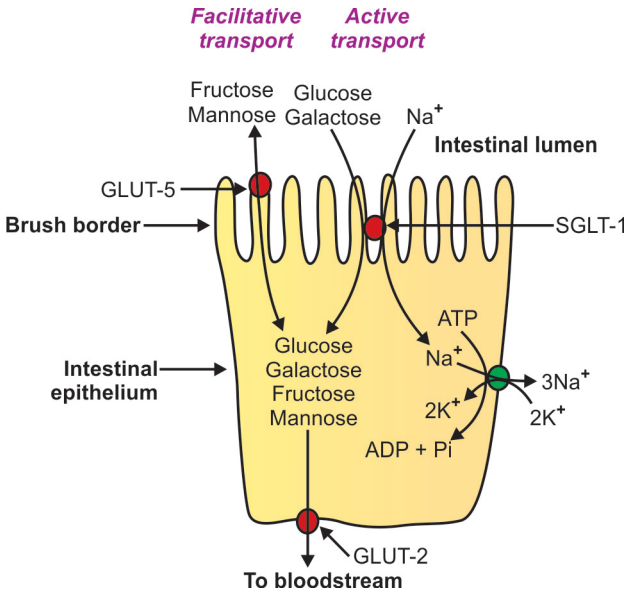


Figure 12.2: Transport of glucose, fructose, galactose and mannose

transports them both through the plasma membrane of the intestinal cell.

- The Na^+ is transported down its concentration gradient (higher concentration to lower concentration) and at the same time glucose is transported against its concentration gradient.
- The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a **sodium pump** that expels Na^+ from the cell in exchange of K^+ **(Figure 12.2)**.

Facilitative Transport

- ***Fructose*** and ***mannose*** are transported across the brush border by a Na^+ independent facilitative diffusion process, requiring specific glucose transporter, GLUT-5.
- Movement of sugar in facilitative diffusion is strictly from a higher concentration to a lower one until it reaches an equilibrium.
- The same transport can also be used by glucose and galactose if the concentration gradient is favorable.

Transport of Carbohydrates

The sodium independent transporter, GLUT-2 that facilitates transport of sugars out of the mucosal cells, thereby entering the portal circulation and being transported to the liver.

Lactose Intolerance

- Intolerance to lactose (the sugar of milk) not to milk. This is the most common disorder due to deficiency of enzyme ***lactase.***
- In this condition, lactose accumulates in the gut which undergoes bacterial fermentation in the large intestine with the production of H_2 and CO_2 gases and low molecular weight acids like acetic acid, propionic acid and butyric acid which are osmotically active.
- Abdominal cramps and flatulence results from the accumulation of gases and the osmotically active products that draw water from the intestinal cells into the lumen resulting in ***diarrhea*** and ***dehydration.***
- Treatment for this disorder is simply to remove lactose from the diet.

METABOLIC FATE OF CARBOHYDRATES

The major metabolic pathways of carbohydrates are :

- **Glycolysis:** The oxidation of glucose to pyruvate and lactate.
- **Citric acid cycle:** (Krebs cycle or tricarboxylic acid cycle) oxidation of acetyl-CoA to CO_2 and water.
- **Gluconeogenesis:** Synthesis of glucose from non-carbohydrate substances such as lactate, glycerol, glucogenic amino acids, etc.
- **Glycogenesis:** Synthesis of glycogen from glucose.
- **Glycogenolysis:** Breakdown of glycogen to glucose.
- **Hexose monophosphate Shunt**(HMP Shunt): It is an alternative pathway for oxidation of glucose. Some pentoses can also be oxidized through this pathway.
- **Uronic acid pathway:** Glucose is oxidized to glucuronic acid.
- **Galactose metabolism:** Galactose is converted to glucose.
- **Fructose metabolism:** Fructose is converted to glucose or metabolized in liver.

GLYCOLYSIS

Definition

Glycolysis is the sequence of reactions that converts glucose into pyruvate in the presence of oxygen (aerobic) or lactate in the absence of oxygen (anaerobic) with the production of ATP. This pathway is also called ***Embden Meyerhof pathway.***

It is a unique pathway since it can utilize oxygen if available, or it can function in the total absence of oxygen.

Location

Glycolysis is the major pathway for the utilization of glucose and is found in **cytosol** of all cells.

Reactions of Glycolysis (Figure 12.3)

The breakdown of glucose (6-carbon compound) to two moles of pyruvate (3-carbon compound) is brought about by sequential action of ten enzymes which can be divided into two phases.

1. Ist phase: Energy requiring phase or preparative phase
2. IInd phase: Energy generating phase.

Ist phase: Energy requiring phase or preparative phase

1. Glucose is phosphorylated to glucose-6-phosphate by **hexokinase** or **glucokinase** and ATP is required as a phosphate donor. Hexokinase and glucokinase are isoenzymes. The difference

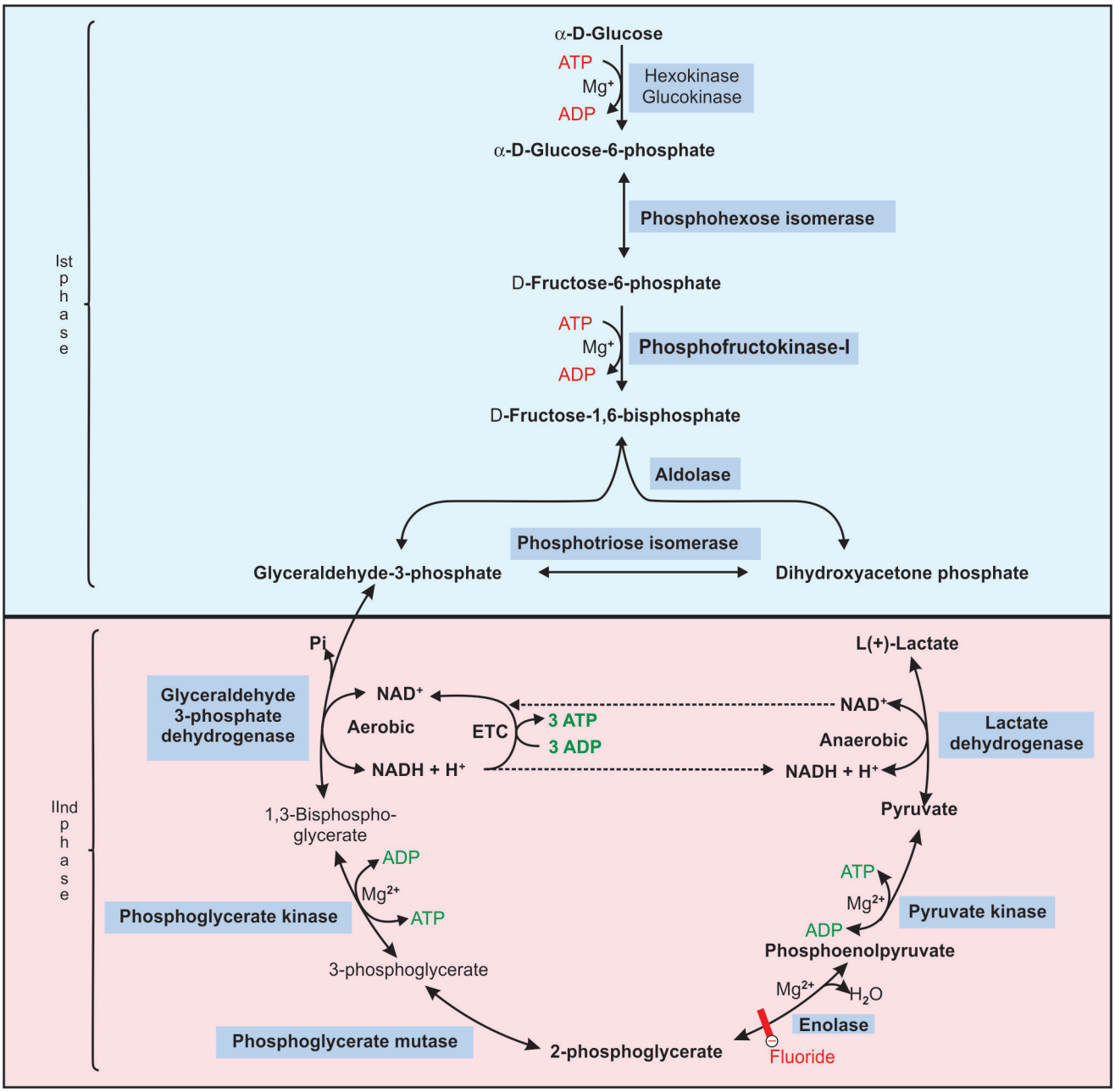


Figure 12.3: The reactions of glycolysis

Table 12.1: Difference between hexokinase and glucokinase

Hexokinase	*Glucokinase*
Present in extrahepatic tissue	Present in liver
High affinity for its substrate glucose (low K_m)	Low affinity for its substrate glucose (high K_m)
Inhibited by its product glucose-6-phosphate	No inhibition by its product glucose-6-phosphate
Its function is to ensure supply of glucose for the tissues, irrespective of blood glucose concentration	Its function is to remove glucose from the blood, when the blood glucose level increases (following meal)
Catalyze the phosphorylation of other hexoses like fructose, galactose, etc.	Specific for glucose
Its activity is not affected by insulin	It is an inducible enzyme that increases its synthesis in response to insulin

between glucokinase and hexokinase is given in **Table 12.1.** This is an **irreversible** reaction.

2. Conversion of glucose-6-phosphate to fructose-6-phosphate by an enzyme ***phosphohexose isomerase*** which involve isomerization and is freely **reversible** reaction.
3. Fructose-6-phosphate to fructose 1, 6-bisphosphate, second phosphorylation reaction requiring ATP catalyzed by an enzyme ***phosphofructokinase-I.*** This step is ***irreversible*** under physiological conditions. **Phosphofructokinase-I** is ***regulatory enzyme of glycolysis.***
4. Fructose-1,6-bisphosphate is cleaved by ***aldolase*** to two three carbon compounds, glyceraldehyde-3-phosphate and dihydroxy acetone phosphate (DHAP).
5. DHAP is isomerized to glyceraldehyde-3-phosphate by the enzyme ***phosphotriose isomerase,*** so that, 2-molecules of glyceraldehyde-3-phosphate are formed from one molecule of glucose.

IInd phase: Energy generating phase

6. Oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate by ***glyceraldehyde-3-phosphate dehydrogenase,*** is a NAD dependent reversible reaction. The reducing equivalents NADH+ H^+ formed, are reoxidized by electron transport chain, to generate 3 ATP molecules per NADH+H^+.
7. 1, 3-bisphosphoglycerate to 3-phosphoglycerate is catalyzed by ***phosphoglycerate kinase.*** This is the step in glycolysis that generates ATP at ***substrate level phosphorylation.*** Since two molecules of glyceraldehyde-3-phosphate are formed per molecule of glucose undergoing glycolysis, two molecules of ATP are generated at this stage per molecule of glucose.
8. 3-phosphoglycerate to 2-phosphoglycerate is a reversible reaction catalyzed by ***phosphoglycerate mutase.***
9. 2-phosphoglycerate to phosphoenol pyruvate. This reaction is catalyzed by ***enolase. Enolase is inhibited by fluoride, a property that can be used when it is required to prevent glycolysis in blood prior to the estimation of glucose.***
10. Phosphoenol pyruvate to pyruvate is an ***irreversible*** reaction catalyzed by **pyruvate kinase.** This is the second step in glycolysis that generates ATP at **substrate level phosphorylation.** Enol pyruvate formed in this reaction is converted spontaneously to the keto form of pyruvate.

Under aerobic condition, pyruvate is taken up into mitochondria and after conversion to acetyl-CoA is oxidized to CO_2 and H_2O by citric acid cycle.

Anaerobic Glycolysis

- In anaerobic conditions, the reoxidation of NADH (formed in the glyceraldehyde-3-phosphate step) by respiratory chain is prevented and gets reoxidized by conversion of pyruvate to lactate by **lactate dehydrogenase (Figure 12.3).**
- Tissues that function under hypoxic conditions produce lactate, e.g. skeletal muscle, smooth muscle and erythrocytes.
- In erythrocytes even under aerobic conditions, glycolysis terminates in lactate because of absence of mitochondria.

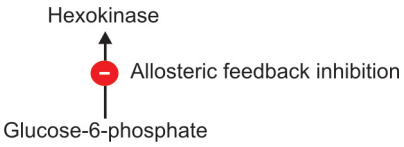
Regulation of Glycolysis

Glycolysis is regulated at 3 steps which are ***irreversible.*** These reactions are catalyzed by:

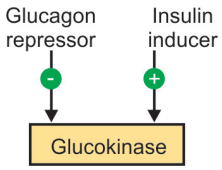
1. Hexokinase and glucokinase
2. Phosphofructokinase-I
3. Pyruvate kinase.

Hexokinase and glucokinase

- Hexokinase is an allosteric enzyme, that is inhibited by its product glucose-6-phosphate.

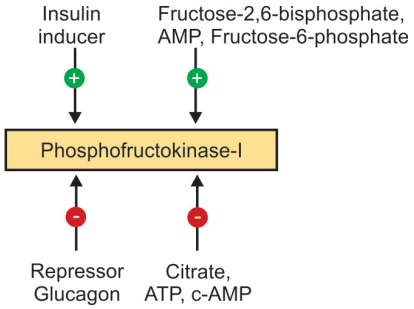


- Liver glucokinase is an inducible enzyme that increases its synthesis in response to insulin and decreases in response to glucagon.



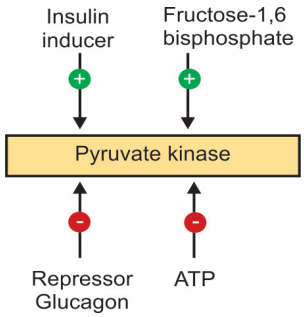
Phosphofructokinase-I

- Phosphofructokinase-l is activated by:
  - Fructose-6-phosphate (substrate)
  - AMP (which signals low energy state)
  - Fructose 2,6-bisphosphate.
- **Phosphofructokinase-I** is inhibited by **citrate, c-AMP** and **ATP** (which signals high energy state slowing down the glycolysis).
- Phosphofructokinase-I is an inducible enzyme that increases its synthesis in response to **insulin** and decreases in response to **glucagon.**



Pyruvate kinase

- Pyruvate kinase is an inducible enzyme that increases in concentration with high insulin levels and decreases with glucagon.
- It is activated by fructose-1, 6-bisphosphate and inactivated by ATP.



Significance of Glycolysis

- Glycolysis is the principal route for glucose metabolism for the production of ATP molecules.
- An important biochemical significance is the ability of glycolysis to provide ATP in the absence of oxygen and allows tissues to survive anoxic episodes.
- It generates precursors for biosynthetic pathway, e.g.
  - Pyruvate may be transaminated to amino acid alanine. In the liver, pyruvate provides substrate, acetyl-CoA for fatty acid biosynthesis.
  - Glycerol-3-phosphate, which is required for the synthesis of triacylglycerol is derived from glycolytic pathway.
- In erythrocytes, glycolysis supplies 2,3-BPG which is required for the transport of oxygen by Hb.

Energetics of Glycolysis

- The details of ATP generation in glycolysis are given in **Table 12.2.** Under aerobic conditions, 8 molecules of ATP are produced.
- In anaerobic glycolysis, on the other hand, only 2 moles of ATP are produced per molecule of glucose.

RAPOPORT LUEBERING CYCLE

In Rapoport luebering cycle, production of ATP by substrate phosphorylation from 1,3-BPG is bypassed in the erythrocyte by taking a diversion pathway **(Figure 12.4).**

- In rapoport lubering cycle 1,3-BPG is converted to 2,3-BPG by an enzyme ***bisphosphoglycerate mutase.***
- Then 2,3-BPG is converted to 3-phosphoglycerate by ***2,3-bisphosphoglycerate phosphatase,*** with a loss of high energy phosphate (energy is dissipated as heat) and there is no net production of ATP when glycolysis takes this route.

Table 12.2: Production of ATP in glycolysis aerobically

Reaction	*Reaction catalyzed by*	*Number of ATP formed or consumed/ glucose molecule*
Glucose to glucose-6-phosphate	Hexokinase, glucokinase	– 1
Fructose-6-phosphate to fructose 1,6-bisphosphate	Phosphofructokinase-I	– 1
Glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate	Glyceraldehyde-3-phosphate dehydrogenase	+6*
1, 3-bisphosphoglycerate to 3-phosphoglycerate	Phosphoglycerate kinase	+2
Phosphoenolpyruvate to pyruvate	Pyruvate kinase	+ 2

- Net production of ATP in aerobic glycolysis = Number of ATP produced minus number of ATPs consumed = 10 – 2 = 8
- *It is assumed that NADH formed in glycolysis uses malate shuttle to produce 6 ATPs
- Total ATP per molecule of glucose under anaerobic glycolysis = 2

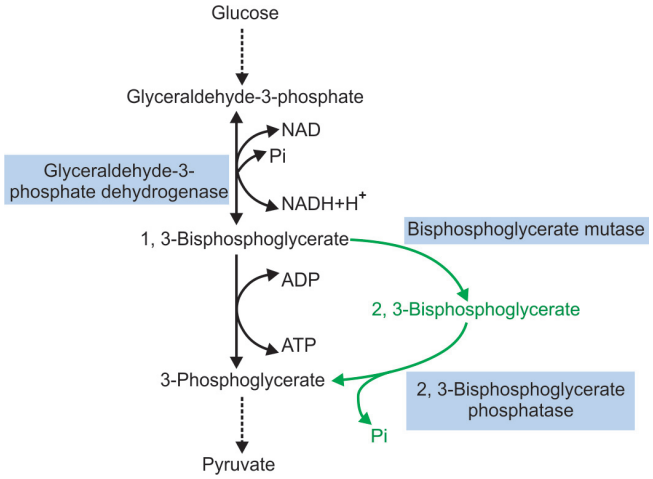


Figure 12.4: Rapoport luebering cycle of erythrocytes shown in green color

Significance of Rapoport Luebering Cycle

- It prevents accumulation of ATP not needed by the erythrocyte.
- It supplies 2,3-BPG required for the transport of oxygen by hemoglobin. 2,3-BPG regulates the binding and release of oxygen from hemoglobin.
- 2, 3-BPG present in erythrocytes acts as a buffer.

CONVERSION OF PYRUVATE TO ACETYL-CoA

- Pyruvate is converted to acetyl CoA by **oxidative decarboxylation.** This step occurs only in mitochondria. This is an irreversible reaction catalyzed by a multienzyme complex known as **pyruvate dehydrogenase complex (PDH) (Figure 12.5).**
- The enzyme pyruvate dehydrogenase requires five coenzymes, namely thiamine pyrophosphate (TPP), lipoate, coenzyme-A, FAD and NAD^+.

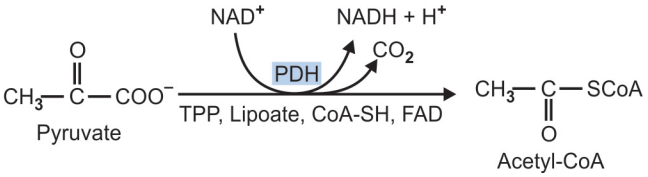


Figure 12.5: Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase (PDH) complex

Energetics in conversion of pyruvate to Acetyl CoA

As a result of oxidation of pyruvate to acetyl CoA catalyzed by pyruvate dehydrogenase, one molecule of NADH is produced for each molecule of pyruvate. Oxidation of NADH by electron transport chain results in synthesis of **3 ATP molecules.**

Significance

- The conversion of pyruvate to acetyl-CoA is a central step, linking the glycolytic pathway with citric acid cycle.

- Acetyl-CoA is also an important precursor in fatty acid biosynthesis and cholesterol biosynthesis.

CITRIC ACID CYCLE

Citric acid cycle is also called ***Krebs cycle*** or ***tricarboxylic acid (TCA) cycle.***

- It is called citric acid cycle because citrate was one of the first compounds known to participate.
- It is called Krebs cycle, because its reactions were formulated into a cycle by ***Sir Hans Krebs.***
- The most common name for this pathway is, the **tricarboxylic acid** or **TCA cycle,** due to involvement of the tricarboxylates—**citrate** and **isocitrate.**

Definition

The citric acid cycle is a series of reactions in mitochondria that brings about the catabolism of acetyl-CoA to CO_2 and H_2O with generation of ATP.

Location of Citric Acid Cycle

The reactions of citric acid cycle are located in the **mitochondrial matrix.**

Reactions of Citric Acid Cycle (Figure 12.6)

1. First reaction of the citric acid cycle is the condensation of acetyl-CoA with oxaloacetate to yield citrate, catalyzed by ***citrate synthase.***
2. Citrate is converted to isocitrate by an enzyme ***aconitase.*** This conversion takes place in two steps:
   - Dehydration to cis-aconitate
   - Rehydration to isocitrate.
3. Isocitrate undergoes dehydrogenation in the presence of ***isocitrate dehydrogenase*** to form oxalosuccinate. There follows a decarboxylation to α-ketoglutarate, also catalyzed by isocitrate dehydrogenase. ***The formation of NADH and liberation of CO_2 occurs at this stage.***
4. Next α-ketoglutarate undergoes oxidative decarboxylation, catalyzed by a multi-enzyme complex, ***α-ketoglutarate dehydrogenase,*** an α-ketoglutarate dehydrogenase complex requires thiamine pyrophosphate (TPP), Lipoate, NAD, FAD and coenzyme-A and results in the formation of succinyl-CoA, a high energy compound, this reaction is physiologically ***irreversible.*** At this stage, ***second NADH is produced*** along with ***liberation of second CO_2 molecule.***
5. Succinyl-CoA is converted to succinate by the enzyme ***succinate thiokinase.*** This reaction is coupled with the phosphorylation of GDP to GTP. This is a **substrate level phosphorylation.** This GTP is converted to ATP.
6. Succinate is oxidized further by ***succinate dehydrogenase*** to fumarate with the production of $FADH_2$.
7. Next, ***fumarase*** catalyzes the addition of water to fumarate to give malate.
8. Malate is converted to oxaloacetate by ***malate dehydrogenase,*** and requires NAD^+. ***The synthesis of third NADH occurs at this stage.*** The oxaloacetate is regenerated which can combine with another molecule of acetyl-CoA and continue the cycle.

Energetics of Citric Acid Cycle

- As a result of oxidation of acetyl-CoA to H_2O and CO_2 by citric acid cycle, ***three molecules of NADH*** and **one *$FADH_2$*** are produced.
- Oxidation of 3 NADH by electron transport chain results in the synthesis of 9 ATP, whereas $FADH_2$ generates 2 ATP molecules.
- One molecule of ATP is generated at substrate level during the conversion of succinyl-CoA to succinate. Thus, a total of 12 ATP are generated from one molecule of acetyl-CoA. **(Table 12.3).**

Significance of Citric Acid Cycle

- The primary function of the citric acid cycle is to provide energy in the form of ATP.
- Citric acid cycle is the final common pathway for the oxidation of carbohydrates, lipids, and proteins as glucose, fatty acids and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle.
- Citric acid cycle is an **amphibolic process.** Citric acid cycle has a dual function, it functions in both catabolism (of carbohydrates, fatty acids and amino acids) and anabolism. **(Figure 12.7)**. Some metabolic pathways end in the constituent of the citric acid cycle while other pathways originate from the cycle, such as:
  - Gluconeogenesis
  - Transamination
  - Fatty acid synthesis
  - Heme synthesis.
- **Gluconeogenesis:** All major members of the citric acid cycle from citrate to oxaloacetate are glucogenic. They can give rise to glucose by gluconeogenesis.
- **Transamination :** Oxaloacetate and α-ketoglutarate respectively, serve as precursors for the synthesis of aspartate and glutamate by transamination which in turn are used for the synthesis of other nonessential amino acids, purines and pyrimidines.
- **Fatty acid synthesis :** Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl-CoA for the biosynthesis of fatty acids and steroids.
- **Heme synthesis :** Succinyl-CoA (intermediate of TCA cycle) together with glycine is used for the synthesis of heme.

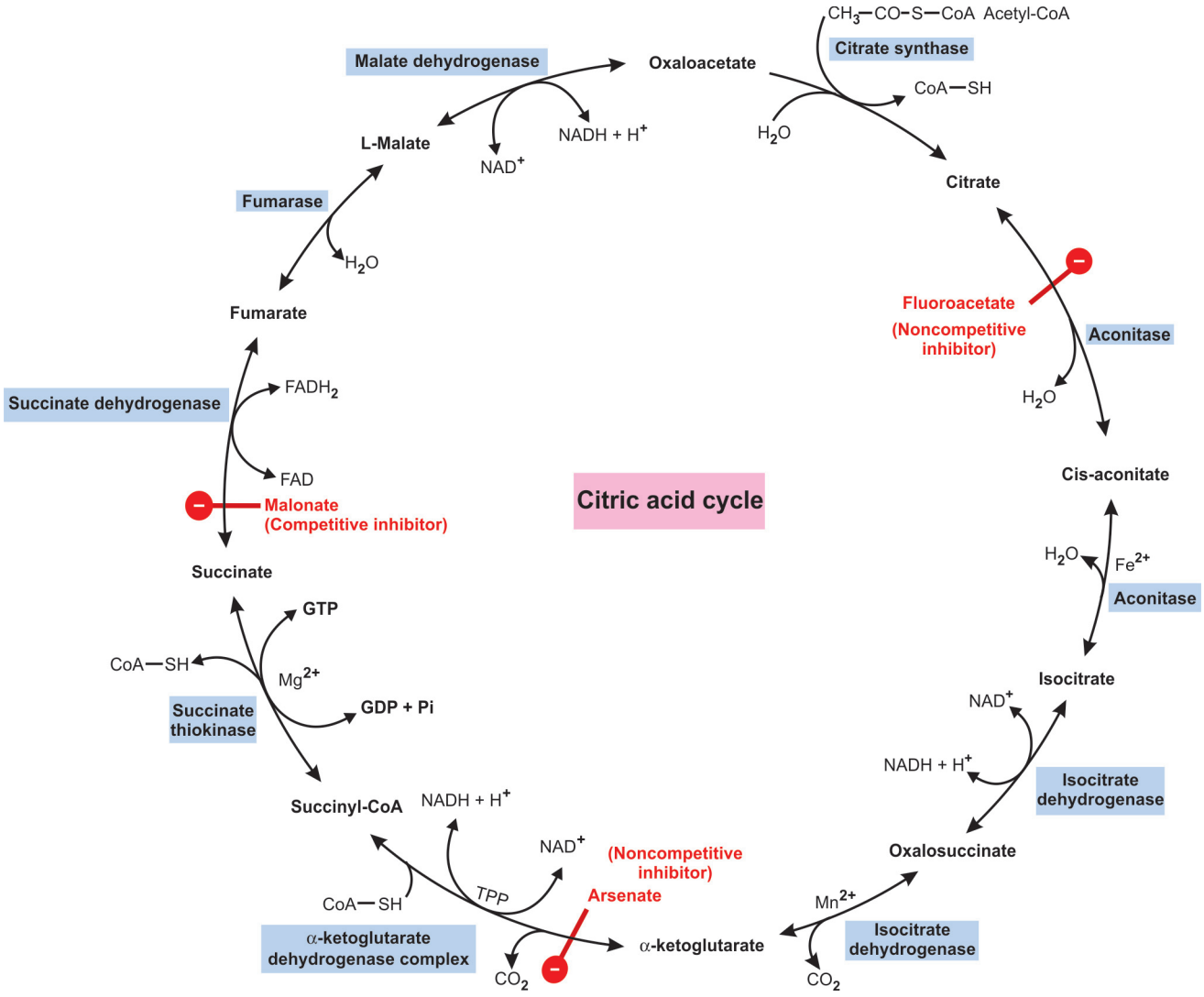


Figure 12.6: Reactions of citric acid cycle

Table 12.3 : Production of ATP in citric acid cycle

Reaction	*Reaction catalyzed by*	*No. of ATP formed per acetyl-CoA molecule*
Isocitrate to α-ketoglutarate	Isocitrate dehydrogenase	+3
α-Ketoglutarate to succinyl-CoA	α-Ketoglutarate dehydrogenase	+3
Succinyl-CoA to succinate	Succinyl thiokinase	+1
Succinate to fumarate	Succinate dehydrogenase	+2
Malate to oxaloacetate	Malate dehydrogenase	+3
Number of ATPs formed per acetyl-CoA molecule in citric acid cycle = 12		

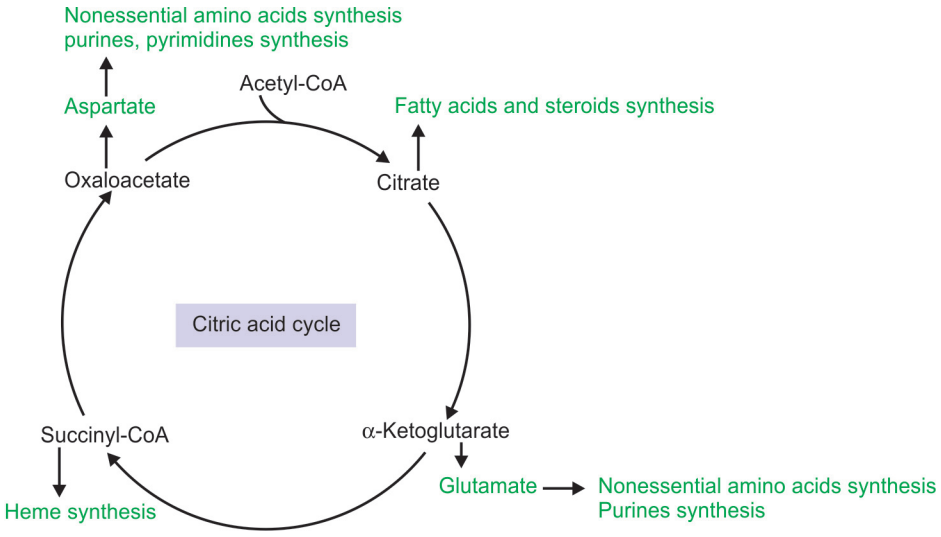


Figure 12.7: Amphibolic role of the citric acid cycle

Regulation of Citric Acid Cycle

- Citric acid cycle is regulated at three steps. These are catalyzed by:
  1. Citrate synthase
  2. Isocitrate dehydrogenase
  3. α-ketoglutarate dehydrogenase.
- Activities of these enzymes are dependent on the energy status of the cycle.
- Excess of ATP, NADH and succinyl-CoA, which signals high energy status of the cell, inhibit these enzymes.
- High level of ADP which signals low energy status of the cell stimulates the operation of the cycle.

GLUCONEOGENESIS

Definition

The synthesis of glucose from noncarbohydrate precursors is called ***gluconeogenesis*** (i.e. synthesis of new glucose).

Precursors for gluconeogenesis

The major noncarbohydrate substrates for gluconeogenesis are:

- Lactate
- Glycerol
- Glucogenic amino acids
- Propionate
- Intermediates of the citric acid cycle.

1. **Lactate :** Lactate is formed from glucose by anaerobic glycolysis in the muscle. It is transported to the liver by **Cori's cycle** (discussed later) and is converted to glucose by gluconeogenesis.
2. **Glycerol :** Glycerol is formed in adipose tissue by hydrolysis of triacylglycerol. Glycerol cannot be utilized by adipose tissue due to poor content of enzyme **glycerol kinase.** Therefore, it is delivered to the liver where it is converted to glucose by gluconeogenesis.
3. **Glucogenic amino acids and intermediates of TCA cycle:** The carbon skeleton of glucogenic amino acids are converted to pyruvate or intermediates of TCA cycle, which are then converted to glucose by gluconeogenesis.
4. **Propionate:** Fatty acids with an odd number of carbons and carbon skeleton of some amino acids produce propionate. Propionate enters the gluconeogenic pathway via citric acid cycle after conversion of succinyl-CoA **(see Figure 13.8)**.

Location of Gluconeogenesis

Gluconeogenesis occurs mainly in the cytosol although some precursors are produced in the mitochondria. **Liver** is the major tissue for gluconeogenesis. During starvation, the **kidney** is also capable of making glucose by gluconeogenesis. Certain enzymes required in gluconeogenesis are present only in these organs.

Characteristics of Gluconeogenesis

- Gluconeogenesis involves **glycolysis,** the **citric acid cycle** plus some **special reactions.**
- Glycolysis and gluconeogenesis share the same pathway but in opposite direction.
- Seven of the reactions of glycolysis are reversible and are used in the synthesis of glucose by gluconeogenesis.
- However, three of the reactions of glycolysis are irreversible and must be circumvented by four special reactions which are unique to gluconeogenesis and catalyzed by:
  1. Pyruvate carboxylase
  2. Phosphoenol pyruvate carboxykinase
  3. Fructose-1,6-bisphosphatase
  4. Glucose-6-phosphatase.

Reactions of Gluconeogenesis (Figure 12.8)

1. **Carboxylation of pyruvate to oxaloacetate:** In gluconeogenesis, pyruvate is first carboxylated to oxaloacetate. ***Pyruvate carboxylase*** which in presence of ATP, vitamin biotin and CO_2 converts pyruvate to oxaloacetate in mitochondria.
   Transport of oxaloacetate to cytosol: Oxaloacetate, formed in mitochondria, must enter the cytosol, where the other enzymes of gluconeogenesis are located. However, as oxaloacetate is unable to cross the inner mitochondrial membrane directly, it must be reduced to malate which can be transported from the mitochondria to the cytosol. In the cytosol, malate is reoxidized to oxaloacetate.
2. **Decarboxylation of cytosolic oxaloacetate to phosphoenol pyruvate (PEP):** Oxaloacetate is decarboxylated and phosphorylated in the cytosol by ***phosphoenol pyruvate carboxykinase.*** High energy phosphate in the form of GTP is required in this reaction. PEP then enters the reversed reaction of glycolysis until it reaches fructose-1, 6-bisphosphate.
3. **Dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate:** Hydrolysis of fructose-1, 6-bisphosphate to fructose-6-phosphate by **fructose-1,6-bisphosphatase** bypasses the irreversible phosphofructokinase-I reaction of glycolysis. Fructose-6-phosphate is then converted to glucose-6-phosphate by reversed reaction of glycolysis.
4. **Dephosphorylation of glucose-6-phosphate to glucose:** Hydrolysis of glucose-6-phosphate to glucose by ***glucose-6-phosphatase*** bypasses the irreversible glucokinase and hexokinase reaction of glycolysis.

Regulation of Gluconeogenesis

Gluconeogenesis is regulated by four key enzymes.

1. Pyruvate carboxylase
2. Phosphoenolpyruvate carboxykinase
3. Fructose-1,6-bisphosphatase
4. Glucose-6-phosphatase.

- The hormones **glucagon** and **epinephrine** stimulate gluconeogenesis by inducing the synthesis of the key enzymes, while **insulin inhibits** the gluconeogenesis by repressing their synthesis.
- During starvation and in diabetes mellitus, a high level of glucagon stimulates gluconeogenesis. However in well-fed state, insulin suppresses the gluconeogenesis.
- Pyruvate carboxylase is an allosteric enzyme, which is stimulated by acetyl-CoA and inhibited by ADP.
- Fructose-1,6-bisphosphatase stimulated allosterically by c-AMP and inhibited by AMP.

Significance of Gluconeogenesis

- Gluconeogenesis maintains blood glucose level when carbohydrate is not available in sufficient amounts from the diet.
- During starvation when hepatic glycogen reserve is totally depleted, glucose is provided by gluconeogenesis to the brain and other tissues like erythrocytes, lens, cornea of the eye and kidney medulla. They require a continuous supply of glucose as a source of energy.
- Gluconeogenesis is used to clear the products of the metabolism of other tissues from the blood, for example,
  - Lactate, produced by muscle and erythrocytes
  - Glycerol produced by adipose tissue
  - Propionyl-CoA produced by oxidation of odd carbon number fatty acids and carbon skeleton of some amino acids.

CORI CYCLE OR LACTIC ACID CYCLE

Lactate is produced in skeletal muscles during anaerobic oxidation of glucose. The lactate thus produced cannot be further metabolized in skeletal muscles. Through blood,

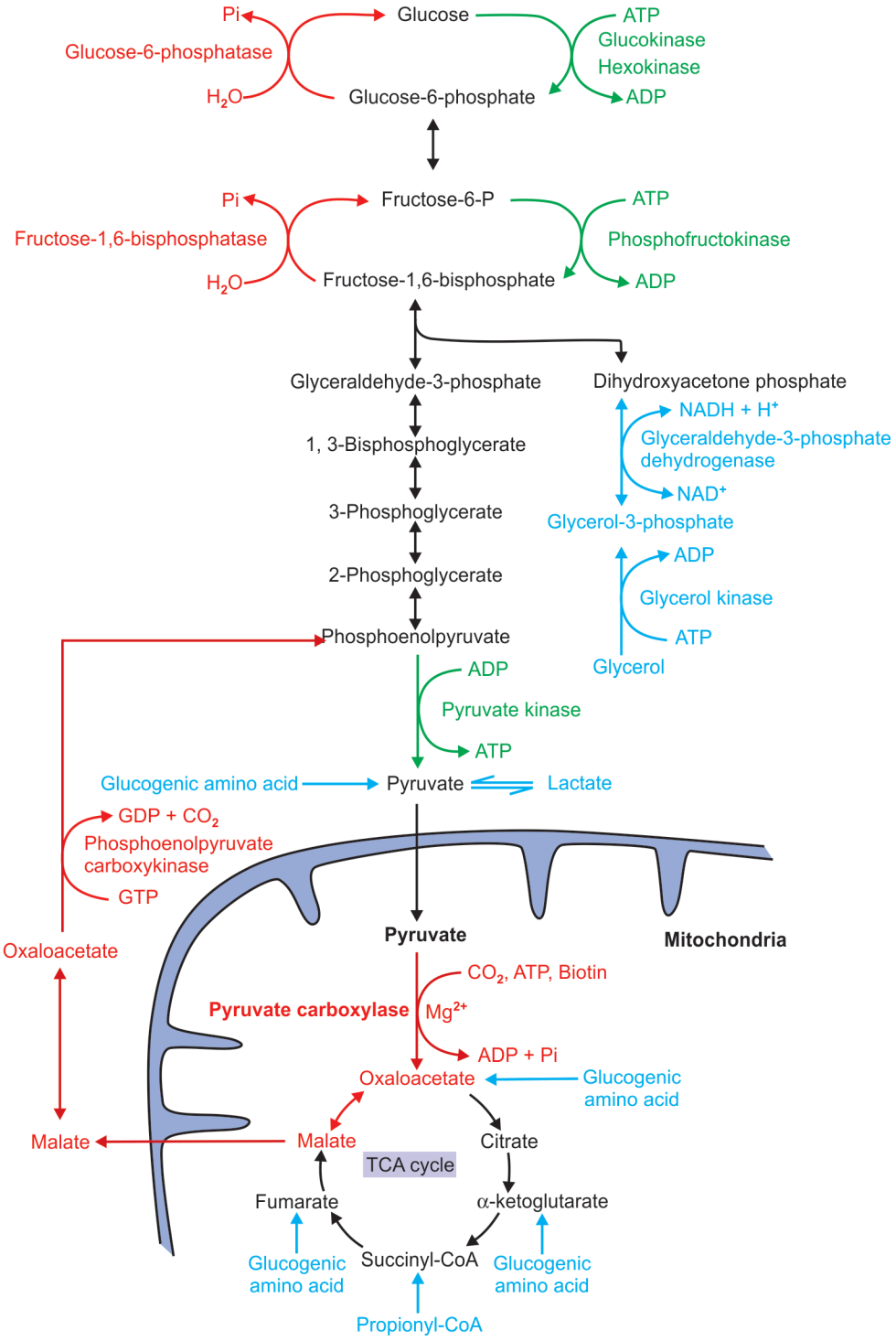


Figure 12.8: Pathway of gluconeogenesis

- Special reactions and enzymes of gluconeogenic pathway are shown in red.
- Irreversible reactions of glycolysis are shown in green.
- Remaining reactions which are common to glycolysis and gluconeogenesis are shown in black.
- The entry points of substrate are shown in blue.

lactate is transported to the liver where it is oxidized to pyruvate. Pyruvate so produced, is converted to glucose by gluconeogenesis, which is then transported to the muscle. The glucose thus reformed from lactate again becomes available for energy purpose in skeletal muscle.

This cycling of lactate between muscle and liver is known as the **Cori *Cycle*** or ***Lactic acid cycle*** **(Figure 12.9).**

GLUCOSE-ALANINE CYCLE

- Because muscle is incapable of synthesizing urea, most of the ammonia formed by protein catabolism is transferred to pyruvate to form **alanine** by transamination reaction.
- Alanine enters the blood and is taken up by the liver.
- In the liver, the amino groups of alanin is removed to form urea, and the resulting pyruvate is converted to glucose by gluconeogenesis which is then transported to the muscle, where it is oxidized to pyruvate.
- The pyruvate acts again as the acceptor for another amino group.
- These reactions transport amino groups from muscle to the liver in the from of **alanine**. This cycle is called the **glucose-alanine cycle** or **the Cahill cycle (Figure 12.9).**

Alanine is the predominant amino acid released from muscle to liver during fasting.

GLYCOGEN METABOLISM

- Glycogen is the major storage form of glucose mainly in the liver and muscle.
- The concentration of liver glycogen (up to 6%) is greater than in muscle (1%) tissues. However, because muscle tissue comprises a large mass, its total capacity to storage is three to four times that of the liver.
- The synthesis, ***glycogenesis*** and degradation, **glycogenolysis** occur via different pathways. Glycogenesis and glycogenolysis are both cytosolic processes.

Glycogenesis

Definition

Glycogenesis is the pathway for the formation of glycogen from glucose. This process requires energy, supplied by ATP and uridine triphosphate (UTP). It occurs in muscle and liver.

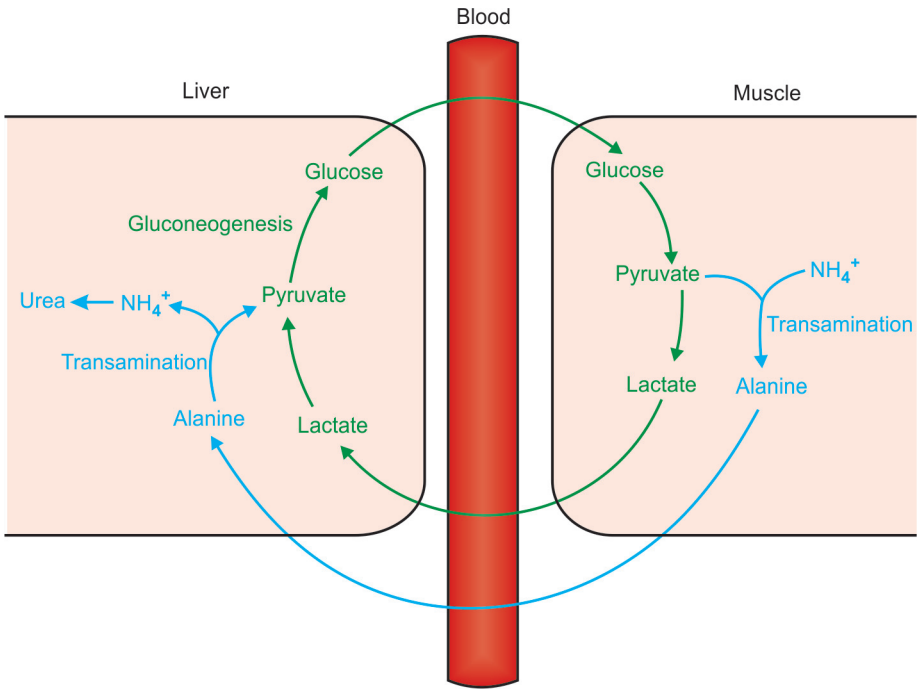


Figure 12.9: Cori cycle or lactic acid cycle and glucose alanine cycle.
The pathway of Cori cycle is shown in green and glucose alanine cycle in blue

Reactions of Glycogenesis (Figure 12.10)

1. Glucose is phosphorylated to glucose-6-phosphate catalyzed by ***hexokinase*** in muscle and ***glucokinase*** in liver.
2. Glucose-6-phosphate is converted to glucose-1-phosphate by the enzyme ***phosphoglucomutase***.
3. Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDP-GIc). The reaction is catalyzed by the enzyme ***UDP-glucose pyrophosphorylase***.
4. By the action of the enzyme ***glycogen synthase,*** the C_1 of the glucose of UDP-Glc forms a glycosidic bond with C_4 of a terminal glucose residue of pre-existing glycogen molecule (glycogen primer), liberating uridine diphosphate (UDP). Thus, pre-existing glycogen molecule must be present to initiate this reaction.
5. In the above reaction, a new **α-1,4 linkage** is established between carbon atom 1 of incoming glucose and carbon 4 of the terminal glucose of a glycogen primer.
6. When the chain has been lengthened to a minimum of 11 residues, a second enzyme, the **branching enzyme,** transfers a part of the 1,4-chain (minimum length of 6-glucose residues) to a neighboring chain to form α-1,6-linkage, thus establishing a branching point in the molecule **(Figure 12.11)**. The branches grow by further additions of glucose units and further branching.

Glycogenolysis

Definition and Location

Glycogenolysis is the degradation of glycogen to glucose-6-phosphate and glucose in muscle and liver respectively. Glycogenolysis is not the reverse of glycogenesis but is a separate pathway.

Reactions of Glycogenolysis (Figure 12.10)

1. Glycogenolysis occurs primarily by phosphorolytic breaking of α-1,4-glycosidic bonds of glycogen to

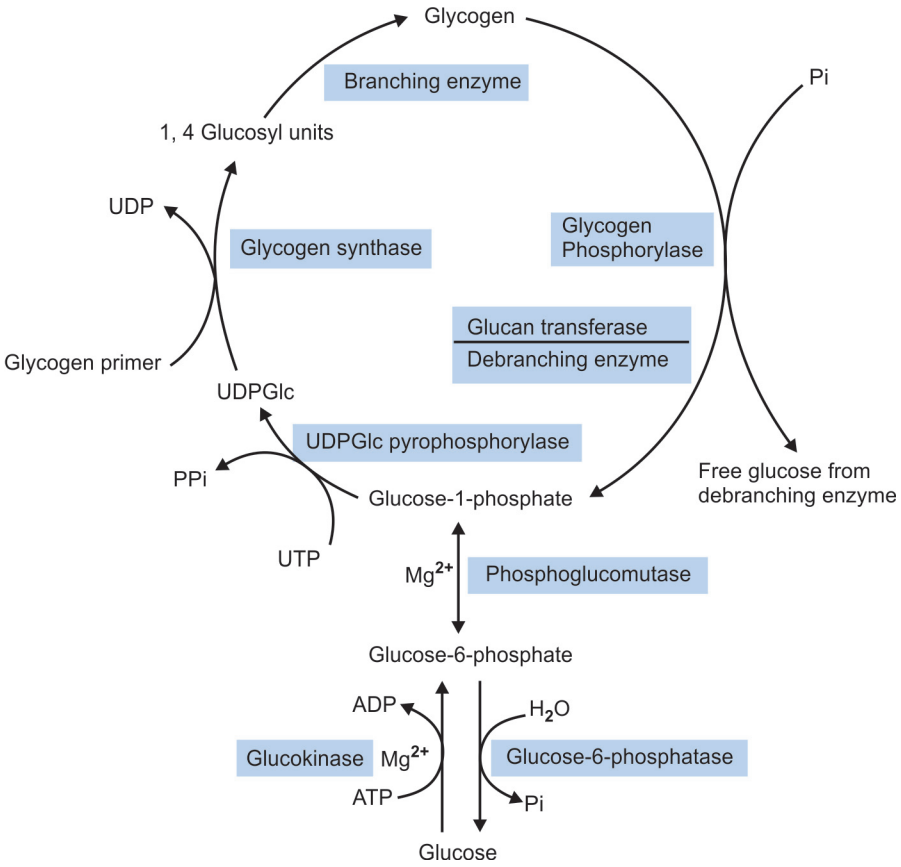


Figure 12.10: Pathway of glycogenesis and glycogenolysis in liver
where, UTP = Uridine triphosphate, UDP = Uridine diphosphate, UDPGlc = Uridine diphosphate glucose

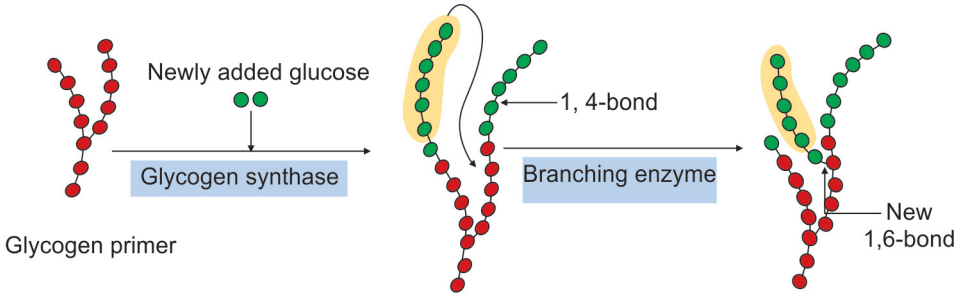


Figure 12.11: Schematic representation of glycogenesis (mechanism of branching)

yield glucose-1-phosphate and residual glycogen molecule. This process is catalyzed by the enzyme ***glycogen phosphorylase***. The glucose residues from outermost chain of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a branch point having α-1,6 linkage **(Figure 12.12)**.

2. Phosphorolysis cannot continue until the branch is removed. This is accomplished by ***debranching enzyme***. It has two catalytic activities—**glucan transferase** and **1,6-glucosidase.**
   - First, it acts as a ***glucan transferase*** and transfers three of the remaining residues from one branch to the other. This exposes the α-1,6 branch point.
   - In the second step, the hydrolytic splitting of the α-1,6 linkages occurs by the action of ***1,6-glucosidase***. This step releases free glucose. Further splitting of the glycogen can then proceed by the actions of phosphorylase until another branch point is reached. The action of glucan transferase and 1,6-glucosidase are repeated.

   The combined action of ***phosphorylase*** and ***debranching enzyme*** leads to the complete breakdown of glycogen with the formation of glucose-1-phosphate and free glucose (from hydrolytic cleavage of the 1,6-glycosidic bond).
3. Next, glucose-1-phosphate is converted to glucose-6-phosphate by ***phosphoglucomutase***. This is a reversible reaction.
4. In the liver but ***not in the muscle***, there is a specific enzyme, ***glucose-6-phosphatase***, that cleaves glucose-6-phosphate to glucose and diffuse from the hepatic cell into the blood. As glucose-6-phosphatase is absent in muscle, free glucose cannot be produced from glucose-6-phosphate in muscle. Moreover, glucose-6-phosphate cannot diffuse out of the muscles. Therefore, muscle cannot provide glucose to maintain blood glucose level.

Lysosomal Degradation of Glycogen

A small amount of glycogen is continuously degraded by the lysosomal enzyme ***α-1,4-glucosidase (acid maltase)***. The significance of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in the cytosol resulting in ***glycogen storage disease type II Pompe's disease***.

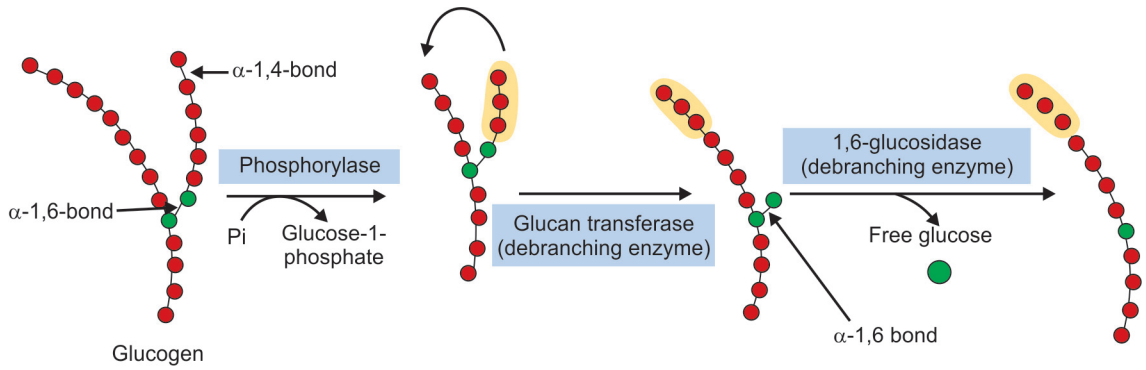


Figure 12.12: Schematic representation of glycogenolysis (mechanism of debranching)

Significance of Glycogenolysis and Glycogenesis

The functional role of glycogen differs considerably from tissue to tissue, as we can see in the case of liver and muscle.

In liver

Following a meal, excess glucose is removed from the portal circulation and stored as glycogen by glycogenesis. Conversely, between meals, blood glucose levels are maintained within the normal range by release of glucose from liver glycogen by glycogenolysis.

In muscle

The function of muscle glycogen is to act as a readily available source of glucose within the muscle itself during muscle contraction. ***The muscle cannot release glucose into the blood, because of the absence of glucose-6-phosphatase*** that hydrolyzes glucose 6-phosphate to glucose. Therefore, muscle glycogen stores are used exclusively by muscle.

Regulation of Glycogenesis and Glycogenolysis

The principal enzymes controlling glycogen metabolism are ***glycogen phosphorylase*** and ***glycogen synthase*** which are regulated reciprocally. Regulation of these enzymes involve:

- Hormonal regulation
- Allosteric regulation.

Hormonal Regulation

- **Epinephrine** and **glucagon** regulate glycogen breakdown and glycogen synthesis.
- Epinephrine (in liver and muscle) and glucagon (in liver) stimulates glycogen breakdown (glycogenolysis) and inhibits glycogen synthesis (glycogenesis).

Regulation of glycogenesis ***(Figure 12.13)***

- **Glycogen synthase** is the regulatory enzyme of glycogenesis. It exists in two forms: **Glycogen synthase-a**, an active or dephosphorylated form and **Glycogen synthase-b**, an inactive or phosphorylated form.
- Glucagon in liver and epinephrine in liver and muscle activates **adenylate cyclase** enzyme that catalyzes the synthesis of **c-AMP.** c-AMP in turn activates **c-AMP dependent protein kinase.**
- c-AMP dependent protein kinase then **phosphorylates glycogen synthase** and thereby inactivates glycogen synthase and synthesis of glycogen is inhibited.

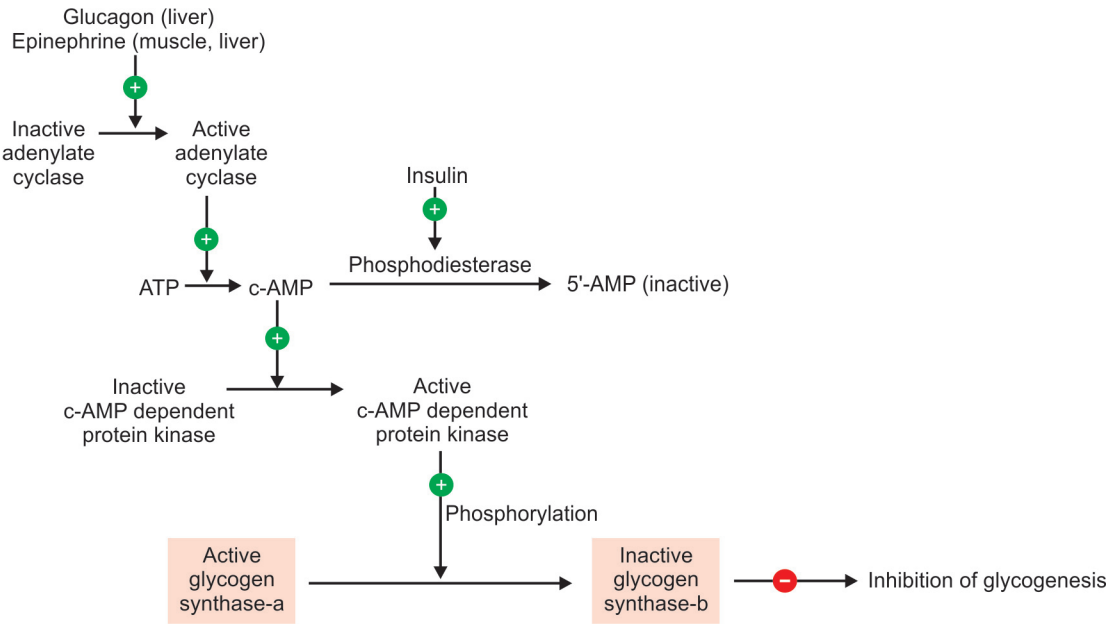


Figure 12.13: Hormonal regulation of glycogenesis

- The hormone **insulin** increases the phosphodiesterase activity in liver and lowers the c-AMP levels and inhibits the action of glucagon and epinephrine.

Regulation of glycogenolysis ***(Figure 12.14)***

- **Glycogen phosphorylase** is the regulatory enzyme of glycogenolysis. It exists in two forms: **Glycogen phosphorylase-a,** an active or phosphorylated form and **Glycogen phosphorylase-b,** an inactive or dephosphorylated form.
- Degradation of glycogen is stimulated by epinephrine in the muscle and by glucagon in the liver via activation of ***adenylate cyclase*** that catalyzes the synthesis of c-AMP**.**
- The consequent increase in levels of c-AMP in turn activates **c-AMP dependent protein kinase.** Active c-AMP dependant protein kinase phosphorylates the inactive form of **phosphorylase kinase** to its active form.
- Active phosphorylase kinase eventually activates inactive form of glycogen phosphorylase to its active form. Active form of **glycogen phosphorylase** stimulates breakdown of glycogen to glucose-1-P **(Figure 12.14).**

Allosteric Regulation (Figure 12.15)

- **Glycogen synthase** is allosterically activated by glucose-6-phosphate when it is present in elevated concentrations.
- In contrast, **glycogen phosphorylase** is allosterically inhibited by glucose-6-phosphate.

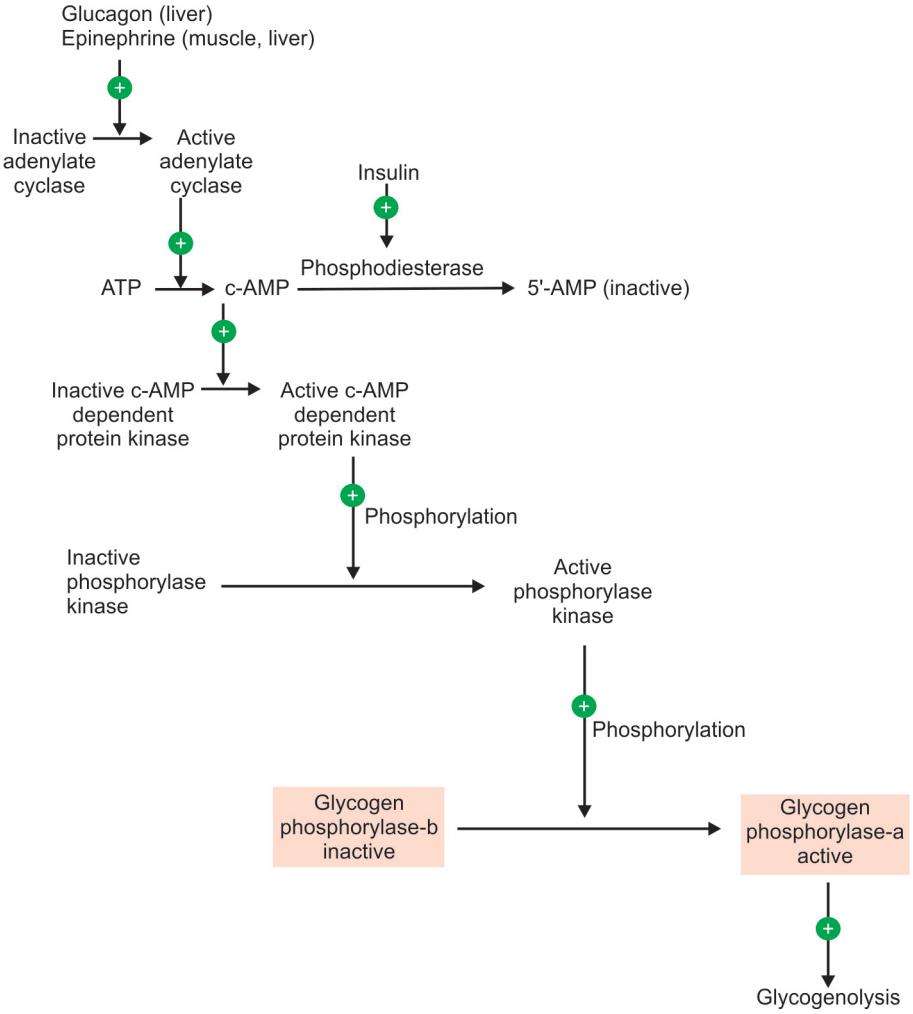


Figure 12.14: Hormonal regulation of glycogenolysis

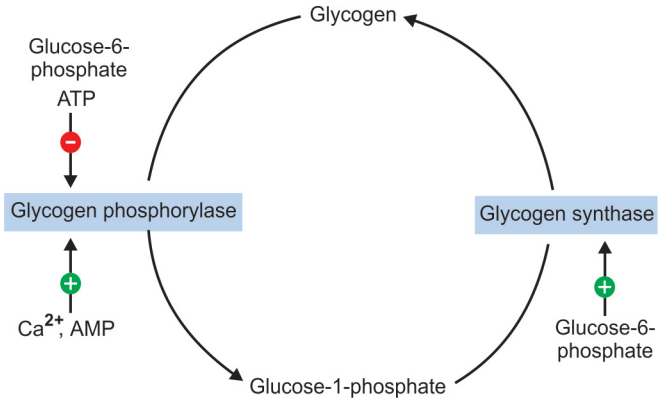


Figure 12.15: Allosteric regulation of glycogenesis and glycogenolysis

- The Ca^{2+} ions stimulate the glycogenolysis by activation of glycogen phosphorylase.
- Increased level of AMP in vigorously contracting muscles stimulates glycogen breakdown by stimulating glycogen phosphorylase allosterically. However, in resting muscles ATP inhibits the glycogen breakdown by allosteric inactivation of glycogen phosphorylase.

Glycogen Storage Disease

This is a group of genetic diseases, that result from a defect in an enzyme required for either glycogen synthesis or degradation and characterized by deposition of either normal or abnormal glycogen in the specific tissues. Some of the more common forms of these diseases and their characteristics are summarized in **Table 12.4.**

PENTOSE PHOSPHATE PATHWAY

Definition

The pentose phosphate pathway is an alternative route for the oxidation of glucose. It is the pathway for formation of pentose phosphate. It is also called ***hexose monophosphate shunt.***

Characteristics of Pentose Phosphate Pathway

- It is a multicyclic process in which three molecules of glucose-6-phosphate give rise to three molecules of CO_2 and three molecules of 5-carbon sugars, (ribulose-5-phosphate).
- The three molecules of ribulose-5-phosphate are arranged to generate two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate.
- It does not generate ATP.

The difference between glycolysis and pentose phosphate pathway is shown in **Table 12.5.**

Table 12.4: Glycogen storage diseases

Type	*Name*	*Enzyme affected*	*Primary organ involved*	*Manifestations*
I	Von Gierke's disease	Deficiency of glucose-6-phosphatase	Liver or kidney	Hypoglycemia, lactic acidemia, hyperlipemia ketosis and hyperuricemia
II	Pompe's disease	Deficiency of lysosomal α-1, 4 glucosidase (acid maltase)	All organs with lysosomes	Infantile form, early death, cardiac failure, accumulation of glycogen in lysosomes
III	Limit dextrinosis, Forbe's or Cori's disease	Absence of debranching enzyme	Liver, skeletal muscle, heart	Accumulation of abnormal glycogen having short outer chains, hypoglycemia
IV	Amylopectinosis, Andersen's disease	Absence of branching enzyme	Liver	Accumulation of abnormal glycogen having few branches, early death due to cardiac or liver failure
V	Mc-Ardle syndrome	Absence of muscle glycogen phosphorylase	Skeletal muscle	Excessive induced muscular pain, cramps, decrease serum lactate after exercise
VI	Her's disease	Deficiency of liver glycogen phosphorylase	Liver	High content of liver glycogen, mild hypoglycemia and ketosis
VII	Taruis disease	Deficiency of phosphofructokinase in muscle and erythrocytes	Muscle and RBC	As in type V, in addition hemolytic anemia

Location

The enzymes of pentose phosphate pathway are present in cytosol. The pathway is found in all cells.

Reactions of the Pentose Phosphate Pathway (Figure 12.16)

The reactions of the pathway are divided into two phases:

1. Phase I : Oxidative irreversible phase
2. Phase II : Nonoxidative reversible phase.

Reactions of phase I (oxidative irreversible phase)

In the first phase, glucose-6-phosphate undergoes dehydrogenation and decarboxylation to give pentose, ribulose-5-phosphate with generation of NADPH.

1. Dehydrogenation of glucose-6-phosphate to 6-phosphogluconolactone, catalyzed by ***glucose-6-phosphate dehydrogenase*** which is an NADP dependent enzyme.
2. 6-phosphogluconolactone is hydrolyzed by **6-phosphogluconolactone *hydrolase*** to 6-phosphogluconate.
3. The subsequent oxidative decarboxylation of 6-phosphogluconate is catalyzed by ***6-phosphogluconate*** **dehydrogenase,** which also requires NADP as hydrogen acceptor. This irreversible reaction produces ribulose-5-phosphate, CO_2 and second molecule of NADPH.

Reactions of phase II (nonoxidative, reversible phase)

In the second phase, ribulose-5-phosphate is converted to fructose-6-phosphate by a series of reactions.

4. Ribulose-5-phosphate formed in the phase I now serves as substrate for two differerent enzymes:
   i. **Ribulose-5-phosphate epimerase** catalyzes the epimerization of ribulose-5-phosphate to xylulose-5-phosphate.
   ii. **Ribulose-5-phosphate isomerase** catalyzes the isomerization of ribulose-5-phosphate to ribose-5-phosphate.
5. **Transketolase** catalyzes the transfer of two carbon units from xylulose-5-phosphate to ribose-5-phosphate, producing a 7-carbon, sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. The reaction requires coenzyme ***thiamine pyrophosphate (TPP)*** and Mg^{2+} ions.
6. ***Transaldolase*** catalyzes the transfer of a three carbon dihydroxyacetone group from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and the 4-carbon, erythrose-4-phosphate.
7. Further reaction again involves ***transketolase,*** which catalyzes the transfer of the two carbon units from xylulose-5-phosphate to erythrose-4-phosphate producing fructose-6-phosphate and glyceraldehyde-3-phosphate.
8. Fructose-6-phosphate and glyceraldehyde-3-phosphate can be further catabolized through glycolysis and citric acid cycle.

Significance of Pentose Phosphate Pathway

- The pentoses (ribose-5-phosphate) required for the biosynthesis of nucteotide and nucleic acids (RNA and DNA) are provided by pentose phosphate pathway.
- It provides a route for the interconversion of pentoses and hexoses.
- It generates NADPH which plays important role in several other biological processes, as given below.
  - NADPH is required for the biosynthesis of fatty acids, cholesterol, steroid hormones and neurotransmitters.
  - It is required for oxidation-reduction reactions involved in detoxification, e.g.for detoxification of drugs by microsomal **cytochrome P_{450} mono-oxygenase** and for reduction of oxidized glutathione.
  - In RBC, NADPH is required to maintain the level of **reduced glutathione.** The reduced glutathione protects the RBC membrane from toxic effect of H_2O_2 by reducing H_2O_2 to H_2O **(Figure 12.17).**
  - NADPH also keeps iron of hemoglobin in reduced ferrous (Fe^{2+}) state and prevents the formation of **methemoglobin.**
  - NADPH is necessary for phagocytosis carried out by white blood cell.

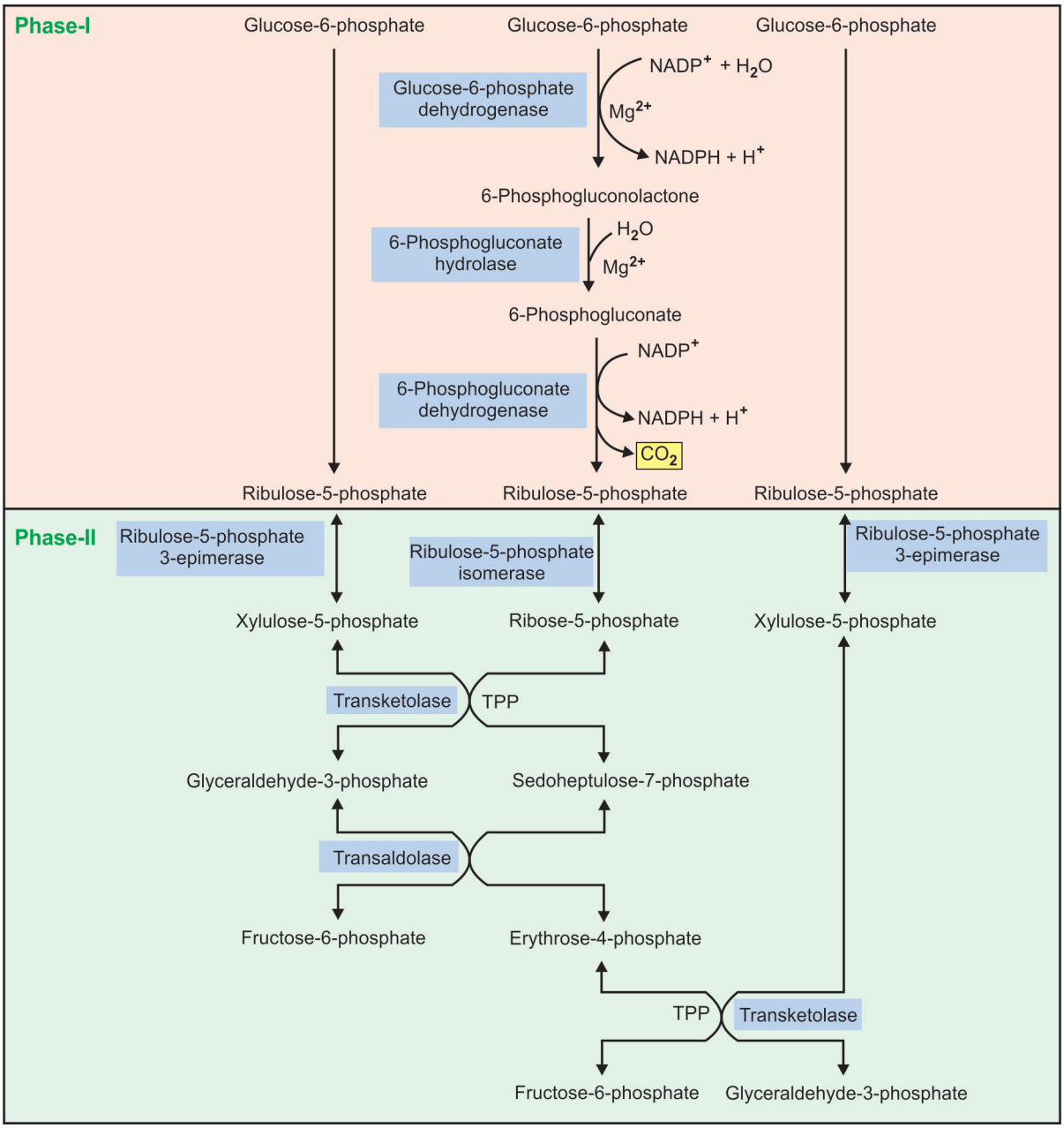


Figure 12.16: Pentose phosphate pathway, where, TPP: Thiamine pyrophosphate

Table 12.5: Difference between glycolysis and pentose phosphate pathway

Glycolysis	*Pentose phosphate pathway*
Oxidation occurs utilizing NAD^+ as an H-acceptor	Oxidation occurs utilizing NADP as an H-acceptor
Aerobic as well as anaerobic process	Anaerobic process
CO_2 is not produced at all	CO_2 is a characteristic product
ATP is generated, where it is a major function	ATP is not generated
Ribose phosphates are not generated	Ribose phosphates are generated
80-90% of glucose oxidized by glycolysis	10-20% glucose oxidized by pentose phosphate pathway

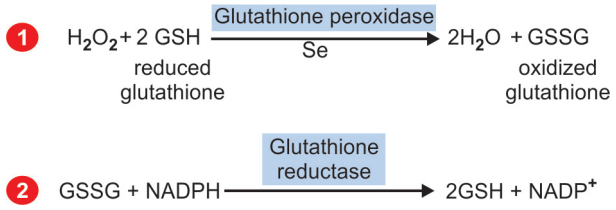


Figure 12.17: Role of NADPH in disposal of H_2O_2

Regulation of Pentose Phosphate Pathway

- The first step in the pathway, catalyzed by ***glucose-6-phosphate dehydrogenase (G-6-PD)*** is the rate limiting step.
- The activity of this enzyme is regulated by cellular concentration of NADPH. **NADPH is a competitive inhibitor of the enzyme G-6-PD.**
- An increased concentration of NADPH decreases the activity of G-6-PD, for example:
  - Under well-fed condition, the level of NADPH decreases and pentose phosphate pathway is stimulated.
  - However, in starvation and diabetes, the level of NADPH is high and inhibits the pathway.
- **Insulin** is also involved in the regulation of pentose phosphate pathway. It enhances the pathway by inducing the enzyme G-6-PD and 6-phosphogluconolactone dehydrogenase.

Disorders of Pentose Phosphate Pathway

Deficiency of Glucose-6-phosphate dehydrogenase (G-6-PD)

Glucose 6-phosphate dehydrogenase deficiency is X-linked inherited disorder, characterized by **hemolytic anemia,** due to excessive hemolysis.

- This enzyme catalyzes the first step in the pentose phosphate pathway and is needed for the formation of NADPH.
- The NADPH is required for the detoxification of H_2O_2 in red blood cell **(Figure 12.17).** In deficiency of G-6-PD, the production of NADPH is inadequate both to restore the reduced glutathione level and to maintain the RBC cell membrane. The consequence is destruction of the red blood cells and severe hemolytic anemia.
- Most of the patients of G-6-PD deficiency are asymptomatic and do not show hemolytic anemia under normal condition. However, they develop severe hemolytic anemia when they are exposed to certain antibiotic, antimalarial (primaquine) or antipyretic drugs.

G-6-PD deficiency and resistance to malaria

The malarial parasite, *Plasmodium falciparum* infects the red blood cell, where it depends on the reduced glutathione and the products of the pentose phosphate pathway for its optimum growth. Therefore, persons with G-6-PD deficiency cannot support growth of this parasite and thus are less prone to malaria than the normal person.

Wernicke-Korsakoff syndrome

- This is a genetic disorder due to reduced activity of the **TPP-dependent transketolase** enzyme. The reduced activity of transketolase is due to reduced affinity for TPP, whereas the other TPP dependent enzymes are normal. Therefore, in the chronic thiamine deficiency the transketolase enzyme has a much reduced activity leading to the **Wernicke-Korsakoff syndrome.**
- The symptoms of Wernicke-Korsakoff syndrome include weakness, mental disorder, loss of memory, partial paralysis, etc.

URONIC ACID PATHWAY (GLUCURONIC ACID CYCLE)

Definition

A pathway in liver for the conversion of glucose to glucuronic acid, ascorbic acid (except in humans and other primates as well as in guinea pigs) and pentoses is referred to as the uronic acid pathway. It is also an alternative oxidative pathway for glucose but does not generate ATP.

Reactions of Uronic Acid Pathway (Figure 12.18)

1. Glucose-6-phosphate is converted to glucose-1-phosphate catalyzed by ***phosphoglucomutase.***
2. Glucose-1-phosphate then reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDP-GLc). This reaction is catalyzed by the enzyme ***UDP-glucose pyrophosphorylase.***
3. UDP-Glucose is oxidized to glucuronate via UDP-glucuronate, catalyzed by an NAD dependent ***UDP-Glucose dehydrogenase.***

4. Glucuronate is reduced to L-gulonate by the NADPH dependent enzyme ***gulonate dehydrogenase***.
5. L-gulonate is the precursor of ascorbate (vitamin C) in those animals capable of synthesizing this vitamin. In humans and other primates, as well as in guinea pigs, ascorbic acid cannot be synthesized because of the **absence of the enzyme L-gulonolactone oxidase.**
6. L-gulonate is oxidized and decarboxylated to the pentose L-xylulose by the enzyme ***L-gulonate decarboxylase.***
7. L-xylulose is reduced to xylitol catalyzed by NADPH dependent ***L-xylulose dehydrogenase.***
8. Xylitol is oxidized to D-xylulose (isomer of L-xylulose) by an NAD dependent ***D-xylulose dehydrogenase*** enzyme**.**
9. D-xylulose, in turn, is phosphorylated by ATP in the presence of ***xylulose kinase*** to yield xylulose-5-phosphate, which is further metabolized in pentose phosphate pathway and leads to formation of glucose.

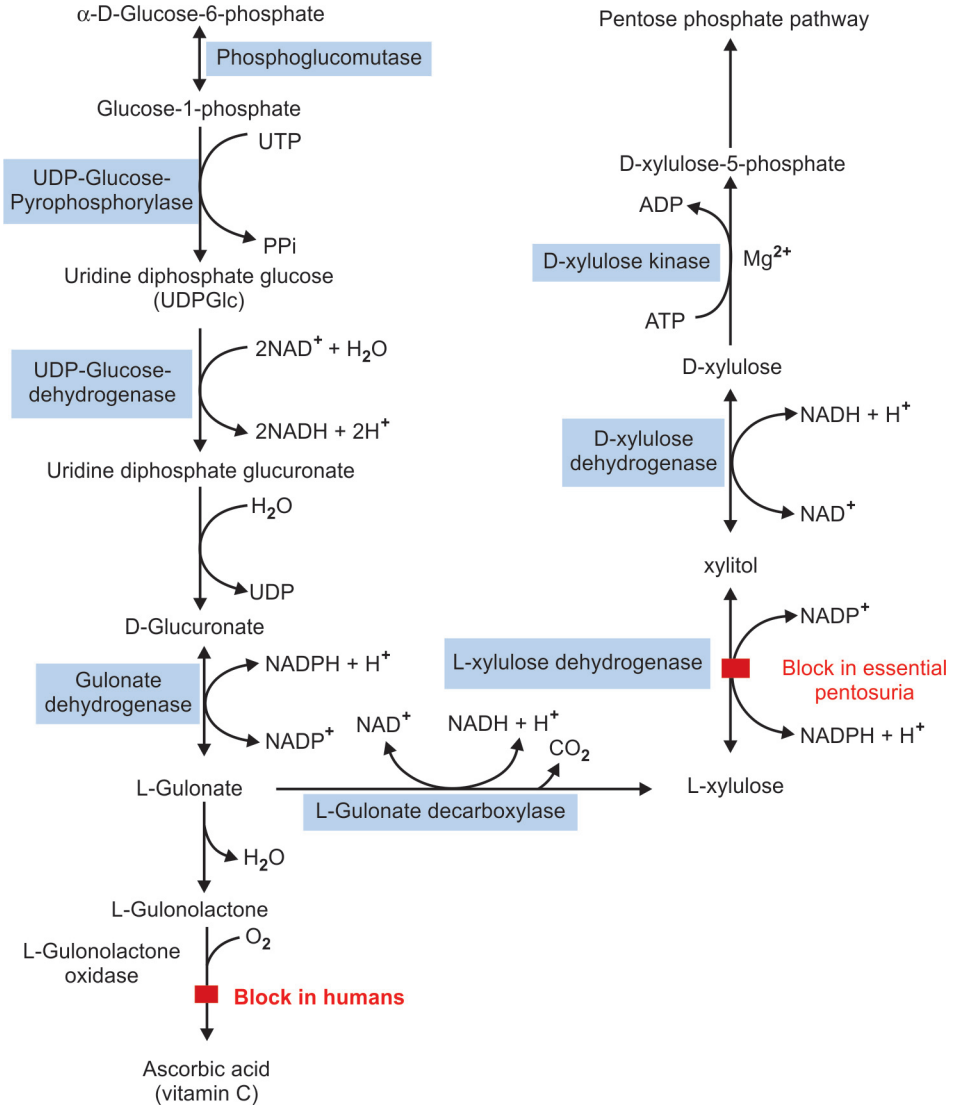


Figure 12.18: Uronic acid pathway

Significance of Uronic Acid Pathway

- The uronic acid pathway is a source of UDP-glucuronate.
- UDP-glucuronate is a precursor in biosynthesis of proteoglycans (glycosaminoglycans) and glycoproteins.
- UDP-glucuronate is involved in detoxification reactions that occur in liver. Many naturally occurring waste substances (like bilirubin and steroid hormones) as well as many drugs (like morphine, methanol, salicylic acid, etc.) are eliminated from the body by conjugating with UDP-glucuronate **(Figure 12.19).**
- The uronic acid pathway is a source of UDP-glucose, which is used for glycogen formation.
- The uronic acid pathway provides a mechanism by which dietary D-xylulose can enter the central metabolic pathway.

Disorder of Glucuronic Acid Pathway

Essential pentosuria

It is a benign (harmless) inborn error of metabolism in which the sugar L-xylulose is excreted in the urine in excess due to defect in $NADP^+$ linked ***L-xylulose dehydrogenase,*** one of the enzymes in the glucuronic acid pathway **(Figure 12.18)**. L-xylulose dehydrogenase is necessary to accomplish reduction of L-xylulose to xylitol.

GALACTOSE METABOLISM AND GALACTOSEMIA

Galactose is derived from disaccharide, lactose (the milk sugar) of the diet. It is important for the formation of:

- Glycolipids
- Glycoproteins

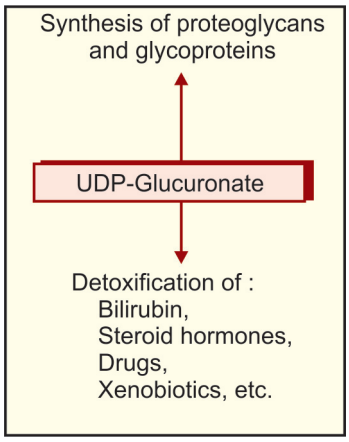


Figure 12.19: Metabolic significance of UDP-glucuronate

- Proteoglycans
- Lactose during lactation.

Galactose is readily converted in the liver to glucose. ***The ability of the liver to convert galactose to glucose is used as a liver function test (galactose tolerance test).***

Reactions of the Pathway (Figure 12.20)

1. The first reaction in galactose metabolism in the liver is phosphorylation of galactose to galactose-1-phosphate, by the enzyme ***galactokinase,*** using ATP as phosphate donor.
2. Galactose-1-phosphate reacts with UDP-glucose to form UDP-galactose and glucose-1-phosphate, catalyzed by ***galactose-1-phosphate uridyl transferase***. In this reaction, galactose displaces a glucose of UDP-glucose.

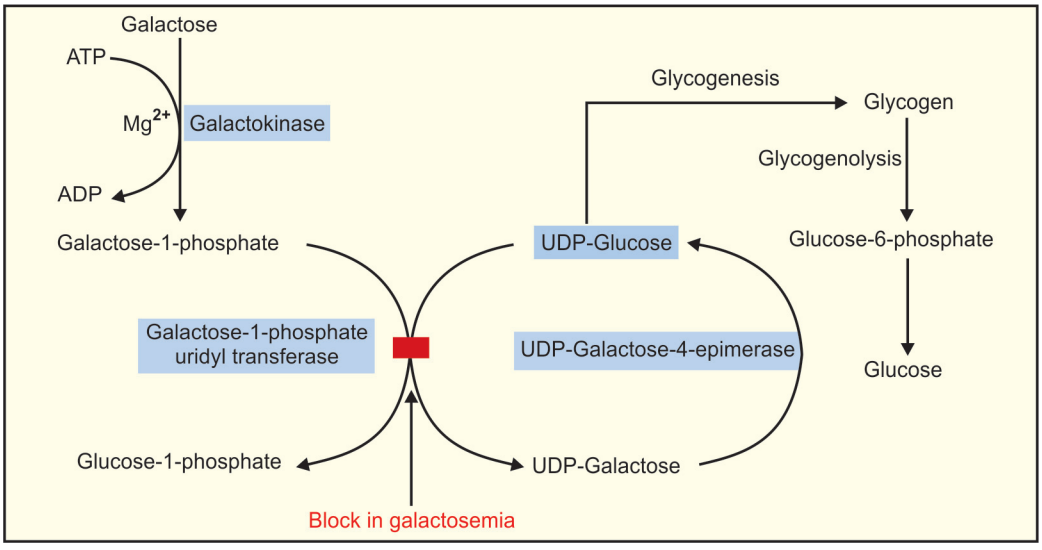


Figure 12.20: Pathway for conversion of galactose to glucose in the liver

3. The conversion of UDP-galactose to UDP-glucose is catalyzed by an ***UDP-galactose-4-epimerase.***
4. Finally, glucose is liberated from UDP-glucose via formation of glycogen by glycogenesis followed by glycogenolysis.

Disorder of Galactose Metabolism

Galactosemia

- It is an inborn error of galactose metabolism, caused by deficiency of enzyme **galactose-1-phosphate uridyl transferase (Figure 12.20).** The inherited deficiencies of **galactokinase** and **UDP-galactose-4-epimerase** also lead to minor types of galactosemia.
- They all interfere with the normal metabolism of galactose, causing a rise in blood and urine galactose.
- The commonest and most severe enzymatic defect is due to ***galactose-1-phosphate uridyl transferase*** deficiency which prevents conversion of galactose to glucose and leads to accumulation of galactose and galactose-1-phosphate in blood, liver, brain, kidney and eye lenses. In these organs, the galactose is reduced to galactitol (dulcitol) by the enzyme **aldose reductase.**

Clinical findings: The accumulation of galactitol and galactose-1-phosphate in liver, brain and eye lenses causes liver failure (hepatomegaly followed by cirrhosis), mental retardation and cataract formation respectively.

Treatment: Galactose in milk and milk products should be eliminated from the diet. Sufficient galactose for the body's need can be synthesized endogenously as UDP-galactose.

METABOLISM OF FRUCTOSE

Liver is the main site of fructose metabolism.

Reactions of Fructose Metabolism (Figure 12.21)

- Fructose is phosphorylated to fructose-1-phosphate by ***fructokinase*** in the liver.
- Fructose-1-phosphate is cleaved by liver ***aldolase (aldolase-B)*** to dihydroxyacetonephosphate (DHAP) and D-glyceraldehyde.
- D-glyceraldehyde is phosphorylated by glyceraldehyde kinase (or triokinase) to D-glyceraldehyde-3-phosphate. These two triose phosphates (DHAP and glyceraldehyde-3-phosphate) then may enter the glycolytic pathway, gluconeogenesis or glycogenesis according to the metabolic status of the tissue.

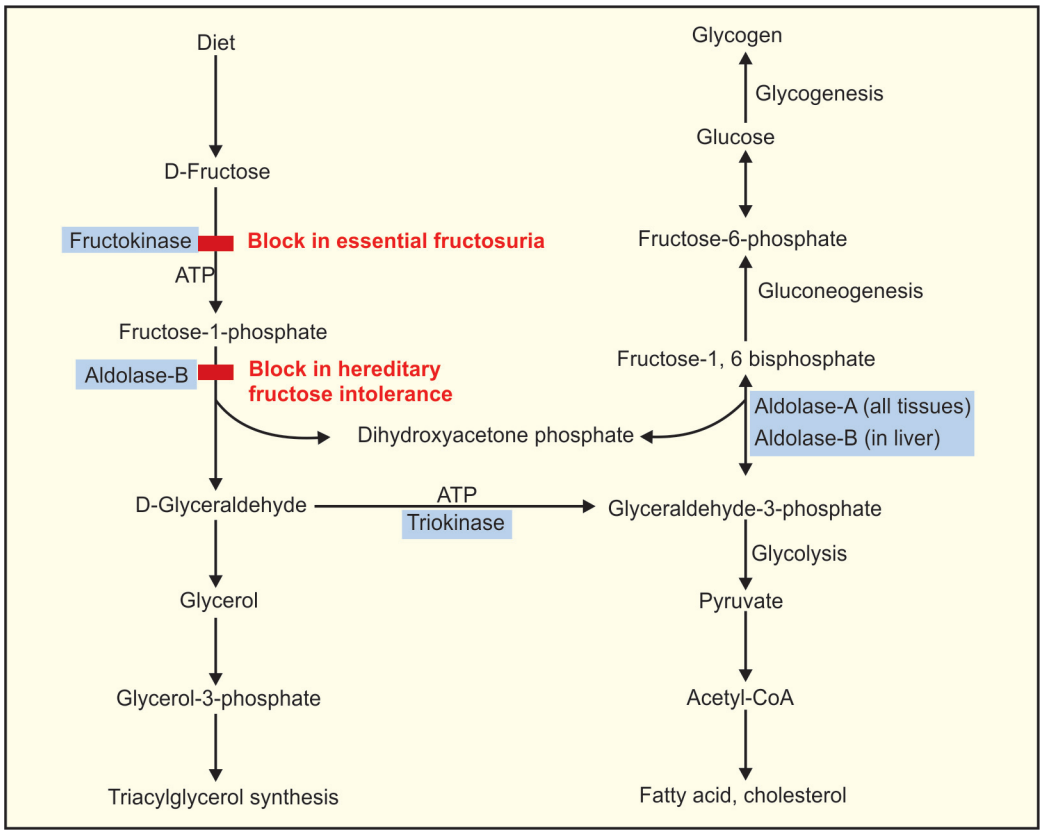


Figure 12.21: Metabolic pathway of fructose

Sorbitol or Polyol Pathway for Formation of Fructose

The sorbitol (polyol) pathway is for formation of fructose from glucose **(Figure 12.22).**

- ***Aldose reductase*** reduces glucose to sorbitol (glucitol).
- In the liver, ovaries and sperm cells, there is a second enzyme, ***sorbitol dehydrogenase*** that can oxidize the sorbitol to fructose.
- The pathway from sorbitol to fructose in the liver provides a mechanism by which dietary sorbitol is converted into fructose.

The Effect of Hyperglycemia on Sorbitol Metabolism

Because insulin is not required for entry of glucose into the cells such as retina, lens and nerve, large amounts of glucose may enter these cells during hyperglycemia, e.g. in uncontrolled diabetes.

- Elevated intracellular glucose concentrations cause increase in the amount of sorbitol and therefore accumulates inside the cell.
- This causes osmotic damage, leading to cataract formation, peripheral neuropathy, retinopathy and nephropathy.

Drugs inhibiting aldose reductase improve the peripheral nerve function in diabetes.

Disorders of Fructose Metabolism

Essential fructosuria

- Essential fructosuria is a rare and benign genetic disorders caused by a deficiency of the enzyme ***fructokinase.***
- In this disorder, fructose cannot be converted to fructose-1-phosphate.
- This is benign because no toxic metabolites of fructose accumulate in the liver and the patient remains nearly asymptomatic with excretion of fructose in urine.

Hereditary fructose intolerance

- It is due to deficiency of the enzyme ***Aldolase-B.***
- Fructose-1-phosphate cannot be converted to dihydroxyacetonephosphate and glyceraldehyde and therefore fructose-1-phosphate accumulates.
- This results in the inhibition of ***fructokinase*** and an impaired clearance of fructose from the blood.

Clinical findings

- Accumulation of fructose-1-phosphate leads to liver and kidney damage.
- Hypoglycemia due to inhibition of glycogenolysis and gluconeogenesis.

Treatment

Elimination of fructose containing foods from the diet.

BLOOD GLUCOSE LEVEL AND ITS REGULATION

The blood glucose level must be maintained within the narrow limits of **70-100** ***mg/dl.*** Levels above the normal range are termed ***hyperglycemia,*** those below are called ***hypoglycemia.*** After the ingestion of a carbohydrate meal, it may rise to 120-140 mg/dl.

Factors involved in the ***homeostasis (regulation)*** of blood glucose are:

- Hormones
- Metabolic processes
- Renal mechanism.

The liver is the organ primarily responsible for controlling the concentration of glucose in the blood. It can rapidly take up and release glucose in response to the concentration of blood glucose. The uptake and release of glucose by liver is regulated by hormones. The two major hormones controlling blood glucose levels are **(Figure 12.23)**:

1. Insulin (hypoglycemic hormone)
2. Glucagon (hyperglycemic hormone).

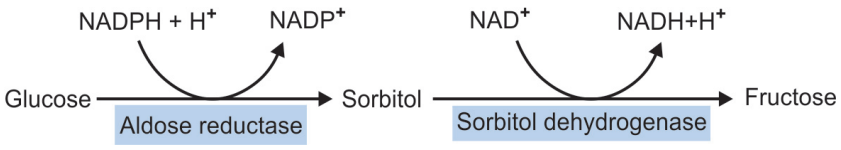


Figure 12.22: Sorbitol or polyol pathway

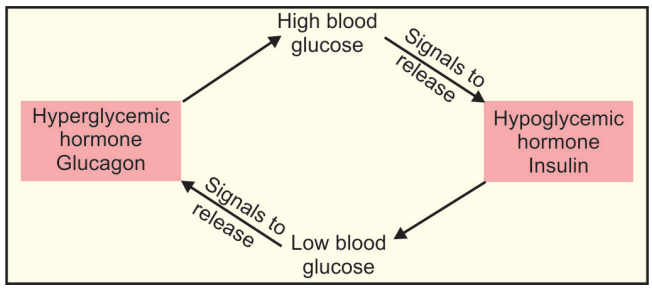


Figure 12.23: Regulation of blood glucose level by insulin and glucagon

Maintenance of Glucose in Fed State (Hyperglycemic condition)

Normally, there is an increased blood glucose level shortly after each meal, a ***postprandial hyperglycemia.*** Increased level of blood glucose releases ***insulin*** by β-cells of the Islets of the Langerhans. This hormone reduces the blood glucose level in a number of ways **(Figure 12.24)** as follows:

1. By stimulating the active transport of glucose across cell membranes of muscle and adipose tissue but not the liver. ***Glucose is rapidly taken up into liver as it is freely permeable to glucose.***
2. In the liver, insulin increases the use of glucose by ***glycolysis*** by inducing the synthesis of key glycolytic enzymes:
   - Glucokinase
   - Phosphofructokinase
   - Pyruvate kinase.
3. In the muscle and the liver, insulin stimulates ***glycogenesis*** by stimulating ***glycogen synthase*** and thereby leading to suppression of glycogenolysis.
4. Insulin inhibits ***gluconeogenesis*** by suppressing the action of key enzymes of gluconeogenesis, e.g.
   - Pyruvate carboxylase
   - Phosphoenol pyruvate carboxykinase
   - Fructose 1,6-bisphosphatase
   - Glucose-6-phosphatase.
5. In adipose tissue, glucose is converted to the glycerol-3-phosphate, needed for the formation of triacylglycerol (lipogenesis).
6. Insulin increases protein synthesis and decreases protein catabolism, thereby releasing amino acids. All these mechanisms are responsible for a drop in blood glucose level (hypoglycemia).

Maintenance of Blood Glucose in Fasting State (Hypoglycemic Condition)

Decreased level of blood glucose, hypoglycemia causes a release of hyperglycemic hormones, e.g.

- Glucagon
- Epinephrine or adrenaline
- Glucocorticoids
- Growth hormone and adrenocorticotropic hormone (ACTH)
- Thyroxine.

Glucagon

Glucagon is the hormone produced by the α-cells of the islets of Langerhans of the pancreas. Glucagon opposes the actions of insulin. It acts primarily in the liver as follows:

- In the liver, it stimulates glycogenolysis by activating enzyme ***glycogen phosphorylase*** and inhibits glycogen synthesis. Unlike epinephrine, glucagon does not have an effect on muscle phosphorylase due to lack of receptors.

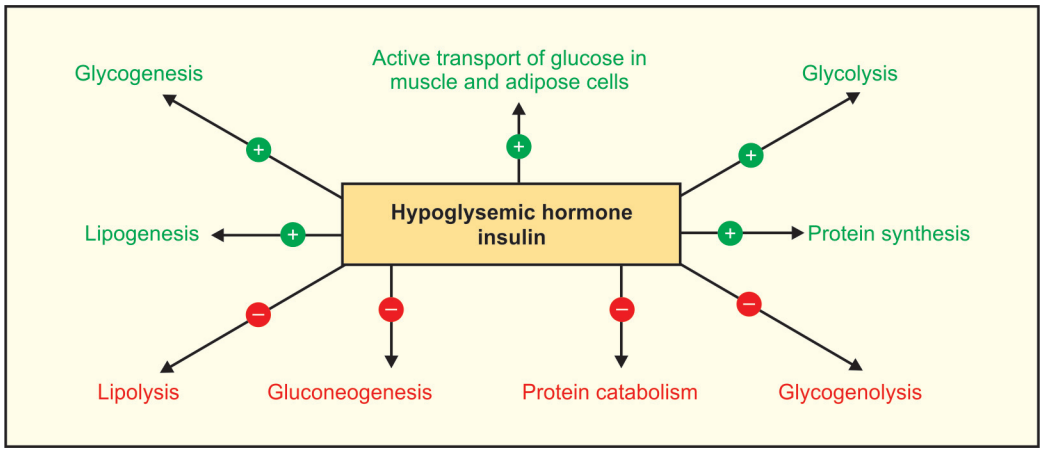


Figure 12.24: Role of insulin in regulation of blood glucose level

- Glucagon enhances gluconeogenesis from amino acids and lactate by inducing the action of key enzymes of gluconeogenesis.
  - Alanine is the predominant amino acid released from muscle to liver by **glucose alanine cycle (see Figure 12.9).**
  - Lactate formed by oxidation of glucose in skeletal muscle is transported to the liver by **lactic acid (Cori) cycle (see Figure 12.9).**

Epinephrine or Adrenaline

- It is secreted by adrenal medulla.
- It stimulates **glycogenolysis** in the liver and the muscle by stimulating **glycogen phosphorylase** activity via c-AMP.
- In muscle as a result of the **absence of glucose-6-phosphatase**, glycogenolysis results with the formation of lactate, whereas in the liver, glucose is the main product, leading to increase in blood glucose.

Glucocorticoids

- These hormones are secreted by adrenal cortex, which causes increased:
  - Gluconeogenesis by increasing the activity of enzymes of gluconeogenesis.
  - Protein catabolism to provide glucogenic amino acid for gluconeogenesis.
  - Hepatic uptake of amino acids for gluconeo genesis.
- In addition, glucocorticoids inhibit the utilization of glucose in extrahepatic tissues. Thus, all the actions of glucocorticoids are antagonistic to insulin.

Growth hormone and anterior pituitary hormones

- Growth hormone and ACTH antagonise the action of insulin by elevating the blood glucose level.
- Growth hormone decreases glucose uptake in the muscle and ACTH decreases glucose utilization by the tissue.

Thyroxine

- It is secreted by thyroid gland.
- Thyroxine accelerates hepatic glycogenolysis with consequent rise in blood glucose.
- It may also increase the rate of absorption of hexoses from the intestine.

Renal Control Mechanism

- When blood glucose rises to relatively high levels, the kidney also exerts a regulatory effect. Glucose is continously filtered by the glomeruli but is normally reabsorbed completely in renal tubules. The capacity of the tubular system to reabsorb glucose is limited.
- If the blood glucose level is raised above ***180 mg/100 ml,*** complete tubular reabsorption of glucose does not occur and the extra amount appears in the urine causing ***glycosuria.***
- 180 mg/100 ml is the limiting level of glucose in the blood, above which tubular reabsorption does not occur which is known as ***renal threshold value for glucose.***
- Thus, by excreting extra amount of sugar in the urine during hyperglycemic state and reabsorbing sugar during the hypoglycemic state, the kidney helps in regulating the level of glucose in blood.

GLYCOSURIA

Normally, the urine contains about 0.05 gm% of sugar. Such a small quantity cannot be detected by Benedict's test, but under certain circumstances, a considerable amount of glucose or other sugar may be excreted in the urine.

- Excretion of detectable amount of sugar in urine is known as ***glycosuria.***
- Glycosuria results from the rise of blood glucose above its renal threshold level (180 mg%)
- Glycosuria may be due to various reasons on the basis of which it is classified into the following groups:
  1. Alimentary (lag storage) glycosuria
  2. Renal glycosuria
  3. Diabetic glycosuria.

Alimentary (Lag Storage) Glycosuria

- The blood sugar level of some individuals after meal rises rapidly above the normal renal threshold (180 mg/dL) and results in glycosuria and known as **alimentary glycosuria.** This is due to an increased rate of absorption of glucose from the intestine. This is called alimentary glycosuria since alimentary canal (GI-tract) is involved.
- Characteristic feature of this glycosuria is that usually high blood glucose level returns to normal at 2 hours after a meal. This type of glycosuria is benign (harmless).

Renal Glycosuria

- This is observed due to impaired tubular reabsorption of glucose and have lowered renal threshold (may be 130-150 mg%) for glucose.

- In such cases, the blood glucose level is below 180 mg%, i.e. below normal renal threshold for glucose, but glucose appears in the urine due to lowered renal threshold.
- Renal glycosuria is a benign condition, unrelated to diabetes and it may occur temporarily in pregnancy without symptoms of diabetes.
- Renal glycosuria may result from inherited defects in the kidney or it may be acquired as a result of kidney disease.

Diabetic Glycosuria

- Diabetic glycosuria is a pathological condition and is due to deficiency or lack of insulin which causes diabetes mellitus.
- Although the renal threshold is normal, as blood glucose level exceeds the renal threshold, the excess glucose passes into the urine to produce glycosuria.

DIABETES MELLITUS

Definition

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism, caused by either:
- Lack of insulin secretion or
- Decreased sensitivity of the tissues to insulin.

Classification of Diabetes Mellitus

Diabetes mellitus is broadly divided into two groups namely:
1. Type I diabetes mellitus or insulin dependent diabetes mellitus (IDDM).
2. Type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM).

Type I diabetes mellitus

Type l diabetes is also called **insulin dependent diabetes mellitus (IDDM)** or juvenile onset diabetes.

Cause

It is caused by lack of insulin secretion due to destruction of pancreatic beta cells. The destructions of β-cells may be due to:
1. Viral infection
2. Autoimmune disorder (destruction of tissues by body's own antibodies)
3. There may be hereditary tendency for β-cell degeneration.

Onset

The usual onset of type-l diabetes occurs at about 14 years of age and for this reason it is called **juvenile** diabetes mellitus ('juvenile' means teenage in Latin).

Symptoms
- It develops symptoms very abruptly with:
  - Polyuria (frequent urination)
  - Polydypsia (excessive thirst)
  - Polyphagia (excessive hunger).
- These symptoms are accompanied by loss of body weight, weakness, and tiredness.
- Hyperglycemia with **glycosuria** and **ketoacidosis** are the metabolic changes.
- The patients of type-I diabetes mellitus are not obese.

Treatment
- Since patients of IDDM (type-I) fail to secrete insulin, administration of exogenous insulin is required.

Type II diabetes mellitus

Type II or non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes mellitus.

Cause

It is caused by decreased sensitivity of target tissues to insulin. This reduced sensitivity of insulin is often referred to as ***insulin resistance.*** This is perhaps due to ***inadequate insulin receptors*** on the cell surfaces of the target tissues. This syndrome is often found in an obese person.

Onset

Onset of the type II diabetes occurs after age 40 and the disorder develops gradually. Therefore, this syndrome is referred to as adult onset diabetes.

Symptoms

In type II diabetes mellitus, the symptoms are developed gradually which are similar to that of type-I **except ketoacedosis** is usually not present in type II diabetes mellitus.

Treatment
- NIDDM (type-II) can be treated in early stages by diet control, exercise and weight reduction and no exogenous insulin administration is required.
- Drugs that increase insulin sensitivity such as ***thiazolidinedions*** and ***metformin*** or drugs that cause additional release of insulin by the pancreas such as ***sulfonylureas*** may also be used.

- However, in the later stages insulin administration is often required to control blood glucose.

The comparison between two types of diabetes mellitus is given in **Table 12.6.**

Metabolic changes in diabetes mellitus

- In diabetes mellitus, the metabolic changes occur due to a deficiency of **insulin** and relative excess of **glucogon.** These changes in hormonal levels most profoundly affect metabolism in three tissues; liver, muscle and adipose tissue.
- Elevated levels of **blood glucose** and **ketone bodies** are the characterisitc feature of untreated diabetes mellitus.
- The lack of insulin activity in diabetes mellitus results in failure of transfer of glucose from the blood into cells and leads to **hyperglycemia.** The body responds as it were in the fasting state (**see chapter 15**) with stimulation of:
  - Glycogenolysis
  - Gluconeogenesis
  - Lipolysis
  - Proteolysis.
- An increase in glucogon favors lipolysis in adipose tissue. Increased lipolysis leads to increased mobilization of fatty acids from adipose tissue to liver, where they are converted to **ketone bodies** (β-hydroxybutyrate and acetoacetate), causing the **ketoacidosis.**
- Due to lack of insulin, the synthesis of the enzyme **lipoprotein lipase,** required for the degradation of VLDL is decreased. Decreased synthesis of lipoprotein lipase leads to elevated levels of plasma VLDL, resulting in **hypertriglycerolemia.**
- The other metabolic changes that occur in diabetes mellitus is increased mobilization of amino acids due to increased rate of proteolysis. The amino acids released from muscle are converted to glucose by gluconeogenesis.

GLUCOSE TOLERANCE TEST (GTT)

Glucose tolerance test (GTT) is performed to assess the ability of the body to utilize glucose. GTT can be performed by two ways:

1. Oral GTT
2. Intravenous GTT.

Oral Glucose Tolerance Test

- The patient who is scheduled for oral GTT is instructed to eat a high carbohydrate diet for at least 3 days prior to the test, and come after an overnight fast on the day of the test.
- A fasting blood glucose sample is first drawn.
- Then 75 gm of glucose (or 1.75 gm per kg body weight) dissolved in 300 ml of water is given orally.
- After giving glucose, blood and urine specimens are collected at half hourly intervals for at least 2 hours.
- Blood glucose content is measured and urine is tested for glycosuria.
- A curve is plotted for time against blood glucose concentration and is called **glucose tolerance curve (Figure 12.25).**

Intravenous Glucose Tolerance Test

- The intravenous glucose tolerance test is often used for persons with malabsorptive disorders or previous gastric or intestinal surgery.

Table 12.6: Comparison of two types of diabetes mellitus

Features	*Type-I:Insulin dependent diabetes mellitus*	*Type-II:Non-insulin dependent diabetes mellitus*
Frequency	5-10%	90-95 %
Age of onset	Early during childhood or puberty usually < 20 years	Later after age of 40 years
Onset of symptoms	Abrupt and severe	Gradual, insidious
Plasma insulin	Low or absent	Normal to high
Body weight	Low to normal	Obese
Blood glucose	Increased	Increased
Insulin sensitivity	Normal	Reduced
Ketosis	Common	Rare
Acute complications	Ketoacidosis	Hyperosmolar coma
Treatment with insulin	Necessary	Usually not required

- Glucose is administered intravenously over 30 minutes using 20% glucose solution. A glucose load of 0.5 gm/kg of body weight is used.

Types of Glucose Tolerance Curves

There are three types of Glucose Tolerance Curves:

1. Normal glucose tolerance curve
2. Decreased glucose tolerance
3. Increased glucose tolerance.

Normal glucose tolerance curve (Figure 12.25)

Normal response to glucose load is as follows:

- Initial fasting glucose is within the normal fasting limits (70 to 100 mg%).
- Blood glucose level rises to a peak (120 to 140 mg%) at half to 1 hour after ingestion of glucose.
- The blood glucose level then returns rapidly to the fasting normal limits in about 2 hours.
- Glucose should not be present in any of the urine specimens collected for 2 hours.

Decreased glucose tolerance (Figure 12.25)

Decreased glucose tolerance means decreased ability of the body to utilize glucose. In decreased glucose tolerance:

- Fasting glucose is higher than normal limits.
- The blood glucose level rises above 180 mg/100 ml (renal threshold) after ingestion of glucose.
- The blood glucose remains high for a longer time and may not return to fasting level even after 3 hours.
- The urine samples corresponding to blood glucose level over 180 mg/100 ml may show urine Benedict's test positive (glycosuria).
- Decreased glucose tolerance occurs in **diabetes mellitus** and certain endocrine disorders like:
  - Hyperthyroidism
  - Hyperpituitarism
  - Hyperadrenalism (Cushing's syndrome).

Increased glucose tolerance (Figure 12.25)

Increased glucose tolerance means increased ability of the body to utilize glucose. In increased glucose tolerance:

- Fasting blood glucose is lower than normal.
- Only small rise in blood glucose level may be observed (not more than 100 mg%) even after glucose administration.
- A flatter type of curve is obtained.
- No appearance of glucose in urine.
- This type of curve is obtained in endocrine hypoactivity like:
  - Hypothyroidism (myxedema, cretinism)
  - Hypoadrenalism (Addison's disease)
  - Hypopituitarism.

Significance of GTT

GTT is not necessary in symptomatic or in known cases of diabetic patients. In such cases, determination of fasting or postprandial glucose is usually sufficient for the diagnosis.

- GTT is most important in the investigation of asymptomatic hyperglycemia or glycosuria such as renal glycosuria and alimentary glycosuria.
- This test may give useful information in some cases of endocrine dysfunctions.
- It is also helpful in recognizing milder cases of diabetes.

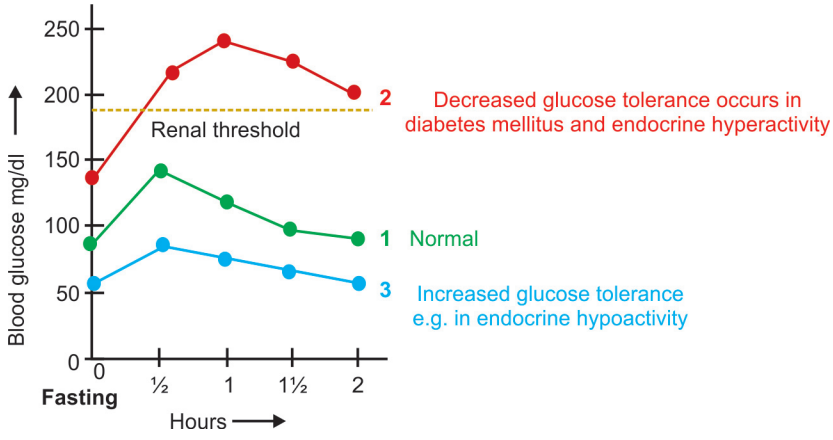


Figure 12.25: The glucose tolerance curves

SUMMARY

- Glycolysis is the pathway found in the cytosol for the conversion of glucose into two molecules of pyruvate with generation of ATP.
- Under anaerobic condition, e.g. in exercising muscles and in erythrocytes, the pyruvate is reduced to lactate.
- In aerobic condition, cell pyruvate is oxidized to acetyl-CoA and CO_2 by multienzyme complex pyruvate dehydrogenase, instead of being reduced to lactate.
- In erythrocytes, the first site for generation of ATP in glycolysis may be bypassed leading to the formation of 2,3 bisphosphoglycerate.
- Citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid and protein.
- The citric acid cycle is amphibolic since it has other metabolic roles in addition to oxidation. It takes part in gluconeogenesis, transamination, synthesis of heme and fatty acids.
- Gluconeogenesis is the synthesis of new glucose from noncarbohydrate sources, such as lactate, glucogenic amino acids, glycerol, and propionate. It provides glucose to the body when carbohydrate is not available from the diet.
- Glycogenesis is the synthesis of glycogen from glucose. Glycogen is broken down by a separate pathway known as glycogenolysis.
- The pentose phosphate pathway, which is present in the cytosol, generates NADPH, ribose-5-phosphate, and CO_2. The pathway does not generate ATP. NADPH is used in reductive biosynthesis, e.g. lipogenesis, or steroidogenesis, whereas ribose-5-phosphate is used in the synthesis of RNA and DNA.
- In erythrocytes, the HMP pathway has a major function in preventing hemolysis by providing NADPH. Impairment of the pentose phosphate pathway due to deficiency of glucose-6-phosphate dehydrogenase leads to hemolytic anemia.
- The uronic acid pathway is the source of UDP-glucuronate which functions to detoxify (by conjugation) many endogenous and exogenous substances.
- Inability to metabolize galactose to glucose occurs in the galactosemia, which may be due to inherited defects in, uridyl transferase.
- The blood glucose level is regulated within the narrow limits of 70-100 mg/dl by metabolic, hormonal and renal mechanism.
- Insulin (hypoglycemic hormone) and glucagon (hyperglycemic hormone) play a central role in regulating blood glucose. Other hyperglycemic hormones involved in blood glucose regulation are growth hormone, ACTH, glucocorticoids, epinephrine and thyroxine.
- Glycosuria occurs when the renal threshold for glucose is exceeded.
- Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by absolute or relative insulin deficiency and characterized by decreased glucose tolerance that leads to hyperglycemia.

EXERCISE

Solve the Following

Case History 1

A 12-year-male had complained of abdominal discomfort, a feeling of being bloated, increased passage of urine and development of diarrhea after taking milk.

Questions

a. Name the probable disorder.
b. Cause of disorder.
c. What will you suggest the patient to relieve the symptoms?

Case History 2

The following are the findings in a patient brought to the hospital in a coma state.

Findings	*Patient*	*Normal*
Blood sugar (Fasting)	270 mg%	70-100 mg%
Urine Benedict's test	Positive	Negative
Urine Rothera's test	Positive	Negative
Plasma pH	7.20	7.35 to 7.45

Questions

a. Name the disorder.
b. Why is patient's plasma pH lower than normal?
c. What does positive Rothera's test indicate?
d. What is the renal threshold value for glucose?

Case History 3

A 20-year-old male suffering from malaria was treated with chloroquine and manifested as hemolytic anemia. Provisional diagnosis of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was made.

Questions

a. Which reaction is catalyzed by the enzyme G-6-PD?

b. How does deficiency of G-6-PD produce hemolytic anemia?
c. Name the pathway in which this reaction occurs.

Case History 4

A chronically cranky, irritable and lethargic baby girl has an extended abdomen, resulting from an enlarged liver and was diagnosed of having Von Gierke's disease.

Questions

a. Which enzyme is deficient in Von Gierke's disease?
b. Name the pathways where the enzyme is required.
c. Give manifestations of the disorder.

Case History 5

A 6-month-old infant was presented with elevated blood and urine galactose.

Questions

a. Name the disease.
b. Give the biochemical steps related to the disease and point out the metabolic defect.
c. What are the clinical manifestation of the disease?

Case History 6

An obese person came to the hospital with complaints of polyuria, thirst, weakness and increased appetite. On investigations, he was diagnosed having diabetes mellitus.

Questions

a. What is the cause of diabetes mellitus?
b. Give names of different types of diabetes mellitus.
c. What is glucosuria? Name different types of glucosuria.
d. What is the normal blood sugar level?

Case History 7

A 28-year-old man has complained of chronic leg muscle pains and cramps during exercise. This patient suffers from McArdle syndrome.

Questions

a. What is McArdle syndrome? To metabolism of which biomolecule is it related?
b. What is the cause of this syndrome?
c. Name different types of disorders related to the concerned biomolecule.

Case History 8

A 13-year-old diabetic boy visits a diabetic clinic for a check-up. He tells the doctor that he complies with all the dietary advice and never misses insulin. His random blood glucose level is within normal limit but his HbA_1c concentration is 10% (normal 4-6%). He has no glycosuria or ketone bodies in his urine.

Questions

a. What does normal blood glucose and urine glucose indicate?
b. What does elevated level of HbA_1c suggest?
c. Name the type of diabetes the boy is suffering.
d. What is HbA_1C?

Case History 9

A 3-year-old patient with mild mental retardation was found to have cataract. Biochemical investigations show high blood concentrations of a sugar alcohol and galactose.

Questions

a. Name the probable disease.
b. Name enzyme most likely to be defective.
c. What is the cause of development of cataracts?
d. What is the treatment?

Multiple Choice Questions (MCQs)

1. Which of the following enzymes produce a product used for synthesis of ATP by substrate level?
a. Phosphofructokinase b. Aldolase
c. 1,3-bisphosphate mutase d. Enloase

2. 2,3-bisphosphoglycerate is:
a. A high energy substrate
b. Involved in substrate level phosphorylation
c. An intermediate in pentose phosphate pathway
d. An allosteric effector that decreases the O_2 affinity of Hb

3. Muscle glycogen is not available for maintenance of blood glucose concentration because:
a. Muscle lacks glucose-6-phosphatase activity
b. There is insufficient glycogen in muscle
c. Muscle lacks glucose transporter GLUT-4
d. Muscle lacks glucagon receptors

4. The primary metabolic fate of lactate released from muscle during intense exercise is:
a. Excretion of lactate in urine
b. Transported to liver for replenishment of blood glucose by *gluconeogenesis*

c. Conversion to pyruvate
d. Gradual reuptake in muscle during the recovery phase following exercise

5. Which of the following statement is true of TCA cycle?
a. It requires coenzyme biotin, FAD, NAD and coenzyme A
b. Two NADH are produced per turn
c. It participates in the synthesis of glucose from pyruvic acid
d. Enzymes are located in cytosol

6. Which of the following vitamins does not participate in the oxidative decarboxylation of pyruvate to acetyl-CoA?
a. Thiamine b. Biotin
c. Niacin d. Riboflavin

7. The following enzymes catalyze a decarboxylation reaction, *except:*
a. α-ketoglutarate dehydrogenase
b. Pyruvate dehydrogenase
c. Pyruvate carboxylase
d. Isocitrate dehydrogenase

8. The principal source of glucose after an overnight fast is:
a. Gluconeogenesis b. Glycolysis
c. Glycogenolysis d. HMP pathway

9. Which of the following cannot take place in the human body?
a. Transformation of lactate into glucose
b. Transformation of glycerol into glucose
c. Transformation of propionyl-CoA into glucose
d. Transformation of acetate into glucose

10. In a patient with galactosemia who is on a galactose free diet, the D-galactose that is required for cell membrane and other biopolymers is formed by:
a. Isomerization of glucose-1-phosphate
b. Epimerization of UDP-glucose
c. Epimerization of D-fructose
d. Epimerization of glucose-6-phosphate

11. Gluconeogenesis, must bypass irreversible reactions of glycolysis, *except:*
a. Hexokinase
b. Phosphohexose isomerase
c. Pyruvate kinase
d. Phosphofructokinase

12. In skeletal muscle, glycogen synthesis occurs during:
a) Contraction
b) Relaxation
c) Well-fed state
d) Increased level of insulin

13. The following hormones have hyperglycemic effect, *except:*
a. Glucagon b. Thyroid
c. Epinephrine d. Insulin

14. Rate controlling steps in the citric acid cycle include all of the following, *except:*
a. Isocitrate dehydrogenase
b. Citrate synthase
c. Fumarase
d. α-ketoglutarate dehydrogenase

15. Gluconeogenesis can proceed from all of the following, *except:*
a. Pyruvate b. Palmitic acid
c. Propionyl-CoA d. Oxaloacetate

16. The following are the functions of pentose phosphate pathway, *except:*
a. Interconverts hexoses and pentoses
b. Produces NADPH
c. Supplies ribose-5-phosphate
d. Converts glucose to galactose

17. People with diabetes mellitus are prone to develop cataracts because their elevated blood glucose concentration:
a. Inhibit gluconeogenesis
b. Increase glycosylate hemoglobin
c. Increase glycogen synthesis within the lens
d. Allow aldose reductase to reduce glucose to sorbitol

18. Both glycolysis and gluconeogenesis involve which of the following enzymes:
a. Pyruvate carboxylase
b. Hexokinase
c. Aldolase
d. Phosphofructokinase

19. Glucose-6-phosphate is involved in which of the following pathways:
a. Glycolysis
b. Gluconeogenesis
c. Pentose phosphate pathway
d. All of the above

20. Gluconeogenesis occurs in which of the following:
a. Heart b. Erythrocytes
c. Liver d. Lungs

21. Von Gierke's disease is characterized by deficiency of the enzyme:
a. Glucokinase
b. Glucose-6-phosphatase
c. Phosphoglucomutase
d. Glycogen synthase

22. McArdle syndrome involves a deficiency of which of the following enzymes:
a. Hepatic phosphorylase
b. Muscle phosphorylase
c. Debranching enzyme
d. Hepatic glycogen synthase

23. Deficiency of glucose-6-phosphate dehydrogenase causes:
a. Cataract
b. Hypoglycemia
c. Hemolytic anemia
d. Galactosemia

24. Essential pentosuria is due to metabolic defect in:
a. Glycolysis b. HMP-shunt
c. Uronic acid pathway d. Glycogenolysis

25. Rapoport leubering cycle in RBC produces:
a. ATP
b. NADPH
c. 2,3-bisphosphoglycerate
d. 1,3-bisphosphoglycerate

26. The first loss of carbon in the metabolism of glucose takes place as CO_2 in the formation of:
a) Acetyl-CoA
b) Pyruvate
c) 2,3 BPG
d) Fructose 1,6 bisphosphate

27. Anaerobic glycolysis from glycogen generates:
a) 3 moles of ATP b) 2 moles of ATP
c) 8 moles of ATP d) 6 moles of ATP

28. The net production of ATP when glycolysis occurs via Rapoport Lubering route:
a) Two b) Six
c) Eight d) Zero

29. In erythrocytes, the end product of glycolysis is:
a) Pyruvate
b) Acetyl-CoA
c) Lactate
d) 2,3 Bisphosphoglycerate

Correct Answers for MCQs

1-d	2-d	3-a	4-b
5-c	6-b	7-c	8-a
9-d	10-b	11-b	12-b
13-d	14-c	15-b	16-d
17-d	18-c	19-d	20-c
21-b	22-b	23-c	24-c
25-c	26-a	27-a	28-d
29-c			

CHAPTER

13

Lipid Metabolism

INTRODUCTION

Lipids include a wide variety of chemical substances such as:

- Neutral fat (triacylglycerol or triglycerides)
- Fatty acids and their derivatives
- Phospholipids
- Glycolipids
- Sterols
- Fat soluble vitamins (A, D, E and K).

An adult ingests about 60–150 gm of lipids per day, of which more than 90% fat intake in the diet is as **triacylglycerols,** with the remainder consisting of cholesterol, cholesterol ester, phospholipid and free fatty acid.

DIGESTION AND ABSORPTION OF LIPIDS

Digestion

Little or no digestion occurs in the mouth or stomach. The major site of lipid digestion is the small intestine, where dietary lipid undergoes its major digestive processes using enzymes secreted by pancreas.

Digestion in Small Intestine

- The acidic stomach contents called ***chyme,*** containing dietary fat leaves the stomach and enters the small intestine, in the duodenum. Digestion of fat occurs in the duodenum by **emulsification of fat.**
- In the duodenum the dietary fat is **emulsified** by the action of **bile salts.**

Hydrolysis of dietary triacylglycerols

Emulsified triacylglycerols are hydrolyzed by ***pancreatic lipase.*** Lipase hydrolyses fatty acid in the 1 and 3 positions of the triacylglycerol, producing 2 monoacylglycerols and two molecules of fatty acids **(Figure 13.1).**

Hydrolysis of dietary phospholipids

Dietary glycerophospholipids are digested by pancreatic ***phospholipase-A_2.*** This enzyme catalyzes the hydrolysis of fatty acid residues at the 2-position of the phospholipid, leaving lysophospholipids and a molecule of fatty acid **(Figure 13.1).**

Hydrolysis of cholesterol ester

Cholesterol esters are hydrolyzed by pancreatic ***cholesterol ester hydrolase (cholesterol esterase),*** which produces cholesterol plus free fatty acid.

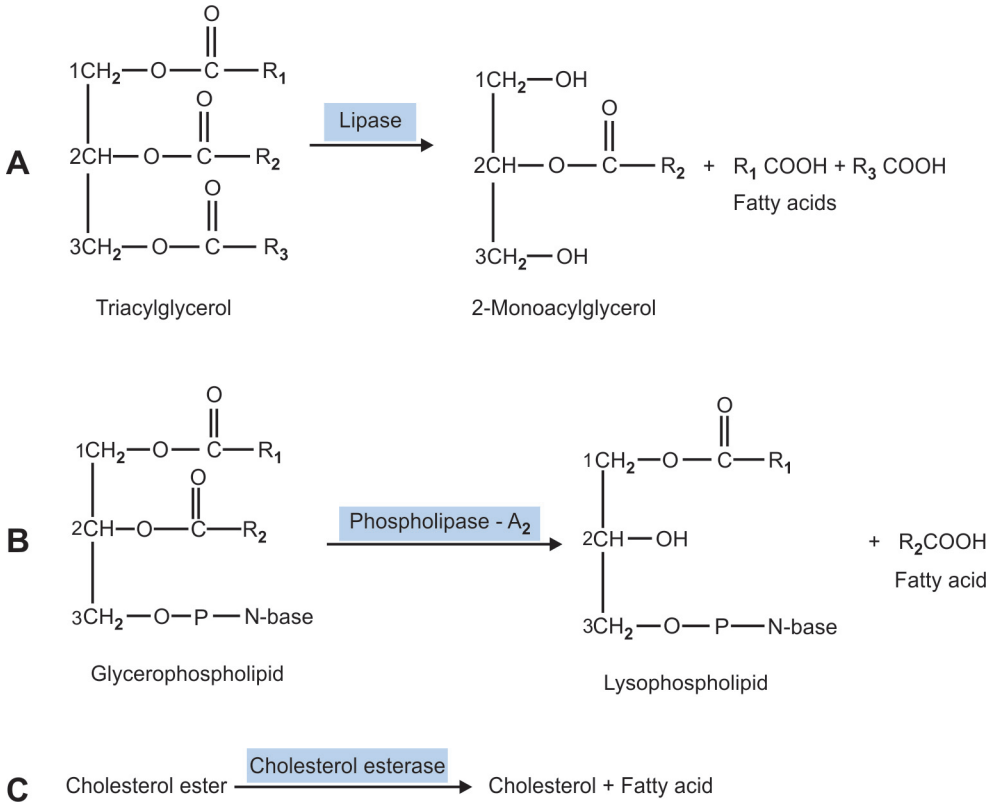


Figure 13.1: Hydrolysis of dietary triacylglycerol, phospholipid and cholesterol ester in intestine

Products of Lipid Digestion (Micelle Formation)

Free fatty acids, free cholesterol, 2-monoacylglycerol, and lysophospholipid are the primary products of dietary lipid digestion. These, together with bile salts, form ***mixed micelles***. Fat soluble vitamins A, D, E and K are also packaged in these micelles and are absorbed from the micelles along with the products of dietary lipid digestion.

Absorption of Lipids by Intestinal Mucosal Cells

- The mixed micelles approach the brush border membrane of the intestinal mucosal cells.
- There the lipid components from mixed micelles are absorbed into the mucosal cells by diffusion.
- The net result is the transfer of monoacylglycerol, fatty acids, cholesterol, and lysophospholipid molecules into the mucosal cell.
- After absorption within the mucosal cell, the following events occur **(Figure 13.2):**
  - 2-monoacylglycerols are reconverted to triacylglycerols. The fatty acids absorbed from the lumen are utilized for this synthesis. Fatty acids are utilized after its conversion to its active form, acyl- CoA.
  - The absorbed lysophospholipids and cholesterol are also reconverted to phospholipids and cholesterol esters.
  - The triacylglycerol resynthesized in intestinal cells combine with cholesterol, phospholipids and proteins to form **chylomicrons.**

Transport

- Triacylglycerol, phospholipid, cholesterol esters resynthesized in the intestinal mucosa and absorbed

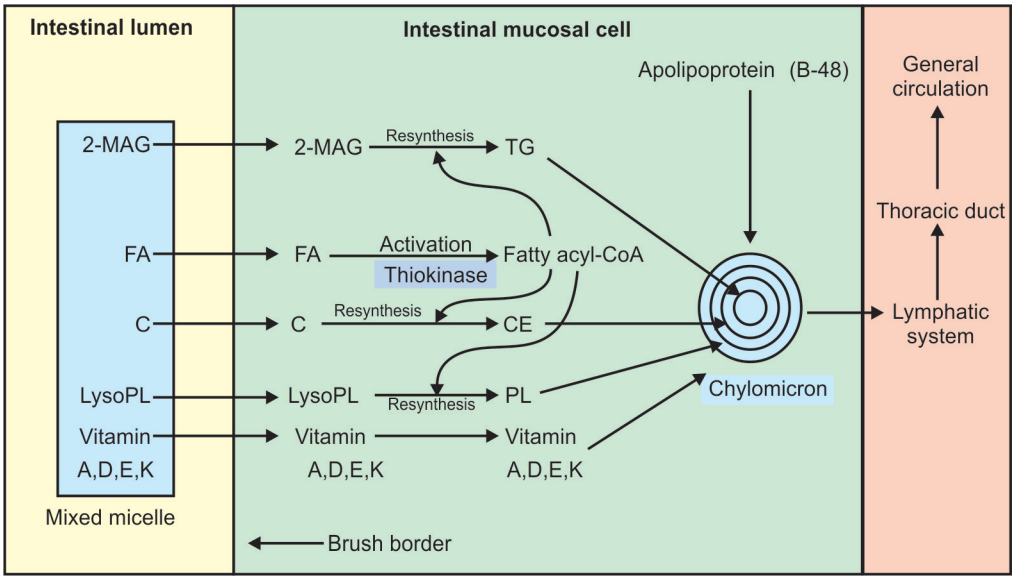


Figure 13.2: Absorption and transport of lipid from intestinal lumen
where, 2-MAG: 2 Monoacylglycerol; FA: Fatty acid; C: Cholesterol; LysoPL: Lysophospholipid; TG: Triacylglycerol; CE: Cholesterol ester

fat soluble vitamins are transported from the mucosal cells into the lymph in the form of lipoprotein known as ***chylomicrons*** **(Figure 13.2).** Chylomicrons are composed of:

- Triacylglycerols (85 – 90%)
- Cholesterol and cholesterol ester (5%)
- Phospholipids (7%)
- Protein (apolipoprotein B-48, 1–2%).

- The chylomicrons pass from the lymph into the blood through the thoracic duct. ***After a fatty meal, the plasma is milky in appearance due to the presence of chylomicrons.***

FATTY ACID OXIDATION

Fatty acids are oxidized mainly by a process called **β-oxidation,** in which two carbon units sequentially removed beginning from the carboxyl end of the fatty acid in the form of **acetyl-CoA**. It is called β-oxidation because oxidation of fatty acids occurs at the β-carbon atom. β-oxidation pathway occurs in **mitochondria.** It involves following three steps:

1. Activation of fatty acid to acyl-CoA
2. Transfer of acyl CoA into mitochondria by carnitine transport system
3. Reactions of β-oxidation in mitochondria.

Activation of Fatty Acid

- Before being catabolized, free fatty acids are converted to an active form called **acyl-CoA.** It occurs in the cytosol in the presence of ATP, coenzyme-A (CoA-SH) and the enzyme **acyl-CoA synthetase** also called **thiokinase (Figure 13.3).** Subsequent steps of β-oxidation occur in the mitochondria of the liver and other tissue cells.

Transport of Acyl-CoA into Mitochondria by Carnitine Transport System

Activation of fatty acids occur in the cytosol, whereas they are oxidized in the mitochondrial matrix. The mitochondrial inner membrane is impermeable to fatty acids. So a special transport mechanism is needed.

Activated long chain fatty acids are carried across the inner mitochondrial membrane by ***carnitine, (β-hydroxy γ-trimethyl ammonium butyrate)***, formed from ***lysine*** and ***methionine*** in liver and kidney. This occurs in four steps **(Figure 13.4).**

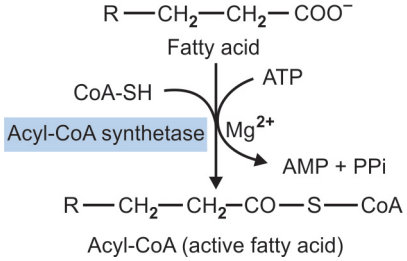


Figure 13.3: Activation of fatty acid

1. The acyl group of acyl-CoA is transferred to the carnitine to form acyl-carnitine. This reaction is catalyzed by ***carnitine acyltransferase-I (CAT-I).*** which is located on the cytosolic face of the inner mitochondrial membrane.
2. Acyl-carnitine is then transported across the inner mitochondrial membrane by an enzyme **translocase**.
3. The acyl group is transferred back to CoA in the mitochondrial matrix by the enzyme ***carnitine acyl transferase-ll (CAT-II),*** located on the inside of the inner mitochondrial membrane.
4. Acyl-CoA is reformed in the mitochondrial matrix with liberation of carnitine which is returned to the cytosolic side by the **translocase** in exchange for an incoming acyl-carnitine.

Reactions of β-oxidation of Fatty Acid

After the penetration of the acyl-CoA into mitochondria, it undergoes β-oxidation.

Sequence of Reactions of β-oxidation

A saturated acyl-CoA is degraded by a repeated sequence of four reactions **(Figure 13.5)**.

1. Oxidation by FAD
2. Hydration
3. Oxidation by NAD
4. Cleavage.

1. **Oxidation *by FAD:*** The first reaction is the oxidation of acyl-CoA by an ***acyl-CoA dehydrogenase*** to give an Δ^2-trans enoyl-CoA (a trans double bond between C_2 and C_3). The coenzyme for the dehydrogenase is FAD which is converted to $FADH_2$.
2. ***Hydration:*** The next step is the hydration (addition of water) of the double bond between C_2 and C_3 by ***Δ^2-enoyl-CoA hydratase*** to form β-hydroxy acyl-CoA.
3. ***Oxidation by NAD:*** The β-hydroxy derivative undergoes second oxidation reaction catalyzed by ***β-hydroxyacyl-CoA dehydrogenase*** to form β-ketoacyl-CoA and generates NADH.
4. ***Cleavage:*** Finally β-ketoacyl-CoA is split at the β-carbon by *thiolase* to yield acetyl-CoA and an acyl-CoA which is shorter by two carbon atoms than the original acyl-CoA that underwent oxidation.

The new acyl-CoA, containing two carbons less than the original, re-enters the β-oxidation pathway at reaction catalyzed by acyl-CoA dehydrogenase **(Figure 13.5).** The process continues till the fatty acid degraded completely to acetyl-CoA.

Acetyl-CoA can be oxidized to CO_2 and H_2O via citric acid cycle in mitochondria and thus oxidation of fatty acids is completed.

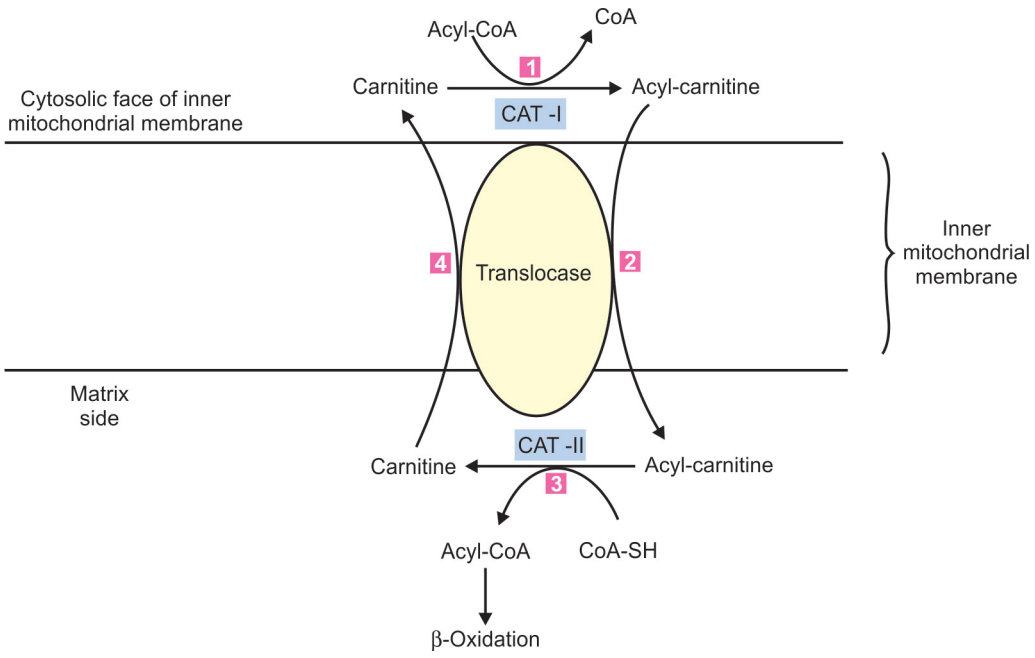


Figure 13.4: Carnitine transport system
where, CAT I: Carnitine acyl transferase-I; CAT II: Carnitine acyl transferase-II

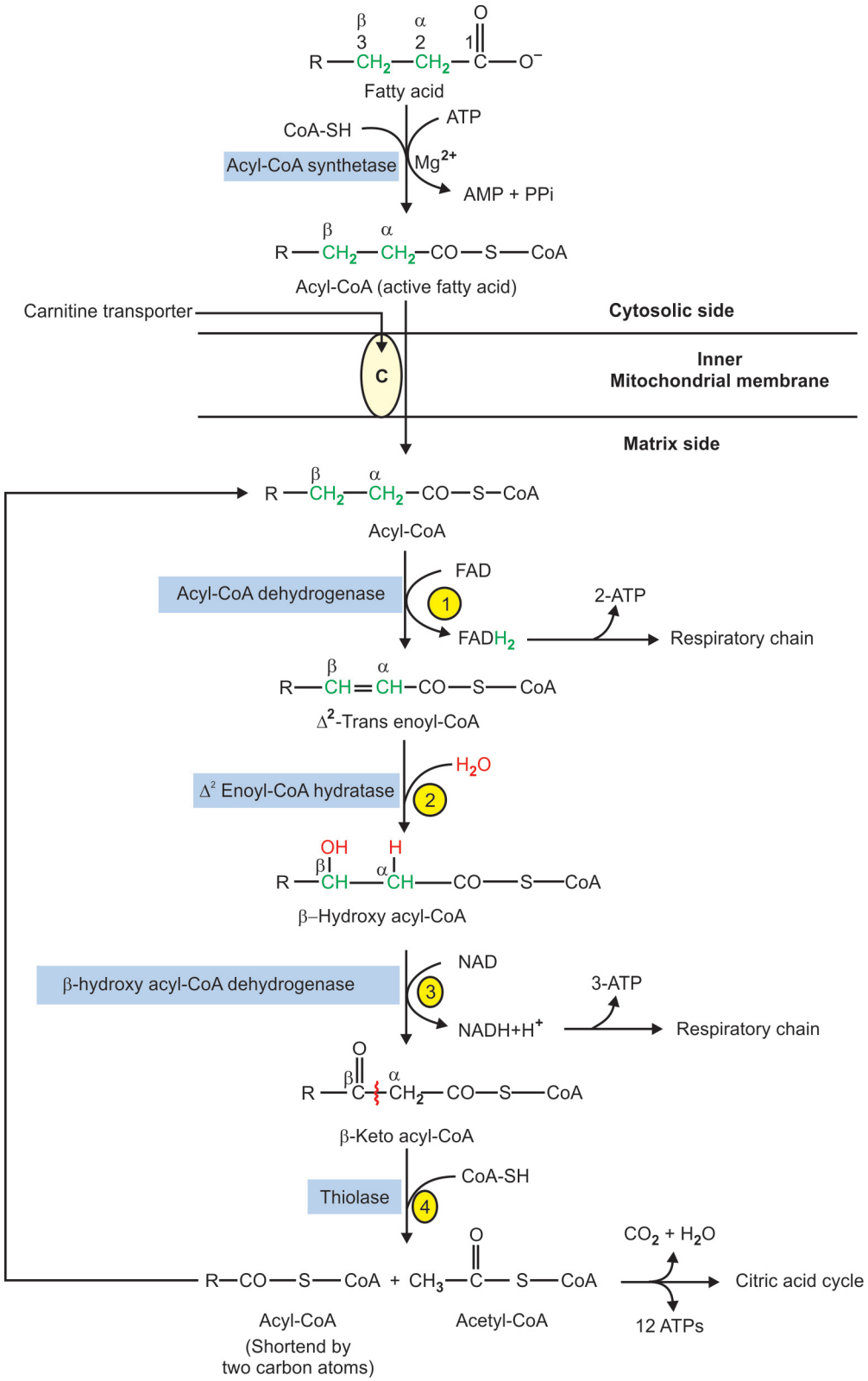


Figure 13.5: β-oxidation of fatty acids

Energy yield from the β-oxidation of fatty acids

- Complete β-oxidation of palmitoyl CoA (16 carbon acid) occurs through 7 cycles of β-oxidation yielding finally 8 acetyl-CoA, 7 $FADH_2$ and 7 NADH **(Figure 13.6).**
- Three ATPs are generated when each of these NADH is oxidized by respiratory chain.
- 2 ATPs are formed for each $FADH_2$.
- The oxidation of acetyl-CoA by the citric acid cycle yields 12 ATPs. Therefore the number of ATPs formed in the oxidation of palmitoyl-CoA is:
  - 14 ATPs from the 7 $FADH_2$
  - 21 ATPs from the 7 NADH
  - 96 ATPs from the oxidation of 8 molecules of acetyl-CoA in TCA cycle.
  - Total of 131 ATPs.
- Two high energy phosphate bonds are consumed in the activation of palmitate, in which ATP is split into AMP and PPi.
- Thus, the net yield from the complete oxidation of palmitate is 129 ATPs.

Regulation of β-oxidation

- Rate limiting step in the β-oxidation pathway is the formation of **acyl-carnitine** which is catalyzed by ***carnitine-acyl transferase-I (CAT-I).*** CAT-I is an allosteric enzyme. **Malonyl-CoA** is an inhibitor of CAT-I.
  - In well-fed state due to increased level of **insulin,** concentration of malonyl-CoA increases which inhibits ***CAT-I*** and leads to decrease in fatty acid oxidation.
  - In starvation, due to increased level of glucagon concentration of malonyl-CoA decreases and stimulates the fatty-acid oxidation **(Figure 13.7).**

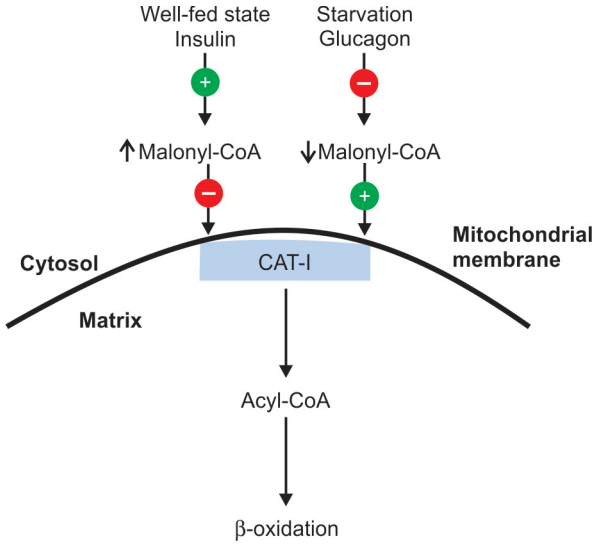


Figure 13.7: Regulation of β-oxidation of fatty acid

β-oxidation of a Fatty Acid with an Odd Number of Carbon Atoms

- Fatty acids, having an odd number of carbon atoms, are oxidized by β-oxidation in the same way as fatty acids, having an even number, except in the last and final β-oxidation cycle, of odd carbon fatty acids a three carbon fragment **propionyl CoA** is formed rather than two carbon fragment **acetyl-CoA**.
- Propionyl-CoA is converted to succinyl-CoA **(Figure 13.8)**, a constituent of citric acid cycle.
- Propionyl-CoA is carboxylated at the expense of an ATP to yield the D-methylmalonyl-CoA; catalyzed by ***propionyl-CoA carboxylase,*** **a** ***biotin*** enzyme.
- The D-methylmalonyl-CoA is converted to the L-methylmalonyl-CoA by the enzyme ***methylmalonyl-CoA epimerase.***

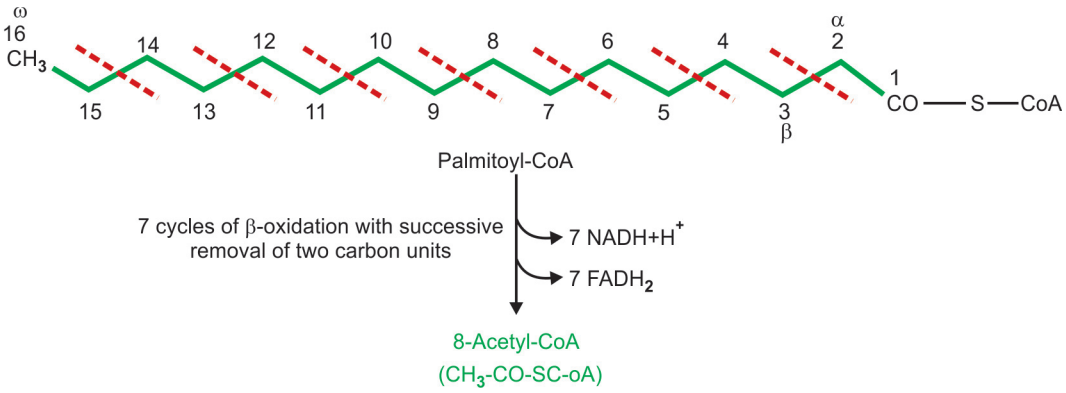


Figure 13.6: Overall process of β-oxidation

- Succinyl-CoA is formed from L-methylmalonyl-CoA by ***methylmalonyl-CoA mutase,*** which requires ***vitamin*** $\mathbf{B_{12}}$ as a coenzyme.

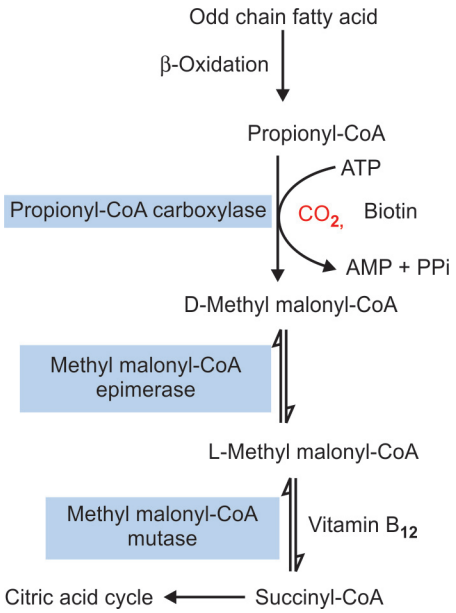


Figure 13.8: Conversion of propionyl-CoA to succinyl-CoA

Peroxisomal Fatty Acid Oxidation (Figure 13.9)

Peroxisomes are able to conduct oxidation of long chain fatty acids. Oxidation of very long chain fatty acids, which contain 20–26 carbons begins in peroxisomes by a process very similar to β-oxidation (and is completed in the mitochondria) with significant differences.

- The action of first enzyme ***acyl-CoA dehydrogenase*** differs, however, in that, it produces H_2O_2 rather than $FADH_2$. In peroxisomes the $FADH_2$ is not linked to ETC for generation of ATP but it transfers reducing equivalents directly to O_2, yielding H_2O_2. There is no ATP synthesized in peroxisomal β-oxidation of fatty acids.
- **Catalase** an enzyme located in peroxisomes converts H_2O_2 to water and molecular oxygen.

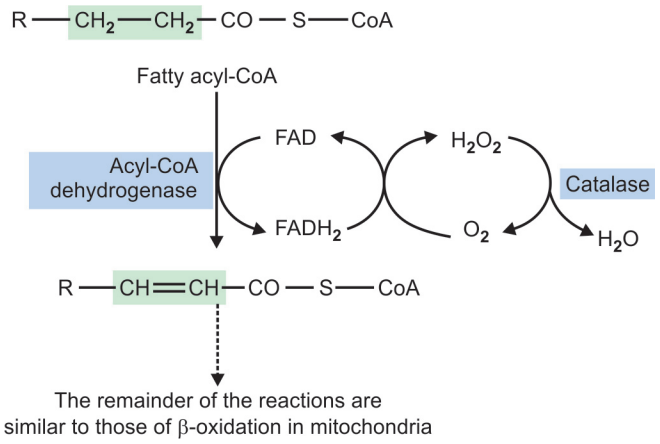


Figure 13.9: Oxidation of fatty acids in peroxisomes

Disorder of Peroxisomal Fatty Acid Oxidation

Zellweger syndrome

- A rare inborn error of peroxisomal fatty acid oxidation is due to inherited absence of **functional peroxisomes** in all tissues.
- As a result, the long chain fatty acids are not oxidized in peroxisomes and accumulate in tissues, particularly in brain, liver, kidney and muscle and usually results in death by age six.

α-oxidation

- Oxidation occurs at α-carbon of fatty acid in which one carbon is removed from the carboxyl end of the fatty acid chain and released as CO_2.
- The remaining carbons of the fatty acid can repeat the process.
- It does not require CoA intermediates and does not generate ATP.
- This process occurrs in brain and other nervous tissues.

> *Role of α-oxidation*
> In α-oxidation, oxidative decarboxylation eliminates one carbon and converts the even numbered fatty acid to odd numbered chains, which are constituent of complex lipids of the brain.

Disorders Associated with Impairment of α-fatty Acid Oxidation

Refsum's disease (phytanate storage disease)

A rare neurologic inborn error of lipid metabolism results from a genetic deficiency in **phytanic acid** α-***hydroxylase*** required for the hydroxylation of phytanic acid by α-oxidation. Persons with Refsum's disease have an inherited defect in α-oxidation that leads to accumulation of phytanic acid in the nerve tissue.

Symptoms

Clinical symptoms include ***retinitis pigmentosa*** (progressive dengeneration of retina), ***peripheral neuropathy*** and **ataxia.** Treatment involves elimination of dietary sources of phytol.

ω-oxidation

Fatty acids may be, oxidized at the ω-carbon of the chain by enzymes in the ***endoplasmic reticulum.*** ω-carbon is a carbon farthest from the carboxyl end.

- The ω-methyl group is first oxidized to an alcohol (CH_2OH) by hydroxylase that uses cytochrome P_{450}, molecular oxygen and NADPH in the endoplasmic reticulum.
- The alcoholic group is subsequently oxidized to –COOH by dehydrogenase and thus forming a dicarboxylic acid.
- Short chain dicarboxylic acid such as ***pimelic acid,*** a precursor of ***biotin*** formed by ω-oxidation.

METABOLISM OF KETONE BODIES

Acetoacetate, β-hydroxybutyrate and **acetone,** these three substances are collectively known as ketone bodies **(Figure 13.10)**. These are water soluble energy yielding substances. Acetone is, however, an exception, since it cannot be metabolized and is readily exhaled through lungs. Acetoacetate and acetone are true ketones, while β-hydroxybutyrate does not possess a keto group.

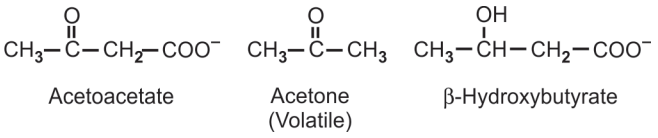


Figure 13.10: Structure of ketone bodies

Ketogenesis (Figure 13.11)

Ketogenesis means the formation of ketone bodies. Liver is the only organ that synthesizes ketone bodies. The

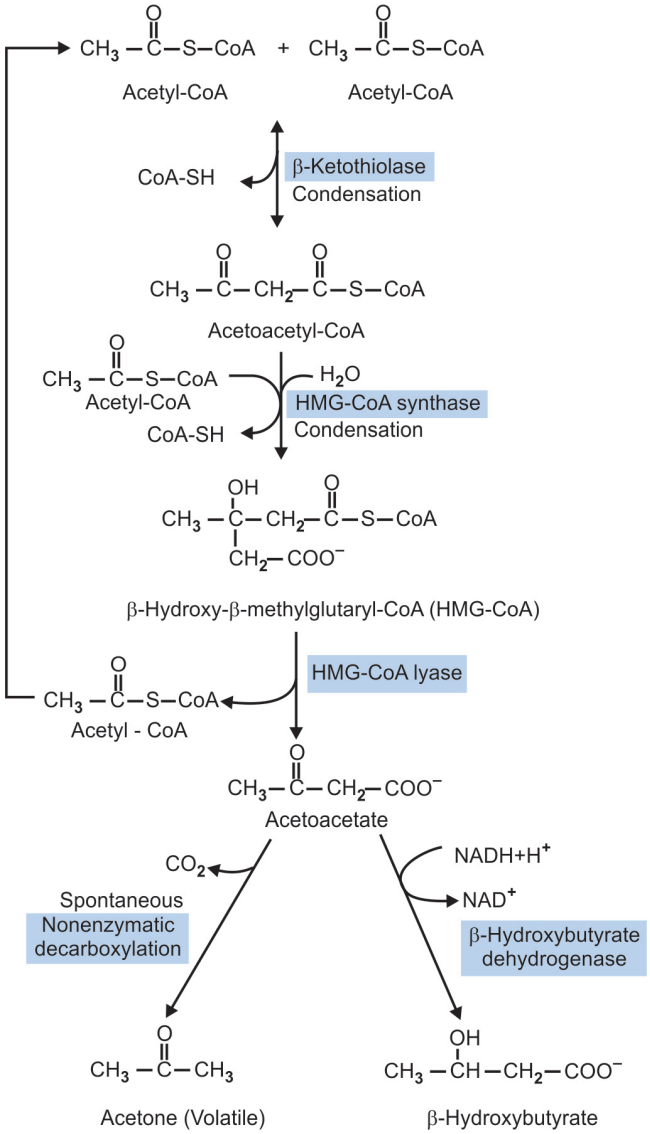


Figure 13.11: Formation of ketone bodies

synthesis of ketone bodies occurs in mitochondrial matrix of hepatic cells. Ketogenesis occurs by the following reactions:

- Two molecules of acetyl-CoA condense to form **aceto-acetyl-CoA.** This reaction, catalyzed by thiolase.
- Acetoacetyl-CoA then condenses with one more molecule of acetyl-CoA to give **β-hydroxy-β-methyl-glutaryl-CoA (HMG-CoA),** catalyzed by HMG-CoA synthase.
- β-HMG-CoA is then cleaved to acetyl-CoA and **acetoacetate** by the enzyme HMG-CoA lyase present in the mitochondria.
- **β-hydroxy butyrate** is formed by the reduction of acetoacetate in the mitochondrial matrix by the enzyme β-Hydroxy butyrate dehydrogenase.
- Acetoacetate also undergoes a slow, nonenzymatic spontaneous decarboxylation to **acetone.**
- The concentration of total ketone bodies in the blood of well-fed condition does not normally exceed ***0.2 mmol/L.***

Utilization of Ketone Bodies

The site of production of ketone bodies is the ***liver***. But the liver cannot utilize ketone bodies because it lacks the particular enzyme ***CoA-transferase*** which is required for the activation of ketone bodies.

Acetoacetate, β-hydroxybutyrate and acetone diffuse from the liver mitochondria into the blood and are transported to peripheral tissues.

- In peripheral tissues the β-hydroxybutyrate is first reconverted to acetoacetate and the acetoacetate is then reactivated to acetoacetyl-CoA **(see Figure 13.12).**
- Activation of acetoacetate occur by ***CoA-transferase*** in the presence of succinyl-CoA. Acetoacetate is activated by the transfer of CoA from succinyl-CoA.
- Acetoacetyl-CoA, formed by this reaction, is then cleaved by ***thiolase*** to yield two molecules of acetyl-CoA which can be oxidized in the citric acid cycle to H_2O and CO_2.
- Further metabolism of acetone does not occur. Because acetone is volatile, it is expired by the lungs.

Schematic representation of overall metabolism (synthesis and breakdown) of ketone bodies is shown in **Figure 13.13.**

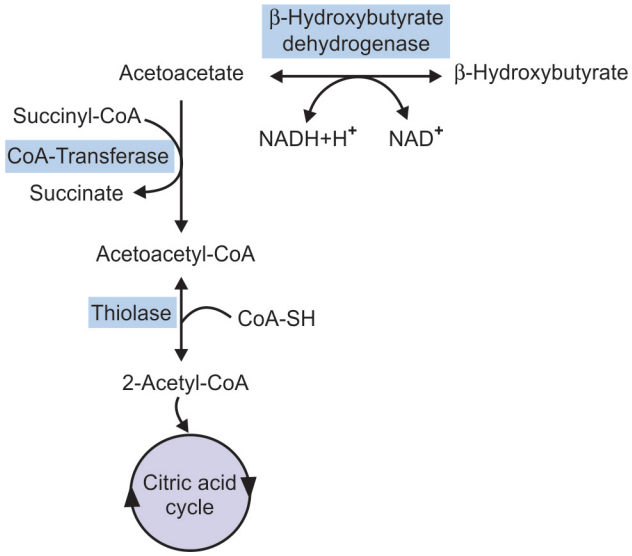


Figure 13.12: Utilization of ketone bodies

Significance of Ketogenesis

- Ketogenesis is a mechanism that allows the liver to oxidize increasing quantities of fatty acids.
- During deprivation of carbohydrate as in starvation and diabetes mellitus, acetoacetate and β-hydroxybutyrate serve as an alternative source of energy for extrahepatic tissues such as skeletal muscle, heart muscle, renal cortex, etc.
- In prolonged starvation 75% of the energy needs of the brain are supplied by ketone bodies reducing its need for glucose.

Regulation of Ketogenesis

The ketone body formation is regulated at the following three important levels **(Figure 13.14)**.

1. **Factors regulating lipolysis:** Free fatty acids the precursors of ketone bodies arise from lipolysis of triacylglycerol in adipose tissue. So factors regulating lipolysis also regulate ketogenesis. The hormone **glucagon** stimulates lipolysis which in turn stimulates ketogenesis, whereas **insulin** inhibits lipolysis and ketogenesis.
2. **Factors regulating β-oxidation of fatty acids**
   - **Carnitin acyl transferase** I (CAT I) regulates the fatty acid oxidation which, in turn, regulates ketogenesis as well.
   - Its activity is high in **starvation** leading to increased fatty acid oxidation and formation of ketone bodies.
3. **Factors regulating oxidation of acetyl-CoA:** In turn, the acetyl-CoA, formed in β-oxidation in liver mitochondria, has two possible fate.

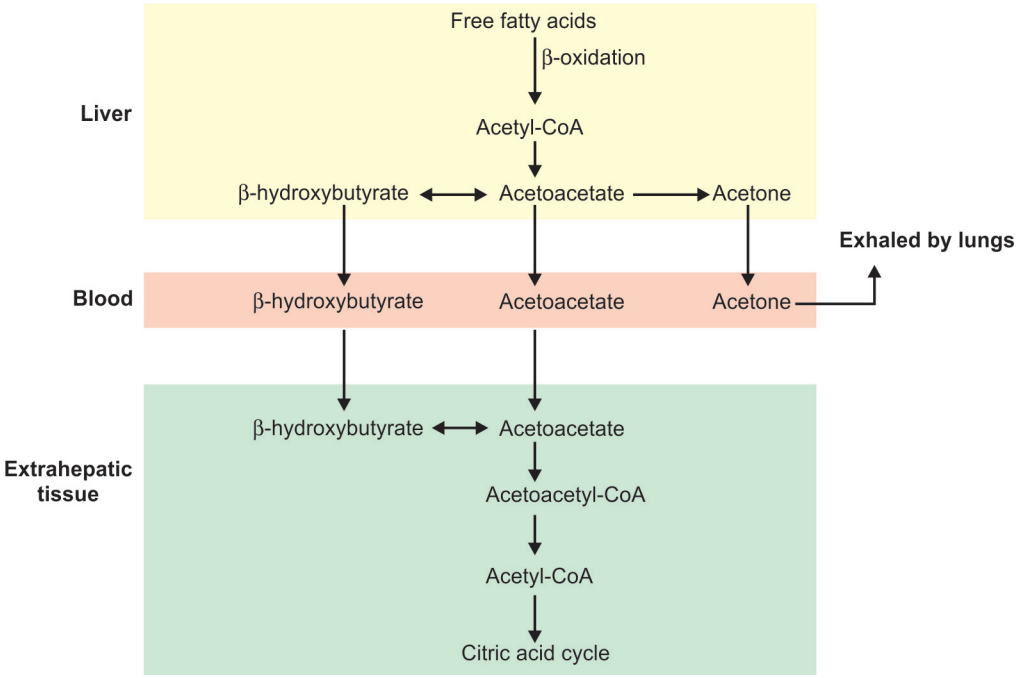


Figure 13.13: Schematic representation of metabolism (formation and fate) of ketone bodies

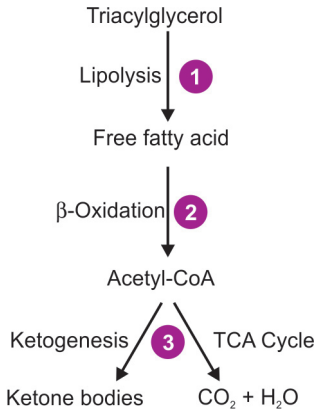


Figure 13.14: Steps of regulation of ketogenesis

- It may be oxidized to CO_2 via the citric acid cycle or it enters the pathway of ketogenesis to form ketone bodies.
- When oxaloacetate concentration is very low, little acetyl-CoA enters the citric acid pathway, and ketone body formation is then favored.
- Concentration of oxaloacetate is lowered if carbohydrate is unavailable or improperly utilized, e.g. in fasting or in diabetes. Under these conditions, acetyl-CoA is diverted to the formation of acetoacetate.

Disorders of Ketone Body Metabolism

Ketosis

- Normally the concentration of ketone bodies in blood is very low, (less than 0.2 mmol/L) but in fasting and in diabetes mellitus it may reach extremely high levels.
- During fasting or in diabetes mellitus, when exogenous glucose is unavailable, insulin secretion is inhibited. The plasma insulin concentration is, therefore, low which causes increased lipolysis and therefore increased production of free fatty acids and hence ketone bodies production is increased **(Figure 13.15).**
- When the rate of formation of the ketone bodies by liver exceeds the capacity of the peripheral tissues to use them up, their levels begin to rise in blood. An increase in concentration of ketone bodies in blood is called ***ketonemia*** and eventually leads to excretion of ketone bodies into the urine called ***ketonuria***. The overall condition (ketonemia and ketonuria) is called **ketosis.**
- In addition to β-hydroxybutyrate and acetoacetate, the blood of diabetics also contains acetone. Acetone is very volatile and is present in the breath of diabetics, to which it gives sweet fruity odor.

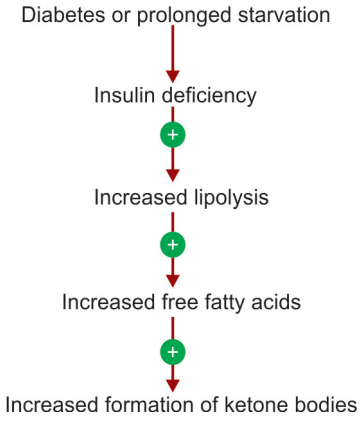


Figure 13.15: Increased formation of ketone bodies in diabetes or prolonged starvation

Ketoacidosis

- Since acetoacetate and β-hydroxybutyrate are moderately strong acids, increased levels of these ketone bodies decrease the pH of the bood and cause **metabolic acidosis.** The acidosis caused by over production of ketone bodies is termed as **ketoacidosis.**
- Acetoacetate and β-hydroxybutyrate acids when present in high concentration in blood, are buffered by HCO_3^- (akali) fraction of bicarbonate buffer. The excessive use of HCO_3^- **depletes the alkali reserve** causing **ketoacidosis.**
- Ketoacidosis is seen in type I diabetes mellitus, whereas in type II diabetes ketoacidosis is relatively rare.

DE NOVO SYNTHESIS OF FATTY ACIDS (LIPOGENESIS)

In humans, fatty acid synthesis occurs mainly in the ***liver*** and ***lactating mammary glands*** and, to a lesser extent, in ***adipose tissue, kidney*** and ***brain.*** *De novo* synthesis means new synthesis. Fatty acid synthesis occurs in three phases:

1. Transport of acetyl-CoA from mitochondria to cytosol.
2. Carboxylation of acetyl-CoA to malonyl-CoA.
3. Reactions of fatty acid synthase complex.

Transport of acetyl-CoA from mitochondria to cytosol

Fatty acids are synthesized in the ***cytosol,*** whereas acetyl-CoA is formed in mitochondria, hence, acetyl-CoA must be transferred from mitochondria to the cytosol. Since acetyl CoA is impermeable to inner mitochondrial membrane, it is transferred in the form of **citrate** from mitochondrial matrix to the cytosol as shown in **Figure 13.16.**

- Citrate is formed in the mitochondrial matrix by the condensation of acetyl-CoA with oxaloacetate (first reaction in the citric acid cycle).
- Then citrate is transported to the cytosol by ***translocase,*** where it is cleaved by ***citrate lyase*** to oxaloacetate and acetyl-CoA.

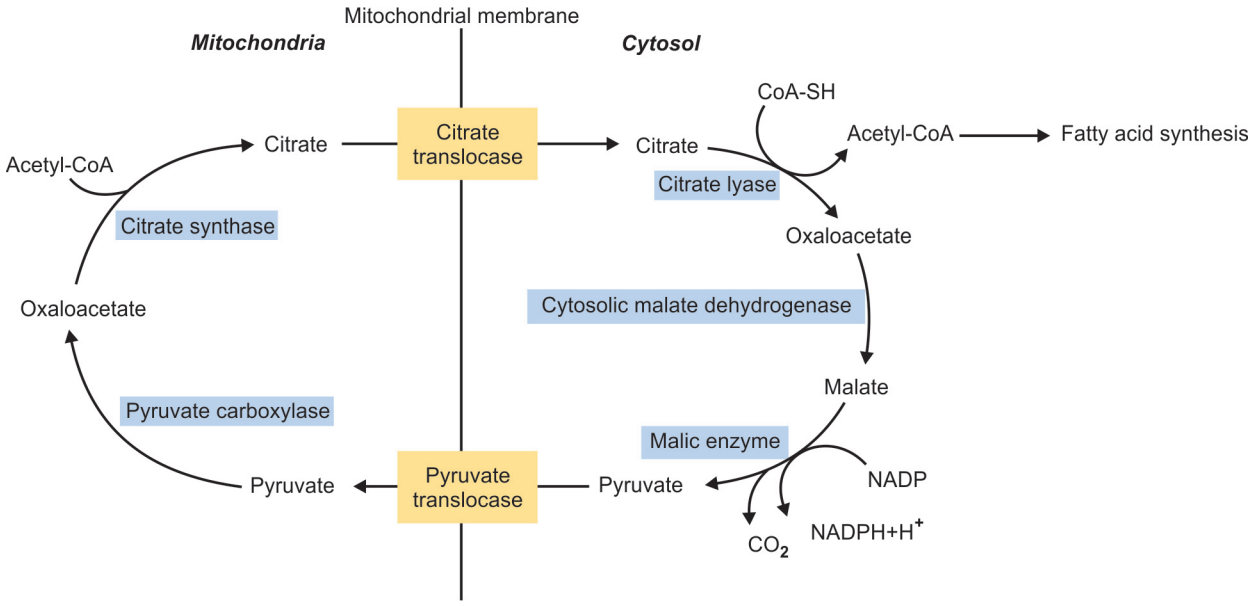


Figure 13.16: Transfer of acetyl-CoA from mitochondria to the cytosol

- Oxaloacetate formed in this reaction must be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Therefore, oxaloacetate is reduced to malate, catalyzed by cytosolic ***malate dehydrogenase.*** Then malate is oxidatively decarboxylated to pyruvate by ***NADP-linked malic enzyme. NADPH and CO_2 are generated in this reaction. Both of them are utilized for fatty acid synthesis.***
- The pyruvate formed in this reaction readily diffuses into mitochondria, where it is carboxylated to oxaloacetate by ***pyruvate carboxylase.***

Carboxylation of acetyl-CoA to malonyl-CoA

- Carboxylation of acetyl-CoA to malonyl-CoA is the initial and ***rate limiting reaction in fatty acid synthesis.***
- This reaction is catalyzed by an enzyme complex, ***acetyl-CoA carboxylase,*** that contains **biotin** and utilizes ***bicarbonate*** (as a source of CO_2) in presence of ATP **(Figure 13.17)**.

Reactions of fatty acid synthase complex

- Once malonyl CoA is formed from acetyl CoA, the *De novo* synthesis of palmitic acid is carried out in cytosol by **fatty acid synthase complex**.
- Fatty acid synthase complex is a multienzyme complex possessing 7 different enzymes and one **acyl carrier protein (ACP)** molecule **(Figure 13.18).** The fatty acid synthase complex has two active **-SH groups.** One -SH group is of 4-phosphopantetheine moiety of **ACP.** Another -SH group is of cysteine residue of enzyme **ketoacyl synthase.** Both -SH groups participate in fatty acid biosynthesis.

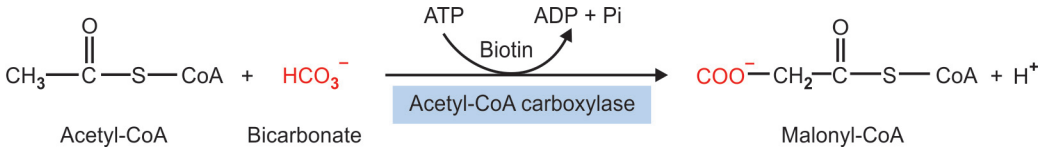


Figure 13.17: Biosynthesis of malonyl-CoA

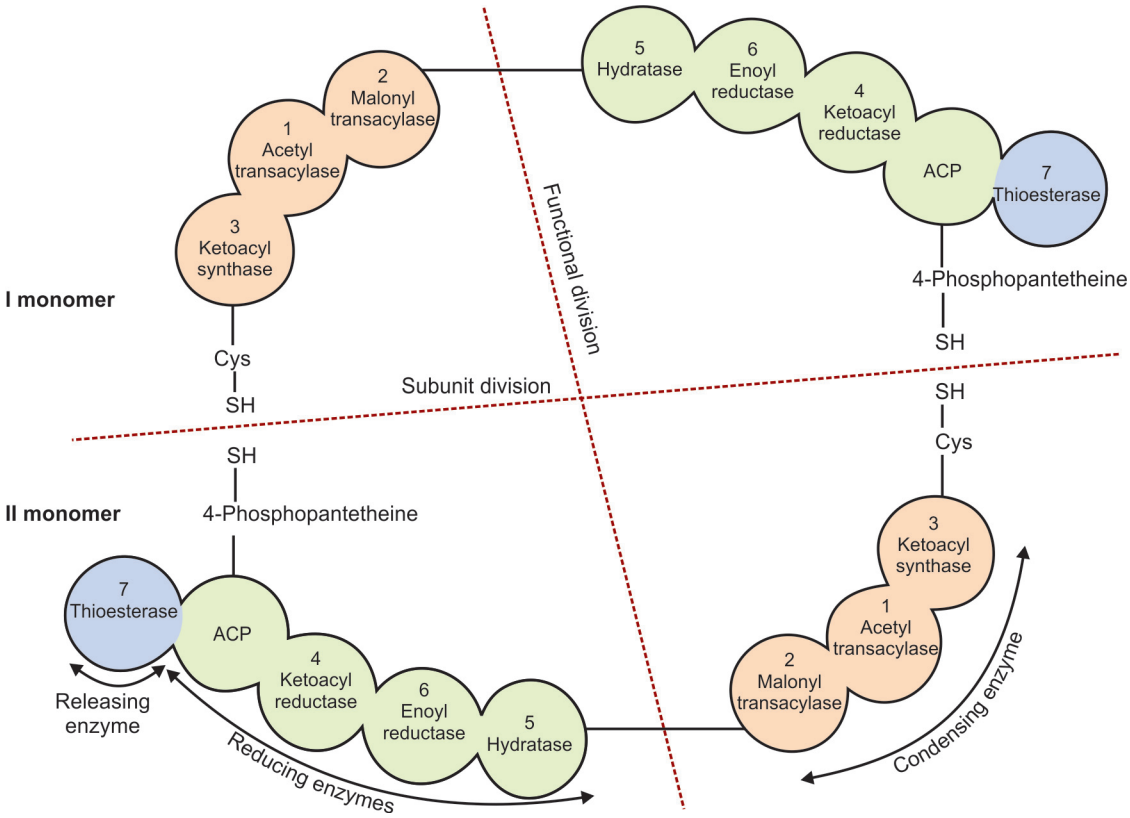


Figure 13.18: Schematic diagram of fatty acid synthase multienzyme complex

- Fatty acid synthase complex is a dimer **(Figure 13.18).** Each monomer is identical consisting of all seven enzyme activities of fatty acid synthase and an ACP.
- The two subunits associate in a head-to-tail arrangement, so that the phosphopantetheine-SH group of one subunit and a cysteinyl-SH group of another subunit are closely aligned.
- Though each monomer contains all the enzyme activities but only the dimer is functionally active. This is because the functional unit consists of half of each subunit interacting with the complementary half of the other. The two functional subunits of fatty acid synthase can synthesize two fatty acids simultaneously.

The fatty acid synthase complex catalyzes following reactions **(Figure 13.19).**

- In the first reaction, catalyzed by ***acetyl transacylase*** the acetyl group of acetyl-CoA is transferred to the cysteine -SH group of the 3-ketoacyl synthase of fatty acid synthase complex (designated E).
- In the second reaction, the malonyl group of malonyl-CoA is transferred to the phosphopantetheine -SH group (Pan-SH) of ACP catalyzed by ***malonyl transacylase*** to form acetyl-malonyl enzyme.
- The acetyl and malonyl groups, covalently bonded to -SH groups of the synthase, undergo a condensation reaction. This reaction is catalyzed by ***3-ketoacyl synthase.*** The acetyl group attached to cysteine -SH is transferred to malonyl group bound to -SH pantetheine. Simultaneously the malonyl moiety loses CO_2 forming 3-ketoacyl enzyme.
- The 3-ketoacyl enzyme undergoes reduction at the 3-keto group, at the expense of NADPH as electron donor to form 3-Hydroxyacyl enzyme, catalyzed by ***3-ketoacyl reductase.***
- 3-hydroxyacyl enzyme is dehydrated by ***3-hydroxy-acyl hydratase*** to yield 2, 3 unsaturated acyl enzyme.
- 2,3 unsaturated acyl enzyme is reduced to form acyl enzyme containing 4-carbon, by the action of ***enoyl reductase*** and NADPH is the electron donor.
- This acyl group (4 carbon) is now transferred from pantetheine -SH group to the cysteine -SH group.
- To lengthen the chain by another 2-carbon unit, the sequence of reactions is repeated, a new malonyl residue being incorporated during each sequence, until a saturated 16-carbon acyl radical (palmityl) has been assembled.
- Thus, after a total of seven such cycles, palmitoyl enzyme is formed, which is liberated from the enzyme complex by the activity of a seventh enzyme in the complex ***thioesterase (deacylase).***

Regulation of Fatty Acid Synthesis

De novo fatty acid synthesis is regulated by **Acetyl-CoA Carboxylase** enzyme **(Figure 13.20).**

- Acetyl-CoA carboxylase which catalyzes the formation of malonyl-CoA is the rate limiting enzyme in the fatty acid synthesis. It is regulated by following ways:
  1. **Allosteric regulation:** Acetyl-CoA carboxylase is allosterically activated by **citrate** and inhibited by its own product **palmitoyl-CoA.**
  2. **Hormonal regulation:** The activity of acetyl-CoA carboxylase is also controlled by hormones. **Glucagon** and **epinephrine** inactivate the enzyme. In contrast, **insulin** activates the enzyme. Thus, fatty acid synthesis is stimulated by insulin and inhibited by glucagon and epinephrine.
  3. **Nutritional regulation:** Synthesis of **acetyl-CoA carboxylase** is increased with high carbohydrate and low fat diet, which promote fatty acid synthesis. By contrast, sythesis of acetyl-CoA carboxylase decreases during starvation, diabetes mellitus or high fat diet.

SYNTHESIS OF LONG CHAIN FATTY ACIDS FROM PALMITATE

The major product of fatty acid synthesis is palmitate. Longer fatty acids are formed by elongation reactions either in **endoplasmic reticulum (microsomes)** or in **mitochondria.**

Microsomal elongation of fatty acids

The microsomal enzyme **fatty acid elongase** elongates palmitate by the addition of 2-carbon fragments derived from malonyl-CoA and NADPH provides the reducing equivalents. The elongation reaction resembles those of fatty acid synthesis, except that the fatty acyl chain is attached to coenzyme-A rather than to phospho-pantetheine group of ACP.

Mitochondrial elongation of fatty acids

Fatty acids can be elongated in mitochondria, but mitochondrial elongation of fatty acids is less active. In this case, the source of the 2-carbon units is acetyl-CoA and the substrates are usually fatty acids containing less than 16-carbons, mainly short and medium chain fatty acids.

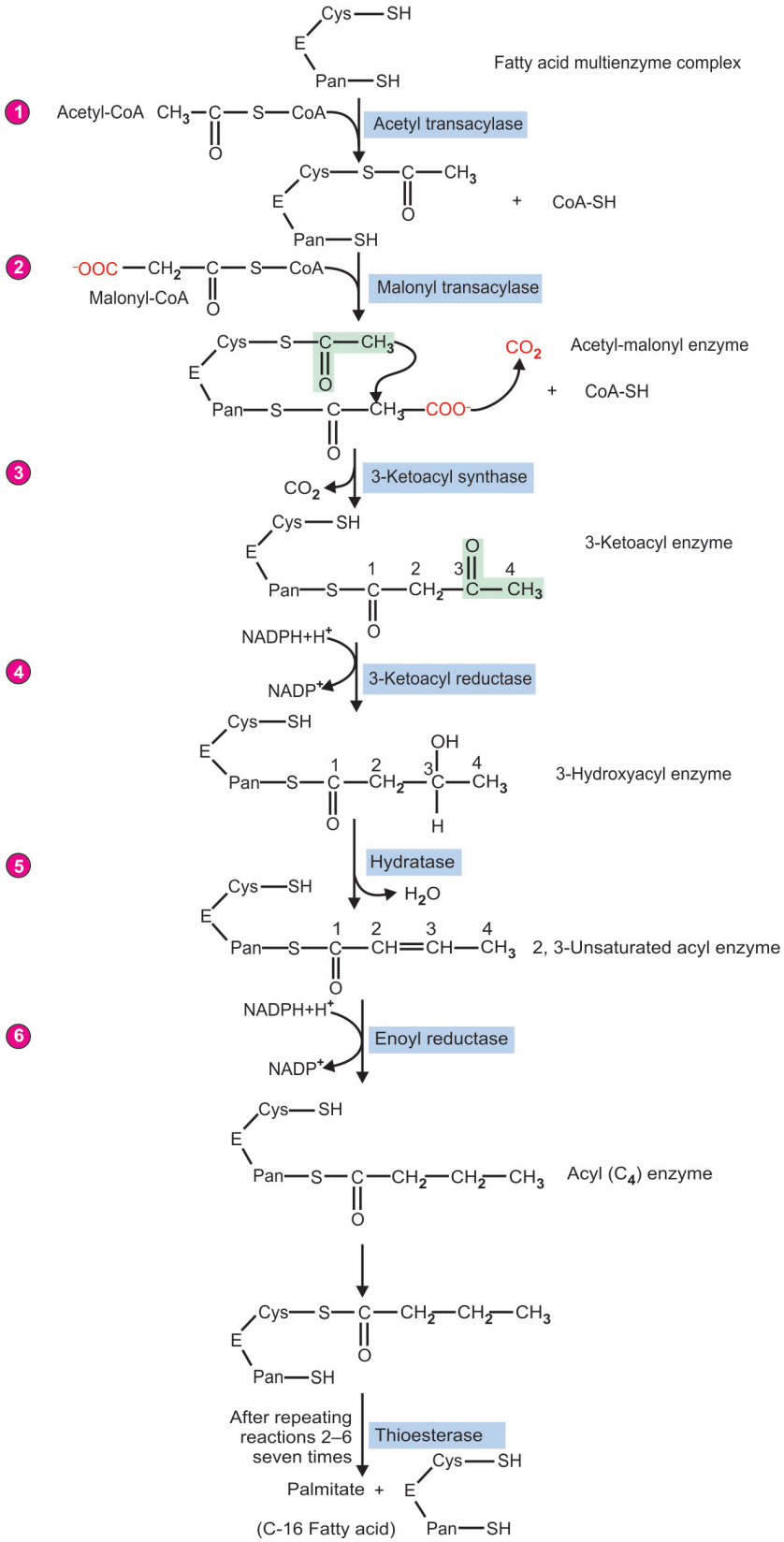


Figure 13.19: *De novo* synthesis of fatty acid. E: Fatty acid synthase complex

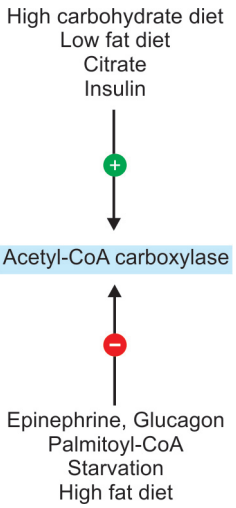


Figure 13.20: Regulation of fatty acid synthesis

TRIACYLGLYCEROL METABOLISM

Triacylglycerols are esters of the alcohol glycerol and fatty acids. Fatty acids derived from endogenous synthesis or from the diet are stored in the adipose tissue in the form of triacylglycerol, called *"neutral fat."*

Triacylglycerol serves as the body's major fuel storage reserve.

Biosynthesis of Triacylglycerols

In both liver and adipose tissue, triacylglycerols are synthesized **(Figure 13.21).**

- First fatty acids are activated to acyl-CoA **(see Figure 13.3).**
- Then two molecules of acyl-CoA combine with glycerol-3-phosphate to form phosphatidic acid (1,2-diacylglycerol phosphate) via formation of lysophosphatidic acid.
- Phosphatidic acid is the common precursor in the biosynthesis of the triacylglycerols and many glycerophospholipids and cardiolipin.
- Dephosphorylation of phosphatidic acid produces diacylglycerol.
- A further molecule of acyl-CoA is esterified with diacylglycerol to form triacylglycerol.
- The sources of glycerol-3-phosphate, which provides the glycerol moiety for triacylglycerol synthesis, differ in liver and adipose tissue.
- In liver glycerol-3-phosphate is produced from the phosphorylation of glycerol by ***glycerol kinase*** or from the reduction of dihydroxyacetone phosphate (DHAP), derived from glycolysis.
- Adipose tissue lacks glycerol kinase and can produce glycerol-3-phosphate only from glucose via DHAP.

Fate of Triacylglycerol Formed in Liver and Adipose Tissue

The fate of triacylglycerol in liver and adipose tissue is different.

- In the liver, little triacylglycerol is stored; instead most is exported in the form of very low density lipoprotein (VLDL). Once released into the blood stream, triacylglycerol of VLDL is hydrolyzed by ***lipoprotein lipase*** enzyme, which is located on the walls of blood capillaries. It clears the triacylglycerol in VLDL, forming free fatty acids and glycerol.
- In adipose tissue triacylglycerol is stored in the cells. It serves as *"depot fat"* ready for mobilization when the body requires it for fuel.

PHOSPHOLIPID METABOLISM

Phospholipids are the major class of ***membrane lipids.*** There are two classes of phospholipids:

1. Those that have glycerol, a 3-carbon alcohol, as a backbone, called ***glycerolphospholipids*** or ***phosphoglycerides,*** **e.g.**
   - phosphatidylserine
   - phosphatidylinositol
   - phosphatidylcholine (Lecithin)
   - phosphatidylethanolamine (Cephalin)
   - cardiolipin
   - plasmalogens
2. Those that contain **sphingosine,** a more complex amino alcohol called ***sphingophospholipid,*** **e.g.**
   - Sphingomyelin.

Biosynthesis of Glycerophospholipids

The initial steps in the synthesis of glycerophospholipids are similar to those of triacylglycerol synthesis **(see Figure 13.21).** Phosphatidate (diacylglycerol-3-phosphate) is a common intermediate in the synthesis of glycerophospholipids and triacylglycerols.

Synthesis of Phosphatidylserine and Phosphatidylinositol (Figure 13.21)

- Synthesis of phosphatidylserine and phosphatidylinositol starts with the formation of cytidine diacylglycerol (CDP-diacylglycerol) an activated phosphatidyl unit, from phosphatidate and cytidine triphosphate (CTP).
- The activated phosphatidyl unit then reacts with the hydroxyl group of alcohol.
  - If alcohol is serine, it forms **phosphatidylserine**
  - Likewise if the alcohol is inositol, the product is **phosphatidylinositol.**

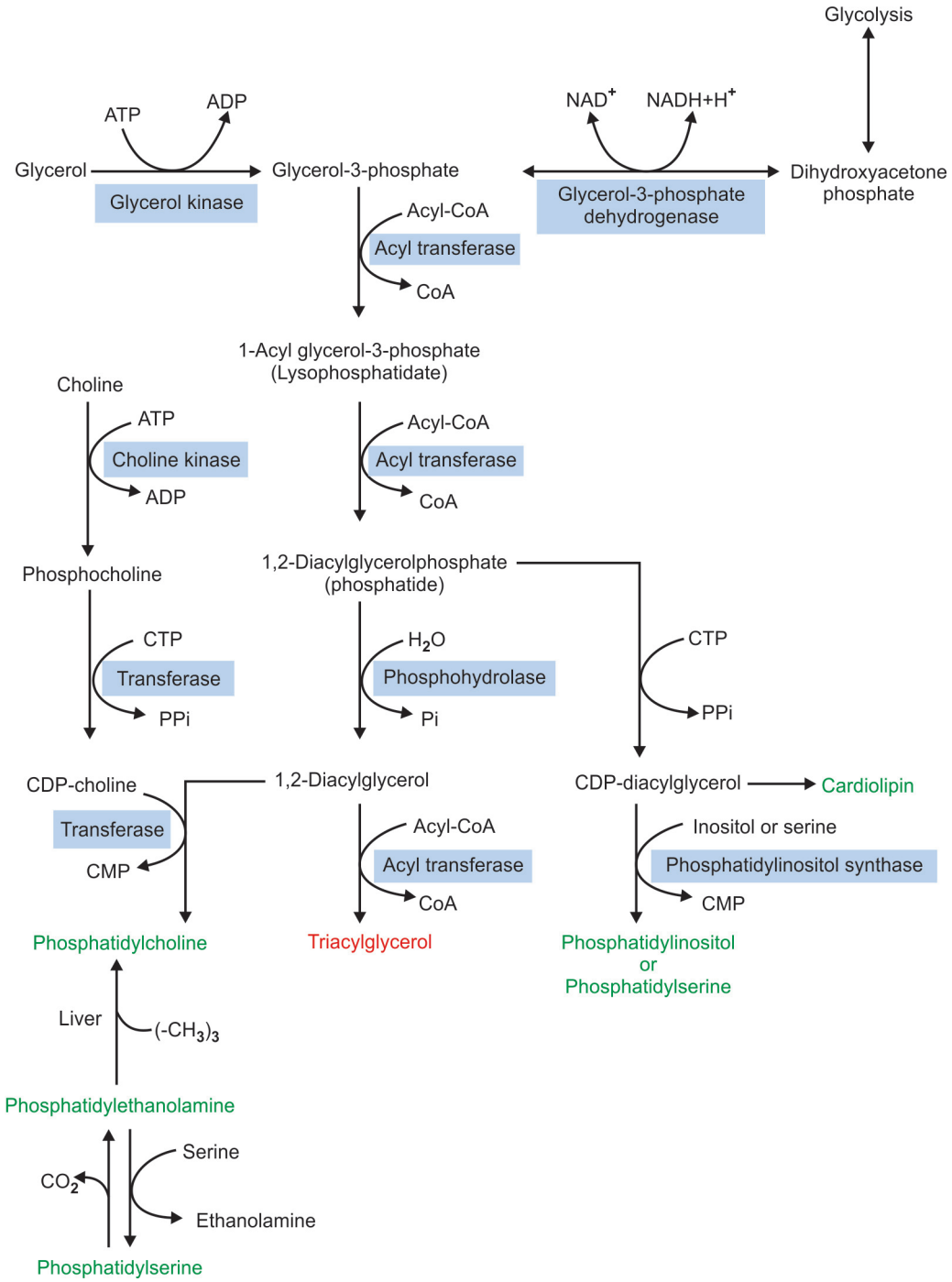


Figure 13.21: Synthesis of triacylglycerol and glycerophospholipids

- Phosphatidylserine can also be formed from phosphatidylethanolamine directly by reactions with serine.

Synthesis of Phosphatidylcholine (Lecithin) and Phosphatidylethanolamine (Cephalin)

Phosphatidylcholine is synthesized by a pathway that utilizes choline obtained from the diet.

- Choline must first be converted to active choline. This is a stage process, first is the phosphorylation with ATP to form phosphocholine, which then reacts with CTP to form CDP-choline (active form).
- The phosphorylcholine unit of CDP-choline is then transferred to a diacylglycerol to form phosphatidylcholine **(see Figure 13.21).**
- Likewise phosphatidylethanolamine can be synthesized from ethanolamine by forming a CDP-ethanolamine intermediate by analogous reactions.
- Phosphatidylserine may form phosphatidylethanolamine by decarboxylation.
- An alternative pathway in liver enables phosphatidylethanolamine to give rise directly to phosphatidylcholine by methylation of the ethanolamine residue utilizing S-adenosyl-methionine (SAM) as the methyl donor.

Synthesis of Cardiolipin (Figure 13.22)

In the synthesis of cardiolipin first CDP-diacylglycerol combines with glycerol-3-phosphate to form phosphatidyl glycerol which, in turn, reacts with another molecule of CDP-diacylglycerol to produce cardiolipin (diphosphatidyl-glycerol).

Synthesis of Plasmalogens

Plasmalogens differ from other glycerophospholipids in that the fatty acid at carbon 1 of glycerol bound by an ether linkage instead of ester linkage. The biosynthesis of plasmalogen occurs in peroxisomes and is shown in **(Figure 13.23).**

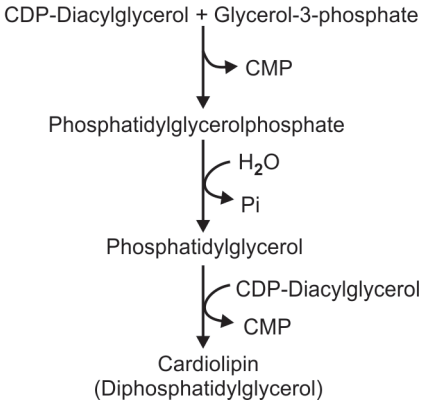


Figure 13.22: Synthesis of cardiolipin

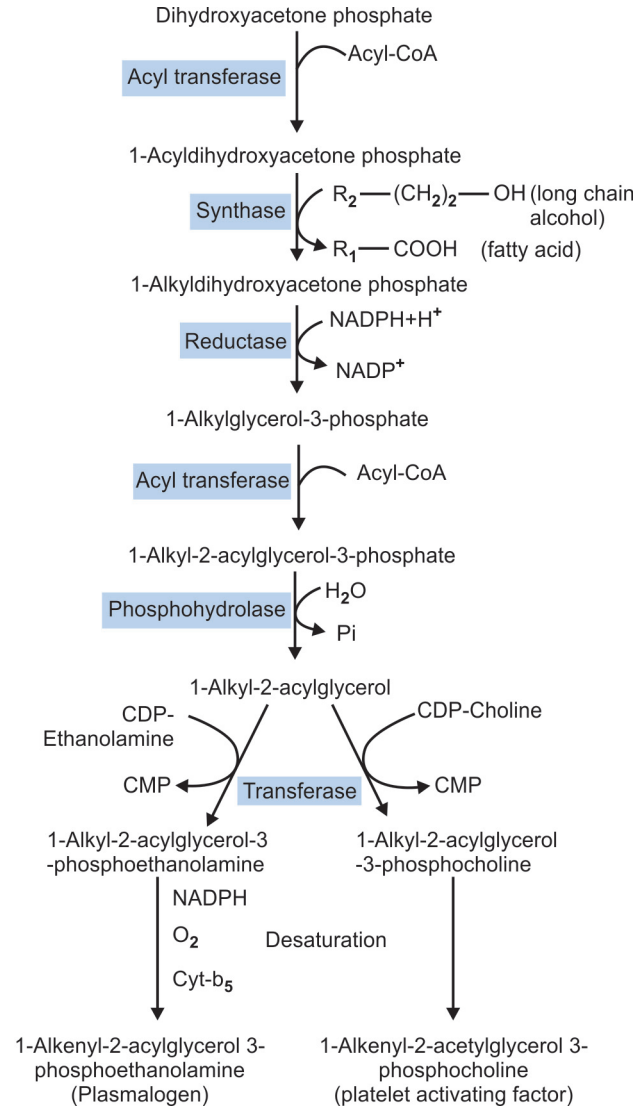


Figure 13.23: Synthesis of plasmalogen and platelet activating factor

Biosynthesis of Sphingomyelin

- These phospholipids contain a complex amino alcohol **sphingosine** instead of glycerol.
- Palmitoyl-CoA and serine condense to form 3-keto-sphinganine. The enzyme catalyzing this reaction requires **pyridoxal phosphate.**
- 3-ketosphinganine is then converted to sphingosine.
- In all sphingolipids, a long chain acyl-CoA reacts with sphingosine to form **ceramide.**
- Ceramide reacts with phosphatidylcholine to form sphingomyelin **(Figure 13.24).**

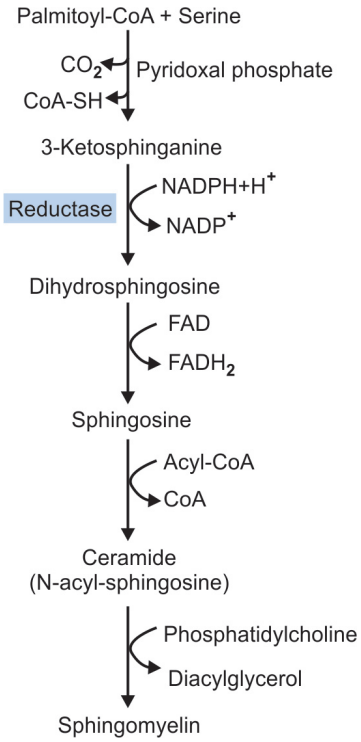


Figure 13.24: Synthesis of sphingomyelin

Degradation of Glycerophospholipids

Phospholipases located in cell membranes or in lysosomes degrade glycerophospholipids. Several specific phospholipases have been isolated which are designated as A_1, A_2, B, C and D. The bonds hydrolyzed by phospholipases ***A_1, A_2, B, C*** and **D** are shown in **(Figure 13.25).**

- **Phospholipase-A_1** removes the fatty acyl group on C_1 of the glycerol moiety.
- **Phospholipase-A_2** catalyzes the hydrolysis of the ester bond in position 2 of glycerophospholipids to form a free fatty acid and lysophospholipid, which is attacked by lysophospholipase removing the remaining 1-acyl group.
- ***Phospholipase-B*** hydrolyzes both acyl groups on C_1 and C_2.
- ***Phospholipase-C*** cleaves the bond, between phosphate and glycerol of phospholipids.
- The bond between the phosphate and the nitrogen base is cleaved by ***phospholipase-D***.

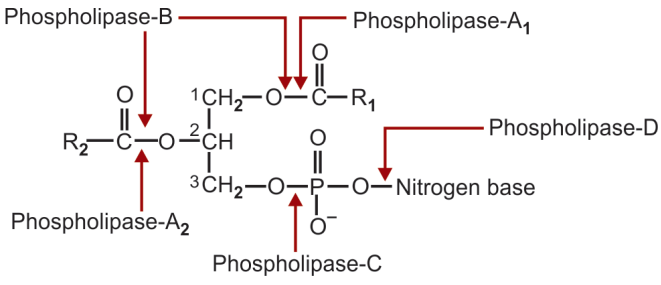


Figure 13.25: Sites of hydrolytic cleavage of glycerophospholipid by phospholipases

Degradation of Sphingomyelin

The sphingomyelins are hydrolyzed by lysosomal enzyme **sphingomyelinase** to ceramide and phosphorylcholine. The ceramide so formed is further hydrolyzed by another lysosomal enzyme **ceramidase** into sphingosine and free fatty acid.

GLYCOLIPID METABOLISM

Glycolipids, as their name implies, are sugar containing lipids. Glycolipids like sphingomyelin are derived from sphingosine. Sphingosine reacts with acyl-CoA to form **ceramide.** ***Cerebroside*** and ***gangliosides*** are the different types of glycolipids.

Biosynthesis of Glycolipids

Cerebroside is the simplest glycosphingolipid. In a cerebroside, glucose or galactose is linked to the terminal hydroxyl group of ceramide to form ***glucocerebroside*** or ***galactocerebroside.***

Galactocerebroside is a major lipid of myelin, whereas glucocerebroside is the major glycolipid of extraneural tissues and a precursor of most of the more complex glycolipids.

- Ceramide reacts with UDP-glucose or UDP-galactose to form ***glucocerebroside*** or ***galactocerebroside*** respectively.
- **Gangliosides** are the more complex glycolipids, contain a branched chain oligosaccharide of as many as seven sugar residues.
- Gangliosides are produced from ceramide by the stepwise addition of activated sugar, e.g. UDP-glucose, UDP-galactose and sialic acid usually N-acetylneuraminic acid (NANA) **(Figure 13.26).**

Degradation of Glycolipids

- The glucocerebrosides and galactocerebrosides are hydrolyzed by lysosomal enzymes β-**glucocerebrosidase** (β-glucosidase) and **β-galactocerebrosidase** (β-glactosidase), respectively to ceramide and hexose residues. The ceramide so formed is further cleaved by another lysosomal enzyme **ceramidase** to sphingosine and free fatty acid.

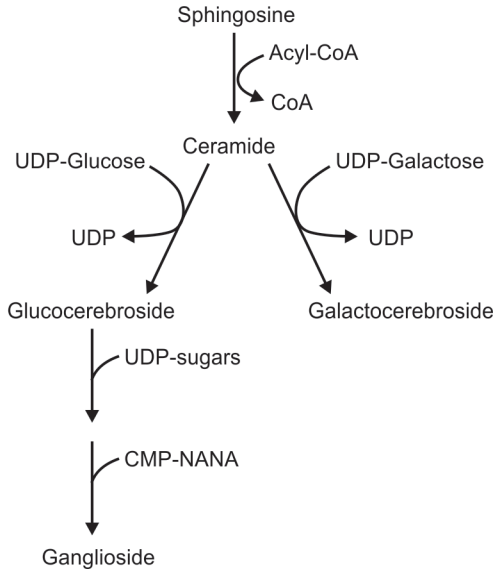


Figure 13.26: Biosynthesis of Glycolipids
where, CMP-NANA: Cytidine monophosphate-N-acetyl neuraminic acid

- The different gangliosides are degraded by a set of lysosomal enzymes, **β-glucosidase, β-hexosaminidase, β-galactosidase, neuraminidase,** etc.

Sphingolipidoses (Table 13.1) (Sphingolipid Storage Disease)

- Spingolipidoses are a group of inherited diseases that result from defective degradation and accumulation of any one of the sphingolipids (sphingomyelins or glycolipids).
- Depending on the enzyme affected several types of sphingolipidoses have been recognized. Some major types of sphingolipidoses are discussed here **(Figure 13.27).**

1. **Niemann Pick disease:** The degradation of sphingomyelins is impaired in Niemann Pick disease due to inherited deficiency of **sphingomyelinase.** As a result sphingomyelins accumulate in liver, brain and spleen. The clinical findings are:
   - Enlarged liver and spleen
   - Mental retardation and death may occur in early childhood.
2. **Tay-Sach's disease:** It is due to inherited deficiency of **hexosaminidase A** required for the degradation of ganglioside GM_2. Since degradation of GM_2 of ganglioside is impaired, the GM_2 accumulates in brain and nervous tissues.
   - The infants with Tay-Sach's disease suffer from muscular weakness, mental retardation, blindness and death occur in early childhood.
3. **Gaucher's disease:** The inherited deficiency **β-glucosidase** impairs the hydrolysis of glucocerebrosides, which results in accumulation of glucocerebrosides in brain, liver, spleen, and bone marrow.
   - This disorder is associated with mental retardation and enlargement of liver and spleen.
4. **Krabbes' disease:** The inherited deficiency of the enzyme **β-galactosidase** impairs the hydrolysis of galactocerebrosides, which results in accumulation of galactocerebrosides in brain and other nervous tissues.
   - There is almost complete absence of myelin in nervous tissue. The clinical features include mental retardation, blindness and deafness. Krabbe's disease is fatal in early life.
5. **Farber's disease:** The inherited deficiency of enzyme **ceramidase** impairs the hydrolysis of ceramides which results in accumulation of ceramides in the body tissue.
   - The symptoms include skeletal deformities, and mental retardation. The disease is fatal and death occurs in early childhood.

Table 13.1: Common form of sphingolipidoses

Disease	*Lipid accumulation*	*Enzyme deficiency*	*Clinical symptoms*
Niemann Pick	Sphingomyelin	Sphingomyelinase	Enlarged liver and spleen, mental retardation in infants
Gaucher's	Glucocerebroside	β-Glucosidase	Same as Niemann-Pick disease
Tay-Sach's	GM_2—Ganglioside	Hexosaminidase	Mental retardation, blindness, muscular weakness
Krabbe's	Galactocerebroside	β-Galactosidase	Mental retardation, blindness and deafness, myelin almost absent
Farber's	Ceramide	Ceramidase	Mental retardation, skeletal deformities

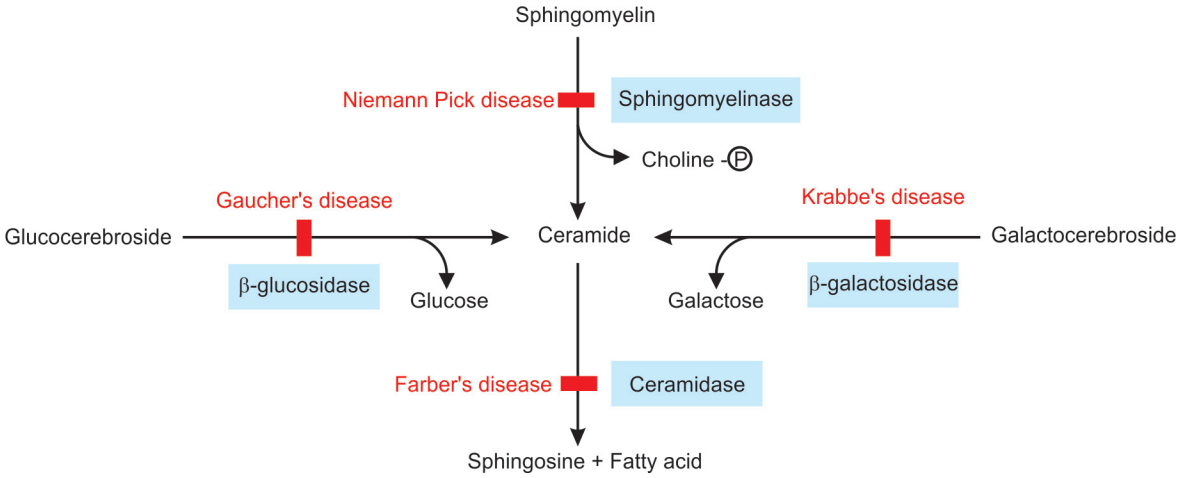


Figure 13.27: Degradation of sphingomyelin and cerebroside

LIPOPROTEIN METABOLISM

- Fat absorbed from the diet and fat synthesized by the liver must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, these are transported in the form of **lipoprotein.**
- Four main types of lipoproteins are, ***(Refer Chapter 3, Lipid Chemistry for structure).***
  1. **Chylomicrons:** These are principal form in which dietary lipids (exogenous lipid) are carried to the tissues.
  2. **Very low-density lipoproteins (VLDL):** These are triacylglycerol rich particles, in which endogenous triacylglycerol are carried to the tissues from the liver.
  3. **Low density lipoproteins (LDL):** These are cholesterol rich particles, formed from VLDL by the removal of triacylglycerol from VLDL.
  4. **High density lipoproteins (HDL):** They transport cholesterol from peripheral cells back to the liver, (reverse cholesterol transport).

Lipoprotein metabolisms include **chylomicrons, VLDL, LDL, HDL.**

Metabolism of Chylomicrons (Figure 13.28)

- Cholesterol and fatty acids released from dietary fats by digestion are absorbed into intestinal mucosal cells where they are re-esterified to form cholesterol esters and triacylglycerols. These together with phospholipids and apo B-48 form **chylomicrons.**
- Chylomicron shortly after entering the circulation they acquire apo C-II and apo E from circulating HDL. The apo C-II then activates **lipoprotein lipase** in the tissues and triacylglycerol is gradually removed from chylomicrons by the action of lipoprotein lipase. This enzyme is present on the walls of the blood capillaries of a number of tissues, predominantly adipose tissue and skeletal muscles.
- Lipoprotein lipase hydrolyzes triacylglycerol to fatty acid and glycerol. The fatty acids are taken up by adipose cells (for storage) and muscle cells as an energy source. The glycerol component enters the hepatic glycolytic pathway. During hydrolysis of chylomicron triacylglycerol, the apo C-II are transferred back to HDL.
- As the chylomicrons loses triacylglycerol, (approximately 90% of the triacylglycerol of chylomicrons), the resulting chylomicron becomes smaller and relatively enriched in cholesterol and cholesterol esters called **chylomicron remnants**.
- These remnants are taken up by the liver. In the liver the cholesterol esters and triacylglycerols of chylomicron remnants are hydrolyzed and metabolized.

Metabolism of VLDL

- The hepatic triacylglycerol and cholesterol are transported in the form of **nascent VLDL (Figure 13.29)**.
- This triacylglycerol rich particle contains apo B-100, circulating HDL donates apo C-II and apo E to convert nascent VLDL to VLDL.
- Like chylomicrons VLDL-triacylglycerol is hydrolyzed by lipoprotein lipase in the peripheral tissues

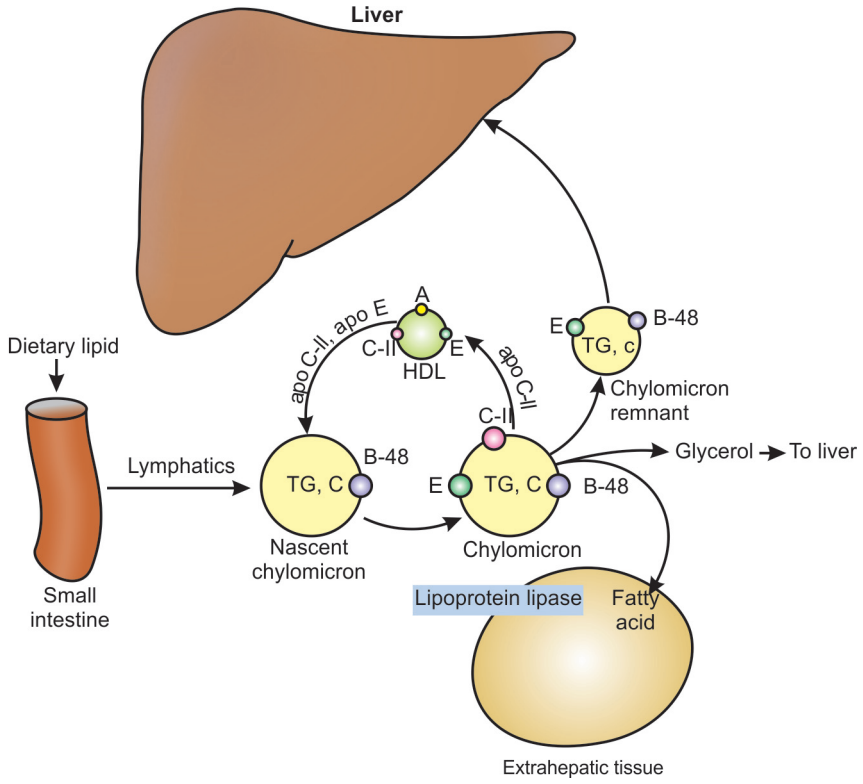


Figure 13.28: Metabolism of chylomicron, where TG: Triacylglycerol; C: Cholesterol

with the release of free fatty acid. During the hydrolysis of VLDL triacylglycerol, the apo C-II are transferred back to HDL.

- This results in the formation of VLDL remnant an intermediate density lipoprotein (IDL), which contains cholesterol and triacylglycerol as well as apo B and apo E. Formation of IDLs are transient which are not normally present in plasma.
- VLDL remnant or IDL undergoes a further hydrolysis, in which most of the remaining triacylglycerol are removed and all apolipoproteins except B-100 are transferred to other lipoproteins and results with formation of cholesterol rich LDL.

Metabolism of LDL (Figure 13.29)

- LDL is formed from VLDL metabolism as discussed above. LDL is a small cholesterol rich lipoprotein containing only apo B-100.
- LDL is removed from the circulation by the binding of LDL to specific LDL receptor present on the cell of liver and other peripheral tissue. This receptor is defective in familial hypercholesterolemia.

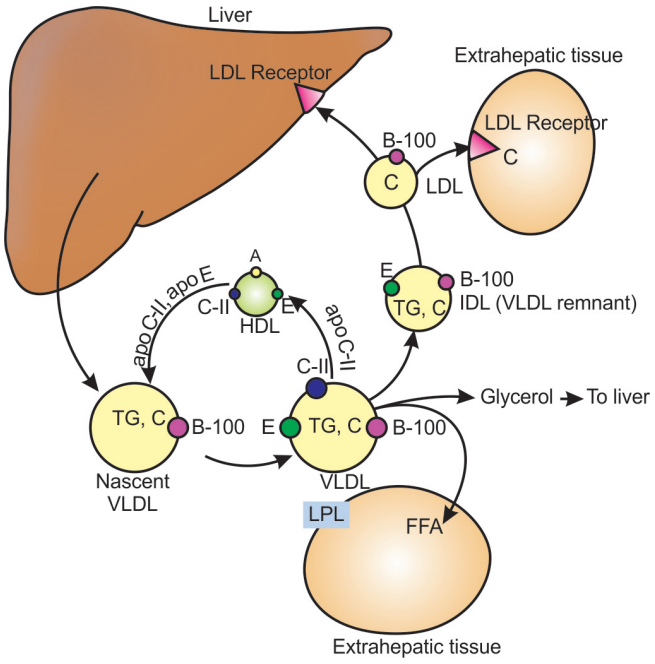


Figure 13.29: Metabolism of VLDL and LDL
where, FFA: Free fatty acid; LPL: Lipoprotein lipase; TG: Triacylglycerol; C: Cholesterol

- Inside the cell the cholesterol esters are hydrolyzed, thereby making unesterified cholesterol available to the cell.
  - The cells may use this cholesterol to maintain their cell membranes.
  - In specific tissue such as adrenal cortex, or gonads this cholesterol is utilized in steroid hormone synthesis.
  - Liver uses this cholesterol for synthesis of bile acids.
- Approximately 30% of LDL cholesterol is degraded in extrahepatic tissues and 70% in the liver.

Metabolism of HDL (Figure 13.30)

- HDLs are synthesized and secreted from liver as disk shaped nascent HDL particles that consist primarily of phospholipids (largely phosphatidyl-choline also known as lecithin), free cholesterol and apo A-1 as the main apolipoproteins together with apo C-II and apo E. These nascent HDL particles are nearly devoid of cholesterol ester and triacylglycerol.
- After secretion of nascent HDL into the plasma, the enzyme synthesized by liver, **lecithin: Cholesterol acyltransferase** (LCAT, pronounced 'el cat;) binds to the nascent HDL.
- Nascent HDL picks up cholesterol from other lipoproteins and from cell membranes of peripheral tissue. The cholesterol picked up by nascent HDL is converted to cholesterol esters by the action of LCAT in the presence of its activator apo A-I within the HDL particle.
- As cholesterol in HDL becomes esterified by LCAT activity, it creates a concentration gradient and draws in free cholesterol from tissues and from other lipoproteins.

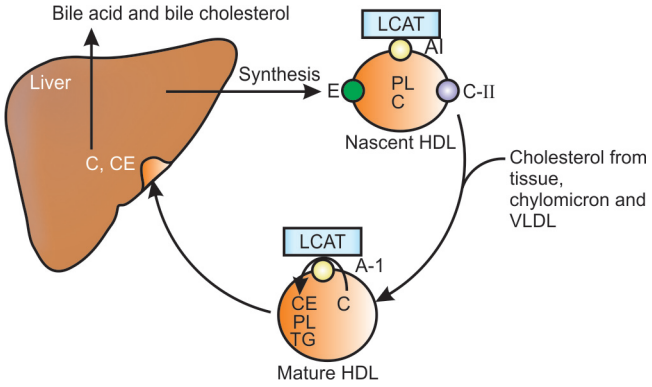


Figure 13.30: Metabolism of high density lipoprotein (HDL) and its role in reverse cholesterol transport
where, CE: Cholesterol ester; C: Cholesterol; TG: Triacylglycerol; PL: Phospholipid; LCAT: Lecithin:Cholesterol acyltransferase

- As the nascent HDL fill with cholesterol ester they become spherical in shape. These spherical HDL which is enriched in cholesterol ester enter the liver. In the liver the cholesterol esters are degraded to cholesterol which is utilized for synthesis of bile acids and lipoproteins or excreted into bile as cholesterol.

Reverse Cholesterol Transport

- This is the process whereby excess cholesterol contained in extrahepatic tissue is taken to the liver, through HDL metabolic cycle **(Figure 13.30)** for utilization or excretion through the bile.
- The LCAT esterifies the cholesterol content of HDL to prevent it from re-entering the cells. If the cholesterol is not esterified within the HDL particle, the free cholesterol can leave the particle by the same route that it entered. Thus esterification by LCAT serves to trap cholesterol within the lipoprotein, preventing it from deposition in the tissues.

Significance of reverse cholesterol transport

- By reverse cholesterol transport cellular and lipoprotein cholesterol is delivered back to the liver. This is important because the steroid nucleus of cholesterol cannot be degraded; and the liver is the only organ that can remove excess cholesterol by secreting it in the bile for excretion in the feces.
- Revese cholesterol transport prevents deposition of cholesterol in the tissues and is thought to be anti-atherogenic. An elevated HDL cholesterol (good cholesterol) level decreases the risk of coronary heart disease.

Disorders of Lipoprotein Metabolism

Hyperlipoproteinemia

In hyperlipoproteinemia, there is elevation of circulating lipoprotein levels. This disorder may be **primary** or **secondary.** Secondary hyperlipoproteinemia is due to some other disease, e.g. diabetes mellitus, nephrotic syndrome, hypothyroidism or alcoholism. The Fredrickson or World Health Organization classification of the hyperlipoproteinemia based on electrophoretic pattern of plasma lipoprotein is the most accepted one. It is given in **Table 13.2** and briefly discussed below.

1. **Type I (Hyperchylomicronemia):** This is due to either deficiency of the enzyme, **lipoprotein lipase** or **lack of apo C** which is required as an activator of lipoprotein lipase. The enzyme defect leads to impaired hydrolysis of chylomicrons resulting in increased level of plasma chylomicron and triacylglycerol.

Table 13.2 Fredrickson (WHO) classification of hyper lipoproteinemia

Type	*Elevated plasma lipoprotein*	*Elevated plasma lipid*	*Metabolic defect*	*Risk*
Type I	Chylomicron	Triacylglycerol	Deficiency of lipoprotein lipase or apo C-II	Pancreatitis
Type IIa	LDL	Cholesterol	Deficiency of functional LDL-receptors	Coronary heart disease
Type IIb	LDL and VLDL	Triacylglycerol Cholesterol	Overproduction of apo B	Coronary heart disease
Type III	Chylomicron remnants, VLDL remnants	Triacylglycerol Cholesterol	Abnormal apo E	Vascular disease
Type IV	VLDL	Triacylglycerol Cholesterol	Overproduction of triacylglycerol	Coronary heart disease
Type V	VLDL, Chylomicron	Triacylglycerol	Seondary to other disease	Coronary heart disease

2. **Type IIa (Familial hypercholesterolemia):** Due to deficiency of functional **LDL receptors** in liver and extrahepatic tissues, cellular uptake of LDL is impaired resulting in high LDL and hypercholesterolemia.
3. **Type IIb (Hypercholesterolemia):** There is elevation of both **LDL** and **VLDL** resulting in elevated level of cholesterol and triacylglycerol. This is believed to be due to excessive production of apo B. Secondary type II hyperlipoproteinemia is seen in obesity, diabetes mellitus, hypothyroidism and nephrotic syndrome.
4. **Type III (Dysbetalipoproteinemia):** There is accumulation of **chylomicron remnants** and **VLDL remnants** in plasma resulting in hypercholesterolemia and hypertriglyceridemia. This is believed to be due to a genetic defect in apo E, which cannot bind to the hepatic apo E receptors.
5. **Type IV (Familial endogenous hypertriglyceridemia):** This is due to overproduction of **triacylglycerol** by liver with a concomitant elevation in plasma VLDL. It may be associated with diabetes mellitus, obesity, alcoholism and impaired glucose tolerance.
6. **Type V:** There is an increase in both **chylomicrons** and VLDL, so triacylglycerol levels are increased. This is usually secondary to other disorders like obesity, diabetes, alcoholism and renal disease.

Hypolipoproteinemia

In hypolipoproteinemia concentration of one or more lipoproteins in plasma is decreased. The commonest of these disorders are discussed below.

Abetalipoproteinemia

- Abetalipoproteinemia is an autosomal recessive defect that results from a genetic defect in the synthesis of **apo B** and the synthesis of lipoproteins that contain apo B. Both chylomicron and VLDL are affected. As a result, LDL (β-lipoprotein) which is formed from VLDL in the plasma is totally absent.
- Both apo B-48 and B-100 are affected because they are inherited from the same gene.
- Fat malabsorption occurs in abetalipoproteinemia because chylomicrons cannot be formed by the intestine due to lack of apo B-48.
- Absorption of fat soluble vitamins is severely impaired resulting in degenerative changes in retina which may lead to blindness.

Tangier disease or an alphalipoproteinemia

- Alphalipoproteinemia also known as Tangier disease as it was first described in patients from Tangier island.
- Tangier disease is due to an inability to synthesize **apo A.** Apo A is the major apolipoprotein of HDL. This results in decreased level of HDL in plasma and impair reverse transport of cholesterol. This leads to the accumulation of cholesterol esters in tissue.

ADIPOSE TISSUE METABOLISM

Triacylglycerol is the major storage form of lipid in humans. Adipose tissue is the main store of triacylglycerol in the body. The triacylglycerol stored in adipose tissue is continually undergoing **lipolysis** (hydrolysis) and **resynthesis.**

Synthesis of Triacylglycerol in Adipose Tissue

- In adipose tissue triacylglycerol is synthesized from acyl-CoA and glycerol-3-phosphate.
- For provision of glycerol-3-phosphate, the tissue is dependent on glycolysis and a supply of glucose. The transport of glucose into adipose tissue is stimulated by insulin.
- Some fatty acids, that are incorporated into the triacylglycerol, are synthesized within the adipose tissue from glucose. The remainder is taken up from the blood, which is formed by the action of lipoprotein lipase on the triacylglycerol of chylomicrons and VLDL. Insulin stimulates this process by stimulating lipoprotein lipase.

Figure 13.31 summarises the process of triacylglycerol synthesis in adipose tissue.

Degradation of Triacylglycerols in Adipose Tissue

To leave the adipose tissue, the triacylglycerol must be hydrolyzed to fatty acids and glycerol.

- Triacylglycerol undergoes hydrolysis by a ***hormone sensitive lipase*** to form free fatty acids and glycerol.
- This lipase is distinct from lipoprotein lipase, that catalyzes hydrolysis of triacylglycerol before its uptake into extrahepatic tissues.
- The glycerol produced by lipolysis cannot be used by adipose tissues because they lack the enzyme ***glycerol kinase.***

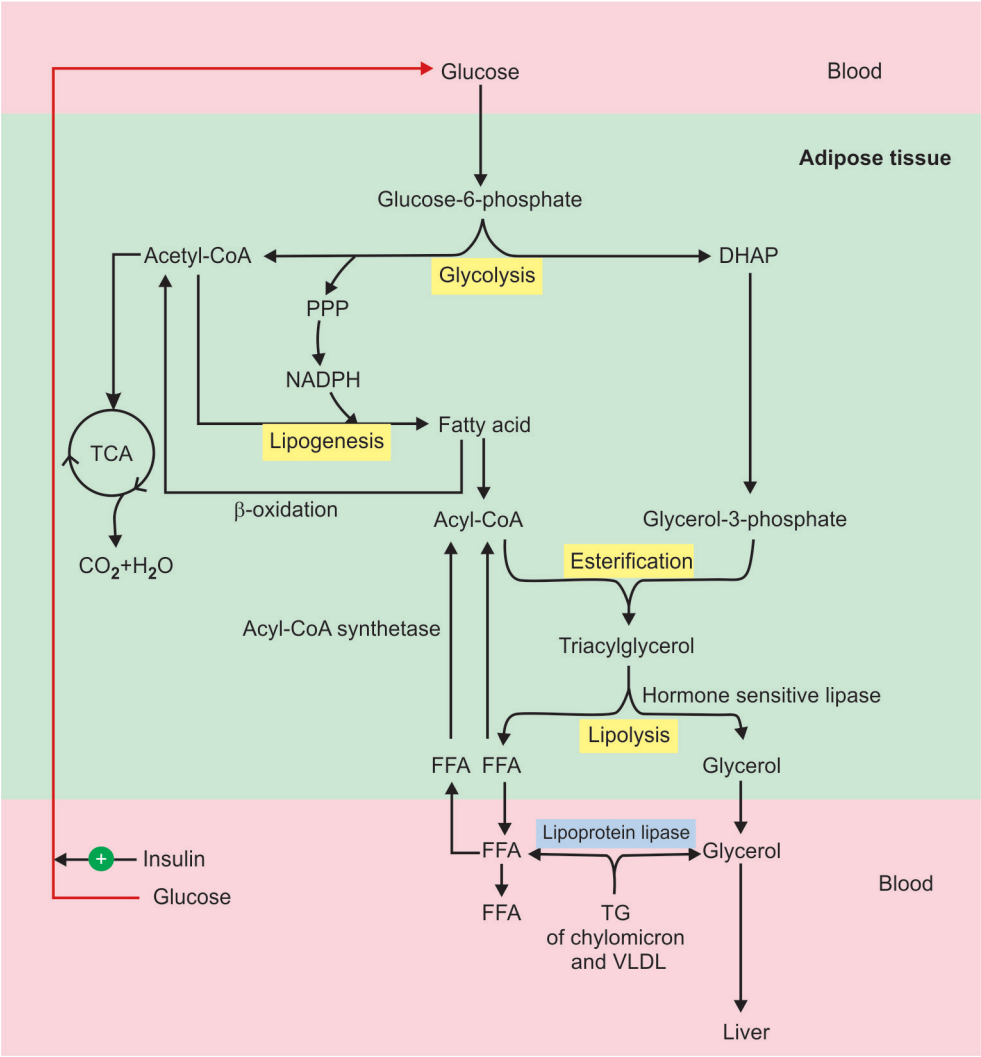


Figure 13.31: Adipose tissue metabolism
where, PPP: Pentose-phosphate pathway; TG: Triacylglycerol;
FFA: Free fatty acid; DHAP: Dihydroxy acetone phosphate

- Therefore, the glycerol is transported to the liver which contains, ***glycerol kinase*** to metabolize glycerol to glucose by the process of gluconeogenesis.
- The free fatty acid formed by lipolysis can be reconverted in the adipose tissue to acyl-CoA by ***acyl-CoA synthetase*** and re-esterified with glycerol-3-phosphate to form triacylglycerol.

Thus, there is a continuous cycle of lipolysis and resynthesis (re-esterification) within the adipose tissue. However, when the rate of re-esterification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma and raise the concentration of plasma free fatty acids, which occurs during fasting, diabetes mellitus, anxiety or physical exertion.

Significance of Adipose Tissue Metabolism

Fat is stored in the adipose tissue when food is plentiful and the individual is calm and resting. Conversely, fat is made available from adipose tissue in postabsorptive state or in stressful situations like starvation and diabetes mellitus.

Regulation of Adipose Tissue Metabolism (Figure 13.32)

- High levels of insulin in the blood stimulate triacylglycerol formation in adipose tissue and inhibits the lipolysis by inhibiting hormone sensitive lipase.
- Conversely, low insulin concentrations enhance the mobilization of fatty acid from adipose tissue
- Other hormones like epinephrine and glucagon accelerate the rate of lipolysis of triacylglycerol stores. These hormones activate hormone sensitive lipase.

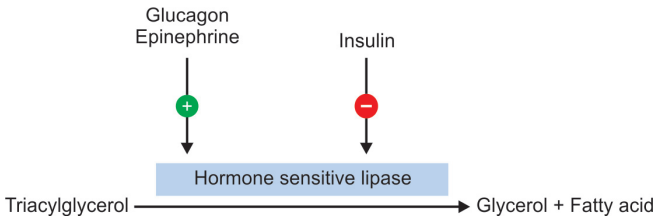


Figure 13.32 Regulation of lipolysis in adipose tissue

FATTY LIVER

Fatty liver is the excessive accumulation of fat primarily ***neutral fat, triacylglycerol*** in the liver. Fat is mainly stored in adipose tissue. Liver is not a storage organ. It contains about 5% fat. In pathological conditions, this may go up to 25–30% and is known as ***fatty liver*** or ***fatty infiltration*** of liver.

When accumulation of lipid in the liver becomes chronic, fibrotic changes occur in cells which may finally lead to ***cirrhosis*** and impairment of liver function.

Causes of Fatty Liver

Fatty liver may occur due to:
1. Overproduction of triacylglycerol in liver.
2. Impaired synthesis of VLDL.

- **Overproduction of triacylglycerol:** Fatty liver is associated with increased level of plasma free fatty acids from the diet (high fat diet) or from the adipose tissue during starvation, diabetes mellitus and alcoholism. The increasing amounts of fatty acids are taken up by the liver and esterified to triacylglycerol. VLDL produced in liver carries this triacylglycerol from liver to the peripheral tissues. But the production of VLDL does not keep pace with the formation of triacylglycerol, allowing triacylglycerol to accumulate in liver.
- **Impaired synthesis of VLDL:** Impairement in the biosynthesis of VLDL, in turn, impairs the transport of triacylglycerol from liver, thus allowing triacylglycerol to accumulate in the liver. The defect in the synthesis of VLDL may be due to:
  1. A block in apolipoprotein synthesis
  2. Defect in the synthesis of phospholipids that are found in lipoproteins.
  3. A failure in the formation mechanism of lipoprotein itself from lipid and apoprotein.

Factors that Cause Fatty Liver

1. **High fat diet:** Due to increased supply of free fatty acids from the diet, capacity of liver for lipoprotein formation is outweighed.
2. **Starvation or uncontrolled diabetes mellitus or insulin insufficiency:** Due to increased mobilization of free fatty acids from adipose tissue.
3. **Alcoholism:** Due to increased hepatic triacylglycerol synthesis and decreased fatty acid oxidation.
4. Dietary deficiency of:
   i. **Lipotropic factors:** Deficiency of lipotropic factors like choline, betaine, methionine, lecithin may cause fatty liver. Choline is required for the formation of phospholipid lecithin, which in turn, is an essential component of lipoprotein. Betain and methionine possessing methyl groups can be used to synthesize choline.

ii. **Essential fatty acids:** Essential fatty acids are required for the formation of phospholipid. A deficiency of essential fatty acids leads to decreased formation of phospholipids.
iii. **Essential amino acids:** Essential amino acids are required for the formation of apolipoprotein and lipotropic factor choline.
iv. **Vitamin E and selenium:** Deficiency of vitamin E or selenium enhances the hepatic necrosis. They have protective effect against fatty liver.
v. **Protein deficiency:** For example, in ***kwashiorkor***, deficiency of protein impairs formation of apolipoprotein.
vi. **Vitamin deficiency:** Deficiency of pyridoxine and pantothenic acid decrease the availability of ATP, needed for protein biosynthesis.

5. **High cholesterol diet:** Excess amount of cholesterol in diet competes for essential fatty acids for esterification.
6. **Use of certain chemicals:** For example, puromycin, chloroform, carbon tetrachloride, lead and arsenic inhibit protein biosynthesis and impair formation of apolipoprotein.

Lipotropic Factors

- The substances that prevent the accumulation of fat in the liver are known as ***lipotropic factors.*** Dietary deficiency of these factors can result in fatty liver. The various lipotropic agents are:
  - choline
  - methionine
  - betaine, etc.
- Choline is the principal lipotropic factor, and other lipotropic agents act by producing choline in the body, e.g. betaine and methionine possessing methyl groups are donated to ethanolamine to form choline.
- Choline is required for the formation of phospholipid, lecithin, which in turn, is an essential component of lipoprotein. And formation of lipoprotein is important in the disposal of triacylglycerol.
- Vitamin B_{12} and folic acid are also able to produce lipotropic effect, as these are involved in the formation of methionine from homocysteine.
- Casein and other proteins also possess lipotropic activity.

CHOLESTEROL METABOLISM

Cholesterol is the major sterol in human and has ***cyclopentanoperhydrophenanthrene*** ring system as a parent structure **(Figure 13.33).**

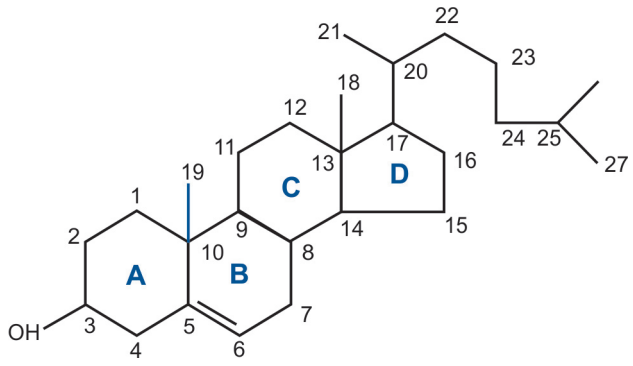


Figure 13.33: Structure of cholesterol

Cholesterol is an **amphipathic** lipid which can be synthesized by most cells of the body and it is obtained from the diet in foods of animal origin. ***It is not synthesized in plants.*** The major sources of dietary cholesterol are egg yolk and meat, particularly liver.

De Novo Synthesis of Cholesterol

Cholesterol is synthesized by a pathway that occurs in most cells of the body. ***Liver*** and ***intestine*** are major sites of cholesterol synthesis.

All 27-carbon atoms of cholesterol are derived from the **acetyl-CoA.** The enzyme system of cholesterol synthesis present in ***cytosolic*** and ***microsomal*** (endoplasmic reticulum) fractions. The reactions of cholesterol biosynthesis occurs into 5 stages **(Figures 13.34 and 13.35).**

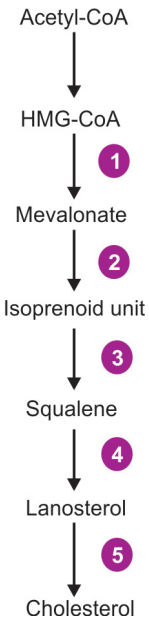


Figure 13.34: Five stages of cholesterol biosynthesis

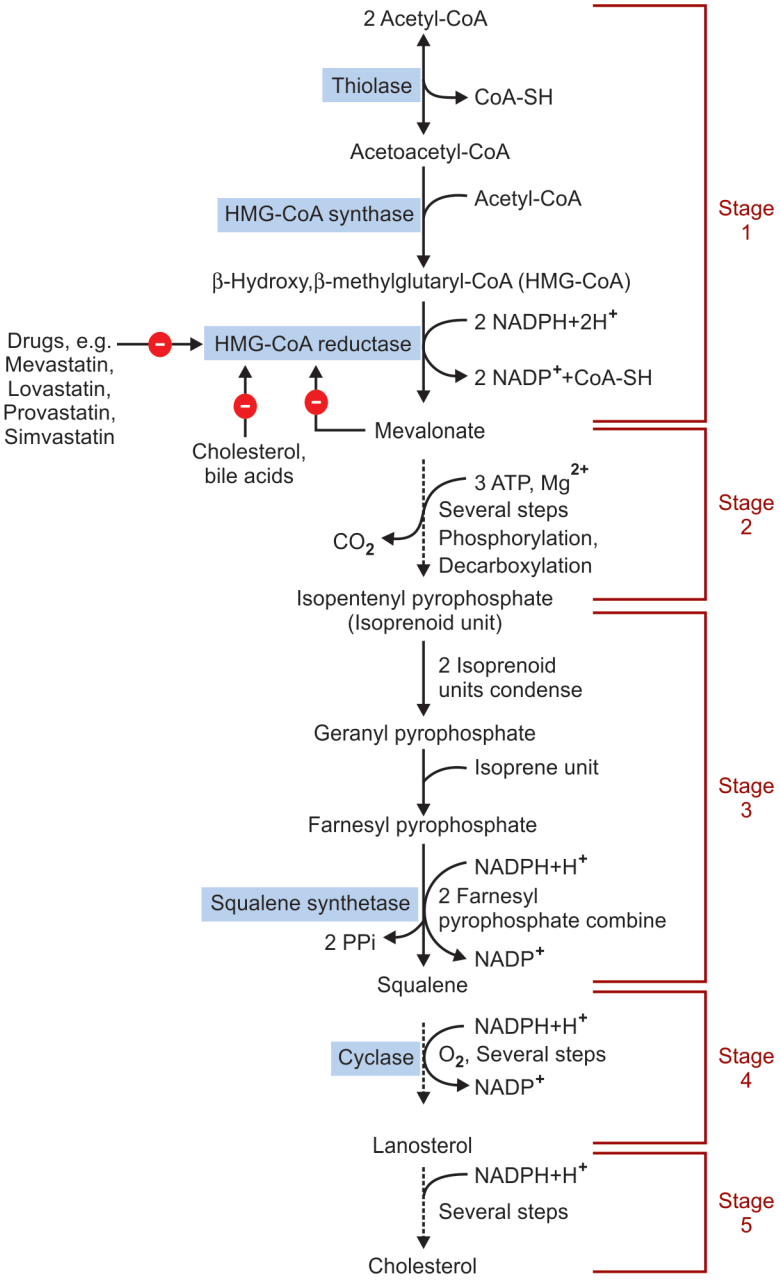


Figure 13.35: Biosynthesis of cholesterol, showing its five stages

Stage 1: Synthesis of Mevalonate from Acetyl-CoA through HMG-CoA

- First, two molecules of acetyl-CoA condense to form **acetoacetyl-CoA,** catalyzed by a cytosolic ***thiolase*** enzyme.
- Next, a third molecule of acetyl-CoA condenses with acetoacetyl-CoA catalyzed by ***HMG-CoA synthase*** *to* form **HMG-CoA.**
- This sequence of reactions in the cholesterol synthesis is similar to those for the synthesis of ketone bodies **(see Figure 13.11)** except that ketone body synthesis occurs in mitochondria and cholesterol is in the cytosol.
- The next step is catalyzed by ***HMG-CoA reductase.*** This enzyme uses two molecules of NADPH and converts HMG-CoA to **mevalonate.** This is the rate limiting step in the cholesterol synthesis.

Stage 2: Formation of Isoprenoid Unit by Decarboxylation of Mevalonate

Mevalonate is phosphorylated by ATP and subsequently decarboxylated to form a five carbon isoprene unit, ***isopentenyl pyrophosphate (IPP).***

Stage 3: Formation of squalene from condensation of six isoprenoid units

Six isopentenyl pyrophosphate molecules condense with loss of their pyrophosphate groups to yield the ***squalene*** (30-carbon atoms compound) through the formation of geranyl pyrophosphate and farnesyl pyrophosphate. ***Squalene was first isolated from the liver of sharks of genus Squalus.***

Stage 4: Cyclization of squalene to lanosterol

Squalene undergoes a series of complex enzymatic reactions, in which its linear structure is folded and cyclized to form lanosterol, which has the four condensed rings that form the steroid nucleus of cholesterol.

Stage 5: Formation of cholesterol from Lanosterol

The conversion of lanosterol to cholesterol is a multistep process, resulting in the

- Shortening of the carbon chain from 30 to 27
- Removal of the three methyl groups at C_4
- Migration of the double bond from C_8 to C_5
- Reduction of the one double bond between C_{24} and C_{25} by NADPH.

Regulation of De Novo Synthesis of Cholesterol (Figure 13.36)

HMG-CoA reductase is the rate limiting enzyme in cholesterol biosynthesis and is subjected to different kinds of metabolic control. The following are the different kinds of metabolic control.

Feedback Regulation

- *Cholesterol* the end product of the pathway, as well as ***mevalonate,*** repress the synthesis of ***HMG-CoA reductase***.
- It is also repressed by bile salts or bile acids, thus decreasing the cholesterol synthesis.

Hormonal Regulation

- Cholesterol synthesis is increased by **insulin** and **thyroid** hormone and is decreased by **glucagon** and **glucocorticoid** by stimulating and inhibiting HMG-CoA reductase enzyme respectively.

Nutritional Regulation

- Dietary cholesterol suppresses the synthesis of the HMG-CoA reductase in the liver.

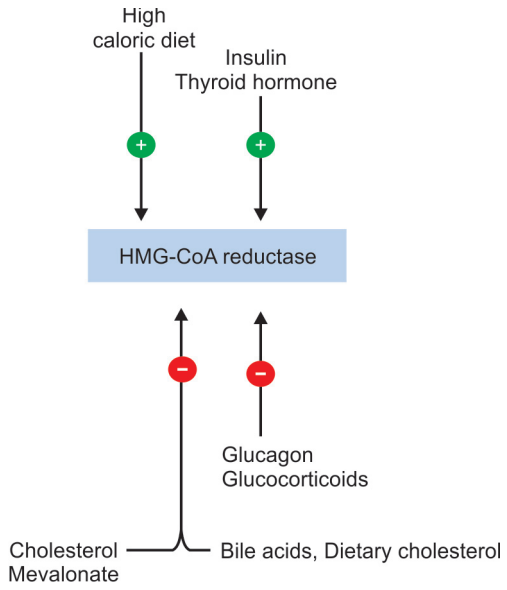


Figure 13.36: Regulation of cholesterol biosynthesis

- Increased caloric intake stimulates cholesterol synthesis primarily by increasing the availability of acetyl-CoA and NADPH.

Drugs like **mevastatin** and **lovastatin** inhibit cholesterol synthesis by acting as **competitive inhibitors** of HMG-CoA reductase. These drugs are used to decrease the serum cholesterol level in patients having elevated serum cholesterol concentration.

Transport of Cholesterol (Figure 13.37)

Transport of Dietary Cholesterol from Intestine

- After digestion, dietary cholesterol is absorbed into the intestinal mucosal cells, where much of it is subsequently reconverted into cholesterol esters.
- Cholesterol esters that are synthesized in the mucosal cells, together with some unesterified cholesterol are incorporated into ***chylomicrons,*** which transports cholesterol and other dietary lipids from intestine.
- When triacylglycerol of chylomicrons hydrolyzed by **lipoprotein lipase** in the peripheral tissue, only about 5% of the cholesterol ester is lost. The rest is taken up by the liver in the form of chylomicron remnants and is hydrolyzed to cholesterol.

Transport of Cholesterol from Liver to Peripheral Tissue

- Cholesterol from liver is transported in the form of VLDL into the plasma.

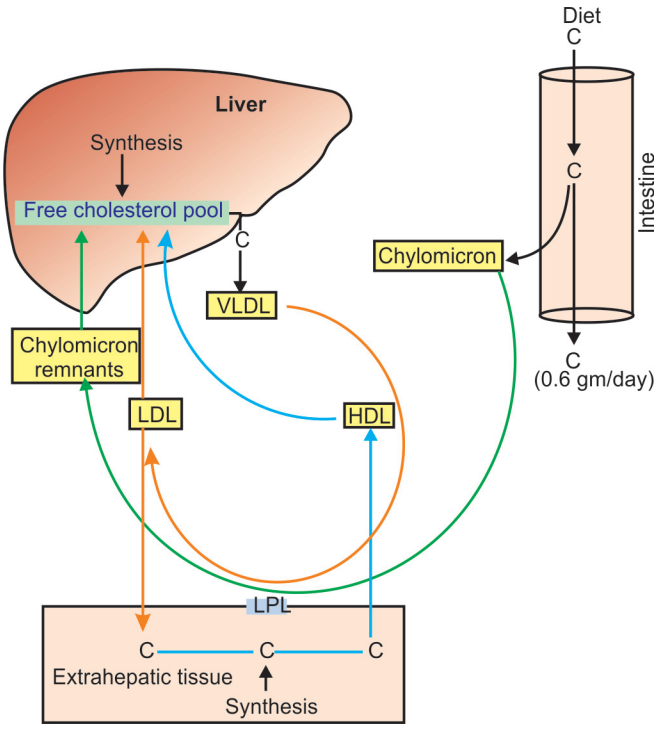


Figure 13.37: Transport of cholesterol
where, C:Cholesterol; LPL:Lipoprotein lipase

- Like chylomicrons VLDL triacylglycerol is hydrolyzed by lipoprotein lipase in the peripheral tissues and results with formation of cholesterol rich LDL.
- LDL cholesterol is taken up by the liver or extra-hepatic tissues by LDL receptors.

Transport of Cholesterol from Peripheral Tissue to Liver

- HDL picks up cholesterol from the peripheral tissues and converts it to cholesterol esters by LCAT enzyme
- These cholesterol esters are ultimately returned to the liver and thus HDL is said to participate in **"reverse *cholesterol transport.*"**

Degradation of Cholesterol

Cholesterol can undergo degradative reactions in humans with conversion of cholesterol to physiologically important products such as:

- Bile acids which is the main catabolic pathway
- Steroid hormones
- Vitamin D

Formation of Bile Acids

The primary bile acids ***cholic acid*** and ***chenodeoxy-cholic acid*** are synthesized in the liver from cholesterol (Figure 13.38).

Importance of bile acids

- The bile acids required for the emulsification of the dietary lipids and facilitate the enzymatic digestion and absorption of dietary lipids.
- Conversion of cholesterol into bile acid in the liver prevents the body from becoming overloaded with cholesterol.
- As steroidal ring of cholesterol cannot be degraded in the body, the excretion of bile salts serves as a major route for removal of the steroid ring from the body.

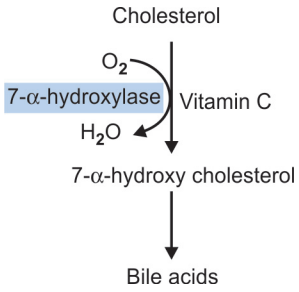


Figure 13.38: Synthesis of bile acid and its regulation

Formation of Steroid Hormones (Figure 13.39)

Cholesterol is the precursor of the five classes of steroid hormones:

1. Progesterone
2. Glucocorticoids
3. Mineralocorticoids
4. Sex hormones androgens
5. Estrogens.

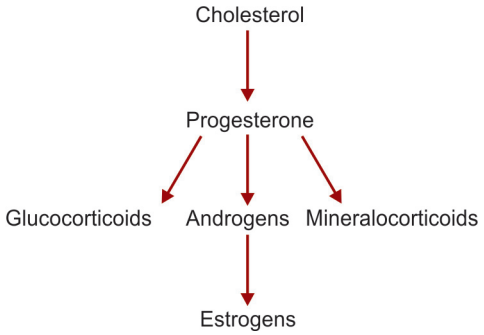


Figure 13.39: Formation of steroid hormones from cholesterol

Formation of Vitamin D

Cholesterol is also the precursor of vitamin D **(Figure 13.40)** which regulates calcium and phosphorus metabolism.

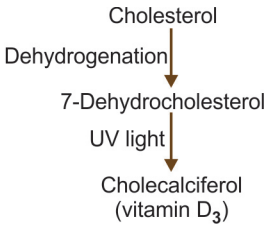


Figure 13.40: The formation of vitamin D_3 from cholesterol

Excretion of Cholesterol

- Cholesterol is excreted in feces
- Unlike many other metabolites, cholesterol cannot be destroyed by oxidation to CO_2 and H_2O, because of absence of enzymes capable of catabolizing the steroid ring.
- It is excreted in the bile either as cholesterol or after conversion to bile acids. About 1 gm of cholesterol is eliminated from the body per day. Roughly, half is excreted in the form of bile acids and half is in the form of cholesterol.
- Moreover, some dietary cholesterol is excreted in feces without being absorbed.
- Some of the cholesterol in the intestine is acted on by intestinal bacterial enzymes and converted to neutral sterols, coprostanol, cholestanol and excreted through feces.

Blood Cholesterol

The normal total plasma cholesterol lies between **150–250 mg per 100 ml.** Cholesterol present in blood occurs both free and as ester form. The hydroxyl group at position three of cholesterol can be esterified with fatty acids producing cholesterol esters. About 70% of the plasma cholesterol is esterified with fatty acids to form cholesterol ester, remaining 30% is free cholesterol.

Good cholesterol and bad cholesterol

- HDL cholesterol is considered to be the good cholesterol, because it removes excess cholesterol from peripheral tissues and transport it to the liver where it is degraded or excreted in the bile. HDL thus tends to lower blood cholesterol level.
- On the other hand LDL cholesterol is called bad cholesterol, because it transports cholesterol from liver

Contd.

to the peripheral tissue. Whereas for excretion, cholesterol must enter the liver **(Figure 13.37).** The risk of coronary heart disease (CHD) is related to plasma levels of total cholesterol and LDL-cholesterol.

Hypercholesterolemia

In a normal adult, the total plasma cholesterol ranges form **150–250 mg/100 ml.** An increase in plasma cholesterol more than 250 mg/100 ml is known as **hypercholesterolemia** and is seen in the following conditions:

1. **Diabetes mellitus:** This is due to deficiency of insulin, rate of lipolysis is increased. Increased rate of lipolysis results in more release of free fatty acids in circulation which increases acetyl-CoA. Excess of acetyl-CoA are diverted for cholesterol biosynthesis.
2. **Hypothyroidism:** This is due to decrease in the **HDL receptors** on hepatocyte in hypothyroidism and due to decreased synthesis of **7-α-hydroxylase** that requires for the conversion of cholesterol to bile acids. Thyroid hormone induces the synthesis of 7-α-hydroxylase.
3. **Obstructive jaundice:** Due to decreased excretion of cholesterol through bile.
4. **Familial hypercholesterolemia:** It is a genetic disease caused by deficiency or malfunction of the LDL receptors. In the absence of these receptors, the liver cannot take LDL and there is no release of cholesterol of LDL into the liver. Therefore, they do not cause feedback inhibition of cholesterol synthesis and lead to increased cholesterol formation.

ATHEROSCLEROSIS

High level of serum cholesterol results in atherosclerosis. The **atherosclerosis** is characterized by hardening and narrowing of the arteries due to deposition of cholesterol and other lipids in the inner arterial wall. Deposition of cholesterol and other lipids in the inner arterial wall leads to formation of plaque (sticky deposite) and results in the endothelial damage and norrowing of the arterial lumen. The hardening and narrowing of coronary arteries results in coronary heart disease (CHD).

Factors responsible for development of atherosclerosis

Age

Aging brings about changes in the blood vessel wall due to decreased metabolism of cholesterol. As age advances, the elasticity of the vessel wall decreases and formation of plaques progresses. The plaques are composed of smooth muscle cells, connective tissue, lipids and debris that accumulate in the inner side of the arterial wall. Formation of plaque leads to narrowing of the lumen and invites atherosclerosis.

Sex
Males are affected more than females, female incidence increases after menopause. Suggesting that male sex hormone might be atherogenic or conversely that female sex hormones might be protective.

Genetic factor
Hereditary genetic derangement of lipoprotein metabolism leads to high blood lipid level and familial hypercholesterolemia.

Hyperlipidemia
Increased levels of the following components of plasma lipids are associated with increased risk of atherosclerosis

- Total serum cholesterol and triacylglycerol.
- Low density lipoprotein (LDL) is richest in cholesterol and deposits it in tissues and has maximum association with atherosclerosis.
- **Lipoprotein(a) (LPa):** Some people have a special type of abnormal LDL called LP(a) containing an additional protein, ***apoprotein-a.*** Elevated LPa levels are associated with an increased risk of coronary heart disease.

Level of HDL
Low level of HDL is associated with atherosclerosis. HDL has protective effect against atherosclerosis. HDL participates in reverse transport of cholesterol, i.e. it transports cholesterol from cells to the liver for excretion in the bile. The higher the levels of HDL, the lower is the risk of ischemic heart disease. Consequently, high ratio of HDL/LDL reduces the development of atherosclerosis. There is an inverse relationship between cardiovascular risks and HDL concentration.

Hypertension
Hypertension is the major risk factor in patients over 45 years of age. It acts probably by mechanical injury of the arterial wall due to increased blood pressure.

Cigarette smoking
Ten cigarettes per day increase the risk three-fold due to reduced level of HDL and accumulating carbon monoxide that may cause endothelial cell injury. Cessation of smoking decreases risk to normal after 1 year.

Diabetes mellitus
The risk is due to the coexistence of other risk factors such as obesity, hypertension, and hyperlipidemia.

Minor or soft risk factors
These include lack of exercise, stress, obesity, high caloric intake, diet containing large quantities of saturated fats, use of oral contraceptive, alcoholism and hyperuricemia, etc. The risk is due to increased LDL and decreased HDL levels.

Prevention of atherosclerosis

- The most important preventive measure against the development of atherosclerosis is to eat a low fat diet that contains mainly unsaturated fat with low cholesterol content.
- Natural antioxidants such as vitamin E, C or β-carotene may decrease the risk of cardiovascular disease by protecting LDL against oxidation.
- Moderate consumption of alcohol and exercise appears to have a slightly beneficial effect by raising the level of HDL.
- Drug therapy, e.g. Lovastatin, clofibrate, cholestyramine which inhibit cholesterol synthesis.

ALCOHOL METABOLISM

Alcohol, i.e. ethanol is a drug. It has high energy content, yielding about 7.1 kcal/g on oxidation. Liver is the major site of ethanol oxidation. At least three enzyme systems are capable of ethanol oxidation **(Figure 13.41).**

1. Alcohol dehydrogenase (zinc dependent enzyme).
2. Microsomal ethanol oxidizing system (MEOS).
3. Catalase of peroxisomes.

Only 2–10% of ethanol is excreted unoxidized in the urine and lungs.

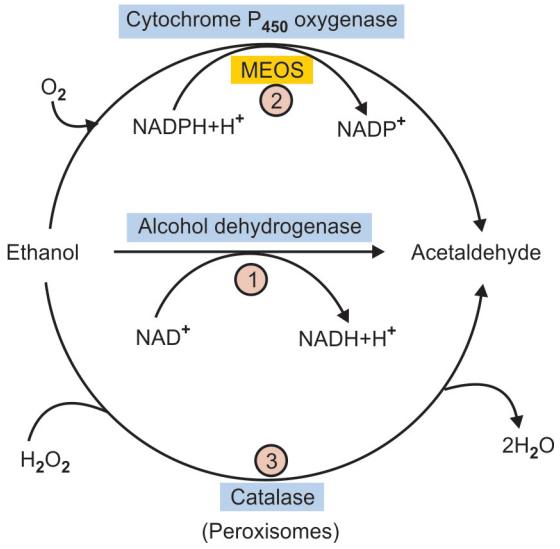


Figure 13.41: The metabolism of ethanol
where, MEOS: Microsomal ethanol oxidizing system

- ***Alcohol dehydrogenase (ADH)*** appears to be the principal pathway for ethanol oxidation. This route is operational for acute intoxication when the blood alcohol concentration is in the range 1–5 mmol/L.
- Above this, most of the ethanol is metabolized via mixed function oxidase system known as ***microsomal ethanol oxidizing system (MEOS)***, which involves ***cytochrome P_{450}***. MEOS appears to be a secondary enzyme system for ethanol clearance. In the chronic alcoholic, there is an increase in MEOS activity.
- The other ethanol oxidizing system is the ***catalase*** present in ***peroxisomes***. The role of catalase in biological oxidation of ethanol is controversial.
- The product of all three oxidation pathways is ***acetaldehyde,*** which is rapidly oxidized to acetate by liver ***aldehyde dehydrogenase (ALDH)*** **(Figure 13.42).** ALDH enzyme is inhibited by ***disulfiram*** and this property is used in the treatment of alcoholism.
- About 80% of the acetate produced by ALDH leaves the liver and undergoes further metabolism in tissues such as heart and skeletal muscle. Acetate is converted to acetyl-CoA, which is oxidized to CO_2 through citric acid cycle yielding ATP.

Biochemical Alterations due to Alcohol

Excess intake of alcohol leads to excessive production of NADH, with a concomitant decrease in NAD^+, produces metabolic changes as follows:

- The increased availability of NADH favors the ***reduction of pyruvate to lactate*** and ***oxaloacetate to malate*** and decreasing its availability for gluconeogenesis, resulting in decreased rate of gluconeogenesis and decreased synthesis of glucose. This can lead to ***hypoglycemia***.
- Increased lactic acid production also can result in hyperlactacidemia. The hyperlactacidemia reduces the capacity of the kidney to excrete uric acid, leading to secondary hyperuricemia and causes aggravation of gout.
- The increased ratio of NADH/NAD^+ also inhibit β-oxidation of fatty acids and citric acid cycle and promotes lipogenesis, triacylglycerol synthesis and cholesterol synthesis from acetyl-CoA.
- Accumulation of lipid in most tissues results in fatty liver, fatty myocardium, fatty renal tubules and so on.

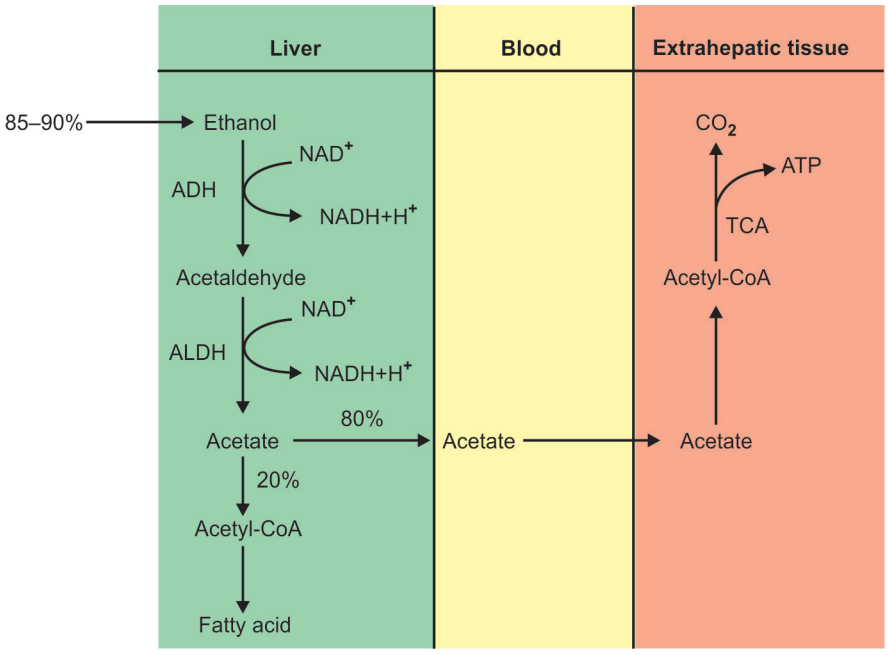


Figure 13.42: Alcohol metabolism
where, ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase

SUMMARY

- Dietary fat consists of triacylglycerol, cholesterol, cholesterol esters, phospholipids and free fatty acids.
- Little or no digestion occurs in the mouth or stomach. The major site of lipid digestion is the small intestine.
- Fatty acids are activated to acyl-CoA, transported across the inner mitochondrial membrane by carnitine and degraded in mitochondrial matrix by a process called β-oxidation, that cleaves acetyl-CoA units sequentially from acyl-CoA chain and leads to the generation of ATP. Acetyl-CoA formed, enters the citric acid cycle, generating further ATP.
- Odd carbon fatty acids are oxidized by the same basic pathway but yield acetyl-CoA plus one molecule of propionyl-CoA.
- The ketone bodies, acetoacetate, β-hydroxybutyrate and acetone are formed in the liver and are carried to other tissues, where they are oxidized via citric acid cycle and thus serve as a fuel in extrahepatic tissues.
- Overproduction of ketone bodies, which accumulate in the blood and urine, constitutes ketonemia and ketonuria respectively. The overall condition is called ketosis.
- Fatty acids are synthesized in the cytosol which is carried out by acetyl-CoA carboxylase and fatty acid synthase.
- Triacylglycerols are highly concentrated energy stores, whereas phosphoglycerols and sphingolipids (sphingomyelin and glycolipids) are structural components of cell membrane.
- Cholesterol and other lipids are transported in the plasma as lipoproteins. Four major groups of lipoproteins are:
  1. Chylomicrons, transport dietary lipids after digestion and absorption from intestine.
  2. Very low density lipoprotein (VLDL), transport endogenously synthesized triacylglycerol from the liver.
  3. Low density lipoprotein (LDL) are cholesterol rich lipoproteins resulting from the metabolism of VLDL and taken up by liver and peripheral tissue by LDL receptors.
  4. High density lipoproteins (HDL) are involved in transport cholesterol from the tissues to the liver.
- Imbalance in rate of triacylglycerol formation and VLDL formation in the liver leads to fatty liver. The substance that prevents the accumulation of fat in the liver are known as lipotropic factor.
- Adipose tissue is the main store of triacylglycerol, which is continually undergoing hydrolysis (lipolysis) and re-esterifications.
- Acetyl-CoA is the source of all carbon atoms in cholesterol. Cholesterol synthesis is controlled by regulation of HMG-CoA reductase. Cholesterol is the precursor of all steroid hormones, bile acids and vitamin D. Cholesterol is excreted by the liver in the bile as cholesterol or bile salts.
- Elevated levels of cholesterol present in LDL is associated with atherosclerosis, whereas high levels of HDL have a protective effect.
- Liver is the major site of ethanol oxidation. Three enzyme systems are cytochrome P_{450} oxygenase, alcohol dehydrogenase and catalase. They are capable of ethanol oxidation. Excessive intake of alcohol leads to toxic effect.

EXERCISE

Solve the Following

Case History 1

Despite strict dietary control, a 55-year-old man had elevated serum cholesterol level. He started to take simvastatin and 3 months later his cholesterol was normal.

Questions

a. Which enzyme of cholesterol biosynthesis is inhibited by simvastatin?
b. What is the role of that enzyme in cholesterol biosynthesis?
c. What is the normal serum cholesterol level?
d. Name precursors of cholesterol synthesis.

Case History 2

A 32-year-old heavy smoker developed a sudden crushing chest pain. He was admitted to the casualty department. Myocardial infarction was confirmed.

Questions

a. Is there any relationship between smoking and myocardial infarction? If yes, what is it?
b. What are the preventive measures against the development of heart disease?
c. Name the lipoproteins that have protective effect against development of myocardial infarction.
d. Name the enzymes likely to be elevated in myocardial infarction.

Case History 3

A 50-year-old male, with history of chronic alcoholism, had fatty liver.

Questions

a. What is fatty liver?
b. What are the causes of fatty liver?
c. What are lipotropic factors? Name any two.
d. What is the nutritional therapy for fatty liver?

Case History 4

A 19-year-old girl was referred to a medical center because of poor exercise tolerance and muscle weakness. After biochemical investigations carnitine deficiency was confirmed.

Questions

a. What is carnitine?
b. What is the role of carnitine?
c. What is cause of muscle weakness?

Case History 5

A 60-year-old woman was referred to a hospital. She was noted to have hypertension. The plasma cholesterol level was 390 mg/dL. An angiogram of the right carotid artery demonstrated a narrowed lumen and the concentration of LDL was elevated.

Questions

a. What is your probable diagnosis?
b. What is the normal plasma cholesterol level?
c. By which enzyme is cholesterol biosynthesis regulated?
d. Which lipoprotein has protective effect against the disorder?

Case History 6

A 35-year-old female was admitted to the hospital. Analysis of her plasma lipid levels revealed an elevated amount of cholesterol and almost no measurable cholesterol esters. No lecithin-cholesterol acyltransferase (LCAT) activity was detected in the patient's plasma.

Questions

a. Where does the LCAT reaction occur?
b. What is the role of LCAT?
c. What is the normal percentage of plasma cholesterol ester and free cholesterol?
d. Name the other enzyme that esterifies cholesterol in tissues.

Case History 7

A 30-year-old woman was hospitalized, with an acute myocardial infarction. Her plasma cholesterol and LDL level were highly elevated. She was found to have familial hypercholesterolemia.

Questions

a. How are LDL formed?
b. What apoprotein does LDL contain?
c. What is the function of LDL?
d. Why LDL is called bad cholesterol?

Case History 8

A male infant was admitted to the hospital. Examination revealed the presence of Gaucher's disease.

Questions

a. What is sphingolipidoses?
b. What is the cause of Gaucher's disease?
c. Name the defective enzyme.
d. What are the clinical symptoms?

Multiple Choice Questions (MCQs)

1. Rate controlling step of cholesterol biosynthesis is:
a) Lanosterol → Cholesterol
b) HMG-CoA → Mevalonic acid + CoA
c) Acetoacetyl-CoA + Acetyl-CoA → HMG-CoA + CoA
d) Squalene → Lanosterol

2. Which one of the following transfers fatty acids from cytosol to mitochondria?
a) Carnitine b) Creatine
c) Citrate d) ACP

3. Number of b-oxidation cycles undergo a 14-carbon saturated fatty acid:
a) 8 b) 7
c) 6 d) None of the above

4. b-oxidation of fatty acids in liver:
a) Requires fatty acids with an even number of carbon atoms
b) Produces only acetyl-CoA
c) Occurs in the mitochondria
d) Degrades fatty acids into CO_2 and H_2O

5. How many ATPs are produced when palmitoyl-CoA, a 16-carbon saturated fatty acid is oxidized completely to CO_2 and H_2O?
a) 96 b) 131
c) 129 d) 135

6. Which of the following intermediates in the oxidation of odd-chain fatty acids is likely to appear in the urine in vitamin B_{12} deficiency?
a) Succinic acid b) Methylmalonic acid
c) Propionic acid d) Butyric acid

7. Which of the following statements is correct for fatty acid synthesis?
a) Occurs in mitochondria
b) Requires NADPH as a cofactor
c) Requires NADH as a cofactor
d) Intermediates are linked to coenzyme A

8. The following are the features of the fatty acid synthase complex, *except:*
a) It is a dimer
b) It is found within cytosol
c) It requires pantothenic acid as a constituent
d) It requires biotin as a cofactor

9. Which of the following is not true for LDL?
a) Transport cholesterol to cells
b) Contains apo B-100
c) Contains apo C-II
d) Is a marker for cardiovascular risk

10. Free fatty acids are transported in plasma as a:
a) Component of VLDL
b) Component of chylomicrons remnants
c) Part of LDL
d) Ligand bound to albumin

11. The concentration of the following is inversely related to the risk of cardiovascular disease:
a) HDL b) LDL
c) VLDL d) IDL

12. Which of the following is an abnormal form of lipoprotein?
a) LDL
b) IDL
c) Lp(a)
d) Chylomicron remnant

13. Which of the following is true in fatty acid elongation?
a) Occurs in mitochondria and microsome
b) Requires provision of essential fatty acids
c) Requires biotin as a cofactor
d) Occurs only in cytosol

14. A key intermediate in the biosynthesis of both glycerophospholipid and triacylglycerol is:
a) Diacylglycerol
b) CDP-choline
c) Phosphatidyl choline
d) Phosphatidic acid

15. NADPH, utilized for synthesis of fatty acid, can be generated from:
a) Citrate lyase
b) Mitochondrial malate dehydrogenase
c) Malic enzyme
d) Citrate synthase

16. Ethanol is converted in the liver to:
a) Acetone
b) Acetaldehyde
c) Methanol
d) Lactate

17. The risk of heart attack can be decreased by, *except*:
a) Cessation of smoking
b) Lowered level of HDL
c) Control of plasma cholesterol
d) Lowered level of LDL

18. The form in which dietary lipids are transported from intestinal mucosal cells is:
a) VLDL b) HDL
c) Chylomicrons d) LDL

19. All of the following are amphipathic lipids, *except:*
a) Triacylglycerol b) Phospholipids
c) Cholesterol d) Fatty acids

20. The triacylglycerol present in adipose tissue is hydrolyzed by:
a) Lipoprotein lipase
b) Pancreatic lipase
c) Hormone sensitive lipase
d) Phospholipase

21. During electrophoretic separation, the fastest moving lipoprotein is:
a) HDL b) LDL
d) VLDL d) Chylomicrons

22. Ketosis occurs in all of the following conditions, *except:*
a) Diabetes mellitus
b) Marasmus
c) Prolonged starvation
d) High fat diet

23. Bile acids are derived from:
a) Phospholipids b) Triacylglycerol
c) Fatty acids d) Cholesterol

24. Which of the following pathways of oxidation of fatty acids does not require CoA intermediates?
a) α-oxidation
b) β-oxidation
c) Peroxisomal fatty acid oxidation
d) Oxidation of unsaturated fatty acid

25. The following are the ketone bodies, *except:*
a) Acetoacetyl-CoA
b) β-hydroxy butyrate
c) Acetone
d) Acetoacetate

26. Satiety value of fat is due to which of the following hormone?

a) Insulin b) Glucagon
c) Thyroxine d) Enterogastron

27. Which of the following ketone bodies cannot be metabolized in human body?

a) Acetoacetate b) Acetone
c) β-hyrodxybutyrate d) None of the above

Correct answers for MCQs

1-b	2-a	3-c	4-c
5-c	6-b	7-b	8-d
9-c	10-d	11-a	12-c
13-a	14-d	15-c	16-b
17-b	18-c	19-a	20-c
21-a	22-b	23-d	24-a
25-a	26-d	27-b	

CHAPTER

14

Protein Metabolism

INTRODUCTION

Proteins, the primary constitutents of the body, may be structural or functional. A regular and adequate supply of protein in diet is essential for cell integrity and function. Dietary proteins are the primary sources of the nitrogen that is metabolized by the body. Adult man requires ***70 to 100 gm*** protein per day. Dietary proteins serve three broad functions:

1. Their constituent amino acids are used for synthesis of the body's proteins.
2. The carbon skeletons of the amino acids can be oxidized to yield energy.
3. Their carbon and nitrogen atoms may be used to synthesize other nitrogen containing cellular constituents as well as many non-nitrogen containing metabolites.

DIGESTION AND ABSORPTION OF PROTEINS

Proteolytic enzymes (also called proteases) break down dietary proteins into their constituent amino acids. These enzymes are produced by three different organs; the ***stomach,*** the ***pancreas*** and the ***small intestine.***

Digestion in Mouth

There is no digestion of protein in mouth. It starts in stomach.

Digestion in Stomach

When protein enters the stomach, it stimulates the secretion of the hormone ***gastrin,*** from gastric mucosal cells, which in turn, stimulates the release of gastric juice containing **hydrochloric acid,** proenzyme (zymogen) **pepsinogen** and **rennin** in infants.

- **Hydrochloric acid:** Denatures proteins making their internal peptide bonds more accessible to subsequent hydrolysis by proteoses and provides an acid environment for the action of pepsin.
- **Pepsin:** It is secreted as the proenzyme ***pepsinogen,*** an inactive form.
- It is converted into active pepsin in the gastric juice by the enzymatic action of pepsin itself or by high hydrogen ion concentration **(Figure 14.1)**.
- Pepsin cleaves those peptide bonds of protein involving the:
  - ***Aromatic amino acids*** (phenylalanine, tyrosine and tryptophan)

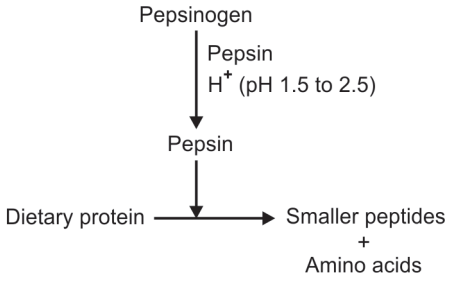


Figure 14.1: Activation of pepsinogen and action of pepsin

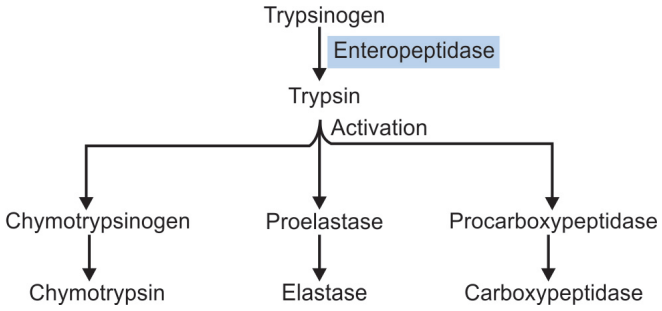
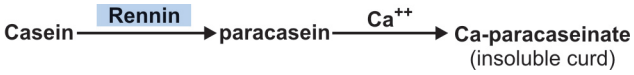


Figure 14.2: Activation of pancreatic proenzymes

– ***Acidic amino acids*** (aspartic acid and glutamic acid).

Thus, pepsin cleaves long polypeptide chains into a mixture of smaller peptides and some free amino acids.

- **Rennin** is important in the digestive processes of infants. It is absent in adults. Rennin is also called **chymosin** or **rennet.**
- Action of rennin is to clot milk. This is accomplished by the slight hydrolysis of the casein of milk to produce **paracasein,** which coagulates in the presence of calcium ions, resulting in an insoluble **calcium-paracaseinate** curd. Calcium paracaseinate is then acted on by **pepsin.**



The purpose of this reaction is to convert milk into a more solid form to prevent the rapid passage of milk from the stomach of infants.

Rennin and renin are different. Renin is secreted by kidney and is involved in regulation of water and electrolyte balance and blood pressure.

Digestion in Intestine by Pancreatic Enzymes

There are two types of peptidase enzymes secreted by pancreas:

1. Endopeptidase
2. Exopeptidase

Endopeptidase

- Endopeptidases cleave internal peptide bonds. This results into formation of smaller peptides from large polypeptides.
- Endopeptidases secreted by pancreas, are ***trypsin, chymotrypsin*** and ***elastase***. These are secreted in proenzyme (inactive) forms, ***trypsinogen, chymotrypsinogen*** and **proelastase**.

Exopeptidase

- Exopeptidase which hydrolyze the peptide bonds of terminal amino acids. Exopeptidase are of two types:
  – **Carboxypeptidase** secreted by pancreas act on C-terminal amino acid.
  – **Aminopeptidases** secreted by mucosal cell act on N-terminal amino acid.

Activation of pancreatic proenzymes

Activation of the pancreatic proenzymes occurs by the action of ***enteropeptidase (enterokinase),*** secreted by duodenal epithelial cells. Enteropeptidase activates trypsinogen to trypsin and the activated trypsin in turn activates more trypsinogen. Trypsin also activates the chymotrypsinogen, proelastase and procarboxypeptidase **(Figure 14.2)**. The specificity of these proteolytic enzymes is given below **Table 14.1**.

Table 14.1: Specificity of proteolytic enzymes

Enzyme	*Peptide bond specificity*
Pepsin	Aromatic amino acids (tyrosine, phenylalanine, tryptophan) Acidic amino acids (glutamic acid and aspartic acid)
Trypsin	It's carboxyl groups are contributed by lysine and arginine
Chymotrypsin	Aromatic amino acids (phenylalanine, tyrosine and tryptophan), leucine, methionine, aspargine, histidine
Elastase	Small nonpolar amino acids alanine, serine and glycine
Carboxypeptidase	Successive C-terminal amino acids
Aminopeptidase	Successive N-terminal amino acids

- **Trypsin** hydrolyzes those peptide bonds whose carboxyl groups are contributed by ***lysine*** and ***arginine*** residues.
- **Chymotrypsin** preferentially cleaves peptide bonds involving the ***carboxyl group of aromatic*** **amino** ***acids.*** It also splits peptide linkages of ***leucine, methionine, aspargine*** and ***histidine.***
- **Elastase** hydrolyzes those peptide bonds, formed by ***small nonpolar amino acid*** residues, such as, ***alanine, serine*** and ***glycine.***

Trypsin, chymotrypsin and elastase, thus, hydrolyze polypeptides, resulting from the action of pepsin in the stomach into smaller peptides. Degradation of short peptides formed in the small intestine is continued by an **exopeptidase**.

- **Carboxypeptidase** (zinc containing enzyme), an exopeptidase removes the successive carboxyl terminal amino acid residues from peptide.

Digestion in Intestine by Intestinal Proteoses

The digestion products of hydrolysis by pepsin, trypsin, elastase, chymotrypsin and carboxypeptidase is completed by enzymes, secreted by the mucosa of the small intestine such as **aminopeptidases** and **dipeptidases.**

- Aminopeptidase is an exopeptidase, hydrolyze peptide bonds next to N-terminal amino acids of the short peptides.
- The dipeptidases complete digestion of dipeptides to free amino acids. These dipeptidases can then finally convert all ingested protein into free amino acids. Dipeptidases require ***cobalt*** or ***manganese*** ions for their activity.

The hydrolysis of most proteins is thus completed to their constituent amino acids which are then ready for absorption into the blood.

Absorption of Amino Acids

- The absorption of most amino acids involves an ***active transport mechanism,*** requiring ATP and specific transport proteins in the intestinal mucosal cells.
- Many transporters have Na^+ dependent mechanisms, coupled with Na^+ K^+ pump, similar to those described for glucose absorption **(Figure 14.3)**.
- Several Na^+ independent transport proteins are found in the brush-border membrane that are not specific for each amino acid but rather for the groups of structurally similar amino acids.

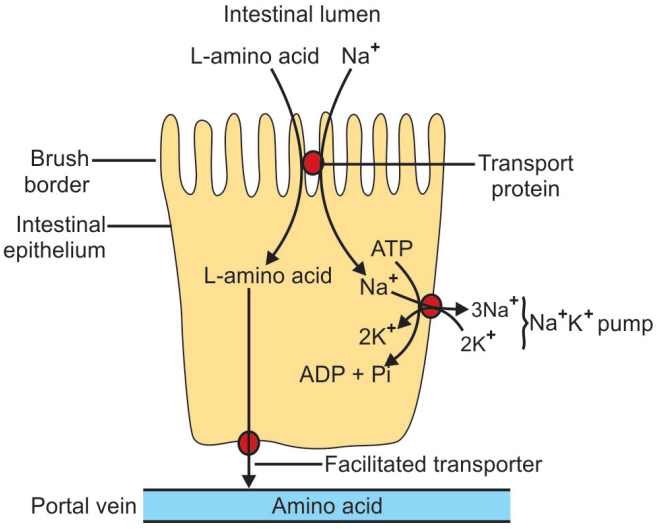


Figure 14.3: Transport of L-amino acid across the intestinal epithelium

- All are specific for only L-amino acid. D-amino acids are transported by passive diffusion.
- Thus, amino acids, released by digestion, pass from the gut through hepatic portal vein to the liver.
- Alton Meister proposed that glutathione (γ-glutamyl cysteinylglycine) participates in absorption of amino acids in **intestine, kidneys** and **brain** and the cycle is called **gammaglutamyl cycle** or **Meister cycle.**

Absorption of Intact Protein

- Small intestinal cells of fetal and newborn infants are able to absorb intact proteins, **e.g.** ***immunoglobulin IgA from colostrum of maternal milk are absorbed intact without loss of biologic activity, so that they provide passive immunity to the infant.***
- The intact proteins are not absorbed by the adult intestine. However, in some adult individuals, small amount of intact proteins may be absorbed through the intestinal mucosa. These proteins often cause formation of antibodies against the foreign protein and are responsible for the symptoms of food allergies.

AMINO ACID POOL (FIGURE 14.4)

- Amino acids, released by hydrolysis of dietary protein, and tissue proteins together constitute the ***amino acid pool***.

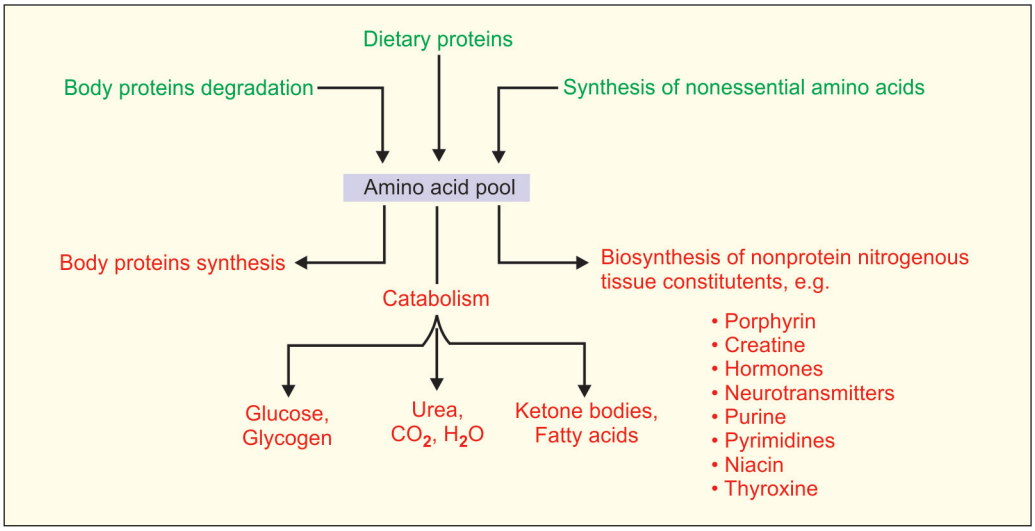


Figure 14.4: Amino acid pool

- In contrast to carbohydrates and fat whose major function is to provide energy, the primary role of amino acids is to serve as building blocks of synthesis of tissue protein and other nitrogen containing compounds **(Figure 14.4)**.
- Amino acids, in excess of those needed for the synthesis of proteins and other biomolecules cannot be stored in contrast with fatty acids and glucose, nor are they excreted. Surplus amino acids are oxidized for energy. Liver is the major site of amino acid oxidation.

Protein Turnover

Most proteins in the body are constantly being synthesized and then degraded, In healthy adults, the total amount of protein in the body remains constant because the rate of protein synthesis is just sufficient to replace the protein that is degraded. This process is called ***protein turnover,*** which leads to the hydrolysis and resynthesis of ***300 to 400 gm of body proteins each day.*** The turnover is high in infancy and decreases with age advance.

NITROGEN BALANCE

Catabolism of amino acids leads to a net loss of nitrogen from the body. This loss must be compensated by the diet in order to maintain a constant amount of body protein. Nitrogen balance studies evaluate the relationship between the nitrogen intake (in the form of protein) and nitrogen excretion.

Three situations of nitrogen balance are possible as follows:

1. Nitrogen equilibrium
2. Positive nitrogen balance
3. Negative nitrogen balance.

Nitrogen Equilibrium

- In normal adults, nitrogen intake is equal to nitrogen excretion. The subject is said to be in nitrogen equilibrium or balance.
- In this situation, the rate of body protein synthesis is equal to the rate of degradation.

Positive Nitrogen Balance

- In this situation, nitrogen intake > nitrogen excretion, i.e. intake of nitrogen is more than excretion.
- It shows that nitrogen is retained in the body, which means that protein is laid down.
- This occurs in growing ***infants*** and ***pregnant women.***

Negative Nitrogen Balance

- In this situation, nitrogen intake < nitrogen excretion, i.e. nitrogen output exceeds input and this occurs during ***serious illness*** and **major *injury*** and ***trauma,*** in ***advanced cancer*** and following failure to ingest adequate or sufficient high quality protein, e.g. in ***kwashiorkor*** and ***marasmus.***
- If the situation is prolonged, it will ultimately lead to death.

CATABOLISM OF AMINO ACIDS

The complete catabolism of amino acids includes following stages:

1. The removal of ***α-amino group*** in the form of ***ammonia*** by following reactions:
   i. **Transamination** by the enzyme ***aminotransferase*** also called ***transaminases***.
   ii. **Deamination** may be oxidative or nonoxidative
      a. Oxidative deamination is by glutamate dehydrogenase or amino acid oxidase.
      b. Nonoxidative deamination is by amino acid dehydratase.
2. Disposal of **ammonia** in the form of **urea** in the liver by reactions of the **urea cycle**.
3. Disposal (catabolism) of the remaining carbon skeleton of amino acid to carbon dioxide and water by reactions of **citric acid cycle**.

FORMATION OF AMMONIA

Transamination

Transamination involves the transfer of α-amino group of α-amino acid to an α-keto acid to form new amino acid and a new keto acid. The enzymes that catalyze these reactions are called ***aminotransferases*** or ***transaminases*** **(Figure 14.5)**.

- Most transaminases use α-ketoglutarate (α-keto acid) as a common acceptor of amino groups.
- All transaminases require **pyridoxal phosphate (PLP)** as a coenzyme. Some of the most important transaminases are ***alanine transaminase (ALT)*** and ***aspartate transaminase (AST)*** **(Figure 14.6)**.
- Alanine transaminase (ALT) also called ***glutamate pyruvate transaminase (GPT),*** catalyzes the transfer of amino group of alanine to α-ketoglutarate resulting in the formation of **pyruvate** and L-glutamate.
- Aspartate transaminase (AST), also called ***glutamate oxaloacetate transaminase (GOT),*** catalyzes the transfer of the amino group of aspartate to α-ketoglutarate, resulting in the formation of **oxaloacetate** and L-glutamate.

Most amino acids undergo transamination reaction except ***lysine, threonine,*** **proline** and ***hydroxyproline***.

It will be noted that there is no net loss of amino groups in transamination reactions.

Metabolic significance of transamination reactions

- This reaction provides a mechanism for collecting the amino groups from many different amino acids into one common product L-glutamate. This is important because glutamate is the only amino acid whose α-amino group can be directly removed at a high rate by oxidative deamination.
- Since transamination reactions are readily reversible, this permits transaminases to function both in amino acid catabolism and biosynthesis. L-glutamate, produced by transamination, can be used as an amino group donor in the synthesis of nonessential amino acids.

Clinical significance of transaminase enzyme

Serum levels of some transaminases are elevated in some disease state and measurement of these are useful in medical diagnosis, e.g. ALT (GPT) and AST (GOT) are important in the diagnosis of liver and heart damage (Refer Chapter 6: Enzyme).

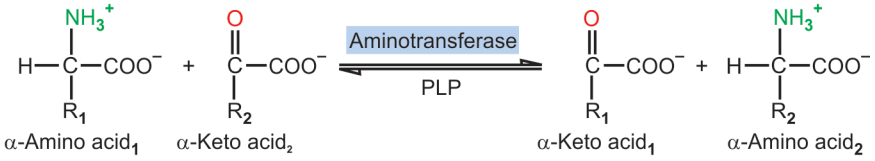


Figure 14.5: Transamination reaction

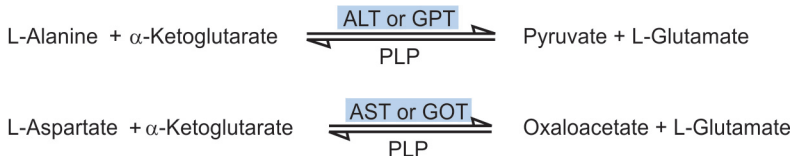


Figure 14.6: Reactions catalyzed by alanine transaminase and aspartate transaminase

Deamination

Oxidative deamination by glutamate dehydrogenase

- The α-amino groups of most amino acids are ultimately transferred to α-ketoglutarate by transamination forming L-glutamate.
- Then L-glutamate undergoes oxidative deamination by the action of **L-glutamate dehydrogenase,** which requires NAD^+ or $NADP^+$ as an oxidizing agent **(Figure 14.7)**.
- ***Thus, the net removal of α-amino groups to ammonia, requires the combined action of glutamate transaminase and glutamate dehydrogenase.***

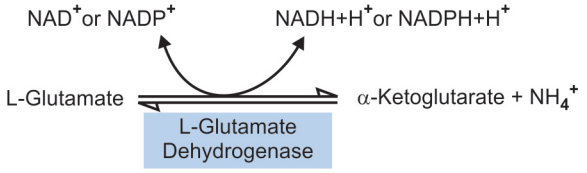


Figure 14.7: Oxidative deamination by L-glutamate dehydrogenase

Metabolic significance

- This freely reversible reaction functions both in amino acid catabolism and biosynthesis.
- Catabolically, it channels nitrogen from glutamate to ammonia and anabolically it catalyzes amination of α-ketoglutarate by free ammonia to form glutamate.

Clinical significance of glutamate dehydrogenase (GLD)

GLD is present in normal serum in trace amount only, but increased activities are observed in cases of liver disease.

Oxidative deamination by amino acid oxidases

- Both L- and D-amino acid oxidases occur in the kidneys and the liver. However, their activities are low. The reaction catalyzed is given in **Figure 14.8**.
- Amino acid oxidases use auto-oxidizable flavins (FMN or FAD) as coenzyme, which oxidize amino acids to an ***α-imino acid.*** α-Imino acid is an unstable compound which decomposes to the corresponding α-keto acid with release of ammonium ion. In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase.

Metabolic significance

D-amino acids present in the diet are metabolized by D-amino acid oxidase in the liver.

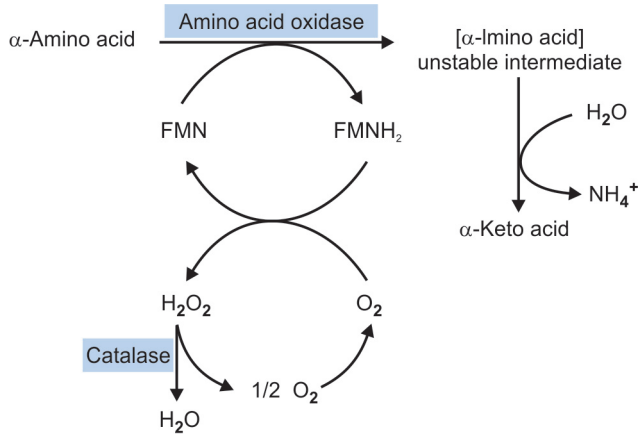


Figure 14.8: Oxidative deamination by amino acid oxidase

Nonoxidative deamination by amino acid dehydratase

- The α-amino groups of ***serine*** and ***threonine*** can be directly deaminated into NH_4^+ ion. These amino acids contains hydroxy group attached to its β-carbon atom.
- These direct deaminations are catalyzed by ***serine dehydratase*** and ***threonine dehydratase***, in which pyridoxal phosphate (PLP) is the coenzyme **(Figure 14.9)**.

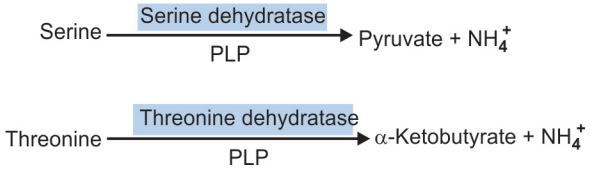


Figure 14.9: Nonoxidative deamination by amino acid dehydratase

METABOLIC FATE OF AMMONIA

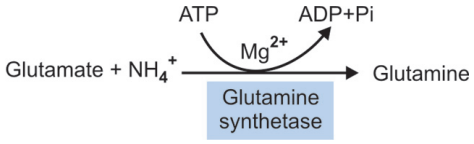
Ammonia is produced in most tissues. Since ammonia is extremely toxic, it is immediately converted to non-toxic metabolites such as, **glutamate** or **glutamine** or **alanine** and ultimately to **urea.** For the ultimate conversion of ammonia to urea, ammonia is transported to the liver.

Transport of ammonia to the liver

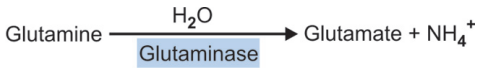
Since free ammonia is highly toxic, it is never transported in free form in blood. Two mechanisms are available in humans for the transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea.

Transport of Ammonia in the Form of Glutamine

- In many tissues (liver, kidney and brain), ammonia is enzymatically combined with glutamate to yield glutamine by the action of **glutamine synthetase.**



- The glutamine, so formed is a neutral nontoxic major transport form of ammonia. The glutamine is transported by blood to the liver, where it is cleaved by **glutaminase** to yield glutamate and free ammonia.



- The ammonia so formed is converted by the liver into urea.

Transport of Ammonia in the Form of Alanine

- Alanine transports ammonia from muscles to the liver through **glucose alanine cycle** as shown in **Figure 12.9**.
- In muscle, glutamate is formed from ammonia and α-ketoglutarate by reversal of the glutamate dehydrogenase reaction **(Figure 14.7)**. L-glutamate then transfers its α-amino group to pyruvate by transamination reaction to form **alanine.**
- Alanine so formed in muscle is transported to liver where it is converted to pyruvate and glutamate again by transamination reaction.
- In the liver, glutamate undergoes oxidative deamination to release free ammonia, which is converted to urea. Whereas, pyruvate is converted to glucose by gluconeogenesis.

Formation of Urea

- Urea is the end product of **protein metabolism.** The nitrogen of amino acids removed in the form of ammonia is detoxified by converting it to **urea.**
- Formation of urea by ***"Kreb's Henseleit urea cycle"*** is an ultimate route for the metabolic disposal of ammonia.
- Urea is produced exclusively by the liver and then is transported through blood to the kidneys for excretion in the urine.
- Urea is formed from ammonia, carbon dioxide and α-amino nitrogen of aspartate, which requires ATP.
- Enzymes catalyzing the urea cycle reactions are distributed between the ***mitochondria*** and the ***cytosol*** of the liver **(Figure 14.10)**.

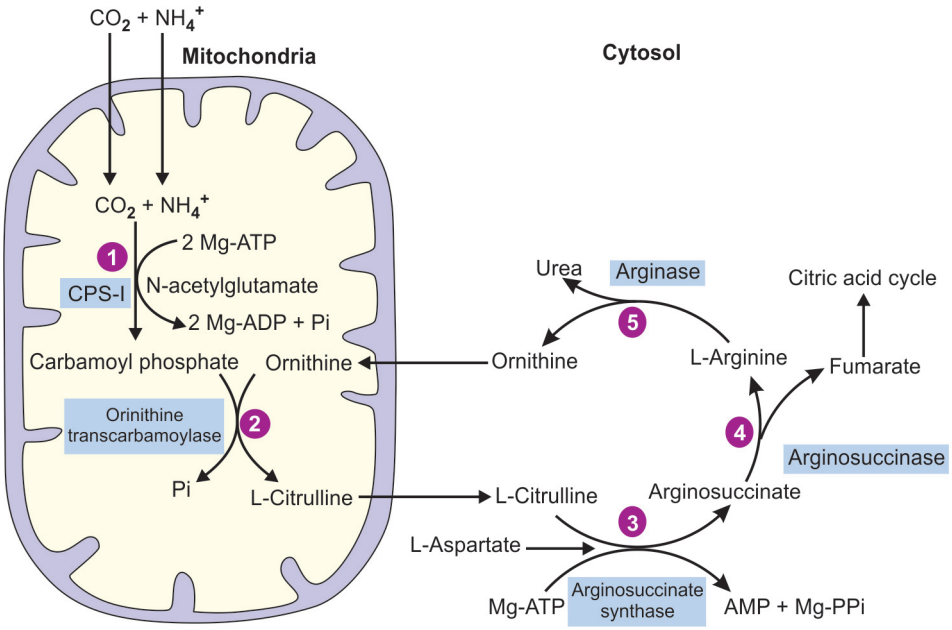


Figure 14.10: Reactions of urea cycle
where, CPS-I: Carbamoyl phosphate synthase-I

The first two reactions of urea cycle occur in the mitochondria, whereas the remaining reactions occur in the cytosol.

UREA CYCLE

The sequence of reactions involved in the biosynthesis of urea, summarized in five steps as follows:

1 Formation of Carbamoyl Phosphate: The biosynthesis of urea begins with the condensation of carbon dioxide, ammonia and ATP to form **carbamoyl phosphate**, a reaction catalyzed by mitochondrial ***carbamoyl phosphate synthase-I (CPS-I)***. Human tissues contain two forms of carbamoyl phosphate synthase:

- ***Carbamoyl phosphate synthase-I*** is a hepatic mitochondrial enzyme functional in urea synthesis.
- ***Carbamoyl phosphate synthase-II*** a cytosolic enzyme that uses glutamine rather than ammonia as a nitrogen source and functions in pyrimidine nucleotide biosynthesis ***(See Chapter 19)***.

- Formation of carbamoyl phosphate requires 2 molecules of ATP. One ATP serves as a source of phosphate and second ATP is converted to AMP and PPi.

2 Formation of Citrulline: Carbamoyl phosphate donates its carbamoyl group to ornithine to form **citrulline** and release phosphate, in a reaction catalyzed by ***ornithine transcarbamoylase***, a Mg^{2+} requiring mitochondrial enzyme.

- The citrulline so formed now leaves the mitochondria and passes into the cytosol of the liver cell.

3 Formation of Arginosuccinate: The transfer of the second amino group (from aspartate) to citrulline occurs by a condensation reaction between the amino group of aspartate and citrulline in the presence of ATP to form **arginosuccinate**.

- This reaction is catalyzed by ***arginosuccinate synthase*** of the liver cytosol, a Mg^{2+} dependent enzyme.

4 Formation of Arginine and Fumarate: Arginosuccinate is cleaved by arginosuccinate lyase (arginosuccinase) to form free arginine and fumarate.

- The fumarate so formed returns to the pool of citric acid cycle intermediates.
- Though fumarate urea cycle is linked with the citric acid cycle, the two Kreb's cycles together have been referred to as the ***Kreb's bi cycle***.

5 Formation of Urea and Ornithine: In the last reaction of urea cycle the liver hydrolytic enzyme ***arginase***, cleaves arginine to yield urea and ornithine.

- Ornithine is thus regenerated and can enter mitochondria again to initiate another round of the urea cycle.

The urea thus formed is excreted in the urine. Human beings excrete about 10 kg of urea per year.

The Energy Cost of Urea Cycle

Four ATPs are consumed in the synthesis of each molecule of urea as follows:

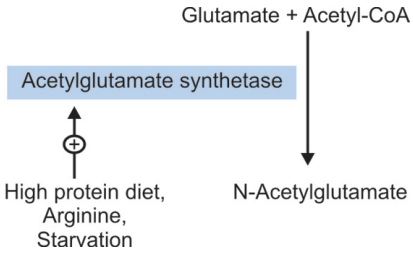
- Two ATP are needed to make carbamoyl phosphate.
  - One ATP serves as a source of phosphate
  - Second ATP is converted to AMP + PPi.
- One ATP is required to make arginosuccinate.
- One ATP is required to restore AMP to ATP.

Significance of Urea Cycle

- The toxic ammonia is converted into the harmless nontoxic urea.
- It disposes off two waste products, ammonia and CO_2.
- It forms **semiessential** amino acid, **arginine.**
- It participates in the regulation of blood pH, which depends upon the ratio of dissolved CO_2, i.e. H_2CO_3 to HCO_3^-.
- Ornithine which is formed in urea cycle can form nonessential amino acid proline **(see Figure 14.39)**.
- Ornithine is a precursor for the formation of polyamines like putrescine, spermidine and spermine **(See Figure 14.46)**.

Regulation of Urea Cycle

- ***Carbamoyl phosphate synthetase-I*** is an allosteric regulatory enzyme of urea cycle, which is allosterically activated by ***N-acetylglutamate (NAG).*** NAG is synthesized from acetyl-CoA and glutamate by NAG-synthase to activate CPS-I. It has no other function.
- The synthesis of NAG increases after intake of protein rich diet, by arginine and during starvation, which ultimately increases the urea formation.



Metabolic Inborn Errors of Urea Cycle

- Five disorders associated with each of the five enzymes of urea cycle have been reported **(Table 14.2)**.
- Since urea synthesis converts toxic ammonia to non-toxic urea, all defects in urea synthesis result in ***hyperammonemia*** and ***ammonia intoxication.***
- This intoxication is more severe when the metabolic block occurs at reaction I or II, since it accumulates ammonia itself.
- Deficiency of later enzymes result in the accumulation of other intermediates of the urea cycle, which are less toxic and therefore severity of symptoms is less.

Symptoms

Clinical symptoms, common to all urea cycle, disorders include:

- Ammonia intoxication
- Protein induced vomiting
- Intermittent ataxia
- Irritability
- Lethargy
- Mental retardation.

Treatment

- Low protein diet, food intake should be in frequent small meals to avoid sudden increase in blood ammonia levels.

Ammonia Intoxication

- Symptoms of ammonia intoxication includes:
  - An impaired function of the brain
  - Tremor
  - Ataxia
  - Convulsions
  - Lethargy
  - Nausea
  - Vomiting
  - Slurred speech
  - Blurred vision
  - In severe cases, coma and death.
- The toxic effect of ammonia may be due to:
  - A decrease in the formation of ATP by citric acid cycle because of diversion of excessive amounts of α-ketoglutarate from citric acid cycle intermediates to form glutamate and glutamine (to detoxify ammonia) in the brain and thus lowering the rate of oxidation of glucose, the major fuel of the brain.
  - By increased formation of GABA from glutamate leads to impaired neural transmission process.
  - One more possibility is that elevated levels of glutamine, formed from NH_4^+ and glutamate produce osmotic effect that leads directly to swelling of the brain.

Blood Urea

- Normal range of blood urea for a healthy adult is **20 to 40 mg/dL**. High protein diet shows increase in level of blood urea concentration.
- In clinical practice, blood urea level is taken as an indicator of **renal function**.
- The term **uremia** is used to indicate increased blood urea levels. For convenience, the causes of high blood urea are subdivided into three classes **(Table 14.3)**.

Table 14.2: Disorders caused by genetic defects of urea cycle enzymes

Disorders	*Defective enzyme*	*Products accumulated*
Hyperammonemia type-I	Carbamoyl phosphate synthase-I	Ammonia
Hyperammonemia type-II	Ornithine transcarbamoylase	Ammonia
Citrullinemia	Arginosuccinate synthase	Citrulline
Argininosuccinic aciduria	Argininosuccinate lyase	Argininosuccinate
Argininemia	Arginase	Arginine

Table 14.3: Causes of high blood urea (Uremia)

Types	*Causes*
Prerenal uremia	High protein diet Any cause of increased protein catabolism, e.g. trauma, surgery, starvation, diabetes mellitus. Any cause of impaired renal perfusion, e.g. cardiac failure
Renal uremia	Any cause that leads to reduced GFR and leads to urea retention
Postrenal uremia	Any cause of obstruction to urine outflow, e.g. benign prostatic hypertrophy, malignant stricture or obstruction, stone

CATABOLISM OF CARBON SKELETON OF AMINO ACIDS

The catabolism of 20 amino acids of proteins involves the removal of α-amino groups followed by the breakdown of the resulting carbon skeletons. The carbon skeletons of 20 amino acids are converged into seven products. These are:

- Pyruvate
- Acetyl-CoA
- Acetoacetyl-CoA
- α-Ketoglutarate
- Succinyl-CoA
- Fumarate
- Oxaloacetate.

All these enter the citric acid cycle and are used for either the synthesis of glucose or lipid or in the production of energy through their oxidation to CO_2 and H_2O.

- Amino acids that are degraded to acetyl-CoA or acetoacetyl-CoA are termed ***ketogenic,*** because they give rise to ketone bodies. Among 20 amino acids, only ***leucine*** and ***lysine*** are purely ketogenic.
- Amino acids, that are degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate or oxaloacetate, are termed ***glucogenic*** because synthesis of glucose from these amino acids is possible.
- ***Isoleucine, phenylalanine, tryptophan*** **and** ***tyrosine*** are both glucogenic and ketogenic. The other fourteen amino acids are classed as purely glucogenic.

Metabolic fate of carbon skeleton of 20 amino acids is described in **Figure 14.11.**

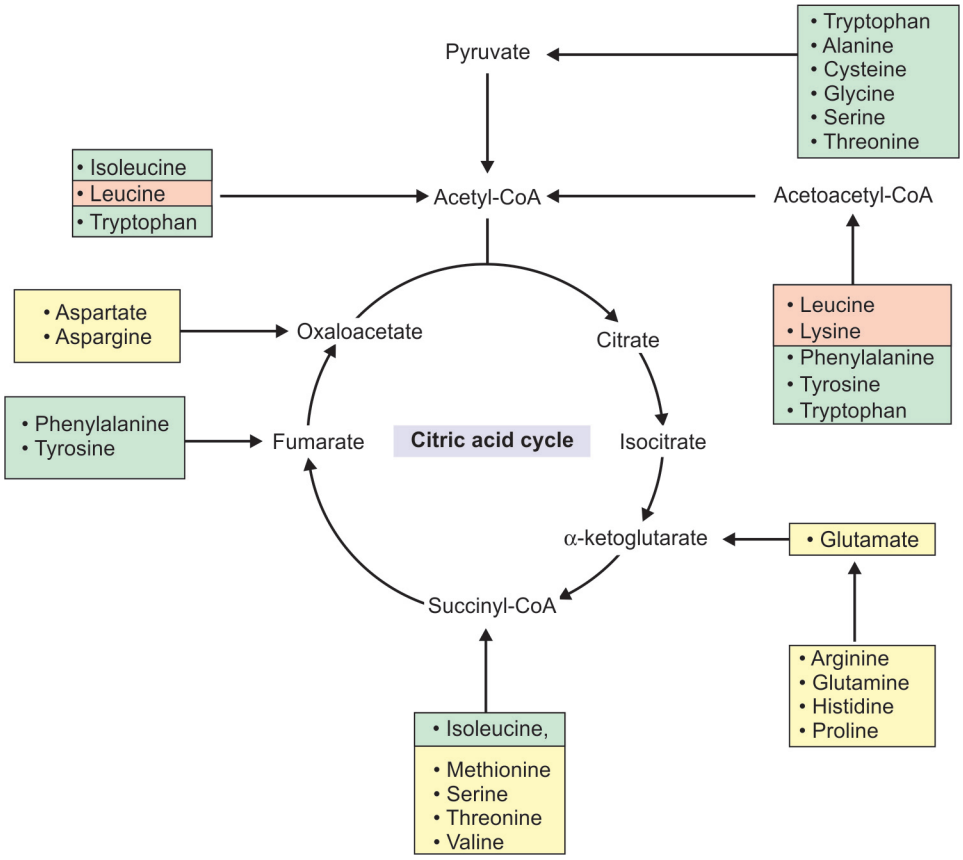


Figure 14.11: Metabolic fate of carbon skeleton of amino acids
Yellow shade: Glucogenic amino acids, Green shade: Glucogenic and ketogenic amino acids, Red shade: Ketogenic amino acids

METABOLISM OF GLYCINE

Glycine is a nonessential or dispensable amino acid.

Synthesis of Glycine

- It is synthesized from **serine** by removal of **hydroxymethyl** group from the side chain of serine.
- The methylene carbon group of hydroxymethyl is transferred to tetrahydrofolate and the hydroxy group is released as water **(Figure 14.12)**. This reaction is reversible, allowing glycine and serine to be interconverted.

Catabolism of Glycine

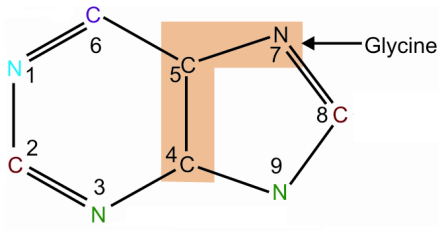
Glycine is catabolized by following three ways:

1. It can be converted into serine, a 3-carbon amino acid, by addition of hydroxy methyl group carried by the coenzyme tetrahydrofolate (THF) **(Figure 14.12)**.
2. The major pathway of glycine degradation is oxidative cleavage of glycine into CO_2 and NH_4^+ and a methylene group (CH_2) which is accepted by THF. This reversible reaction is catalyzed by glycine synthase **(Figure 14.13).** N^5,N^{10}-methylene THF is used in certain biosynthetic pathways.
3. Glycine may be oxidatively deaminated by ***glycine oxidase*** to glyoxylic acid **(Figure 14.13)**.
   - Glyoxylic acid formed:
     - May be decarboxylated to yield formaldehyde (formate) and CO_2
     - May also be converted to malate and then metabolized by the citric acid cycle
     - May be oxidized to oxalate and excreted.
   - Metabolic defect of glyoxylate metabolism, associated with failure to convert glyoxylate to formate, as a result excess of glyoxylate is oxidized to oxalate.

Metabolic Importance of Glycine (Figure 14.14)

Glycine is necessary for the formation of following products:

1. **Synthesis of heme:** Glycine along with succinyl CoA serves as a precursor for heme synthesis **(See Figure 18.2)**.
2. **Synthesis of glutathione:** Glycine is required for the formation of biologically important peptide, glutathione (γ-glutamyl-cysteinyl-glycine).
3. **Formation of purine ring:** Glycine is required for the formation of purine ring. It provides C_4, C_5 and N_7 of the purine ring.



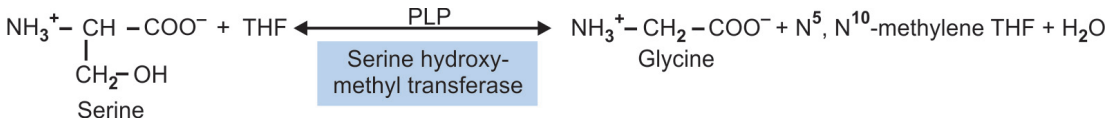


Figure 14.12: Synthesis of glycine from serine (THF: Tetrahydrofolate)

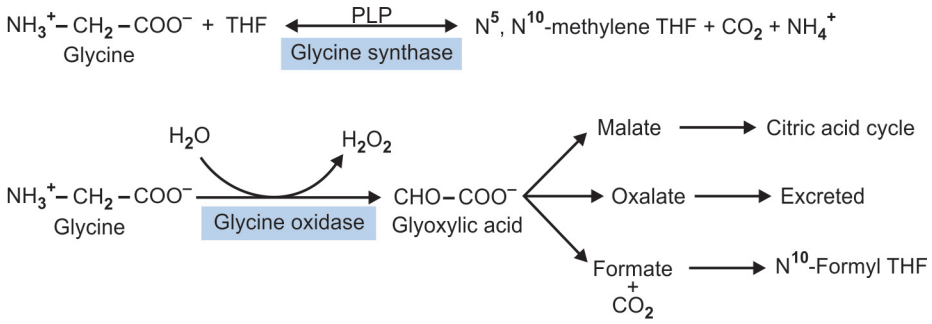


Figure 14.13: Catabolic pathways of glycine

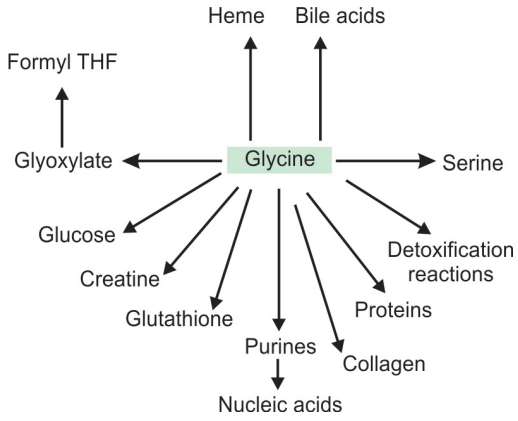


Figure 14.14: Metabolic importance of glycine

4. **Formation of bile acids:** The bile acids cholic acid and chenodeoxy cholic acid are conjugated with glycine to form glycocholic acid and glyco-chenodeoxy cholic acid respectively. Conjugation lowers the pK of the bile salts, making them better detergents.
5. Glycine is involved in **detoxification reactions,** e.g. benzoic acid is detoxicated by conjugating with glycine to hippuric acid.
6. **Synthesis of creatin:** Glycine is necessary for the formation of creatine. Creatin is present in muscle and brain tissue as the high energy compound **creatin phosphate**. Phosphocreatine functions as a store of high energy phosphate in muscle.
7. **Collegen formation:** In collagen glycine occurs at every third position of a chain of the triple helix.
8. Glysine is **glucogenic** amino acid, thus involved in the synthesis of glucose.
9. Glycine is constituent of various tissue proteins, hormones and enzymes.
10. By way of glyoxylate, it may form formate, which by THF may be incorporated into wide variety of biologically active compounds.

Metabolic Disorders of Glycine

Primary hyperoxaluria

- It is an inborn error, characterized by high urinary excretion of oxalate.
- The metabolic defect involves a failure to catabolize glyoxylate, which therefore gets oxidized to **oxalate.** Increased level of oxalate results in **urolithiasis** (stone in urinary tract), **nephrocalcinosis** (presence of Ca deposits in kidneys) and **recurrent infections** of urinary tract. Death occurs in childhood or early adult life from renal failure or hypertension.

Glycinuria

- It is an inborn error characterized by increased excretion of glycine through urine, despite plasma concentration of glycine is normal.
- Since plasma levels are normal, glycinuria occurs probably due to a ***defect in renal tubular reabsorption of glycine.***
- Glycinuria is characterized by increased tendency for the formation of oxalate renal stones.

METABOLISM OF AROMATIC AMINO ACIDS

- **Phenylalanine, tyrosine** and **tryptophan** are the aromatic amino acids.
- Phenylalanine and tryptophan are nutritionally essential amino acids but tyrosine is not as it can be synthesized from phenylalanine.

Metabolism of Phenylalanine and Tyrosine

Catabolism of Phenylalanine and Tyrosine

Phenylalanine metabolism is initiated by its oxidation to tyrosine which then undergoes oxidative degradation. Thus, catabolic pathway for phenylalanine and tyrosine is same as follows **(Figure 14.15)**:

1. The first step is the hydroxylation of phenylalanine to tyrosine, a reaction catalyzed by ***phenylalanine hydroxylase.*** This enzyme requires tetrahydro-biopterin (H_4-biopterin) as a cofactor. The cofactor is oxidized to dihydrobiopterin (H_2-biopterin) during this reaction. The dihydrobiopterin produced is reduced back to H_4-biopterin by ***dihydrobiopterin reductase***.
2. The next step is transamination of tyrosine with α-ketoglutarate to P-hydroxyphenyl pyruvate, catalyzed by ***tyrosine transaminase.***
3. P-Hydroxyphenyl pyruvate then reacts with O_2 to form **homogentisate.** This reaction is catalyzed by ***P-hydroxy-phenyl-pyruvate*** **hydroxylase,** is called a ***dioxygenase*** because both atoms of O_2 become incorporated into product.
4. Homogentisate is then cleaved by O_2 to yield 4-maleylacetoacetate. This reaction is catalyzed by ***homogentisate oxidase.***
5. 4-Maleylcetoacetate is then isomerized to 4-fumaryl-acetoacetate, by an enzyme maleylacetoacetate isomerase that uses ***glutathione*** as a cofactor.
6. Finally, 4-fumarylacetoacetate is hydrolyzed by ***fumaryl acetoacetate hydrolase*** to fumarate, a glucogenic intermediate and acetoacetate, a ketogenic intermediate. Pheyalanine and tyrosine are therefore, both glucogenic and ketogenic.

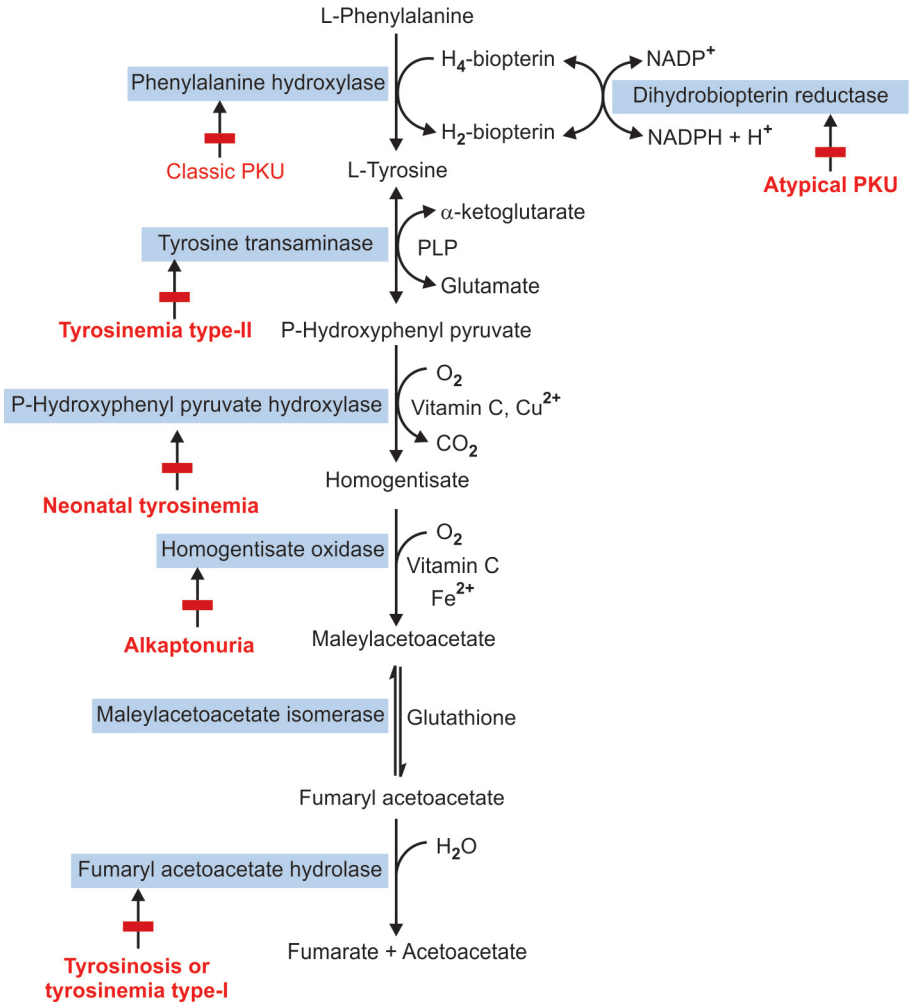


Figure 14.15: Catabolic pathway for phenylalanine and tyrosine

Metabolic Disorders of Phenylalanine and Tyrosine

Phenylketonuria (PKU)

- It is an inborn error of phenylalanine metabolism, associated with the inability to convert **phenylalanine to tyrosine**.
- The phenylketonuria is inherited in an autosomal recessive manner. The incidence of phenylketonuria is about 1 in 20,000 newborns.
- In phenylketonuria, there is an accumulation of phenylalanine in tissues and blood and results in its increased excretion in urine.
- Since phenylketonuric patients cannot convert phenylalanine to tyrosine, by normal pathway, some minor pathway of phenylalanine becomes prominent in phenylketonurics **(Figure 14.16)** and accumulation of toxic metabolites of phenylalanine such as, ***phenylpyruvate, phenylacetate, phenyllactate*** and ***phenylacetyl glutamine*** occurs.
- The disease acquired its name (PKU) from the high levels of the keto acid, phenylpyruvate in urine.
- Almost all untreated phenylketonurics are severely **mentally retarded.**
- Untreated phenylketonuria is life threatening, half are dead by age 20 and three quarters by age 30.

Classification of PKU

Phenylketonuria may be classified into three broad groups **(Table 14.4)**. PKU caused by deficiency of ***phenylalanine hydroxylase,*** is the most commonly encountered error.

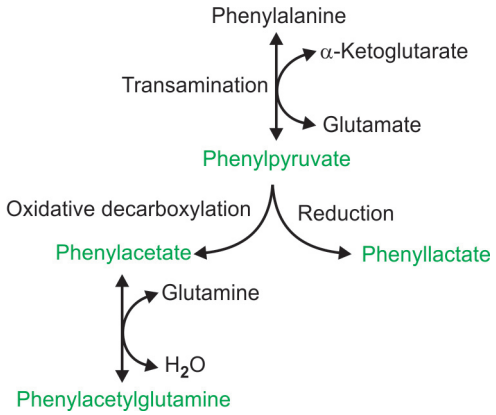


Figure 14.16: Alternative pathways of phenylalanine catabolism in phenylketonuria

Table 14.4: Types of phenylketonuria with their defects

Type of PKU	*Defect*
Classic phenylketonuria or Hyperphenylalaninemia type-I	Defect in phenylalanine hydroxylase
Atypical phenylketonuria or Hyperphenylalaninemia type-II and III	Defect in dihydrobiopterin reductase
Hyperphenylalaninemia type-IV and V	Defect in dihydrobiopterin synthesis

Characteristics of PKU

- **Increased level of:** Phenylalanine, phenylacetate, phenyllactate, phenylpyruvate and phenylacetylglutamine, in tissues, plasma and urine. Phenylacetate gives the urine a **mousy odor**.
- **Neurological symptoms:** Mental retardation, failure to walk, to talk, seizures, psychoses, tremor and failure to grow.
- **Hypopigmentation:** Phenylketonurics have a lighter skin color, fair hair and blue eyes due to deficiency of pigment melanin. The hydroxylation of tyrosine by ***tyrosinase*** is the first step in the formation of the pigment melanin is competitively inhibited by the high levels of phenylalanine in PKU **(Figure 14.20).**

Treatment of PKU

The therapy for PKU is a low phenylalanine diet. The aim is to provide just enough phenylalanine to meet the needs for growth and replacement.

- Proteins that have a low content of phenylalanine such as casein from milk, are hydrolyzed and phenylalanine is removed by adsorption.
- A low phenylalanine diet must be started very soon after birth to prevent irreversible brain damage.

Diagnostic tests for PKU

- In the past years, the urine of newborns was assayed by the addition of ***$FeCl_3$*** which gives an ***olive color*** in the presence of phenylpyruvate.
- The phenylalanine level in blood is detected by screening by using ***Guthrie test.***
- The gene for human ***phenylalanine hydroxylase*** has been ***cloned,*** so that prenatal diagnosis of PKU is now possible with DNA probes.

Tyrosinemia

There are three types of tyrosinemia:

1. Tyrosinemia type-I (Tyrosinosis/Hepatorenal tyrosinemia)
2. Tyrosinemia type-II (Richner-Hanhart syndrome)
3. Neonatal tyrosinemia.

Tyrosinemia type-I

- Tyrosinemia type-I, also called **tyrosinosis** or **hepatorenal tyrosinemia.** It is caused by a genetic deficiency of **fumarylacetoacetate hydroxylase.**
- This defect results in accumulation and excretion of tyrosine and its metabolites such as P-hydroxyphenylpyruvate, P-hydroxyphenyllactate, P-hydroxyphenylacetate, N-acetyltyrosin and tyramine.
- The deficiency of enzyme fumarylacetoacetate hydrolylase causes liver failure, kidney dysfunction, polyneuropathy and vitamin D ressistant rickets.

Classification of tyrosinemia type-I

1. Acute tyrosinosis
2. Chronic tyrosinosis.

Clinical features

- In acute tryosinosis, the infant exhibits dirrhea, vomiting and cabbage like odor. Death may occur in infancy due to acute liver failure (within first year of life).
- Whereas in chronic tyrosinosis in later life it develops liver cirrhosis and death occurs by age 10.

Treatment

The patient should be kept on diet low in phenylalanine and tyrosine.

Tyrosinemia Type-II (Richner-Hanhart Syndrome)

Cause

Tyrosinemia type-II is caused by genetic deficiency of hepatic enzyme **tyrosine aminotransferase** (tyrosine transaminase).

Clinical features

- Due to deficiency of hepatia enzyme **tyrosine aminotransferase,** the tyrosine cannot be metabolized by its routine pathway. As a result, the tyrosine and its toxic metabolites accumulates in blood and tissues and appears in urine.
- The accumulation of tyrosine produces lesions in eye and skin and causes mild to moderate mental retardation.

Treatment

Diet low in tyrosine and phenylalanine is recommended.

Neonatal tyrosinemia

It is caused by absence of the enzyme **P-hydroxyphenylpyruvate hydroxylase** (dioxygenase).

In neonatal tyrosinemia, serum tyrosine levels are high in premature infants resulting from an immature liver and its limited ability to synthesize the enzyme, **p-hydroxyphenylpyruvate hydroxylase.**

As the liver matures, the accumulated tyrosine is metabolized and serum levels decrease to adult levels within 4 to 8 weeks of age. It is benign condition and responds well to ascorbic acid.

Alkaptonuria

Cause

This inherited metabolic disorder is due to defect in the enzyme ***homogentisate*** **oxidase,** that catalyzes oxidation of homogentisate **(Figure 14.15).** As a result, the homogentisate accumulates in blood and body tissues and is excreted in large amounts in urine.

Clinical features

- The urine of alkaptonuric patients becomes dark after being exposed to air. In presence of oxygen, the colorless homogentisate present in urine undergoes spontaneous oxidation to yield **benzoquinone acetate,** which polymerize to form black brown pigment **alkapton (Figure 14.17)**. The alkapton imparts a characteristic black-brown color to urine.
- Alkaptonuria is a harmless condition. Later in life patients may suffer from deposition of dark colored alkapton pigments in connective tissues and bones. This results in black pigmentation of the sclera, ear, nose and cheeks and the clinical condition is known as **ochronosis** (because ochre color of the deposit). Ochronosis leads to tissue damage and may develop joint pain, arthritis and backache.

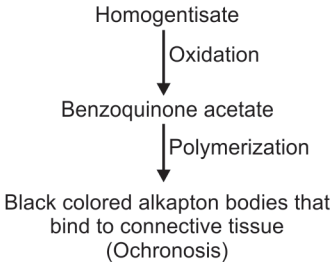


Figure 14.17: Formation of alkapton bodies

Treatment

Since alkaptonuria is not considered life threatening, this condition is not treated. Later in life, the symptoms of arthritis may be treated but the condition itself is not.

Diagnosis

The urine sample of patients of alkaptonuria turns dark on standing in air. The urine gives positive test with **ferric chloride** and **silver nitrate** due to reducing activity of homogentisate.

Albinism

Cause

It is an inborn error of tyrosine catabolism. It is due to inherited deficiency of enzyme **tyrosinase.** The inherited deficiency of tyrosinase impairs the synthesis of melanine from tyrosine **(Figure 14.20).** Melanins are dark pigment of skin, hair, iris, and retinal epithelial cells. Melanin protects the body from harmful radiation of sunlight.

Clinical features

- Impaired synthesis of melanin results in either hypopigmentation or no pigmentation of hair, skin and eyes and leads to white hair and skin.
- Albinos are highly sensitive to sunlight and can lead to skin cancer.
- The lack of melanin pigment in eyes is responsible for photophobia (intolerance to light) and nystagmus.

Biologically Important Compounds Derived from Tyrosine

Tyrosine serves as a precursor for following several biologically important compounds **(Figure 14.18).**

1. Catecholamines
   - Dopamine
   - Norepinephrine
   - Epinephrine

2. Melanin pigment
3. Thyroxine.

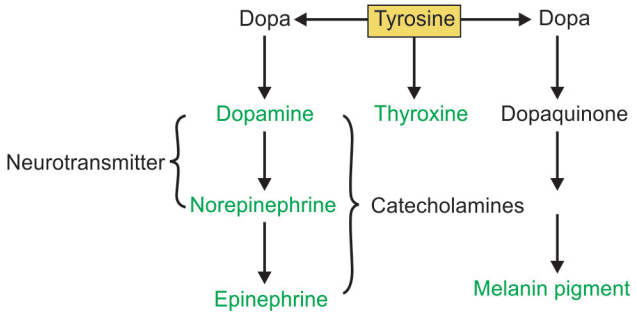


Figure 14.18: Biosynthesis of biologically important compounds from tyrosine

Biosynthesis of catecholamines (Figure 14.19)

- Epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine are collectively called **catecholamines.** They are synthesized from tyrosine.
- Epinephrine and norepinephrine are produced by adrenal medulla and serve as **hormones,** whereas dopamine and norepinephrine produced in the CNS and postganglionic sympathetic nerves act as **neurotransmitter.** In **Parkinson's disease,** dopamine levels in the CNS are decreased because of a deficiency of cells that produce dopamine.

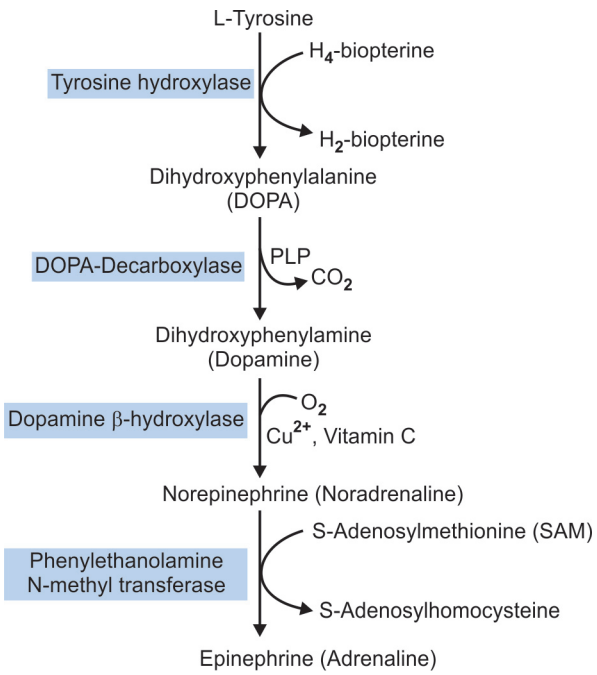


Figure 14.19: Synthesis of catecholamines

Biosynthesis of melanin pigment (Figure 14.20)

Melanin is a pigment. The synthesis of melanin occurs only in pigment producing cells called ***melanocytes.***

- The first step is the conversion of tyrosine to DOPA.
- In melanocyte, a different enzyme ***tyrosinase*** catalyzes this reaction.
- Tyrosinase also catalyzes the subsequent oxidation of dopa to dopaquinone.
- Dopaquinone is then converted to melanin. Tyrosinase does not require tetrahydrobiopterin but it utilizes copper as a cofactor.

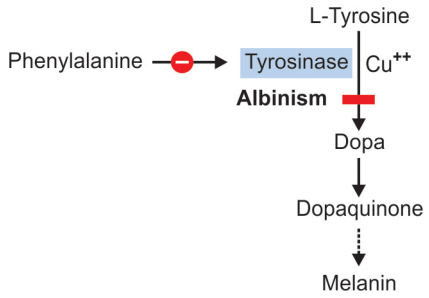


Figure 14.20: Biosynthesis of melanin

Biosynthesis of thyroxine and tri-iodothyroxine

The hormones ***thyroxine*** **(T_4)** and ***tri-iodothyroxine (T_3)*** are formed in the follicle cells of the thyroid gland by iodination of tyrosine residues of protein **thyroglobulin.** Monoiodo and diiodotyrosine residues are first formed and these then react to form T_3 and T_4 **(Figure 14.21)**.

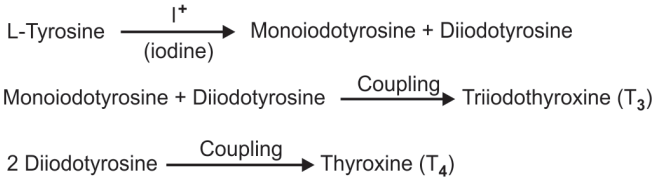


Figure 14.21: Biosynthesis of T_3 and T_4

METABOLISM OF TRYPTOPHAN

- Tryptophan is an essential amino acid, containing ***indol ring.*** Tryptophan is oxidized to produce alanine, which is glucogenic and acetyl-CoA, which is ketogenic. ***Thus, tryptophan is both glucogenic and ketogenic.***
- Tryptophan is a precursor for the synthesis of:
  - Vitamin niacin (vitamin B_3)
  - Neurotransmitter serotonin
  - Hormone melatonin.

- Tryptophan is metabolized by two pathways:
  1. Kynurenine pathway
  2. Serotonin pathway.

Kynurenine Pathway

In this pathway tryptophan is oxidized to kynurenine and alanine. Kynurenine is then converted to either **vitamin niacin** or **acetyl-CoA (Figure 14.22)**.

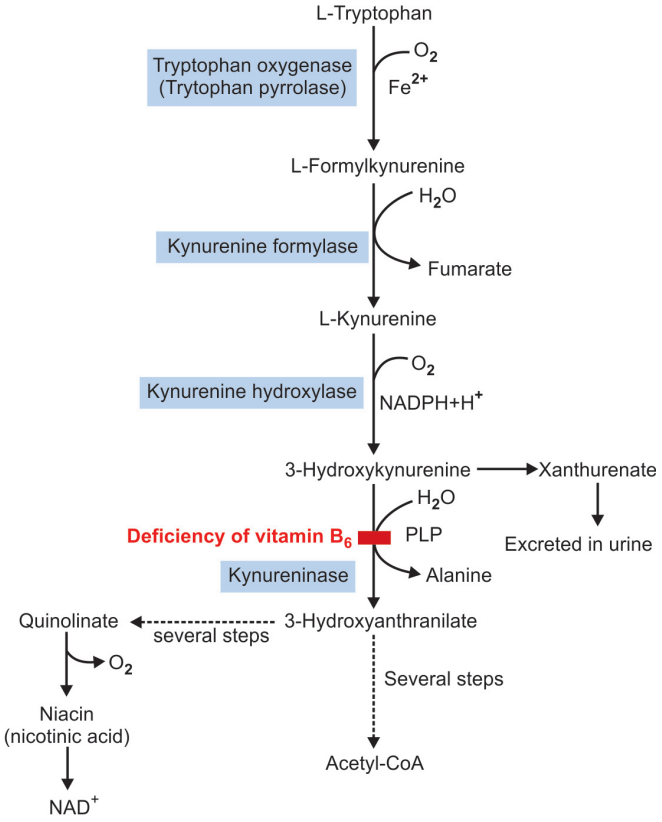


Figure 14.22: Kynurenine metabolic pathway of tryptophan and biosynthesis of vitamin niacin

- The initial reaction is an oxidation of tryptophan to formylkynurenine, catalyzed by the enzyme ***tryptophan oxygenase*** also called ***tryptophan pyrrolase***, which is feedback inhibited by nicotinic acid derivatives, e.g. NADH or NADPH.
- Formylkynurenine is converted to kynurenine by removal of formyl group with the enzyme ***kynurenine formylase***.
- Kynurenine is then metabolized to 3-hydroxy kynurenine by ***kynurenine hydroxylase***.
- Next 3-hydroxykynurenine is converted to 3-hydroxyanthranilate and alanine by ***kynureninase***, a PLP-dependent enzyme. A deficiency of ***vitamin B_6 (pyridoxin)*** results in failure to catabolize the hydroxykynurenine, forming ***xanthurenate*** **(Figure 14.22)** and excreted in urine in vitamin B_6 deficiency.
- Next 3-hydroxyanthranilate undergoes decarboxylation forming vitamin ***niacin*** which can be converted to ***NAD***$^+$ and **NADP**$^+$ or 3-hydroxyanthranilate can also be converted through a number of steps to acetyl-CoA.

For every 60 mg of tryptophan, 1 mg equivalent of niacin can be generated.

Serotonin Pathway (Figure 14.23)

- Tryptophan is first oxidized to 5-hydroxytryptophan by ***tryptophan hydroxylase***, which requires, tetrahydrobiopterin as a cofactor.
- 5-hydroxytryptophan undergoes decarboxylation to yield ***serotonin*** (5-hydroxytryptamine)

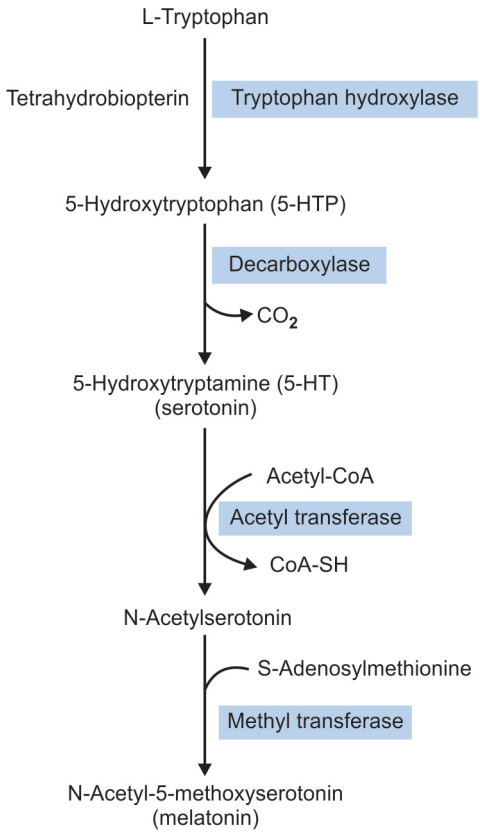


Figure 14.23: Serotonin metabolic pathway of tryptophan and formation of melatonin

- Acetylation of serotonin followed by methylation in the pineal gland forms a hormone ***melatonin.***

Serotonin

- Serotonin is synthesized from tryptophan by neurons, pineal glands and intestinal argentaffin cells. In normal adult, about 1% of tryptophan is converted to serotonin.

Functions of serotonin

- Serotonin is a **neurotransmitter** and stimulates cerebral activity. Therefore, serotonin deficiency causes a decrease in cerebral (brain) activity, which leads to depression.
- In humans, serotonin is involved in a variety of behavioral patterns, including sleep, body temperature and blood pressure.
- Serotonin produced in intestinal cells stimulates the release of gastrointestinal peptide hormones.
- Serotonin serves as precursor of **melatonin** in the pineal gland.
- Serotonin is also a powerful **vasoconstrictor** and stimulator of smooth muscle contraction.

Argentaffinomas or carcinoid syndrome

Agentaffinomas or carcinoid syndrome is the cancer of argentaffin cells of the gastrointestinal tract. Normally, about 1% of tryptophan is converted to **serotonin,** however, in carcinoid syndrome about 60% of tryptophan is diverted to serotonin formation resulting in decreased synthesis of **vitamin niacin.** Therefore, these patients develop the symptoms of **pellagra**.

Melatonin

- Melatonin is a hormone produced from serotonin by the pineal gland **(Figure 14.23)**.
- Synthesis of melatonin is regulated by **light dark cycle**. It is synthesized mostly at night.
- It is an inhibitor of **melanocyte stimulating hormone (MSH)** and **adrenocorticotropic hormone (ACTH)**.
- Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body. It may also be involved in regulating **reproductive functions**.

Metabolic Disorder of Tryptophan

Hartnup's disease

- Hartnup's disease is an inherited disorder of tryptophan metabolism. This disorder was first of all reported in the family of **Hartnup,** therefore, named Hartnup's disease.
- It is due to defect in the intestinal and renal **transport of tryptophan** and other neutral amino acids and leads to tryptophan deficiency. Tryptophan deficiency ultimately leads to decreased synthesis of **vitamin niacin** and **serotonin.**
- Decreased synthesis of niacin leads to **pellagra** like symptoms and decreased serotonin synthesis is responsible for **neurological symptoms** observed in Hartnup's disease.
- In addition to the neurological and pellagra like symptoms, there is **amino aciduria** due to failure of transport of amino acids from kidney.
- As well, any unabsorbed tryptophan remaining in the intestine is metabolized by intestinal bacteria to **indolacetic acid** and **indolpyruvic acid** which are subsequently excreted in urine.

Therapy

- Oral nicotinic acid supplementation will permit adequate synthesis of NAD^+ in patients with Hartnup disease. This corrects pellagra like symptoms of the disorder. The aminoaciduria remains unaltered.

METABOLISM OF SULFUR CONTAINING AMINO ACIDS

The three sulfur containing amino acids are ***cystine, cysteine,*** and ***methionine.*** Among these methionine is an essential amino acid whereas cysteine and cystine are non-essential amino acids. Cysteine and cystine are synthesized from two amino acids, **methionine,** which is nutritionally essential and **serine** is non-essential. Cystine and cysteine are readily interconvertible in the body.

METABOLISM OF METHIONINE

Methionine is metabolized by:

1. Transfer of methyl group of methionine by transmethylation reactions.
2. Conversion of demethylated portion of the methionine to cysteine and cystine.

Transfer of Methyl Group of Methionine (Transmethylation reactions)

Transfer of methyl group ($-CH_3$) from methionine to an acceptor molecule is termed as **transmethylation**. The methyl group of methionine becomes available for transmethylation only in an active form of methionine, **S-adenosylmethionine (SAM)**.

- **ATP** and an enzyme **methionine adenosyltransferase** are required for the activation of methionine to S-adenosylmethionine. **(Figure 14.24)**.
- The methyl group of active methionine (SAM) is very reactive and can be enzymatically transferred in the synthesis of many compounds. Some important transmethylation reactions are given below:
  - Norepinephrine to epinephrine
  - Phosphatidylethanolamine to phosphatidylcholine
  - Gunaidoacetoacetate to creatine
  - Ethanolamine to choline
  - Acetyl serotonin to melatonin
  - Nucleotides to methylated nucleotides

Conversion of Demethylated Portion of the Methionine to Cysteine and Cystine (Figure 14.25)

- Removal of the methyl group from SAM forms **S-adenosyl homocysteine**.
- Hydrolytic cleavage of S-adenosylhomocysteine occurs to **homocysteine** and **adenosine**.
- Homocysteine then condenses with serine forming **cystathionine** by the enzyme **cystathionine synthase**.
- Hydrolytic cleavage of cystathionine forms **homoserine** plus **cysteine** by the enzyme **cystathionine lyase,** which requires cofactor pyridoxal phosphate (PLP).
- The cysteine then may be oxidized to **cystine.**
- Next homoserine is converted to α-ketobutyrate by **homoserine deaminase**.
- Then α-ketobutyrate undergoes oxidative decarboxylation to form propionyl-CoA which is catabolized by way of methylmalonyl-CoA and succinyl CoA.

It should be noted that cysteine is synthesized from two amino acids, **methionine** and **serine.** Methionine frunishes the sulfur atom and serine furnishes the carbon skeleton.

Synthesis of methionine from homocysteine (Figure 14.25)

- Methionine can be regenerated by remethylation of homocysteine, which requires both **tetrahydrofolate (THF)** and **vitamin B_{12}**.
- In this reaction methyl group of **N^5-methyl THF** is transferred to vitamin B_{12} forming **methyl B_{12}.** Then methyl B_{12} transfers the methyl group to homocysteine which is converted to methionine. It should be noted that there is no net synthesis of methionine because homocysteine which serves as precursor for methionine has to be derived from methionine.

METABOLISM OF CYSTEINE AND CYSTINE

Synthesis of Cysteine and Cystine

- Cysteine is a nutritionally non-essential, glucogenic amino acid.
- As dissussed above, it is synthesized from two amino acids, ***methionine,*** which is nutritionally essential and ***serine,*** which is not. Methionine furnishes sulfur atom and serine furnishes the carbon skeleton **(Figure 14.25)**.
- Cystine is formed by oxidation of cysteine.

Catabolism of Cystine and Cysteine

- The major catabolic fate of cystine is conversion of cystine to cysteine **(Figure 14.26)** a reaction catalyzed by ***cystine reductase***.
- Catabolism of cystine then occurs simultaneously with that of cysteine.

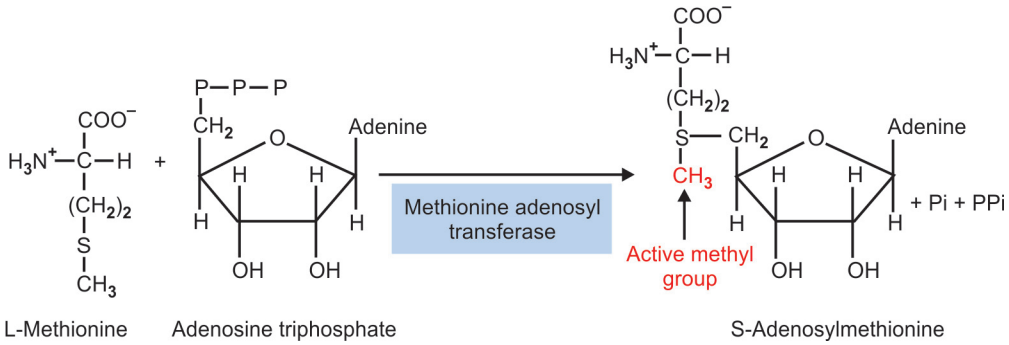


Figure 14.24: Formation of active methionine

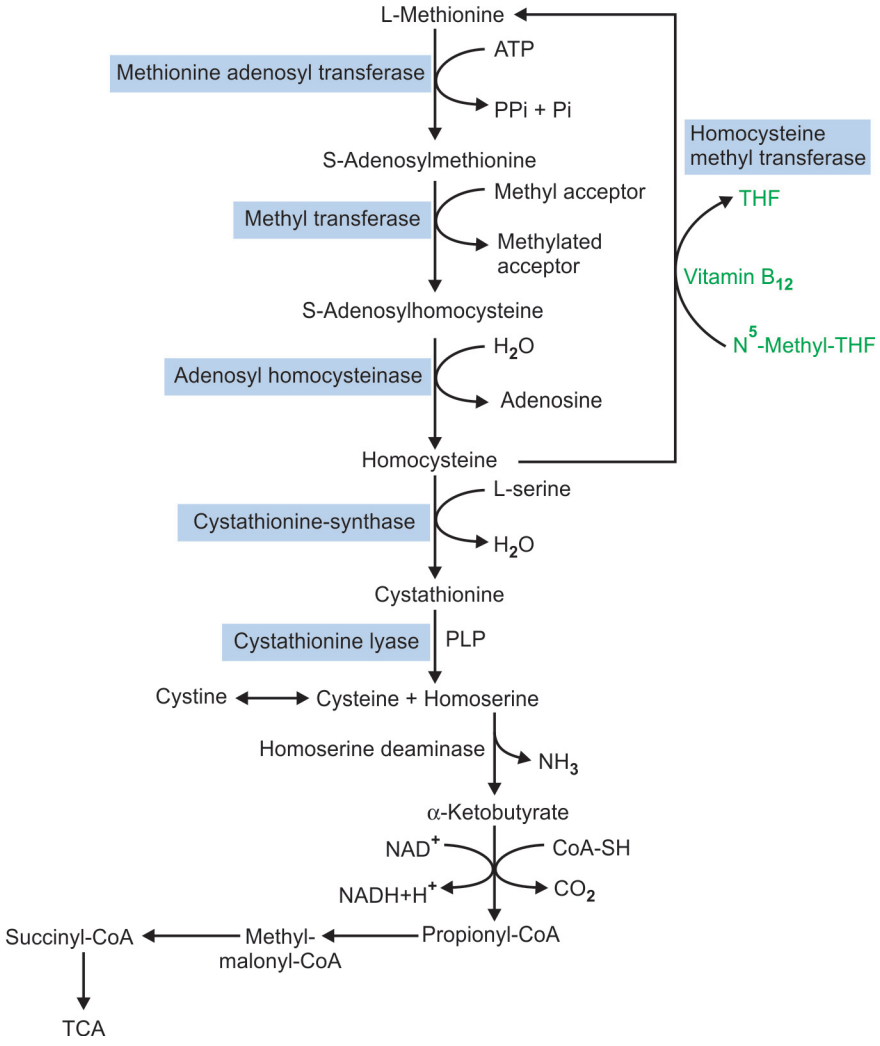


Figure 14.25: Catabolic pathway of methionine or biosynthetic pathway of cysteine where, THF: Tetrahydrofolate

- Cysteine can be catabolized by two routes as follows:
  1. The transamination pathway
  2. Direct oxidative pathway

The Transamination Pathway (Figure 14.27)

- One principal pathway is its transamination in the presence of α-ketoglutarate by the enzyme ***cysteine transaminase*** to form β-mercaptopyruvate and glutamate.
- β-Mercaptopyruvate then undergoes desulfuration with ***sulfur transferase*** to form pyruvate and H_2S, which is converted to sulfide by reduced glutathione.

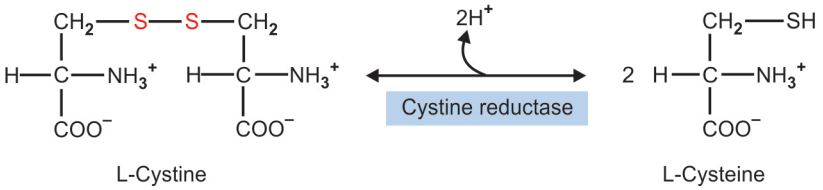


Figure 14.26: Conversion of cystine to cysteine

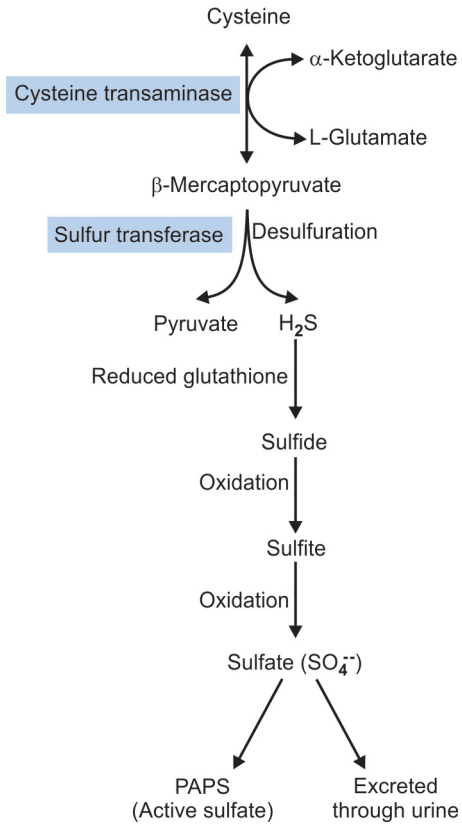


Figure 14.27: Transamination pathway for the catabolism of cysteine where, PAPS: Phosphoadenosyl phosphosulfate

- The sulfide formed in the reaction is converted to sulfite and then to sulfate (SO_4^{--}).
- The sulfate thus formed is either excreted in the urine or converted to active sulfate, ***phosphoadenosyl phosphosulfate (PAPS)*** by using ATP. PAPS is used as a source of sulfur in the synthesis of ***polysaccharides, glycolipids*** and ***sulpholipids*** or used for ***detoxification*** reactions.

Direct Oxidative Pathway (Figure 14.28)

- Cysteine is oxidized to cysteine sulfinate by ***cysteine dioxygenase*** which is then decarboxylated to **hypotaurine.** Hypotaurine may be oxidized to taurine (an inhibitory neurotransmitter), that conjugates with bile acid ***cholic acid*** to form taurocholic acid.
- Cysteine sulfinate may undergo transamination and give rise to sulfate.

Importance of cysteine

- The conversion of cysteine to pyruvate accounts for glucogenic nature and involved in formation of glucose.
- Cysteine is most important dietary source of sulfur.
- Besides taurine, other physiologically important sulfur containing compounds such as ***insulin, coenzyme-A, glutathione*** and ***vasopressin*** are derived from cysteine.
- It is also involved in detoxification mechanisms.

Metabolic Disorders of Sulfur Containing Amino Acids

Cystinuria (Cystin-lysinuria)

- Cystinuria is the most common inborn error of amino acid transport.
- Cystinuria is an inherited disorder in which kidney tubules fail to reabsorb the amino acids cystine, ornithine, arginine and lysine (the mnemonic is COAL).
- This is characterized by massive urinary excretion of cystine, ornithine, arginine and lysine.

Cause

- Normally, these amino acids are filtered by the glomerulus and reabsorbed in the proximal renal tubule by specific carrier proteins.
- In cystinuria defect in this carrier system leads to the excretion of all these four amino acids.

Clinical features

Since cystine is the least soluble its overexcretion often leads to precipitation and formation of **cystine calculi** (stones) in the renal tubules and leads to obstruction, infection and renal insufficiency in cystinuric patient.

Treatment

Treatment involves ingestion of large amounts of water, which increases cystine solubility through maintenance of alkaline urine.

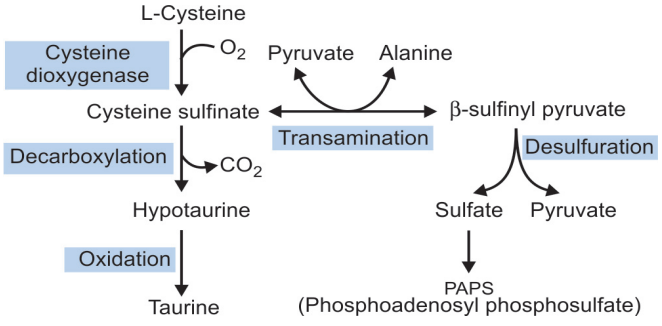


Figure 14.28: Direct oxidative pathway for catabolism of cysteine

Cystinosis (cystine storage disease)

Cystinosis is a rare but serious lysosomal disorder.

Cause

It is caused by a defective carrier that transports cystine across the lysosomal membrane from lysosomal vesicles to the cytosol.

Clinical features

- Cystine accumulates in the lysosomes in many tissues and forms crystals impairing their function.
- Cystinosis is usually accompanied by a generalized aminoaciduria.
- Patients usually die within 10 years due to acute renal failure.

Homocystinuria

Homocystinurias are a group of disorder of methionine metabolism. It is characterized by high blood and urinary levels of **homocysteine** and **methionine**. Four metabolic defects cause four types of homocystinuria. **(Table 14.5)**.

Homocystinuria type-I

- It is due to defect in the enzyme **cystathionine synthase,** which converts homocysteine to cysthathionine. **(Figure 14.25)**. As a result, homocysteine accumulates in blood and appears in urine.
- The accumulation of homocysteine causes skeletal abnormalities, ectopia lentis (dislocation of the lenses in the eyes), osteoporosis, mental retardation and thrombosis.
- Thrombosis may result in myocardial infarction, pulmonary embolism or stroke.
- A deficiency of cystathionine synthase is the most common cause of homocystinuria. Other types of homocystinuria are due to defects in the remethylation of homocysteine to form methionine.

Homocystinuria type-II and III

- In type-II and III, there is deficiency in synthesis of **N^5-methyltetrahydrofolate** and **methyl B_{12}** respectively. Both N^5-methyl THF and methyl B_{12} are required for the remethylation of homocysteine to form methionine.

Homocystinuria type-IV

It is due to defective intestinal absorption of vitamin B_{12}.

Treatment

- The biochemical defect in cystathionine synthase can be corrected in some cases by providing pyridoxine (vitamin B_6). Pyridoxine is needed to activate the enzyme cystathionine synthase.
- Those with complete enzyme deficiency should be treated with a diet low in methionine and supplemented with cysteine.
- Vitamin B_{12} may be given in instances of vitamin B_{12} deficiency.

Table 14.5: Different types of homocystinuria with their defects

Type	*Defect*
Homocystinuria-I	Cystathionine-β-synthase
Homocystinuria-II	Synthesis of N^5 methyl THF
Homocystinuria-III	Deficiency of methyl B_{12}
Homocystinuria-IV	Defective intestinal absorption of vitamin B_{12}

ONE CARBON METABOLISM

- Groups, containing a single carbon atoms are called one carbon groups. One carbon groups are formed from several amino acids during their metabolism. These include **serine, glycine, histidine** and **tryptophan.** One carbon groups formed during metabolism are:
  - Methyl (CH_3)
  - Methylene (CH_2)
  - Methenyl (CH)
  - Formyl (CHO)
  - Formimino (CH=NH)
- These one carbon groups are transferred by way of **tetrahydrofolate (THF)**. Tetrahydrafolate is formed from vitamin folic acid **(Figure 14.29)**. It should be noted that CO_2 is also a one carbon group, is carried by vitamin **biotin**. However, biotin is not considered as a member of one carbon pool.

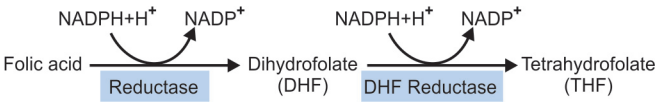


Figure 14.29: Formation of tetrahydrofolate from folic acid

- One carbon groups carried by THF are attached either to nitrogen N^5 or N^{10} or to both N^5 and N^{10}. **(Figure 14.30)**.
- The different one carbon derivatives of THF are as follows:
  - N^5-methyl THF
  - N^5,N^{10}-methylene THF
  - N^5,N^{10}-methenyl THF
  - N^5-formyl THF
  - N^5-formimino THF.

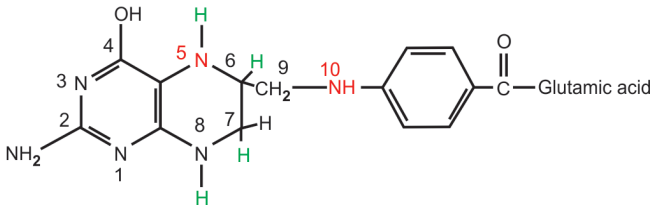


Figure 14.30: Structure of tetrahydrofolate

These different derivatives of THF are interconvertible. **Figure 14.31** shows sources of one carbon groups and their utilization.

Sources of one carbon groups (Figure 14.31)

1. Formate produced in the degradation of glycine and tryptophan reacts with THF to form **N^{10}-formyl THF**.
2. Histidine during its degradation produces **formimino glutamate (FIGLU),** which reacts with THF forming **N^5-formimino THF** which on releasing ammonia generates **N^5,N^{10}-methenyl THF**.
3. Serine is the major one carbon source. When serine is converted to glycine **N^5, N^{10}-methylene THF** is formed.
4. Methyl groups of choline and betaine reacts with THF to form **N^5-methyl THF**.
5. Active form of methionine, **S-adenosylmethionine (SAM)** carries methyl groups and acts as a methyl group donor.

Utilization of one carbon groups (Figure 14.31)

One carbon groups carried by THF are used for the synthesis of other biologically important compounds, e.g.

1. Synthesis of serine from glycine.
2. Synthesis of pyrimidine nucleotide thymidylate (TMP).
3. Synthesis of purine bases.
4. Synthesis of methionine from homocysteine.
5. Methyl group carried by SAM is used for the synthesis of choline, creatine, epinephrine, carnitine, t-RNA, DNA, etc.

> ***Importance of one carbon group metabolism***
> One carbon groups at different levels of oxidation are transferred and made available by way of the tetrahydrofolate and vitamine B_{12} coenzymes for use in a wide variety of vital anabolic processes.

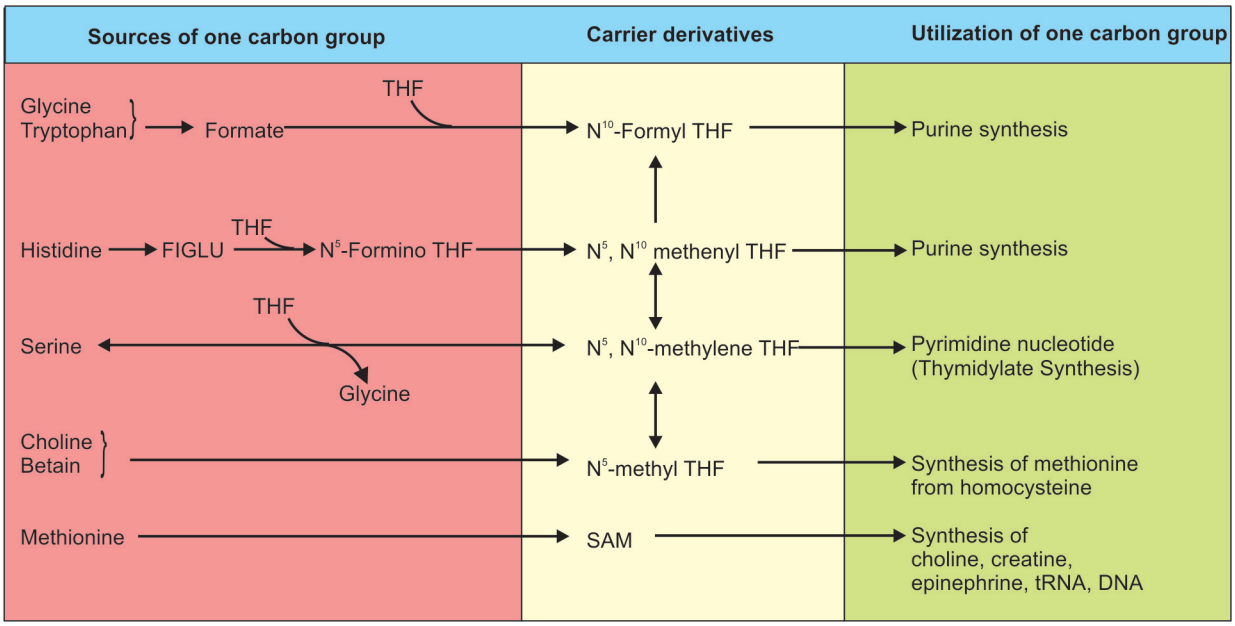


Figure 14.31: One carbon metabolism showing sources of one carbon groups and utilization of one carbon groups

METABOLISM OF BRANCHED CHAIN AMINO ACIDS (FIGURE 14.32)

Valine, isoleucine and leucine are branched essential amino acids.

Catabolism of Branched Chain Amino Acids

The first three metabolic reactions for these branched chain amino acids are similar and are catalyzed by common enzymes. Therefore, the metabolism of three branced chain amino acids are considered together.

1. **Transamination :** The initial step in the degradation of the branched chain amino acids is a transamination reaction to yield corresponding α-keto acids.
2. **Oxidative decarboxylation :** In the second step, the α-keto acids are oxidatively decarboxylated to the corresponding acyl-CoA thioesters by branched-chain α-keto acid dehydrogenase complex. TPP, FAD, Lipoic acid, NAD^+ and CoA are required as coenzymes in this reaction.
3. **Dehydrogenation :** The third reaction is a FAD dependent dehydrogenation, a reaction that resembles the first step of β-oxidation.
4. The subsequent reactions of all the three branched chain amino acids differs and is catabolized as follows:
   - Valine is converted to succinyl-CoA accounting for the glucogenic nature of the valine.
   - Isoleucine is converted to succinyl-CoA and acetyl-CoA, accounting for the ketogenic and glucogenic nature of the isoleucine.
   - Leucine forms acetoacetate and acetyl-CoA but does not produce succinyl-CoA, which accounts for exclusively the ***ketogenic nature of the leucine.*** Leucine produces hydroxymethyl glutaryl-CoA (HMG-CoA) intermediate product which is a precursor of cholesterol biosynthesis and ketone body formation.

Metabolic Disorder of Branched Chain Amino Acids

Maple syrup urine disease (MSUD) or branched chain keto aciduria

- It is an inborn error or branched chain amino acids namely leucine, isoleucine and valine catabolism **(Figure 14.32)**.

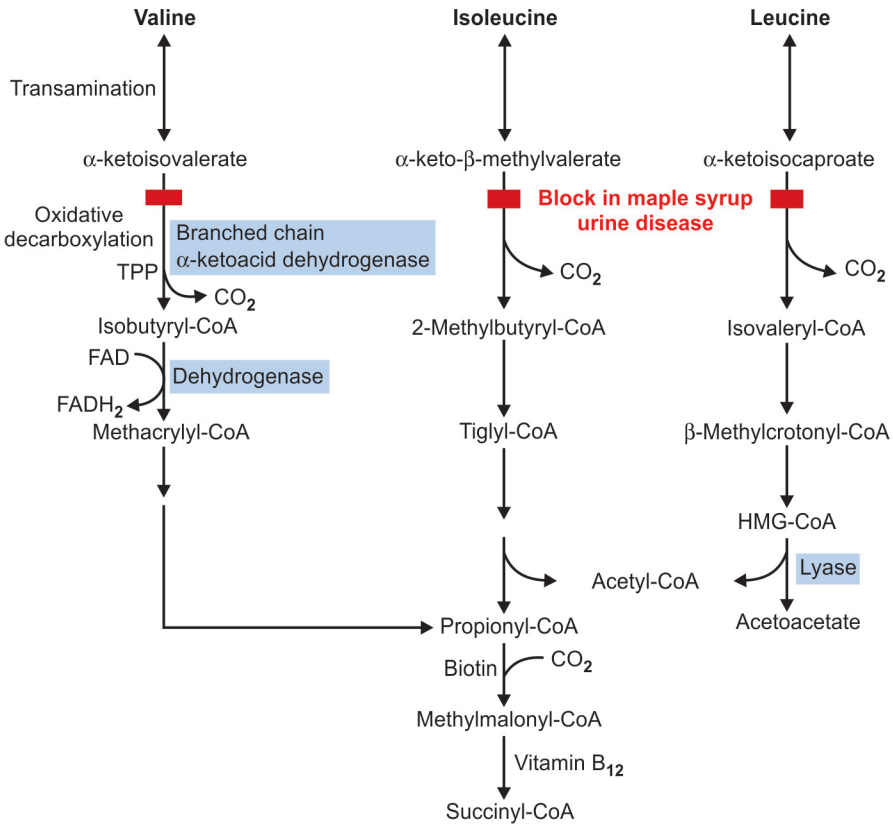


Figure 14.32: Degradation of the branched chain amino acids where, HMG-CoA: Hydroxymethylglutaryl-CoA

Biochemical cause

- Maple syrup urine disease is due to inherited defect in the **branched chain α-keto acid dehydrogenase.** Due to this defect α-keto acids of leucine, isoleucine and valine cannot be further metabolized. As a result, the branched chain amino acids, leucine, isoleucine and valine, and their α-keto acids accumulate in blood, urine and CSF.
- α-keto acids impart a characteristic sweet odor to the urine of the affected individuals which resembles with maple syrup or burnt sugar hence the name.

Symptoms

- Maple syrup urine disease is characterized by vomiting, dehydration severe metabolic acidosis and a characteristic **maple syrup** odor to the urine.
- If untreated, it leads to mental retardation, coma and even death within one year after birth.

Diagnosis

- Maple syrup disease is diagnosed by estimating increased levels of branched chain amino acids and their keto acids in plasma and urine.

Treatment

- Treatment involves replacing dietary protein by mixture of amino acids that contain low or no leucine, isoleucine and valine.
- To monitor the effectiveness of the dietary treatment, plasma and urinary levels of branched chain amino acids should be measured constantly.

METABOLISM OF HYDROXY GROUP CONTAINING AMINO ACIDS

Serine and threonine are the hydroxy group containing amono acids.

Metabolism of Serine

Serine is a non-essential amino acid.

Synthesis of Serine

- Serine is synthesized from glycine by transfer of hydroxymethyl group from N^5N^{10}-methylene THF **(Figure 14.12)**.

Catabolism of Serine

- In humans, serine is catabolized via glycine and N^5, N^{10}-methylene tetrahydrofolate. The reaction is catalyzed by ***serine hydroxy methyl transferase*** **(Figure 14.12)**. Further catabolism of serine merges with that of glycine.
- In many mammals, serine is degraded by ***serine dehydratase,*** an enzyme that requires pyridoxal phosphate (PLP) and produces NH_4^+ and pyruvate **(Figure 14.33)**. This pathway appears to be a minor one in humans.

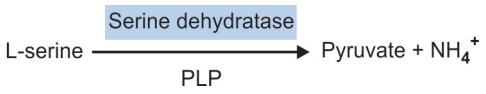


Figure 14.33: Degradation of serine

Importance of Serine

- Serine is a constituent of phospholipid, ***phosphstidylserine,*** found in brain.
- After decarboxylation serine give rise to **ethanolamine** which is the constituent of another phospholipid, ***phosphatidylethanolamine*** (cephalin).
- Serine also takes part in the synthesis of cysteine **(Figure 14.25)**.

Metabolism of Threonine

Threonine is an essential and glucogenic amino acid.

Catabolism of Threonine

Threonine is degraded by two pathways:

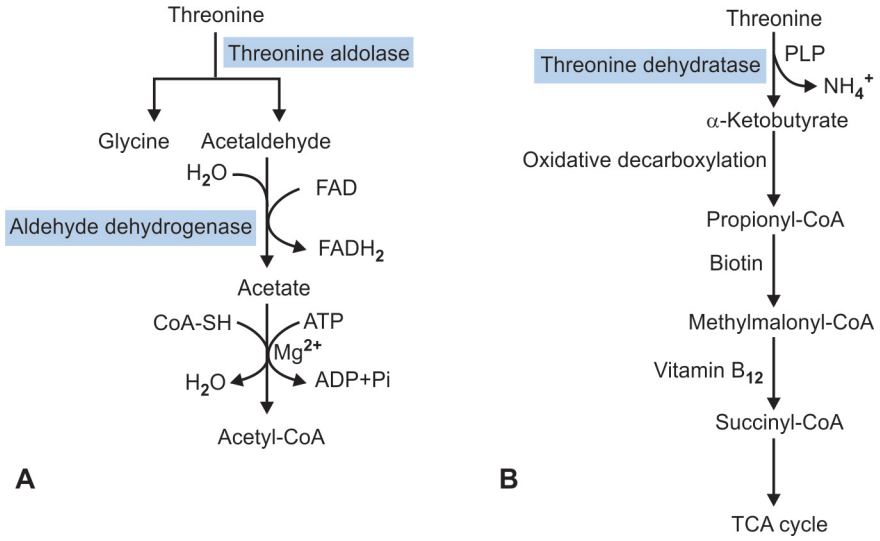
1. Threonine is cleaved to acetaldehyde and glycine by ***threonine aldolase.*** Acetaldehyde is then oxidized to acetate, which then is converted to acetyl-CoA **(Figures 14.34A and B)**.
2. ***Threonine dehydratase*** produces α-ketobutyrate. Subsequently, α-ketobutyrate is oxidatively decarboxylated to yield propionyl-CoA, which is then carboxylated to methylmalonyl-CoA, which, in turn, is isomerized to succinyl-CoA. Succinyl-CoA enters the Kreb's cycle, **(Figures 14.34A and B)** and gives rise to pyruvate. Threonine is thus glucogenic amino acid.

Metabolism of Alanine

Alanine is a nonessential amino acid.

Synthesis and Catabolism of Alanine

It is produced from pyruvate by a transamination reaction catalyzed by alanine transaminase (ALT) and



Figures 14.34 A and B: Catabolic pathways of threonine

may be metabolized back to pyruvate by a reversal of the same reaction **(Figure 14.35)**. Alanine is a major glucogenic amino acid.

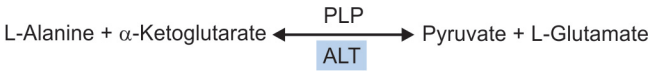


Figure 14.35: Synthesis and catabolism of alanine

Role of alanine in regulation of blood glucose

- In fasting, alanine plays a key role in hepatic gluconeogenesis.
- Alanine is released from muscle (for the transport of ammonia to the liver) during fasting and undergoes transamination to form pyruvate.
- The pyruvate is then converted to glucose by the gluconeogenesis.
- Thus, alanine has an important role in maintaining the fasting blood glucose level by way of gluconeogenesis.

METABOLISM OF ACIDIC AMINO ACIDS

Metabolism of Glutamic Acid

Glutamic acid is a nonessential, glucogenic amino acid.

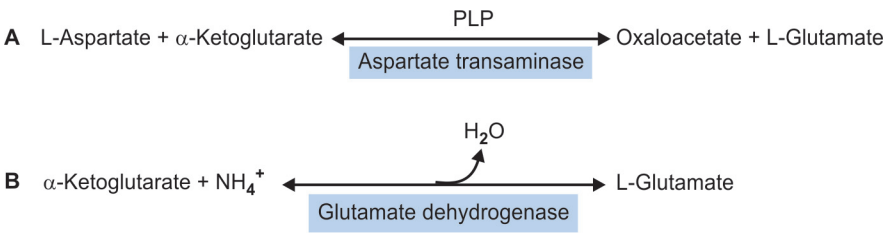
Synthesis of Glutamic Acid

It is synthesized from α-ketoglutarate an intermediate of citric acid cycle by:

- Transamination **(Figure 14.36A)**
- By reductive amination of a ketoglutarate by NH_4^+, catalyzed by ***glutamate dehydrogenase*** **(Figure 14.36B)**.

Catabolism of Glutamic Acid

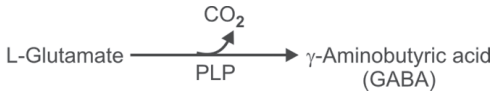
When glutamate is degraded, it is converted back to α-ketoglutarate either by transamination or by glutamate dehydrogenase reaction **(Figures 14.36A and B)**.



Figures 14.36 A and B: Synthesis and degradation of L-glutamate

Importance of Glutamic Acid

- A number of other amino acids like glutamine, proline and arginine are derived from glutamate.
- Glutamate involved in the synthesis of glutathione, (γ-glutamyl-cysteinyl glycine) which is involved in the reduction of H_2O_2 to H_2O and transport of amino acids into cells of kidney and intestine.
- Glutamate is decarboxylated at C-1 to form amine ***gamma aminobutyric acid (GABA),*** which serves as a neurotransmitter.



Metabolism of Glutamine

Glutamine is an amide of glutamate.

Synthesis of Glutamine

It is produced from glutamate by ***glutamine synthetase***, which adds NH_4^+ to the carboxyl group of the side chain, forming an amide **(Figure 14.37)**.

Degradation of Glutamine

Glutamine is reconverted to glutamate by a different enzyme, ***glutaminase***, which is particularly important in the kidney.

Importance of Glutamine

- **Glutamine is the major transport form of ammonia**.
- Glutamine is the principal source of ammonia in the kidney, the ammonia it produces enters the urine and decreases its acidity ($NH_3 + H^+ \rightarrow NH_4^+$) and in this way it plays an important role in the ***maintenance of acid-base balance.***

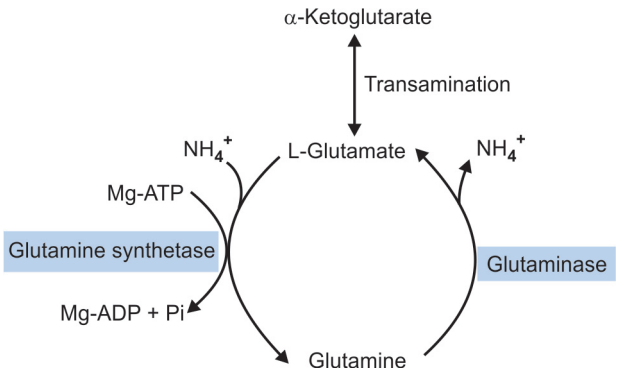


Figure 14.37: Synthesis and degradation of glutamine

- Glutamine participates in a number of biosynthetic reactions, usually by supplying amino or ammonia nitrogen, e.g. in the formation of arginine, carbamoyl phosphate, purines, etc.

Metabolism of Aspartic Acid

Aspartic acid is a nonessential, glucogenic amino acid.

Synthesis of Aspartic Acid

Aspartate is synthesized from Kreb's citric acid cycle intermediate, oxaloacetate by transamination reaction **(Figure 14.36A)**.

Degradation of Aspartic Acid

Because the transamination reaction is readily reversible, aspartate can be converted to oxaloacetate, an intermediate of citric acid cycle **(Figure 14.36A)**.

Functions of Aspartic Acid

- In the urea cycle, aspartate reacts with citrulline to form arginosuccinate, which is cleaved, forming an essential amino acid *arginine* and fumarate.
- Aspartate reacts with inosine monophosphate (IMP) to form ***AMP.***

Metabolism of Aspargine

Aspargine is an amide of aspartate.

Synthesis of Aspargine

- Aspargine is formed from aspartate by a reaction in which glutamine provides the nitrogen for formation of the amide group **(Figure 14.38).**

Degradation of Aspargine

- Hydrolytic release of the amide nitrogen of aspargine as ammonia and aspartate is catalyzed by **asparginase (Figure 14.38)**.

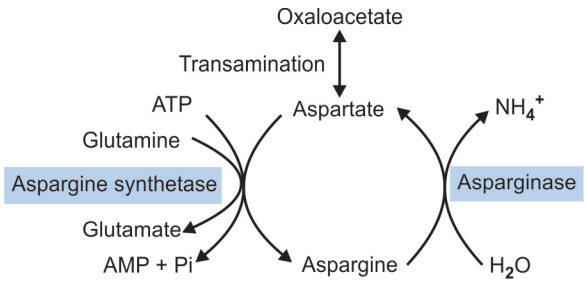


Figure 14.38: Synthesis and degradation of aspartate and aspargine

Clinical Importance of Asparginase

- Certain types of tumor cells, particularly leukemic cells, require aspargine. Therefore, asparginase has been used as an antitumor agent.
- It acts by converting aspargine to aspartate, decreasing the amount of aspargine available for tumor cell growth.

METABOLISM OF IMINO ACID

Metabolism of Proline

Proline is a non-essential, glucogenic amino acid.

Synthesis of Proline

- Proline is formed from glutamate by reversal of the reactions of proline catabolism **(Figure 14.39)**.
- Glutamate is first phosphorylated and then converted to glutamate γ-semialdehyde by reduction.
- This semialdehyde spontaneously cyclizes and reduction of this cyclic compound yields proline.

Degradation of Proline

- Proline rather than undergoing direct transamination is oxidized to dehydroproline, which adds water, forming glutamate γ-semialdehyde.

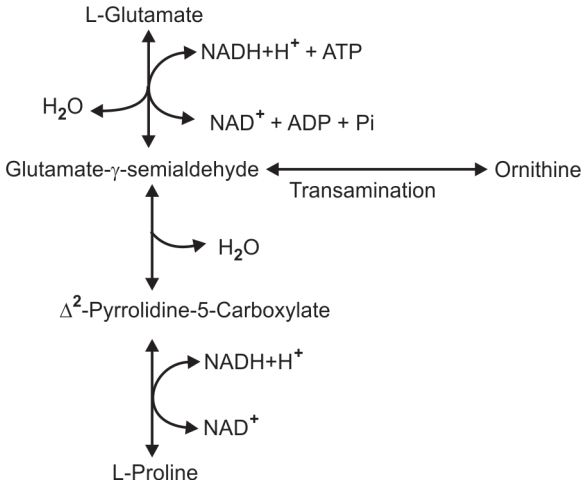


Figure 14.39: Synthesis of proline from glutamate

- This is further oxidized to glutamate and transaminated to α-ketoglutarate **(Figure 14.40)**.

Importance of Proline

- Proline serves as a precursor of hydroxyproline. Hydroxyproline is an important constituent of collagen which stabilizes the collagen triple helix.

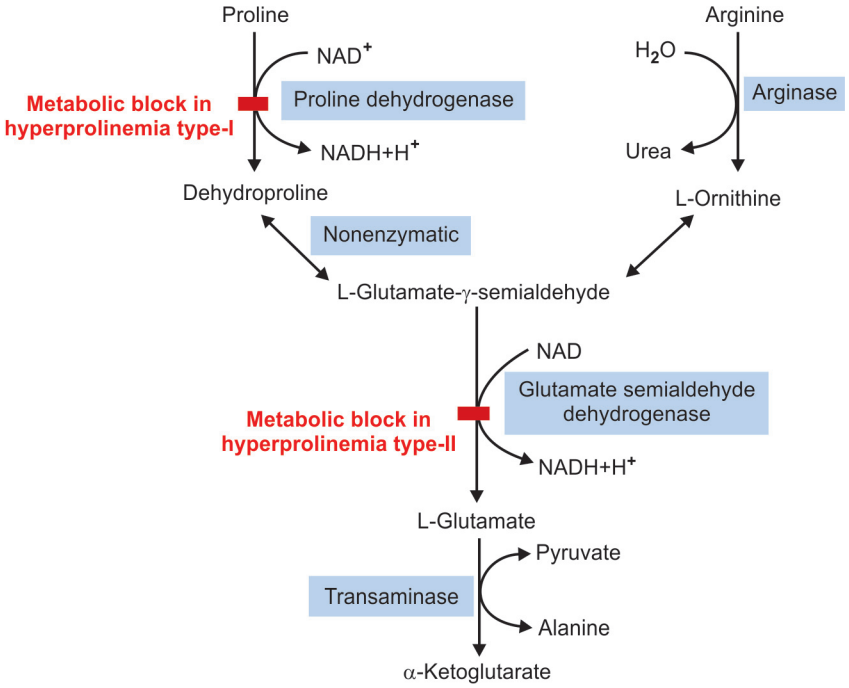


Figure 14.40: Degradation of L-proline and L-arginine to α-ketoglutarate

Collagen contains about one-third glycine and one-third proline plus hydroxyproline.

- Proline and ornithine are readily interconverted in the body. Thus, proline may yield ornithine for urea synthesis or ornithine may be broken down via proline **(Figure 14.39)**.

Metabolic Disorders of Proline

- Two autosomal recessive hyperprolinemias have been reported. Mental retardation occurs in half of the known cases but neither type is life-threatening. Two types of hyperprolinemia, called ***type-I*** and ***type-II,*** resulting in increase in blood and urine levels of proline are as follows:

Hyperprolinemia type-I

- The metabolic block in type-I is at ***proline dehydrogenase*** **(Figure 14.40)**.
- There is no associated impairment of hydroxyproline catabolism.

Hyperprolinemia type-II

- The metabolic block occurs at ***glutamate γ-semialdehyde dehydrogenase*** **(Figure 14.40)**.
- Since the same dehydrogenase functions in hydroxyproline catabolism, both proline and hydroxyproline catabolism are affected.
- The urine contains the hydroxyproline catabolites.

METABOLISM OF BASIC AMINO ACIDS

Metabolism of Arginine

- Arginine is considered to be a semi-essential amino acid. It can be synthesized in the body but not in quantities sufficient to permit normal growth. It is thus an amino acid, which is essential for growth but not for maintenance.

Synthesis of Arginine

- Arginine is synthesized from glutamate **(Figure 14.41)**.
- Glutamate is reduced to glutamate-γ-semialdehyde, which is then transaminated to yield ornithine, an intermediate of urea cycle.
- The reactions of the urea cycle convert ornithine to arginine.

Degradation of Arginine

- Arginine is cleaved by **arginase** to form urea and ornithine.

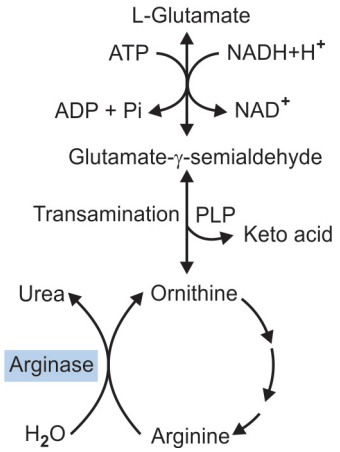


Figure 14.41: Synthesis and degradation of arginine

- If ornithine is present in amounts in excess of those required for the urea cycle, it is transaminated to glutamate semialdehyde which is reduced to glutamate **(Figure 14.41).**

Importance of Arginine

- Arginine takes part in the formation of urea.
- Arginine is involved in the synthesis of creatine, an important constituent of muscle. In the formation of creatine, the guanidinium group of arginine is transferred to glycine.
- Nitric oxide is synthesized from arginine **(Figure 14.42)**. Nitric oxide is a biological messenger in a variety of physiological responses including vasodilation, neurotransmission and the ability to kill tumor cells and parasites.

Metabolic Disorders of Arginine

The five hereditary defects in the biosynthesis of urea, mentioned earlier **(Table 14.2),** may be considered inherited disorders in the metabolism of arginine. Since they share common biosynthetic and cataboic pathways.

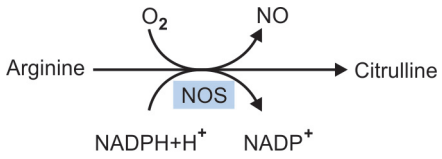


Figure 14.42: Synthesis of nitric oxide
where, NOS: Nitric oxide synthase, NO: Nitric Oxide

Metabolism of Histidine

Histidine like arginine is a nutritionally semi-essential amino acid and is glucogenic.

Degradation of Histidine

Degradation of histidine occurs mainly in the liver. The major pathway and principal intermediates are shown in **Figure 14.43**.

- In a series of steps, histidine is converted to formiminoglutamate (FIGLU).
- The subsequent reactions transfer one carbon group, i.e. formimino group of FIGLU to the tetrahydrofolate (THF) to form N^5-formimino THF leaving L-glutamate.
- N^5-formimino THF may be used in one carbon metabolism **(Figure 14.31)**.

Clinical Significance of FIGLU

- In folic acid deficiency transfer of formimino group of FIGLU to THF reaction is partially or totally blocked, and FIGLU is excreted in the urine.
- Excretion of FIGLU, therefore, provides a diagnostic test for folic acid deficiency.

Importance of Histidine

Histidine has several other important functions in addition to the general role of amino acids in tissue protein formation as follows:

- Upon decarboxylation, it forms ***histamine***, which reduces blood pressure, is a vasodilator and increases the secretion of gastric juice. Allergic reactions stimulate an excessive liberation of histamine.
- Histidine is involved in the formation of biologically important peptides like ***carnosine*** and ***anserine*** present in muscle, ***ergothioneine*** present in erythrocytes, liver and brain.

Metabolic Disorders of Histidine

Two benign hereditary disorders of histidine catabolism are:

- **Histidinemia:** Histidinemia is characterized by elevated blood and urine histidine. The defective enzyme is ***histidase***, resulting in impaired conversion of histidine to urocanate.
- **Urocanic aciduria:** It is characterized by elevated excretion of urocanate, it results from a defective ***urocanase*** enzyme.

Metabolism of Lysine

- Lysine is a nutritionally essential amino acid.
- Lysine is both glucogenic and ketogenic amino acid.
- It contains two amino groups, neither of which can undergo direct transamination.

Catabolism of Lysine

Lysine is degraded by a complex pathway in which ***saccharopine***, ***α-ketoadipate*** and ***crotonyl-CoA*** are

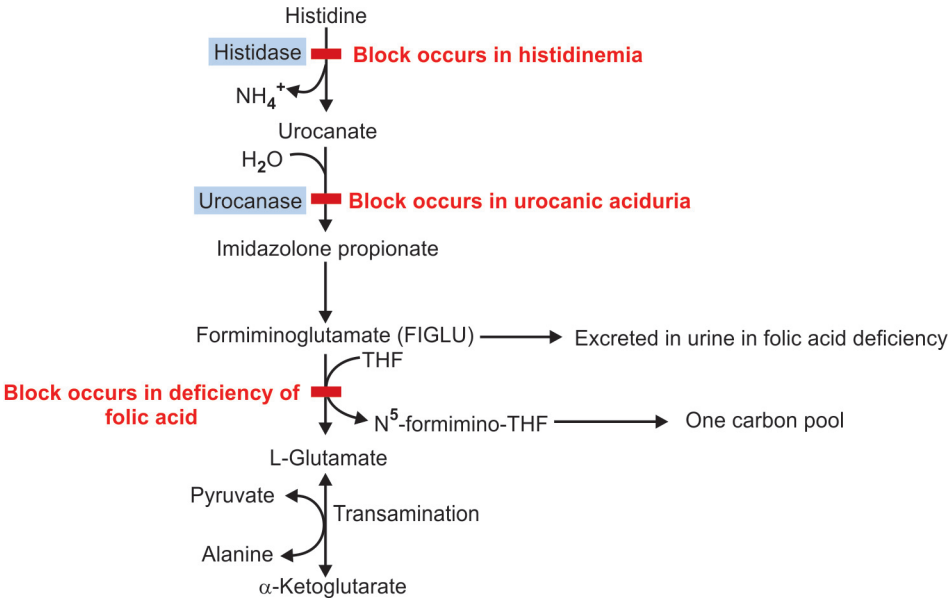


Figure 14.43: Degradation of histidine

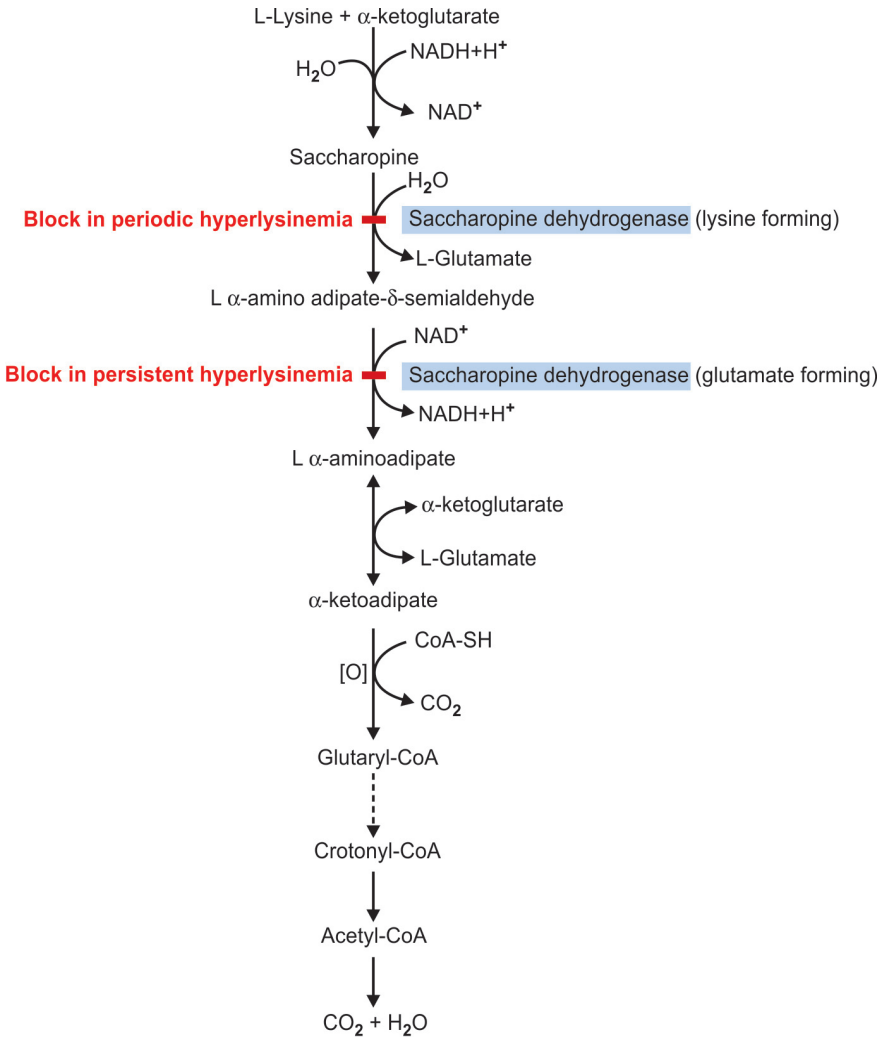


Figure 14.44: Degradation of L-lysine

intermediates **(Figure 14.44)**. Ultimately, lysine generates acetyl-CoA.

Importance of Lysine

Lysine is involved in the synthesis of ***carnitine,*** that serves to shuttle fatty acyl groups across mitochondrial membrane.

Metabolic Disorder of Lysine

Two inherited disorders of lysine metabolism have been reported:

- Periodic hyperlysinemia
- Persistent hyperlysinemia.

Periodic hyperlysinemia

- It is characterized by hyperammonemia and elevated plasma lysine.
- Elevated liver lysine levels competitively inhibit liver arginase, causing hyperammonemia and shows the clinical symptoms of ammonia intoxication.
- The biochemical cause of hyperlysinemia is uncertain.

Persistent hyperlysinemia

- Persistent hyperlysinemia is believed to be inherited as an autosomal recessive trait.

- In addition to impaired conversion of lysine and α-ketoglutarate to saccharopine, some patients cannot cleave saccharopine.
- Persistent hyperlysinemia is not associated with hyperammonemia.
- Some patients are mentally retarded.

BIOGENIC AMINES

Decarboxylation of amino acids results in the formation of amines. These amines are called **biogenic amines.** They have diverse biological function. Decarboxylation reactions are catalyzed by **PLP dependent decarboxylases.** The important biogenic amines formed by decarboxylation of amino acids are given below **(Figure 14.45)**.

- **γ-amino butyric acid (GABA) :** It is an inhibitory neurotransmitter derived from glutamate on decarboxylation. In vitamin B_6 deficiency, under-production of GABA leads to convulsions (epileptic seizures) in infants and children.
- **Serotonin and melatonin :** Serotonin and melatonin are produced from tryptophan. Serotonin is a neurotransmitter and stimulates the cerebral activity. Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body.
- **Histamine:** Histamine is produced by decarboxylation of histidine. It is a vasodilator and lowers blood pressure. It is involved in allergic reactions.
- **Catecholamines** (dopamine, norepinephrine and epinephrine). Synthesis of catecholamines from tyrosine requires PLP-dependent DOPA decarboxylase. Catecholamines are neurotransmitters and involved in metabolic and nervous regulation. Some of the most important biogenic amines and their functions are given in **Table 14.6**.

Polyamines

- Biological amines made up of multiple amino acids called polyamines, e.g.
  - Putrescine
  - Spermidine
  - Spermine.
- Polyamines are positively charged at physiological pH and associate with negatively charged nuclear

Table 14.6: Some important biogenic amines and their function

Amine	*Amino acid precursor*	*Function*
Dopamine	Tyrosine	Neurotransmitter
Norepinephrine	Tyrosine	Neurotransmitter
Epinephrine	Tyrosine	Hormone
Tyramine	Tyrosine	Vasoconstrictor
Serotonin	Tryptophan	Vasoconstrictor
Melatonin	Tryptophan	Vasoconstrictor
GABA	Glutamate	Neurotransmitter
Histamine	Histidine	Vasodilator
Taurine	Cysteine	Neurotransmitter
Spermine	Ornithine and methionine	Growth factor, Regulator of transcription and translation

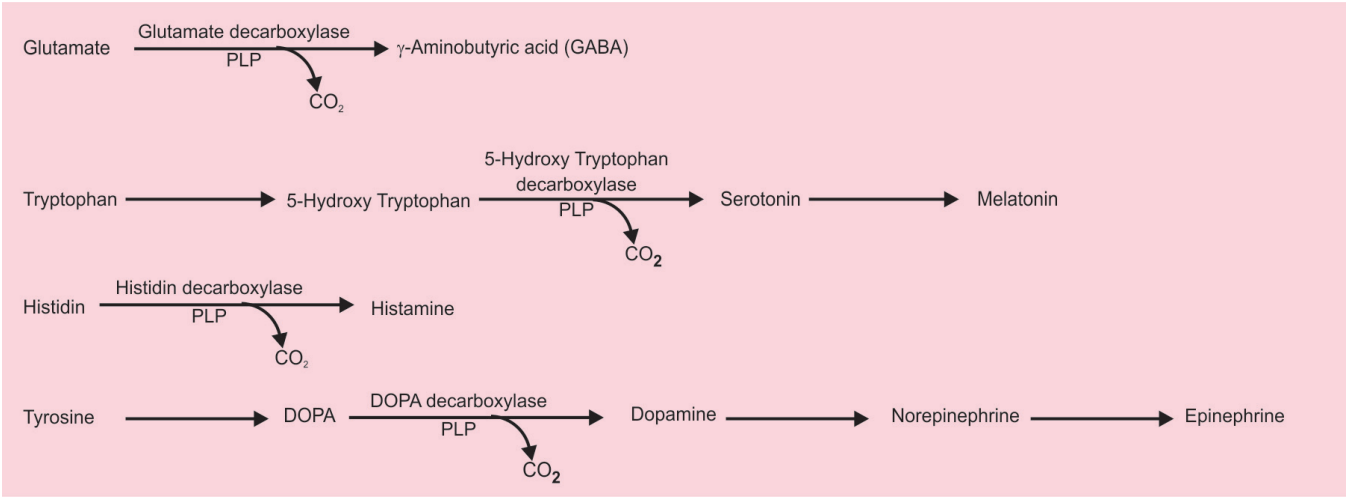


Figure 14.45: Synthesis of biogenic amines by PLP dependent decarboxylation of amino acids

DNA. These are present in high concentration in semen. The concentration of polyamines in brain is about 2 mM.

Functions of polyamines

- Polyamines are involved in regulation of transcription and translation.
- They act as a growth factor and function in cell proliferation and growth.
- Polyamines are involved in stabilization of intact cells, subcellular organelles and membranes.

Biosynthesis of polyamines

- **Putrescine, spermidine** and **spermine** are derived from ornithine and methionine.
- Ornithine is derived from arginine. Arginine undergoes a decarboxylation to form **putrescine** and carbon dioxide **(Figure 14.46)** by an enzyme **ornithine decarboxylase**.
- S-Adenosylmethionine undergoes a decarboxylation to form 5-adenosylmethiopropylamine, by an enzyme S-adenosylmethionine decarboxylase.
- S-Adenosylmethiopropylamine donates aminopropyl group to putrescine and then to spermidine to form spermine.
- It is presumed that the 15% of methionine which cannot be used for cysteine synthesis in minimal diets is used for polyamine synthesis.

Catabolism and excretion of polyamines

- The enzyme **polyamine oxidase** present in liver peroxisomes oxidizes spermine to spremidine and spermidine to putrescine.
- Putrescine is then oxidized by a copper containing diamine oxidase to CO_2 and NH_3.
- Major portions of putrescine and spermidine are excreted in urine after conjugation with acetyl-CoA as acetylated derivatives.

Clinical significance of polyamines

- Polyamines and their derivatives have application in diagnosis and treatment of cancer.
- Their levels have been shown to increase in response to cell growth and differentiation.
- Their concentration is elevated in body fluids of cancer patients.
- Assays of urinary and blood polyamines have been used to detect cancer and to determine the success of therapy (diagnostic indicator).

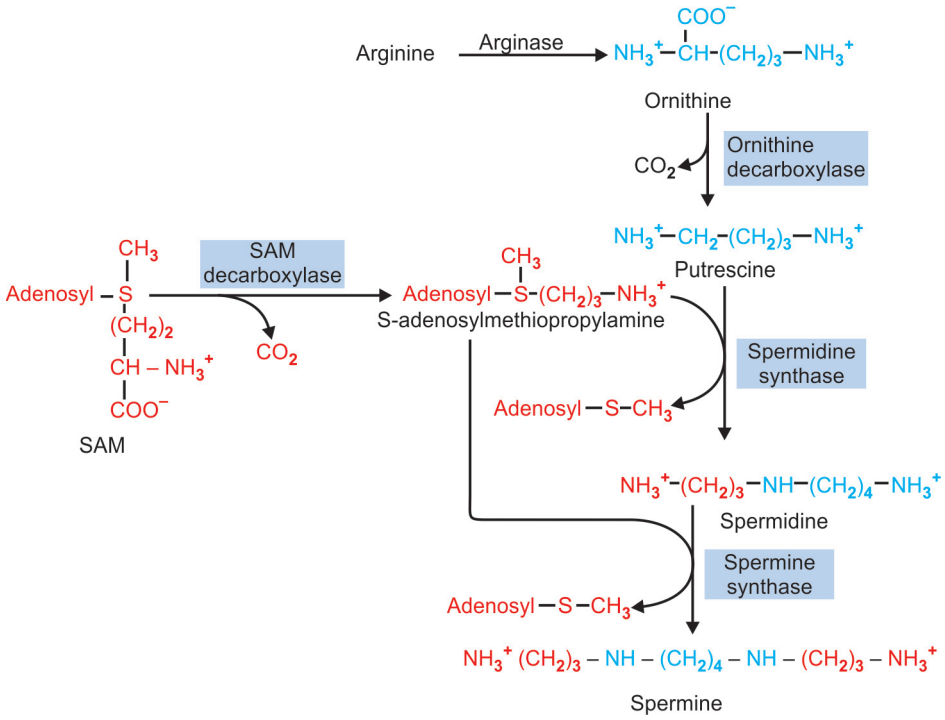


Figure 14.46: Biosynthesis of polyamines
where, SAM: S-Adenosylmethionine

SUMMARY

- Ingested proteins are degraded in the stomach and small intestine by proteases. Most proteases are initially synthesized as inactive zymogens; which are activated in the stomach or intestine.
- Absorption of most amino acid involves an active transport mechanism requiring ATP and specific transport proteins.
- Amino acids released by hydrolysis of dietary protein and tissue proteins together constitute an amino acid pool.
- Protein turnover is the continuous degradation and resynthesis of all cellular proteins.
- Nitrogen balance studies evaluate the relationship between the nitrogen intake and nitrogen excretion.
- First step in the catabolism of amino acids is removal of the amino group in the form of ammonia from the carbon skeleton.
- Ammonia, formed in other tissues, is transported to liver mitochondria in the form of glutamine or alanine.
- Glutamine is the major transport and temporary storage form of ammonia. Ammonia is highly toxic, it is excreted as urea formed in the liver by the urea cycle.
- Inborn errors of urea cycle are associated with each reaction of urea cycle. These include hyperammonemia-I, hyperammonemia-II, citrullinemia, argino-succinic aciduria, and hyperargininemia.
- After removal of amino groups, the carbon skeletons of amino acids undergo oxidation to compounds that can enter the citric acid cycle for further oxidation to CO_2 and H_2O.
- Glycine participates in the biosynthesis of heme, purines and creatine.
- Tyrosine forms epinephrine, norepinephrine, thyroid hormone and skin pigment melanin. Tryptophan metabolites include serotonin, melatonin and vitamin niacin.
- Active form of methionine (SAM) is the methyl group donor for the synthesis of creatine, carnitine, DNA, RNA, epinephrine, melatonin, etc.
- Cysteine is involved in the synthesis of coenzyme-A and taurine of taurocholic acid.
- Serine participates in the synthesis of phospholipid, sphingosine and provides carbon 2 and 8 of the purines and methyl group of thymine via tetrahydrofolate.
- Glutamate forms the neurotransmitter γ-amino butyric acid (GABA). Decarboxylation of histidine forms histamine.
- Arginine is involved in creatine synthesis. It also serves as the nitrogen source for synthesis of nitric oxide.

EXERCISE

Solve the following

Case History 1

A 3-year-old boy was admitted to hospital with the symptoms of pellagra, accompanied by mental retardation and excessive excretion of neutral amino acids. He was diagnosed as having Hartnup disease.

Questions

a. What is the cause of Hartnup disease?
b. Why does it show mental retardation and pellagra like symptoms?
c. How will you treat pellagra like symptoms?
d. Can aminoaciduria be treated?

Case History 2

A 5-month-old female infant was admitted to hospital with a complaint of vomiting and a failure to gain weight. The mother also reported that the child would oscillate between periods of irritability and lethargy, biochemical investigations of the patient indicated markedly increased concentration of plasma ammonia (550 μg/dL).

Questions

a. Name the probable disorder.
b. What is the cause of the disorder?
c. Name the transport form of ammonia.
d. What will be the nutritional therapy for the patient.

Case History 3

A full term infant was observed to have a lack of pigmentation, blue eyes, white hair and confirmed as a case of albinism.

Questions

a. Name the deficient pigment.
b. Name the enzyme responsible for the defect.
c. Write biochemical reaction catalyzed by the enzyme.
d. Name the amino acid, from which the pigment is synthesized.

Case History 4

A 20-year-old man came to the emergency room with severe pain in his right side and back. Subsequent examination and evaluation indicated a kidney stone

and increased excretion of cystine, arginine and lysine in the urine and diagnosed as a case of cystinuria.

Questions

a. What is the cause of cystinuria?
b. Why is there formation of kidney stone?
c. How is the condition to be treated?
d. Name the biosynthetic precursor of cysteine.

Case History 5

A 5-month-old female infant was hospitalized. A diagnosis of classic phenylketonuria (PKU) was made.

Questions

a. Name the defective enzyme of classic phenylketonuria.
b. Name the other types of PKU with their defective enzymes.
c. What are the characteristics of PKU?
d. Name diagnostic test for PKU.

Case History 6

A patient was diagnosed as having alkaptonuria.

Questions

a. Outline the biochemical pathway and point out metabolic defect which leads to this condition.
b. State the changes in urine, on standing, in such patients. Why?
c. What is ochronosis?
d. What is the treatment?

Multiple Choice Questions (MCQs)

1. Histidine is converted to histamine by:
a) Transamination
b) Decarboxylation
c) Hydroxylation
d) Reduction

2. In Maple-syrup urine disease, which of the following compounds is accumulated?
a) Homogentisate
b) Methylmalonyl-CoA
c) Branched chain α-keto acids
d) Homocysteine

3. The methylene group, transferred to glycine in converting it to serine, comes from:
a) S-Adenosylmethionine
b) Methylene-B_{12}
c) Carboxybiotin
d) N^5,N^{10}-methylene-THF

4. Which of the following does not take part in the human urea cycle?
a) Arginine b) Aspartate
c) Arginosuccinate d) Urease

5. Which type of reaction is conversion of norepinephrine to epinephrine?
a) Transamination
b) Decarboxylation
c) Transmethylation
d) Phosphorylation

6. Phenylketonuria results due to the absence of the enzyme:
a) Phenylalanine oxidase
b) Phenylalanine hydroxylase
c) Phenylalanine transaminase
d) Phenylalanine oxygenase

7. Which of the following amino acid cannot undergo transamination?
a) Lysine b) Alanine
c) Aspartic acid d) Glutamic acid

8. The amino acid required for synthesis of heme is:
a) Glutamine b) Glutamic acid
c) Glycine d) Lysine

9. All of the following are synthesized from tyrosine, *except*:
a) Melanin b) Serotonin
c) Dopamine d) Epinephrine

10. The fate of ammonia in brain is:
a) Conversion to urea
b) Conversion to glutamate
c) Conversion to aspartate
d) Remains as such

11. Which of the followng amino acids can undergo deamination by dehydration?
a) Threonine b) Alanine
c) Tryptophan d) Glycine

12. Transamination of oxaloacetate results in the formation of:
a) Aspartic acid b) Valine
c) Alanine d) Serine

13. All of the following are intermediates formed by amino acid degradation, *except*:
a) α-Ketoglutarate
b) Oxaloacetate
c) Fumarate
d) Citrate

14. In alkaptonuria, which of the following accumulates abnormally in the urine?
a) Phenylalanine b) Acetoacetate
c) Homogentisate d) Fumarate

15. The reactions of urea cycle occur in:
a) The cytosol
b) The mitochondria
c) The mitochondrial matrix and the cytosol
d) Lysosomes

16. All of the following statements are true in the transamination of amino acids, *except*:
a) Ammonia is neither consumed nor produced
b) Requires pyridoxal phosphate
c) The amino group acceptor is α-keto acid
d) All amino acids can undergo transamination

17. Tetrahydrobiopterine is used as a coenzyme in the metabolism of:
a) Folic acid b) Phenylalanine
c) Glycine d) Aspargine

18. Which of the following pathways occurs in part in the mitochondria and in part in the cytoplasm?
a) Urea cycle
b) TCA cycle
c) Glycolysis
d) Oxidative phosphorylation

19. Which of the following amino acid is involved in the synthesis of carnitine?
a) Lysine b) Phenylalanine
c) Tryptophan d) Threonine

20. The following enzymes are involved in digestion and absorption of protein, *except*:
a) Trypsinogen
b) Amylase
c) Pepsin
d) Chymotrypsin

21. Norepinephrine is converted to epinephrine by:
a) Transamination b) Transmethylation
c) Transcarboxylation d) Decarboxylation

22. Following enzymes are endopeptidase, *except:*
a) Pepsin b) Trypsin
c) Chymotrypsin d) Aminopeptidase

23. Which of the follwing enzyme is secreted by mucosal cell?
a) Trypsin b) Proelastase
c) Aminopeptidase d) Carboxypeptidase

24. Carboxypeptidase contains which of the following mineral?
a) Fe b) Cu
c) Ca d) Zn

Correct Answers for MCQs

1-b	2-c	3-d	4-d
5-c	6-b	7-a	8-c
9-b	10-b	11-a	12-a
13-d	14-c	15-c	16-d
17-b	18-a	19-a	20-b
21-b	22-d	23-c	24-d

CHAPTER

15

Integration of Metabolism and Metabolism in Starvation

INTRODUCTION

Carbohydrates, lipids and proteins are the principal foods providing necessary nutrients to the body. The metabolism of all of them are inter-related, and occur simultaneously. The deficiency of one is made up by another to some extent.

INTEGRATION OF METABOLISM

Definition

The various ***anabolic*** and ***catabolic*** pathways by which carbohydrates, lipids and proteins are processed for energy supply or as precursors for the biosynthesis of compounds required by the cell for maintenance or growth are closely co-ordinated. ***This coordination between three metabolites is called integration of metabolism.***

Integration of metabolism is considered at two levels:

1. Cellular level
2. Tissue or organ level.

Integration of Metabolism at Cellular Level (Figure 15.1)

Integration of metabolism at cellular level includes the different metabolic pathways of glucose, fatty acids, glycerol and amino acids, at the cellular level which results in:

1. Conversion of carbohydrates into fats and fats into carbohydrates
2. Conversion of carbohydrates into proteins and proteins into carbohydrates
3. Conversion of proteins into fats and fats into proteins.

Conversion of Carbohydrates into Fats

- Carbohydrates (glucose) are metabolized via glycolytic pathway to pyruvate and then pyruvate to acetyl-CoA.
- Acetyl-CoA is the starting material for synthesis of ***fatty acids.*** Fatty acids, that are produced, combine with glycerol to form ***triacylglycerol***.
- Glycerol may also be supplied from the glucose by glycolysis as glycerol-3-phosphate. Thus, carbohydrates can easily form fat.

Conversion of Fatty Acids to Carbohydrate

- There cannot be a net conversion of fatty acids having an **even number** of carbon atoms (which form acetyl-CoA) to glucose or glycogen.
- Only a fatty acid having an **odd number** of carbon atoms is glucogenic (i.e. can form glucose) as it forms a molecule of propionyl-CoA upon β-oxidation.
- **Propionyl-CoA** can be converted to succinyl-CoA, an intermediate of citric acid cycle, which can be converted to glucose by gluconeogenesis.

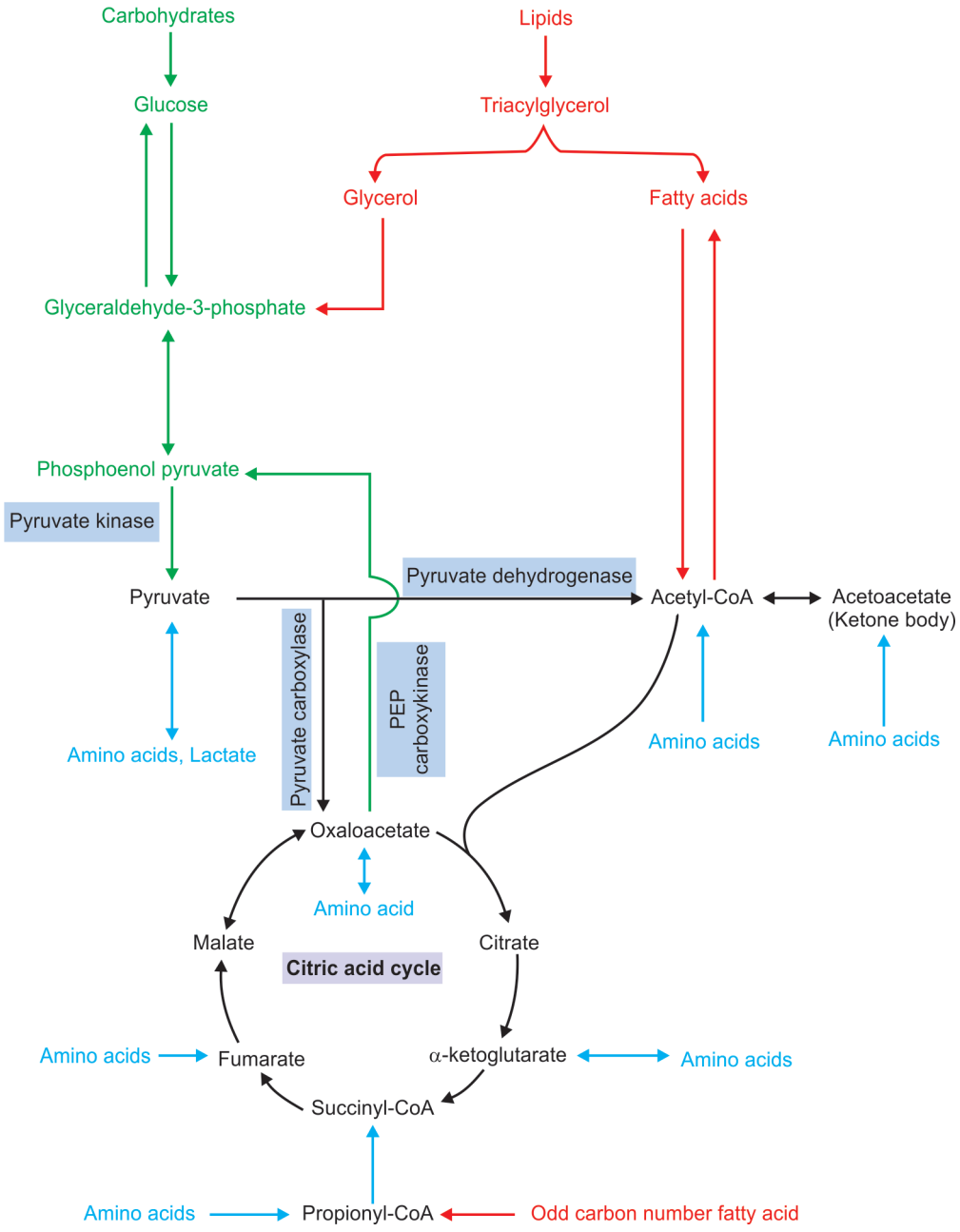


Figure 15.1: Integration of three metabolism at cellular level

- The glycerol moiety of triacylglycerol is converted to glucose by gluconeogenesis after activation to glycerol-3-phosphate and this is an important source of glucose in starvation.

Conversion of Carbohydrates into Proteins and Proteins into Carbohydrates

- Many carbon skeletons of the nonessential amino acids can be produced from carbohydrate via the intermediates of citric acid cycle and transamination.

- By reversal of these processes, glucogenic amino acids yield intermediates of the citric acid cycle. They are therefore readily converted by gluconeogenesis to glucose and glycogen.

Conversion of Proteins to Fats

- Conversion of carbon skeletons of glucogenic amino acids to fatty acids is possible either by formation of pyruvate and acetyl-CoA.
- Generally, however, the net conversion of amino acids to fat is not a significant process.

Conversion of Fats into Proteins

It is not possible for a net conversion of fatty acids to amino acids to take place.

Integration of Metabolism at Tissue or Organ Level

Integration of metabolism at tissue or organ level includes the inter-relationship of different tissues and organs to meet metabolic demands for the whole body. The major organs along with their most important metabolic functions are discussed here **(Figure 15.2)**.

Role of Liver

Major roles of the liver include the following:

- Maintenance of blood glucose levels.
- During the fed state, the liver takes up excess glucose and stores it as glycogen or converts it to fatty acids.
- During the fasting state, liver provides glucose for the body by the **glycogenolysis** and **gluconeogenesis**.
- The liver serves as the major site of fatty acid synthesis.
- The liver synthesizes ketone bodies during starvation and supplies to the peripheral tissues as a source of energy.

Role of Skeletal Muscle

- Skeletal muscle maintains large stores of glycogen, which provide energy during exertion.
- During starvation, free fatty acids and ketone bodies supplied by liver are oxidized in preference to glucose in muscle.
- The protein present in muscle may be used as a fuel source, if no other fuel is available.
- Pyruvate, the product of glycolysis in the skeletal muscle, may be converted to either lactate or alanine

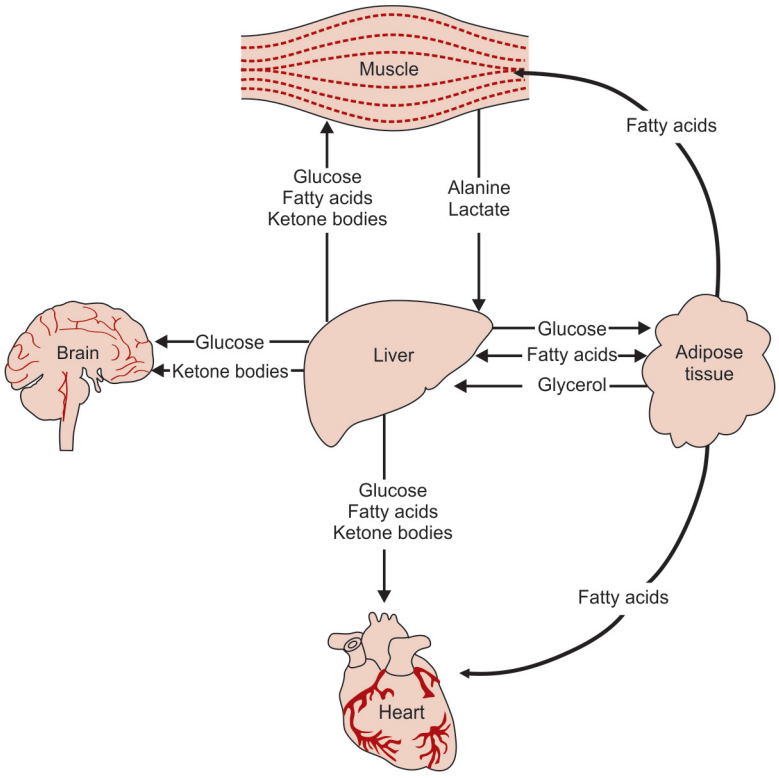


Figure 15.2: Integration of metabolism among major tissues of the body

and transported to the liver, where it is used to regenerate glucose via gluconeogenesis ***(See Figure 12.9)***.

Role of Adipose Tissue

- The primary function of the adipose tissue is the storage of metabolic fuel in the form of triacylglycerols.
- During the fed state, the adipose tissue synthesizes triacylglycerols from glucose and free fatty acids.
- During the fasting state, triacylglycerols are converted to glycerol and fatty acids, which are exported to the liver and other tissues.

Role of Heart Muscle

Heart muscle contains essentially no fuel reserves and must be continuously supplied with fuel from liver and adipose tissue.

Role of Brain

- Brain tissue normally uses glucose as an exclusive fuel, except during starvation, when it can adapt to use ketone bodies as an energy source.
- The brain contains essentially no fuel reserves and must be continuously supplied with fuel from the liver.

Significance of Integration of Metabolism

- Integration of metabolism ensures a supply of suitable fuel for all tissues, at all times from the fully fed state to the totally starved state.
- Under positive caloric balance, i.e. in well fed state, a significant proportion of the food energy intake is stored as either glycogen or fat.
- Under negative caloric balance, i.e. in starvation, fatty acids are oxidized in preference to glucose, to spare glucose for those tissues, (e.g. brain and erythrocytes) that require it under all conditions.

METABOLISM IN STARVATION

Definition

- Starvation is the deprivation of the food and thereby deprivation of exogenous supply of calories to meet the energy demands of the body for basal metabolism and other activities.
- Starvation is not always the result of unavailability or scarcity of food.
- Any medical condition, which prevents consumption or utilization of available food will lead to starvation, e.g. trauma, surgery, cancer cachexia, infections, malabsorption, etc.

Fuel Reserve of a Normal Healthy Person

- In starvation, energy has to be derived from the body's own stores. A typical well nourished 70 kg man has fuel reserve of:
  - 1600 kcal in glycogen
  - 2400 kcal in mobilizable protein and
  - 135000 kcal in triacylglycerols.
- The energy, needed for 24-hr period, ranges from about ***1600 kcal in the basal state to 6000 kcal*** depending on the extent of activity.
- Thus, stored fuels are enough to meet caloric needs in starvation for one to three months and in the case of some obese individuals, much longer.

Phases of Starvation

Starvation can be divided into two phases characterized by distinct metabolic changes. In starvation, the changes in metabolism are not abrupt but gradual **(Figure 15.3)**

1. ***Short-term starvation,*** which covers the 12 hr overnight fast and can extend to 24 hr.
2. ***Prolonged starvation,*** which lasts longer than 24 hr and can extend to several days or weeks.

Metabolic Changes Occur During Short-term Starvation (Figure 15.3)

Changes in carbohydrate metabolism and role of liver

- The first phase of starvation begins four to five hours after a meal.
- Within about 1 hr after a meal, blood glucose levels begin to fall.
- Consequently, **insulin levels decline** and **glucagon, epinephrine levels rise**.
- The brain, the erythrocytes, the bone marrow, the renal medulla and peripheral nerves have to be supplied with glucose, for their energy needs.
- However, the tissues such as muscle can readily use free fatty acids, released from adipose tissue.

The **first priority** of metabolism in starvation is to provide sufficient glucose to the brain and other tissues that are absolutely dependent on glucose.

- In this phase, the main source of blood glucose is ***liver glycogen***.
- The liver first uses glycogenolysis (glycogen degradation) then gluconeogenesis to maintain blood glucose levels and provide sufficient glucose to the brain and other glucose requiring tissues.

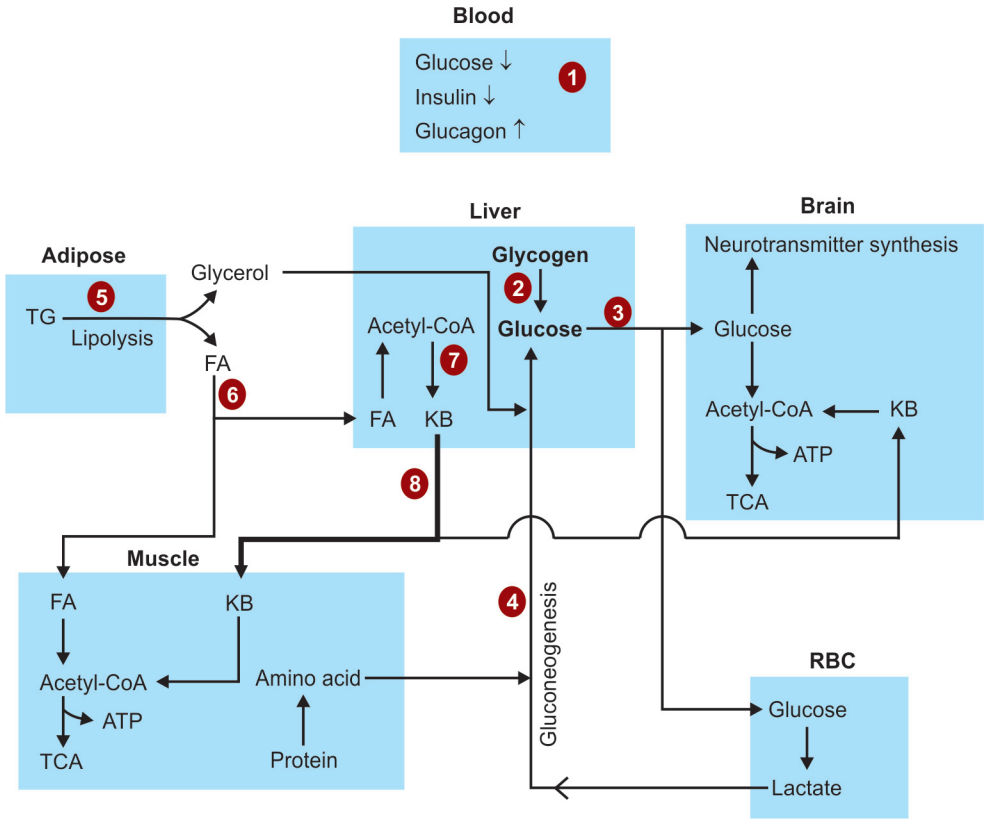


Figure 15.3: Metabolic changes occurring during starvation.
The circled number indicates the approximate order in which processes begin to occur
TG: Triacylglycerol; FA: Fatty acid; KB: Ketone bodies; AA: Amino acid

- The fall of **blood glucose** and **insulin concentration** and rise of **glucagon** stimulates glycogenolysis to maintain the blood glucose levels.
- Glucagon stimulates c-AMP formation, essential for degradation of glycogen

The liver glycogen is capable of maintaining the blood glucose concentration at normal values for 12 to 16 hours.

- As the liver glycogen store begins to be depleted, gluconeogenesis becomes active, which ensures a continuous supply of glucose to the brain and other tissues.
- The source of substrates for gluconeogenesis are:
  - Pyruvate
  - Lactate which is a product of glycolysis in red blood cell and exercising muscle
  - Glucogenic amino acids released from muscle
  - Glycerol released by degradation of triacylglycerols.

Gluconeogenesis plays an essential role in maintaining blood glucose during both short-term and prolonged starvation.

Changes in protein metabolism and role of muscles

- The decrease in plasma insulin and increase in epinephrine inhibits glycolysis and the uptake of glucose by muscle, whereas fatty acids enter freely in muscle.
- Increased proteolytic activity occurs due to decreased concentration of insulin.
- The main precursor for gluconeogenesis is, however, tissue protein, in the form of amino acids, released after proteolytic degradation.
- During short-term starvation there is a rapid breakdown of muscle protein, providing amino acids that are used by the liver for synthesis of glucose by gluconeogenesis.
- The major gluconeogenic amino acids, coming from muscle, are ***alanine*** and ***glutamine.***

Changes in fat metabolism and role of adipose tissue

- Increased levels of epinephrine stimulates breakdown of triacylglycerol of adipose tissue to free fatty acids and glycerol.
- Free fatty acids can be used by some tissues such as skeletal muscle, as an alternative source of fuel, and glucose is spared for the brain and other glucose requiring tissues.
- The glycerol derived from the cleavage of triacylglycerol is a source for the synthesis of glucose by gluconeogenesis by the liver.

Metabolic Changes Occur During Prolonged Starvation

- The processes which take place in short-term starvation cannot go on indefinitely, because, although gluconeogenesis provides glucose efficiently for the body's energy requirements, it will soon deplete the substantial proportion of body protein and it is known that death ensues when 30 to 50% of the body protein is lost.
- Adjustments to metabolism are made after 24 to 48 hr, which conserve body protein.

Thus, the second priority of metabolism in starvation is to preserve protein. This is accomplished by using fatty acids and ketone bodies in place of glucose as a fuel.

Changes in carbohydrate metabolism

- The blood glucose levels drop from about 100 mg/dl to about 70 mg/dl after 24 h but, are thereafter maintained **(Figure 15.4)**.
- Conservation of body proteins is accomplished by a reduction in glucose production by gluconeogenesis.

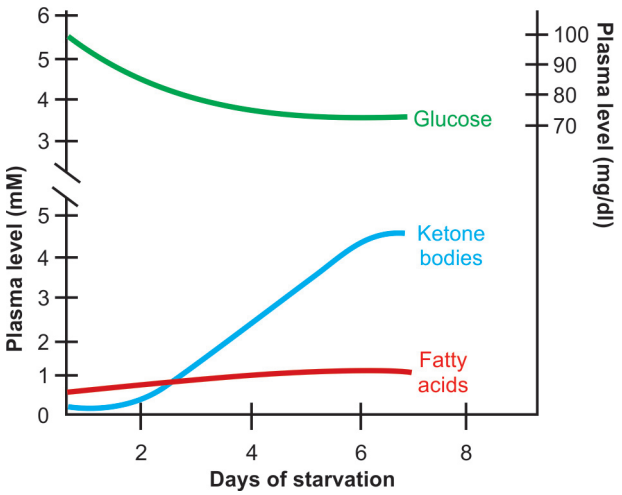


Figure 15.4: Changes in the concentration of fuels in the blood during prolonged fasting (1 mM glucose = 18 mg/dl glucose)

Changes in fat metabolism

- During prolonged fasting, adipose tissue continues to breakdown its triacylglycerol store to fatty acids and glycerol.
- These fatty acids serve as the major source of fuel for the body. The glycerol is converted to glucose while fatty acids are oxidized to CO_2 and H_2O by muscle and in the liver where they are converted into ketone bodies.
- The synthesis of ketone bodies from acetyl-CoA increases markedly producing the state of **ketosis (Figure 15.4)**. As the citric acid cycle is unable to oxidize all of the acetyl-CoA generated by the degradation of fatty acids, these are converted to kenone bodies. Gluconeogenesis depletes the supply of oxaloacetate, which is essential for the entry of acetyl-CoA into the citric acid cycle. Consequently, liver produces large quantities of ketone bodies.

Ketosis is a metabolic adaptation to starvation, arises as a result of deficiency in available carbohydrate.

- As the supply of ketone bodies increases and the supply of glucose diminishes, the brain reduces its utilization of glucose and begins to consume appreciable amounts of ketone bodies in place of glucose. Although the brain still has a residual glucose requirement, not only for provision of energy, but also for synthesis of neurotransmitters.
- After several weeks of starvation, ketone bodies become the major fuel of the brain which diminishes the need for glucose.
- Therefore, in prolonged starvation, less muscle is degraded than in the first days of starvation.
- This sequence of events leads to at least partial preservation of the protein stores of the body.

Changes in protein metabolism

- During the first few days of starvation, there is a rapid breakdown of muscle protein, providing amino acids, alanine and glutamine for gluconeogenesis.
- After several weeks of starvation, the rate of muscle breakdown decreases due to decreased need of glucose as a fuel for brain which has began using ketone bodies as a source of energy.
- Because of these adaptive mechanisms the duration of starvation of the adult human is determined by the size of the triacylglycerol depot. When the triacylglycerol stores are completely exhausted, muscle proteins, the largest single source of energy is heavily drained.
- At that time, the protein stores once again enter a stage of rapid depletion. Because proteins are also

essential for the maintenance of cellular function, death ordinarily ensues when the proteins of the body have been depleted to about half their normal level.

- During the conversion of amino acid to glucose by gluconeogenesis, the nitrogen of the amino acid is converted to urea and so production of urea decreases during prolonged starvation, as compared to its production in short-term starvation **(Figure 15.5)**.

Figure 15.5: Changes in urea excretion during starvation

Effect on BMR

- Apart from increase in the ratio of glucagon to insulin concentrations thyroxine (T_3), production is reduced and that leads to the decreased:
  - BMR
  - Body temperature
  - Pulse rate
  - BP.
- The body is more vulnerable to infections, all these lead death in prolonged starvation.

SUMMARY

- Nearly all products of digestion of carbohydrates, fats and proteins are metabolized to a common metabolite, acetyl-CoA, which, in turn, is degraded to CO_2 and H_2O with formation of ATP.
- Many of the major foodstuffs are interconvertible.
- Carbohydrate is converted to fatty acids. Glucose provides glycerol moiety of fat.
- Conversion of fatty acids to carbohydrate cannot take place. Nor can there be any net conversion of acetyl-CoA to glucose via citric acid cycle. Only a fatty acid, having an odd number of carbon atoms, is glucogenic, as it forms a molecule of propionyl-CoA, upon β-oxidation.
- Many nonessential amino acids can be produced from carbohydrate via the citric acid cycle and transamination. By reversal of the processes, glucogenic amino acids can be converted to glucose and glycogen.
- Conversion of glucogenic amino acids to fatty acids is possible, but conversion of fatty acid to amino acid is not possible.
- Starvation describes the situation of total food deprivation, and it can be divided into two phases: short-term and prolonged starvation which are characterized by distinct metabolic patterns.
- The first priority of metabolism in starvation is to provide sufficient glucose to the brain and other tissues, such as red blood cell that are absolutely dependent on this fuel.
- The second priority of metabolism in starvation is to preserve protein. This is accomplished by using the fatty acids and ketone bodies as a fuel instead of glucose.
- Ketosis is metabolic adaptation to starvation.
- During starvation, fatty acids and ketone bodies are oxidized in preference to glucose, which is spared for those tissues such as the brain and other tissues that require glucose at all times.
- The metabolic adaptations in starvation are designed to minimize protein degradation.

EXERCISE

Multiple Choice Questions (MCQs)

1. The first priority of metabolism in starvation is to provide which of the following substances to the brain and other tissue?

a) Fatty acids b) Ketone bodies
c) Glucose d) Cholesterol

2. During starvation, blood or tissue levels of all of the following are elevated, *except*:

a) Free fatty acids
b) Ketone bodies
c) Glucagon
d) Insulin

3. Which of the following processes plays an essential role in maintaining blood glucose during short-term and prolonged starvation?

a) Glycolysis b) Gluconeogenesis
c) Ketogenesis d) Glycogenolysis

4. In starvation, muscle protein is spared by:
a) Using fatty acids and ketone bodies
b) By increasing rate of gluconeogenesis
c) By inhibiting glucose utilization
d) By stimulating protein synthesis

5. In prolonged starvation, the main energy source of brain is:
a) Glucose
b) Ketone bodies
c) Fructose
d) Fatty acids

6. The major glucogenic amino acid coming from muscle to liver for gluconeogenesis during starvation is:
a) Threonine
b) Methionine
c) Alanine
d) Glycine

7. Under conditions of carbohydrate shortage, available fuels are oxidized in the following order of preference:
a) i) Ketone bodies
ii) Free fatty acids
iii) Glucose
b) i) Glucose
ii) Ketone bodies
iii) Free fatty acids
c) i) Free fatty acids
ii) Glucose
iii) Ketone bodies
d) i) Glucose
ii) Free fatty acid
iii) Ketone bodies

Correct Answers for MCQs

1-c	2-d	3-b	4-a
5-b	6-c	7-a	

CHAPTER

16

Water Metabolism

INTRODUCTION

Water is the most abundant constituent of the human body accounting approximately 60 to 70% of the body mass in a normal adult. Water content of the body changes with age. It is about 75% in the newborn and decreases to less than 50% in older individuals. Water content is greatest in brain tissue and least in adipose tissue.

IMPORTANCE OF WATER

- It is a medium in which body solutes, both organic and inorganic, are dissolved and metabolic reactions take place.
- It acts as a vehicle for transport of solutes.
- Water itself participates as a substrate and a product in many chemical reactions, e.g. in glycolysis, citric acid cycle and respiratory chain.
- The stability of subcellular structures and activities of numerous enzymes are dependant on adequate cell hydration.
- Water is involved in the regulation of body temperature because of its highest latent heat of evaporation.
- Water also acts as a lubricant in the body so as to prevent friction in joints, pleura, peritonium and conjuctiva.
- Both a relative deficiency and an excess of water impair the function of tissues and organs.

TOTAL BODY WATER (TBW) AND ITS DISTRIBUTION

Total body water, includes water both inside and outside of cells and water normally present in the gastrointestinal and genitourinary systems.

Total body water can be theoretically divided into two main compartments:

1. Extracellular water (ECW) and
2. Intracellular water (ICW).

- The ECW includes all water external to cell membranes. The ECW can be further subdivided into:
  - Intravascular water, i.e. plasma
  - Extravascular water, i.e. interstitial fluid.
- The ICW includes all water within cell membranes and constitues the medium in which chemical reactions of cell metabolism occur.

Distribution of Water (Table 16.1)

In a 70 kg adult the total body water is about **42 L.** About **28 L** of intracellular water (ICW) and **14 L** of extracellular water (ECW). The ECW is distributed as **3.5 L** plasma water (intravascular water) and **10.5 L** interstitial water (extravascular).

Factors Affecting Distribution of Water

- Two important factors influence the distribution of water between intracellular and extracellular compartments are:

Table 16.1: Distribution of water

Compartment	*Percentage of TBW*	*Volume in normal adult*
Total body water (TBW)	–	42 L
Extracellular water (ECW)	33%	14 L
a. Plasma	8%	3.5 L
b. Interstitial water	25%	10.5 L
Intracellular water (ICW)	67%	28.0 L

  - Osmolality or Osmolarity
  - Colloidal osmotic pressure.
- Osmolarity or osmolality is a measure of solute particles present in fluid medium.
- Osmolarity is the number of moles per liter of solution and osmolality is the number of moles per kg of solvent.
- All molecules dissolved in the body water contribute to the **osmotic pressure.** Thus, osmolarity or osmolality determines the osmotic pressure exerted by a solution across a membrane. However, for biological fluids, the osmolality is more commonly used.
- The osmotic pressure of a solution is directly proportional to the concentration of osmotically active particles in that solution.
- In a normal person, the osmotic pressure of ECF (mainly due to Na+ ions) is equal to the osmotic pressure of ICF (which is mainly due to K+ ions). Due to this osmotic equilibrium there is no net movement of water in or out of the cells.
- A change in the concentration of osmotically active ions in either of the water compartments creates a difference of osmotic pressure and consequently movement of water between compartments occur.
- Water diffuses from a compartment of low osmolality to one of high osmolality until the osmotic pressures are identical in both of them.

NORMAL WATER BALANCE

- The body water is maintained within the fairly constant limits by a regulation between the **intake** and **output** of water as shown in **Table 16.2**.
- Average daily water turnover in the adult is approximately 2500 ml. However, the range of water turnover depends on **intake**, **environment** and **activity**.

Water intake

Under normal conditions:

- Approximately, one-half to two-thirds of water intake is in the form of **oral fluid intake,** and
- Approximately, one-half to one-third is in the form of oral **intake of water in food**.
- In addition, a small amount of water (150 to 350 ml/day) is produced during metabolism of food called **metabolic *water***.
- Oral water intake is regulated by a **thirst center** located in hypothalamus. Increase in the osmolality of plasma causes increased water intake by stimulating thirst center.

Water output

Water is lost from the body by following routes.

- Urinary water loss via kidney
- Insensible water loss via skin and lungs
- Sensible perspiration (sweating)
- Gastrointestinal water loss through stool.

Urinary water loss

In a normal individual, 1200 to 1500 ml of water is lost in urine per day. Urine volume varies in response to changes in ECW volume and osmolality.

Table 16.2: Average water balance in normal adult

Daily intake of water		*Daily output of water*	
Source	*ml*	*Source*	*ml*
Drinking water	1200	Urine	1400
Water from food	1000	Insensible water loss	
Water derived during metabolism of food (metabolic water)	300	through skin	400
		through lungs	400
		Sensible perspiration water loss	100
		Gastrointestinal water loss through stool	200
Total	2500		2500

Insensible water loss

Loss of water by diffusion through skin and through the lungs is known as insensible water because it is not apparent. It is the only route by which water is lost without solute. Normally, half of the insensible water loss occurs through the skin (about 400 ml) and half through the lungs (about 400 ml). Insensible water loss increases with increase in surrounding temperature, body temperature and physical acitivity.

Sensible perspiration

Sensible perspiration via skin is negligible in cool environment but increases with surrounding temperature, body temperature or physical activity. An increase in plasma osmolality causes a decrease in the rate of sensible perspiration.

Gastrointestinal water loss

Water loss from the gastrointestinal tract through stool is approximately 200 ml/day.

ELECTROLYTES

Electrolytes are the inorganic substances which are readily dissociated into **positively charged (cations)** and **negatively charged (anions)** ions.

Normal cellular functions and survival requires electrolytes which are maintained within narrow limits. The concentration of electrolytes are expressed as **milli-equivalent per liter (mEq/L) rather than milligrams.**

Distribution of Electrolytes

- The electrolytes are well distributed in body fluids and play an important role in distribution and retention of body water by regulating the osmotic equilibrium.
- Total concentration of cations and anions in each compartment (ECF and ICF) is equal to maintain electrical neutrality. The concentration of electrolytes in extracellular and intracellular fluid is shown in **Table 16.3**. There are striking differences in composition between the two fluids.
- **Sodium** is the principal cation of the **extracellular fluid** and comprises over 90% of the total cations, but has a low concentration in intracellular fluid and constitutes only 8% of the total cations
- **Potassium** by contrast, is the principal cation of **intracellular fluid** and has a low concentration in extracellular fluid.
- Similar differences exist with the anions. **Chloride (Cl^-)** and **bicarbonate (HCO_3^-)** predominate in the **extracellular fluid,** while **phosphate** is the principal anion within the cells.

Table 16.3: Electrolyte content of ECF and ICF

Ions	*Extracellular fluid mEq/L*	*Intracellular fluid mEq/L*
Cations		
Na^+	142	10
K^+	5	150
Ca^{++}	5	2
Mg^{++}	3	40
Total	155	202
Anions		
Cl^-	103	2
HCO_3^-	27	10
HPO_4^{--}	2	140
SO_4^{--}	1	5
Organic acids	6	5
Protein	16	40
Total	155	202

The term electrolytes applied in medicine to the four ions in plasma, (Na^+, K^+, Cl^- and HCO_3^-) that exert the greatest influence on water balance and acid-base balance.

REGULATION OF WATER AND ELECTROLYTE BALANCE

Water and electrolyte balance are regulated together. It is regulated through following hormones:

- Antidiuretic hormone (ADH), or vasopressin
- The renin-angiotensin-aldosteron system (RAAS)
- Atrial natriuretic factor (ANF).

Antidiuretic hormone (ADH)

- Water **intake** is normally controlled by the sensation of **thirst** and its **output** by the action of hormone **vasopressin,** also known as antidiuretic hormone (ADH). The major role of ADH is to increase the reabsorption of water from the kidney.
- An increase in plasma osmolality (due to deficiency of water) causes sensation of thirst and stimulates hypothalamic thirst center, which results in an increase in water intake. An increase in plasma osmolality also stimulates hypothalamus to release ADH. ADH then increases water reabsorption by the kidney. All these events ultimately help to restore the plasma osmolality **(Figure 16.1)**.
- Conversely, a large intake of water causes fall in osmolality, suppresses thirst and reduces ADH

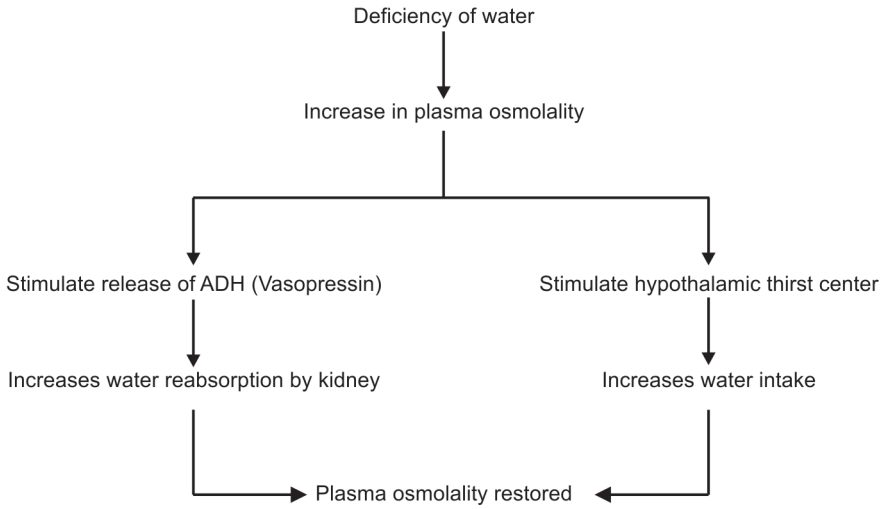


Figure 16.1: Regulation of water balance

secretion, leading to a diuresis, producing large volume of dilute urine.

Renin-angiotensin-aldosterone system (RAAS) (Figure 16.2)

Renin is secreted in response to a decreased level of Na^+ in the fluid of the distal tubule. Renin converts angiotensinogen in plasma to angiotensin I, which in turn is converted to **angiotensin II** by angiotensin converting enzyme (ACE). Angiotensin II stimulates aldosterone secretion, thirsting behavior and ADH secretion.

Aldosterone stimulates Na^+ reabsorption in the renal tubules in the exchange of H^+ and K^+. As a consequence of Na^+ reabsorption, water is retained by the body.

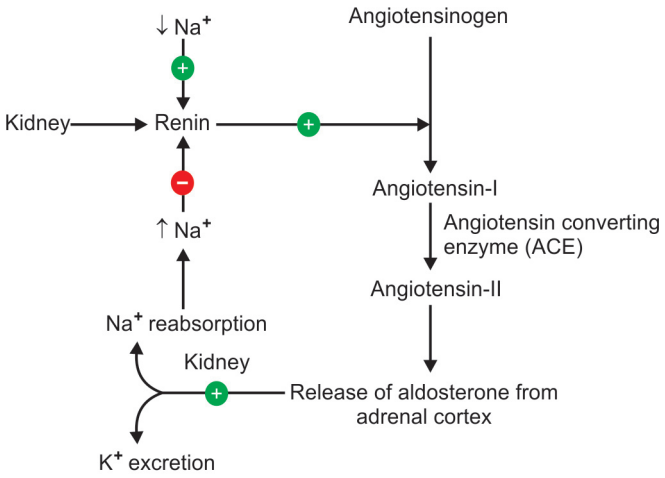


Figure 16.2: Renin-angiotensin-aldosterone system (RAAS) in regulation of water and electrolyte balance

Atrial natriuretic factor (ANF)

ANF is a polypeptide **hormone** secreted by the right atrium of the heart. It **increases Na^+** and **water excretion** by the kidney. Thus kidney plays an important role in maintenance of electrolyte and water balance.

DISORDERS OF WATER AND ELECTROLYTE BALANCES

Dehydration and **overhydration** are the disorders of water balance, which are due to an imbalance of water intake and output or sodium intake and output.

Dehydration

Dehydration may be defined as a state in which loss of water exceeds that of intake, as a result of which body's water content gets reduced and the body is in negative water balance. Dehydration may be of two types:

1. **Dehydration due to pure water deficiency,** without loss of electrolytes, called **simple dehydration**
   - Simple dehydration or pure water deficiency is due to deprivation of water without corresponding loss of electrolytes. Simple dehydration is associated with **hypernatremia,** i.e. increased level of sodium and **increase in ECW osmolality** due to loss of water from the body.
2. **Dehydration due to combined deficiency of water and electrolyte, sodium.**
   - Dehydration due to combined water and electrolyte sodium deficiency is more common than simple dehydration.

Causes of dehydration

- Simple dehydration results from deprivation of water either due to no or inadequate intake of water or due to excessive loss of water from body, e.g. in diabetes insipidus.
- Dehydration due to combined deficiency of water and electrolyte occur as a result of vomiting, diarrhea, excessive sweating, salt wasting renal disease, and adrenocortical insufficiency (Addison's disease).

Symptoms of dehydration

- Symptoms of simple dehydration are intense thirst, mental confusion, fever and oliguira (decreased urine output).
- Symptoms of dehydration due to combined deficiency of water and electrolytes are wrinkled skin, dry mucous membranes, muscle cramps, sunken eyeballs and increased blood urea nitrogen. With increasing severity, weakness, hypotension and shock may occur.

Treatment

- **Treatment of simple dehydration :** The patient is asked to drink plenty of water. If oral administration is not possible, an isotonic solution of 5% dextrose is given intravenously.
- **Treatment of dehydration due to combined deficiency of water and electrolyte :** An isotonic solution of sodium chloride (normal saline) is given intravenously.

Overhydration or Water Intoxication

Overhydration is a state of pure **water excess** *or* **water intoxication.** More often, water intoxication results due to the retention of excess water in the body, which can occur due to:

- Renal failure
- Excessive administration of fluids parenterally
- Hypersecretion of ADH (syndrome of inappropriate ADH secretion, SIADH).

This results in reduced plasma electrolytes with decreased osmolality.

Symptoms of overhydration

Nausea, vomiting, headache, muscular weakness confusion and in severe cases convulsions, coma and even death.

SUMMARY

- Water is the most abundant constituent of the human body, accounting for approximately 60 to 70% of the body mass in a normal adult.
- The total body water is divided into two main compartments, the intracellular fluid and extracellular fluid.
- The body water balance is maintained within the fairly constant limits by regulation between water intake (by drinking, from food and metabolic water) and output (through urine, skin, lungs and stool).
- The ICF is twice as large as the ECF.
- Osmolality and colloidal osmotic pressure influence the distribution of water between ECF and ICF.
- Total concentration of cations and anions in each compartment is equal.
- Sodium is the main ECF cation and potassium is the main ICF cation.
- Water and electrolyte balance are regulated together by thirst, antidiuretic hormone, renin angiotensin-aldosterone system, atrial natriuretic peptide and kidney.
- Dehydration (water loss) and overhydration (water excess) are the disorders of water and electrolyte balance which occur due to an imbalance of water intake and output or sodium intake and output.

EXERCISE

Solve the Following

Case History

A 40-year-old female was brought to the hospital with complaints of persistent vomiting, loose motions, cramps and extreme weakness, sunken eyes and dry tongue.

Questions

a. Name the condition arising due to the above symptoms.
b. What are the causes for the condition?
c. Which are the different types of the condition?
d. Suggest the treatment.

Multiple Choice Questions (MCQs)

1. Chief anion of ECF is:
a) Cl^- b) HCO_3^-
c) HPO_4^{--} d) Protein

2. In ICF, main cation is:
a) Na^+ b) K^+
c) Ca^{++} d) Mg^{++}

3. Which of the following is correct about intracellular water (ICW)?
a) Amount less than ECW
b) Amount more than ECW

c) Amount equal to ECW
d) None of the above

4. Which of the following hormones affects fluid and electrolyte balance?
a) Epinephrine
b) Glucagon
c) Thyroxine
d) Aldosterone

5. Distribution of water between intracellular and extracellular compartments depends on all of the following, *except*:
a) Osmolality
b) Osmolarity
c) Colloidal osmotic pressure
d) Surface tension

6. Source of daily output of water is:
a) Urine
b) Insensible water (skin and lungs)
c) Sensible water (sweat and stool)
d) All of the above

7. Metabolic water is:
a) Water from food
b) Drinking water
c) Water derived from metabolism
d) Total body water

8. Water and electrolyte balance is regulated by, *except*:
a) ADH
b) Renin-angiotensin-aldosterone system (RAAS)
c) Atrial natriuretic factor (ANF)
d) Insulin

9. Main anions of ICF is:
a) Cl^-
b) HPO_4^{--}
c) HCO_3^-
d) SO_4^{--}

10. Main cation of ECF is:
a) Na^+
b) K^+
c) Ca^{++}
d) Mg^{++}

11. Which of the following has greatest water content?
a) Liver
b) Adipose tissue
c) Brain
d) Kidney

12. Which of the following has least water content?
a) Pancreas
b) Brain
c) Liver
d) Adipose tissue

13. In a 70 kg adult, the total body water content is
a) 42 L
b) 28 L
c) 14 L
d) 3.5 L

Correct Answers for MCQs

1-a	2-b	3-b	4-d
5-d	6-d	7-c	8-d
9-b	10-a	11-c	12-d
13-a			

CHAPTER

17

Mineral Metabolism

INTRODUCTION

Minerals are inorganic elements, required for a variety of functions. The minerals required in human nutrition can be grouped into ***macrominerals*** *and* ***microminerals (trace elements)*** **(Table 17.1)**.

- The macrominerals are required in excess of 100 mg/day.
- The microminerals or trace elements are required in amounts less than 100 mg/day.
- The principal functions and deficiency manifestations of each of the macro and microminerals are summarized in **Table 17.2**.

Table 17.1: Minerals required in human nutrition

Macrominerals	*Microminerals or Trace elements*
Sodium	Chromium
Potassium	Cobalt
Chlorine	Copper
Calcium	Fluoride
Phosphorus	Iodine
Magnesium	Iron
Sulfur	Manganese
	Molybdenum
	Selenium
	Zinc

METABOLISM OF SODIUM, POTASSIUM AND CHLORIDE

Sodium

Sodium is the major cation of extracellular fluids.

Dietary food sources

Table salt (NaCl), salty foods, animal foods, milk and some vegetables.

Recommended dietary allowance per day

- 1–5 gm
- 5 gm NaCl per day is recommended for adults without history of hypertension and 1 gm NaCl per day with history of hypertension.

Absorption and excretion

Sodium readily absorbed from the gut and is excreted from the body via urine. There is normally little loss of sodium occur through skin (sweat) and in the feces. Urinary excretion of sodium is regulated by aldosterone, which increases sodium reabsorption in kidney.

Metabolic functions

- It maintains the ***osmotic pressure*** and ***water balance***.
- It is a constituent of ***buffer*** and involved in the maintenance of ***acid-base balance***.
- It maintains ***muscle*** and nerve ***irritability*** at the proper level.

Table 17.2: Principal functions and deficiency manifestations of macrominerals and microminerals

Element	*Metabolic function*	*Deficiency manifestation*
Macrominerals		
Sodium	Principal extracellular cation, buffer constituent, water and acid base balance, cell membrane permeability	Dehydration, acidosis, excess leads to edema and hypertension
Potassium	Principal intracellular cation, buffer constituent, water and acid base balance, neuromuscular irritability	Muscle weakness, paralysis and mental confusion, acidosis
Chloride	Principal extracellular anion, electrolyte balance, osmotic balance, and acid base balance, gastric HCl formation	Deficiency secondary to vomiting and diarrhea
Calcium	Constituent of bone and teeth, blood clotting, regulation of nerve, muscle and hormone function	Tetany, muscle cramps, convulsions, osteoporosis, rickets
Phosphorus	Constituent of bone and teeth, nucleic acids, and NAD, FAD, ATP, etc. Required for energy metabolism	Growth retardation, skeletal deformities, muscle weakness, cardiac arrhythmia
Magnesium	Cofactor for phosphate transferring enzymes, constituent of bones and teeth, muscle contraction, nerve transmission	Muscle spasms, tetany, confusions, seizures
Sulfur	Constituent of proteins, bile acid, glycosoaminoglycans, vitamins like thiamine, lipoic acid, involved in detoxication reactions	Unknown
Microminerals or trace elements		
Chromium	Potentiate the effect of insulin	Impaired glucose metabolism
Cobalt	Constituent of vitamin B_{12}	Macrocytic anemia
Copper	Constituent of oxidase enzymes, e.g. tyrosinase, cytochrome oxidase, ferroxidase and ceruloplasmin, involved in iron absorption and mobilization	Microcytic hyporchromic anemia, depigmentation of skin, hair. Excessive deposition in liver in Wilson's disease
Fluoride	Constituent of bone and teeth, strengthens bone and teeth	Dental caries
Iodine	Constituent of thyroid hormones (T_3 and T_4)	Cretinism in children and goiter in adults
Iron	Constituent of heme and non-heme compounds and transport, storage of O_2	Microcytic anemia
Manganese	Cofactor for number of enzymes, e.g. arginase, carboxylase, kinases, etc.	Not well defined
Molybdenum	Constituent of xanthine oxidase, sulfite oxidase and aldehyde oxidase	Xanthinuria
Selenium	Antioxidant, cofactor for glutathione peroxidiase, protects cell against membrane lipid peroxidation	Cardiomyopathy
Zinc	Cofactor for enzymes in DNA, RNA and protein synthesis, constituent of insulin, carbonic anhydrase, carboxypeptidase, LDH, alcohol dehydrogenase, alkaline phosphatase, etc.	Growth failure, impaired wound healing, defects in taste and smell, loss of apetite

- Sodium is involved in ***cell membrane permeability***.
- Sodium is required for intestinal absorption of glucose, galactose and amino acids.

Plasma Sodium

The plasma concentration of sodium is **135-145 mEq/L.** Whereas blood cell (intracellular) contain only about **35 mEq/L**.

Clinical Conditions Related to Plasma Sodium Level Alterations

Hypernatremia

Hypernatremia is an increase in serum sodium concentration above the normal range of 135 – 145 mEq/L.

Causes of hypernatremia

- ***Water depletion,*** may arise from a decreased intake or excessive loss with normal sodium content, e.g. diabetes insipidus.
- ***Water and sodium depletion,*** if more water than sodium is lost, e.g. diabetes mellitus (osmotic diuresis), excessive sweating or diarrhea in children
- ***Excessive sodium intake or retention*** in the ECF due to excessive aldosterone secretion, e.g. **Cohn's syndrome** and in **Cushing's syndrome**.

Symptoms of hypernatremia

It is due to water loss, then the symptoms are therefore those of dehydration and if it is due to excess salt gain, leads to hypertension and edema.

Hyponatremia

It is a significant fall in serum sodium concentration below the normal range 135 to 145 mEq/L.

Causes of hyponatremia

- **Retention of water:** Retention of water dilutes the constituents of the extracellular space causing hyponatremia, e.g. in heart failure, liver disease, nephrotic syndrome, renal failure, syndrome of inappropriate ADH secretion (SIADH).
- **Loss of sodium:** Such losses may be from gastrointestinal tract, e.g. vomiting, diarrhea, or in urine. Urinary loss may be due to aldosterone deficiency (Addison's disease).

Symptoms of hyponatremia are constant thirst, muscle cramps, nausea, vomiting, abdominal cramps, weakness and lethargy.

Potassium

Potassium is the main intracellular ***cation.*** About 98% of total body potassium is in cells (150–160 mEq/L), only 2% in the ECF (3.5–5 mEq/L).

Dietary food sources

Vegetables, fruits, whole grain, meat, milk, legumes and tender coconut water.

Recommended dietary allowance per day

2–5 gm.

Absorption

Potassium is absorbed readily by passive diffusion from gastrointestinal tract.

Excretion

- Potassium excretion occurs through three primary routes, the ***gastrointestinal tract***, the ***skin*** and the ***urine***. Under normal conditions, loss of potassium through gastrointestinal tract and skin is very small. The major means of K^+ excretion is by the kidney.
- When sodium is reabsorbed by distal tubule cations (e.g. K^+ or H^+) in the cell move into the lumen to balance the charge. ***Thus during the sodium reabsorption there is an obligatory loss of potassium.***

Serum potassium

The concentration of potassium in serum is around ***3.5–5 mEq/L.*** Serum potassium concentration does not vary appreciably in response to water loss or retention.

Metabolic functions

- Potassium maintains the intracellular ***osmotic pressure, water balance*** and ***acid-base balance***.
- It influences activity of cardiac and skeletal muscle.
- Several *glycolytic enzymes* need potassium for their formation.
- Potassium is required for ***transmission of nerve impulses***.
- ***Nuclear activity*** and ***protein synthesis*** are dependent on potassium.

Clinical Conditions Related to Plasma Potassium Level Alterations

Hyperkalemia

Hyperkalemia is a clinical condition associated with elevated plasma potassium above the normal range (3.5–5 mEq/L).

Causes of hyperkalemia

- **Renal failure:** The kidney may not be able to excrete a potassium load when GFR is very low.

- **Mineralocorticoid deficiency:** For example, in Addison's disease.
- **Cell damage:** For example, in trauma and malignancy.

Symptoms of hyperkalemia

First manifestation is cardiac arrest, changes in electrocardiogram, cardiac arrhythmia, muscle weakness which may be preceded by parasthesia (abnormal tingling sensation).

Hypokalemia (low plasma concentration)

Causes of hypokalemia

- **Gastrointestinal losses:** Potassium may be lost from the intestine due to vomiting, diarrhea.
- **Renal losses:** Due to renal disease, administration of diuretics.

Symptoms of hypokalemia

Muscular weakness, tachycardia, electrocardiographic (ECG) changes (flattering of ECG waves), lethargy, and confusion.

Chloride

Chloride is the major **anion** in the **extracellular fluid** space.

Dietary food sources

Table salt, leafy vegetables, eggs and milk.

Recommended dietary allowance (RDA) per day

2–5 gm.

Absorption

Rapidly and almost totally absorbed in the gastrointestinal tract.

Excretion

Under normal conditions chloride excretion occurs by way of three routes; the **gastrointestinal tract,** the skin and **urinary tract.** Chloride is excreted, mostly as sodium chloride and chiefly by way of the kidney.

Plasma chloride

The concentration of chloride in plasma is **95–105 mEq/L**.

Functions

- As a part of sodium chloride, chloride is essential for water balance, regulation of osmotic pressure, and acid-base balance.
- Chloride is necessary for the formation of HCl by the gastric mucosa and for activation of enzyme amylase.
- It is involved in ***chloride shift.***

Clinical Conditions Related to Plasma Chloride Level Alterations

Hyperchloremia

- An increased chloride concentration occurs in dehydration, metabolic acidosis and Cushing's syndrome.

Hypochloremia

- A decreased chloride concentration is seen in severe vomiting, metabolic alkalosis, excessive sweating and Addison's disease.

METABOLISM OF CALCIUM, PHOSPHORUS AND MAGNESIUM

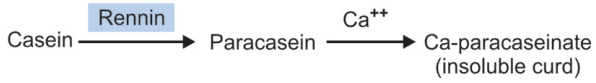
Calcium

Calcium is the most abundant mineral in the body. The adult human body contains about 1 kg of calcium. About 99% the body's calcium is present in bone together with phosphate as the mineral ***hydroxyapatite*** **[Ca_{10} (PO_4)$_6$ (OH)$_2$]**, with small amounts in soft tissue and extracellular fluid.

Functions

1. ***Formation of bone and teeth***: 99% of the body's calcium is located in bone in the form of **hydroxyapatite crystal [$3Ca_3$ (PO_4)$_2$ Ca (OH)$_2$]**. The hardness and rigidity of bone and teeth are due to hydroxyapatite.
2. ***Blood coagulations***: Calcium present in platelets involved in blood coagulation, the conversion of an inactive protein prothrombin into an active **thrombin** requires calcium ions.
3. **Muscle contraction:** Muscle contraction is initiated by the binding of calcium to troponine**.**
4. **Release of hormones:** The release of certain hormones like parathyroid hormone, calcitonin, etc. requires calcium ions.
5. **Release of neurotransmitter:** Influx of Ca^{2+} from extracellular space into neurons causes release of neurotransmitter.
6. **Regulation of enzyme activity:** Activation of number of enzymes requires Ca^{2+} as a specific cofactor. For example:
   - Activation of enzyme **glycogen phosphorylase kinase** which then triggers glycogenolysis.
   - Activation of salivary and pancreatic α-amylase.
7. **Second messenger:** Calcium acts as a second messenger for hormone action. For example, it acts as a **second messenger** for epinephrine or glucagon. Ca also functions as a **third messenger** for some hormones such as antidiuretic hormone (ADH).

8. ***Membrane excitability***: **Calcium ions** activate the sodium channels. Deficiency of calcium ions lead to decreased activity of Na-channels, which ultimately leads to decrease in membrane potential so that the nerve fiber becomes highly excitable causing muscle tetany.
9. ***Cardiac activity***: Cardiac muscle depends on extracellular Ca^{2+} for contraction. Myocardial contractility increases with increased Ca^{2+} concentration and decreases with decreased calcium concentration.
10. ***Membrane integrity and permeability:*** Calcium is required for maintenance of integrity and permeability of the membrane.
11. ***Hydrolysis of casein of milk***: Calcium is required for the formation of Ca-paracaseinate (insoluble curd).



The significance of this reaction is to convert milk into a more solid form to increase its retention in the stomach for a longer period of time and facilitate its gastric digestion in infants.

Dietary sources

The main dietary sources of calcium are milk and dairy products, (half a liter of milk contains approximately 1,000 mg of calcium) cheese, cereal grains, legumes, nuts and vegetables.

Recommended dietary allowance (RDA) per day

- Adults: 800 mg/day
- Women during pregnanacy: 1200 mg/day and lactation and for teenagers.
- Infants: 300–500 mg/day.

Absorption

Factors affecting absorption: The absorption of calcium from the intestine depends on several factors. Some of these are discussed below:

Factors that stimulate calcium absorption

1. **Vitamin D** stimulates absorption of calcium from intestine by inducing the synthesis of calcium binding protein, necessary for the absorption of calcium from intestine.
2. **Parathyroid hormone** (PTH) stimulates calcium absorption indirectly via activating vitamin D.
3. **Acidic pH:** Since, calcium salts are more soluble in acidic pH, the acidic foods and organic acids (citric acid, lactic acid, pyruvic acid, etc.) favor the absorption of calcium from intestine.
4. **High protein diet** favors the absorption of calcium. Basic amino acids, lysine and arginine derived from hydrolysis of the dietary proteins increase calcium absorption.
5. **Lactose** is known to increase the absorption of calcium, by forming soluble complexes with the calcium ion.

Factors that inhibit calcium absorption

1. **Phytates** and **Oxalates** bind dietary calcium forming insoluble salts which cannot be absorbed from the intestine. Phytates present in many cereals and oxalates present in green leafy vegetables.
2. **High fat diet** decreases the absorption of calcium. High amounts of fatty acids derived from hydrolysis of dietary fats react with calcium to form insoluble calcium soaps which cannot be absorbed.
3. **High phosphate content** in diet causes precipitation of calcium as calcium phosphate and thereby lower the ratio of Ca: P in the intestine. The Ca: P ratio should be 1:2–2:1 for optimum absorption of calcium. Absorption of calcium is maximum when food contains almost equal amounts of calcium and phosphorus.
4. **High fiber diet** decreases the absorption of calcium from intestine.

Excretion

The excretion of calcium is partly through the kidneys but mostly by way of the small intestine through feces. Small amount of calcium may also be lost in sweat.

PLASMA CALCIUM

The plasma calcium concentration in normal individual is **9–11 mg%.**

Regulation of Plasma Calcium Level (Figure 17.1)

Homeostasis of plasma calcium is dependent on the:

- Function of three main organs:
  1. Bone
  2. Kidney
  3. Intestine.
- Function of three main hormones:
  1. Parathyroid hormone (PTH)
  2. Vitamin D or cholecalciferol or calcitriol
  3. Calcitonin.
- The four major processes are **(Figure 17.1):**
  1. Absorption of calcium from the intestine, mainly through the action of vitamin D.
  2. Reabsorption of calcium from the kidney, mainly through the action of parathyroid hormone and vitamin D.

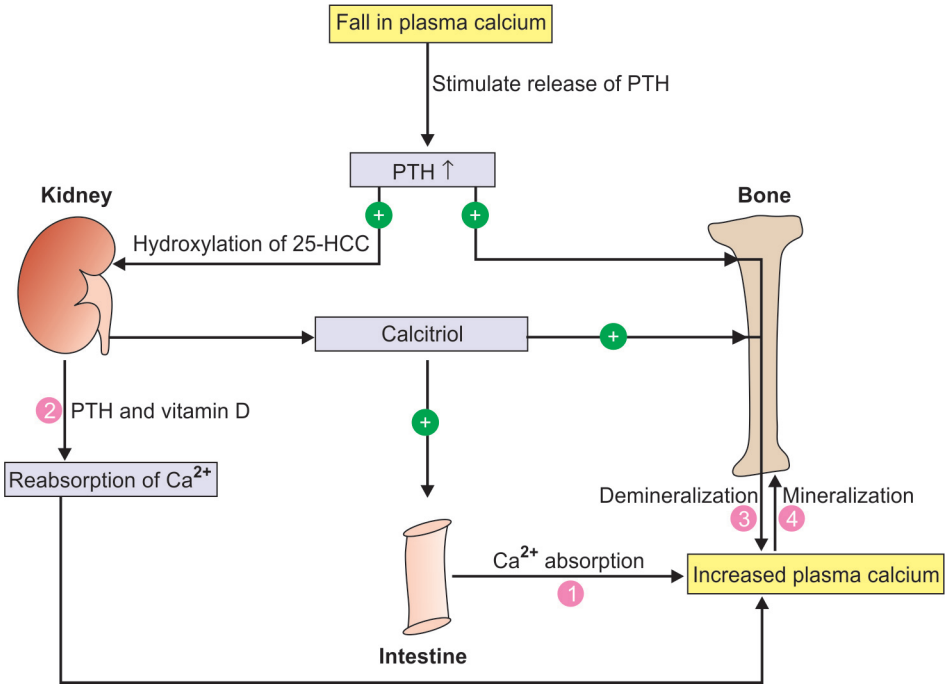


Figure: 17.1: Regulation of plasma calcium
where, 25-HCC: 25-Hydroxycholecalciferol

3. Demineralization of bone mainly through action of parathyroid hormone, but facilitated by vitamin D.
4. Mineralization (calcification) of bone through the action of calcitonin.

Role of parathyroid hormone (PTH): It is secreted by the parathyroid in response to drop in the blood calcium level. It acts on two main target organs, bone and kidney and indirectly via the activation of vitamin D on the intestine to increase the plasma calcium concentration.

Action on bone: PTH stimulates mobilization of calcium and phosphate from bones by stimulating osteoclast activity. Osteoclast activity results in demineralization of the bone.

- Uptake of calcium and phosphate by bone is also decreased by PTH resulting in an increase in blood calcium and phosphate level.

Action on kidney: In kidney PTH increases the tubular reabsorption of calcium and decreases renal excretion of calcium. PTH increases excretion of phosphate by inhibiting its renal reabsorption.

Action on intestine: Action of PTH on intestine is indirect via the formation of calcitriol, active form of vitamin D. PTH stimulates the production of calcitriol. Calcitriol then increases absorption of calcium from intestine.

Role of vitamin D (calcitriol): It is the active form of vitamin D, which causes the increase in plasma calcium and phosphate concentration by stimulating the following processes:

- Absorption of calcium and phosphorus from intestine by inducing synthesis of calcium binding protein necessary for the absorption of calcium from intestine.
- Reabsorption of calcium and phosphorus from the kidney.
- Mobilization of calcium and phosphorus from the bone.
- Thus, overall effects of PTH and calcitriol elevate plasma calcium and phosphate level.

Role of Calcitonin: The secretion of calcitonin is stimulated by increase in blood calcium level.

- Action of calcitonin on the bones is opposite to that of the PTH. It inhibits calcium mobilization from bone and increases bone calcification (mineralization) by increasing the osteoblasts activity.
- In the kidney it stimulates the excretion of calcium and phosphorus, thereby decreasing the blood calcium level.

Clinical Conditions Related to Plasma Calcium Level Alterations

Hypocalcemia

Hypocalcemia is characterized by lowered levels of plasma calcium. The causes of hypocalcemia include:

- **Hypoparathyroidism:** The commonest cause of hypoparathyroidism is neck surgery, or due to magnesium deficiency (See functions of magnesium).
- **Vitamin D deficiency:** This may be due to dietary deficiency, malabsorption or little exposure to sunlight. It may lead to bone disorders, **osteomalacia** in adults and **rickets** in children ***(See Chapter 7).***
- **Renal disease:** The diseased kidneys fail to synthesize calcitriol due to impaired hydroxylation.

Clinical features of hypocalcemia

The clinical features of hypocalcemia include:

- Neuromuscular irritability
- Neurologic features such as tingling, tetany, numbness (fingers and toes).
- Muscle cramps
- Cardiovascular signs such as an abnormal ECG.
- Cataracts.

Hypercalcemia

Hypercalcemia is characterized by increased plasma calcium level. The commonest causes of hypercalcemia are:

- Hyperparathyroidism
- Malignant disease.

Clinical features of hypercalcemia

- Neurological symptoms such as depression, confusion, inability to concentrate.
- Muscle weakness
- Gastrointestinal problems such as anorexia, abdominal pain, nausea and vomiting and constipation.
- Renal features such as polyuria and polydypsia.
- Cardiac arrhythmias.

Phosphorus

Adults contain about **400–700 gm** of phosphorus, about 80% of which is combined with calcium in bones and teeth.

Functions

- **Constituent of bone and teeth:** Inorganic phosphate is a major constituent of **hydroxyapatite** in bone, thereby playing an important role in structural support of the body.
- **Acid-base regulation:** Mixture of HPO_4^{--} and $H_2PO_4^-$ constitutes the phosphate buffer which plays a role in maintaining the pH of body fluid.
- **Energy storage and transfer reactions:** High energy compounds, e.g. ATP, ADP, creatin phosphate, etc. which play a role of storage and transport of energy, contain phosphorus.
- **Essential constituent:** Phosphate is an essential element in phospholipid of cell membrane, nucleic acids (RNA and DNA), nucleotides (NAD, NADP, c-AMP, c-GMP, etc.)
- **Regulation of enzyme activity:** Phosphorylation and dephosphorylation of enzymes modify the activity of many enzymes.

Dietary sources

The foods rich in calcium are also rich in phosphorus, i.e. milk, cheese, beans, eggs, cereals, fish and meat.

Recommended dietary allowance per day

- The recommended dietary allowance for both men and women is **800 mg/day**.
- The amount during pregnancy and lactation is **1200 mg/day**.

Absorption

- Like calcium, phosphorus is absorbed from small intestine and the degree of absorption is similarly affected by different factors as that of calcium.
- Vitamin D stimulates the absorption of phosphate along with calcium.
- Acidic pH favors the absorption of phosphorus.
- Phytate and oxalates decrease absorption of phosphate from intestine.
- Optimum absorption of calcium and phosphate occurs when dietary Ca: P ratio is 1:2 – 2:1.

Excretion

Phosphates are mainly excreted by the kidneys as NaH_2PO_4 through urine (unlike calcium). PTH decreases the reabsorption of phosphorus from the tubules and cause increased excretion of phosphorus in urine.

Plasma phosphorus

Plasma contains **2.5–4.5 mg/dL** of inorganic phosphate. Plasma phosphate concentration is controlled by the kidney, where tubular reabsorption is reduced by PTH. ***The phosphate which is not reabsorbed in the renal tubule acts as an important urinary buffer.***

Clinical Conditions Related to Plasma Phosphorus Concentration Alterations

Hypophosphatemia

In hypophosphatemia serum inorganic phosphate concentration is less than 2.5 mg/dL.

Causes of hypophosphatemia

- **Hyperparathyroidism:** High PTH increases phosphate excretion by the kidneys and this leads to low serum concentration of phosphate.
- **Congenital defects** of tubular phosphate reabsorption, e.g. **Fanconi's syndrome,** in which phosphate is lost from body.

Clinical symptoms of hypophosphatemia

- As phosphate is an important component of ATP, cellular function is impaired with hypophosphataemia and leads to muscle pain and weakness and decreased myocardial output.
- If hypophosphatemia is chronic; rickets in children or osteomalacia in adults may develop.

Hyperphosphatemia (High serum phosphate concentration)

Causes of hyperphosphatemia

- **Renal failure:** This is the commonest cause in which phosphate excretion is impaired.
- **Hypoparathyroidism:** Low PTH decreases phosphate excretion by the kidney and leads to high serum concentration.

Clinical symptoms of hyperphosphatemia

Elevated serum phosphate may cause a decrease in serum calcium concentration; therefore ***tetany*** and ***seizures*** may be the presenting symptoms.

Magnesium

The body contains about **25 gm** of magnesium, most of which (55%) is present in the bones in association with calcium and phosphorus, a small proportion of the body's content is in the ECF.

Functions

- Magnesium is essential for the activity of many enzymes. Magnesium is a cofactor for more than 300 enzymes in the body, in addition, magnesium is an allosteric activators of many enzyme systems. It plays an important role in oxidative phosphorylation, glycolysis, cell replication, nucleotide metabolism, protein synthesis and many ATP dependent reactions
- Magnesium influences the secretion of PTH by the parathyroid glands.
- Hypomagnesemia may cause hypoparathyroidism.
- Magnesium along with sodium, potassium and calcium controls the neuromuscular irritability.
- It is an important constituent of bone and teeth.

Dietary sources

Cereals, pulses, nuts, green leafy vegetables, meat, eggs and milk.

Recommended dietary allowance per day

- RDA of the adult man is 350 mg/day and for women 300 mg/day.
- More magnesium is required during pregnancy and lactation (450 mg/day).

Absorption

- About 30–40% of the dietary magnesium is absorbed from the small intestine.
- Vitamin D and PTH increase the absorption of magnesium from intestine.
- Large amounts of calcium and phosphate in diet reduce the absorption of magnesium from intestine.

Excretion

Magnesium is excreted mainly by way of intestine. All unabsorbed magnesium as well as that in biliary excretion and intestinal secretion are excreted through feces. A fraction of absorbed magnesium is excreted by the kidneys through urine.

Serum Magnesium

Human blood serum magnesium concentration is **1–3.5 mg/dL**.

The mechanism of control is poorly understood

- Renal conservation of magnesium is partly controlled by PTH and aldosterone.
- PTH increases tubular reabsorption of magnesium similar to that of calcium.
- Aldosterone increases its renal excretion as it does for potassium.

Clinical Conditions Related to Plasma Magnesium Concentration Alterations

Hypomagnesemia

Hypomagnesemia is an abnormally low serum magnesium level.

- It is usually associated with magnesium deficiency.
- Since magnesium is present in most common food stuffs, low dietary intakes of magnesium are associated with general nutritional insufficiency, accompanied by intestinal malabsorption, severe vomiting, diarrhea or other causes of intestinal loss.
- The symptoms of hypomagnesemia are very similar to those of hypocalcemia, impaired neuromuscular function such as tetany, hyperirritability, tremor, convulsions and muscle weakness.

Hypermagnesemia

Hypermagnesemia is uncommon but is occasionally seen in renal failure.

Depression of the neuromuscular system is the most common manifestation of hypermagnesemia.

METABOLISM OF SULFUR

The body receives sulfur through the proteins, as sulfur containing amino acids, e.g. methionine and cysteine.

Food Sources

Plant and animal proteins, legume, eggs, cereals and cauliflower.

Functions

- Sulfur is a constituent of:
  - Protein
  - Glycosaminoglycans, e.g. heparin and chondroitin sulfate.
  - Vitamins, e.g. thiamine, biotin, lipoic acid, CoA of pantothenic acid.
  - Bile acids, e.g. taurocholic acid.
  - Active form of sulfate, phosphoadenosine phosphosulfate (PAPS) is involved in detoxication reactions, e.g. some phenolic compounds are detoxified by conjugating with PAPS and eliminated from the body in the form of etheral sulfate.
  - Non-heme iron enzyme such as mitochondrial NADH dehydrogenase and Fe-S protein.
  - Compounds like glutathione and insulin.

Excretion

Sulfur is excreted by the kidneys in urine in the form of inorganic, organic and etheral sulfate.

Deficiency manifestation

Not well defined.

METABOLISM OF TRACE ELEMENTS (MICROMINERALS)

Microminerals or trace elements are present in the body in very small amount (micrograms to miligrams) that are essential for certain biochemical processes. Trace elements required by humans are:

- Chromium
- Cobalt
- Copper
- Fluoride
- Iodine
- Iron
- Manganese
- Molybdenum
- Selenium
- Zinc.

Chromium (Cr)

The adult human body contains only **6 mg** of chromium.

Dietary food sources

Yeast, molasses, meat products, cheese, whole grains.

Recommended dietary allowance per day

For healthy adults it is **0.05–20 mg**.

Functions

- Chromium functions in the control of glucose and lipid metabolism.
- It acts as a ***cofactor for insulin*** in increasing glucose utilization and transport of amino acids into cells.
- ***Chromium is also reported to lower the cholesterol levels.***

Absorption and excretion

The biologically active form (Cr^{3+}) is absorbed poorly from the diet. The majority of orally absorbed chromium is excreted through urine.

Deficiency manifestation

Deficiency of chromium can develop symptoms of glucose intolerance and weight loss, that are reversed with complementary chromium.

Toxicity

Chromium has toxic properties. The hexavalent (Cr^{6+}) form of chromium has strong oxidizing properties and is much more toxic than the trivalent (Cr^{3+}) form. Chromium toxicity is known to result in:

- Inflammation and necrosis of the skin and nasal passages.
- Allergic contact dermatitis and lung cancer.
- Oral ingestion can result in damage to the gastrointestinal tract and renal failure.

Cobalt (Co)

Cobalt is necessary for biological activity of vitamin B_{12}. Cobalt fits into the corrin ring of vitamin B_{12} **(See Figure 7.14)**.

Dietary food sources

Liver, pancreas and vitamin B_{12}.

Recommended dietary allowance per day

Not established.

Functions
The only known function of cobalt is that it is an integral part of vitamin B_{12}.

Absorption and excretion
Dietary cobalt is poorly absorbed and is stored in the liver, probably as vitamin B_{12}. Cobalt is excreted in bile.

Deficiency manifestation
A cobalt deficiency is accompanied by all the signs and symptoms of a vitamin B_{12} deficiency. The most important is anemia.

Toxicity
An excess of cobalt can lead to **polycythemia.**

Copper (Cu)

A 70 kg human adult body contains approximately 80 mg of copper. It is present in all tissues. The highest concentrations are found in liver and kidney, with significant amount in cardiac and skeletal muscle and in bone.

Dietary food sources
Shellfish, liver, kidneys, egg yolk and some legumes are rich in copper.

Recommended dietary allowance per day
2–3 mg.

Functions
- Copper is an essential constituent of many enzymes including:
  - Ceruloplasmin (ferroxidase)
  - Cytochrome oxidase
  - Superoxide dismutase
  - Dopamine β-hydroxylase
  - Tyrosinase
  - Tryptophan dioxygenase
  - Lysyl oxidase.
- Copper plays an important role in iron absorption. Ceruloplasmin, the major copper containing protein in plasma has ferroxidase activity that oxidizes ferrous ion to ferric state before its binding to transferrin (tranport form of iron).
- Copper is required for the synthesis of hemoglobin. Copper is a constituent of **ALA synthase** enzyme required for heme synthesis.
- Being a constituent of enzyme **tyrosinase,** copper is required for synthesis of **melanin pigment**.
- Copper is required for the synthesis of **collagen** and **elastin. Lysyl oxidase,** a copper containing enzyme converts certain lysine residues to allysine needed in the formation of collagen and elastin.

Absorption and excretion
About 10% of the average daily dietary copper is absorbed mainly from the duodenum. Absorbed copper is transported to the liver bound to albumin and exported to peripheral tissues mainly (about 90%) bound to ceruloplasmin and to a lesser extent (10%) to albumin. The main route of excretion of copper is in the bile into the gut.

Plasma copper
Normal plasma concentrations are usually between **100** to **200 mg/dl** of which 90% is bound to ceruloplasmin.

Deficiency manifestation
Signs of copper deficiency include:
- ***Neutropenia*** (decreased number of neutrophils) and ***hypochromic anemia*** in the early stages.
- ***Osteoporosis*** and various bone and joint abnormalities, due to impairment in copper-dependent cross-linking of bone collagen and connective tissue.
- ***Decreased pigmentation of skin*** due to depressed copper dependent **tyrosinase** activity, which is required in the biosynthesis of skin pigment melanine.
- In the later stages ***neurological abnormalities*** probably caused by depressed **cytochrome oxidase** activity.

Inborn errors of copper metabolism
There are two inborn errors of copper metabolism:
1. Menkes syndrome
2. Wilson's disease.

Menkes syndrome or Kinky-hair disease
It is very rare, fatal, X-linked recessive disorder. The genetic defect is in absorption of copper from intestine. Both serum copper and ceruloplasmin and liver copper content are low. Clinical manifestations occur early in life and include:
- Kinky or twisted brittle hair (steely) due to loss of copper catalyzed disulfide bond formation.
- Depigmentation of the skin and hair.
- Mental retardation.

Wilson's disease
- Wilson's disease is an inborn error of copper metabolism. It is an autosomal recessive disorder in which excessive accumulation of copper occurs in tissues.

The possible causes are:
- An impairment in binding capacity of copper to ceruloplasmin or inability of liver to synthesize ceruloplasmin or both.
- An impairment in excretion of copper in bile.

Symptoms

- Accumulation of copper in liver, brain, kidney and eyes leading to copper toxicosis.
- Excessive deposition of copper in brain and liver leads to neurological symptoms and liver damage leading to cirrhosis.
- Copper deposition in kidney leads to renal tubular damage and those in cornea form yellow or brown ring around the cornea, known as **Kayser-Fleisher (KF)** rings.
- The disease is also characterized by low levels of copper and ceruloplasmin in plasma with increased excretion of copper in urine.

Treatment

The excess copper is removed from the body by treatment with penicillamine.

Fluorine (F)

In the form of fluoride, fluorine is incorporated into the structure of teeth and bone.

Dietary food sources

The body receives fluorine mainly from drinking water. Some sea fish and tea also contain small amount of fluoride.

Recommended dietary allowance per day

1.5–4 mg per day or **1–2 ppm** (since it is present in water it is expressed as ppm).

Absorption and excretion

Inorganic fluoride is absorbed readily in the stomach and small intestine and distributed almost entirely to bone and teeth. About 50% of the daily intake is excreted through urine.

Functions

Fluoride is required for the proper formation of **bone** and **teeth.** Fluoride becomes incorporated into hydroxyapatite, the crystalline mineral of bones and teeth to form **fluoroapatite.** Fluoroapatite increases hardness of bone and teeth and provides protection against dental caries and attack by acids.

Deficiency symptoms

Deficiency of fluoride leads to ***dental caries*** and ***osteoporosis.***

Toxicity

- Excessive amounts of fluoride can result in ***dental fluorosis.*** This condition results in teeth with a patch, dull white, even chalk looking appearance. A brown mottled appearance can also occur.
- It is known to ***inhibit*** several enzymes especially ***enolase*** of glycolysis.

Iodine (I_2)

The adult human body contains about **50 mg** of iodine. The blood plasma contains 4–8 µg of protein bound iodine (PBI) per 100 ml.

Dietary food sources

Seafood, drinking water, iodized table salt, onions, vegetables, etc.

Recommended dietary allowance per day

100–150 µg for adults

Functions

The most important role of iodine in the body is in the synthesis of thyroid hormones, **triiodothyronine (T_3)** and **tetraiodothyronine (T_4),** which influence a large number of metabolic functions.

Absorption and excretion

Iodine in the diet absorbed rapidly in the form of iodide from small intestine. Normally, about 1/3rd of dietary iodide is taken up by the thyroid gland, a little by the mammary and salivary glands. The rest is excreted by the kidneys.

Nearly 70–80% of iodine is excreted by the kidneys, small amounts are excreted through bile, skin and saliva. Milk of lactating women also contains some iodine.

Deficiency manifestation

Deficiency of iodine occurs in several regions of the world, where the iodine content of soil and therefore of plants is low. A deficiency of iodine in children leads to ***cretinism*** and in adults endemic ***goiter.***

- ***Cretinism:*** Severe iodine deficiency in mothers leads to intrauterine or neonatal hypothyroidism results in cretinism in their children. Cretinism is characterized by mental retardation, slow body development, dwarfism and characteristic facial structure.
- ***Goiter:*** A goiter is an enlarged thyroid with decreased thyroid hormone production. An iodine deficiency in adults stimulates the proliferation of thyroid epithelial cells, resulting in enlargement of the thyroid gland. The thyroid gland collects iodine from the blood and uses it to make thyroid hormones. In iodine deficiency, the thyroid gland undergoes compensatory enlargement in order to extract iodine from blood more efficiently.

Iron (Fe)

A normal adult possesses **3–5 gm** of iron. This small amount is used again and again in the body. Iron is called a ***one way substance,*** because very little of it is excreted. Iron is not like vitamins or most other organic or even inorganic substances which are either inactivated or excreted in course of their physiological function.

Dietary food sources

The best sources of food iron include liver, meat, egg yolk, green leafy vegetables, whole grains and cereals. There are two types of food iron:

- **Heme iron:** Iron associated with porphyrin is found in green leafy vegetables.
- **Non-heme iron:** Iron without porphyrin, and is found in meat, poultry and fish.

Recommended dietary allowance per day

- Adult men and post menopausal women: 10 mg
- Premenopausal women: 15–20 mg
- Pregnant women: 30–60 mg.

Women require greater amount than men due to the physiological loss during menstruation.

Functions

Iron is required for:

- Synthesis of heme compound like hemoglobin, myoglobin, cytochromes, catalase and peroxidase. Thus iron helps mainly in the ***transport, storage*** and ***utilization of oxygen.***
- Synthesis of non-heme iron (NHI) compounds, e.g. iron-sulfur proteins of flavoproteins, succinate dehydrogenase and NADH dehydrogenase.

Absorption **(Figure 17.2)**

The normal intake of iron is about 10–20 mg/day. Normally, about 5–10% of dietary iron is absorbed. Most absorption occurs in the duodenum.

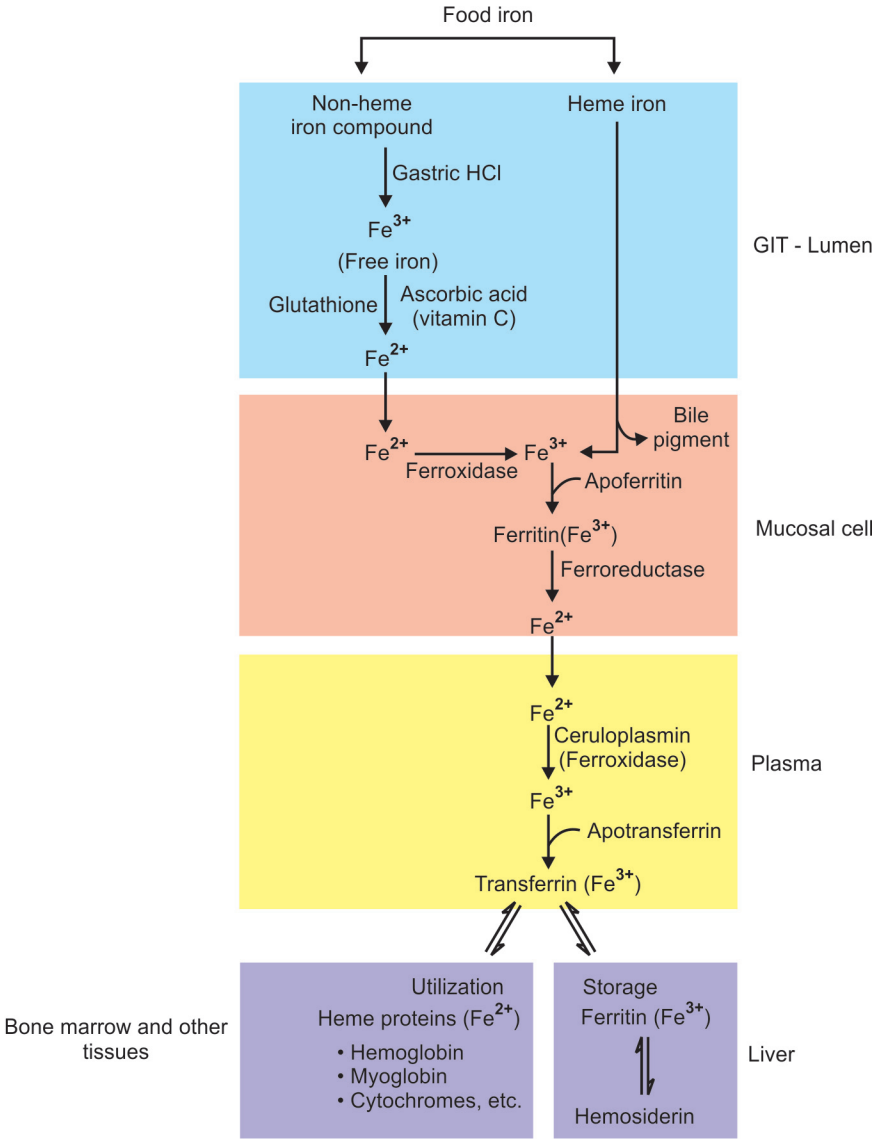


Figure 17.2: Absorption, storage and utilization of iron

- Non-heme iron bound to organic acids or proteins is absorbed in the ferrous (Fe^{2+}) state into the mucosal cell as follows:
  - The gastric acid, HCl and organic acids in the diet convert bound non-heme compound of the diet into **free ferric (Fe^{3+})** ions.
  - These free ferric ions are reduced with ascorbic acid and glutathione of food to more soluble ferrous (Fe^{2+}) form which is more readily absorbed.
  - After absorption Fe^{2+} is oxidized in mucosal cells to Fe^{3+} by the enzyme **Ferroxidase,** which then combines with **aproferritin** to form **ferritin.** Ferritin is a temporary storage form of iron.
- Heme of food is absorbed as such by the intestinal mucosal cells. It is subsequently broken down and iron is released with the cells.

Transport

- The transfer of iron from the storage ferritin (Fe^{3+} form) to plasma involves reduction of Fe^{3+} to Fe^{2+} in the mucosal cell with the help of ***ferroreductase***.
- Fe^{2+} then enters the plasma where it is reoxidized to Fe^{3+} by a copper protein, ***ceruloplasmin*** (ferroxidase).
- Fe^{3+} is then incorporated into **transferrin** by combining with **apotransferrin**.
- Apotransferrin is a specific iron binding protein. Each apotransferrin can bind with two Fe^{3+} ions.

Storage

Iron in plasma is taken up by cells and either incorporated into heme or stored as ***ferritin*** or ***hemosiderin.*** Storage of iron occurs in most cells but predominantly in cells of liver, spleen and bone marrow.

- Ferritin is the major iron storage compound and readily available source of iron. Each apoferritin molecule can take up about 4500 **iron atoms.**
- In addition to storage as ferritin, iron can also be found in a form of ***hemosiderin***. The precise nature of hemosiderin is unclear. Normally very little hemosiderin is to be found in the liver, but the quantity increases during iron overload.

Excretion

- Iron is not excreted in the urine, but is lost from the body via the ***bile, feces*** and in ***menstrual blood***.
- Iron excreted in the feces is exogenous, i.e. dietary iron that has not been absorbed by the mucosal cells is excreted in the feces.
- In male, there is an average loss of endogenous iron of about 1 mg/day through desquamated cells of the skin and the intestinal mucosa.
- Females may have additional losses due to menstruation or pregnancy.

Factors affecting iron absorption

- **State of iron stores in the body:** Absorption is increased in iron deficiency and decreased when there is iron overload.
- **Rate of erythropoiesis** (the process of red blood cell production). When rate of erythropoiesis is increased, absorption may be increased even though the iron stores are adequate or overloaded.
- **The contents of the diet:** Substances that form soluble complexes with iron, e.g. ascorbic acid (vitamin C) facilitates absorption. Substances that form insoluble complexes, e.g. phosphate, phytates and oxalates inhibit absorption.
- **Nature of gastrointestinal secretions and the chemical state of the iron:** Iron in the diet does not usually become available for absorption unless released in free form during digestion. This depends partly on gastric acid (HCl) production. Ferrous (Fe^{2+}) is more readily absorbed than ferric form (Fe^{3+}) and the presence of HCl, helps to keep iron in the Fe^{2+} form.

Disorders of iron metabolism

Iron deficiency and **iron overload** are *the major disorders of iron metabolism.*

Iron deficiency

A deficiency of iron causes a reduction in the rate of hemoglobin synthesis and erythropoiesis, and can result in **iron deficiency anemia**.

Iron deficiency anemia is the commonest of all single nutrient deficiencies. The main causes are:

- **Deficient intake:** Including reduced bioavailability of iron due to dietary fiber, phytates, oxalates, etc.
- **Impaired absorption:** For example, intestinal malabsorptive disease and abdominal surgery.
- **Excessive loss:** For example, menstrual blood loss in women and in men from gastrointestinal bleeding (in peptic ulcer, diverticulosis or malignancy).

Iron deficiency causes low hemoglobin resulting in ***hypochromic microcytic anemia*** in which the size of the red blood cells are much smaller than normal and have much reduced hemoglobin content.

Clinical features of anemia: Weakness, fatigue, dizziness and palpitation. Nonspecific symptoms are nausea, anorexia, constipation, and menstrual irregularities. Some individuals develop pica, a craving for unnatural articles of food such as clay or chalk.

Iron overload

Hemosiderosis and hemochromatosis are the conditions associated with iron overload.

- ***Hemosiderosis:*** Hemosiderosis is a term that has been used to imply an increase in iron stores as hemosiderin without associated tissue injury. Hemosiderosis is an initial stage of iron overload.
- ***Hemochromatosis:*** Hemochromatosis is a clinical condition in which excessive deposits of iron in the form of hemosiderin are present in the tissues, **with injury** to involved organs as follows:
  - **Liver:** Leading to cirrhosis
  - **Pancreas:** Leading to fibrotic damage to pancreas with diabetes mellitus
  - **Skin:** Skin pigmentation, bronzed diabetes
  - **Endocrine organ:** leading to hypothyroidism, testicular atrophy
  - **Joints:** Leading to arthritis
  - **Heart:** Leading to arrhythmia and heart failure.

Manganese (Mn)

The adult human body contains about **15–20 mg** of manganese. The liver and kidney are rich in Mn. Mn mainly found in the nuclei, where it gives stability to the nucleic acid structure.

Dietary food sources

Meat (liver and kidney), wheat germs, legumes and nuts.

Recommended dietary allowance per day

2.5–5.0 mg.

Functions

- Manganese acts as a cofactor or activator of many enzymes such as arginase, pyruvate carboxylase, glucosyl transferase, mitochondria superoxide dismutase, decarboxylase, etc.
- Manganese is required for synthesis of glycoproteins, proteoglycans, Hb, and cholesterol.
- Manganese is required for the physical growth and reproductive functions.
- It plays important role in formation of connective and bony tissue.
- Manganese also functions with vitamin K in the formation of prothrombin.

Absorption and excretion

Dietary manganese is absorbed poorly from the small intestine. Most of the manganese is excreted rapidly in the bile and pancreatic secretion in the feces.

Deficiency manifestation

Because of wide distribution of manganese in plant and animal foods, the deficiency of manganese is not known in humans. However, in animals manganese deficiency leads to sterility and bone deformities.

Molybdenum (Mo)

Dietary food sources

Liver and kidney are good meat sources, whole grains, legumes and leafy vegetables serve as vegetable sources.

Recommended dietary allowance per day

0.15–0.5 mg.

Functions

Molybdenum is a constituent of the following enzymes:

- Xanthine oxidase
- Aldehyde oxidase
- Sulphite oxidase.

Absorption and excretion

Dietary molybdenum is readily absorbed by the intestine and is excreted in urine and bile.

Deficiency manifestation

Deficiency of molybdenum has been reported to cause ***xanthinuria*** with low plasma and urinary uric acid concentration.

Selenium (Se)

Dietary food sources

Liver, kidney, seafoods and meat are good sources of selenium. Grains have a variable content depending on the region where they are grown.

Recommended dietary allowance per day

50–200 µg for normal adults.

Functions

- Selenium functions as an ***antioxidant*** along with vitamin E.
- Selenium is a constituent of ***glutathione peroxidase***. Glutathione peroxidase has a cellular ***antioxidant*** function, that protects cell membrane, against oxidative damage by H_2O_2 and a variety of hydroperoxides.
- Selenium, as a constituent of glutathione peroxidase is important in preventing lipid peroxidation and protecting cells against superoxide (O_2^-) and some other free radicals.
- Selenium also is a constituent of ***iodothyronine deiodinase,*** the enzyme that converts thyroxine to triiodothyronine.

Absorption and excretion

The principal dietary forms of selenium ***selenocysteine*** and **selenomethionine** are absorbed from gastrointestinal tract. Selenium homeostasis is achieved by regulation of its excretion via urine.

Deficiency manifestations

Selenium deficiency has been associated in some areas of China with ***Keshan disease***, a cardiomyopathy, that primarily affects children and women of child bearing age. Its most common symptoms include dizziness, loss of appetite, nausea, abnormal electrocardiograms, and congestive heart failure.

Selenium toxicity (selenosis)

Excessive selenium intake results in ***alkali disease***, characterized by loss of hair and nails, skin lesions, liver and neuromuscular disorders that are usually fatal.

Zinc (Zn)

Total zinc content of the adult body is about 2 gm. In blood, RBCs contain very high concentration of zinc as compared to plasma.

Dietary food sources

Meat, liver, seafoods, and eggs are good sources. Milk including breast milk also is a good source of zinc. ***The colostrum is an especially rich source.***

Recommended dietary allowance per day

15 mg per day for adults with an additional 5 mg during pregnancy and lactation.

Functions

- Zinc is a constituent of a number of enzymes. For example,
  - Carbonic anhydrase
  - Alkaline phosphatase
  - DNA and RNA-polymerases
  - Porphobilinogen (PBG) synthase of heme synthesis.
- Because it is required by many of the enzymes needed for DNA and RNA synthesis, ***zinc is necessary for the growth and division of cells.***
- Zinc is an important element in wound healing as it is a necessary factor in the biosynthesis and integrity of connective tissue.
- Zinc stabilizes structure of protein and nucleic acids.
- Zinc is required for the secretion and storage of insulin from the β-cells of pancreas.
- Gustin, a Zn containing protein present in saliva is required for the development and functioning of taste buds. Therefore, zinc deficiency leads to loss of taste acuity.

Absorption and excretion

Approximately 20–30% of ingested dietary zinc is absorbed in small intestine. It is transported in blood plasma mostly by albumin and α_2-macroglobulin. Zinc is excreted in urine, bile, in pancreatic fluid and in milk in lactating mothers.

Deficiency manifestation

Zinc deficiency has many causes, but malnutrition and malabsorption are the most common. Clinical symptoms of zinc deficiency include:

- Growth failure
- Hair loss
- Anemia
- Loss of taste sensation
- Impaired spermatogenesis
- Neuropsychiatric symptoms.

Acrodermatitis enteropathica: A rare inherited disorder of zinc metabolism is due to an inherited defect in zinc absorption that causes low plasma zinc concentration and reduced total body content of zinc, it is manifested in infancy as skin rash.

SUMMARY

- Minerals are inorganic elements, having vital structural and functional roles in the human body. They are classified into two groups, macrominerals (that are required in amounts greater than 100 mg/day) and microminerals or trace elements (that are required in amounts less than 100 mg/day).
- The macrominerals include, calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfur.
- The microminerals or trace element required by humans include chromium, cobalt, copper, fluoride, iodine, iron, manganese, molybdenum, selenium and zinc.
- Sodium is the major cation of extracellular fluids, while potassium is of intracellular fluids. Chloride is principal anion of the body.
- Sodium, potassium and chloride have important role in maintaining osmotic pressure, water and electrolyte balance.
- Calcium is the most abundant mineral in the body. About 99% of the body's calcium is present in bone together with phosphorus as the mineral hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$].
- Homeostasis of plasma calcium is dependent on the function of three main organs, bone, kidney and intestine, and three main hormones, PTH, vitamin D and calcitonin.

- Phosphorus occurs predominantly in the skeleton, but is also involved in acid-base balance, energy storage and transfer reactions and enzyme activity.
- Magnesium occurs both in bone and a cofactor for enzymes.
- Sulfur is a constituent of proteoglycans, bile acid and involved in detoxication.
- Chromium potentiates the effect of insulin.
- Cobalt is a constituent of vitamin B_{12}.
- Copper is a constituent of oxidase enzymes and required for iron absorption and mobilization.
- Fluoride is required for proper formation of bone and teeth and increases hardness of bone and teeth.
- Iodine is a constituent of thyroid hormone (T_4 and T_3).
- Iron is a constituent of heme and non-heme compounds and thus involved in transport and storage of oxygen.
- Selenium, manganese, molybdenum and zinc are involved in the function of several enzymes.

EXERCISE

Solve

Case History 1

A patient in the hospital, had seizures and usually appeared weak and tired. Physical finding was deposition of copper in the eyes as brown pigment (the Kayser-Fleisher ring) and hepatomegaly. A diagnosis of Wilson's disease was made.

Questions

1. What is the biochemical problem in Wilson's disease?
2. Name two copper containing enzymes.
3. Give functions and sources of copper.

Case History 2

A 35-year-old man, who required total intravenous feeding (with no assessment of his trace metal status), for four months, developed a skin rash, with accompanying hair loss, reduced taste acuity and delayed wound healing. He was clearly diagnosed zinc deficient.

Questions

1. Give food sources of zinc.
2. RDA for zinc.
3. Functions of zinc.
4. Name two enzymes having zinc as a constituent.

Case History 3

A 40-year-old woman complains of tiredness and appears pale. She is experiencing a heavy and prolonged monthly menstrual flow and her hemoglobin concentration is 90 g/L (normal range 120–160 g/L).

Questions

1. What is your probable diagnosis?
2. How can the complaints be relieved?
3. Give RDA and factors affecting absorption of the deficient biochemical substance.

Multiple Choice Questions (MCQs)

1. Normal serum sodium level is:
a) 135–145 mEq/L b) 150–160 mEq/L
c) 120–130 mEq/L d) 170–180 mEq/L

2. In wound healing the following trace element is involved:
a) Iron b) Copper
c) Zinc d) Selenium

3. The mineral having sparing action of vitamin E:
a) Chromium b) Iron
c) Iodine d) Selenium

4. Wilson's disease is characterized by impaired:
a) Copper excretion into bile
b) Reabsorption of copper in the kidney
c) Hepatic incorporation of copper into ceruloplasmin
d) All of the above

5. Glutathione peroxidase contains:
a) Calcium b) Iron
c) Selenium d) Chromium

6. Hemochromatosis is due to excessive deposition of:
a) Iron in the form of hemosiderine
b) Copper
c) Zinc
d) Iodine

7. Transferrin is involved in:
a) Hormone metabolism
b) Diagnosis of Wilson's disease
c) Transport of iron
d) Transport of bilirubin

8. The major storage form of iron is:
a) Transferrin
b) Ceruloplasmin
c) Ferritin
d) Hemosiderin

9. The element that prevents the development of dental caries:
a) Fluorine b) Calcium
c) Phosphorus d) Selenium

10. Carbonic anhydrase contains mineral:
a) Copper b) Iodine
c) Zinc d) Iron

11. Molybdenum is a constituent of all of the following, *except*:
a) Xanthine oxidase
b) Aldehyde oxidase
c) Sulphite oxidase
d) Cytochrome oxidase

12. Transport form of iron is:
a) Transferrin b) Ferritin
c) Hemosiderin d) Ceruloplasmin

13. Iodine is required for the formation of:
a) Vitamin B_{12} b) Thyroxine
c) Insulin d) Calcitonin

14. Which of the following minerals is known as glucose tolerance factor (GTF)?
a) Chromium b) Cobalt
c) Calcium d) Copper

15. Intestinal absorption of iron is enhanced by:
a) Phytic acid b) Ascorbic acid
c) Oxalic acid d) Alkaline pH

16. Element called "one way substance" is:
a) Iodine b) Iron
c) Copper d) Calcium

17. Which of the following minerals stimulates secretion of PTH?
a) Iodine b) Magnesium
c) Copper d) Sodium

Correct Answers for MCQs

1-a	2-c	3-d	4-d
5-c	6-a	7-c	8-c
9-a	10-c	11-d	12-a
13-b	14-a	15-b	16-b
17-b			

CHAPTER

18

Hemoglobin Metabolism

INTRODUCTION

Heme is the prosthetic group of several proteins and enzymes including hemoglobin, myoglobin, cytochrome, cytochrome P_{450}, enzymes like catalase, certain peroxidase and tryptophan pyrrolase. Heme is synthesized from **porphyrin** and **iron.** Porphyrin ring is coordinated with an atom of **iron** to form heme **(Figure 18.1)**.

- Porphyrins are cyclic molecule formed by the linkage of 4-pyrrole rings through methen bridges.
- Eight side chains serve as substituents on the porphyrin ring, two on each pyrrole.
- These side chains may be acetyl (A), propionyl (P), methyl (M) or vinyl (V) groups.
- The side chains of the porphyrin can be arranged in four different ways. Designated by Roman numerals I to IV. Only type III isomer is physiologically important in humans.

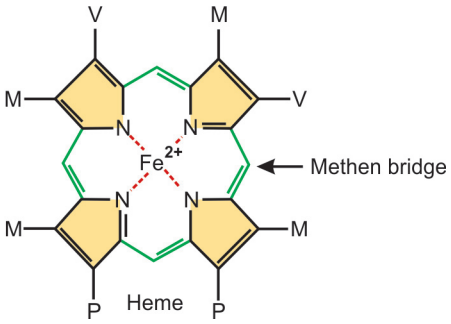


Figure 18.1: Structure of heme molecule

SYNTHESIS OF HEME

Heme synthesis takes place in all cells, but occurs to the greatest extent in the ***bone marrow*** and **liver**.

Stages of Heme Synthesis

Biosynthesis of heme may be divided into three stages **(Figure 18.2)**:

1. Biosynthesis of **δ-aminolevulinic acid (ALA)** from the precursor glycine and succinyl-CoA
2. Formation of **porphobilinogen (PBG)** from δ-aminolevulinic acid
3. Formation of porphyrins and heme from porphobilinogen.

Biosynthesis of δ-Aminolevulinic Acid (δ-ALA)

- The first step in the biosynthesis of porphyrins is the condensation of glycine and succinyl-CoA to form **δ-aminolevulinic acid,** which occurs in mitochondria. This reaction is catalyzed by **δ-aminolevulinic acid synthase (δ-ALAS)** and PLP (pyridoxal phosphate) is also necessary in this reaction. **This is the rate controlling step in heme synthesis.**

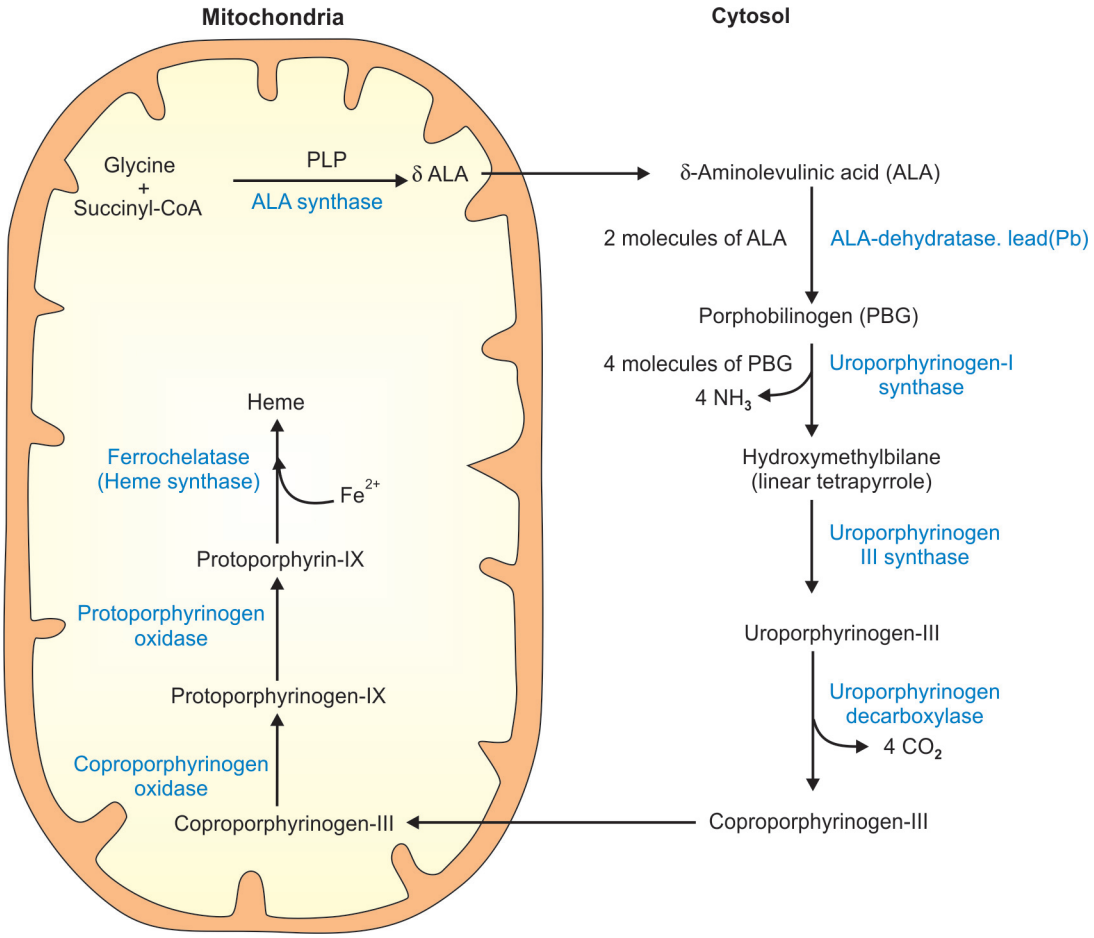


Figure 18.2: Biosynthetic pathway of heme

Formation of Porphobilinogen (PBG)

- In the cytosol, two molecules of δ-ALA condense to form **porphobilinogen.** This reaction is catalyzed by ***δ-aminolevulinate dehydratase,*** also called ***porphobilinogen synthase (PBGS),*** which is a ***zinc*** containing ***enzyme.***
- δ-aminolevulinate dehydratase enzyme is inhibited by relatively low concentrations of lead (Pb). This accounts for the large excretion of ALA in the urine of a person who is affected with lead poisoning.

Indeed, ***the quantitative determination of ALA in urine is one of the better analytical means of monitoring the severity and control of lead poisoning in human subjects.***

Formation of Porphyrins and Heme

- Four porphobilinogens condense head-to-tail to form a linear tetrapyrrole, hydroxy-methylbilane. The reaction is catalyzed by ***uroporphyrinogen-I synthase,*** also known as ***PBG deaminase.***
- Hydroxymethylbilane cyclizes to form uroporphyrinogen-III by the action of ***uroporphyrinogen-III synthase.*** *At this point basic ring structure (porphyrin skeleton) is formed.* Under normal conditions, the uroporphyrinogen formed is almost ***exclusively the III isomer*** but in certain of the porphyrias (discussed later), the type I isomers of porphyrinogens are formed in excess.
- Uroporphyrinogen-III is converted to coproporphyrinogen III by decarboxylation. The reaction is catalyzed by **uroporphyrinogen decarboxylase.**
- Coproporphyrinogen-III then enters the mitochondria, where it is converted to **protoporphyrinogen-IX** by the mitochondrial enzyme **coproporphyrinogen oxidase.**
- ***This enzyme is able to act only on type-III coproporphyrinogen; that is why type-I protoporphyrins do not generally occur in nature.***

- The oxidation of protoporphyrinogen-IX to protoporphyrin-IX is catalyzed by another mitochondrial enzyme, **protoporphyrinogen oxidase.**
- The final step in heme synthesis involves the incorporation of **ferrous iron** into protoporphyrin-IX in a reaction catalyzed by mitochondrial ***heme synthase*** or ***ferrochelatase***.

Regulation of Heme Synthesis

- ***δ-aminolevulinic acid synthase (ALAS),*** a ***mitochondrial allosteric enzyme*** that catalyzes first step of heme biosynthetic pathway, is a regulatory enzyme. It is feedback inhibited by ***heme***.
- Regulation also occurs at the level of enzyme synthesis. Increased level of heme represses the synthesis of δ-aminolevulinic acid synthase.

DISORDER OF HEME BIOSYNTHESIS

Porphyrias

Porphyrias are rare inherited (or occasionally acquired) disorder due to deficiencies of enzymes in heme synthesis. This leads to accumulation and increased excretion of ***porphyrins*** or ***porphyrin precursors*** (ALA and PBG).

Classification of porphyria

The hereditary porphyrias are classified into two categories on the basis of the organs or cell that are mostly affected. Thus, porphyria are classified into:

1. **Erythropoietic porphyria :** Enzyme deficiency occur in erythropoietic cells of the bone marrow.
2. **Hepatic porphyria :** Enzyme deficiency occur in hepatic cell.

Different types of hereditary porphyrias that fall into these two classes are given in **Table 18.1.**

Clinical symptoms of porphyrias

- Acute abdominal pain
- Neuropsychiatric symptoms
- Photosensitivity and skin lesions (in some porphyrias only).

Where the enzyme deficiency occurs early in the pathway prior to the formation of porphyrinogen, ALA and PBG will accumulate in the body tissues and fluids. These compounds can impair the function of abdominal nerve and central nervous system, resulting in **abdominal pain** and **neuropsychiatric** symptoms. The "madness" of George III, king of England during the American Revolution, is believed to have been due to this porphyria.

On the other hand, enzyme deficiency occurs later in the pathway, results in the accumulation of the **porphyrinogens** which on exposure to light auto-oxidized to corresponding porphyrin derivatives, causes ***photosensitivity*** and ***skin lesions.***

Acquired porphyria

- The most common acquired form of porphyria is due to lead poisoning. **δ-ALA dehydratase** and **ferrochelatase** are inactivated by lead.
- Thus, in lead poisoning, δ-ALA and protoporphyrin, accumulate, and the production of heme is decreased.
- Anemia results from lack of hemoglobin and energy production decreases due to lack of cytochromes required for the electron transport chain.

BREAKDOWN OF HEMOGLOBIN

After approximately 120 days, red blood cells are degraded by reticuloendothelial (RE) system, particularly in the liver and spleen.

- First hemoglobin is dissociated into heme and globin.

Table 18.1: Types of porphyrias

Classes	*Types*	*Enzyme defect*	*Signs and symptoms*
Hepatic	Acute intermittent porphyria	Uroporphyrinogen-I synthase	Abdominal pain, neuropsychiatric symptoms
Erythropoietic	Congenital erythropoietic	Uroporphyrinogen-III synthase	Photosensitivity
Hepatic	Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Photosensitivity
Hepatic	Hereditary coproporphyria	Coproporphyrinogen oxidase	Photosensitivity, abdominal pain neuropsychiatric symptoms
Hepatic	Variegate porphyria	Protoporphyrinogen oxidase	Photosensitivity, abdominal pain neuropsychiatric symptoms
Erythropoietic	Protoporphyria	Ferrochelatase	Photosensitivity

- Globin is degraded to its constituent amino acids, which are reused.
- The catabolism of heme is carried out in the microsomal fractions of cells by a complex enzyme system called ***heme oxygenase***, in the presence of NADPH and O_2. Ferric ion and carbon monoxide (CO) are released with production of the green pigment **biliverdin**.
- Biliverdin is reduced to red orange colored **bilirubin**. This reaction is catalyzed by an NADPH dependent **biliverdin reductase (Figure 18.3).** Approximately, 250 to 350 mg of bilirubin is produced per day in human adults. Bilirubin and its derivatives are collectively termed ***bile pigments***.

FATE OF BILIRUBIN

The further metabolism and excretion of bilirubin occurs in the **liver** and **intestine.** It can be divided into four processes:

1. Uptake of bilirubin by liver
2. Conjugation of bilirubin in the liver
3. Secretion of conjugated bilirubin into the bile
4. Excretion of bilirubin.

Uptake of Bilirubin by Liver

- Since bilirubin is hydrophobic and insoluble in aqueous plasma, it is transported to the liver by binding noncovalently to **plasma albumin**.

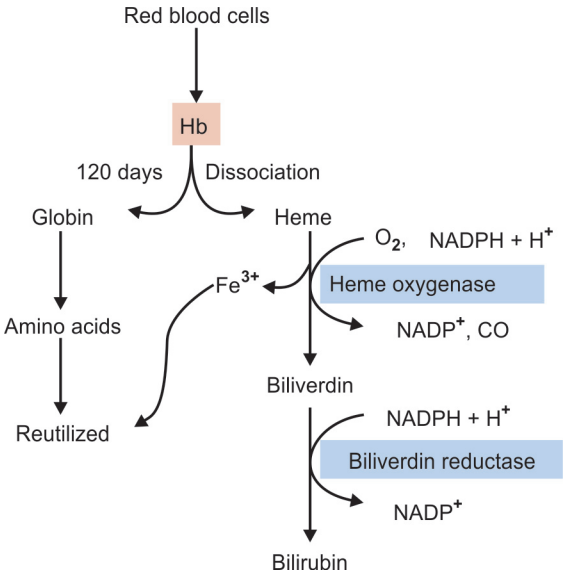


Figure 18.3: Catabolic pathway of hemoglobin

- In the liver, the bilirubin is removed from albumin and taken up by hepatocytes.

Conjugation of Bilirubin

- Hepatocytes convert insoluble bilirubin to a soluble form by conjugation with two molecules of glucuronate supplied by UDP-glucuronate. This reaction is catalyzed by **bilirubin glucuronyl transferase**.
- Bilirubin monoglucuronide is an intermediate and is subsequently converted to the bilirubin diglucuronide **(Figure 18.4)**.

Secretion of Bilirubin into Bile

Bilirubin diglucuronide (conjugated bilirubin) formed in the liver is secreted in the bile and is a rate limiting step for the entire process of hepatic bilirubin metabolism. Unconjugated bilirubin is not secreted into bile.

Excretion of Bilirubin

- Following secretion into bile conjugated bilirubin passes through the hepatic and common bile ducts into the intestinal lumen.
- Bilirubin diglucuronide is hydrolyzed in the intestine by bacterial enzymes ***β-glucuronidase*** to liberate free bilirubin. The free bilirubin so formed is further reduced to a colorless **urobilinogen.** A part of which absorbed from the gut into the portal circulation **(Figure 18.5)**.
- The urobilinogen which is absorbed into portal circulation can take two alternative routes.
  1. A part of it enters the systemic circulation and transported to the kidneys, where it is oxidized to **urobilin** (orange yellow pigment) and excreted in urine. The normal umber yellow color of urine is due to urobilin.

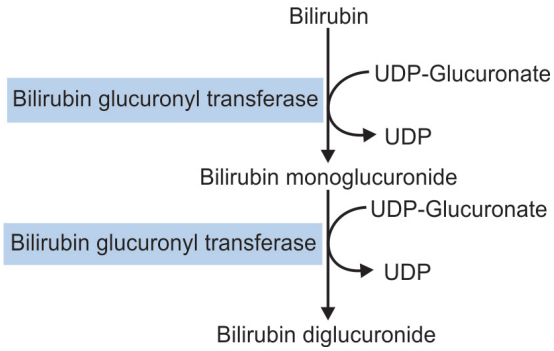


Figure 18.4: Conjugation of bilirubin with glucuronic acid in the liver

2. A part of the urobilinogen is returned to the liver and re-excreted through liver to the intestine, known as **enterohepatic urobilinogen cycle.**

- The major portion of urobilinogen which remains within intestinal lumen is reduced further in the intestine to **stercobilinogen,** which is excreted as an oxidized brown pigment **stercobilin** in the feces. The characteristic brown color of stool is due to stercobilin. **Figure 18.5** shows the four major processes involved in the metabolism of bilirubin.

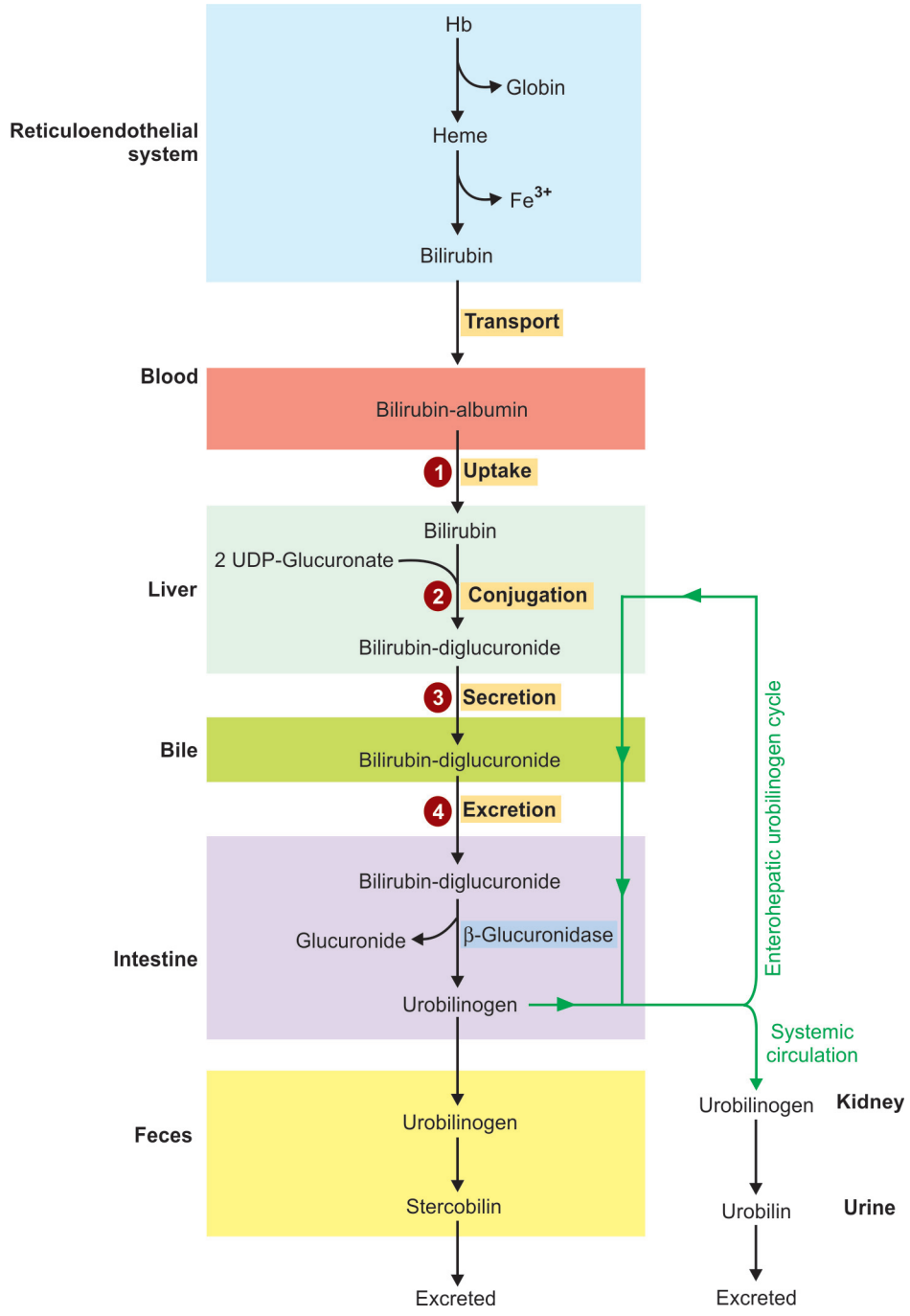


Figure 18.5: Schematic representation of normal bilirubin metabolism

Serum Bilirubin

The normal concentration of serum bilirubin is:

1 Total bilirubin = 0.1 to 1.0 mg/dL
2 Conjugated (direct) bilirubin = 0.1 to 0.4 mg/dL
3 Unconjugated (indirect) bilirubin = 0.2 to 0.7 mg/dL.

JAUNDICE

When bilirubin in blood exceeds 1mg/dL is called **hyperbilirubinemia** and when it reaches a certain concentration approximately 2.2 to 5 mg/dL it diffuses into the tissues. The skin and sclera appear yellowish due to deposition of bilirubin in the tissues. This clinical condition is called **jaundice** (French : jaune = yellow) or **icterus.**

Classification of Jaundice

Jaundice can be classified into three types:

1. Hemolytic or prehepatic
2. Hepatocellular or hepatic
3. Obstructive or posthepatic.

Prehepatic or hemolytic jaundice

- In prehepatic or hemolytic jaundice, there is increased breakdown of hemoglobin to bilirubin at a rate in excess of the ability of the liver cell to conjugate and excrete it. Excess hemolysis may be due to:
  - Sickle hemoglobin
  - Deficiency of enzyme glucose-6-phosphate dehydrogenase
  - Incompatible blood transfusion.
- In hemolytic jaundice, more than normal amounts of bilirubin are excreted into the intestine, resulting in an increased amount of **urobilinogen** in feces and urine.
- The main biochemical features of the hemolytic jaundice are:
  - Increased plasma concentration of unconjugated bilirubin
  - Increased amount of urobilinogen in urine and feces
  - Absence of bilirubin in the urine. Since the excess bilirubin is unconjugated, it is not excretable in the urine.

Hepatocellular or hepatic jaundice

- In this kind of jaundice, there is some disorder of the liver cells. Hepatic parenchymal cell damage impairs uptake and conjugation of bilirubin and results in ***unconjugated hyperbilirubinemia.*** Liver damage is usually caused by:
  - Infections (viral hepatitis)
  - Toxic chemicals (such as alcohol, chloroform, carbon tetrachloride, etc.)
  - Drugs
  - Cirrhosis.
- Patients with jaundice due to hepatocellular damage commonly have obstruction of the liver biliary tree that leads to increased plasma level of conjugated bilirubin also.
- The main biochemical features of hepatocellular jaundice are:
  - Increased plasma concentration of conjugated and unconjugated bilirubin
  - Decreased amount of urobilinogen in urine and feces
  - Presence of bilirubin in the urine
  - Raised level of alanine transaminase (ALT) and aspartate transaminase (AST) enzymes.

Posthepatic or obstructive jaundice

- This occurs when there is an obstruction in the common bile duct that prevents the passage of conjugated bilirubin from the liver cells to the intestine. The obstruction may be due to:
  - Blockage of the common bile duct by gallstones
  - Carcinoma of the head of the pancreas
  - Carcinoma of the duct itself.
- The main biochemical manifestations of obstructive jaundice are:
  - Increased plasma concentration of conjugated bilirubin
  - Absence of urobilinogen in feces and urine
  - The presence of bilirubin and bile salts in the urine
  - Raised level of plasma alkaline phosphatase (ALP). ALP is normally excreted through bile. Obstruction to the flow of bile causes regurgitation of enzyme into the blood resulting in increased serum concentration.

Table 18.2 summarizes laboratory findings in the differential diagnosis of jaundice.

Neonatal or Physiologic Jaundice

- Mild jaundice in the first few days after birth is common and physiological.
- It results from an **increased hemolysis** and **immature liver enzyme system** for conjugation of bilirubin in the new born.
- The liver of new borns is deficient in enzyme ***UDP-glucuronyl transferase,*** necessary for conjugation.
- The enzyme deficiency is more in premature infants.

Table 18.2: Laboratory findings in the differential diagnosis of jaundice

Condition	Serum bilirubin		Urine urobilinogen	Urine bilirubin	Fecal urobilinogen
	Conjugated (Direct)	Unconjugated (Indirect)			
Normal	0.1-0.4 mg/dL	0.2-0.7 mg/dL	0.4 mg/24 h	Absent	40-280 mg/24 h
Hemolytic	Normal	Increased	Increased	Absent	Increased
Hepatocellular	Increased	Increased	Decreased	Present	Decreased
Obstructive	Increased	Normal	Absent	Present	Trace to absent

- Since the increased bilirubin is unconjugated, it is capable of penetrating the blood-brain barrier when its concentration in plasma exceeds 20 to 25 mg/dL. This results in a ***hyperbilirubinemic toxic encephalopathy*** or ***kernicterus,*** which can cause mental retardation.

INHERITED HYPERBILIRUBINEMIAS

Gilbert's syndrome

Gilbert's syndrome, is an inherited disease characterized by mild benign (harmless) unconjugated hyperbilirubinemia due to:

- Impaired hepatic uptake of bilirubin
- Partial conjugation defect due to reduced activity of **UDP-glucuronyl transferase**.

Crigler-Najjar syndrome

This is a rare autosomal recessive disorder due to deficiency of **hepatic glucuronyl transferase** enzyme. There are two forms of this condition:

- **Type-I** is characterized by complete absence of the conjugating enzyme **glucuronyl transferase** and therefore no conjugated bilirubin is formed. It causes severe jaundice with kernicterus and early death.
- **Type-II** is a less severe form in which the enzyme deficiency is partial and is compatible with more prolonged survival.

Dubin-Johnson syndrome

This is harmless autosomal recessive disorder and is due to defective hepatic secretion of **conjugated bilirubin** into the bile and is characterized by slightly raised plasma conjugated bilirubin level. It is characterized by abnormal black pigment in the hepatocytes, imparting a dark brown to black color to the liver.

SUMMARY

- Biosynthesis of heme occurs in mitochondria and cytosol. It is synthesized from glycine and succinyl-CoA.
- δ-aminolevulinic acid synthase, the enzyme catalyzing the committed step in heme synthetic pathway is feedback inhibited by heme.
- The porphyrias are a group of disorders (genetic or acquired) due to abnormalities in the pathway of biosynthesis of heme.
- Heme is degraded by monoxygenase that converts it into biliverdin. Reduction of biliverdin yields bilirubin.
- Bilirubin is transported by albumin from peripheral tissues to the liver.
- In the liver, bilirubin is made water soluble by conjugation with two molecules of glucuronic acid and is secreted into the bile as bilirubin diglucuronide. The action of bacterial enzymes in the gut produces urobilinogen and stercobilinogen, which are excreted in the urine and feces respectively.
- Hyperbilirubinemia causes jaundice. Jaundice can be classified according to cause as hemolytic (prehepatic), hepatocellular (hepatic), and obstructive (posthepatic).
- Measurement of plasma total and unconjugated bilirubin, urinary urobilinogen and bilirubin help in the differential diagnosis of jaundice.

EXERCISE

Solve

Case History 1

A 53-year-old woman developed a hyperpigmentation and rash on her neck and photosensitive nature, a diagnosis of porphyria cutanea tarda was considered.

Questions

1. What is porphyria?
2. What are the types of porphyria?
3. Which enzyme is defective in porphyria cutanea tarda?

Case History 2

A 45-year-old woman complains of acute abdominal pain and vomiting following fatty food. The biochemical investigations are:

a. Raised serum conjugated bilirubin.
b. Significantly raised serum alkaline phosphatase.
c. Excretion of dark yellow colored urine.
d. Fouchet's test on fresh urine shows green color.

Questions:

a. Name the disease.
b. Give differential diagnosis of the condition.

Multiple Choice Questions (MCQs)

1. **Acute intermittent porphyria is accompanied by increased urinary excretion of:**
   a) Porphobilinogen
   b) Heme
   c) Bilirubin
   d) Biliverdin
2. **The normal brown red color of feces results from the presence of:**
   a) Stercobilin
   b) Bilirubin
   c) Biliverdin
   d) Bilirubin diglucuronide
3. **The end product of catabolism of heme is:**
   a) Bile acids
   b) Bile salts
   c) Bile pigment
   d) Uric acid
4. **Unconjugated bilirubin is raised mostly in:**
   a) Hemolytic jaundice
   b) Obstructive jaundice
   c) Carcinoma of pancreas
   d) Stone in gallbladder
5. **The rate limiting reaction in heme synthesis is catalyzed by:**
   a) δ-ALA dehydratase
   b) δ-ALA synthase
   c) Ferrochelatase
   d) Uroporphyrinogen decarboxylase

Correct Answers for MCQs

1-a 2-a 3-c 4-a 5-b

CHAPTER

19

Nucleic Acid Metabolism

INTRODUCTION

Purines and pyrimidines are **dietary nonessential** components. Dietary nucleic acids and nucleotides do not provide essential constituents for the biosynthesis of endogenous nucleic acids. Humans can synthesize purine and pyrimidine nucleotides *de novo*, i.e. from amphibolic intermediates.

BIOSYNTHESIS OF PURINE NUCLEOTIDES

- The two purine nucleotides of nucleic acids are:
  1. Adenosine monophosphate, AMP
  2. Guanosine monophosphate, GMP
- Purine nucleotides can be synthesized by two pathways:
  1. ***De novo pathway*** (New synthesis from amphibolic intermediates).
  2. **Salvage pathway**.

DE NOVO BIOSYNTHESIS OF PURINE NUCLEOTIDES

In de novo pathway, the purine ring formed from variety, of precursors **(Figure 19.1)** is assembled on ribose-5-phosphate.

Precursors for the De Novo *Synthesis of Purine*

- Glycine provides C_4, C_5 and N_7

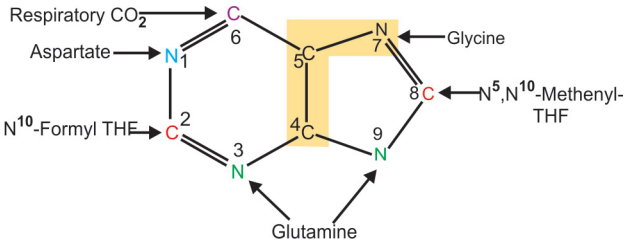


Figure 19.1: Source of carbon and nitrogen atoms in the purine ring

- Aspartate provides N_1
- Glutamine provides N_3 and N_9
- Tetrahydrofolate derivatives furnish C_2 and C_8
- Carbon dioxide provides C_6.

Major Steps of De Novo *Synthesis of Purine Nucleotides*

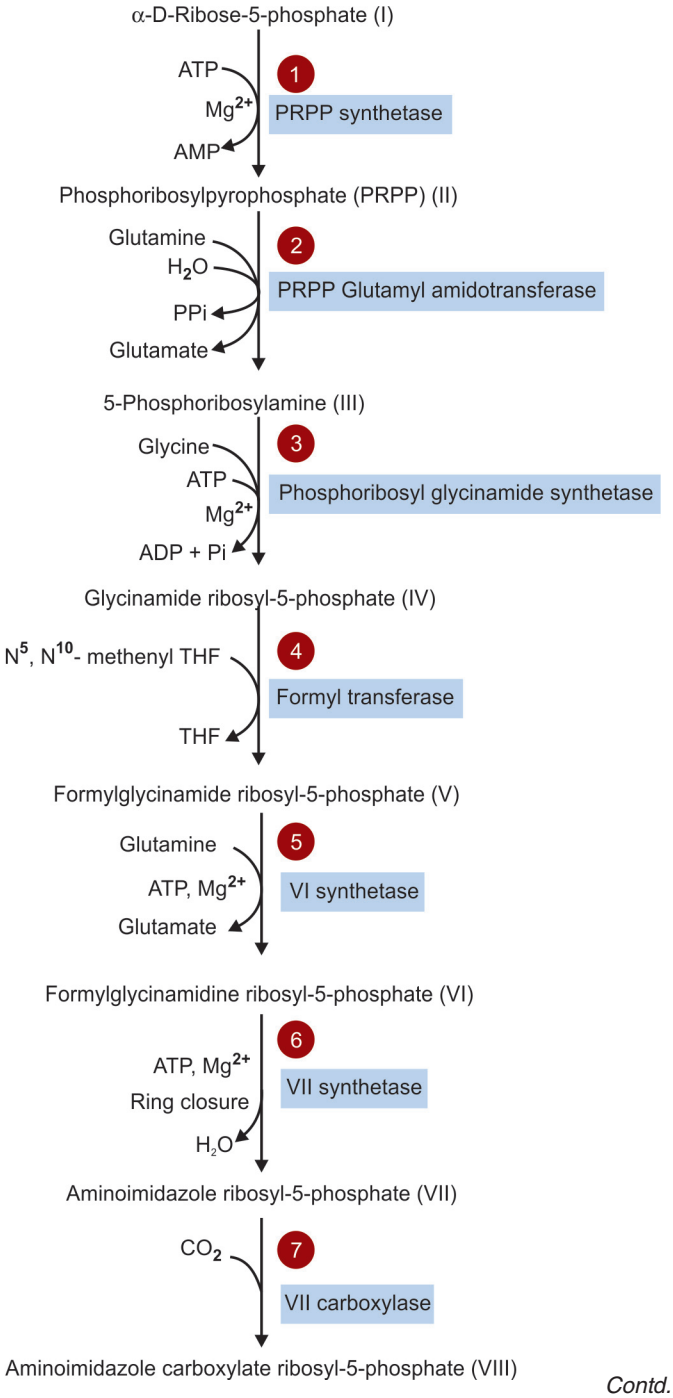
In **Figure 19.2** different steps of *de novo* synthesis of purine nucleotides are numbered by Arabic numerals and Roman numerals designate structures in the pathway for convenience.

1. The biosynthesis of purine begins with ribose-5-phosphate (I), derived from pentose phosphate pathway, which is converted to phosphoribosyl-pyrophosphate, PRPP (II) by the transfer of pyrophosphate group from ATP to C-1 of the ribose.

This reaction is catalyzed by the enzyme ***PRPP synthetase.***

2. PRPP is aminated by the addition of the amide group from **glutamine** to form amino sugar 5-phosphoribosylamine (III), The enzyme that catalyzes the transfer of the amide nitrogen is called **PRPP-amidotransferase.** The amino nitrogen of phosphoribosylamine provides $\mathbf{N_9}$ of the purine ring.

The synthesis of phosphoribosylamine from PRPP is the **first committed (rate limiting) step** in the formation of inosine monophosphate (IMP).



Contd...

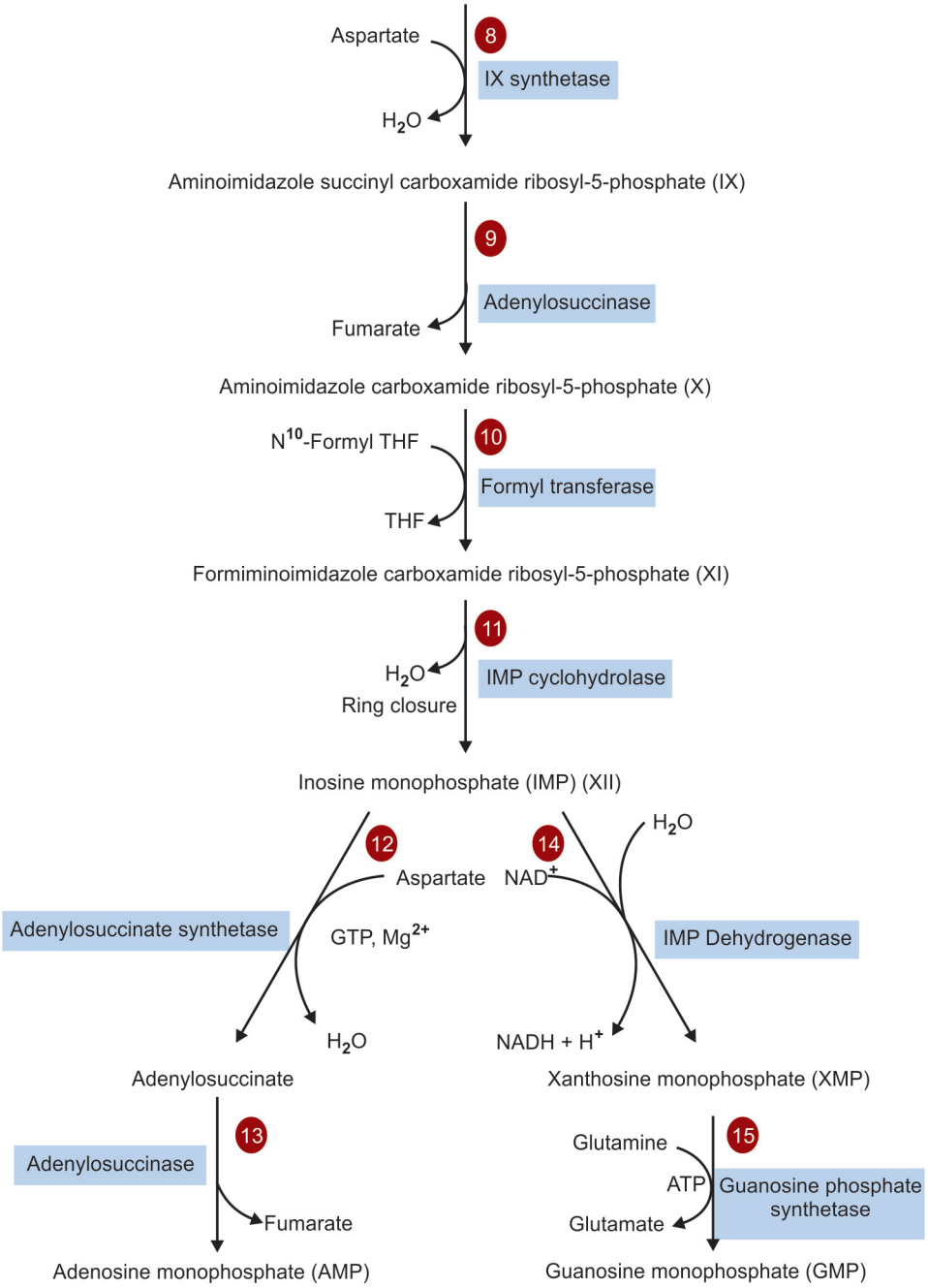


Figure 19.2: *De novo* pathway for synthesis of purine nucleotides

3. **C_4, C_5,** and **N_7** are next provided by the addition of amino acid **glycine** to form glycinamide ribosyl-5-phosphate (IV). ATP is consumed in this reaction and the enzyme ***phosphoribosyl glycinamide synthetase*** is required.
4. A one carbon unit is next transferred to the free amino group of (IV) by an *enzyme* ***glycinamide ribosyl-5-phosphate formyl transferase*** to form formylglycinamide ribosyl-5-phosphate (V). **N^5, N^{10}-methenyl tetrahydrofolate** serves as the carrier of 1-C-unit. This reaction adds a carbon that will become **C_8** of the purine ring.
5. The **N_3** of the purine structure is introduced by another amination using **glutamine** and ATP to

form formylglycinamidine ribosyl-5-phosphate *(VI)* catalyzed by ***formylglycinamidine ribosyl-5-phosphate synthetase (VI synthetase).***

6. In a reaction catalyzed by ***aminoimidazole ribosyl-5-phosphate synthetase (VII synthetase),*** loss of water accompanied by ring closure forms *amino-imidazole ribosyl-5-phosphate (VII).*
7. Addition of CO_2 to (VII) adds the atom that will become C_6 of the purine structure. The reaction, catalyzed by ***aminoimidazole ribosyl-5 phosphate carboxylase (VII carboxylase),*** requires neither ATP nor biotin and forms aminoimidazole carboxylate ribosyl-5-phosphate (VIII).
8. Condensation of **aspartate** with (VIII), catalyzed by ***succinyl carboxamide ribosyl-5-phosphate synthetase (IX synthetase),*** forms aminoimidazole succinyl carboxamide ribosyl-5-phosphate (IX).
9. Liberation of the succinyl group of (IX) as fumarate, catalyzed by **adenylosuccinase,** forms aminoimi-dazole carboxamide ribosyl-5- phosphate (X). Reactions 8 and 9, add the atom that becomes **nitrogen-1** of the purine structure.
10. **Carbon-2** of the purine is added by **N^{10}-formyl tetrahydrofolate** to form formiminoimidazole carboxamide ribosyl-5-phosphate (XI).
11. Ring closure of (XI) catalyzed by **IMP cyclohydro-lase** forms the first purine nucleotide, inosine monophosphate, IMP (XII).
12. Addition of aspartate to IMP forms adenylosucci-nate in the presence of ***adenylosuccinate synthetase*** and GTP.
13. ***Adenylosuccinase*** in turn catalyzes the removal of fumarate to yield AMP.
14. To produce GMP, the IMP must first be oxidized to xanthosine monophosphate (XMP) by NAD^+ linked enzyme ***IMP dehydrogenase***.
15. XMP then accepts an amino group from glutamine in the presence of ATP and the enzyme ***guanosine phosphate synthetase.***

Regulation of De Novo *Synthesis of Purine Nucleotide (Figure 19.3)*

- The synthesis of purine nucleotides is controlled by:
  - Concentration of PRPP
  - Feedback regulation at several sites.
- Increased concentration of PRPP stimulates the purine nucleotides synthesis. Concentration of PRPP depends on :
  - Availability of ribose-5-phosphate
  - On the activity of PRPP synthase.

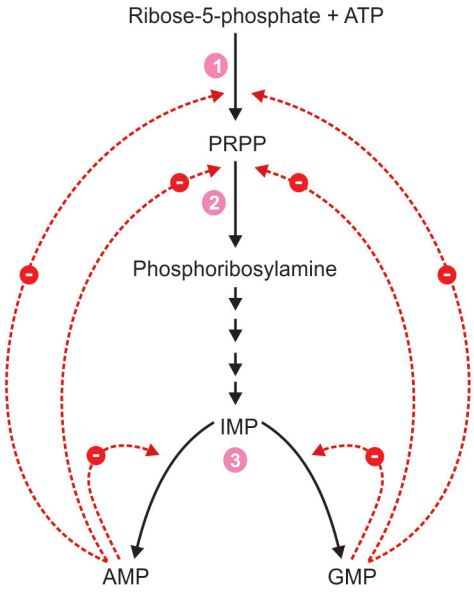


Figure 19.3: Regulation of *de novo* synthesis of purine nucleotides

- Three major feedback mechanisms regulate the overall rate of *de novo* purine nucleotide synthesis.
  1. The step leading to formation of PRPP. This reaction is catalyzed by an allosteric enzyme ***PRPP* synthetase,** which is feedback inhibited by purine nucleotides, AMP and GMP **(Figure 19.3)**.
  2. The committed step in purine nucleotide biosynthesis is the conversion of PRPP into phosphoribosylamine by ***PRPP glutamyl-amido transferase*** which is feedback inhibited by AMP and GMP.
  3. AMP and GMP feedback regulate their formation from IMP. AMP feedback regulates **adenylo-succinate synthase** and GMP feedback regulates **IMP dehydrogenase**.

SYNTHESIS OF PURINE NUCLEOTIDES BY SALVAGE PATHWAY

- The pathway involved in the conversion of free purines to nucleotides is called **salvage pathway** (Salvage means property saved from loss).
- Free purine bases (adenine, guanine and hypoxan-thine) are formed in cells during the metabolic degradation of nucleic acids and nucleotides. However, free purines are salvaged and used over again to remake purine nucleotides. This occurs by a pathway that is quite different from the *de novo* biosynthesis of purine nucleotides described earlier,

in which the purine ring system is assembled step by step on ribose-5-phosphate in a long series of reactions.

- The salvage pathway is much simpler and requires far less energy than does *de novo* synthesis. It consists of a single reaction.

Significance of Salvage Pathway

Salvage pathway provides purine nucleotides for tissues, which are incapable to synthesize purine nucleotides by *de novo* pathway, e.g. human brain, erythrocytes and polymorphonuclear leukocytes.

Salvage Reaction (Figure 19.4)

In salvage reaction ribose phosphate moiety of PRPP is transferred to the purine to form the corresponding nucleotide. There are two salvage enzymes with different specificities as follows:

- Adenine phosphoribosyl transferase (APRTase) catalyzes the formation of adenine nucleotide (AMP) from adenine.
- Whereas, **Hypoxanthine Guanine phosphoribosyl transferase (HGPRTase),** catalyzes the formation of ionosine (IMP) from hypoxanthine and guanine nucleotide (GMP) from guanine **(Figure 19.4)**.

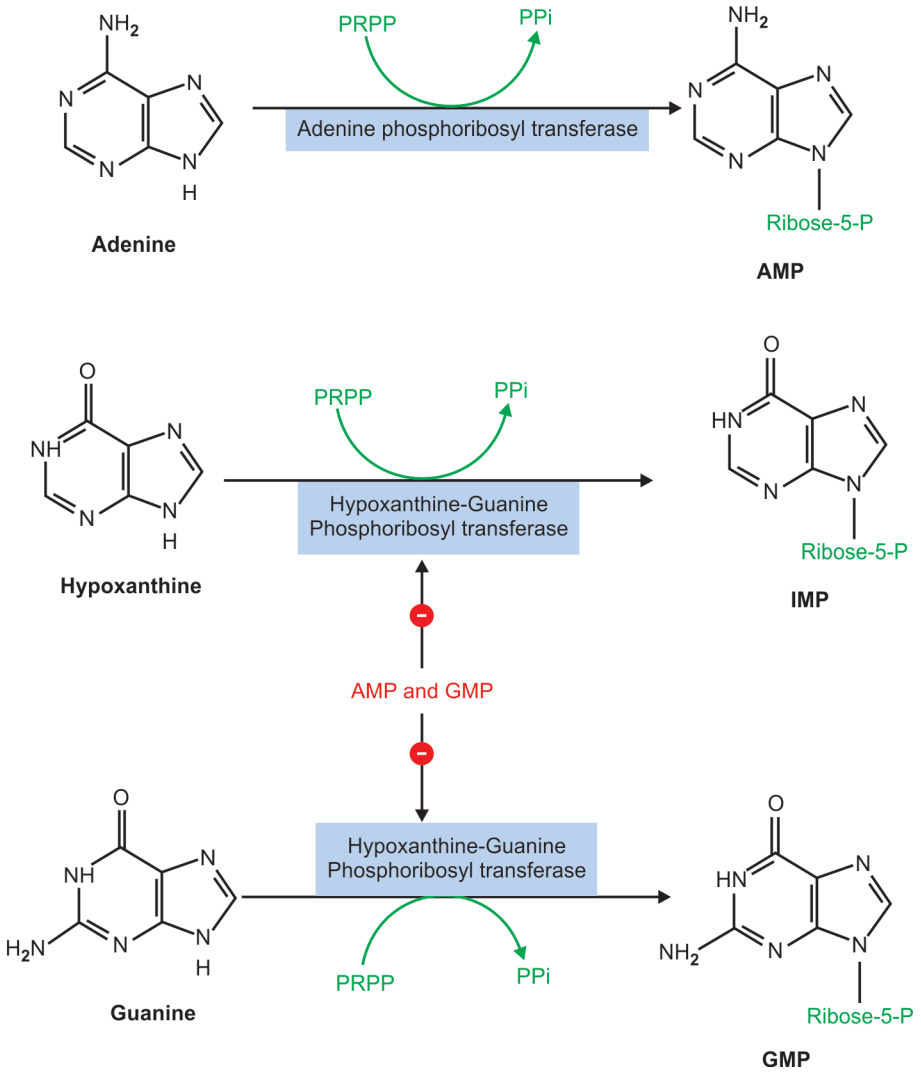


Figure 19.4: Salvage pathway of purine nucleotide synthesis

CATABOLISM OF PURINE NUCLEOTIDES

The end product of purines (adenine and guanine) in humans is sparingly soluble uric acid **(Figure 19.5)**.

- Purine nucleotides (AMP and GMP) are degraded by a pathway, in which the phosphate group is removed by the action of **nucleotidase;** to yield the nucleoside, adenosine or guanosine.
- Adenosine is then deaminated to **inosine** by **adenosine deaminase**.
- Inosine is then hydrolyzed by ***purine nucleoside phosphorylase*** to yield its purine base **hypoxanthine** and ribose-1-phosphate.
- Hypoxanthine is oxidized successively to **xanthine** and then **uric acid,** by ***xanthine oxidase,*** *a* molybdenum and iron containing flavoprotein. In this reaction, molecular oxygen is reduced to H_2O_2, which is decomposed to H_2O and O_2, by catalase.
- Guanosine is cleaved to **guanine** and ribose-1-phosphate by **phosphorylase** enzyme.
- Guanine undergoes hydrolytic removal of its amino group by **guanase** to yield **xanthine,** which is converted to **uric acid** by **xanthine oxidase**.

DISORDERS OF PURINE CATABOLISM

The catabolism of the purines, adenine and guanine produces **uric acid.** At physiological pH, uric acid is mostly ionized and present in plasma as ***sodium urate.***

An elevated serum urate concentration is known as ***hyperuricemia.*** Uric acid and urate are relatively insoluble molecules which readily precipitate out of aqueous solutions such as urine or synovial fluid. The consequence of this is the condition, ***gout.***

The average normal blood serum level of uric acid is **4 to 7 mg per 100 ml.**

Gout

Gout is a metabolic disorder associated with an elevated level of **uric acid** in the serum. The increased serum uric acid is due to either increased formation of uric acid or its decreased renal excretion. *Whatever be the cause,* **gout is associated with hyperuricemia but hyperuricemia is not always associated with gout**.

Classification of gout

Gout is classified into two broad types:

1. Primary gout
2. Secondary gout

Primary gout

- Primary gout is an inborn error of metabolism due to **overproduction of uric acid.** In primary gout,

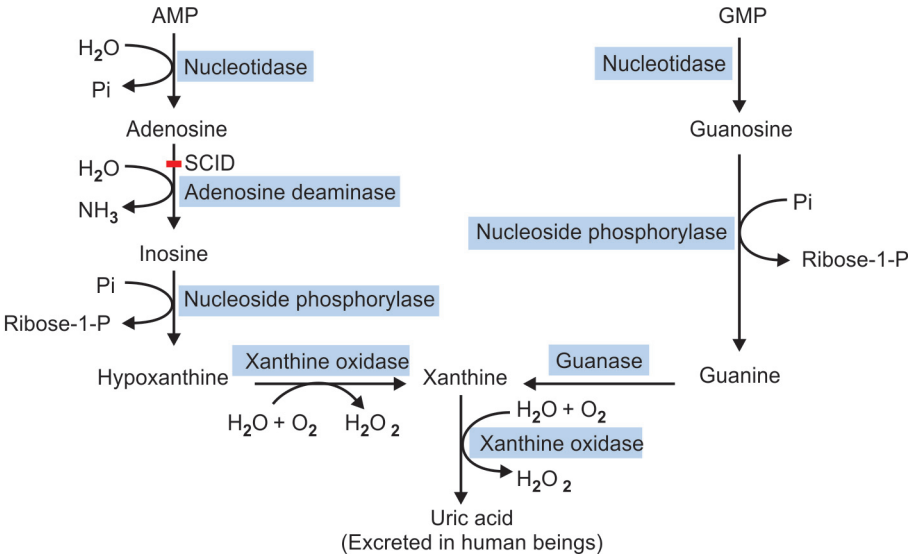


Figure 19.5: Catabolism of purine nucleotides

increased level of uric acid is associated with increased synthesis of purine nucleotides. Increased synthesis of purine nucleotides is caused by defective enzymes of purine nucleotide biosynthesis, such as:
  - PRPP synthetase
  - PRPP glutamyl amidotransferase
  - HGPRTase
  - Glucose-6-phosphatase
- In normal course **PRPP synthetase** and **PRPP glutamyl amidotransferase** are allosterically feedback regulated by its own product AMP and GMP. But due to loss of allosteric feedback regulation, abnormally high level of PRPP synthetase and PRPP glutamyl amidotransferase results in excessive production of PRPP, which in turn accelerates the rate of *de novo* synthesis of purine nucleotides. Increased synthesis is associated with increased break down to uric acid.
- **HGPRTase deficiency:** The enzyme HGPRTase catalyzes the synthesis of GMP and IMP by salvage pathway **(Figure 19.4).** Deficiency of HGPRTase leads to reduced synthesis of IMP and GMP by salvage pathway and increases the level of PRPP. Increased level of PRPP accelerates the purine nucleotide biosynthesis by *de novo* pathway.
- **Glucose-6-phosphatase deficiency:** Glucose-6-phosphatase enzyme is not directly involved in purine synthesis. It is involved indirectly with biosynthesis of purine nucleotide.

In type-I glycogen storage disease, **Von Gierkes disease** due to deficiency of **glucose-6-phosphatase**, glucose-6-phosphate cannot be converted to glucose. Accumulated glucose-6-phosphate is then metabolized via HMP shunt, which in turn generates large amounts of ribose-5-phosphate, a precursor of PRPP. The increased synthesis of PRPP then enhances de novo synthesis of purine nucleotides.

Symptoms of primary gout

- Patients with primary gout often show deposition of urate as tophi (clusters of urate crystals) in soft tissues **(Figure 19.6)** that affects the joints and leads to painful arthritis.
- The kidneys are also affected, since excess urate is also deposited in the kidney tubules **(Figure 19.7)** and leads to renal failure.

Secondary gout

Secondary gout results from a variety of diseases that cause an ***elevated destruction of cells*** or ***decreased elimination of uric acid*** as follows:

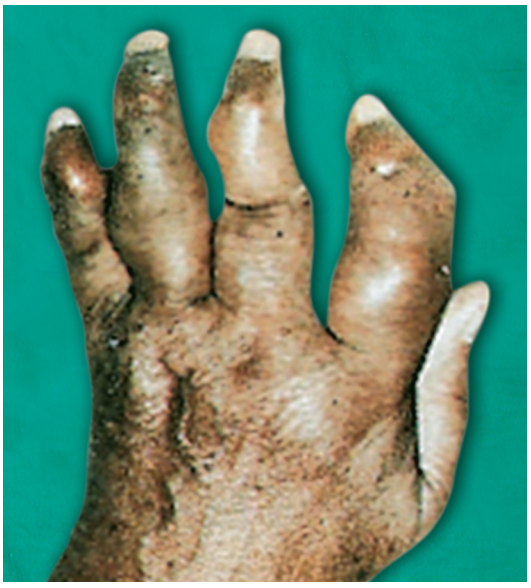


Figure 19.6: Deposition of urate as tophi in soft tissues

Figure 19.7: Urate crystals deposited in kidney tubules

- Elevated destruction of cells is accompanied by increased degradation of nucleic acids to uric acid, which occurs in cancers (leukemia, polycythemia), psoriasis and hyper catabolic states (starvation, trauma, etc.).
- Decreased elimination of uric acid occurs in chronic renal disease due to reduced glomerular filtrate rate.

Treatment of gout

Gout can be treated by a combination of nutritional therapy and drug therapy.

- Foods especially rich in nucleotides and nucleic acids such as liver or coffee and tea, which contain the purines **caffeine** and **theobromin** are withheld from the diet. Restriction in intake of alcohol is also advised.
- Major improvement follows the use of the drug ***allopurinol,*** an analog of hypoxanthine which inhibits **xanthine oxidase competitively**, the enzyme responsible for converting purines into uric acid. This leads to reduced formation of uric acid and accumulation of xanthine and hypoxanthine, which are more soluble and thus easily excreted.

Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome is an inherited X-linked disorder, that affects only males. It was first described by Michael Lesch and Willian Nyhan. It is caused by a ***complete deficiency of HGPRTase,*** an enzyme which is involved in purine salvage pathway **(Figure 19.4)**.

- In the absence of HGPRTase, the salvage pathway is inhibited and purines cannot be reconverted to nucleotides; instead they are degraded to uric acid.
- The lack of HGPRTase also causes an overproduction of PRPP, which stimulates purine biosynthesis. Because of increased purine synthesis, the degradation product uric acid also increases. This results in increased concentration of uric acid in plasma and urine.

Symptoms

The symptoms include:

- Hyperuricemia
- Gout
- Urinary tract stones
- The neurological symptoms or mental retardation
- Spasticity (resistance to the passive movement of a limb)
- Self-mutilation (self painful, destructive behavior of biting of fingers and lips).

The basis for neurologic symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzyme (HGPRTase) than other cells. Brain normally use salvage pathway for the synthesis of IMP and GMP, because brain is incapable to synthesize purine nucleotides by *de novo* pathway. In Lesch-Nyhan syndrome due to lack of HGPRTase, synthesis of purine nucleotides (which are precursors of DNA) by brain is decreased.

Treatment

Allopurinol reduces uric acid formation but does not alleviate the neurologic symptoms.

Xanthinuria

Deficiency of the enzyme, ***xanthine oxidase,*** due to either genetic defect or severe liver damage results in **hypouricemia** and increased urinary excretion of xanthine and hypoxanthine.

Xanthine lithiasis (formation of stones) and secondary renal damage may occur in severe xanthine oxidase deficiency.

Immunodeficiency Disorders of Purine Metabolism

Adenosine deaminase (ADA) deficiency

- ADA deficiency causes severe combined immunodeficiency (SCID).
- In ADA deficiency both thymus-derived lymphocytes (T-cells) and bone marrow derived lymphocytes (B-cells) are dysfunctional.
- A deficiency of ADA results in an accumulation of adenosine which in turn results in accumulation of deoxyadenosine and d ATP.
- As d ATP levels rise, ribonucleotide reductase is inhibited which is required for the formation of deoxyribonucleotides. Consequently, cells cannot make DNA and divide.
- Elevated levels of d ATP are toxic to lympocytes. Thus, lymphocytes are killed resulting in impairment of both cell and humoral immunity.
- The disease is fatal and victims usually die in early childhood (within 2 years of age).

DE NOVO BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

The pyrimidine nucleotides are:

1. Cytidine monophosphate CMP
2. Uridine monophosphate UMP
3. Thymidine monophosphate TMP.

Unlike the synthesis of purine nucleotide, six membered pyrimidine ring is made first and then attached to ribose phosphate, which is donated by PRPP.

Precursors for the De Novo Synthesis of Pyrimidine (Figure 19.8)

- Glutamine provides N_3
- Aspartic acid furnishes C_4, C_5, C_6 and N_1
- Carbon dioxide provides C_2.

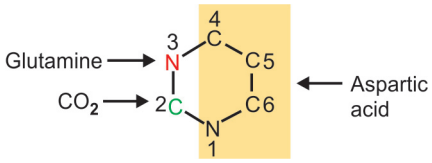


Figure 19.8: Sources of the carbon and nitrogen atoms in the pyrimidine ring

Major Steps for De Novo *Synthesis of Pyrimidine Nucleotide (Figure 19.9)*

1. Pyrimidine biosynthesis starts with the formation of carbamoyl phosphate from glutamine, ATP and CO_2. This reaction is catalyzed by **cytosolic carbamoyl phosphate synthase-l (CPS-II)** an enzyme different from mitochondrial carbamoyl phosphate synthase-l (CPS-I) required in the synthesis of urea.
2. Condensation of carbamoyl phosphate with aspartate forms carbamoyl aspartate in a reaction catalyzed by ***aspartate transcarbamoylase and is the committed step in the biosynthesis of pyrimidine.***
3. By removal of water from carbamoyl aspartate, catalyzed by ***dihydro-orotase,*** the pyrimidine ring is closed with formation of dihydro-orotic acid.
4. Dihydro-orotic acid is now oxidized to yield pyrimidine derivative orotic acid, a reaction catalyzed by mitochondrial NAD^+ dependent **dihydro-orotate dehydrogenase.** All other enzymes of pyrimidine biosynthesis are cytosolic.
5. The next step is the acquisition of a ribose phosphate group. Transfer of ribose phosphate moiety from PRPP to orotate, forming orotidine monophosphate, OMP (orotidylate). This reaction is catalyzed by **orotate phosphoribosyl transferase.**
6. Orotidylate is then decarboxylated to yield uridylate (UMP).
7. UMP is phosphorylated to UDP by kinase using ATP as a phosphate donor. UDP serves as a precursor for the synthesis of UTP, CTP, dUMP and dTMP.
8. UDP undergoes an ATP dependent kinase reaction to produce UTP.
9. UTP is aminated by accepting amino group from glutamine to form cytidine triphosphate (CTP).
10. Ribonucleotide reductase converts UDP to dUDP.
11. Deoxyuridine diphosphate (dUDP) is dephosphorylated to dUMP which acts as a substrate for thymidine monophosphate (TMP).

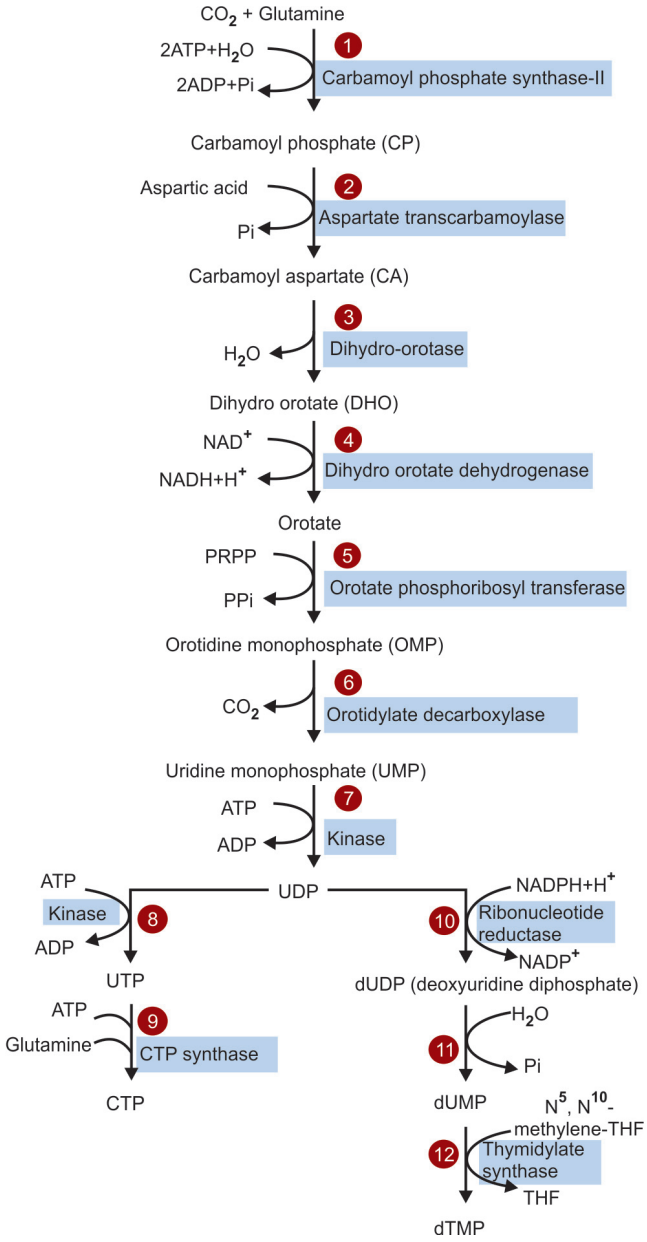


Figure 19.9: *De novo* pathway for the synthesis of pyrimidine nucleotide

12. Methylation of dUMP by N^5N^{10}-methylene tetrahydrofolate, catalyzed by ***thymidylate synthase,*** forms deoxythymidine monophosphate (dTMP).

Regulation of De Novo *Synthesis of Pyrlmidine Nucleotides*

The first two enzymes **carbamoyl phosphate synthase-II** and **aspartate transcarbamoylase** are allosteric enzymes and are regulated allosterically **(Figure 19.10)**.

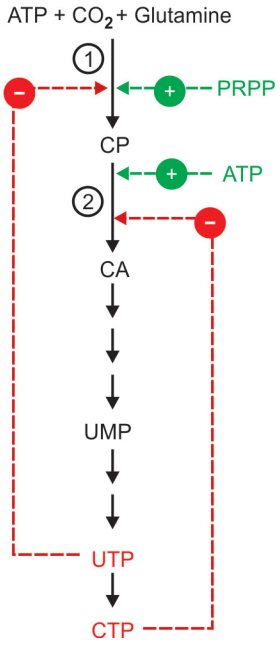


Figure 19.10: Regulation of *de novo* pyrimidine nucleotide synthesis
CP: Carbamoyl phosphate; CA: Carbamoyl aspartate

- Carbamoyl phosphate synthase-II, reaction 1 is feed- back inhibited by UTP and activated by PRPP.
- Aspartate transcarbamoylase, reaction 2, is feedback inhibited by CTP and activated by ATP.

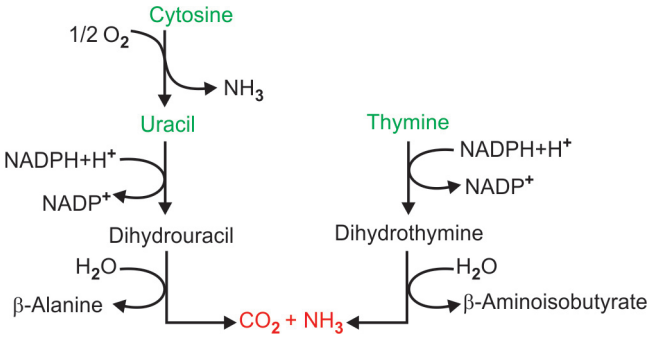


Figure 19.11: Catabolism of pyrimidines

CATABOLISM OF PYRIMIDINE NUCLEOTIDES (FIGURE 19.11)

Unlike the purines which degraded to sparingly soluble product, uric acid, the end products of pyrimidine catabolism are highly water soluble:

- CO_2
- NH_3
- β-alanine
- β-aminoisobutyrate

Humans probably transaminate β-aminoisobutyrate to methylmalonate semialdehyde which is then converted to succinyl-CoA via methylmalonyl-CoA. β-alanine can serve as precursor of acetyl-CoA.

DISORDERS OF PYRIMIDINE CATABOLISM

Since the end products of pyrimidine catabolism are highly water soluble, overproduction of pyrimidine catabolites is rarely associated with clinically significant abnormalities.

Disorder of Pyrimidine Synthesis

Orotic aciduria

- Orotic aciduria is a hereditary disorder which can result from a defective enzyme in pyrimidine synthesis.
- A defect in the multifunctional enzyme ***UMP synthase*** that converts orotic acid to UMP results in the excretion of orotic acid in the urine.
- UMP synthase is a multifunctional enzyme containing both orotate phosphoribosyltransferase and orotidylate decarboxylase activity **(Figure 19.9)**.
- There are two types of orotic acidemia:
  i. **In type-I,** there is deficiency of both enzymes, orotate phosphoribosyltransferase and orotidylate decarboxylase.
  ii. **In type-II,** only orotidylate decarboxylase is deficient.

The deficiency in UMP and other pyrimidine nucleotides results in inhibition of DNA and RNA synthesis, which causes ***megaloblastic anemia*** and ***failure to thrive*** (growth retardation).

Reye's syndrome

Reye's syndrome is a secondary orotic aciduria, which may be due to inability of severely damaged mitochondria to utilize carbamoylphosphate in the formation of urea which then may be diverted for cytosolic overproduction of orotic acid.

SUMMARY

- Purine ring is synthesized from a variety of precursors; glutamine, glycine, aspartate, methenyl tetrahydrofolate, N^{10} formyl tetrahydrofolate and CO_2.
- Purine ribonucleotides can also be synthesized by a salvage pathway in which a preformed base reacts directly with PRPP.
- The pyrimidine ring is assembled first and then linked to ribose phosphate to form a pyrimidine nucleotide in contrast with the sequence in the *de novo* synthesis of purine nucleotides.
- The sources of carbon and nitrogen atoms in the pyrimidine ring are glutamine, CO_2 and aspartic acid.
- Humans catabolize purines to the uric acid.
- Gout and Lesch-Nyhan syndrome (hyperuricemic) and xanthinuria (hypouricemic) are the disorders of purine catabolism.
- Unlike uric acid, the relative insoluble products of purine catabolism, the end products of pyrimidine catabolism CO_2, NH_3, β-aminoisobutyrate and β-alanine are highly water-soluble, and therefore overproduction of pyrimidine catabolites is generally not associated with clinically significant abnormalities.
- Orotic aciduria can result from a defective enzyme in pyrimidine synthesis. Reye's syndrome is secondary to orotic aciduria.

EXERCISE

Solve

Case History 1

A 50-year-old female patient complains of pain and swelling in joints and shows hyperuricemia.

Questions

1. Name the probable disease.
2. Name the enzyme defect.
3. What is the normal value of serum uric acid?
4. Suggest nutritional therapy.

Case History 2

A 4-year-old boy suffered from pain in joints, showed signs of mental retardation and had self mutilation (self destructive behavior of biting fingers and lips). His serum uric acid level was 10 mg/dl.

Questions

1. What is the probable diagnosis?
2. Which enzyme is defective in this disease?
3. What is the normal level of serum uric acid in human?
4. What is the mode of inheritance of this disease?

Multiple Choice Questions (MCQs)

1. Rate controlling step of pyrimidine biosynthesis is catalyzed by:
a) Orotidylate decarboxylase
b) Aspartate transcarbamoylase
c) Carbamoyl phosphate synthase II
d) Orotate phosphoribosyl transferase

2. Which statement for purine biosynthesis is incorrect?
a) Requires vitamin B_{12}
b) Assembled on ribose phosphate
c) Requires PRPP
d) Requires glycine

3. Purines and pyrimidines are:
a) Dietary essential
b) Dietary non-essential
c) Derived from essential fatty acids
d) Derivatives of essential amino acid

4. The two nitrogens of the pyrimidine ring are contributed by:
a) Glutamate and ammonia
b) Glutamate and aspartate
c) Glutamate and glutamine
d) Aspartate and carbamoyl phosphate

5. Nitrogen or carbon atoms are contributed to the structure of the purine ring by amino acids, *except:*
a) Glycine b) Glutamine
c) Aspartate d) Glutamate

6. Lesch-Nyhan syndrome may lead to:
a) Self-destructive behavior
b) Gout
c) Elevated levels of PRPP
d) All of the above

7. Hyperuricemia can result from defect in enzymes, *except:*
a) Carbamoyl phosphate synthase II
b) HGPRTase
c) PRPP synthase
d) Glucose-6-phosphatase

8. The end product of purine metabolism in human is:
a) Creatinine b) Uric acid
c) Urea d) Ammonia

9. The salvage pathway for purines involves enzyme:
a) PRPP amidotransferase
b) PRPP synthase
c) HGPRTase
d) Xanthine oxidase

10. Enzyme involved with immunodeficiency disease of purine metabolism is:
a) Adenosine deaminase b) Xanthine oxidase
c) PRPP synthetase d) HGPRTase

11. Purine salvage occurs in the tissues, *except:*
a) RBC
b) Brain
c) Liver
d) Polymorphonuclear leukocytes

12. Allopurinol is used in the treatment of:
a) Rickets b) Cancer
c) Gout d) Pellagra

13. Lesch-Nyhan syndrome is due to the lack of:
a) HGPRTase
b) APRTase
c) Adenosine deaminase
d) PRPP amidotransferase

14. Hereditary orotic aciduria type II is due to deficiency of the following enzyme:
a) Dihydro-orotate dehydrogenase
b) Orotate phosphoribosyl transferase
c) Aspartate transcarbamoylase
d) Orotidylate decarboxylase

Correct Answers for MCQs

1-b	2-a	3-b	4-b
5-d	6-d	7-a	8-b
9-c	10-a	11-c	12-c
13-a	14-d		

CHAPTER

20

Replication, Transcription and Translation

INTRODUCTION

The important role of DNA in transfer of information in living cells, formulated by Francis Crick, is called **the central dogma of molecular biology (Figure 20.1)**, which defines three major steps in the processing of genetic information.

1. The first is **replication,** the copying of parent DNA to form daughter DNA molecules having nucleotide sequences identical to those of the parent DNA.
2. The second step is **transcription,** the process in which the genetic message in DNA are rewritten in the form of ribonucleic acid (RNA).
3. The third step is **translation** in which the genetic message coded by RNA is translated by the ribosomes into the protein structure.

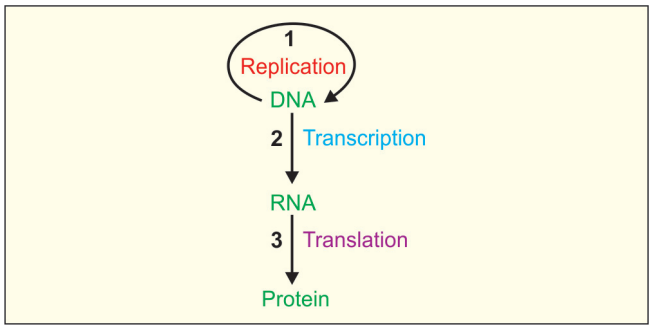


Figure 20.1: The central dogma of molecular biology

This flow of genetic information from **DNA** to **RNA** to **protein** is called **"the central dogma"** of molecular biology.

In this chapter we shall study the successive steps in this sequence.

REPLICATION (DNA SYNTHESIS)

DNA is a major store of genetic information. To transfer this genetic information from a parent cell to a daughter cell during cellular reproduction, the DNA must be duplicated. ***The duplication or synthesis of DNA is called replication.***

This method of DNA replication results in ***semi conservative mechanism,*** in which each replicated duplex daughter DNA molecule contains one parent strand and one newly synthesized strand **(Figure 20.2)**.

Prokaryotic Replication

Replication in prokaryotes is much better understood than replication in eukaryotes. The basic requirements and components of replication are the same for prokaryotes as for eukaryotes. Therefore, an understanding of how prokaryotes replicate provides the understanding of how eukaryotes replicate.

- The DNA synthesis is catalyzed by enzyme called ***DNA dependent DNA polymerase.*** They are called

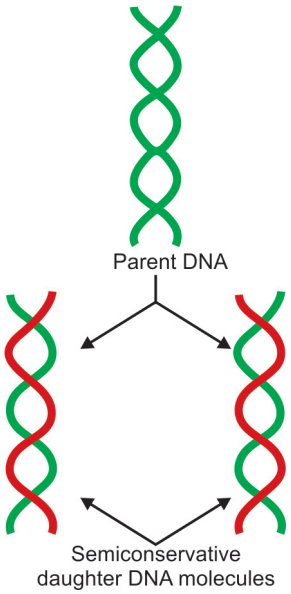


Figure 20.2: Semiconservative DNA molecules

DNA dependent as they require DNA template. They are more commonly called ***DNA polymerases.*** These polymerases, which are required for:
- DNA chain elongation
- DNA repair (5' – 3' exonuclease activity)
- Proofreading (3' – 5' exonuclease activity).

- There are three types of polymerases in prokaryotes
  1 **DNA polymerase I (Pol I**): DNA polymerase I completes chain synthesis between Okazaki fragments on the lagging strand.
  2 **DNA polymerase II (Pol II):** Pol II is mostly concerned with proofreading and DNA repair.
  3 **DNA polymerase Ill (Pol III):** Pol III catalyzes leading and lagging strand synthesis.
- At least five DNA-polymerases exist in eukaryotic cells, α, β, γ, δ and ε **(Table 20.1).**

Table 20.1: A Comparison of prokaryotic and eukaryotic DNA polymerase

Prokaryotic polymerase	*Eukaryotic polymerase*	*Function*
I	α	Gap filling and synthesis between Okazaki fragments of lagging strand
II	ε	DNA proofreading and DNA repair
	β	DNA repair
	γ	Mitochondrial DNA synthesis
III	δ	Leading and lagging strand synthesis

Stages of Replication

The process of replication can be divided into three stages.
1. Initiation
2. Elongation
3. Termination.

Initiation

- Initiation of DNA replication involves unwinding (separation) of two complementary DNA strands and formation of replicating fork.
- Unwinding occurs at a single, specific site at a particular DNA sequence on circular DNA of prokaryotes. The site is called the **origin of replication, "Ori"** where active synthesis occurs. This region is called **replicating fork (Figure 20.3).**
- Replication of double stranded DNA is bidirectional.
- In eukaryotes replication begins at multiple sites composed almost exclusively of A-T base pairs along the DNA helix and is referred to as a ***consensus sequence***.

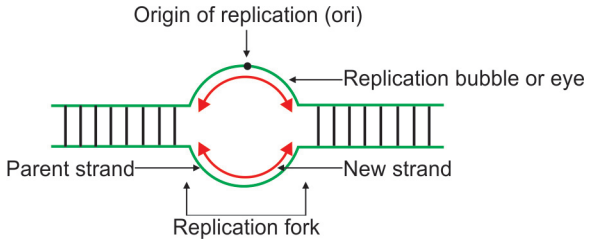


Figure 20.3: Formation of replicating fork

Steps involved in initiation

1. First **DNA A protein** recognizes and binds to the **"ori"** of the DNA and successively denatures the DNA.
2. **DNA B protein (helicase)** then binds to this region and unwinds the parental DNA, and form a "V" where active synthesis occurs. This region is called the **replicating fork (Figure 20.4).**
3. The stress produced due to unwinding by helicase is released by **topoisomerases** by cutting either one or both DNA strands.
4. The **Single stranded binding (SSB)** protein stabilizes the separated strands and prevents their reassociation.
5. To initiate the DNA synthesis by DNA polymerase III, it requires **RNA** primer. The **RNA primers** are short pieces of RNA (some 5–50 nucleotides in

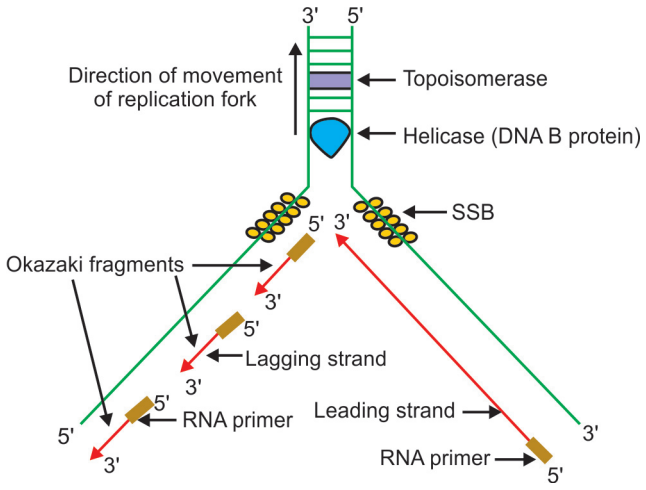


Figure 20.4: Replicating fork

length) formed by the enzyme **primase** (RNA polymerase) using DNA as a template.

Elongation

- Once RNA primer has been synthesized at each of the replicating forks, a **DNA polymerase III** initiates the synthesis of new DNA strand by adding deoxyribonucleotide to the 3' end of the RNA primer. Thus, DNA polymerase III can synthesize a new chain only in the 5' to 3' direction. Both the DNA strands are synthesized simultaneously but in opposite direction one is in direction towards the replication fork, the other in a direction away from the replication fork.
- The DNA chain which runs in the 3' → 5' direction is copied by polymerase III as a continuous strand, requiring one primer. This new strand is known as the ***leading strand.***
- The DNA chain which runs in the 5′ → 3' direction is copied by polymerase III as a discontinuous manner because synthesis can only proceed in the 5' to 3' direction. This new strand is known as the ***lagging strand.*** This requires numerous RNA primers. As the replication fork moves, RNA primers are synthesized at specified intervals. These RNA primers are extended by DNA polymerase III into short pices of DNA called **Okazaki fragments**.
- Upon completion of lagging strand synthesis, the RNA primers are removed from fragments by **DNA polymerase I.** DNA Polymerase I also fills the gaps that are produced by removal of the primer leaving only a nick. It cannot join two polynucleotide chains together, an additional enzyme **DNA ligase** is required to perform this function**.** This enzyme catalyzes the formation of a phosphodiester bond to seal the Okazaki fragments.

Termination

Termination sequences, e.g. ***"ter"***, direct termination of replication. A specific protein, ***ter binding protein,*** binds these sequences and prevents the helicase (DNA B protein) from further unwinding of DNA and facilitates the termination of replication.

Proofreading

- DNA is copied by DNA polymerase with high fidelity (accuracy). Incorrect nucleotides are incorporated with a frequency of one in 10^8–10^{12} bases, which could lead to **mutation**. But the error ratio during replication is kept at a very low level by specific process. This process is known as ***proofreading***.
- Mismatches, occur more frequently but do not lead to stable incorporations because the all three **DNA polymerases** have **3' to 5' exonuclease** activity (proofreading activity).
- DNA polymerase I and II are known to excise mismatched nucleotides before the introduction of the next nucleotide.

Eukaryotic Replication

DNA replication in eukaryotic organisms resembles that in prokaryotic cells and proceeds by a mechanism similar to that of prokaryotic replication but is not identical. Some differences are given in **Table 20.2**.

Inhibitors of DNA Replication

Some **antibacterial, antiviral** and many **chemotherapeutic** drugs inhibit replication.

- Many antibacterial drugs like **Novobiocin**, **Nalidixic acid** and **Ciprofloxacin** inhibit the prokaryotic enzyme, **topoisomerase** (DNA gyrase).
- These topoisomerase inhibitors are widely used for the treatment of urinary tract and other infections.
- **Camptothecin,** an antitumor drug, inhibits human topoisomerase.
- Certain anticancer and antiviral drugs inhibits elongation of DNA chain by incorporating certain nucleotide analogs, e.g. **2, 3 deoxyinosine.**

Table 20.2: Some differences between prokaryotic and eukaryotic DNA replication

Features	*Prokaryotes*	*Eukaryotes*
RNA primer length	~50 nucleotides	9 nucleotides
DNA polymerase	Three types and designated by Roman numerals; I, II, III	Five types and designated by Greek numerals α, β, γ, δ and ε
Number of origins	Single	Multiple
Nucleotide length of Okazaki fragments of lagging strand	1000–2000 nucleotides	~200 nucleotides
Rate of replication	~500 nucleotides per sec	50 nucleotides per sec (10 times slower than prokaryotes)

TRANSCRIPTION (RNA SYNTHESIS)

Transcription is defined as the synthesis of RNA from DNA, that results in the transfer of the information stored in double stranded DNA into a single stranded RNA; which is used to direct the synthesis of its proteins.

Transcription in Prokaryotes

- DNA dependent RNA polymerase, called **RNA polymerase (RNAP)** responsible for the synthesis of RNA using DNA template.
- Prokaryotes have single RNA polymerase that transcribes all three RNAs, i.e. mRNA, t-RNA and r-RNA. RNAP contains four subunits (2 α, β', β) which form the core enzyme. The active enzyme, the holoenzyme contains core enzyme and a fifth subunit called **sigma subunit (Figure 20.5).** The sigma subunit is required for binding of the RNA-polymerase to specific regions (promoter region) of DNA template.

Stages of Transcription

RNA synthesis involves **(Figure 20.6):**

1. Initiation
2. Elongation
3. Termination

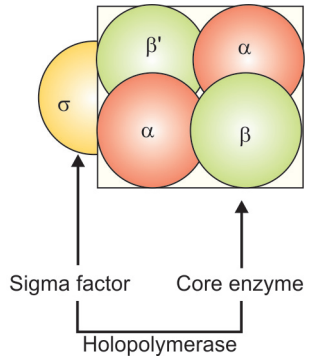


Figure 20.5: Prokaryotic RNA polymerase

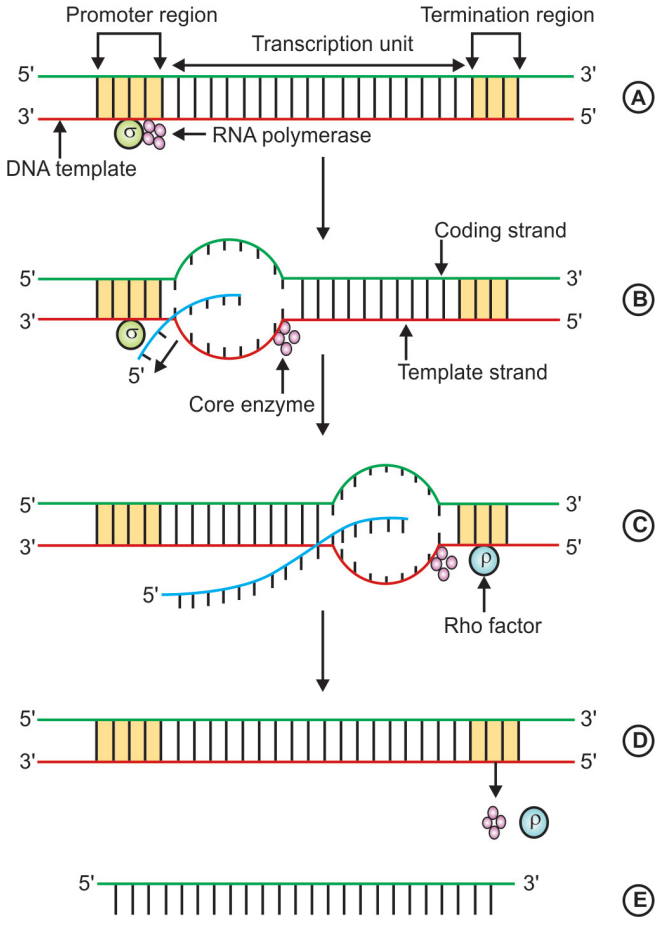


Figure 20.6: Process of transcription in prokaryotes
A: Recognition of promoter by sigma factor
B: Binding of core enzyme and starts the synthesis of RNA
C: Elongation continues until termination region is reached
D: Termination of transcription by Rho factor
E: Newly synthesized RNA (primary transcript)

Initiation

- Initiation of transcription involves the binding of RNA polymerase (core enzyme + σ factor) to the DNA template at the ***promoter site.*** The sigma factor enables the RNA polymerase (holoenzyme) to recognize and bind to promoter sequences. Promoters are characteristic sequences of DNA which are different in prokaryotes and eukaryotes **(Figure 20.7)**.

Prokaryotic promoters

- Prokaryotic genes have two promoter sequences **(Figure 20.7)**.
  1. **Pribnow box** (–10 region) has the nucleotide sequence TATAAT and is usually found 10 base pairs away from (upstream) the start point.
  2. **The –35 region,** has the nucleotide sequence TTGACA. It is named –35 sequence because it is found 35 base pairs away from (upstream) the start point.
- Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a particular point known as **start point** or **initiation site.**
- The binding of the RNA polymerase to the DNA template results in the unwinding of the DNA double helix. The enzyme then catalyzes the formation of phosphodiester bond between the first two ribonucleotides complementary to DNA template sequence. Unlike the initiation of replication, transcriptional initiation does not require a primer.

Elongation

- Elongation proceeds after the formation of the first phosphodiester bond.
- After formation of approximately 10 phosphodiester bonds of the new RNA, sigma (σ) subunit dissociates from the core enzyme.
- RNA polymerase utilizes ribonucleotide triphosphates (ATP, GTP, CTP and UTP) for the formation of RNA.
- The process of elongation of the RNA chain continues until a termination signal is reached.

Termination

In prokaryotes termination of transcription occurs by one of the two well characterized mechanisms:

1. Rho-dependent
2. Rho-independent.

Rho-dependent termination

Rho-dependent termination, requires a protein factor called **rho (ρ)** which recognizes the termination signal and has an ATP dependent helicase activity that displaces the RNA polymerase from template resulting in termination of RNA synthesis.

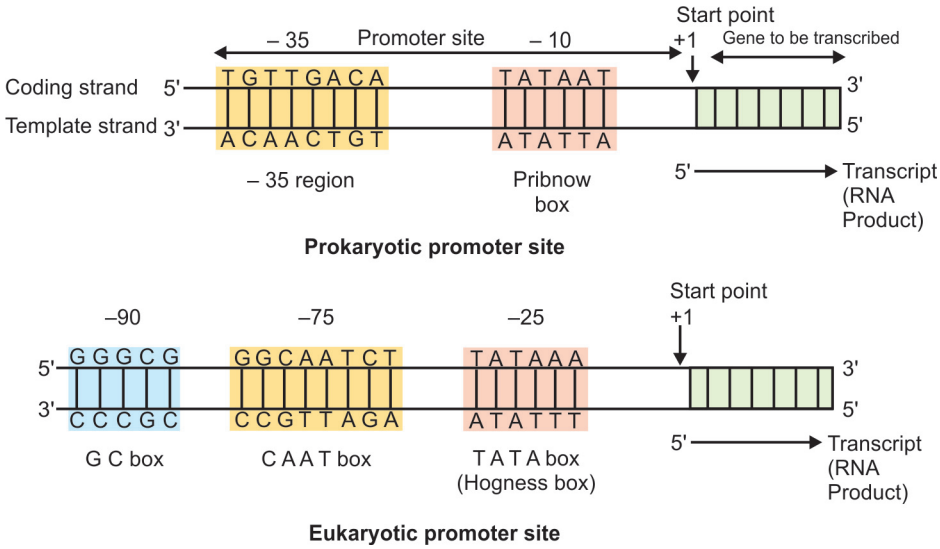


Figure 20.7: Promoter sites for transcription in prokaryotes and eukaryotes

Rho-independent termination

Rho-independent termination brought about by the formation of a secondary structure (hair-pin loop) in the newly synthesized RNA **(Figure 20.8)**, which removes the RNA polymerase from DNA template, resulting in the release of the transcript.

This hairpin loop structure is followed by a sequence of four or more uracil residues, which also are essential for termination. The RNA transcript ends within or just after then.

In eukaryotic cells termination is less well defined. It is believed that it is similar to that described for rho-independent prokaryotic termination.

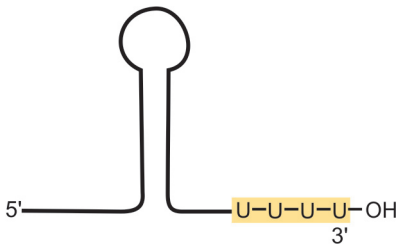


Figure 20.8: A hair-pin loop structure of newly synthesized RNA

Transcription in Eukaryotes

The description of RNA synthesis in prokaryotes is applicable to eukaryotes even though the enzyme involved and regulatory signals are different.

Eukaryotic RNA polymerase

In contrast to prokaryotes eukaryotic cells have three RNA polymerases I, II and III found in nucleus. Each of these RNA polymerase is responsible for the transcription of different sets of genes.

1. **RNA Polymerase I:** It catalyzes the synthesis of ribosomal RNA.
2. **RNA Polymerase II:** It catalyzes the synthesis of m-RNA and small nuclear RNAs (sn-RNA).
3. **RNA Polymerase III:** It catalyzes the synthesis of tRNA.

Besides the three nuclear RNA polymerases, in eukaryotic cell, a fourth type of RNA polymerase is found in mitochondrial matrix known as **mitochondrial RNA polymerase (mtRNAP).** Similar to prokaryotic RNA polymerase, mtRNA polymerase catalyzes the synthesis of all the three types of RNA, i.e. mRNA, tRNA and rRNA.

Eukaryotic promoter sites (Figure 20.7)

Each type of eukaryotic RNA polymerse uses a different promoters. These are:

1. **Hogness box or TATA box:** It is a stretch of six nucleotides and located 25 nucleotides upstream of the transcription starting point.
2. **CAAT box:** It is stretch of eight nucleotides and located about 75 nucleotides upstream of the transcription starting point.
3. **GC box:** It is a stretch of six nucleotides and is located about 90 nucleotides upstream of the transcription starting point.

Post-transcriptional Processing

- The RNAs formed during transcription are called **primary transcript.** The primary transcript normally undergo further enzymatic alteration, called **post-transcriptional processing.** Post-transcriptional processing is required to convert the primary RNAs into functional or active forms. Processing may involve either:
  - Cleavage of large precursor of RNA to a smaller molecule by the action of endonuclease or exonuclease.
  - Splicing
  - Terminal addition of nucleotide
  - Nucleoside modifications.
- Post-transcriptional processing is more extensive in eukaryotes than in prokaryotes.

m-RNA processing

- In prokaryotes mRNA is not post-transcriptionally processed. Prokaryotic mRNA is functional immediately upon synthesis.
- In eukaryotes the primary transcript of mRNA is the hnRNA (heterogeneous nuclear RNA). After transcription hnRNA is extensively modified to form functional mRNA. These modifications are as follows:
  1. **The 5'-capping:** The 5' end of eukaryotic mRNA is capped with **7-methylguanylate**. This cap functions in protein biosynthesis and to stabilize the structure of mRNA.
  2. **Addition of poly A tail:** The 3' end of most eukaryotic mRNAs posses a chain of 200–300 adenine nucleotides and called **Poly A tail.** Poly A tail is not transcribed by DNA but rather is added after transcription. Poly A tail may enhance translation efficiency and involved in stabilization of mRNA.

3. **Removal of introns:** Introns are the nucleotide sequences on mRNA that do not code for proteins. These are the intervening sequences between exons. Exons are the coding sequences that code for proteins. The process by which introns are excised and exons are linked to form functional mRNA is called **splicing.**

t-RNA processing

t-RNA precursors are converted to mature tRNAs by following alterations.

1. Cleavage of a 5' leader sequence.
2. Splicing to remove introns.
3. Replacement of the 3' terminal UU by CCA.
4. Modification of several bases, e.g. Uridylate is modified after transcription to form ribothymidylate and pseudouridylate.

r-RNA processing

The rRNA precursors called preribosomal RNAs are cleaved and trimmed to produce the mature functional r-RNAs.

Inhibitors of Transcription

A number of compounds inhibit RNA synthesis. Those that inhibit RNA synthesis in prokaryotes but not in eukaryotes, serve as therapeutic drugs, antibiotics. For example:

- **Rifampin:** It is an antituberculosis drug, which inhibits the initiation of transcription by binding β-subunit of prokaryotic RNA polymerase. Rifampin has no effect on eukaryotic nuclear RNA polymerases.
- **Dactinomycin (actinomycin D):** Dactinomycin is a therapeutic agent in the treatment of some cancer. It binds tightly and specifically to double helical DNA and thereby prevents the movement of the RNA polymerase.

Reverse Transcription

Viral DNA polymerase is called **reverse *transcriptase*** because it uses RNA as a template to synthesize DNA. It is therefore an ***RNA dependent DNA polymerase***.

Some viruses, referred to as ***'retroviruses'***, carry RNA as their genetic material and can synthesize double stranded DNA from their genomic RNA by a process known as reverse transcription. An example of retrovirus is the ***human immunodeficiency virus (HIV)*** which causes ***AIDS.***

GENETIC CODE

The information needed to direct the synthesis of protein is contained in the mRNA in the form of a **genetic code**.

The genetic code is the system of nucleotide sequences of mRNA that determines the sequence of amino acids in protein.

Codons are a group of three adjacent bases that specify the amino acids of protein.

Characteristics of Genetic Code

- **Number of codons:** There are 64 possible codon sequences. Because four nucleotide bases A, G, C and U are used to produce the three base codons, there are therefore 4^3 or 64 possible codon sequences **(Figure 20.9).**
- **Stop or termination or nonsense codons:** Three of the 64 possible nucleotide triplets, UAA, UAG and UGA do not code for any amino acids, they are called **nonsense codons** that normally signal termination of polypeptide chains. These **nonsense** codons are arbitarily named **amber**, **ochre** and **opal**.
- **The code is degenerate but unambiguous:** As there are 61 codons for 20 amino acids, one amino acid has more than one codon and the code is referred to as **degenerate**, indicating that there are redundancies. Although an amino acid may have more than one codon, each codon specifies only one amino acid. Thus, the genetic code is unambiguous. Degeneracy minimizes the deleterious effects of mutations.
- Codons that designate the same amino acid are called ***synonyms.*** Two amino acids methionine (AUG) and tryptophan (UGC) each have only one codon. The remaining amino acids have multiple codons, e.g. arginine is specified by six different codons **(Figure 20.9).**
- **The code** is **almost universal:** That is, the meaning of each codon is the same in almost all known organisms. Exceptions to the universality of the genetic code are found in human mitochondria, where the code:
  - UGA codes for tryptophan instead of serving as a stop codon
  - AUA codes for methionine instead of isoleucine
  - CUA codes for threonine instead of leucine.
- **The code is non-overlapping and without punctuation:** During translation, the code is read sequentially, without spacer bases, from a fixed starting point, as a continuous sequence of bases, taken 3 at a time, e.g. **A U G C U A G A C U U U** is read as:
  AUG / CUA / GAC / UUU without "punctuation" between the codons.

Wobble Hypothesis for Codon-Anticodon Interactions

- The genetic code assumes that each codon base pairs in antiparallel fashion with the anticodon of the

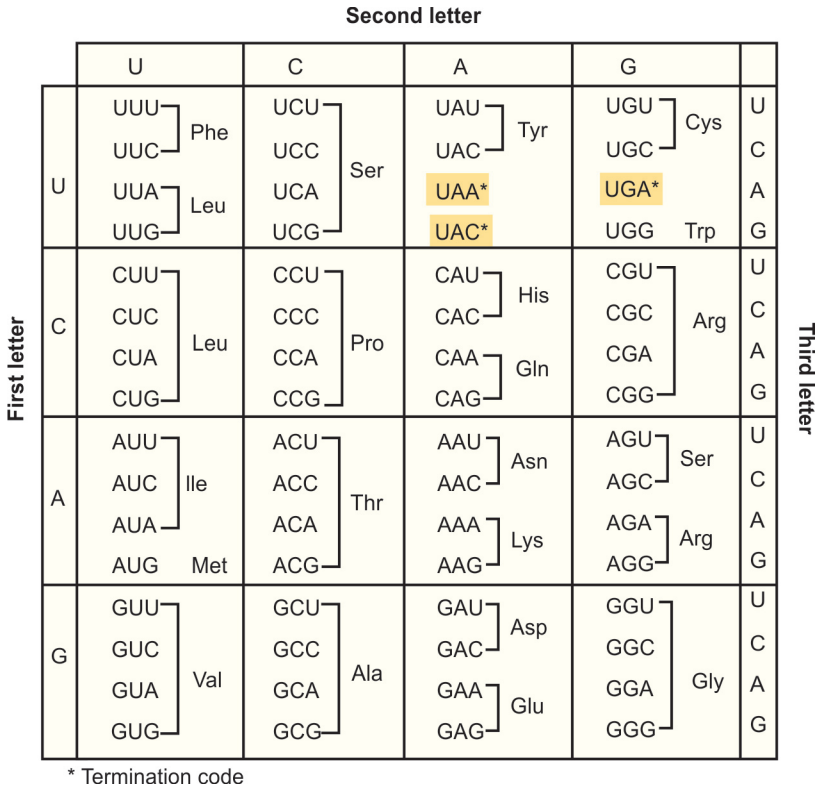


Figure 20.9: The genetic code

tRNAs that are specific for the amino acid corresponding to the codons on mRNA.

- It was found, however, that a single tRNA could recognize several codons.
- For example, the anticodon IGC (some of the tRNAs contain nucleoside inosine) of yeast tRNA for alanine binds to three codons: GCU, GCC and GCA.
- The first two bases of these codons are the same, whereas the third is different, ***"wobble."***
- Wobble allows some tRNAs to recognize more than one codon.
- The non-standard base pairing occurs in the third position of the codon, the position that has the least effect on specifying a particular amino acid **(Figure 20.9)**.

Crick has proposed a hypothesis to explain how a single tRNA can recognize several (degenerate) codons. Crick proposed four rules called ***wobble hypothesis***. These four rules are as follows:

1. The first two bases of a codon pair in the standard way and have normal Watson Crick base pairing.
2. For a given amino acid codons that differ in either of the first two bases must be recognized by different tRNAs. For example, both UUA and CUA code for leucine, but are read by different tRNAs.
3. The first base of an anticodon determines whether a particular tRNA molecule reads more than one codon for a given amino acid:
   i. When the first base of the anticodon is C or A, it can read only one codon.
   ii. When it is U or G, it can read two different codons.
   iii. When the wobble base of an anticodon is I (inosine), it can read three different codons.
4. It is not necessary to have 61 different types of tRNA to read all 61 possible code words. A minimum of 32 tRNAs is required to translate all 61 different codons for the amino acids.

TRANSLATION (PROTEIN BIOSYNTHESIS)

The pathway of protein Biosynthesis is called ***translation***.

- Translation is the process by which ribosomes convert the information carried by mRNA in the form of genetic code to the synthesis of new protein.

- Translation occurs in cytosol on ribosomes and is guided by mRNA.

The basic plan of protein synthesis in eukaryotes is similar to that in prokaryotes. However, eukaryotic protein synthesis involves more protein components and some steps are more intricate. Some differences are listed in **Table 20.3.**

Basic Requirements for the Translation

- mRNA
- tRNAs
- Ribosomes
- Energy in the form of ATP and GTP
- Enzymes and specific protein factors, e.g. initiation factors, elongation factors, etc.

Stages of Eukaryotic Translation

There are four major stages in protein synthesis, each requiring a number of components.

1. Activation of amino acids
2. Initiation
3. Elongation
4. Termination.

Activation of Amino Acid

Activation of amino acid takes place in the cytosol. In activation of amino acid each of the 20 amino acids, is covalently attached to their respective tRNA, at the expense of ATP, by **aminoacyl tRNA synthases (AAS)** enzyme **(Figure 20.10)**.

When an amino acid is attached to the tRNA to form aminoacyl tRNA, the latter can recognize a codon on mRNA by virtue of an anticodon in the tRNA structure.

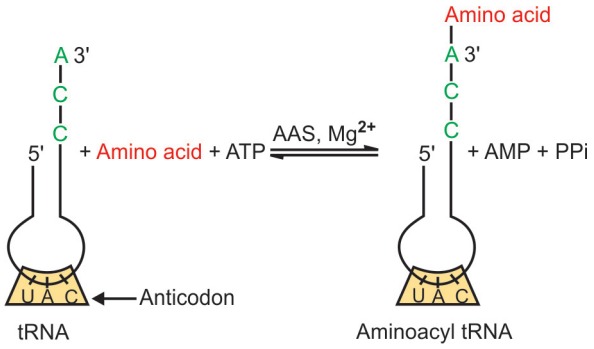


Figure 20.10: Activation of amino acid.
AAS: Aminoacyl tRNA synthase

Initiation (Figure 20.11)

Initiation can be divided into four steps:

1. Ribosomal dissociation
2. Formation of 43S pre-initiation complex.
3. Formation of 48S initiation complex
4. Formation of 80S initiation complex.

Ribosomal dissociation

Eukaryotic ribosomes are 80S particles composed of two subunits of 40S and 60S. The ribosome has two binding sites for tRNA molecules, the **A (aminoacyl)** and **P(peptidyl)** site. 40S subunit of ribosome binds two initiation factors elF_3 and elF-1A and dissociates 80S into two subunits 40S and 60S.

Formation of 43S pre-initiation complex

- *First it involves the binding of GTP with initiation factors elF_2.* This complex then binds to Met-$tRNA_i^{Met}$, (a tRNA specifically involved in binding to the initiation codon AUG on mRNA).

Table 20.3: Differences between eukaryotic and prokaryotic protein synthesis

Characters	*Eukaryotes*	*Prokaryotes*
Ribosome	Larger, 80S, consists of 60S and 40S subunits	Smaller, 70S, consists of 50S and 30S subunits
Initiator tRNA	Met-$tRNA_i^{Met}$	f-met-$tRNA_i^{f\ met}$
Start signal	Initiating AUG of mRNA is preceded by a cap, methylguanosyl triphosphate	Initiating AUG of mRNA is preceded by a purine rich sequence
Initiation factors	Contains more intiation factors than do prokaryotes Nine are known and several consists of multiple subunits The prefix elF denotes a eukaryotic initiation factor	Contain three initiation factors, IF_1, IF_2 and IF_3
Termination factors	Termination is carried out by a single release factor eRF	Termination is carried by three releasing factors RF_1, RF_2 and RF_3

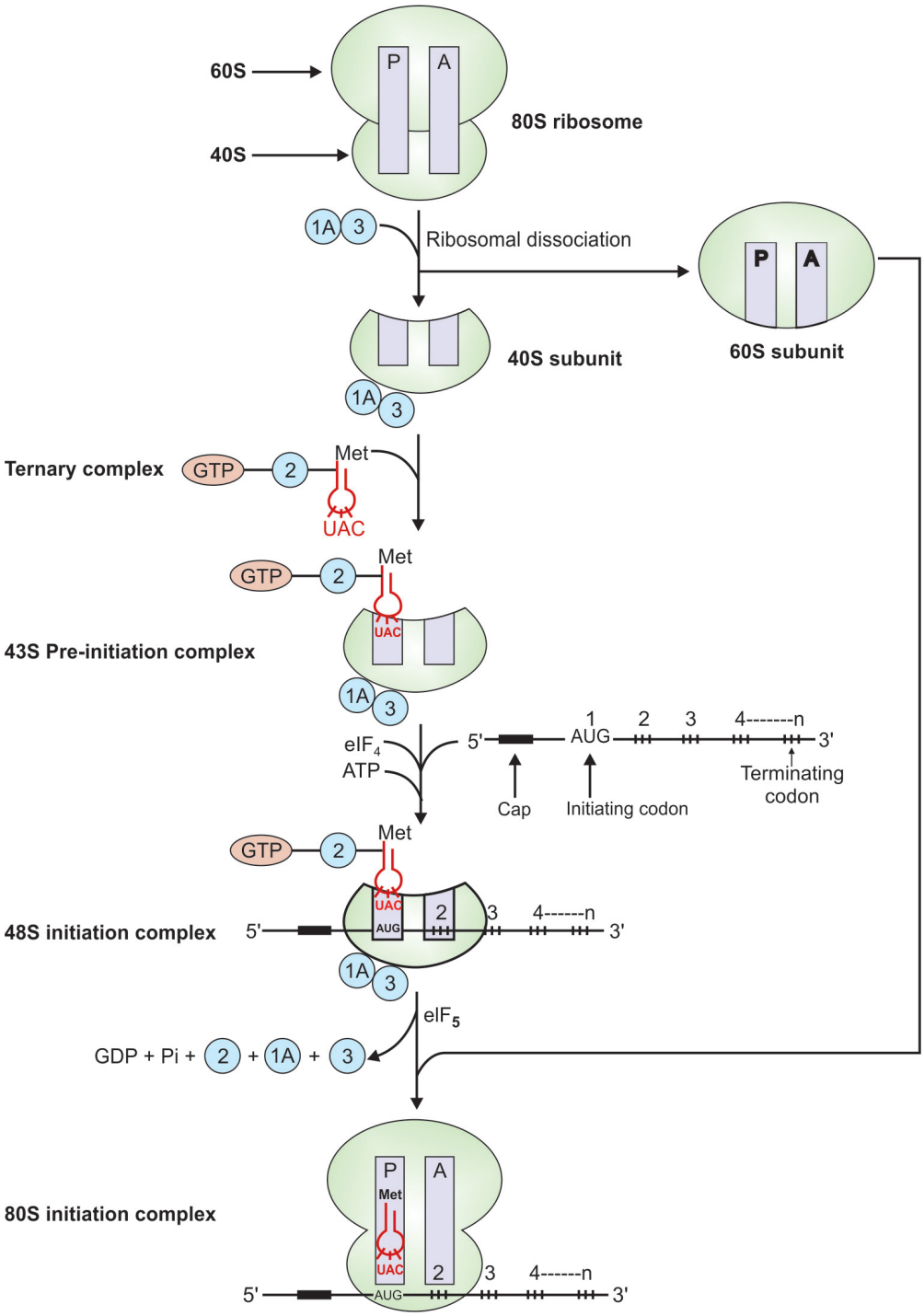


Figure 20.11: Diagrammatic representation of initiation of protein biosynthesis
1A: eIF-IA; 2: eIF_2; 3: eIF_3; CBP: Cap binding protein (eIF_4)

In prokaryotes, the first amino acid methionine is modified by formylation of its amino group to N-formyl-methionine (f Met) and initiator tRNA is fmet-$tRNA_i^{fMet}$.

- *This* **ternary (GTP-elF$_2$-tRNA)** *complex binds to the 40S ribosomal subunit to form* **43S pre-initiation complex,** *which is stabilized by association with elF$_3$ and elF-1A.*

Formation of 48S initiation complex

- Binding of mRNA to the 43S pre-initiation complex forms ***48S initiation complex.***
- The 5' terminals of most mRNA molecules in eukaryotic cells are capped by methylguanosyl triphosphate, which facilitates the binding of mRNA to the 43S pre-initiation complex to form 48S initiation complex.
- The association of mRNA with 43S initiation complex requires **cap binding protein (CBP)** or **eIF$_4$** and **ATP.**

In prokaryotes, a sequence of nucleotide bases on mRNA known as ***Shine-Dalgarno sequence*** facilitates the binding of mRNA to the preinitiation complex.

Formation of 80S initiation complex

- Combination of the 48S initiation complex with 60S ribosomal subunit forms 80S initiation complex.
- The binding of the 60S ribosomal subunit to the 48S initiation complex involves the hydrolysis of the GTP bound to elF$_2$, by elF$_5$ with the release of the initiation factors bound to the 48S initiation complex. These factors are then recycled.
- At this stage, the Met-$tRNA_i^{Met}$, is on the P site of the ribosome so that its anticodon pairs with the initiating AUG codon on the mRNA and is now ready for the elongation process.

Elongation (Figure 20.12)

Elongation can be divided into three steps

1. Binding of next aminoacyl tRNA specified by the next coding triplet in the mRNA to the A site of ribosome.
2. Formation of peptide bond.
3. Translocation.

Binding of aminoacyl tRNA to the A site

- The next aminoacyl tRNA specified by the next codon in the mRNA is first bound to a complex of elongation factor eEf-1α-containing a molecule of bound GTP
- The resulting aminoacyl tRNA-eEF-1α- GTP complex then allows aminoacyl tRNA to enter the A site on the ribosome with the release of eEF-1α-GDP and phosphate.

Formation of peptide bond

- In the second step of the elongation cycle, a new peptide bond is formed between the amino acids whose tRNAs are located on the A and P sites of the ribosome.
- This step occurs by the transfer of the initiating methionine from its tRNA to the amino group of the new amino acid that has just entered the A site.
- This step is catalyzed by ***peptidyl transferase (ribozyme).***
- As a result of this reaction, a dipeptide tRNA is formed on the A site and the ***"empty"*** initiating $tRNA_i^{Met}$ remains bound to P site.

Translocation

- In the third step of the elongation cycle the ribosome moves along the mRNA, towards its 3' end, by a distance of one codon (three bases).
- The movement of the ribosome shifts the dipeptidyl tRNA from the A site to the P site, which causes the release of the preceding tRNA, which is empty, from the P site back into the cytosol.
- Now the third codon of the mRNA is on the A site and second codon on the P site. This shift of ribosome along the mRNA is called the ***translocation.*** It requires elongation factor (translocase) eEF$_2$.
- The hydrolysis of GTP provides energy for the translocation.

The ribosome with its attached dipeptidyl tRNA and mRNA is now ready for another elongation cycle to attach the third amino acid residue, which proceeds in precisely the same way as the addition of the second. Thus, the process of peptide synthesis occurs until a termination codon is reached.

Termination (Figure 20.12)

- The elongation steps are repeated until one of the three (UAA, UAG, UGA) termination or nonsense codon of mRNA appears in the A site.
- Once the ribosome reaches a termination codon, releasing factors are capable of recognizing the termination signal present in the A site.
- Prokaryotes have three release factors, **RF-1, RF-2,** and **RF-3** but eukaryotes have only single release factor, eRF.
- Releasing factors in conjunction with GTP and peptidyl transferase hydrolyze the peptidyl tRNA bond (the bond between the peptide and the tRNA), when a nonsense codon occupies the A site.
- This hydrolysis releases the polypeptide and tRNA from P site.

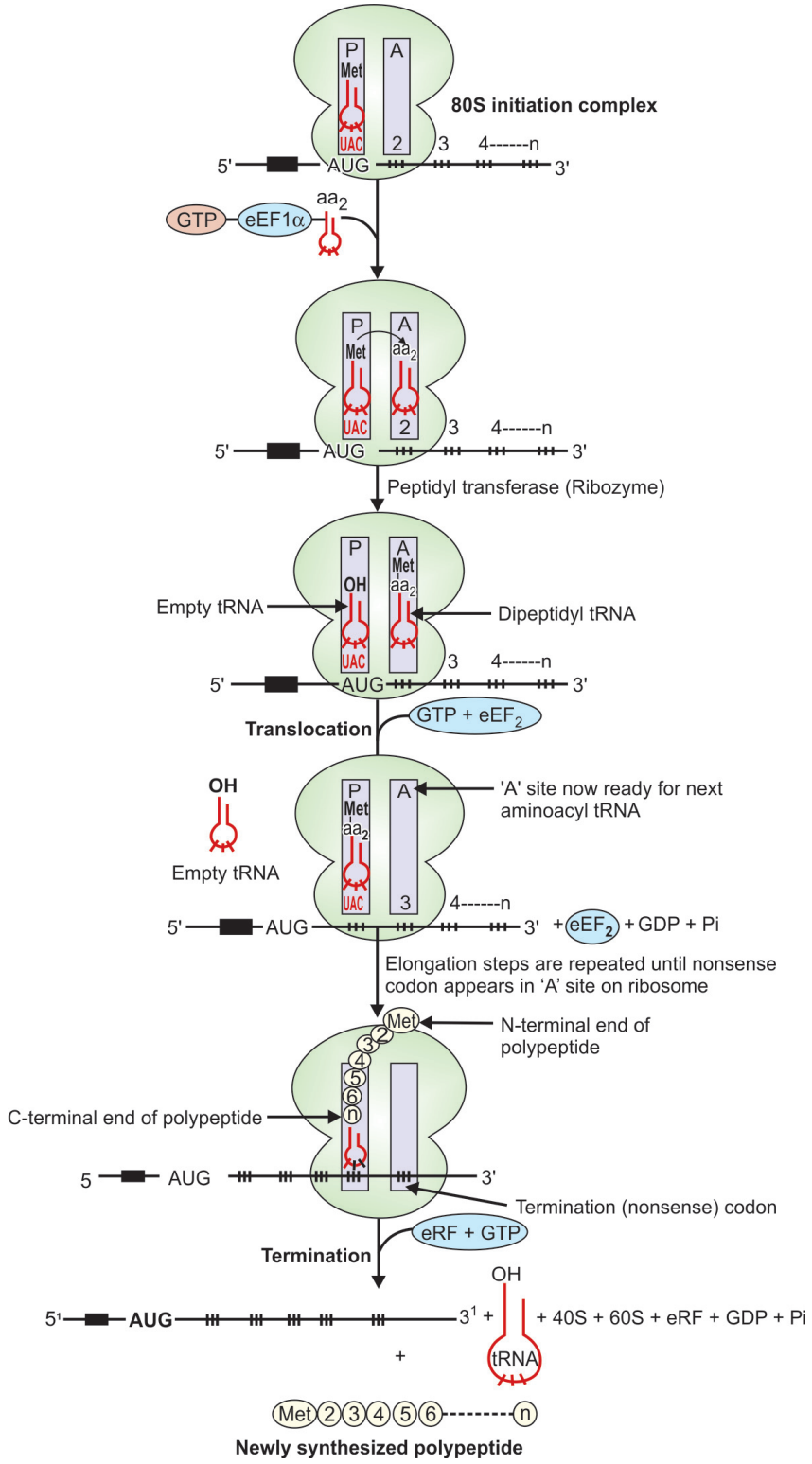


Figure 20.12: Diagrammatic representation of elongation and termination of protein biosynthesis

- Upon hydrolysis and release of polypeptide the mRNA is then released from the ribosome.
- Ribosome then dissociates into 60S and 40S subunits, which are then recycled.

FOLDING AND PROCESSING (POST-TRANSLATIONAL MODIFICATION)

In order to achieve native biologically active form of the polypeptide, it must undergo folding into its proper three-dimensional conformation. The **chaperones** (a group of specialized proteins) ensure the folding of a protein into its native form. Before or, after folding, the polypeptide may undergo processing by enzymatic action. Collectively these alterations are known as ***post-translational modifications.*** These modifications may include removal of part of the translated sequence or the covalent addition of one or more chemical groups required for protein activity. Some types of post-translational modifications are listed below.

1 Amino Terminal Modifications

- Initially, all polypeptides begin with a residue of N-formyl methionine (in prokayotes) or methionine (in eukaryotes).
- The formyl group and the amino terminal methionine residue are removed enzymatically.

2 Loss of Signal Sequence

- 15–30 residues of amino terminal end of some proteins required to direct the proteins to its ultimate destination in the cell. Such signal sequences are ultimately removed by specific peptidase.

3 Covalent Modification of Proteins

- **Glycosylation: Glycoproteins** and **proteoglycans** are attached covalently during or after the synthesis of polypeptide chain.
- **Phosphorylation:** Covalent addition of phosphate group to hydroxyl group of serine, threonine and tyrosine.
- **Carboxylation:** It involves carboxylation of amino acids like glutamic acid residues of prothrombin and other blood clotting factors.
- **Hydroxylation:** During formation of collagen, the prolin and lysine residues are hydroxylated to hydroxylproline and hydroxylysine respectively.
- **Methylation:** In some proteins lysine residues are methylated enzymatically, e.g. methylated lysine residues present in muscle proteins and cytochrome C.
- **Addition of prosthetic group:** It includes covalent attachment of non protein substance to protein, e.g. biotin is covalently bound to acetyl CoA carboxylase and heme group of cytochrome.

4 Proteolytic Processing

Insulin and proteases such as trypsin and chymotrypsin are initially synthesized as larger, inactive precursor proteins. These precursors are proteolytically trimmed to produce their final, active forms.

INHIBITORS OF PROTEIN SYNTHESIS

A number of commonly used variety of antibiotics, act by inhibiting selectively the process of prokaryotic protein biosynthesis. The most useful antibiotics do not interact with eukaryotic protein synthesis and thus are not toxic to eukaryotes. Such antibiotics can be used as therapeutic drugs **(Table 20.4).** Puromycin and cycloheximide are not clinically useful because they inhibit protein biosynthesis in eukaryotes also but are used for research purpose.

Table 20.4: Inhibitors of protein synthesis

Antibiotic	*Action*
Streptomycin	It binds to the 30S subunit of prokaryotes at the A site, thereby inhibits chain elongation by preventing the binding of additional aminoacyl tRNA
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl tRNA to mRNA in prokaryotes
Chloramphenicol	Binds to the 50S ribosomal subunit and blocks the peptidyl transferase reaction in prokaryotes
Erythromycin	Binds to the 50S ribosomal subunit that inhibits the translocation reaction in prokaryotes
Lincomycin and Clindamycin	Binds to the 50S subunit and inhibits peptidyl transferase, thereby, preventing peptide bond formation in prokaryotes
Puromycin	Causes premature chain termination in both prokaryotes and eukaryotes
Cycloheximide	Inhibits peptidyl transferase activity of the 60S ribosomal subunit in eukaryotes

PROTEIN TARGETING AND DEGRADATION

- The pathway by which proteins are sorted and transported to their proper cellular location are often referred to as **protein targeting pathways.**
- The most important element in all of these targeting systems (with the exception of cytosolic and nuclear proteins) is a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the **signal sequence,** which do not have a common amino acid sequence.
- This signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination.

SUMMARY

- Replication is the process of synthesis of DNA.
- DNA synthesis is semiconservative.
- The major enzyme involved in the replication of DNA is DNA polymerase.
- Synthesis of RNA from DNA template is called transcription.
- Transcription is catalyzed by enzymes known as RNA Polymerases.
- The direction of RNA synthesis is 5' → 3' direction as in DNA synthesis.
- In prokaryotes, a single RNA polymerase produces the precursors of mRNA, tRNA and rRNA.
- Eukaryotic RNA is transcribed in the nucleus by three different RNA polymerases; I, II, and III. Type I make rRNA, Type II mRNA and Type III tRNA.
- The compounds that inhibit RNA synthesis in prokaryotes, but not in eukaryotes, use as therapeutic drugs, e.g. rifampin and actinomycin D.
- Amino acids are coded by group of three bases called codons, present on mRNA.
- Sixty-one out of the sixty-four codons specify particular amino acids, whereas the other three codons (UAA, UAG, UGA) are signals for chain termination, called nonsense or termination codons.
- The code is almost universal.
- The code is non-overlapping and without punctuation. The mRNA is read continuously from a start codon AUG to a termination codon.
- Some tRNAs recognize more than one codon because of wobble in base pairing.
- Translation is the process by which ribosomes convert the information carried by mRNA to the synthesis of protein.
- A variety of antibiotics, which act by inhibiting selectively the processes of prokaryotic translation, can be used as therapeutic drugs.

EXERCISE

Multiple Choice Questions (MCQs)

1. A promoter site on DNA:
a) Transcribes represser
b) Codes for RNA polymerase
c) Initiates transcription
d) Regulates termination

2. A codon AUG is a:
a) Chain initiating codon
b) Chain terminating codon
c) Releasing factor for peptide chains
d) Recognition site on the tRNA

3. Release of a polypeptide chain from a ribosome is catalyzed by:
a) Release factors
b) Dissociation of ribosomes
c) Peptidyl transferase
d) Stop codons

4. In contrast to eukaryotic mRNA, prokaryotic mRNA:
a) Has a poly A tail
b) Has 7-methylguanosine at the 5′end
c) Can be polycistronic
d) None of the above

5. Erythromycin prevents synthesis of polypeptide:
a) By inhibiting binding of aminoacyl tRNA to mRNA
b) By inhibiting translocation reaction
c) By inhibiting binding of tRNA to mRNA
d) Blocking mRNA formation from DNA

6. Formation of Okazaki fragments occur in the process of:
a) Transcription
b) Translation
c) Replication
d) Reverse transcription

7. Termination of protein biosynthesis requires presence of:
a) Termination sequences "ter"
b) Rho factor
c) Nonsense codons
d) Sigma factor

8. Which of the following enzyme joins Okazaki fragments?
a) DNA polymerase
b) DNA ligase
c) RNA polymerase
d) Peptidyl transferase

9. Which the following enzyme fill the gap between Okazaki fragments?
a) DNA polymerase b) RNA polymerase
c) Translocase d) Helicase

10. Which of the following nucleotide base is not present in codons?
a) Adenine b) Guanine
c) Thymine d) Cytosine

11. Total number of codons are:
a) 64 b) 61
c) 62 d) 63

12. Which of the following eukaryotic DNA polymerases is required for mitochondrial DNA synthesis?
a) DNA polymerase α
b) DNA polymerase β
c) DNA polymerase γ
d) DNA polymerase δ

13. Degeneracy of the genetic code means that:
a) Codons are not ambiguous
b) A given amino acid can be coded for by more than one base triplet
c) A given codon can code for more than one amino acid
d) There is no punctuation in the code sequence

14. Which of the following inhibits eukaryotic protein synthesis?
a) Tetracycline
b) Erythromycin
c) Streptomycin
d) Puromyrin

15. Consensus sequence is:
a) Initiation site of replication in eukaryotes
b) Initiation site of replication in prokaryotes
c) Initiation site of transcription in eukaryotes
d) Initiation site of transcription in prokaryotes

Correct Answers for MCQs

1-c	2-a	3-c	4-c
5-b	6-c	7-c	8-b
9-a	10-c	11-a	12-c
13-b	14-d	15-a	

CHAPTER

21

Regulation of Gene Expression and Mutation

INTRODUCTION

Gene expression is the combined process of the transcription of a gene into mRNA and its translation into protein. Genetic mutation alter the regulation or expression of gene and results in dysfunctional or non-functional protein synthesis.

The aim of this chapter is to introduce the basic concepts involved in the regulation of genes and how these processes may involved in the causation of mutation.

REGULATION OF GENE EXPRESSION

Information, encoded in DNA, is transcribed into RNA and then translated into protein. It is called the ***gene expression****. Thus, gene is expressed in terms of synthesis of protein.* The regulation of gene expression is essential for metabolic functions, growth and development and differentiation of tissues.

The rate of expression of prokaryotic genes is controlled mainly at the level of transcription, mRNA synthesis.

Eukaryotes, however have a much larger and more complex genome than prokaryotes. Gene expression in eukaryotes is controlled by many ways.

Types of Gene Regulation

There are two types of gene regulation:

- Positive regulation
- Negative regulation.

Positive Regulation

When the expression of genetic information is increased by the presence of a specific regulatory element, regulation is said to be ***positive.*** The element or molecule mediating the positive regulation is said to be an ***activator*** or ***inducer.***

Negative Regulation

When the expression of genetic information is diminished by the presence of a specific regulatory element, regulation is said to be **negative** and element mediating negative regulation is said to be a **repressor.**

Types of Genes

There are two types of genes:

- Inducible gene
- Constitutive gene.

Inducible Gene

Inducible genes are expressed only when a specific positive regulatory substance, i.e an inducer or activator is present, e.g. the production of the enzyme β-galactosidase is induced by the presence of lactose in the prokaryotes or insulin is an inducer of the gene glucokinase of glycolysis in human beings.

Constitutive Gene

Constitutive genes refer to genes whose expression is not regulated. They are expressed at a constant rate. These are often referred to as ***housekeeping genes.***

REGULATION OF GENE EXPRESSION IN PROKARYOTES

In prokaryotes, the genes involved in a metabolic pathway are often present in a linear fashion, called an **operon.** For example:

- Lactose operon (Lac operon for regulation of lactose metabolism)
- Arabinose operon (Ara operon for regulation of arabinose metabolism)
- Galactose operon (Gal operon for regulation of galactose metabolism).

LACTOSE OPERON OR LAC OPERON

Jacob and Monod, in 1961, described their operon model for the regulation of lactose metabolism (lac operon) by *E. coli*.

Definition of Lac Operon

Lac operon is a coordinated unit of gene expression to make the enzymes necessary to metabolize lactose.

Structure of the Lac Operon (Figure 21.1)

The lac operon is a region of DNA in the genome of *E.coli* that contains the following genetic elements:

1. **Regulatory gene (lac i)** produces a repressor protein
2. **A promoter site (P)** for the binding of RNA polymerase. Promoter site contains two specific regions:
   i. Catabolite activator protein binding site (CAP site)
   ii. RNA polymerase entry site, to which RNA polymerase first becomes bound.
3. **An operator site (O)**, a regulatory protein called the **lac repressor protein** binds to this site and blocks initiation of transcription.
4. **Three structural genes, Z, Y and A,** that code for β-galactosidase, galactoside permease and transacetylase respectively, required for lactose metabolism.

This genetic arrangement of the structural genes and their regulatory genes allows for the coordinated expression of the three enzymes concerned with lactose metabolism.

Regulation of Lac Operon

Lac operon is regulated by following mechanism:

1. Regulation in absence of lactose and presence of glucose.
2. Regulation in presence of lactose and absence of glucose.
3. Regulation in presence of both glucose and lactose.

Regulation of gene expression in absence of lactose and presence of glucose

- *E. coli* bacteria usually depend on glucose as their source of energy. However, in absence of glucose, *E. coli* can use lactose as their energy source.

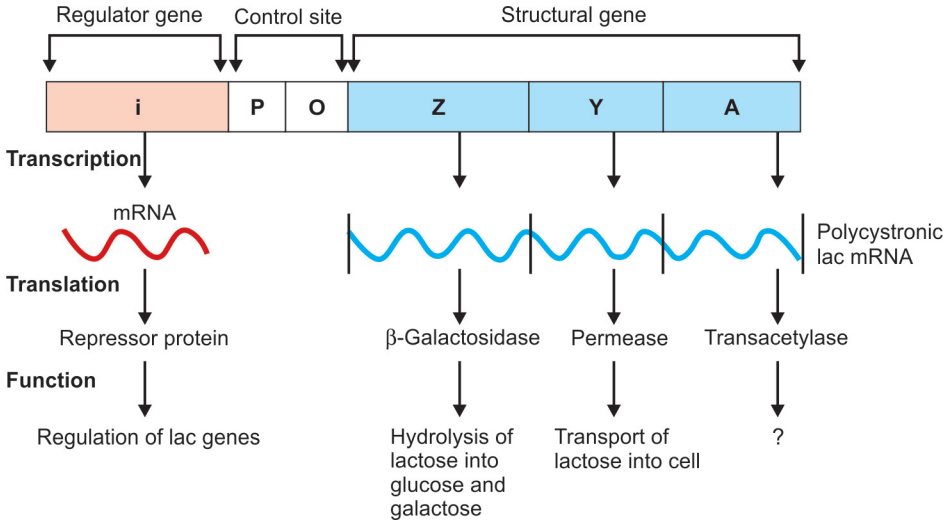


Figure 21.1: Schematic diagram of lac operon
where, P: Promoter, O: Operator, i: Lac i gene, Z: Lac Z gene, Y: Lac Y gene, A: Lac A gene

- In the absence of lactose, the cell has no need for the production of **β-galactosidase, galactoside permease** and **transacetylase** which are required for metabolism of lactose. Hence regulatory molecule, the **lac repressor protein,** prevents expression of the lac operon in the absence of lactose.
- In the absence of lactose, repressor protein (a product of regulatory gene i) can bind to the operator, thus preventing the binding of RNA polymerase and subsequent transcription of the structural genes Z, Y and A. Under these conditions, β-galactosidase and other two enzymes are not made by the cells **(Figure 21.2A)**.

Regulation of gene expression, in presence of lactose and absence of glucose

- In presence of lactose and absence of glucose in the medium, lactose acts as an inducer. It binds to repressor protein and inactivates it.
- The inactive repressor protein no longer binds to the operator.
- RNA polymerase now can bind to the promoter and transcribe the three structural genes of the operon.
- The result is the continued production of the enzymes needed for the metabolism of lactose **(Figure 21.2B)**.

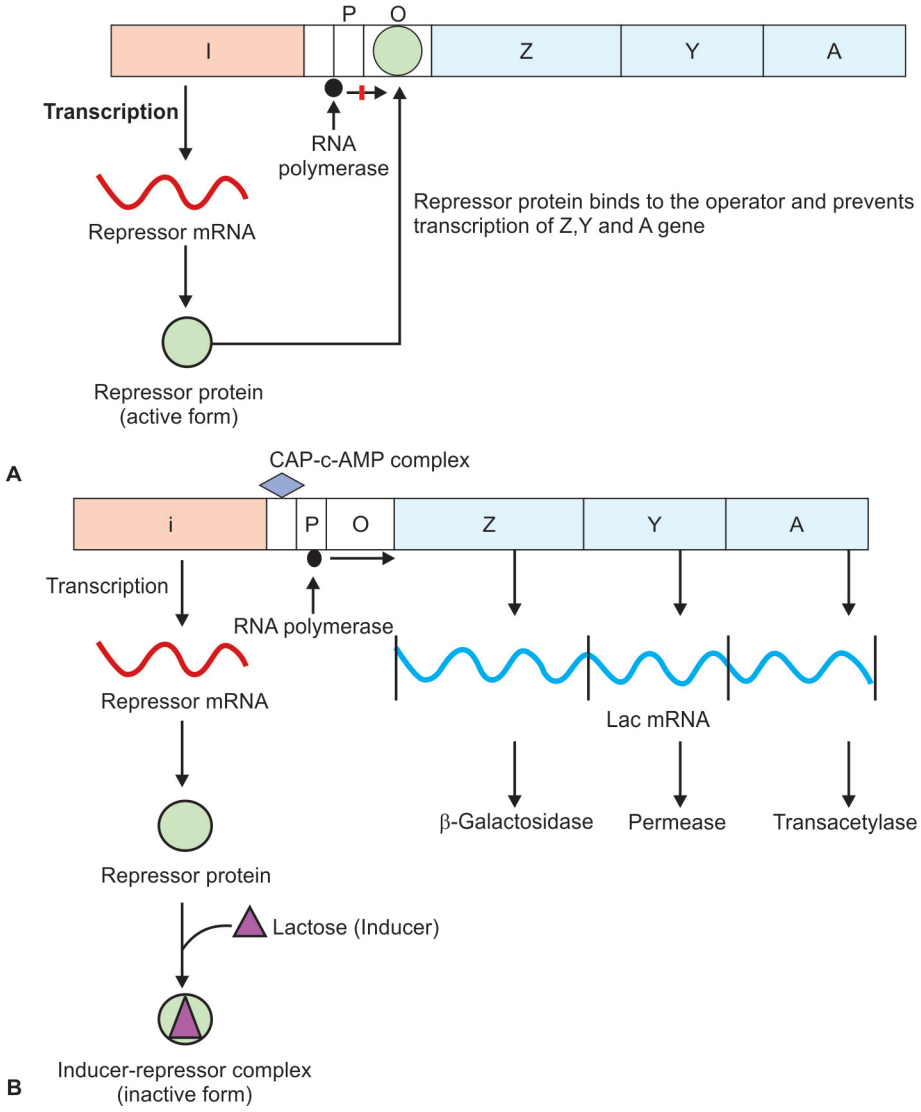


Figure 21.2: Regulation of lac operon
A: In presence of glucose and absence of lactose; B: In presence of lactose and absence of glucose

Regulation of gene expression in presence of both glucose and lactose (Figure 21.2B).

When *E. coli* is exposed to both lactose and glucose, they first metabolize the glucose, although lactose is present. The cell does not induce those enzymes necessary for catabolism of lactose until the glucose has been exhausted.

- As glucose levels decrease, c-AMP levels increase. In the presence of c-AMP, a complex of c-AMP-**Catabolite activator Protein (CAP)** binds to the CAP binding site of promoter, stimulating the binding of the RNA polymerase to the promoter and transcription occurs.
- As glucose levels increase, c-AMP levels decrease and in the absence of c-AMP, CAP does not bind to CAP-binding site of promoter and transcription does not occur. Thus, the enzymes for metabolism of lactose are not produced if cells have an adequate supply of glucose even if the alternative energy source, lactose, is present at very high levels.

MUTATIONS

The term mutation refers to the permanent changes in the DNA sequence.

- Mutations in germ cells are transmitted to the next progeny and may give rise to inherited diseases.
- Mutations in somatic cells are not transmitted to the progeny but are important in the causation of cancers and some congenital malfunctions.

Causes of Mutations

Mutation arises by a number of different means:

- **Errors in replication**, If a mismatch base pair is not corrected during proofreading and post replication repair system.
- **Error due to recombination events**.
- **Spontaneous change in DNA**, e.g. deamination of cytosine to uracil or spontaneous depurination.
- **Environmental factors** like chemical mutagens and irradiations, e.g. UV light or ionizing radiation can alter the structure of DNA.

Types of Mutations (Figure 21.3)

Base Substitution or Point Mutation

- Point mutation occurs when only one base in DNA is altered, which may be transcribed into mRNA and therefore, may result in the translation of a protein with an abnormal amino acid sequence. Single base change can be of **transition type** or **transversion type**.
- **Transition,** in which one purine is replaced by another purine or one pyrimidine is replaced by another pyrimidine **(Figure 21.4)**.
- **Transversion,** in which a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine **(Figure 21.4)**.
- The point mutation can lead to:
  1. Silent mutation
  2. Missense mutation
  3. Nonsense mutation.

Silent mutation

Point mutations are said to be silent when there is no detectable effect. Silent mutation leads to the formation of a codon synonym and no change in the amino acid sequence of the protein occurs due to degeneracy of the codon, e.g. a codon change from CGA to CGG does not affect the proteins because both of these codons specify arginine.

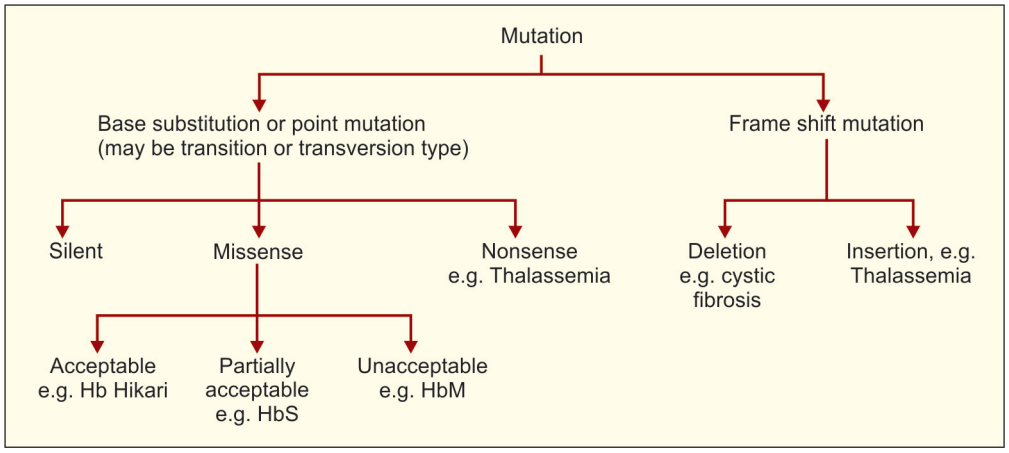


Figure 21.3: Types of mutation

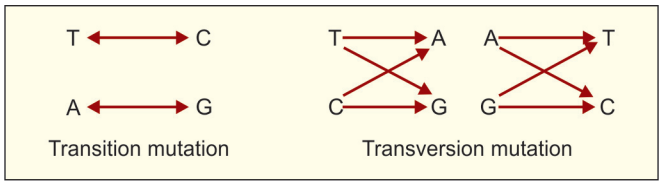


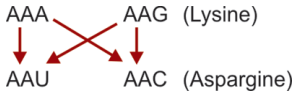
Figure 21.4: Diagrammatic representation of transition and transversion mutations

Missense mutation

Missense mutation will occur when a different amino acid is incorporated at the corresponding site in the protein molecule. Depending upon the location of the mistaken amino acid (missense) in the specific protein, missense mutation might be **acceptable, partially acceptable** or **unacceptable** with respect to the function of that protein.

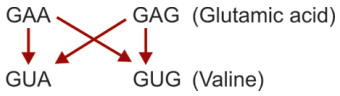
Acceptable missense mutations

For example, ***Hb-Hikari***. This Hb has aspargine substituent for lysine at the 61 position in the β-globin chain. Hb-Hikari is a type of **transversion mutation,** in which either AAA or AAG changed to either AAU or AAC. The replacement of the specific ***lysine with aspargine*** does not alter the normal function of the β-chain in these individuals and is therefore called acceptable missense mutation.



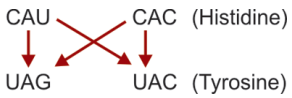
Partially acceptable missense mutation

For example, HbS, ***sickle hemoglobin***, in which the normal amino acid in position 6 of the β-chain, glutamic acid has been replaced by valine. The corresponding transversion might be either GGA or GAG of ***glutamic acid*** *to* GUA or GUG of ***valine***.



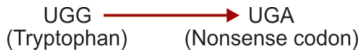
Unacceptable missense mutation

For example, *Hb M (Methemoglobin)* in which normal amino acid in position 58 of α-chain, ***histidine*** has been replaced by ***tyrosine*** and nonfunctional Hb molecule is generated which cannot transport oxygen.



Nonsense mutations

Nonsense mutation leads to the conversion of an amino acid codon to a stop or nonsense codon. Nonsense mutation causes the premature termination of a polypeptide chain, which is usually nonfunctional, e.g. one type of ***thalassemia***, in which codon 17 of the β-chain is changed from UGG to UGA and results in the conversion of a codon tryptophan to a nonsense codon.



Frame Shift Mutations **(Figure 21.5)**

Frame shift mutation occurs when there is insertion or deletion of one or two nucleotides in DNA, that generates altered mRNAs.

Deletion frame shift mutation

- The deletion of a single nucleotide from the coding strand of a gene results in an altered reading frame in the mRNA.
- Since there is no punctuation in the reading of codons, the translating machinery does not recognize that a base was missing.
- Such frame shifts would result in the production of an entirely different protein after transcription and translation or indeed, no protein, at all, if a stop codon is encountered, as in cystic fibrosis of pancreas.

Insertion frame shift mutation

Insertion may be of one or two nucleotides. As with deletions, insertions of nucleotides into genes can lead to severe frame shift mutations, e.g. thalassemia.

If the number of nucleotides involved in deletion or insertion is three or multiples of three, frame shift does not occur. Instead, an abnormal protein, missing one or more amino acids, is synthesized. Such a mutation is likely to be less severe than a frame shift mutation.

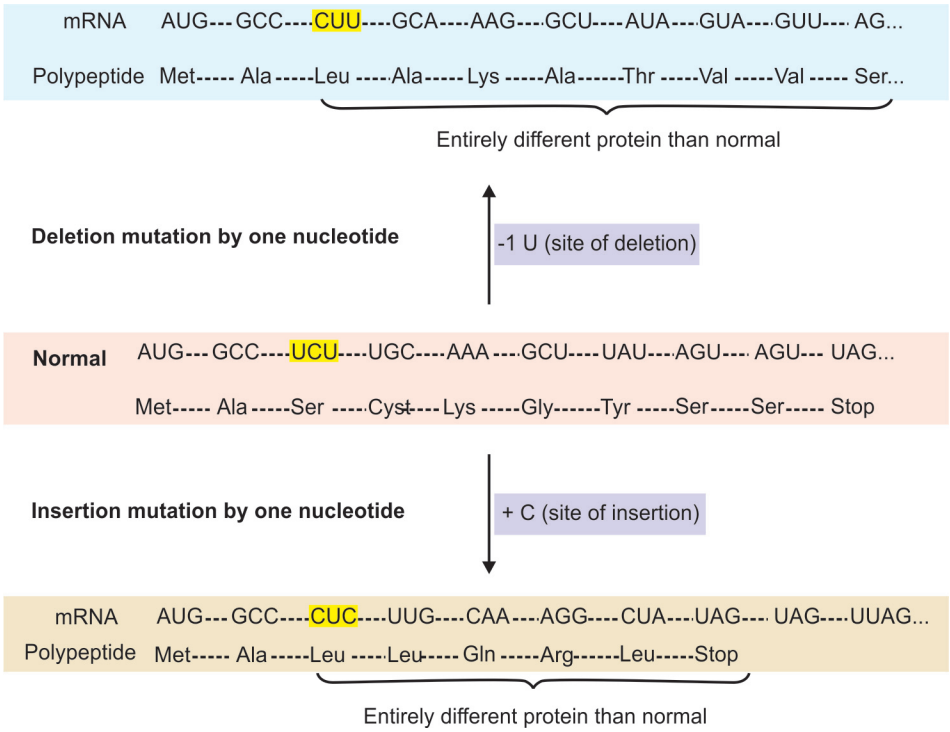


Figure 21.5: Diagrammatic representation of frame shift mutation

SUMMARY

- Mutations result when changes occur in the nucleotide sequence in DNA.
- Point mutations result from single base substitution. These may be transition or transversion type. Types of point mutations are silent mutation, missense mutation and nonsense mutation. Missense mutation might be acceptable or unacceptable to the function of that protein molecule.
- Frame shift mutations result from deletion or insertion of nucleotides in DNA that generates alterered mRNAs.
- An operon is a group of coordinately regulated genes. It consists of control sites (an operator and promoter) and a set of structural genes.
- The rate of expression of prokaryotic genes is controlled mainly at the level of transcription.

CHAPTER

22

Genetic Engineering

INTRODUCTION

Engineering is the designing, construction and repair of structures of nonliving things. Genetic engineering is the construction and repair of the genes of living things. Now, it is possible to synthesize a gene in a laboratory and then introduce it into a living cell which will synthesize protein corresponding to that gene.

RECOMBINANT DNA

- Recombinant DNA is a DNA formed by the hybrid combination of two DNA fragments, derived from different sources.
- *In vivo* genes often undergo recombinations.
- The normal biological exchange or addition of genes from different sources to form an altered chromosome, which can be replicated, transcribed and translated, is called ***genetic recombination.*** It occurs in a number of different biological situations.
- In eukaryotic organisms genetic recombination occurs by the sexual union of egg and sperm cell, in which both parental chromosomes contribute certain genes to the new daughter chromosomes appearing in the progeny cell.
- In this process, the chromosomes of both the sperm and egg cells undergo cleavage at homologous point, and pieces of the chromosomes from the two parent cells are then exchanged and spliced together to yield new combinations of genes.
- Such naturally occurring cutting, reassembly and splicing of genes during sexual conjugation of eukaryotes occur without disturbing the reading frame of the DNA sequence.

Artificial Recombinant DNA

Genes or sets of gene can also be recombined *in vitro* (in the test tube) to produce new combinations that do not occur biologically. ***Recombinant DNA technology involves isolation and manipulation of DNA to make chimeric or hybrid DNA molecule***.

Chimeric DNA

Chimeric DNA is a recombinant DNA containing genes from two different species, e.g. molecules containing both human and bacterial DNA sequences (Chimera was a mythological creature with the head of the lion, the body of goat and the tail of the snake). A number of techniques have been used to construct recombinant DNA.

Construction of Recombinant DNA

Following steps are involved in the construction of recombinant DNA:

1. Fragmentation of DNA by restriction endonuclease enzyme
2. Isolation of specific human DNA
3. Insertion of isolated human DNA into vector to form chimeric or hybrid DNA molecule
4. Joining of two different cut DNA fragments by DNA ligase.

Fragmentation of DNA by Restriction Endonuclease Enzyme

Restriction endonuclease

- Restriction endonuclease also called ***restriction enzyme.*** These are found in wide variety of prokaryotes. These enzymes were originally called restriction enzymes because their presence in a given bacterium restricted the growth of foreign infective bacterial viruses (bacteriophages).
- Restricted enzymes cut DNA of any source in a sequence specific manner in contrast to action of most other endonuclease which break DNA randomly. The restricted enzymes are named according to the bacteria from which they are isolated, e.g.
  - **EcoRI** is from *Escherichia coli*
  - **BamHI** is from Bacillus *Amylo liquefacients*.
- Restriction endonuclease recognizes specific base sequences of double helical DNA, usually 4 to 6 base pairs in length and cleave both strands within this sequence.
- Most of the DNA sequences recognized by restriction endonucleases are ***palindromic*** (in Greek palindromic = running back again), i.e. both strands of DNA have the same sequence when read in 5' to 3' direction **(Figure 22.1)**.

Action of restriction endonuclease

- Some restricted endonucleases cleave both strands of DNA, so as to leave no unpaired bases on either end. These ends are often called ***blunt ends.***
- Some restricted endonucleases make staggered cuts on the two strands, leaving two to four nucleotides of one strand unpaired at each resulting end. These are referred to as **cohesive ends** or ***sticky ends*** **(Figure 22.2).**
- Different restriction fragments of DNA can base-pair with each other if they have sticky ends which are complementary.
- Therefore, two unrelated DNA fragments can base-pair with each other, if they were cleaved by the same restriction endonuclease enzyme.

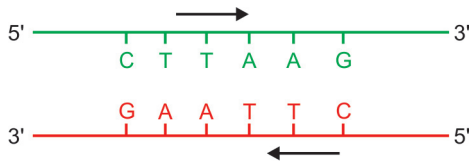


Figure 22.1: Palindromic sequence when read in 5' → 3' direction

Isolation of Specific Human DNA

Once DNA has been cleaved into fragments by restriction endonuclease, a particular fragment of interest can be separated by others, by electrophoresis or HPLC.

Insertion of Isolated Human DNA into Vector to Form Chimeric or Hybrid DNA Molecule (Figure 22.3)

A specific selected fragment of DNA of human genome is inserted into vector (carrier). *A vector is a carrier DNA molecule* to which the fragment of DNA of interest is attached. Normally, foreign DNA fragments cannot self-replicate in a cell. Therefore, they are joined together to a vector, that can replicate within the host cell. Most commonly used cloning vectors are:

- Bacterial plasmids
- Bacteriophages
- Cosmids.

Joining of Two Different Cut DNA Fragments by DNA Ligase

After the fragments of DNA (one from human genome and another from vector DNA) have base paired, the ends are covalently joined by the action of ***DNA ligase.*** Restriction enzymes in conjunction with DNA ligase, therefore, can produce vector containing recombinant or hybrid or chimeric DNA.

CLONING

Cloning is a technique developed for amplifying the quantity of DNA, *in vivo*. Cloning allows for the production of a large number of identical DNA molecules, which can be produced as follows:

- **Introduction of recombinant DNA into appropriate host cell:** The recombinant DNA inserted back into bacterial cell (host cell) by the process called ***transformation***. Host cells that contain recombinant DNA are called ***transformed cells.***

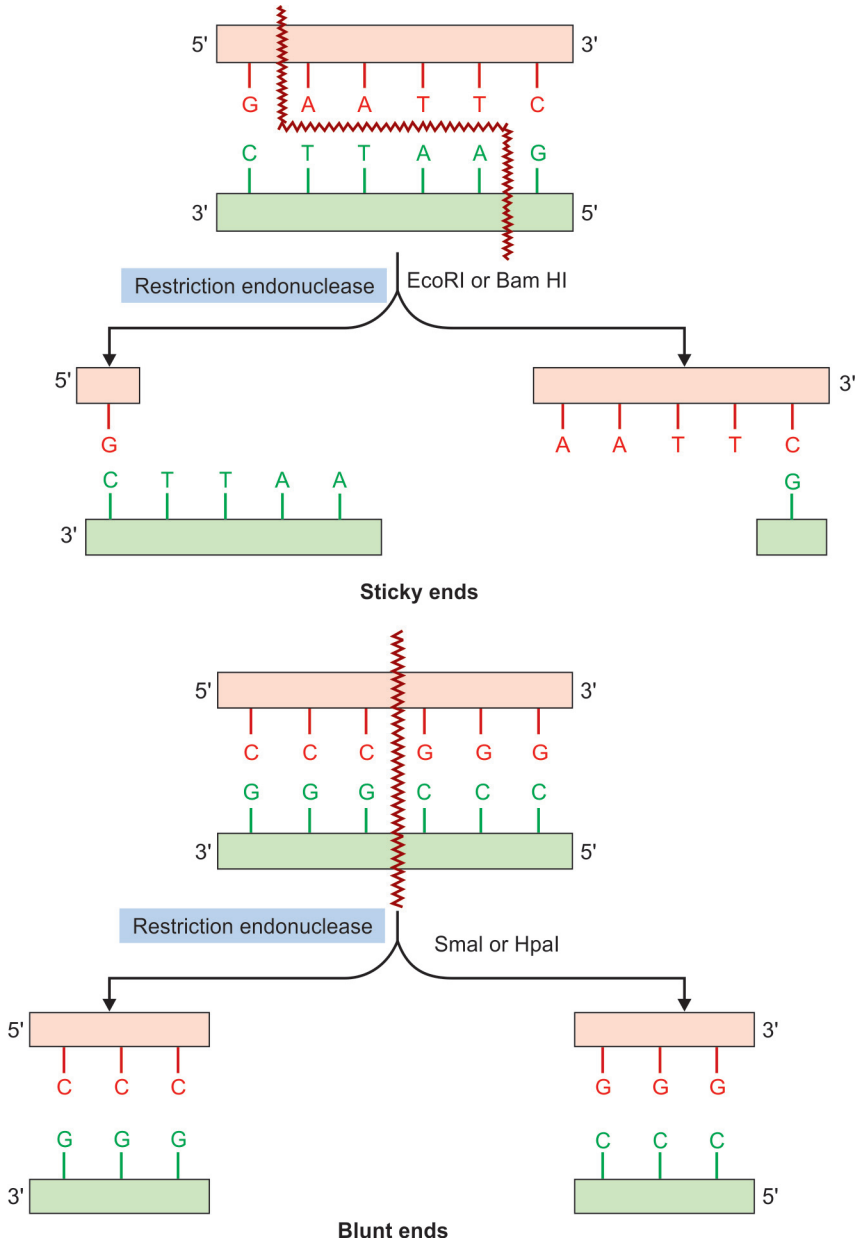


Figure 22.2: Action of restriction endonuclease
• EcoRI or BamHI produces sticky ends
• SmaI or HpaI produces blunt ends

- **Amplification of recombinant DNA:** The host cells containing the recombinant DNA are incubated under conditions in which they replicate rapidly. As the host cell divide in addition to replicating their own DNA, they also replicate the DNA of the vector which includes the human DNA. Subsequently, relatively large quantities of human DNA can be isolated from cells.
- If the host cells are grown under conditions permitting expression of the human DNA, the human protein produced from this DNA can be isolated **(Figure 22.4)**.

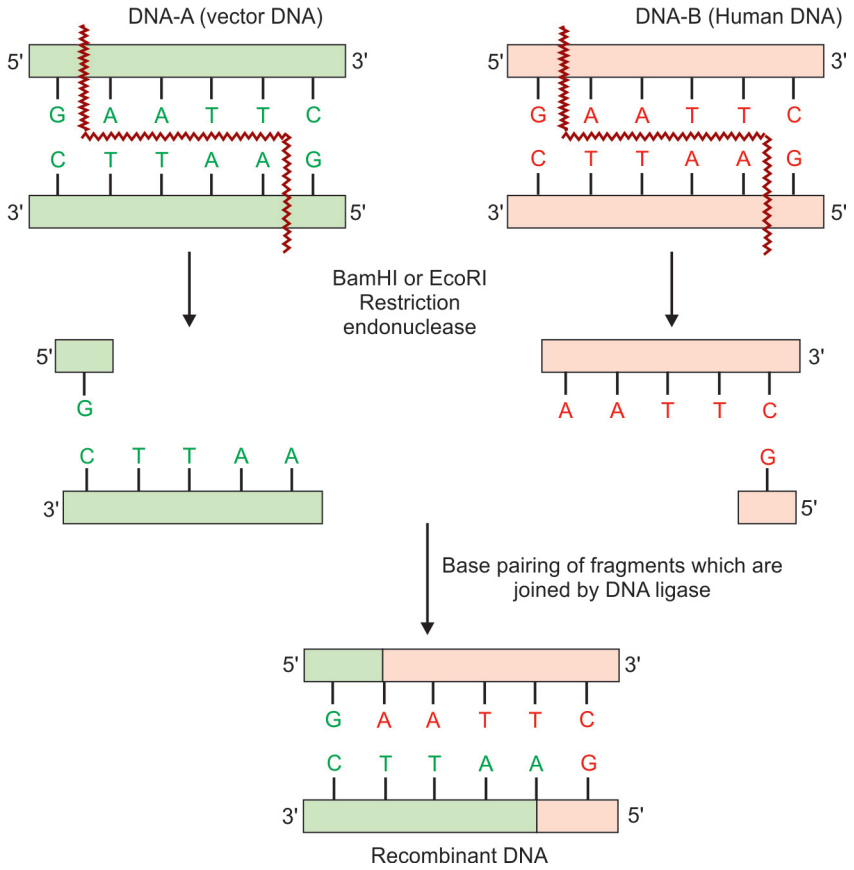


Figure 22.3: Construction of recombinant DNA molecules

APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

- **Molecular basis of disease:** Recombinant DNA technology is used to understand the molecular basis of a number of diseases, for example:
  - Familial hypercholesterolemia
  - Sickle cell disease
  - Thalassemias
  - Cystic fibrosis
  - Muscular dystrophy, etc.
- **Diagnosis of disease:** Recombinant DNA technology is used to diagnose existing diseases.
- **Production of proteins:** Using recombinant technology, human proteins can be produced in abundance for therapy, research and diagnosis, for example:
  - **Anticoagulant:** Tissue plasminogen activator (TPA). Used in treating heart attack victims.
  - **Human growth hormone:** It is used to treat children with growth hormone deficiencies (dwarfism)
  - **Erythropoietin:** Used to treat anemia.
  - **Blood factors:** Factor VIII, used to treat hemophilic patients.
  - **Insulin:** A hormone used to treat diabetes.
  - **Interferons:** Used to treat cancer.
  - **Interleukins:** Used in wound healing, HIV infections, cancer, immune deficiencies.
  - **Monoclonal antibodies:** Used in diagnostic tests.
  - **Superoxide dismutase:** Used during surgery.
  - **Vaccines:** Various vaccincs can be produced by recombinant DNA technology. The first successful recombinant DNA vaccine produced was for the hepatitis B virus.
- **Gene therapy:** Gene therapy is the introduction of normal genes into individuals who have defective genes. Gene therapy for sickle cell disease, thalassemia, adenosine deaminase deficiency and other diseases may be devised. Currently gene therapy is at experimental level.
- **Transgenic animals:** Genes can be introduced into fertilized eggs from which transgenic animals

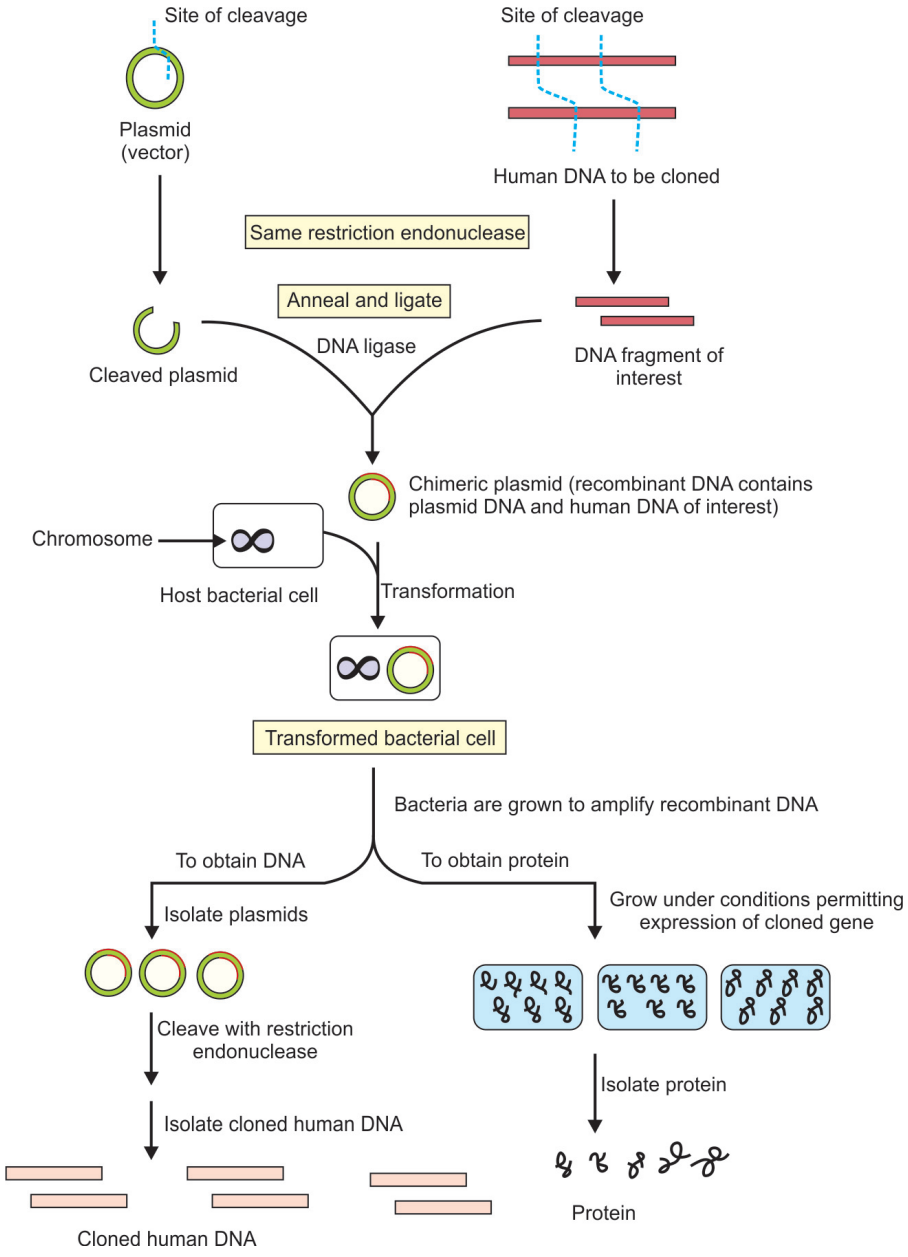


Figure 22.4: Cloning of human DNA in bacteria using recombinant DNA technology

develop and these transgenic animals can produce normal offspring. Transgenic means containing gentic material into which DNA from a different organism has been artificially added.

- **Genetic counseling:** Genetic counseling is the device of preventing passing of defective genes to offspring. Screening tests, based on the recombinant DNA techniques, can be performed on the prospective parents prior to conception. If they have decided to conceive, the fetus can be tested for genetic defect. If the fetus has the defect, treatment can be started at an early stage, even *in utero*.
- **In forensic medicine:** Special techniques, e.g. DNA fingerprint is used in forensic medicine and can be used in criminal cases.
- **Commercial applications**

- **Agricultural application:** In agriculture, plants that are resistant to disease, insects, herbicides, drought and temperature extremes or more efficient at fixing nitrogen are being produced using recombinant DNA technology.
- **Industrial applications:** Industrial applications includes the production of enzymes used in detergents, sugar and cheese.

DNA LIBRARY

DNA library is sometimes called ***shotgun collection.*** DNA library is a collection of cloned restriction fragments of DNA that represents the entire genome. DNA libraries may be either:

1. **Genomic DNA library** in which both introns (non-coding sequences) and exons (coding sequences) are represented.
2. **Complementary DNA (cDNA) library** in which only exons are represented.

Genomic DNA Library

In genomic DNA library both introns and exons are represented and thus, contains every sequence of the genome of a specific organism.

The entire genomic DNA of an organism is cut into small pieces by restriction endonucleases. These cut pieces are then introduced into vectors. A collection of these different recombinant clones is called ***genomic DNA library.***

To produce the complete gene library about 1500 fragments are required in the case of *E. coli* but about 10 lakh fragments for human DNA.

Complementary DNA (cDNA) Library

In cDNA library only exons are represented. It is constructed so as to include only those genes that are expressed. ***cDNA library is a more specialized and exclusive DNA library.***

To construct cDNAs library, the mRNAs from an organism is extracted and complementary double stranded DNAs (cDNAs) are produced from this mRNAs by reverse transcriptase. The resulting DNA fragments are then inserted into a suitable vector and cloned. A collection of these clones is called a cDNA library. Since there will be no introns in the mRNA, the expression of the genes is easier.

BLOT TRANSFER TECHNIQUES

Blot transfer is a technique used for visualization of specific DNA, RNA or proteins among the thousands of molecules. These are:

- Southern blot (for DNA)
- Northern blot (for RNA)
- Western blot (for protein).

EM Southern developed a technique for identifying DNA sequences on gels, which bears his name, i.e. Southern blot and the other two names Northern and Western began as laboratory jargons.

- Southern blot transfer procedure is useful in determining the number of copies of a gene present in a given tissue and mutations in the gene.
- The Northern blot transfer technique is used to size and quantitate specific RNA molecules.
- Western blots technique is used to size and quantitate specific protein molecules. Western blot are currently being used as one of the tests for the AIDS virus. In this case, the presence of viral proteins in the blood is detected by antibodies.

The Blot Transfer Procedures (Figure 22.5)

Southern or DNA Blot Transfer

In Southern blot transfer:

- First DNA is extracted from cells, which is digested into many fragments with restriction enzyme.

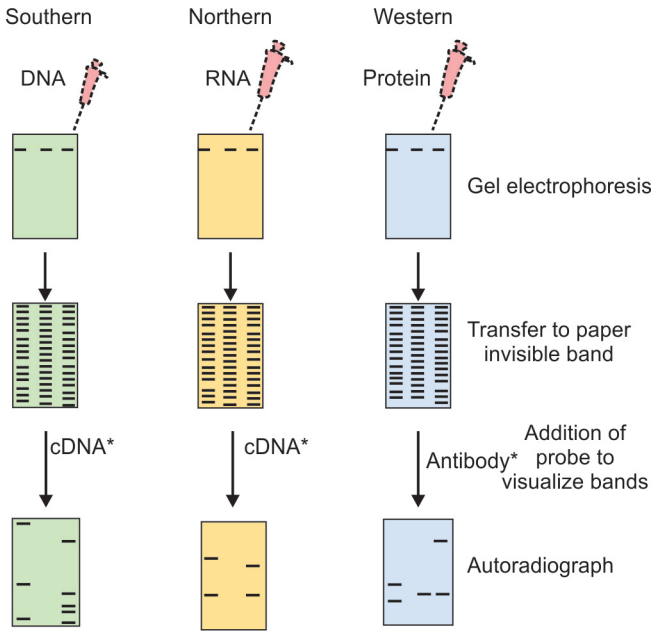


Figure 22.5: The blot transfer procedures (Asterisks signify radiolabeling)

- The resulting fragments are separated by agarose or polyacrylamide gel electrophoresis. The smaller fragments move more rapidly than larger fragments.
- After a suitable time the DNA is denaturated by mild alkali and transferred to nitrocellulose paper in an exact replica of the pattern on the gel by blotting technique, hence the name blot.
- The paper is then exposed to the labeled cDNA probe, which hybridizes to complementary fragments on the paper.
- The paper is exposed to X-ray film, which shows specific bands corresponding to the DNA fragment that recognized the sequence in the cDNA probe.

Northern or RNA Blot Transfer

In Northern blot procedures, RNA is subjected to electrophoresis before blot transfer and treated similarly except that alkali is not used (alkali hydrolyzes RNA). The hybrids formed between RNA and cDNA can be identified by radioautography.

Western or Protein Blot Transfer

In the Western blot procedures, proteins are electrophoresed and transferred to nitrocellulose paper and then probed with labeled antibodies. The probes are labeled to visualize the bands with which they hybridize.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

Variations of DNA sequence is called polymorphism, which occurs approximately once in every 500 nucleotides or about 10^7 times per genome. In normal healthy individual, these alterations occur in non-coding region of DNA or at sites that cause no change in function of encoded protein.

The differences in DNA sequences can result in variations of restriction sites and thus, in the length of restriction fragments. ***An inherited difference in the pattern of restriction sites is known as restriction fragment length polymorphism or RFLP.***

Application of RFLP

- RFLP can be used to detect human genetic defects in prospective parents or in fetal tissue, for example:
  - Sickle cell anemia
  - Thalassemia.
- In forensic medicine, RFLP is used to establish a unique pattern of DNA fragments (fingerprinting) for an individual.

POLYMERASE CHAIN REACTION (PCR)

PCR is an *in vitro* technique for amplification of DNA. It is particularly used for amplifying DNA for clinical or forensic testing procedures. By PCR, DNA can be amplified from a single strand of hair or single drop of blood or semen.

DNA can be amplified *in vivo* by cloning by recombinant DNA (discussed earlier). Amplification of DNA by PCR is faster and technically less difficult than laborious cloning methods using recombinant DNA techniques.

Events of The PCR (Figure 22.6)

- A DNA containing the sequence to be amplified is isolated and two ***"primers"*** (synthetic oligonucleotide of 20-35 sequences) are constructed. The sequences of primer should be complementary to flanking sequences. Flanking sequences are the sequences present at 5' end of the two strands of the target DNA. Flanking sequences bracket the DNA sequences of interest **(Figure 22.6)**.
- The target strands are separated by heating and the mixture of primers is added in the separated strands of DNA to be amplified.
- On cooling the mixture, the primers bind to the separated strands.
- Deoxyribonucleotides and DNA polymerase are added to the mixture to initiate the synthesis. DNA polymerase originally obtained from thermophilic bacterium, thermos aquatics and designated ***Taq polymerase.***
- DNA polymerase (Taq polymerase) then uses the primer for DNA synthesis using target strands as templates.
- The mixture is once more heated to melt or to separate all double stranded structure. There are now four DNA strands.
- A second cycle of cooling, allows further binding of the primers and a second round of polymerization is repeated. The process of heating, cooling and new DNA synthesis is repeated many times until a large number of copies of the DNA of interest is obtained.

Applications of PCR

- PCR is a very powerful diagnostic and research tool. In microbiology, it can be used to pick out and amplify DNA sequences that are unique to invading organisms and enable their identification. For example, viruses, such as HIV, are difficult to detect at the early stage of infection using conventional methods but can be identified by PCR.

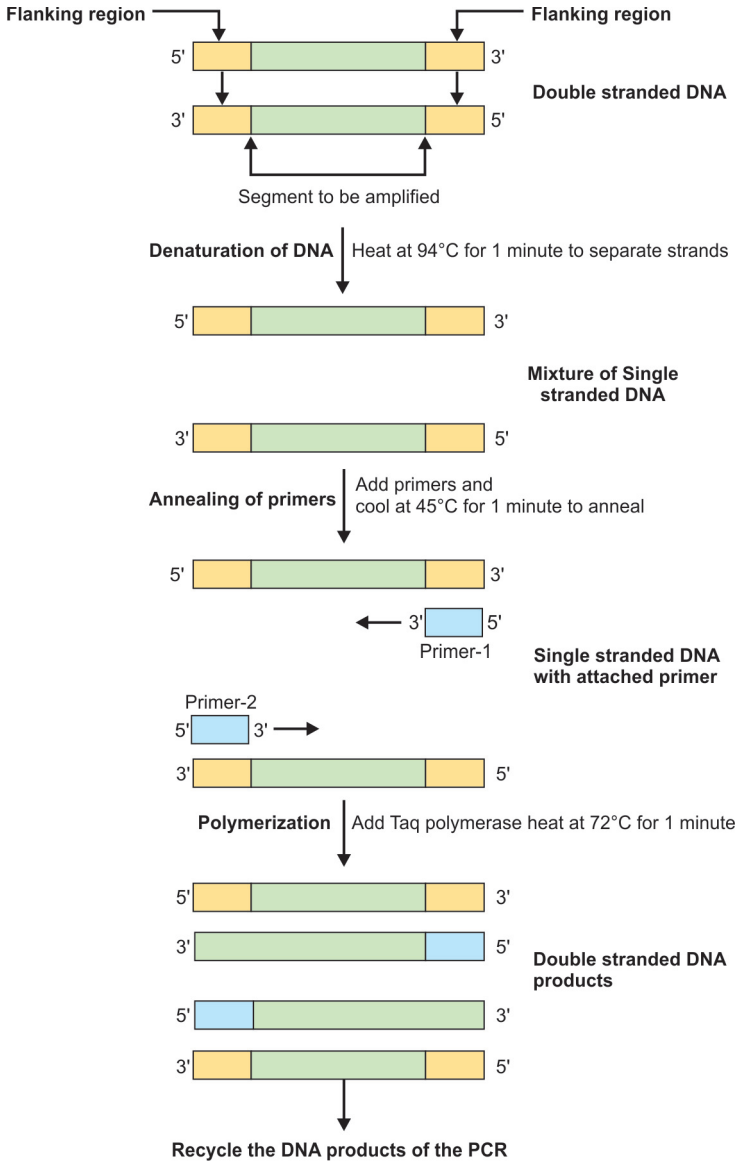


Figure 22.6: The polymerase chain reaction (PCR)

Contd.

- It can also be used to amplify DNA for the purposes of DNA fingerprinting in the forensic laboratory.
- In clinical biochemistry and molecular biology, it is used in the antenatal diagnosis of single gene mutation and in studying structural gene polymorphism.

SUMMARY

- Genetic engineering is the construction and repair of the genes of living things.
- Recombinant DNA technology involves isolation and manipulation of DNA to make chimeric molecules, using restriction endonuclease enzyme.
- Cloning is a technique developed for amplifying the quantity of DNA *in vivo*.
- DNA library is a collection of cloned restriction fragments of DNA that represents the entire genome. Libraries may be either genomic DNA (in which both introns and exones are represented) or c-DNA (in which only exones are represented).

- Southern blot, Northern blot and Western blot techniques are used for the specific identification of DNA, RNA and protein molecules respectively.
- An inherited difference in the pattern of restriction sites is known as restriction fragment length polymorphism or RFLP, and used to detect human genetic defects and in forensic medicine to establish a unique pattern of DNA fragments (fingerprinting) for an individual.
- Polymerase chain reaction (PCR) is an *in vitro* technique for amplification of DNA and used for clinical or forensic testing procedure.
- Genetic engineering is used for understanding the molecular basis of a number of diseases, production of a number of human proteins which are used for therapy, research and diagnosis, in forensic medicine, gene therapy and genetic counseling.

EXERCISE

Multiple Choice Questions (MCQs)

1. Restriction endonuclease has the following characteristics, *except:*
a) Cut DNA in a sequence specific manner
b) Named according to the bacteria from which they are isolated
c) Most of the DNA sequences recognized by it are palindromic
d) Cut DNA randomly

2. Most commonly used cloning vectors are:
a) Bacterial plasmids b) Bacteriophages
c) Cosmids d) All of the above

3. Cloning is a technique developed for:
a) Amplifying the quantity of DNA *in vivo*
b) Amplifying the quantity of DNA *in vitro*
c) To search specific gene from DNA library
d) To visualize specific DNA, RNA or proteins

4. Southern blot is a technique used for visualization of specific:
a) RNA b) DNA
c) Protein d) Carbohydrate

5. Polymerase chain reaction (PCR) is a technique for:
a) Amplification of DNA *in vitro*
b) Amplification of DNA *in vivo*
c) Amplification of protein
d) Amplification of RNA

6. Which one of the following enzymes is required for PCR?
a) DNA ligase
b) Thymidine kinase
c) Taq polymerase
d) Restriction endonuclease

7. Restriction endonucleases are enzymes:
a) Used to joining DNA to cloning vector
b) Which cleaves DNA at specific sequence
c) Which cleaves DNA randomly
d) Which digest DNA molecule from ends

Correct Answers for MCQs

1-d	2-d	3-a	4-b
5-a	6-c	7-b	

CHAPTER

23

Mechanism of Hormone Action

INTRODUCTION

The word hormone is derived from a Greek verb meaning ***to*** **excite.** Hormones are produced by special cells or glands such as adrenals, ovaries, parathyroids, pituitary, testes and thyroid. These glands secrete their hormones directly into the bloodstream and are known as ***endocrine glands.*** Endocrinology is the study of hormones and their interactions.

A hormone is a chemical messenger, secreted in trace amounts by one type of tissue and carried by the blood to a target tissue elsewhere in the body to stimulate a specific biochemical or physiological activity.

Figure 23.1 shows a schematic masterplan of the regulatory relationship between the endocrine glands and their target tissue.

- Nerve impulses received by the hypothalamus cause it to send specific hormones to the pituitary gland, stimulating (or inhibiting) the release of different tropic hormones.
- The anterior pituitary hormones in turn can stimulate other endocrine glands to secrete their characteristic hormones, which in turn, stimulate specific target tissues.

CLASSIFICATION OF HORMONES

Hormones can be classified according to:

- Chemical structure
- Mechanism of hormone action.

Classification Based on Chemical Structure

Hormones are usually classified into three main groups on the basis of their chemical structure as follows:

1. Peptide or protein hormones.
2. Amine hormones or amino acid derivatives.
3. Steroid hormones.

Peptide or Protein Hormones

Most hormones fall into this class. These are water soluble and may have 3 to over 200 amino acid residues, e.g. hormones of the hypothalamus and pituitary. As well as insulin and glucagon of the pancreas.

Amine Hormones or Amino Acid Derivatives

These are small, water soluble compounds containing amino groups. For example:

- Adrenaline of the adrenal medulla
- Thyroid hormones.

Steroid Hormones

These are fat soluble (lipophilic) and all are derivatives of cholesterol. For example:

- Adrenal cortical hormones
- Androgen (male sex hormones)
- Estrogens (female sex hormones).

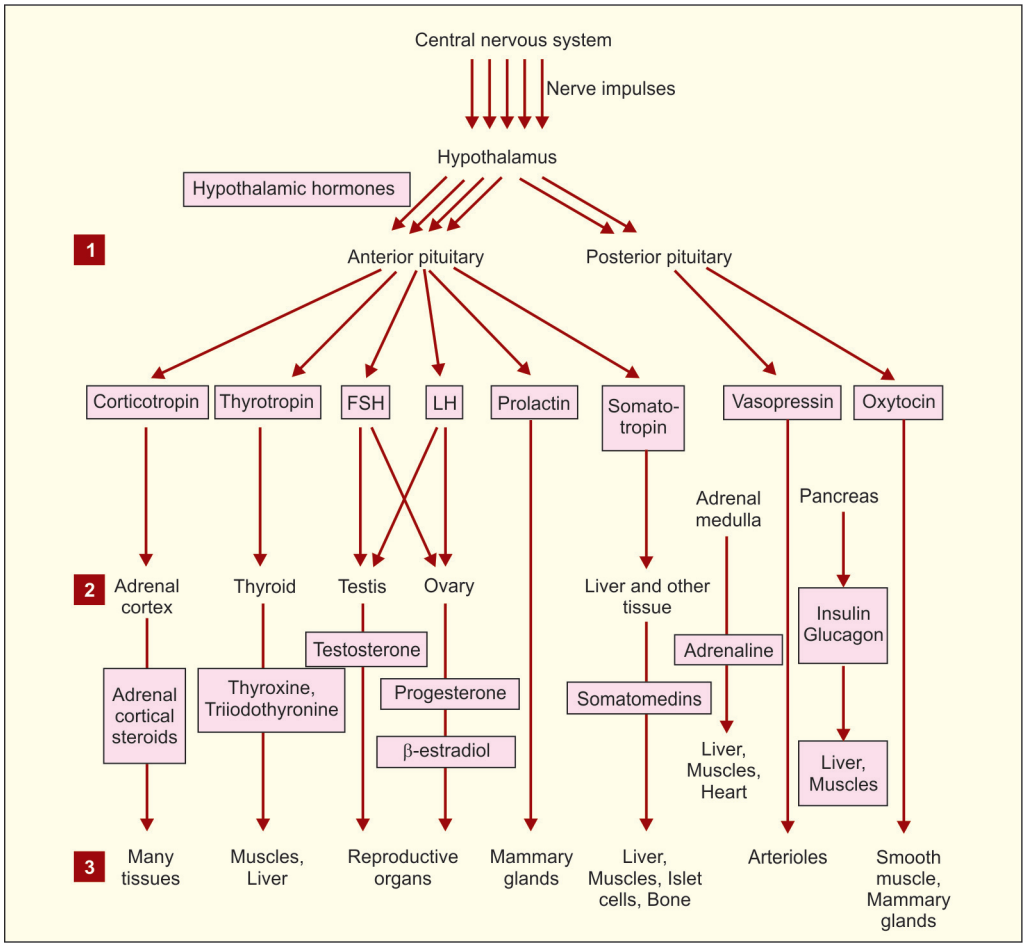


Figure 23.1: Schematic masterplan of the regulatory relationship between the endocrine glands and their ultimate target tissue where, 1: First target tissue; 2: Second target tissue; 3: Third target tissue

Classification Based on Mechanism of Hormone Action

Hormones can be classified according to mechanism of hormone action to:

1. Group I hormones
2. Group II hormones.

This classification is based on location of the hormone receptors.

- The hormones of **Group I** are **lipophilic** which readily pass through the lipophilic plasma membrane of the target cells and interacts with receptors which are located intracellularly in either the **cytosol** or the **nucleus.** The hormones that have intracellular receptors are given in **Table 23.1**.
- The receptors for the different **steroid hormones** are found mainly in the **cytoplasm** and the receptors for the **thyroid hormones** are found in the **nucleus.**
- The hormones of **group II** are **water-soluble peptides** and **amine (except T_3 and T_4)** hormones **(Table 23.1)** which do not penetrate lipophilic cell membrane readily. The receptors for such hormones are located on the outer surface of the target cell (cell surface receptors).

MECHANISM OF HORMONE ACTION

The first step of hormone action is binding of hormone to specific receptors of the target cell. Hormone receptor complex activates the receptor itself and the activated receptor initiates the hormonal effects. Hormonal receptors are large proteins, which are highly specific for a single hormone. Due to this specificity a particular hormone will act on a particular tissue.

Table 23.1: Classification for hormone based on mechanism of action

Class	*Second messenger or mediator*	*Examples*
Group I Cytosolic or nuclear receptor	Hormone-receptor complex	Androgens Estrogens Glucocorticoids Mineralocorticoids Progesterol Thyroid hormones (T_3 and T_4)
Group II Cell membrane receptor	c-AMP	Epinephrine Norepinephrines Glucagon Parathyroid hormone
	Calcium or phosphatidylinositol or both	Vasopressin Oxytocin

MECHANISM OF HORMONE ACTION AT CYTOSOLIC OR NUCLEAR LEVEL (FIGURE 23.2)

- Several hormones, e.g. steroid hormones (adrenal and gonadal) and thyroid hormones bind with receptors inside the cell rather than in the cell membrane. Because these hormones are lipid soluble, they readily cross the cell membrane and interact with receptors in the cytoplasm or nucleus.

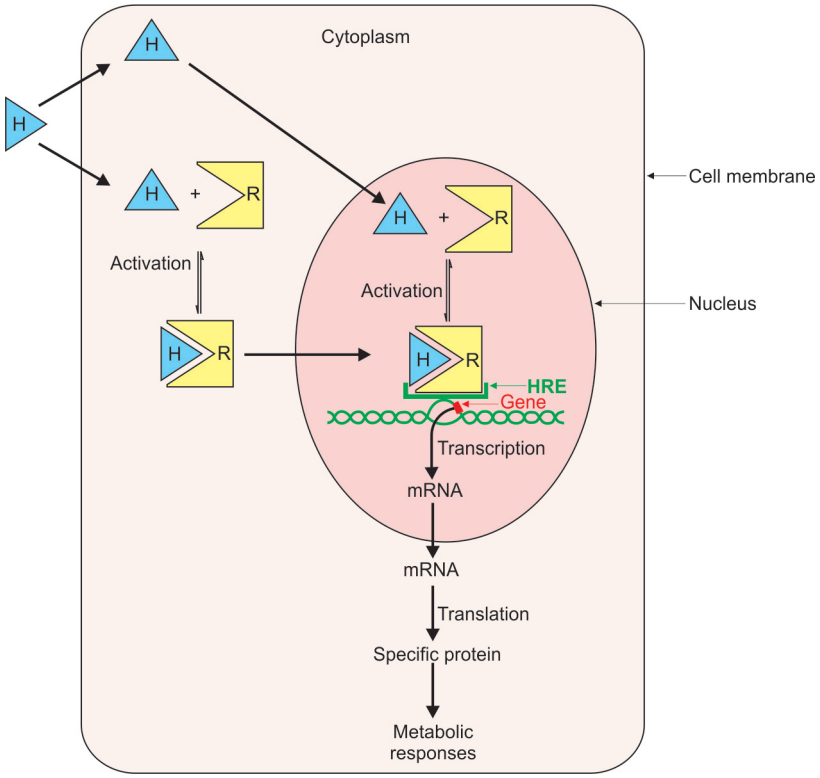


Figure 23.2: Schematic representation of mechanism of hormone action at cytosolic or nuclear level where, H: Hormone; R: Receptor; R-H: Hormone receptor complex; HRE: Hormone response element

- The receptors for the steroid hormones are found mainly in the cytoplasm and the receptors for the thyroid hormones are found in the nucleus.
- The **hormone receptor complex** is assumed to be the **intracellular messenger** for these (Group I) hormones.
- The activated hormone receptor complex undergoes conformational change which then binds with a specific regulatory (promoter) sequence of DNA called the **hormone response element (HRE)** which activates transcription of specific genes and formation of mRNA.
- These mRNA get translate into proteins. Newly formed proteins in the cell controls the cellular metabolic functions.

CELL MEMBRANE RECEPTOR MECHANISM OF HORMONE ACTION (FIGURE 23.3)

- The receptors for group II hormones are located on the **outer surface of the target cell** because these hormones are water soluble which do not penetrate lipophilic cell membrane.
- Hormones that bind to surface receptors of the cells communicate their action through intermediary molecules called **second messenger** (the hormone itself is the first messenger).
- On binding of the hormone to the receptor, a **conformational change** occurs in the receptor, that causes activation of the G-protein (GTP-binding protein). This results from the exchange of GDP to GTP on the α-subunit. G-protein is a group of three subunits α, β, γ. In absence of the hormone the G-protein is in an inactive form. Inactive G-protein is bound to GDP **(Figure 23.3)**.
- Activation of G-protein by hormone leads to dissociation of α-subunit (to which GTP is bound) from β, γ subunits of the G-protein.
- The dissociated α-subunit with bound GTP interacts with other intracellular signaling enzymes such as **adenylate cyclase** or **phospholipase-C** which generates second messenger. Interaction of α-subunit with **adenylate cyclase** generates **c-AMP** as a second messenger and interaction with **phospholipase C** generates **inositol triphosphate (IP_3)** and **diacylglycerol (DAG)** as a second messenger from **phosphatidylinositol**. These second messengers serve as mediators of the hormone action, as discussed below.

c-AMP second messenger

c-AMP derived from ATP through the action of **adenylate cyclase** is the second messenger for many hormones, e.g. epinephrine, glucagon, calcitonin, PTH, etc.

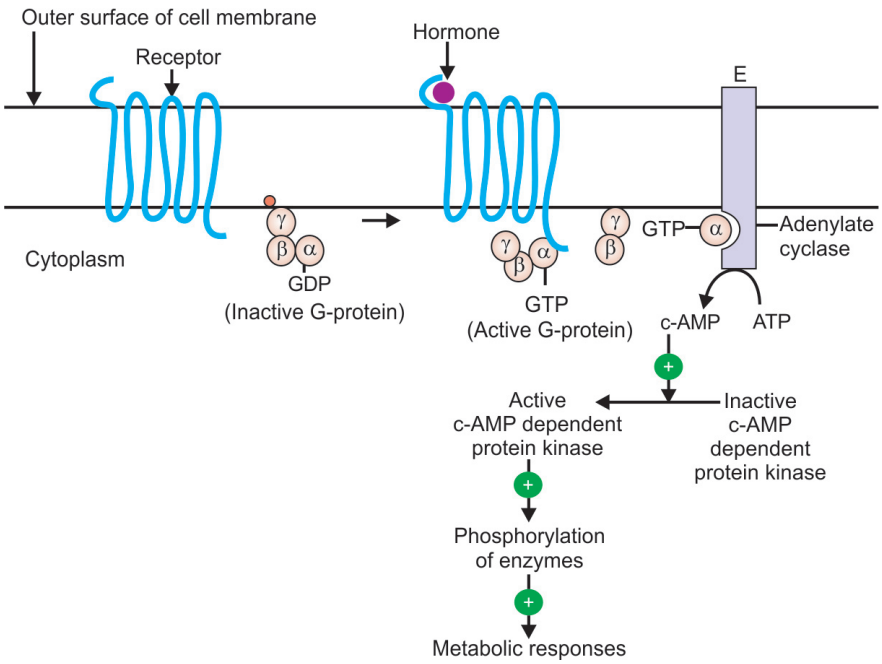


Figure 23.3: Cell membrane receptor mechanism of hormone action through c-AMP as a second messenger

- c-AMP then activates c-AMP dependent **protein kinase,** which phosphorylates specific proteins (enzymes) in the cell. Phosphorylation alters activities of these enzymes, some are activated (e.g. glycogen phosphorylase) while some are inactivated (e.g. glycogen synthase).

Phosphatidylinositol/calcium second messenger

- Certain hormone-receptor interaction result in the activation of the enzyme **phospholipase C** through a specific G-protein **(Figure 23.4)**.
- Phospholipase C enzyme catalyzes the breakdown of phospholipids in cell membrane especially phosphatidylinositol bisphosphate (PIP_2) into two different second messenger products:
  1. Inositol triphosphate (IP_3)
  2. Diacylglycerol (DAG).
- The IP_3 liberates stored intracellular calcium ions from mitochondria and endoplasmic reticulum.
- The calcium in turn acts as a third messenger which influences a variety of biochemical processes through the mediation of calmodulin.
- **DAG,** the other second messenger, activates the enzyme **protein kinase C** (PKC), which then alter physiological processes. The hormones thyrotropin releasing hormone (TRH) gastrain, cholecystokinin act through this second messenger.

SUMMARY

- Hormones are chemical messengers secreted by certain tissues into the blood, serving to regulate the activity of certain other tissues.
- The action of hormones requires the binding of a hormone to its specific receptor.
- Steroid and thyroid hormones have intracellular (cytosolic or nuclear) receptors and affect gene expression.
- The receptors for water soluble peptides and amines (except T_4 and T_3) hormones are located on the outer surface of the cell membrane and use second messengers such as c-AMP, Ca^{2+}, phosphatidylinositol, to communicate their messages.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Binding of the following hormones to receptor activate adenylate cyclase, *except:***
   a) Vasopressin b) Glucagon
   c) Thyroxine d) Epinephrine
2. **The following are the intracellular second messenger for hormone action, *except:***
   a) c-AMP b) c-GMP
   c) Phosphatidylinositol d) NADPH

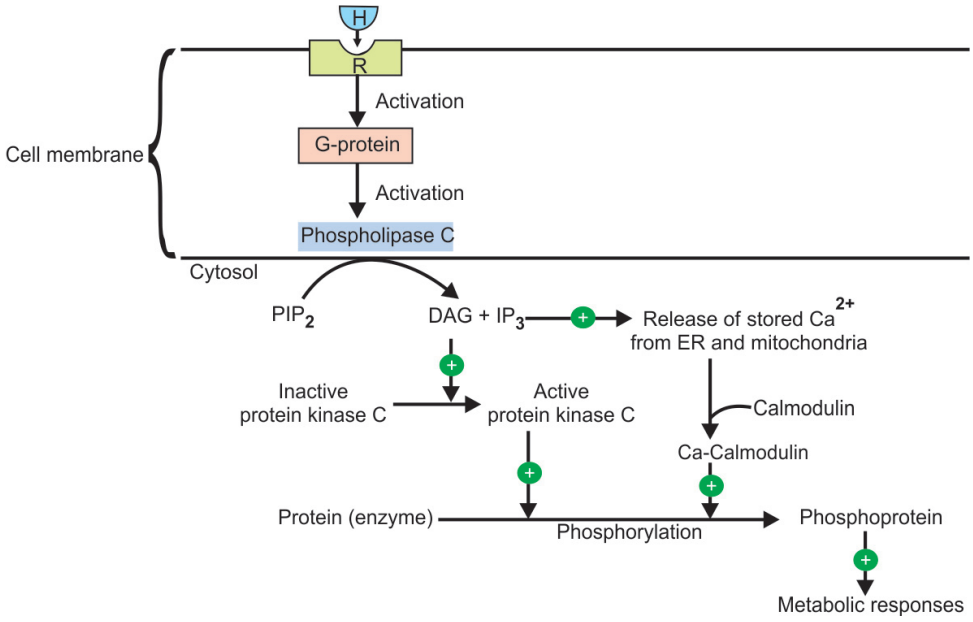


Figure 23.4: Cell membrane receptor mechanism of hormone action through phosphatidylinositol or calcium or calcium calmodulin as a second messenger,
where, H:Hormone, R:Receptor, PIP_2: Phosphatidylinositol; DAG: Diacylglycerol; IP_3: Inositol triphosphate

3. Water soluble hormones have cell surface receptors, *except:*

a) Glucagon
b) Epinephrine
c) Thyroxine
d) Vasopressin

4. Hormone that have intracellular receptor:

a) Glucocorticoids
b) ACTH
c) TSH
d) Glucagon

5. Second messenger that mobilizes intracellular calcium is:

a) Phosphatidic acid
b) Diacylglycerol
c) Inositol triphosphate
d) c-GMP

Correct Answers for MCQs

1-c 2-d 3-c 4-a 5-c

CHAPTER

24

Acid-base Balance

INTRODUCTION

To achieve acid-base balance, there must be a balance between the intake or production of hydrogen ions and net removal of hydrogen ions from the body. The various mechanisms that contribute to the regulation of hydrogen ion concentration are discussed in this chapter.

ACIDS, BASES AND BUFFERS

- **An acid** is defined as a substance that releases protons or hydrogen ions (H^+), e.g. hydrochloric acid (HCl), carbonic acid (H_2CO_3).

$$HCl \rightarrow H^+ + Cl^-$$
$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

- **A base** is a substance that accepts protons or hydrogen ions, e.g. bicarbonate ion (HCO_3^-), and HPO_4^{--},

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$
$$HPO_4^{--} + H^+ \rightarrow H_2PO_4^-$$

Proteins in the body also function as bases, because some of the amino acids accept hydrogen ions, e.g. **hemoglobin** in red blood cells and plasma protein especially **albumin** are the most important of the body's bases.

- Buffer is a solution of weak acid and its corresponding salt which resists a change in pH when a small amount of acid or base is added to it. By buffering mechanism a strong acid (or base) is replaced by a weaker one.

NORMAL pH OF THE BODY FLUIDS

- The normal pH of **arterial blood is 7.4,** whereas the pH of venous blood and interstitial fluids is about 7.35 because of the extra amounts of carbon dioxide (CO_2), released from the tissues to form H_2CO_3 in these fluids. ***Thus, the pH of blood is maintained within a remarkable constant level of 7.35–7.45.***
- Normal pH of body fluids are shown in **Table 24.1**.

Table 24.1: Normal pH of body fluids

Body fluid	*pH*
Extracellular fluid	
– arterial blood	7.40
– venous blood, and interstitial fluid	7.35
Intracellular fluid	6.0–7.4
Urine	4.5–8.0
Gastric HCl	0.8

- The maintenance of a constant pH is important because, the activities of almost all enzyme systems in the body are influenced by hydrogen ion concentration. Therefore, changes in hydrogen ion concentration alter virtually all cell and body functions, the conformation of biological structural components and uptake and release of oxygen.

Metabolic Sources of Acids and Bases Which Tend to Alter pH of the Body Fluids

Metabolic Sources of Acids

During metabolic processes two types of acids are produced:

Fixed acids or non-volatile acids

Fixed acids are non-gaseous acids such as:

- Phosphoric and sulfuric acids, produced from the sulfur and phosphorus of proteins and lipoproteins.
- Organic acids such as pyruvic acid, lactic acid, keto acids (acetoacetic and β-hydroxybutyric acid), and uric acid.

Volatile acids

The physiologically important volatile acid is **carbonic acid (H_2CO_3).** It is equivalent to 36 liters of 1.0 N acid.

Metabolic Sources of Bases

Catabolism of few food materials produce bases. For example:

- Citrate salts of fruit juices may produce bicarbonate salt.
- Deamination of amino acids produces ammonia.
- Formation of biphosphate and acetate also contributes to alkalinizing effect.

REGULATION OF BLOOD pH

To maintain the blood pH at **7.35 –7.45,** there are three primary systems that regulate the hydrogen ion concentration in the body fluids. These are:

1. **Buffer mechanism:** First line of defense.
2. **The respiratory mechanism:** Second line of defense.
3. **Renal mechanism:** Third line of defense.

The first two lines of defense keep the hydrogen ion concentration from changing too much until the more slowly responding third line of defense, the kidneys, can eliminate the excess acid or base from the body.

Buffer Systems and their Role in Acid-base Balance

- The buffer systems of the blood, tissue fluids and cells; immediately combine with acid or base to prevent excessive changes in hydrogen ion concentration.
- Buffer systems do not eliminate hydrogen ions from the body or add them to the body but only keep them tied up until balance can be re-established.

Blood Buffers

Various buffer systems present in human body are given below **(Table 24.2).**

1. Buffers of **extracellular fluid** present in plasma.
   i. Bicarbonate buffer ($NaHCO_3/H_2CO_3$).
   ii. Phosphate buffer (Na_2HPO_4/NaH_2PO_4).
   iii. Protein buffer (Na protein/H protein).
2. Buffers of **intracellular fluid** present in RBCs
   i. Bicarbonate buffer ($KHCO_3/H_2CO_3$).
   ii. Phosphate buffer (K_2HPO_4/KH_2PO_4).
   iii. Hemoglobin buffer (KHb/HHb), ($KHbO_2$/H HbO_2).

The Bicarbonate Buffer System (HCO_3^- /H_2CO_3)

The bicarbonate buffer system is the most important extracellular buffer.

Under physiological conditions, with a plasma pH 7.4, the ratio of bicarbonate to carbonic acid (HCO_3^- / H_2CO_3) is 20:1.

Mechanism of action of bicarbonate buffer

- When a strong acid, such as HCI, is added to the bicarbonate buffer solution, the increased hydrogen ions are buffered by HCO_3^-.

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$

- Thus, hydrogen ions from strong acid HCI react with HCO_3 to form very weak acid H_2CO_3.
- The opposite reactions take place when a strong base such as sodium hydroxide (NaOH), is added to the bicarbonate buffer solution.

$$NaOH + H_2CO_3 \rightarrow NaHCO_3 + H_2O$$

- In this case, the hydroxyl ion (OH^-) from NaOH combines with H_2CO_3 to form weak base HCO_3^-. Thus, strong base NaOH is replaced by a weak base $NaHCO_3$.

Table 24.2: The principal buffers of the blood

Buffer system	*Plasma (extracellular) buffer*	*Erythrocyte (intracellular) buffer*
Bicarbonate	$NaHCO_3/H_2CO_3$	$KHCO_3/H_2CO_3$
Phosphate	Na_2HPO_4/NaH_2PO_4	K_2HPO_4/KH_2PO_4
Protein	Na Protein/ H Protein	KHb/HHb $KHbO_2/HHbO_2$

Plasma bicarbonate is a measure of the base that remains after all acids, stronger than carbonic, have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids and it has been termed as the ***alkali reserve.***

The Phosphate Buffer System (HPO_4^{--}/$H_2PO_4^-$)

- The phsophate buffer system is not important as a blood buffer, it plays a major role in buffering renal tubular fluid and intracellular fluids.
- Its concentration in both plasma and erythrocytes is low, i.e. only 8% of the concentration of the bicarbonate buffer. Therefore, the total buffering power of the phosphate system in the blood is much less than that of the bicarbonate buffering system.

Mechanism of action of phosphate buffer

- The main elements of the phosphate buffer system are HPO_4^{--} and $H_2PO_4^-$. When a strong acid such as HCl is added to a phosphate buffer system, the H^+ is accepted by the base HPO_4^{--} and converted to $H_2PO_4^-$ and strong acid HCl is replaced by a weak acid H_2PO_4 and decrease in pH is minimized.

$$HCl + Na_2HPO_4 \rightarrow NaH_2PO_4 + NaCl$$

- When strong base, such as NaOH, is added to the buffer system, the OH^- is buffered by the $H_2PO_4^-$ to form HPO_4^{--} and water. Thus, strong base NaOH is replaced by weak base HPO_4^{--}, causing slight increase in the pH.

$$NaOH + NaH_2PO_4 \rightarrow Na_2HPO_4 + H_2O$$

At a plasma pH of 7.4 the ratio HPO_4^{--} : $H_2PO_4^-$ is 4:1.

Protein Buffer

Plasma protein buffer (Na protein/H protein)

- In the blood, plasma proteins especially **albumin** act as buffer because:
  - Proteins contain a large number of dissociable *acidic (COOH)* and *basic (NH_2)* groups in their structure.
  - In acid solution they act as a buffer in that, the basic amino group (NH_2) takes up excess H^+ ions forming (NH_3^+).
  - Whereas in basic solutions the acidic COOH groups give up hydrogen ion forming OH^- of alkali to water.
  - Other important buffer groups of proteins in the physiological pH range, are the **imidazole groups of histidine**. Each albumin molecule contains **16 histidine** residues.

Hemoglobin Buffer (KHb/HHb and $KHbO_2$/$HHbO_2$)

- Hemoglobin is the major **intracellular buffer** of blood which is present in erythrocytes.
- It buffers carbonic acid (H_2CO_3) and its anhydride CO_2 from the tissues.

Action of hemoglobin buffer

Hemoglobin works effectively in cooperation with the bicarbonate system. The several reactions occur in regulation of body pH by hemoglobin are given below.

- In the tissues the CO_2 formed by metabolic processes diffuses into red blood cell and is converted to carbonic acid (H_2CO_3) by carbonic anhydrase (CA). The H_2CO_3 thus formed ionizes to form H^+ and HCO_3^- and results in decrease in blood pH.
- The deoxyhemoglobin (KHb) acts as a buffer and accepts these H^+ ions to form HHb (weak acid) and $KHCO_3$. Thus, H^+ ions produced from H_2CO_3 does not cause any change in pH **(Figure 24.1).**
- Now, the increase in bicarbonate concentration in the erythrocyte leads to diffusion of these ions from the erythrocytes into the plasma where its concentration is low.
- The bicarbonate diffused from erythrocyte to plasma is transported to the lungs.
- In the lungs deoxyhemoglobin (HHb) carried from tissue is oxygenated to oxyhemoglobin ($HHbO_2$).
- Since, **oxyhemoglobin ($HHbO_2$)** is a **stronger acid** results in the release of H^+, which is buffered by $KHCO_3^-$ to give H_2CO_3 and $KHbO_2$. This buffering effect reduces the pH change as a result of the oxygenation of HHb.

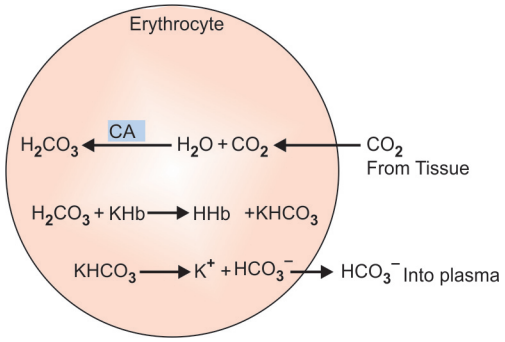


Figure 24.1: Action of hemoglobin buffer in tissue where, CA: Carbonic anhydrase

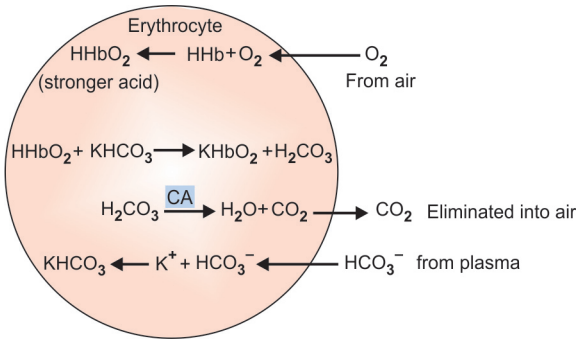


Figure 24.2: Action of hemoglobin buffer in lungs where, CA: Carbonic anhydrase

- As the concentration of HCO_3^- in the erythrocytes is reduced, HCO_3^- from the plasma where its concentration is higher enters into the erythrocyte.
- The carbonic acid formed is converted quickly in the presence of the carbonic anhydrase (CA) to carbon dioxide and water which is eliminated by ventilation **(Figure 24.2).**

Respiratory Mechanism in Acid-base Balance

- The second line of defense against acid-bases disturbances is by regulating the concentration of carbonic acid (H_2CO_3) in the blood and other body fluids by the lungs.
- The respiratory center regulates the removal or retention of CO_2 and thereby H_2CO_3 from the extracellular fluid by the lungs. Thus lungs, function by maintaining one component (H_2CO_3) of the bicarbonate buffer as follows:
  - An increase in (H^+) or (H_2CO_3) stimulates the respiratory center to increase the rate of respiratory ventilation. When the ventilation rate increases, more CO_2 is released from the blood and pH increases.
  - Similarly, an increase in (OH^-) or (HCO_3^-) depresses respiratory ventilation. A decrease in ventilation rate will cause a decrease in release of CO_2 from the blood. The increased blood CO_2 will result in the formation of more H_2CO_3. Thus, there will be decrease in pH.
- Thus, when the rate of ventilation is increased, excess acid (H_2CO_3) in the form of CO_2 is quickly removed. Similarly, when the rate of ventilation is decreased, acid (H_2CO_3) in the form of CO_2 is added to neutralize excess alkali (HCO_3^-).

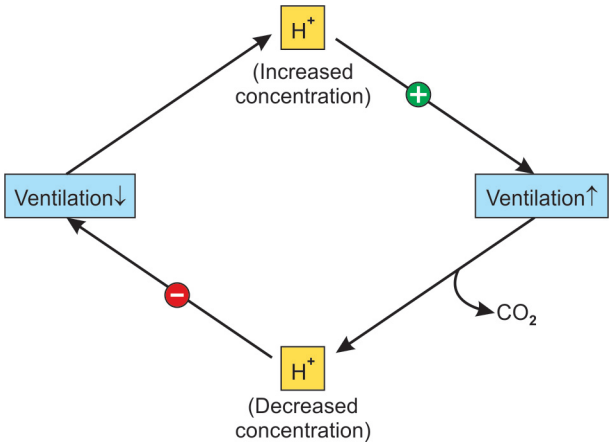


Figure 24.3: Feedback control of H^+ concentration by respiratory system

Respiratory system acts as a **feedback controller** of H^+ concentration **(Figure 24.3).** Increased H^+ concentration stimulates respiratory center and alveolar ventilation. This decreases the concentration of CO_2 in extracellular fluid and reduces H^+ concentration back to normal. Conversely, decreased H^+ concentration below normal depresses respiratory center and alveolar ventilation and H^+ concentration increases back to normal.

Renal Mechanism in Acid-base Balance

Renal mechanism is the third line of defense in acid-base balance. Long-term acid-base control is exerted by renal mechanisms. Kidney participates in the regulation of acid base balance primarily by **conservation of HCO_3^- (alkali reserve)** and excretion of acid as the case may be.

- The pH of the initial glomerular filtrate is approximately 7.4 same as that of plasma, whereas the average urinary pH is approximately 6.0 due to the renal excretion of non-volatile acids produced by metabolic processes.
- The pH of the urine may vary from 4.5 to 8.0 corresponding to the case of acidosis or alkalosis. In acidosis, excretion of acids is increased and base is conserved, in alkalosis, the opposite occurs. This ability to excrete variable amounts of acid or base makes the kidney, the final defense mechanism against change in body pH.
- Renal conservation of HCO_3^- and excretion of acid occur through four key mechanisms **(Figure 24.4)**.
  1. Exchange of H^+ for Na^+ of tubular fluid.
  2. Reabsorption (reclamation) of bicarbonate from tubular fluid.

3. Formation of ammonia and excretion of ammonium ion (NH_4^+) in the urine.
4. Excretion of H^+ as $H_2PO_4^-$ in urine.

Exchange of H^+ for Na^+ of Tubular Fluid

- In renal tubular cells, the carbonic anhydrase catalyzes the formation of carbonic acid (H_2CO_3) from CO_2 and water. The carbonic acid, thus formed dissociates to yield H^+ and HCO_3^-.
- The H^+ ions formed in tubular cells are secreted into the tubular fluid in exchange for Na^+ present in tubular fluid **(Figure 24.4).**
- The bicarbonate anion formed by the dissociation of H_2CO_3 in the tubular cell diffuses into the blood as the accompanying ion to Na^+ and HCO_3^- is thus conserved and increases the **'alkali reserve'** of the body.

Reabsorption of Bicarbonate from Tubular Fluid

- Some H^+ that are secreted into the tubular fluid in exchange of Na^+ react with HCO_3^- in the tubular fluid to form H_2CO_3, which is dehydrated to CO_2 and H_2O by an enzyme carbonic anhydrase.
- The increase in CO_2 in tubular fluid causes carbon dioxide to diffuse into the tubular cell where it reacts with H_2O to form H_2CO_3 and subsequently, H^+ and HCO_3^-. Thus reabsorption of bicarbonate is in terms of diffusion of CO_2 into tubular cells and its subsequent conversion to HCO_3^- **(Figure 24.4).**
- The process of bicarbonate reabsorption is enhanced in states of acidosis and decreased in alkalosis.
- The kidney reabsorbs almost all filtered bicarbonate at plasma bicarbonate concentration below 25 mEq/L. Only when bicarbonate levels become elevated above 25 mEq/L, bicarbonate will be excreted into the urine.

Formation of Ammonia and Excretion of Ammonium Ions (NH_4^+) in the Urine

- Ammonia (the urinary buffer) is produced by deamination of glutamine in renal tubular cell. **Glutaminase** present in tubular cells catalyzes this reaction.

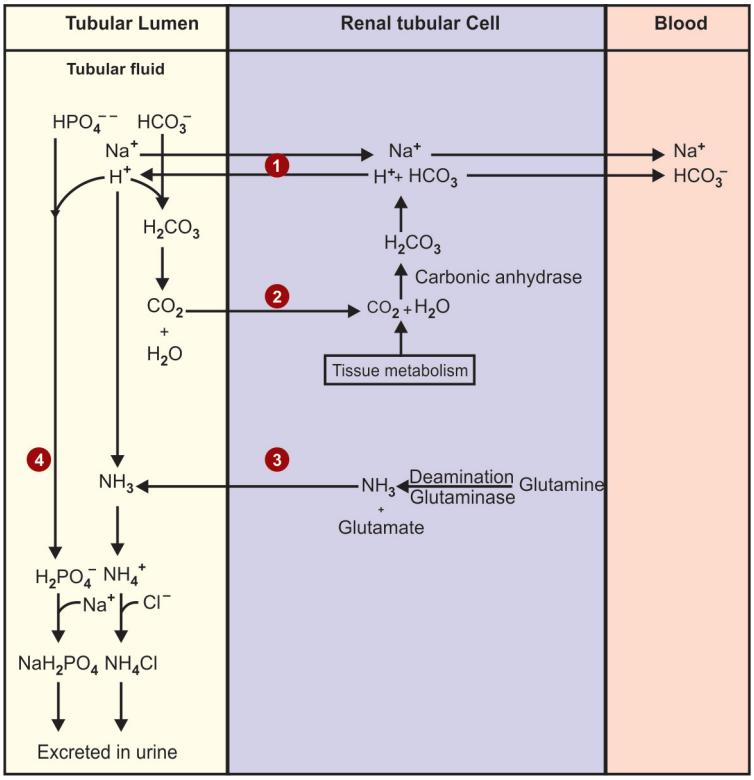


Figure 24.4: Renal mechanism in acid-base balance

- Ammonia is a gas and diffuses readily across the cell membrane into the tubular lumen, where it buffers hydrogen ions to form ammonium (NH_4^+) ions **(Figure 24.4).**
- The NH_4^+ ions formed in the tubular lumen cannot diffuse back into tubular cells and thus, is trapped in the tubular urine and excreted with anions, such as phosphate, chloride or sulphate.
- The rate of glutamine uptake from the blood and its utilization by the kidney depends on the amount of acid which must be excreted to maintain a normal blood pH. As the level of H^+ ions of the blood increases (pH decreases), glutamine uptake increases. During a metabolic acidosis, the excretion of NH_4^+ by the kidney increases several fold.
- The removal of hydrogen ions as NH_4^+ decreases the requirement of bicarbonate to buffer the urine.

Excretion of H^+ Ions as $H_2PO_4^-$ in Urine

- The hydrogen ions secreted into the tubular fluid in exchange of Na^+ are buffered by HPO_4^{--} of phosphate buffer. HPO_4^{--} combines with the secreted H^+ and is converted to $H_2PO_4^-$ and are excreted in the urine as NaH_2PO_4.
- This process depends on the amount of phosphate filtered by the glomeruli and the pH of urine. Acidemia increases phosphate excretion and thus provides additional buffer for reaction with H^+.
- A decrease in the glomerular filtration rate (GFR) with renal disease may result in a decrease of $H_2PO_4^-$ excretion.

ACIDOSIS AND ALKALOSIS

Acid-base balance depends on the ***ratio HCO_3^-/ H_2CO_3*** which is constant at ***20:1*** at physiological pH. Any alteration produced in the ratio between carbonic acid and bicarbonate results in an acid-base imbalance and leads to **acidosis** or **alkalosis.**

- ***Acidosis*** may be defined as an abnormal condition caused by the accumulation of excess acid in the body or by the loss of alkali from the body.
- ***Alkalosis*** is an abnormal condition caused by the accumulation of excess alkali in the body or by the loss of acid from the body.

Acidosis and alkalosis are classified, in terms of their immediate cause, as follows:

1. **Metabolic acidosis:** Decrease in bicarbonate (HCO_3^-) concentration.
2. **Respiratory acidosis:** Increase in H_2CO_3 concentration.
3. **Metabolic alkalosis:** Increase in bicarbonate (HCO_3^-) concentration.
4. **Respiratory alkalosis:** Decrease in H_2CO_3 concentration.

Metabolic Acidosis

A fall in blood pH due to a decrease in bicarbonate levels of plasma is called ***metabolic acidosis.***

- Decrease in bicarbonate levels may be due to:
  - Increased production of acids. In uncontrolled diabetes mellitus and starvation there is an excessive production of acetoacetic acid and β-hydroxybutyric acid. These acids are buffered by utilizing base component (i.e. HCO_3^-) of the bicarbonate buffer. Consequently, the concentration of bicarbonate ions falls, giving rise to bicarbonate deficit and results in metabolic acidosis (ketoacidosis).
  - Excessive loss of bicarbonate occurs in the urine in renal tubular dysfunction and form GI tract in severe diarrhea.

Compensatory mechanisms

- Metabolic acidosis is compensated by:
  1. Increasing rate of respiration to wash out CO_2 (hence H_2CO_3) faster. Consequently, the ratio HCO_3^- : H_2CO_3 is elevated.
  2. Increasing excretion of H^+ ions as NH_4^+ ions.
  3. Increasing elimination of acid ($H_2PO_4^-$) in the urine.

All these compensatory mechanisms tend to reduce carbonic acid to keep the pH in the normal range and a compensated acidosis results.

Respiratory Acidosis

It results from an increase in concentration of carbonic acid (H_2CO_3) in plasma. An increase in concentration of H_2CO_3 is due to decrease in alveolar ventilation, and that leads to retention of CO_2. Decreased alveolar ventilation may occur in following circumstances.

- **Obstruction to respiration:** This may occur in pneumonia, emphysema, asthma, etc.
- **Depression of respiration:** Administration of respiratory depressant toxic drugs, e.g. morphine depresses the respiratory center.

Compensatory mechanisms

1. Increase in renal reabsorption of bicarbonate.
2. Rise in urinary acid ($H_2PO_4^-$) and ammonia.

Metabolic Alkalosis

A rise in blood pH due to rise in the bicarbonate levels of plasma is called **metabolic alkalosis**. This is seen in the following conditions:

- Loss of gastric juice along with H^+ ions in prolonged and severe vomiting.
- Therapeutic administration of large dose of alkali (as in peptic ulcer) or chronic intake of excess antacids.

Compensatory mechanisms

1. Increased excretion of alkali (HCO_3^-) by the kidney
2. Diminished formation of ammonia
3. Respiration is depressed to conserve CO_2.

Respiratory Alkalosis

A rise in blood pH due to lowered concentration of CO_2 or H_2CO_3, due to hyperventilation. This occurs in the following conditions:

- Anxiety or hysteria
- Fever
- Hot baths
- At high altitude
- Working at high temperature, etc.

Compensatory mechanisms

1. Reduction of urinary ammonia formation
2. Increased excretion of bicarbonate.

Mixed Acid-base Disturbances

- Respiratory and metabolic disorders of acid-base balance can occur together and called **mixed acid-base disturbances.**
- For example, some patients with chronic renal failure (which causes a primary metabolic acidosis) may also have chronic obstructive airways disease, which causes a primary respiratory acidosis.
- Plasma (H^+) will be increased in these patients, but the results for plasma CO_2 and concentration of (HCO_3^-) cannot be predicted. The history and clinical findings must be taken into account.

ANION GAP

- The concept of anion gap originally was devised as a quality control rule when it was found that if the sum of the Cl^- and HCO_3^- values was subtracted from the Na^+ and K^+ values the difference or 'gap' averaged 16 mmol/L in healthy individuals.
- The concentration of anions and cations in plasma must be equal to maintain electrical neutrality. Therefore, there is no real anion gap in the plasma. Anion gap is not a physiological reality.
- The anion gap is the difference between **unmeasured anions** and **unmeasured cations** and is estimated as:

$$\begin{aligned}\text{Anion gap} &= ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) \\ &= (142 + 4) - (103 + 27) \\ &= 146 - 130 \\ &= 16 \text{ mEq/L}\end{aligned}$$

- The most important unmeasured cations include **calcium, magnesium,** and the major unmeasured anions are **albumin, phosphate, sulphate** and other organic anions. The anion gap ranges between 8–16 mEq/L.
- Acid base disorders are often associated with alterations in the anion gap.
- In metabolic acidosis the anion gap can increase or remain normal depending on the cause of acidosis.

Clinical Significance of Anion Gap

The anion gap is a biochemical tool which sometimes helps in assessing acid-base problems. It is used for the diagnosis of different causes of metabolic acidosis.

SUMMARY

- The blood remains at a slightly basic level of pH 7.35 – 7.45 in healthy condition.
- The body has three primary mechanisms to regulate $[H^+]$ in the body fluid—the buffer, respiratory and renal mechanism.
- The blood buffers form the first line of defense against alterations in the pH of body fluids and tissues.
- The second line of defense against acid-base disturbances is by regulating the concentration of carbonic acid ($H_2CO_3^-$) in the blood and other body fluids by the lungs.
- Long-term acid-base balance is exerted by renal mechanism is the third line of defense. The kidney contributes to maintenance of the alkali reserve and to a constant level of blood pH by reabsorbing, secreting and excreting acidic or basic substances, as the case may be.
- Acidosis (may be metabolic or respiratory) and alkalosis (may be metabolic or respiratory) are the disorders of acid-base balance.
- Acidosis may be defined as an abnormal condition caused by the accumulation of excess acid in the body or by the loss of alkali from the body.
- Alkalosis is an abnormal condition caused by the accumulation of excess alkali in the body or by the loss of acid from the body.

- Metabolic acidosis results from decreased HCO_3^- concentration.
- Metabolic alkalosis is caused by increased HCO_3^- concentration.
- Respiratory acidosis results from the raised level of CO_2 and therefore H_2CO_3.
- Respiratory alkalosis results from increased ventilation with decreased CO_2.

EXERCISE

Solve

Case History 1

A 38-year-old man reported in the emergency ward of a hospital emergency with complaints of persistent vomiting for one week. He had generalized muscular cramps. On examination, he appeared dehydrated and had shallow respiration. Blood sample was analyzed with the following results:

pH = 7.8
Bicarbonates = 35 mEq/L
pCO_2 = 50 mm Hg
Na^+= 145 mEq/L
K^+ = 2.9 mEq/L.

Questions

1. Identify the nature of acid-base disorder.
2. What could be the cause of this acid-base disorder?
3. What is the cause of shallow respiration?
4. Give reason for development of muscle cramps.

Case History 2

A 50-year-old male was admitted with a history of chronic obstructive airways disease for many years. On examination, he was found cyanosed, and breathless. Blood sample was analyzed with the following results:

Blood pH = below normal
pCO_2 = markedly elevated
(HCO_3^-) = markedly elevated.

Questions

1. Identify the nature of acid-base disorder.
2. What could be the cause of elevated pCO_2?
3. What could be the cause of elevated (HCO_3^-)?

Case History 3

A person presents himself with untreated diabetes mellitus. He is treated for acidosis.

Questions

1. What is the type of acidosis?
2. What is the normal bicarbonate/carbonic acid ratio? What will happen to the ratio in this patient?
3. How will compensation occur?
4. What is the role of kidney in correcting acidosis?

Multiple Choice Questions (MCQs)

1. Metabolic acidosis is primarily due to:
a) Increase in carbonic acid
b) Decrease in carbonic acid
c) Decrease in bicarbonate
d) Increase in bicarbonate

2. In compensated metabolic acidosis plasma:
a) HCO_3^-/H_2CO_3 ratio is increased
b) Total CO_2 content is decreased
c) HCO_3^-/H_2CO_3 ratio is decreased
d) None of the above

3. Important buffer in extracellular fluid is:
a) Hemoglobin b) Bicarbonate
c) Protein d) Phosphate

4. All of the following are associated with metabolic alkalosis, *except:*
a) Rise in blood pH
b) Rise in bicarbonate plasma levels
c) Loss of H^+ ions
d) Decrease in bicarbonate plasma levels

5. Metabolic acidosis is associated with all of the following, *except:*
a) Increased elimination of acid in urine
b) Increased formation of ammonia
c) Increased respiration
d) Diminished formation of ammonia

6. The normal pH of plasma is maintained by all of the following, *except:*
a) Plasma buffer
b) Lung's mechanism
c) Heat mechanism
d) Renal mechanism

7. Normal pH of blood is:
a) 7.0 b) 7.2
c) 7.4 d) 7.6

8. At blood pH 7.4, the ratio of $NaHCO_3/H_2CO_3$ will be:
a) 5:1 b) 10:1
c) 20:1 d) 4:1

9. At blood pH 7.4, the ratio of $HPO_4^-/H_2PO_4^-$ will be:

a) 4:1 b) 5:1
c) 20:1 d) 1:20

10. Respiratory acidosis results from:

a) Obstruction to respiration
b) Diabetes mellitus
c) Starvation
d) Hyperventilation

11. Which of the following is volatile acid?

a) Phosphoric acid b) Carbonic acid
c) Sulfuric acid d) Lactic acid

12. Physiologically important carbonic acid (H_2CO_3) present in the body is equivalent to:

a) 36 liters of 0.1 N acid
b) 56 liters of 1 N acid
c) 36 liters of 1 N acid
d) 36 liters of 0.01 N acid

13. Carbonic acid (H_2CO_3) is buffered by:

a) Bicarbonate buffer b) Phosphate buffer
c) Hemoglobin buffer d) None of the above

Correct Answers for MCQs

1-c	2-c	3-b	4-d
5-d	6-c	7-c	8-c
9-a	10-a	11-b	12-c
13-c			

CHAPTER

25

Organ Function Tests

INTRODUCTION

A large number of biochemical tests are carried out in the investigation of diseases. Many of them are well associated with the impairment of the function of particular organ and called ***organ function tests*.** Thus, organ function tests are the tests carried out to assess whether a particular organ is functioning normally or not. The following organ function tests are most common:

- Liver function tests
- Renal function tests
- Thyroid function tests
- Lipid profile tests.

LIVER FUNCTION TESTS

Classification of Liver Function Tests

Liver function tests can be classified into five classes according to the function of the liver as given below:

Tests based on excretory function

It includes measurement of:

- Serum bilirubin
- Urine bilirubin
- Urine bile salts
- Bromosulphophthalein (BSP) dye tests.

Tests based on detoxification function

It includes determination of:

- Blood ammonia and bilirubin
- Hippuric acid test.

Tests based on synthetic function

It includes determination of:

- Plasma proteins, albumins and globulins
- Prothrombin time.

Tests based on metabolic function

It includes:

- Test related to carbohydrate metabolism
  - Galactose tolerance test.
- Test related to lipid metabolism
  - Determination of serum cholesterol and ratio of free to esterified cholesterol.
- Test related to protein metabolism
  - Serum protein estimation
  - Serum ammonia estimation.

Determination of serum enzymes

- Serum alanine transaminase (ALT)
- Serum aspartate transaminase (AST)
- Serum alkaline phosphatase (ALP).

Liver Function Tests Based on Excretory Function

An important physiologic role of the liver is the removal of toxic endogenous and exogenous substances from the

blood. The tests based on excretory function of liver are related to bilirubin metabolism.

Tests based on bilirubin metabolism

Bilirubin is the excretory end product of heme. It is conjugated in the liver to form bilirubin diglucuronide. Bilirubin is insoluble in water but bilirubin diglucuronide is soluble in water. The bilirubin glucuronide is excreted in the bile and through the bile goes to the intestine. There, it is reduced by to bacterial enzymes to urobilinogen.

- Bilirubin exist in the serum in two forms.
  - Conjugated or direct bilirubin which is water soluble.
  - Unconjugated or indirect bilirubin which is water insoluble.
- The normal concentration of total serum bilirubin is 0.1 to 1 mg/dL of which direct serum bilirubin ranges from 0.1 to 0.4mg/dL and indirect serum bilirubin is 0.2 to 0.7 mg/dL.

Serum bilirubin estimation

Van Den Bergh Reaction

Estimation of serum bilirubin is based on Van Den Bergh reaction. In this reaction, when serum bilirubin is allowed to react with **Van den Bergh's diazo** reagent (sulfanilic acid and sodium nitrite in HCL) a purple colored **azobilirubin** is formed.

- Conjugated bilirubin being water soluble can react directly with aqueous solution of diazo reagent and so called ***direct bilirubin.***
- Whereas the unconjugated bilirubin is water insoluble and does not react in aqueous solution. It requires addition of methyl alcohol to react with diazo reagent in the determination method and called **indirect bilirubin**.
- If both conjugated and unconjugated bilirubin are present in increased amounts, a purple color is produced immediately and the color is intensified on addition of alcohol. Then, the reaction is called biphasic.

Clinical Interpretation

Estimation of **direct** and **indirect bilirubin** is useful for the **differential diagnosis of jaundice.** Bilirubin metabolism is deranged in three important diseases. They are:

- Hemolytic jaundice
- Hepatic jaundice
- Obstructive jaundice.

- In hemolytic jaundice, unconjugated bilirubin is increased. Hence, Van den Bergh test is indirect positive.
- In obstructive jaundice, conjugated bilirubin is elevated and Van den Bergh test is direct positive.
- In hepatic jaundice both conjugated and unconjugated bilirubins are increased hence a biphasic reaction is observed.
- Laboratory results in normal persons and patients with three different types of jaundice are shown in **Table 25.1**.

Urine bilirubin

- In normal individuals, bilirubin is not excreted in the urine. When it is present in the urine, it indicates some disease of the liver.
- Only conjugated bilirubin is soluble in water and is excreted in urine but not the unconjugated which is water insoluble.
- In urine, conjugated bilirubin can be detected by Fouchet's test.

Clinical interpretation

- Conjugated bilirubin appears in urine of patients in obstructive and hepatic jaundice.
- In hemolytic jaundice, unconjugated bilirubin is increased in blood, it does not appear in urine.

Urobilinogen in urine

The amount of urobilinogen present in urine depends on the amount of bilirubin entering the intestine.

Urine urobilinogen is estimated semi-quantitatively, by Ehrlich's aldehyde reagent.

Table 25.1: Laboratory results in normal and patients with three types of jaundice

Condition	*Serum bilirubin*		*Urine urobilinogen*	*Urine bilirubin*
	Conjugated (Direct)	*Uncojugated (Indirect)*		
Normal	0.1-0.4 mg/dL	0.2-0.7 mg/dL	0.4 mg/24 h	Absent
Hemolylic or prehepatic jaundice	Normal	Increased	Increased	Absent
Hepatic jaundice	Increased	Increased	Normal or Decreased	Present
Obstructive or posthepatic jaundice	Increased	Normal	Absent	Present

Clinical Interpretation

- Normally, trace amounts of urobilinogen are present in urine.
- An increase in urobilinogen in urine, is found in hemolytic jaundice due to excess production of bilirubin.
- In hepatitis, the urobilinogen in urine may be normal or decreased.
- In posthepatic obstructive jaundice, due to the complete or almost complete biliary obstruction, no urobilinogen is found in urine because bilirubin is unable to enter the intesine.

Dye excretion test for excretory function

In addition to excreting bilirubin, the liver is capable of eliminating various dyes or drugs by the same excretory pathway as bilirubin.

Bromosulfophthalein excretion test

A 5% solution of BSP is injected intravenously (the dose is 5 mg/kg body wt) and a sample of blood is tested 45 minutes later for percentage of injected dye remaining in the blood.

Clinical interpretation

- In normals, the retention of BSP at 45 minutes is less than 5%. Impairment of liver cell function causes an increase in BSP retention.

Tests Based on the Detoxification Function

The liver is involved in the detoxification and removal of potentially hazardous substances from the body. These may be endogenous, e.g. ammonia and bilirubin (discussed earlier) or exogenous chemicals and drugs.

Hippuric acid test

Hippuric acid test is based on detoxicating function of the liver. The liver removes **benzoic acid** by conjugating it with **glycine** to hippuric acid which is excreted in the urine.

In hippuric acid test, a dose of sodium benzoate is given either orally or intravenously and the amount of hippuric acid excreted in a fixed time is determined.

For the oral test, the patient ingests 6 gm sodium benzoate dissolved in about 250 ml water. Urine collections are made for the next 4 hours and the amount of hippuric acid excreted is estimated.

Clinical Interpretation

- In normal subjects, at least 3 to 3.5 gm hippuric acid should be excreted in the bile during the 4 hours period.
- It is decreased in hepatitis, tumors, cirrhosis and obstructive jaundice.
- Excretion is normal in hemolytic jaundice.

Determination of blood ammonia

Liver detoxicates ammonia to form urea. In a liver disease, the ability to remove ammonia may be impaired. The normal level of blood ammonia is 40-70 μg/100 ml of blood.

Clinical Interpretation

High blood levels of ammonia are found in acute hepatitis and cirrhosis.

Determination of serum bilirubin (discussed earlier)

Tests Based on Synthetic Function

Liver is the main source of synthesis of plasma proteins, e.g. albumin, globulin (except γ-globulins which are synthesized in the reticuloendothelial system), blood clotting factors, e.g. fibrinogen, prothrombin and factors V, VII, IX, X. Impaired function of liver results in decreased protein synthesis.

Determination of serum albumin and globulin

The normal concentrations of serum proteins are given below:

- Total serum protein = 6 to 8 gm/dl
- Serum albumin = 3.5 to 5.5 gm/dl
- Serum globulin = 2 to 3.5 gm/dl
- Albumin/globulin ratio = 1.2:1 to 2.5:1.

Clinical Interpretation

- Hypoalbuminemia may occur in hepatocellular disease, e.g. cirrhosis.
- Hyperglobulinemia may be present in chronic inflammatory disorders such as cirrhosis and in infectious hepatitis.
- In advanced stages of liver disease, albumin is decreased and globulins are increased, so that the A/G ratio may be reversed. The concentration of total globulins increases due to a rise in the γ-globulins (synthesized by reticuloendothelial system and not by the liver) to compensate for a possible fall in the α-globulins by liver.

Determination of prothrombin time

Hepatic synthetic function of several clotting factors can be assessed by a simple coagulation test, e.g. prothrombin time.

Various proteins that participate in blood coagulation are synthesized in the liver, e.g. fibrinogen, prothrombin

(factor II) and factors V, VII, IX and X. If any one of these factors is deficient, the deficiency causes prolonged prothrombin time.

Clinical Interpretation

- An increased prothrombin time indicates the failure of hepatic synthesis of one or more of the above mentioned clotting factors.
- As vitamin K is required for the synthesis of blood clotting factors, deficiency of vitamin K can also cause prolonged prothrombin time, which must be ruled out by estimating the prothrombin time, before and after administration of vitamin K. In case of liver disease, the prothrombin remains prolonged even after administration of vitamin K.

Tests Based on Metabolic Function of Liver

Tests related to carbohydrate metabolism

Galactose tolerance test

Galactose is converted to glucose exclusively in liver, but this metabolic process is impaired in hepatocellular damage. In this test, the person is given 40 gm of galactose dissolved in water and if he has a normal liver, he should not excrete more than 3 gm of galactose in a 5 hour collection of urine after administration of galactose. However, in hepatocellular damage 4 to 5 gm or more of galactose is excreted in urine within 5 hours.

The normal liver is able to convert galactose to glucose. In patients with hepatic disease, this ability is defective.

Tests related to lipid metabolism

Determination of **serum total cholesterol** and ratio of free to esterified cholesterol: Liver is involved in the synthesis, esterification and excretion of cholesterol. In a normal individual, the serum cholesterol level ranges between ***150 to 250 mg/dL,*** approximately 70% of which is esterified. Esterification occurs in liver and blood. Changes in the ratio of free to esterified cholesterol are often found in liver diseases, as the liver is also a site of esterification of cholesterol. Normally, the ester fraction predominates in plasma but in liver damage, it goes down.

Clinical interpretation

- Increase in total blood cholesterol is found in obstructive jaundice due to retention of cholesterol, which is normally excreted in the bile.
- Serum cholesterol is normal or depressed in hepatic jaundice. In severe acute liver necrosis, the total serum cholesterol value is usually low with a marked reduction in the percentage of the esterified cholesterol.

Tests related to protein metabolism

Serum estimation of proteins

The liver is the principal site of metabolism and synthesis of plasma proteins and amino acids. Amino acids are metabolized in the liver to ammonia and urea. Based on these metabolic functions of the liver, serum estimation of proteins, and ammonia (discussed earlier) are employed to assess the liver cell damage.

Enzymes in Diagnosis of Liver Disease

Liver cells contain several enzymes. In liver damage, these enzymes are released into blood and levels of these enzymes increase in blood.

- A large number of different enzymes have been used in the diagnosis of liver disease. But most commonly and routinely employed in laboratory are **(Table 25.2):**
  - Serum aspartate transaminase (AST)
  - Serum alanine transaminase (ALT)
  - Serum alkaline phosphatase (ALP).
- Other enzymes which have been found to be useful but not routinely done in the laboratory are:
  - Serum 5,-nucleotidase
  - Lactate dehydrogenase
  - Isocitrate dehydrogenase
  - γ-Glutamyl transferase.

Serum transaminases

- Liver is the richest source of:
  - **Aspartate transaminase (AST)** which is previously called serum glutamate oxaloacetate transaminase (SGOT).

Table 25.2: Enzyme assays in differential diagnosis of jaundice

Enzyme assays	*Hemolytic or prehepatic jaundice*	*Hepatic jaundice*	*Obstructive or Posthepatic jaundice*
ALT or AST	Usually normal	Marked increase	Increased
ALP	Normal	Increased slightly	Marked increase

  - **Alanine transaminase (ALT)** which is previously called serum glutamate pyruvate transaminase (SGPT).
- The normal range for these enzymes are as follows:
  - AST or SGOT = 4–17 IU/L
  - ALT or SGPT = 3–15 IU/L.
- Although, both AST and ALT are commonly thought of as liver enzymes because of their high concentrations in liver, ***only ALT is markedly specific for liver since AST is widely present in myocardium, skeletal muscle, brain and kidney and may rise in acute necrosis of these organs besides liver cell injury.***

Clinical interpretation

- Alanine transaminase (ALT) estimations are useful in early diagnosis to evaluate severity and prognosis of liver disease.
- In hepatitis, the levels of both these enzymes (ALT and AST) are increased, which go in thousand units, usually 500 to 1500 IU/L.
- In obstructive jaundice also an increase occurs but usually does not exceed 200 to 300 IU/L.
- In hemolytic jaundice, the level of these enzymes are normal.

Alkaline phosphatase (ALP)

ALP is produced by many tissues, especially bone, liver, intestine and placenta and is excreted in the bile. Elevation in activity of the enzyme can thus be found in diseases of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, there are elevated ALP levels generally due to hepatobiliary disease. The normal level of ALP in the plasma is 3-13 KA units/100 ml (King Armstrong units).

Clinical interpretation

- The greatest elevation (3 – 10 times normal) occurs in obstructive jaundice. ***The enzyme ALP is normally excreted through bile. Obstruction to the flow of bile, causes regurgitation of enzyme into the blood resulting in increased serum concentration.***
- Slight to moderate increase is seen in hepatitis and cirrhosis.
- Normal serum ALP values are found in hemolytic jaundice.

RENAL FUNCTION TESTS

Kidney performs following important functions. The functional unit in the kidney is the **nephron**. The component parts of the nephron are given in **Figure 25.1**.

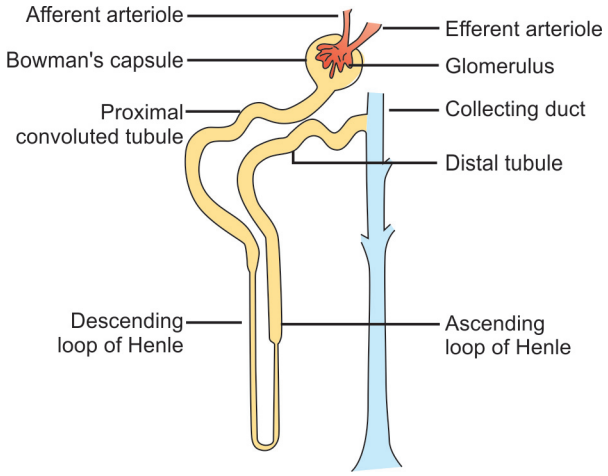


Figure 25.1: Components of nephron

- The chief function of kidney is excretion of water and metabolic wastes in urine.
- The glomerular filtration and renal tubular reabsorption are the two major functions of kidney that are involved in the formation of urine.
- In order to assess kidney function several kidney function tests (renal function tests) are performed. The various renal function tests have been divided into three major groups.

1. Glomerular function tests which include :
   i. Clearance test :
      a. Creatinine clearance test
      b. Urea clearance test
      c. Inulin clearance test.
   ii. Blood analysis of urea and creatinine
   iii. Test for protein in urine.
2. Tubular function tests which include:
   i. Urine concentration test (fluid deprivation test)
   ii. Urine dilution test (excess fluid intake test)
   iii. Acid load test (urine acidification test)
   iv. Phenosulfonaphthalein (PSP) test.
3. Urine analysis which include:
   i. Physical examination
   ii. Chemical examination
   iii. Microscopic examination.

Glomerular Function Tests

These tests are performed to assess the glomerular filtration rate (GFR). GFR provides a useful index of the status of functioning glomeruli. Renal clearance tests are performed to determine GFR.

Clearance test

Clearance test is performed to assess the glomerular filtration rate (GFR). Clearance is defined as the volume of plasma (in ml) that could be completely cleared off a substance per minute and is expressed as milliliter per minute. It may also be defined as that volume of plasma (in ml) which contains the amount of the substance which is excreted by kidney in urine in 1 minute. Renal clearance (C) is calculated by using following formula.

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

where,
C: Renal clearance = GFR of a substance in ml/minute
U: Concentration of substance in urine (mg/100 ml)
V: Volume of urine in ml excreted per minute
P: Concentration of substance in plasma (mg/100 ml).

Creatinine clearance tests

Creatinine clearance test is the renal function test based on the rate of excretion of creatinine by the kidneys. Creatinine is an excretory product derived from creatin phosphate. The excretion of creatinine is not influenced by metabolism or dietary factors.

Creatinine is freely filtered at the glomerulus and is not reabsorbed by the tubules. A small amount of creatinine is secreted by tubules. Because of these properties, the creatinine clearance can be used to estimate the GFR.

> Creatinine clearance is defined as the volume of plasma (in ml) that would be completely cleared off creatinine per minute.

The creatinine clearance is determined by collecting urine over a 24 hour period and a sample of blood is drawn during the urine collection period. The clearance of creatinine from plasma is directly related to the GFR, which is calculated as follows:

$$\text{Creatinine clearance} = \text{GFR} = \frac{U \times V}{P}$$

where,
U is urinary creatinine (mg/dl)
P is plasma creatinine (mg/dl)
V is volume of urine excreted (ml/minute).

Clinical interpretation

- The normal range for creatinine clearance is **90 to 120 ml/minute.**
- A decreased creatinine clearance is a very sensitive indicator of a decreased glomerular filtration rate.
- The reduced filtration rate may be caused by acute or chronic damage to the glomerulus or any of its components.
- Reduced blood flow to the glomeruli may also produce a decreased creatinine clearance.

Urea clearance test

Urea is the end product of protein metabolism. Urea clearance may also be employed as a measure of the GFR. But urea clearance is not as sensitive as creatinine clearance because:

- Unlike creatinine, 40–60% of urea is reabsorbed by the renal tubules after being filtered at glomeruli. Hence, its clearance is less than GFR.
- Moreover, urea clearance is influenced by number of factors, e.g. dietary protein, fluid intake, infection, surgery, etc.

Urea clearance is defined as the volume of plasma (in ml) that would be completely cleared off urea per minute. It is calculated by the formula.

$$\text{Urea clearance} = \frac{U \times V}{P}$$

where,
U is urinary urea (mg/dl)
P is plasma urea (mg/dl)
V is volume of urine in ml excreted per minute.

Clinical interpretation

- The normal value of urea clearance is 75 ml/minute.
- Urea clearance between 40-70 ml/min indicates mild impairment, between 20-40 ml/min indicates moderate impairment and below 20 ml/min indicates severe impairment of renal function.

Inulin clearance test

- Inulin clearance is the method of choice when accurate determination of GFR is required.
- Inulin is a polysaccharide of fructose, which is filtered by the glomerulus but not reabsorbed, secreted or metabolically altered by the renal tubule.
- The normal value of inulin clearance is **120 ml/min**.

Inulin clearance is calculated by the following formula:

$$\text{Inulin clearance} = \frac{U \times V}{P}$$

where,
U is urinary inulin (mg/dl)
P is plasma inulin (mg/dl)
V is volume of urine in ml excreted per minute.

- The main disadvantages in the measurement of inulin clearance is that inulin is required to be injected intravenously.
- From a practical point of view, creatinine clearance (inspite of secretion of small fraction of creatinine) is used in clinical medicine as an assessment of the GFR because:
  – In creatinine clearance test, there is no need of intravenous administration of creatinine, since creatinine is produced endogenously. It fulfils all the requirements of an ideal substance.
  – Creatinine is freely filtered through glomerulus and is not reabsorbed by the tubules.

Blood analysis

- Clearance determination may be most helpful in the early stages of progressive renal disease while blood analysis may be more sensitive when renal failure is advanced.
- An impaired glomerular filtration results in retention of urea and creatinine, which causes in elevation of **blood urea** (normal range 20-40 mg/dl) and **creatinine** (normal range 0.5 to 1.5 mg/dl). An increase of these end products in the blood is called **azotemia.**
- Plasma urea is less reliable than creatinine because it is affected by dietary protein intake and liver function.

Test for proteins in urine

- Protein in urine is an indicator of **leaky glomeruli** and is the first sign of **glomerular injury** before a decrease in GFR.
- The glomeruli of kidney are not permeable to plasma proteins and therefore plasma proteins are absent in normal urine.
- When glomeruli are damaged in diseased conditions, they become more premeable and plasma proteins may appear in urine and the condition is known as **proteinuria**.
- Excretion of albumin more than 300 mg/day is indicative of significant damage to the glomerular membrane.
- Excretion of albumin in the range 30-300 mg/day is termed **microalbuminuria.**

Microalbuminuria is the earliest sign of renal damage due to **diabetes mellitus** and **hypertension.**

Tubular Function Tests

- Assessment of the concentrating and diluting ability of the kidney, can provide the most sensitive means of detecting early impairment in renal function.
- The ability to concentrate or dilute urine is dependent upon renal **tubular reabsorption** function and presence of **antidiuretic hormone (ADH)**.
- The kidneys fail to concentrate urine either due to renal tubular damage or due to ADH deficiency (endocrine disorder).

The urinary ***specific gravity*** and ***osmolality*** are used to measure the concentrating and diluting ability of the tubules.

Urine concentration test (fluid deprivation test)

- In the fluid deprivation test, fluid intake is withheld for 15 hours.
- The first urine sample in the morning is collected and osmolality or specific gravity is measured.
- If it exceeds osmolality 850 mOsmol/kg or specific gravity of 1.025, the renal concentrating ability is considered normal.
- Dehydration maximally stimulates ADH secretion. If kidney is normal, water is selectively reabsorbed resulting in excretion of urine of high solute concentration and urine osmolality should be at least three times that of plasma (286 mOsmol/kg).

Clinical interpretation

In case, the urine does not have specific gravity 1.025 or osmolality 850 mOsmol/kg, it is sure that renal concentrating ability is impaired due to tubular defect.

Urine dilution test

In dilution test, after emptying the bladder, 1,000 to 1,200 ml of water is given to the patient. Urine specimens are then collected every hour for the next 4 hours.

Clinical interpretation

- Under these circumstances, if the functioning of renal tubule is normal, the urinary specific gravity should fall to 1.005 or less or an osmolality of less than 100 mOsm/kg.
- If the renal tubules are diseased, the concentration of solutes in the urine will remain constant irrespective of excess water intake.
- Renal or cardiac failure may be cause of decreased output. If concentrating ability is normal, the cause may be extrarenal.

Acid load test or ammonium chloride loading test

- The acid load test is occasionally used for the diagnosis of **renal tubular acidosis** in which metabolic acidosis arises due to diminished tubular secretion of H^+ ions.
- Ammonium chloride is administered orally in gelatin capsule (100 mg/kg body weight) to cause metabolic acidosis and the capacity of kidneys is

assessed for the production of acidic urine. Urine samples are collected hourly for the following 8 hours.

Clinical interpretation

- In normal subjects, the urine pH falls below 5.5 in at least one sample. Normal urine pH is 5.5 to 7.5.
- In an individual with renal tubular acidosis this decrease does not occur, which remains between 5.7 and 7.0.

Phenolsulfonphthalein (PSP) test or phenol red test

Dyes are widely used for excretion tests. PSP dye is nontoxic and exclusively excreted by kidney and hence, is the dye of choice for excretory function of the kidney.

- The test is conducted by measuring the rate of excretion of the dye following intravenous administration.
- After intravenous injection of 6 mg of PSP in 1 ml of saline. Urine specimen may be collected at 15, 30, 60 and 120 minutes.

Clinical interpretation

- If the 15 minute urine contains 25% or more of the injected PSP, the test is normal.
- Forty to Sixty percent of the dye is normally excreted in the first hour and 20 to 25% in the second.
- Excretion of less than 23% of the dye during the 15 minute urine indicates impaired renal excretory function. Two hour excretion may be normal.

Urine Analysis

Routine urine examination is usually the first test undertaken to assess the renal function and very often it gives some important information like **proteinuria, hematuria** to do further renal investigation. Its analysis, therefore, is important in evaluating kidney function. The standard urine analysis includes:

1. Physical examination
2. Chemical examination
3. Microscopic examination of urine.

Physical Examination

A. Physical examination includes:
   a. The 24 hours urinary output (volume)
   b. Appearance (color)
   c. Specific gravity and osmolality
   d. pH
   e. Odor.

Volume

The daily output of urine in adult is 800 to 2,500 ml with an average of **1,500 ml/day.** The quantity normally depends on the water intake, the external temperature, the diet and the mental and physical state, cardiovascular and renal function.

Polyuria: Volume more then 2,500 ml/day occur in

- Diabetes mellitus, upto 5-6 L/day
- Diabetes insipidus, 10-20 L/day
- Later stages of chronic glomerulonephritis, 2-3 L/day.

Oliguria: Volume 500 ml/day due to: Fever, diarrhea, acute nephritis, early stages of glomerulonephritis, cardiac failure.

Anuria: Complete cessation of urine occurs in: Acute tubular necrosis, bilateral renal stones, surgical shock.

Appearance (color)

Normal urine is transparent pale yellow or amber color. Variation in color may be physiological or pathological.

- Darkening from the normal pale yellow color indicating more concentrated urine or presence of another pigment.
  - Hemoglobin and myoglobin in urine produce a brownish coloration.
  - Turbidity in a fresh sample may indicate infection but also may be due to fat particles in an individual with nephrotic syndrome.
- Reddish coloration in hematuria is due to renal stones, cancer, some injury or disease of kidneys or urinary tract.

Specific gravity and osmolality

- The specific gravity indicates the concentrating ability of the kidney. It normally varies from 1.016 to 1.025 with an average 1.020. It can vary widely depending on diet, fluid intake and renal function. If renal function is impaired, the quantity of eliminated urine will be very less. In this condition increased specific gravity may be seen.
- The urine osmolality of normal individuals varies widely depending on the state of hydration. After excessive intake of fluids, the osmotic concentration may fall as low as 50 mOsm/kg, whereas with restricted fluid intake it is upto 1,200 mOsm/kg have been observed. On average, fluid intakes 300 to 900 mOsm/kg are found.

pH

The urine is normally acidic in reaction with a pH of about 6.0 (range 5.5 to 7.5). Alkaline urine is found in urinary tract infection.

Odor

Fresh urine is normally aromatic. Foul smell indicates bacterial infection.

Chemical examination

Chemical examination includes detection of the following:

- Glucose
- Protein
- Blood.

Glucose

- Normal urine contains small amounts of glucose which cannot be detected by routine test.
- Excretion of detectable amounts of reducing sugar in urine is called **glycosuria.** It may be benign or pathological (Refer glycosuria).

Protein

- Increased amount of protein in urine, i.e. proteinuria can be caused by:
  - Increased glomerular permeability
  - Reduced tubular reabsorption.
- Most common type of proteinuria is due to albumin.

Blood

Presence of blood in urine is called **hematuria** and is commonly seen due to some injury or disease of kidneys or urinary tract. It may be found in renal stones, cancer, tuberculosis, trauma of kidney or acute glomerulonephritis.

Microscopic examination

- Microscopic examination of the centrifuged urinary sediment is done to detect:
  - Cells, e.g. RBC, WBC, pus cells
  - Crystals, e.g. calcium phosphate, calcium oxalate, amorphos phosphates, etc.
  - Casts, e.g. hyaline casts, granular casts, red blood casts, etc.
- Presence of crystals in the urine may be a clue to the diagnosis of a specific type of renal calculus. Various components are observed on microscopic examination of urine in renal disease.

THYROID FUNCTION TESTS

Laboratory determinations of thyroid functions are useful in distinguishing patients with ***euthyroidism*** (having a normally functioning thyroid gland) from those with ***hyperthyroidism*** or ***hypothyroidism.*** To understand the thyroid function tests, it is necessary to understand the following basic concepts:

- The function of the thyroid gland is to take iodine from the circulating blood, combine it with the amino acid tyrosine of thyroglobulin and convert it to the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3).

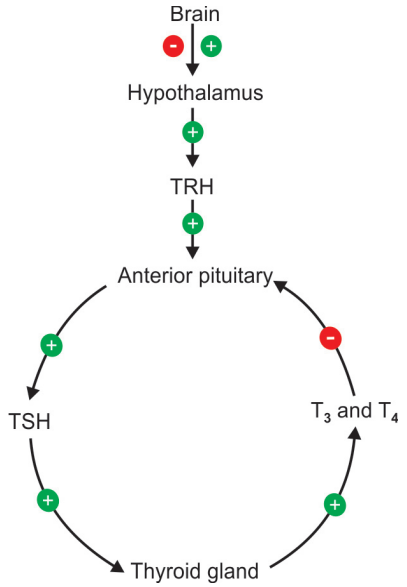


Figure 25.2: Hypothalamic pituitary thyroid axis (HPTA)

- Hormone production by the thyroid gland is tightly regulated through ***hypothalamic pituitary thyroid axis (HPTA)*** **(Figure 25.2)**. Thyroid hormones are released in response to stimulation of the thyroid gland by the pituitary hormone called ***thyroid stimulating hormone (TSH).*** TSH in turn secreted in response to stimulation of the pituitary gland by the hypothalamic, ***thyrotropin releasing hormone (TRH).***
- T_3 and T_4 (70-80%) are transported in plasma by a ***thyroid binding globulin (TBG),*** a plasma protein.
- The remaining 20 to 30% of T_3 and T_4 is transported by ***thyroxine binding prealbumin (TBPA)*** and ***albumin***.
- Only a small amount of the hormone is free which is not bound to protein.

Classification of Thyroid Function Tests

The tests used to investigate thyroid dysfunction can be performed *in vivo* or *in vitro* and classified as **(Figure 25.3)**.

1. *In vitro* thyroid function tests
2. *In vivo* thyroid function tests.

In Vitro Thyroid Function Tests

Serum total T_4 and T_3 by immuno assay

It is a direct measurement of the concentration of total (bound and free) T_4 and T_3 in the blood. The serum T_4 assays are more reliable than T_3, because it is the principal secretory product of thyroid gland. ***This test***

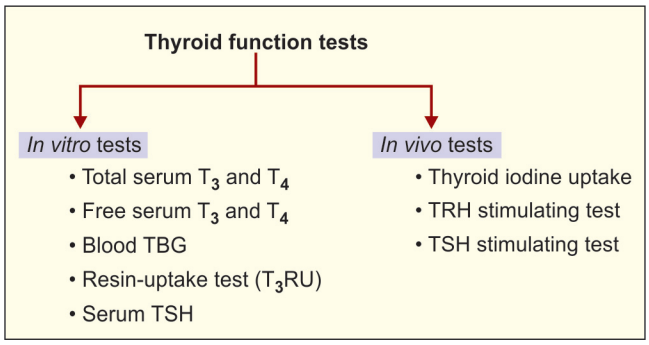


Figure 25.3: Classification of thyroid function tests

***is commonly done to rule out* hyperthyroidism *and* hypothyroidism.**

The normal values of total T_4 and T_3:
- T_4 = 5 to 12.5 µg/dl
- T_3 = 70 to 200 ng/dl.

Clinical interpretation
- Values can be increased in:
  - Hyperthyroidism
  - Increased concentration of TBG
- Values can be decreased in:
  - Hypothyroidism
  - Decreased concentration of TBG
- This test is a good index of thyroid function when TBG is normal.

Free serum T_3 and T_4

Free thyroid hormone concentrations are independent of changes in the concentration and affinity of thyroid binding proteins (TBG) and provides more reliable means of diagnosing thyroid dysfunction than measurement of total T_4 and T_3.

Normal values of free T_4 and T_3
- Free T_4 = 10–27 pmol/L
- Free T_3 = 3–9 pmol/L.

Clinical interpretation
- Increased values are associated with hyperthyroidism and thyrotoxicosis.
- Decreased values are associated with hypothyroidism.

Thyroxin binding globulin (TBG)

Almost all of the thyroid hormones in the blood are bound to protein, TBG. Changes in concentrations of TBG affect the total plasma concentrations of T_4 and T_3.

This measurement is useful in determining congenital excess or deficit of TBG and in confirming abnormalities of thyroxine binding proteins suggested by T_3RU results.

Normal values of TBG
12-28 µg/ml.

Clinical interpretation
The TBG level is increased in hypothyroidism and decreased in hyperthyroidism.

Resin uptake test (T_3RU)

It provides an indirect estimate of binding capacity of the plasma TBG. Serum of the patient is incubated with labeled hormone, ^{125}I-T_3. The labeled hormone becomes firmly attached to all unoccupied binding sites of TBG. The excess unattached ^{125}I-T_3 is removed by the addition of suitable, solid adsorbent resin or antibody and the amount of uptake of radioactive hormone by resin is determined. T_3 uptake (T_3U) is the percentage of labeled hormone taken up by the resin and is inversely related to the unoccupied binding sites on TBG.

Normal values of T_3U
25 to 35%.

Clinical interpretation
- Increased levels are associated with hyperthyroidism because more TBG binding sites occupied by the patients T_3 and T_4.
- Decreased levels are associated with hypothyroidism because of lowered level of thyroid hormone and less saturated TBG.

Serum thyroid stimulating hormone (TSH)

The measurement of plasma TSH in a basal blood sample provides the single most sensitive, specific and reliable test of thyroid status.

Stimulation of the thyroid gland by the TSH, which is produced by the anterior pituitary gland will cause the release of stored thyroid hormones.
- When T_4 and T_3 are too high, TSH secretion decreases.
- When T_4 and T_3 are too low, TSH secretion increases.

This measurement is used in the diagnosis of primary hypothyroidism (thyroid gland failure).

Normal values of serum TSH
2 to 6 µU/ml.

Clinical interpretation
- Increased levels are seen in primary hypothyroidism due to absence of negative feedback control on the pituitary **(Figure 25.2)**.
- Decreased levels are associated with:
  - Primary hyperthyroidism

- Secondary (anterior pituitary failure) hypothyroidism
- Tertiary (hypothalamic failure) hypothyroidism.

In Vivo *Thyroid Function Tests*

Thyroid iodine uptake test

Thyroid iodine uptake test is based on the iodine concentrating property of the thyroid.

- For these studies, tracer amounts of radioiodine (^{123}I or ^{131}I) are administered to the patient.
- Gamma rays emitted by the radioiodine concentrated in the thyroid are detected by specially designed imaging and counting devices and transformed into thyroid radioiodine uptakes and images.
- This parameter measures the percentage of radioiodine that concentrates in the gland. Theoretically, synthesis and secretion of thyroid hormone depends on the degree of iodine uptake in the thyroid and thus, the degree of iodine uptake by thyroid reflects the functional status of the thyroid.

Normal values of thyroid iodine uptake

- 1 to 13% is absorbed by thyroid gland after 2 hours
- 15 to 45% is absorbed by thyroid gland after 24 hours.

Clinical interpretation

- Increased uptake suggests hyperthyroidism.
- Low thyroid uptakes are found in:
  - Hypothyroidism
  - Thyrotoxicosis.

TRH stimulation test

Normally, after the intravenous administration of TRH, there is an increase of TSH level in response to TRH, which in turn increases T_3 and T_4 level in blood.

This test is done to assess the responsiveness of the anterior pituitary gland and to differentiate between the three types of hypothyroidism:

- Primary (thyroid gland failure)
- Secondary (anterior pituitary failure)
- Tertiary (hypothalamic failure).

When TRH is injected, a rise in TSH indicates that the pituitary gland is functioning.

Normal values

- TSH should increase approximately two times baseline and is usually greater in females than in males.

Clinical interpretation

- The TSH level shows a very slight increase or no response in hyperthyroidism.
- In hypothyroidism, differing responses will be seen in the different types of hypothyroidism.
  - In primary, there is an increase of two or more times the normal response.
  - In secondary, there is no response.
  - In tertiary, the TSH rises after a delay.

TSH stimulation test

Intravenous administration of exogenous TSH will stimulate all phases of thyroid function, which increases radioiodine thyroid uptake and blood thyroid hormone level.

This test is performed to differentiate primary hypothyroidism from secondary or tertiary hypothyroidism.

Normal values

- Normally, there is an increase of serum T_4 and radioiodine uptake to more than 1.5 times the base line value in response to TSH administration.

Clinical interpretation

- In secondary hypothyroidism thyroid responds well to exogenous TSH (since the endogenous synthesis is defective).
- No response to TSH is seen in:
  - Primary untreated hypothyroidism
  - Hashimoto's thyroiditis.

LIPID PROFILE TESTS

Lipid profile tests are used to estimate increased risk of cardiovascular disease which includes measurement of:

1. Total serum cholesterol
2. Serum triglycerides
3. HDL cholesterol
4. LDL cholesterol.

Total Serum Cholesterol

Enzymatic method for estimation of cholesterol

- Commercially available cholesterol reagents commonly combine all enzymes and other required components into a single reagent.
- The reagent usually is mixed with 3 µL to 10 µL aliquot of serum or plasma, incubated under controlled conditions for color development and absorbance is measured at about 500 nm.
- The reagents typically use a bacterial **cholesterol ester hydrolase** to hydrolyze cholesterol esters to cholesterol and fatty acids **(Figure 25.4)**.
- The 3-OH group of cholesterol is then oxidized to a ketone derivative and H_2O_2 by cholesterol oxidase.

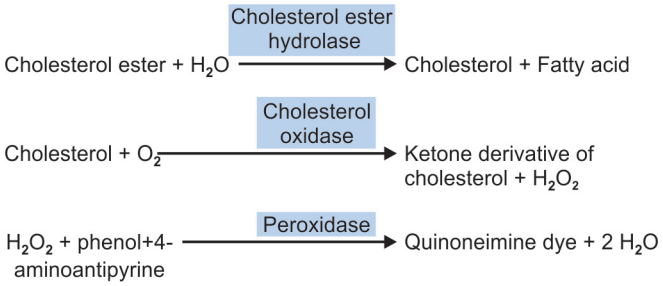


Figure 25.4: Reaction occurs in enzymatic method of total serum cholesterol estimation

- H_2O_2, is then measured in a peroxidase catalyzed reaction that forms dye.

Normal values and interpretation

- The normal range for healthy young adults is 150-270 mg/dL.
- It may be lower in children.
- The concentration increases with age.
- The concentration in the women is generally somewhat lower than in men upto the time of menopause but then increase and may exceed that in men of the same age.

Increased concentration

- The total concentration is increased in:
  - Hypothyroidism
  - Uncontrolled diabetes mellitus
  - Nephrotic syndrome
  - Extrahepatic obstruction of the bile ducts
  - Various hyperlipidemias.
- Long time elevated cholesterol concentration (more than 240 mg/dL) is a serious **risk factor for the development of coronary artery disease**.
- Lowering of plasma cholesterol concentration reduces the incidence of coronary heart diseases.
- National Cholesterol Education Program (NCEP) defined the levels of serum cholesterol believed to be **desirable, tolerable** or a **serious risk factor** for development of coronary artery disease. The report classifies total cholesterol concentration **(Table 25.3)** which is applicable to all individuals over 20 years age and sex.

Decreased concentration

Hypocholesterolemia is usually present in:

- Hyperthyroidism
- Hepatocellular disease
- Certain genetic defects, e.g. abetalipoproteinemia.

Serum Triglycerides (TG)

Enzymatic method for estimation of TG ***(Figure 25.5)***

- Single reagents that consist of all the required enzymes, cofactors and buffers generally is used.
- The first step is the hydrolysis of triglycerides to glycerol and fatty acid by lipase.
- Glycerol is then oxidized to dihydroxyacetone and H_2O_2 by glycerophosphate oxidase enzyme.
- The H_2O_2 formed in the reaction subsequently is measured as described in enzymatic method for total serum cholesterol.

Normal values and clinical interpretation

- The normal range of serum triglycerides is **40-145 mg/dL**. Mean values rise slowly with age after the third decade.
- Values below the normal range are of little clinical significance.

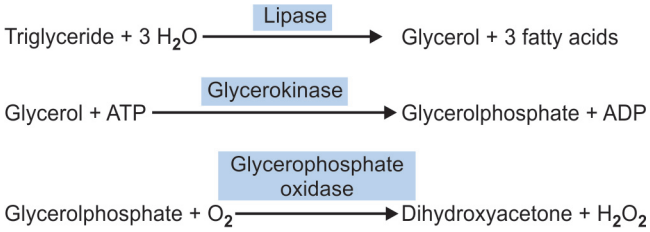


Figure 25.5: Reaction of enzymatic estimation of triglycerides

Table 25.3: Classification of serum cholesterol and serum triglycerides concentration according to the NCEP

Category	*Serum cholesterol mg/dL*	*Serum triglycerides mg/dL*	*Serum LDL-cholesterol mg/dL*
Normal (desirable, safer side)	Below 200	Below 200	Below 130
Borderline high risk	200 to 240	200 to 400	130 to 160
High risk	Above 240	400 to 1000	Above 160

NCEP = National Cholesterol Education Program. All values are in mg/dl; to convert to mml/L, multiply by 0.259

- Elevated concentration are often found in disturbances of **lipid metabolism** and in **atherosclerosis** and **coronary artery disease.** The classification of triglyceride concentration according to the NCEP is listed in **Table 25.3**.
- The serum triglyceride concentration is greatly elevated in hyperlipoproteinemia type I and V and moderately increased in type II b and III.
- The cause of hyperlipoproteinemia is a genetic origin but hypertriglyceridemia occur commonly secondary to the following pathologic conditions:
  - Hypothyroidism
  - Nephrotic syndrome
  - Alcoholism
  - Obstructive liver diseases
  - Acute pancreatitis
  - Uncontrolled diabetes mellitus
  - Glycogen storage disease (type I).

Decreased concentration

The plasma triglyceride concentration is low in the rare disease, abetalipoproteinemia (absence of low density lipoproteins).

HDL Cholesterol

Method for HDL-cholesterol estimation

Commercial kits are available for the HDL cholesterol determination.

Principle

LDL, VLDL and chylomicrons are precipitated by polyanions in the presence of magnesium ions to leave HDL in solution. The cholesterol content of the supernatant fluid is then determined by an enzymatic method.

Normal values and clinical significance of HDL cholesterol

- Serum level of HDL cholesterol for:
  - Men is 30-60 mg/dL
  - For women 40-80 mg/dL which is 20 to 30% higher than men.
- Studies have indicated that when the HDL cholesterol value is lower than 45 mg/dL in men and lower than 55 mg/dL in women there is an increased risk for heart disease and the relative risk increases with lower HDL cholesterol concentrations.
- Higher HDL cholesterol concentrations may be associated with decreased risk of coronary disease.
- Thus, HDL cholesterol levels are inversely related to the risk of cardiovascular disease.
- HDL cholesterol level above 60 mg/dL indicate very low risk for coronary artery disease (CAD).
- HDL below 35 mg/dL increases the risk of CAD.
- The ratio of total cholesterol to HDL cholesterol gives a more accurate and definite assessment of heart disease risk **(Table 25.4)**.
- Decreased levels are associated with stress, obesity, androgens, cigarette smoking and diseases like diabetes mellitus, augments the risk of coronary artery disease. HDL cholesterol is very low in genetic disorder, **Tangier disease.**

LDL Cholesterol

- The value of LDL cholesterol may be calculated, if the concentrations of total and HDL cholesterol and triglycerides are measured.
- In practice, LDL can be measured indirectly by use of **Friedewald equation** assuming that total cholesterol is composed primarily.
  Total cholesterol = Cholesterol in (VLDL + LDL + HDL).
  LDL cholesterol = Total cholesterol – (HDL cholesterol + 1/5 x Triglyceride (TG)).

The concentrations of all constituents should be expressed in the same units mg/dL or mg/L. 1/2.22 x TG is used when LDL cholesterol is expressed in mmol/L.

The factor 1/5 x TG is an estimate of the VLDL cholesterol concentration.

Normal values and clinical interpretation

- The LDL cholesterol in women is somewhat lower than in men but increase after menopause.
- Low levels of LDL cholesterol lower the risk.
- Values above 160 mg/dL indicate high risk.

Table 25.4: Ratio of total cholesterol / HDL cholesterol and LDL cholesterol / HDL cholesterol for the assessment of risk of coronary artery disease

	Total cholesterol / HDL cholesterol		*LDL cholesterol / HDL cholesterol*	
Category	Men	Women	Men	Women
Safer side	3.40	3.25	1.00	1.45
Borderline high risk	4.95	4.45	3.50	3.20
High risk	9.50	7.00	6.25	5.00

- Values between 130-160 mg/dL are in borderline risk.
- Values below 130 mg/dL are safer side **(Table 25.3)**.

Thus, the risk of cardiovascular disease is correlated directly with a high concentration of LDL cholesterol

- The highest correlations have been obtained as a risk factor by the ratio of LDL cholesterol to HDL cholesterol **(Table 25.4)**.

CARDIAC MARKERS

After myocardial infarction, a number of intracellular **enzymes** and **proteins** are released from the damaged cells. They have diagnostic importance and are called **cardiac markers.** Cardiac markers are useful in the detection of acute myocardial infarction (AMI) or minor myocardial injury.

- The cardiac markers of major diagnostic interest includes, enzymes such as:
  - Creatine kinase (CK)
  - Lactate dehydrogenase (LD)
  - Serum aspartate aminotransferase (AST) or serum glutamate transaminase (SGOT).
- Non-enzyme proteins such as:
  - Myoglobin (Mb)
  - Cardiac troponin T and I (cTnT and cTnI).

Creatine Kinase (CK)

Creatine kinase has three isoezymes ***(Refer Chapter 6)*** CK-2 or CK-MB isoenzyme is specific for the heart.

Reference values

- Normal values for total CK ranges from 10 to 100 U/L
- The upper limit for CK-MB activity = 6 U/L.

Clinical interpretation

Increased activity

- There is a rise in total CK activity following a myocardial infarction. The degree of increase varies with the extent of the tissue damage. CK is the first enzyme to appear in serum in higher concentration after myocardial infarction and is probably the first to return to normal levels if there is no further coronary damage.
- The serum total CK activity may be increased in some cases of coronary insufficiency without myocardial infarction. So, the simultaneous determination of CK-MB isoenzyme and LDH_1 isoenzyme help to make the diagnosis.
- The CK-MB isoenzyme starts to increase within 4 hours after an acute myocardial infarction (AMI) and reaches a maximum within 24 hours.
- CK-MB is a more sensitive and specific test for AMI than total CK.

Lactate Dehydrogenase (LDH)

LDH is distributed widely in liver, cardiac muscle, kidney, skeletal muscle, erythrocytes and other tissues. Because LDH is not tissue specific enzyme, serum total LDH is increased in a wide variety of disease including heart disease. LDH has five isoenzymes ***(Refer chapter 6)***. LDH_1 is specific for the heart.

Reference values

Total LDH = 125 to 290 U/L
LDH_1 = 100 U/L
LDH_2 = 115 U/L
LDH_3 = 65 U/L
LDH_4 = 40 U/L
LDH_5 = 35 U/L

The use of LDH and LDH isoenzymes for detection of AMI is declining rapidly. The troponin tests are much more useful, as will be explained later in the chapter.

Clinical interpretation

- For patients having an AMI, serum total LDH values become elevated at 12 to 18 hours after onset of symptoms, peak at 48 to 72 hours and returns to normal after 6 to 10 days **(Figure 25.6)**.
- The LDH_1 increase over LDH_2 in serum after AMI (the so called flipped pattern, in which the LDH_1/LDH_2 ratio becomes greater than 1).
- The combination of an elevated CK-MB and a flipped LDH isoenzyme ratio in a patient suspected of having a myocardial infarct makes the diagnosis certain. The combination never occurs in coronary insufficiency without a myocardial infarction.

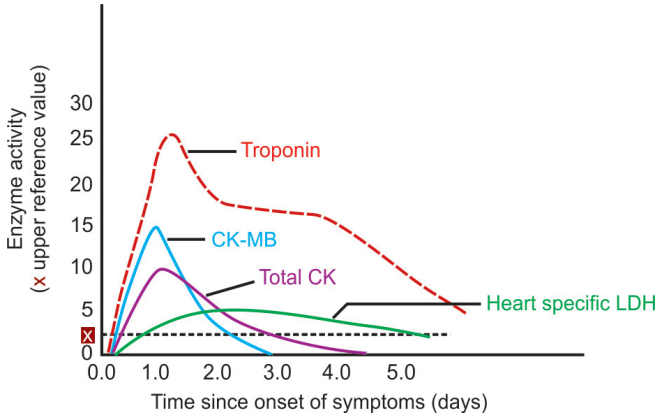


Figure 25.6: Enzyme activity after myocardial infarction (troponin which is not an enzyme is also shown)

Serum Aspartate Aminotransferase (AST)/Serum Glutamate Oxaloacetate Transaminase (SGOT)

AST is found practically in every tissue of the body, including red blood cells. It is in particularly high concentration in cardiac muscle and liver, intermediate in skeletal muscle and kidney.

Reference values

The normal concentration of serum AST is 6 to 25 U/L.

Clinical interpretation

- The serum activity of AST begins to rise about 6 to 12 hours after myocardial infarction and usually reaches its maximum value in about 24 to 48 hours.
- It usually return to normal 4 to 6 days after the infarct.
- The increase in activity is not as great as for CK, nor does it rises as early after the infarct.
- It is much less specific indication of myocardial infarction than the rise in CK, because so many other conditions, e.g. liver, muscle or hemolytic disease, can cause a rise in serum AST. Prolonged myocardial ischemia, congestive heart failure are also associated an increased AST level.

Myoglobin (Mb)

- Myoglobin is an oxygen binding protein of cardiac and skeletal muscle.
- Its low molecular weight probably account for its early appearance in the circulation after muscle injury.
- Increase in serum myoglobin occur after AMI.
- The major advantage offered by myoglobin as a serum marker for myocardial injury is that it is released early from damaged cells.

Clinical interpretation

- As shown in **Figure 25.7**, serum concentrations of myoglobin rise above the normal values as early as 1 hour after the occurrence of an AMI with peak activity in the range of 4 to 12 hours.
- Myoglobin is cleared rapidly and thus has no clinical significance after 12 hours.
- The role of myoglobin in the detection of AMI is within the first 0 to 4 hours, the time period in which CK-2 and cardiac troponin are still within their normal values.

Disadvantages of myoglobin as a cardiac marker

- The measurement of serum myoglobin has not been used extensively in clinical laboratories for the routine analysis of AMI because it is non-specific, since it is raised following any form of muscle damage.

Contd.

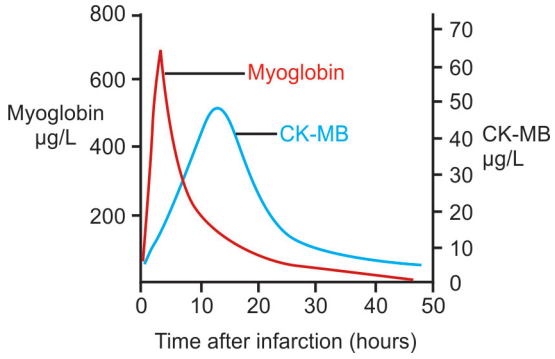


Figure 25.7: Temporal pattern of serum myoglobin and creatine kinase-2 in patients with myocardial infarction

Contd.

Even minor injury to skeletal muscle may result in an elevated concentration of serum myoglobin which may lead to the misdiagnosis of AMI.

Cardiac Troponin

- The contractile proteins include the regulatory protein troponin ***(See Chapter 33)***.
- Troponin is a complex of three protein subunits:
  - Troponin C (TnC, the calcium binding component)
  - Troponin I (TnI, the inhibitory component)
  - Troponin T (TnT, tropomyosin binding component).
- The troponin subunits exist in a number of isoforms. However, cardiac specific **troponin T (cTnT)** and **troponin I (cTnT)** forms are the most useful cardiac markers of acute myocardial infarction.

Clinical interpretation

The initial rise in cardiac troponins (cTnI and cTnT) after myocardial infarction occurs at about the same time as CK and CK–MB but this rise continues for longer than for most of the enzyme.

- For patients having AMI serum cTnT and cTnI values become elevated above the normal level at 4 to 8 hours after the onset of the symptoms.
- Secondly, cTnT and cTnI also can remain elevated up to 5 to 10 days respectively, after an AMI occurs.

SUMMARY

- Unconjugated hyperbilirubinemia with normal ALT activity indicates the presence of hemolytic or prehepatic jaundice.
- Conjugated hyperbilirubinemia with raised activities of the ALT indicates hepatocellular or hepatic jaundice.

On the other hand, conjugated hyperbilirubinemia with marked elevation of ALP activity suggests obstructive jaundice, a blockage in the bile flow.

- The glomerular filtration rate (GFR) is the best single measure of the number of functioning nephrons; and is usually estimated routinely by measuring creatinine clearance.
- Assessment of the concentrating and diluting ability of the kidney can provide the most sensitive means of detecting early impairment in renal function.
- The tests used to investigate thyroid dysfunction can be performed *in vivo* or *in vitro*.
- Serum TSH concentration is usually the single best test for assessing thyroid status. Plasma TSH concentration is elevated in primary hypothyroidism and suppressed in primary hyperthyroidism.
- Serum level of free T_4 or total T_4 can help to assess the severity of thyroid disease. Serum level of free T_3 or total T_3 can help to determine the severity of hyperthyroidism.
- Free thyroid hormone measurements correlate more closely with thyroid status than total hormone measurements, which are influenced by changes in the concentration of thyroid hormone binding protein.
- The most commonly available cardiac markers includes, creatine kinase, lactate dehydrogenase, aspartate transaminase, myoglobin and cardiac troponin I and T.
- The major advantage offered by myoglobin as a serum marker for myocardial injury is that, it is released early from damaged cells as compared to other cardiac markers.
- Tests used to estimate increased risk of cardiovascular disease is lipid profile.
- Increased plasma concentration of cholesterol and triglyceride are associated with increased risk of coronary arterial disease leading to myocardial infarction.
- Elevated HDL cholesterol decreases the risk of cardiovascular disease, high LDL cholesterol has the opposite effect.

EXERCISE

Solve the Following

A 50-year-old woman presented at the accident and emergency department with severe chest pain which had been present for the past hour.

Questions

1. What specific test would you request from the biochemistry laboratory?
2. Explain their clinical interpretation with normal range.

Multiple choice questions (MCQs)

1. Conjugated hyperbilirubinemia with raised alkaline phosphatase levels are found in:
a) Viral hepatitis
b) Obstructive jaundice
c) Hemolytic jaundice
d) Neonatal physiological jaundice

2. Inulin clearance is used to assess:
a) Renal threshold
b) Concentrating ability of tubules
c) GFR
d) Diluting ability of tubules

3. In hemolytic jaundice:
a) Urinary urobilinogen excretion is increased
b) Serum conjugated bilirubin is increased
c) Serum alkaline phosphatase is increased
d) Bilirubin is excreted in urine

4. Prothrombin time in obstructive jaundice:
a) Decreases
b) Increases after parenteral injection of vitamin K
c) Normal
d) Normalizes after parenteral injection of vitamin K

5. Which of the following has protective effect against myocardial infarction?
a) LDL
b) VLDL
c) HDL
d) Chylomicrons

6. Positive direct van den Bergh's reaction indicates presence of:
a) Conjugated bilirubin
b) Unconjugated bilirubin
c) Urobilinogen
d) Bile salts

7. Failure of concentrating capacity of urine is assessed by measurement of:
a) Inulin clearance test
b) Fluid deprivation test
c) Acid load test
d) Creatinine clearance test

8. Which of the following tests measures GFR accurately?
a) Creatinine clearance
b) Urea clearance

c) Inulin clearance
d) None of the above

9. Which of the following is not a feature of obstructive jaundice?
a) Increased level of serum unconjugated bilirubin
b) Increased level of serum conjugated bilirubin
c) Absence of urobilinogen in stool
d) Increased level of alkaline phosphatase

10. Absence of urobilinogen in urine occurs in:
a) Obstructive jaundice
b) Hemolytic jaundice
c) Hepatic jaundice
d) All of the above

11. In obstructive jaundice which of the following enzymes is diagnostically important?
a) Alkaline phosphatase
b) Acid phosphatase
c) Lactate dehydrogenase
d) Creatine phosphokinase

12. Following are the cardiac markers, *except:*
a) Troponin
b) Myoglobin
c) CK-MB
d) Alkaline phosphatase

13. Increased plasma level of which of the following is a risk factor of CHD *except:*
a) LDL cholesterol b) HDL-cholesterol
c) Triglyceride d) Homocysteine

14. Which of the following cardiac marker releases first following myocardial infarction?
a) Myoglobin b) Troponin
c) LDH (heart specific) d) AST

Correct Answers for MCQs

1-b	2-c	3-a	4-d
5-c	6-a	7-b	8-c
9-a	10-a	11-a	12-d
13-b	14-a		

CHAPTER

26

Radioisotopes in Medicine

INTRODUCTION

A radioisotope of an element may be defined as a special kind of atom which has the physical property of giving out radiations. Isotope means **'same *place*'** and implies that these have the same place in ***Mendeleev periodic table*** of the chemical elements.

Isotope is defined as an element with same atomic number but different atomic weight. The radioactive isotope or radioisotope, therefore, would behave in the same way both chemically and metabolically as the stable element with the additional advantage of giving out radiations which can be detected or can be used to destroy the tissue.

WHAT IS RADIOACTIVITY?

An atom is made up of a central core called the nucleus, which consists of closely packed ***protons*** (positively charged particle) and ***neutrons*** (uncharged particle), surrounded by a cloud of ***electrons*** moving around the nucleus in well-defined orbits **(Figure 26.1).** The nucleus has a positive charge, while the electrons have an equivalent negative charge, so that the complete atom is electrically neutral.

The phenomenon of radioactivity is associated with changes in the nucleus which consists of protons and neutrons. The ratio of neutrons to protons in a nucleus of a given element may vary due to variations in the number of neutrons. Since, an atom is electrically neutral, the number of electrons does not change, while the number of protons remains the same. In different isotopes of an element, therefore, the number of protons (Z) remains the same but the number of neutrons (N) and mass number (A) differ.

- Some of these ratios of protons to neutrons give stability to the nucleus and the isotope is then referred to as a ***'stable' isotope;*** while other ratios lead to instability
- An unstable nucleus has excess of energy and it undergoes spontaneous transformation of the number of protons or neutrons or of their internal arrangement, in order to achieve stability. During this process, the excess of energy is given out in the form of radioactivity. Such isotopes are called ***radioactive isotopes*** *or* ***radioisotopes.***

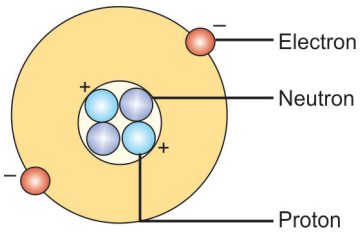


Figure 26.1: Structure of an atom of helium showing two protons, two neutrons and two electrons

Nature of Radioactivity

There are different types of transformations which can take place within the nucleus, resulting into various types of radioactivity. These are **alpha, beta** and **gamma** rays.

Properties of Radioisotopes

Penetrating Ability

Radiations given out can travel through or penetrate the matter.

- Alpha particles have poor penetrability.
- Beta particles can penetrate only a few, 4–5 millimeters in the soft tissue.
- The gamma rays, however, can penetrate deeper into the tissues and have greater penetrating power **(Figure 26.2).**

Alpha radiations cannot penetrate the skin and are not of medical interest. The beta and gamma emissions, however, are useful both for *diagnostic* and *therapeutic* purposes.

Radioactive Decay

Unlike the ordinary therapeutic drugs, radioisotopes do not have an expiry date, but they are continuously losing their activity.

- The time taken for reduction of the activity to half of its zero hour value is known as **physical half-life** of that isotope
- The time taken by the element to reduce its body concentration to half of that administered is known as the **biological half-life.**

Thus, labeled compounds using carbon-14 with half-life of 5,760 years may be metabolized and eliminated by the body within less than one day.

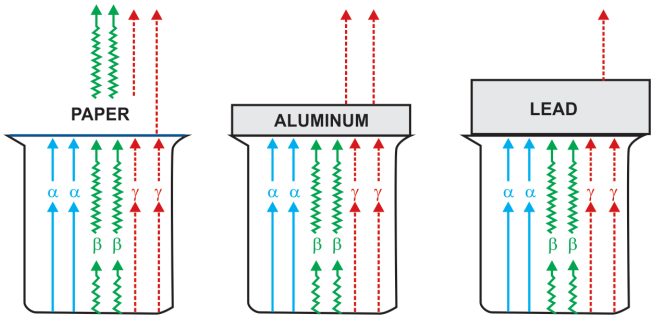


Figure 26.2: Penetrating ability of alpha, beta and gamma radiations

Ionization

Ionization is mainly responsible for the biological effects of radiation and it forms the basis for the measurement of radioactivity with detectors like Geiger Muller (GM) counters.

Unit of Radioactivity

The unit of radioactivity is based, not on the mass of the element, but on the rate of disintegrations per second.

- The unit is the becquerel (Bq). It is the quantity of radioactive material in which one nuclear disintegration occurs in one second.
- The curie (Ci) was formerly used as the unit of activity; $1Ci = 3.7 \times 10^{10}$ Bq.
- In order to express the radiation effect on the tissues, units like *'roentgen'*, or more commonly 'rad' are used. The rad (r) expresses the dosage to the tissue in terms of energy dissipated and one rad is equivalent to 100 ergs of energy absorbed per 1 gm of tissue.

USE OF RADIOISOTOPES IN MEDICINE

Radioisotopes are now extensively used in various branches of medicine for ***diagnostic, research*** and **therapeutic** purposes. The radioisotope may be used as such or by attaching it to an organic compound; the latter is then called a ***labeled*** compound.

Therapeutic Applications of Radioisotopes

When radioisotopes are focused carefully on the malignant tissue, death of the tumor cell is caused mainly due to the direct **ionization effect on its DNA**. The radiation also acts indirectly by creating free radicals that attack the DNA of the tumor cell but the process is slow. The radioisotopes used therapeutically are given below:

- The γ-rays of radio cobalt (^{60}Co) are used to treat cancer of the cervix and many other types of internal cancer.
- Radio phosphorus, ^{32}P is used for the treatment of polycythemia and chronic myeloid leukemia.
- Pleural and intra-abdominal effusions arising as secondary complication of neoplasm of thoracic and abdominal viscera is often treated with radioactive chromic sulfate $^{32}CrSO_4$.
- Superficially involved cancer, as in the cornea of the eye may be treated with ^{32}P or ^{90}Sr.
- Radio cesium ^{137}Cs is used in the treatment of chronic leukemia.

- Radio tantalum ^{182}Ta is used in the treatment of bladder cancer.
- Radio gold ^{198}Au is introduced to treat malignant pleural/peritoneal effusions.
- Radio iodine ^{131}I is used for the treatment of thyroid cancer and thyrotoxicosis.
- Radio yttrium ^{90}Y is used to treat arthritis of hemophilic patients.
- ^{32}P is used in dermatology for the treatment of squamous cell carcinoma.

Diagnostic Applications of Radioisotopes

Radioisotopes can be used in the diagnosis of many diseases. For example:

- Thyroid disorder can be diagnosed with radioiodine ^{131}I.
- ^{24}Na and ^{51}Cr have been used in the determination of blood volume and circulation time.
- ^{32}P is used for the diagnosis of cancer of liver, bone, lymph nodes and spleen.
- Radioactive iron ^{59}Fe has been used to diagnose obscure anemias and some other blood diseases.
- ^{131}I, ^{90}Sr and ^{131}l-hippuran are used in the scanning of organs, thyroid, bone and kidney respectively.
- ^{131}I diodrast (iodopyracet) is used to detect internal hemorrhages.
- ^{24}Na, ^{59}Fe, ^{131}l-labels are used to calculate ECF, circulating blood volume and cardiac output respectively.

RADIATION HAZARDS

Biological effects of radiation may be divided into two groups:

1. Somatic effect
   i. Immediate effect
   ii. Delayed effect.
2. Genetic effect.

Somatic effects are recognizable within the lifespan of the irradiated person, while genetic effects would be manifest in the offspring.

Somatic Radiation Hazards

Immediate Effects

Immediate effects of radiation leads to ***acute radiation syndrome.*** The acute radiation syndrome is characterized by:

- Severe nausea, vomiting and prostration. It is due to necrosis and ulceration of the gastrointestinal tract and may be accompanied by bleeding.
- Large dose causes high fever which is a bad prognostic sign.
- Various blood elements such as red cells, granulocytes, lymphocytes and platelets are markedly decreased and this may cause anemia, bleeding from various sites.
- Radiation decreases antibody formation and lowers the body resistance.

There is general deterioration of health, and death ensues within 2–3 weeks.

Delayed Effects

Chronic exposure to low level radiation can give rise to certain delayed effects. These are:

- **Aging effect:** Radiation can accelerate the physical processes of aging and shorten the lifespan. There is an early
  - Graying of the hair
  - Epilation (removal of hair by its roots)
  - Early development of vascular and degenerative changes
  - Lenticular cataracts.
- **Induction of neoplasms (cancer),** e.g.
  - Carcinoma of the skin following radiation.
  - Osteogenic sarcoma following radium deposition in bones.
  - Lung cancer following the inhalation of radon in uranium mine workers.
  - The incidence of leukemia in children exposed to radiation *in utero*, during the diagnostic radiological procedures in pregnancy.

Genetic Radiation Hazards

Genetic effects result from injury to chromosomes, that leads to mutations.

RADIATION HEALTH SAFETY AND PROTECTION

Basic knowledge of radiation safety is vital to every laboratory worker who has frequent contact with radioactive substances as these are hazardous to health. The protection techniques are designed to prevent radioactive material from entering the body. The protection techniques include:

- Prevention of external exposure
- Prevention of internal exposure
- Proper storage of radioactive substance
- Proper disposal of radioactive substance.

Prevention of External Exposure

Three principal methods that help to reduce exposure from external sources are:

1. **Reduce time:** Minimize the time spent in the vicinity of a source of radiation. Work efficiently, but do not rush.
2. **Increase distance:** The radiation intensity from a source diminishes rapidly as the distance from the source is increased. Maintain as large a distance from the source as practical.
3. **Use shielding:** In case of X-rays, shielding should be used to reduce the exposure.
   - Pregnant women should not be allowed to work in places where there is continuous exposure.

Prevention of Internal Exposure

Prevent the entry of radioactive materials into the body through ***inhalation, ingestion*** or ***absorption*** by the ***skin.***

- **Limiting inhalation:** This is accomplished through proper laboratory design, including attention to adequate air exchange and air flow patterns, use of fume hoods to minimize inhalation.
- **Limiting ingestion:** This is accomplished through good laboratory hygiene, such as wearing of protective gloves and gowns, knowledge of proper glove removal, handwashing before eating, prohibition of pipetting by mouth, as well as eating, drinking, smoking, cosmetics applications in the laboratory.
- **Limiting absorption through skin:** Individuals should exercise extra care to properly cover the hands, forearms and other parts that could become contaminated.
- **Proper storage:** All radioactive material should be stored in special refrigerators or cabinets conspicuously labeled with appropriate signs indicating the presence of radioactive elements.
- **Proper disposal:** Proper disposal of radioactive waste is a must. All waste containers should be clearly marked as containing radioactive material, so that waste will not be incinerated and handed over to the atomic energy authorities.

SUMMARY

- Radioisotope is an unstable element, with excess of energy and it undergoes spontaneous transformation, in order to achieve stability. During this transformation the excess of energy is given out in the form of alpha or beta or gamma rays.
- Radioactivity is the phenomenon of spontaneous emission of accelerated high energy particles or electromagnetic radiation from the unstable atomic nuclei of an element.
- Alpha rays have poor penetrating ability while the beta particles can penetrate only few millimeters. The gamma rays, however, can penetrate deeper into the tissues.
- Ionization is mainly responsible for the biological effects of radiation.
- Radioisotopes are used in various branches of medicine for diagnostic, research and therapeutic purposes.
- Exposure to radioisotopes can be hazardous to living organisms.
- A basic knowledge of radiation safety is important to every worker in the laboratory handling radioisotopes.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Becquerel (Bq) is a:**
   a) Unit of light energy
   b) Unit of radioactivity
   c) Type of radiation
   d) Measure of half-life of radio isotope
2. **The most penetrating rays are:**
   a) α-rays b) γ-rays
   c) β-rays d) X-rays
3. **Radio isotope used for treatment of polycythemia is:**
   a) ^{32}P b) ^{131}I
   c) ^{60}Co d) ^{90}Ya
4. **Which of the following emitters is more suitable for the diagnostic purpose?**
   a) α-rays b) β-rays
   c) γ-rays d) UV-rays.
5. **Unit of activity is based on:**
   a) Mass of the element
   b) Disintegration per sec
   c) Number of protons in the element
   d) Mass number of the element
6. **Unit of radioactivity is:**
   a) Becquerel b) Curie
   c) Rad (r) d) All of the above

Correct Answers for MCQs

1-b	2-b	3-a	4-c
5-b	6-d		

CHAPTER

27

Free Radicals and Antioxidants

INTRODUCTION

Free radicals are atoms or molecules containing one or more unpaired electrons in an outer orbit. A free radical is designated by a superscript dot (R°). They are highly reactive species and have a tendency either to lose an electron, thereby acting as reducing agents or gain an electron, acting as oxidizing agents. Thus, they can initiate chain reactions by extracting an electron from a neighboring molecule to complete its own orbit.

- The most important radicals are derived from molecular oxygen and nitric oxide.
- These free radicals may act as signaling molecules in physiological and biochemical activities or may provide defense against invading microorganisms
- Oxygen derived free radicals and related nonradical compound (H_2O_2) are referred to as **reactive oxygen species (ROS).**
- Not all reactive oxygen species are free radicals, e.g. singlet oxygen and hydrogen peroxide are not free radicals.

FREE RADICALS AND REACTIVE OXYGEN SPECIES (ROS)

When the oxygen molecule (O_2) is introduced into a reducing environment, it may undergo a series of reactions leading to the formation of ROS **(Figure 27.1)**.

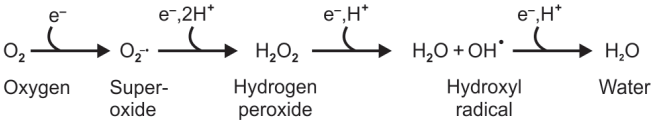


Figure 27.1: Formation of reactive oxygen species (ROS) from molecular oxygen

The reactive intermediates which are formed usually remain tightly bound in the active sites of the enzymes, until the reaction is finished. These free radicals usually have only a transient existence in the catalytic process. But occasionally, they may escape from the active site of the enzyme and lead to a destructive effect.

Free Radicals

Various reactions occurring in cells depend on a supply of oxygen. In such type of reactions, free radicals are generated as byproducts. The enzyme catalyzing oxygen requiring reactions with generation of free radicals are given below.

Oxidases

Catalyzes the reduction of dioxygen to yield water or hydrogen peroxide, e.g. **cytochrome oxidase** of mitochondria and **xanthine oxidase** which generate **superoxide** during reduction of dioxygen.

Mono-Oxygenases

Mono-oxygenases are the enzymes that incorporate one atom of oxygen into the substrate and other is reduced to water, e.g. **cytochrome P_{450} enzyme complex** found in endoplasmic reticulum. It is responsible for the hydroxylation of a wide range of endogenous and exogenous (xenobiotics) molecules and generates **superoxide** as an intermediate.

Dioxygenases

Enzymes that incorporate both atoms of oxygen into the substrate to form derivatives of hydroxyl and peroxy radicals, e.g. the reactions catalyzed by **cyclo-oxygenase** and **lipo-oxygenase** (in the synthesis of prostaglandins from arachidonic acid). Both lead to the formation of **hydroxyl** and **peroxyl radicals**.

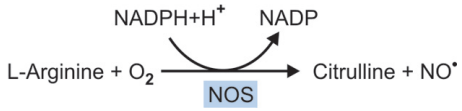
Functional Free Radicals

Functional free radicals are involved in:

1. Cellular signaling in various physiological or biochemical reactions
2. Cellular defense mechanism.

Intracellular Signaling Free Radical

The free radical **nitric oxide** is produced in various cells by a reaction catalyzed by **nitric oxide synthase (NOS).**



Nitrous oxide (NO) produced, acts as an intracellular signaling molecule and performs many biological functions:

- Relaxes smooth muscle and blood vessels
- Acts as a neurotransmitter
- Kills bacterial and tumor cells
- Prevents platelet aggregation by increasing synthesis of c-GMP.

Cellular Defense Mechanism

Phagocytic cells, such as monocytes, macrophages, neutrophils and eosinophils form large quantities of free radical **superoxide** for the destruction and removal of foreign cells by the mechanism, called **respiratory burst**.

Respiratory Burst

During phagocytosis of foreign particles such as bacteria, the phagocytic cells are activated and they exhibit a rapid increase in oxygen consumption known as **respiratory burst (Figure 27.2)**. This phenomenon reflects the rapid utilization of oxygen and production of large amounts of reactive derivatives such as **$O_2^{-\bullet}$, H_2O_2, $OH^{-\bullet}$** and **OCl^-** (hypochlorite ion). Some of these products are potent microbicidal agents.

NADPH oxidase located in plasma membrane, is responsible for the respiratory burst.

- During phagocytosis NADPH oxidase, converts molecular oxygen from the surrounding tissue into **superoxide**.
- Next superoxide is converted into hydrogen peroxide by **superoxide dismutase (SOD)**.
- In the presence of myeloperoxidase (MPO), peroxide plus chloride ions are converted into **hypochlorous acid (HOCl)** that kills the bacteria.

Genetic deficiencies of **NADPH oxidase** system cause **chronic granulomatosis,** a disease characterized by persistent and multiple infections of the skin, lungs, bone, liver and lymphocytes.

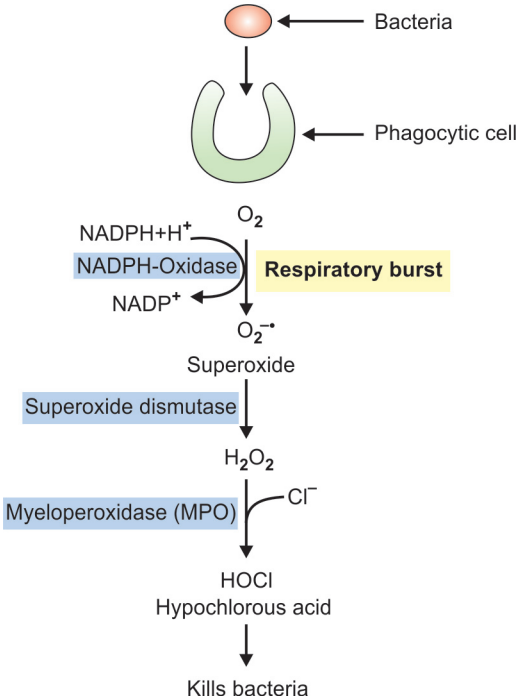


Figure 27.2: The respiratory burst during phagocytosis by neutrophil

Formation of Harmful Free Radical

- Environmental factors responsible for the generation of harmful free radicals are:
  - Exposure to ionizing radiation (X-rays and UV rays)
  - Exposure to toxic chemicals (including pollutants).
- These free radicals are highly reactive and are capable of damaging biological compounds. The main biological compounds that are damaged by free radicals are :
  - Polyunsaturated fatty acids (PUFA)
  - Proteins
  - Nucleic acids especially DNA

Damage to biological compounds leads to many pathological conditions.

Polyunsaturated fatty acids

The hydroxy radical can remove a hydrogen atom from the polyunsaturated fatty acid (PUFA) to produce a lipid free radical ($L^{\bullet}$).

$$LH + OH^{\bullet} \longrightarrow L^{\bullet} + H_2O$$

The interaction of molecular oxygen with these lipid free radicals give rise to a **peroxyl radical ($LOO^{\bullet}$)**.

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

The peroxyl radical can react with another PUFA to produce a lipid hydroperoxide (LOOH) and another lipid free radical ($L^{\bullet}$).

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$$

This new lipid radical can, in turn, be converted into another lipid peroxyl radical and lipid peroxidation proceeds as a chain reaction until the available PUFA gets oxidized with the generation of harmful products.

Protein

Cellular proteins are susceptible to attack by free radical and lead to cellular malfunction, impaired membrane and transport function.

DNA

Damage to DNA results in mutation and thereby leads to cancer.

ANTIOXIDANTS

Antioxidants are the compounds that protect the body against toxic effect of free radicals. The protective mechanisms of antioxidants serve to scavange (remove) the free radicals.

Action of Antioxidant

Different antioxidants act at different levels:

- They may prevent the initiation of chain reactions by removing free radicals.
- They may scavanage free radicals generated in chain reactions, thereby interrupting the chain sequence.
- They may remove peroxides, thereby preventing further generation of reactive oxygen species (ROS).

Types of Antioxidant System

There are three main types of antioxidant systems:

1. Enzymes antioxidant system
2. Vitamins antioxidant system
3. Minerals antioxidant system

Enzymes Antioxidant System

Following antioxidant enzymes destroy the superoxide radical and H_2O_2. The protective action of these enzymes need not be independent. They may function cooperatively.

1. Superoxide dismutase (SOD)
2. Catalase
3. Glutathione peroxidase.

Superoxide dismutase (SOD)

- It has two isomers:
  - SOD-1 is a cytosolic copper and zinc containing enzymes
  - SOD-2 is a mitochondrial manganese containing enzyme.
- These enzymes catalyze the conversion of highly reactive superoxide radical (O_2^-) to relatively less toxic hydrogen peroxide. The reaction catalyzed by these enzymes is given below:

$$2H^+ + 2O_2^{-\bullet} \xrightarrow{SOD} H_2O_2 + O_2$$

(Superoxide) — Hydrogen peroxide

SOD acts as first line of defence to protect the tissues from the toxic effects of superoxide radicals.

Catalase

- Catalase protects the tissues from toxic effect of H_2O_2.
- Catalase is present in peroxisomes. The H_2O_2 which is generated by SOD can be scavenged by catalase.

$$2\,H_2O_2 \xrightarrow{Catalase} H_2O + O_2$$

- Catalase decomposes hydrogen peroxide to water and oxygen.

Glutathione peroxidase

- Glutathione peroxidase is a selenium containing enzyme. It catalyzes the reduction of hydrogen peroxide (H_2O_2) and lipid hydroperoxides ($LOO^{\cdot}$) using reduced glutathione (GSH) as the reducing agents.
- During reduction of H_2O_2 and lipid hydroperoxide, reduced glutathione is converted into oxidized glutathione (G-S-S-G).
- The oxidized glutathione (G-S-S-G) is then reduced by glutathione reductase which requires coenzyme NADPH **(Figure 27.3)**.

Vitamins Antioxidant System

Few vitamins have antioxidant activity and help in detoxification of free radical. These vitamins are:

1. Tocopherol (vitamin E)
2. β carotenes (vitamin A)
3. Ascorbic acid (vitamin C).

- Reduced form of vitamin E (EH) can break the chain process by reacting with lipid peroxide radical and itself forming a free radical tocopheroxy radical ($E^{\bullet}$).

$$\underset{\text{Lipid peroxide radical}}{LOO^{\bullet}} + \underset{\text{Reduced vitamin E}}{EH} \longrightarrow \underset{\text{Tocopheroxyl radical}}{LOOH + E^{\bullet}}$$

- The resulting vitamin $E^{\bullet}$ radical is stable, as it is able to delocalize the unpaired electron within its structure and does not propagate the chain reaction.
- Vitamin C and carotenoids are able to generate vitamin E from $E^{\bullet}$, permitting the vitamin E once more to act as an antioxidant **(Figure 27.4)**.
- Vitamin C, β-carotene, are also able to reduce and detoxify oxygen intermediates in cells.

Minerals Antioxidant System

The activity of the antioxidant enzymes depends on supply of minerals:

1. Manganese
2. Copper
3. Zinc
4. Selenium.

- Manganese, coper and zinc are required for the activity of **superoxide dismutase**.
- Selenium is required for the activity of **glutathione peroxidase**.

Figure 27.4 shows the interrelationship between antioxidant systems and **Table 27.1** shows ROS and their antioxidants.

Table 27.1: ROS and their antioxidants

Reactive species	*Antioxidant*
Superoxide free radical ($O_2^{-\bullet}$) and Hydroxyl free radical ($OH^{\bullet}$)	Superoxide dismutase, Vitamin E, β-Carotene
Hydrogen peroxide (H_2O_2)	Catalase, Glutathione peroxidase
Lipid peroxides ($LOO^{\bullet}$)	Glutathione peroxidase
Peroxy free radical ($ROO^{\bullet}$)	Vitamin E and C

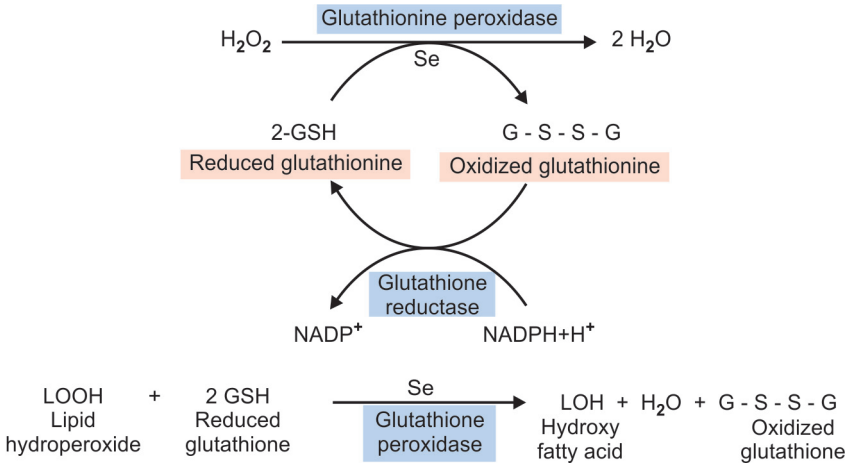


Figure 27.3: Role of glutathione as an antioxidant

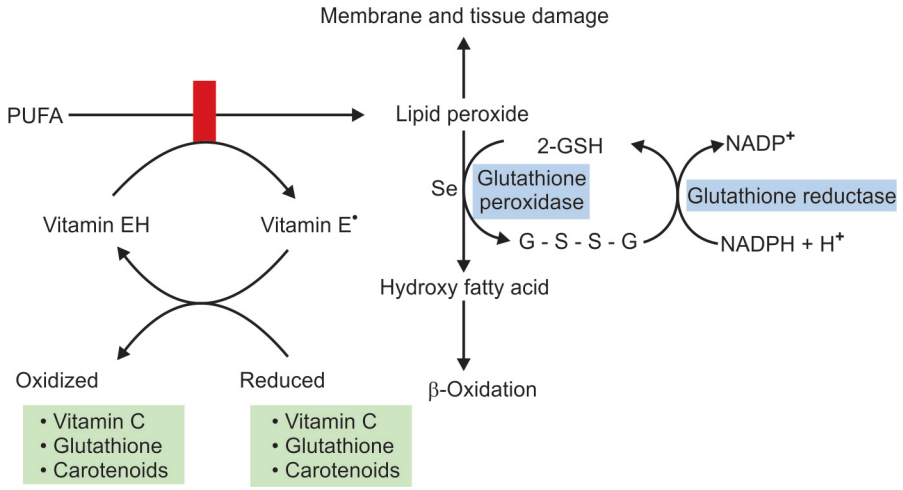


Figure 27.4: Interrelationship between antioxidant system

OXIDATIVE STRESS

In a normal cell, there is an appropriate **pro-oxidant**: **antioxidant balance**. Pro-oxidants are the compounds capable of generating toxic ROS. However, this balance can be shifted towards the pro-oxidants when production of ROS is increased greatly, (e.g. following ingestion of certain chemicals or drugs, exposure to ionizing radiation) or when levels of antioxidants are diminished. This state is called **"oxidative stress"** and can result in serious cell damage if the stress is massive or prolonged.

Free radicals are responsible for the causation and progress of several diseases. These include:
- Atherosclerosis
- Some forms of cancer
- Cataract formation
- Rheumatoid arthritis
- Ulcerative colitis
- Crohn's disease.

SUMMARY

- A free radical is an atom that has an unpaired electron in an outer orbit. It is highly reactive and can initiate chain reactions by extracting an electron from a neighboring molecule to complete its own orbit.
- Oxygen derived free radicals and related non-radical compounds are referred to as reactive oxygen species (ROS).
- In enzyme reactions such as oxidases, mono-oxygenases, and dioxygenases, free radicals are by-products.
- The free radical nitric oxide is generated in a wide range of cells by a reaction catalyzed by nitric oxide synthase (NOS). $NO^{\bullet}$ is a functional free radical and acts as a intracellular signaling molecule for physiological and biochemical activities.
- During phagocytosis of foreign particles such as bacteria, the phagocytic cells are activated and generate a respiratory burst and help to kill bacteria.
- Exposure to ionizing radiation to toxic chemicals and to smoke generates free radicals.
- The main biological targets for attack by free radicals are polyunsaturated fatty acids (PUFA), proteins and nucleic acids especially DNA can lead to many pathological conditions, such as atherosclerosis cancer, cataract, inflammatory diseases such as rheumatoid arthritis, etc.
- Antioxidant defense mechanism is a protective mechanism of the cell that serves to minimize the toxic effect of free radicals.
- There are three main types of antioxidant systems against ROS: Enzymatic, vitamins and minerals.
- Enzymatic antioxidant system includes enzymes such as superoxide dismutase, catalase and glutathione peroxidase.
- Vitamins antioxidant system includes vitamins, vitamin E and C and β-carotenoids and minerals antioxidant system includes, manganese, copper, zinc and selenium.

EXERCISE

Multiple Choice Questions (MCQs)

1. **The following enzymes act as antioxidants, *except:***
   a) Superoxide dismutase
   b) Lactate dehydrogenase
   c) Catalase
   d) Glutathione peroxidase

2. **All of the following are antioxidant *except:***
   a) Vitamin E
   b) Vitamin C
   c) Carotenoid
   d) Phylloquinone

3. **Free radicals are:**
   a) Chemical species with unpaired electron
   b) Ion having both positive and negative charge
   c) Positively charged ion
   d) Negatively charged ion

4. **The minerals function as antioxidants, *except:***
   a) Manganese b) Copper
   c) Zinc d) Sodium

5. **α-Tocopherol prevents rancidity by virtue of this property:**
   a) Antioxidant b) Oxidant
   c) Hydrogenation d) Phosphorylation

6. **During respiratory burst, all the following ROS are formed, *except:***
   a) Superoxide
   b) Hydrogen peroxide
   c) Hydroxy radical
   d) Nitric oxide

7. **The main biological targets for attack by free radicals are, *except:***
   a) PUFA
   b) Protein
   c) DNA
   d) Glycosaminoglycans

8. **Enzyme responsible for respiratory burst is:**
   a) NADPH-oxidase
   b) Nitric oxide synthase
   c) Glutathione peroxidase
   d) Catalase

Correct Answers for MCQs

1-b	2-d	3-a	4-d
5-a	6-d	7-d	8-a

CHAPTER

28

Detoxification (Metabolism of Xenobiotics)

INTRODUCTION

Certain unwanted and harmful chemical agents may enter the human body through various routes such as inhalation, ingestion, skin contact, etc. These foreign compounds are known as ***xenobiotics*** (Greek, Xenos = stranger).

- These foreign compounds may be in the form of food, drugs, chemical carcinogenes, additives, preservatives or pollutants.
- Besides, foreign molecules are also produced in intestine through bacterial enzymatic actions upon normal digestion products. Xenobiotics can produce a variety of biological effects, including pharmacologic responses, toxicity, immunologic reactions, and cancer.

Detoxification and Biotransformation

Most of these foreign compounds are subjected to metabolism (chemical alteration) in the human body. In the past the metabolic processes that lead to the disposition of foreign compounds have been referred to as ***detoxification*** mechanisms. However, the term is not always appropriate, because the detoxified products are sometimes more toxic than the original substance. ***Biotransformation*** has been suggested as a preferable term.

The primary purpose of the biotransformation process is to convert the lipophilic, nonpolar, toxic compound to more polar, water soluble forms to decrease the permeability of the toxic compound through lipid cell membrane, thus, protecting cell interior and to facilitate their excretion from the body through urine or bile.

MECHANISM OF DETOXIFICATION OF XENOBIOTICS

The liver is the principal organ responsible for detoxification of xenobiotics. The pathways for hepatic detoxification include either:

1. Oxidation
2. Reduction
3. Hydrolysis
4. Conjugation
5. Some combinations of all these metabolic reactions.

For convenience, the detoxification reactions of xenobiotics are grouped into two phases **(Figure 28.1):**

1. Phase I reactions
2. Phase II reactions.

PHASE I REACTIONS

Phase I reactions involve **oxidation, reduction** and **hydrolysis** and metabolites may be excreted without further reactions or is subsequently conjugated with conjugating agent (phase II reaction) for excretion.

Oxidation

A large number of foreign substances which include alcohols, aldehydes, amines, aromatic hydrocarbons and

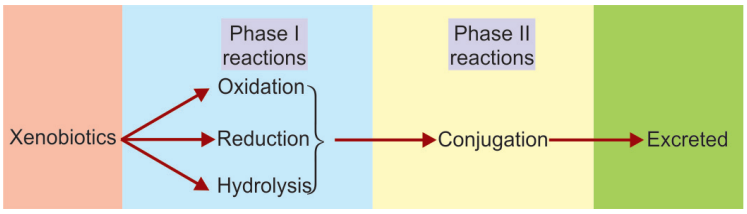


Figure 28.1: Phase I and Phase II reactions of detoxification

certain drugs are destroyed in the body by oxidation. Reactions of this type include.

Hydroxylation

Hydroxylation is the chief reaction involved in oxidative metabolism of foreign compounds, catalyzed by **monoxygenase** or **cytochorme P_{450}** (so named because the enzyme absorbs light at 450 nm, P stands for pigment). Monoxygenase enzyme, is also called **mixed function oxidases (MFOs).**

- Microsomal cytochrome P_{450} enzymes hydroxylate many xenobiotics such as drugs, carcinogens and environmental agents (pollutants). Their hydroxylated products are more water soluble facilitating their excretion.
- The reaction catalyzed by cytochrome P_{450} can be represented as shown in **Figure 28.2**. Approximately, 50% of the drugs that are ingested in humans are metabolized by microsomal cytochrome P_{450} enzyme.

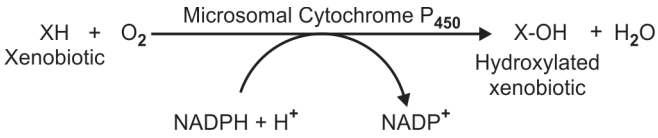


Figure 28.2: Mode of action of cytochrome P_{450}

Oxidation of alcohols and aldehydes to acids

- Alcohols and aldehydes are converted to corresponding acids by oxidation. For example:

Ethanol → Acetic acid

Methanol → Formic acid

Benzaldehyde → Benzoic acid } Conjugated later on

Benzylalcohol → Benzoic acid

- Oxidation of methanol results in the production of formic acid, a more toxic compound than methanol which may be removed by phase II conjugation reaction.

Oxidation of aromatic hydrocarbons

Aromatic hydrocarbons oxidized to phenols. Phenols may be removed by conjugation reaction. For example:

Benzene → Phenol

Acetanilide → Aminophenol } Conjugated later on

Oxidative deamination of amines

Aliphatic amines undergo oxidative deamination to the corresponding acids and nitrogen is converted to urea. For example:

Butylamine → Acetoacetic acid

Benzylamine → Benzoic acid

Oxidative Dealkylation of Hydrocarbons

Aromatic hydrocarbons containing alkyl group is oxidized to acids. For example:

Toluene → Benzoic acid

Reduction

Reduction is less common than oxidation. The major group of compounds which are reduced and detoxified by the liver are **azo compounds** and **nitro compounds.** Some important reactions are:

- Aldehydes may be reduced to alcohol, e.g.

Chloral → Trichloroethanol

- Aromatic nitro compounds are reduced to amines, e.g.

Picric acid → Picramic acid

- Compounds with S-S bonds reduced to -SH, e.g.

Disulfiram (used in the treatment of alcoholism) → Dithiocarbamic acid

Hydrolysis

Foreign compounds that are esters, amides or glycosides are subject to hydrolysis by esterases, amidases and glycosidases, e.g.

Procaine (Anesthetic) → P-Aminobenzoic acid + Diethylaminoethanol

Aspirin → Salicylic acid + Acetic acid

Atropine → Tropic acid + Tropin

Cyclohexane → Cyclohexane diol

PHASE II REACTIONS

In phase II, the hydroxylated or other compounds produced in phase I are converted by specific enzymes to various polar (water soluble) metabolites by **conjugation reactions**.

Conjugation reaction

- Conjugation means chemical combination of one compound with some other.
- Xenobiotics may undergo conjugation reactions without prior metabolism (phase I reaction) but conjugation occurs more frequently as a phase II reaction, after preliminary modification of the molecule by phase I reactions (oxidation, reduction and hydrolysis).
- The effect of conjugation is to make the compound more polar (water soluble) and therefore, more easily excreted in the urine or bile. Many conjugating agents are known. These are as follows:
  - Glucuronic acid
  - Glycine
  - Glutamine
  - Cysteine
  - Glutathione
  - Acetyl-CoA
  - Phosphoadenosyl phosphosulfate (PAPS)
  - S-Adenosyl methionine (SAM)
  - Thiosulfate
  - British antilewisite (BAL).

Conjugation with Glucuronic Acid

- Glucuronic acid is formed from oxidation of glucose by glucuronic acid pathway. Glucuronic acid participates in detoxification reactions as its UDP-derivative, ***UDP-glucuronic acid.*** UDP-glucuronic acid combines with a number of xenobiotics.
- A general conjugation reaction with UDP-glucuronic acid is shown in **Figure 28.3**. ***UDP-glucuronyl transferase*** enzyme catalyzes the formation of glucuronide conjugates, in which glucuronic acid being transferred to various compounds.
- Various drugs that form glucuronides are:
  - Morphine
  - Chloramphenicol
  - Salicylic acid
  - Indomethacin
  - Dapsone
  - Sulfathiazole, etc.

Formation of bilirubin diglucuronide is a normal metabolic reaction for detoxification of bilirubin by phase reactions. (Figure 28.3).

Conjugation with Glycine

- Many aromatic carboxylic acids are conjugated with glycine. The most common example is conjugation of benzoic acid with glycine to form hippuric acid. Some more examples are given below:

Glycine + Benzoic acid → Hippuric acid

Glycine + Salicylic acid → Salicyluric acid

Glycine + Nicotinic acid → Nicotinuric acid

Glycine + Cholic acid → Glycocholic acid

Glycine + Deoxycholic acid → Deoxyglycocholic acid

Cholic acids and deoxycholic acid are conjugated with glycine to form glycocholic acid and deoxyglycocholic acid respectively are the examples of normal metabolic detoxification reactions.

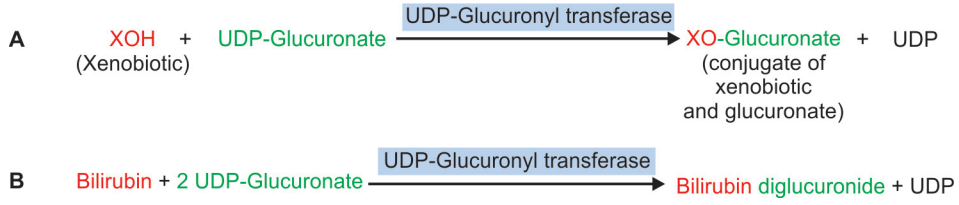


Figure 28.3: A: Representation of the general reaction for conjugation with glucuronic acid; B: Conjugation reaction with bilirubin

Conjugation with Glutamine

In normal metabolic pathway, phenylacetate conjugates with glutamine.

Phenylacetate + Glutamine ⟶ Phenylacetylglutamine

Conjugation with Cysteine

Cysteine is acetylated first with acetic acid whereby acetyl-cysteine is formed which gets conjugated with toxic substances like bromobenzene, chlorobenzene, lodobenzene, naphthalene, anthracene, benzyl chloride and number of other substances which are converted to nontoxic **mercapturic acids**. For example:

Bromobenzene + Cysteine + Acetic acid ⟶ Bromophenyl mercapturic acid

Conjugation with Glutathione (GSH)

- Glutathione (γ-glutamyl-cysteinyl-glycine) is a tripeptide of glutamic acid, cysteine and glycine. A wide range of xenobiotics are known to be excreted following conjugation with glutathione. These include:
  - Aromatic nitro compounds
  - Halogenated compounds
  - Aliphatic halides
  - The epoxide formed in phase I oxidation of hydrocarbons.
- The general reaction can be represented as follows:

$$X + G\text{-}SH \xrightarrow{\text{Glutathione S-transferase}} X\text{-}S\text{-}G$$

where,

X = xenobiotic
GSH = glutathione
X-S-G = Glutathione conjugate.

- Glutathione conjugates are subjected to further metabolism and excreted in the form of mercapturic acid (sulfur containing compound).

Conjugation with Acetic Acid (Acetylation)

- It has just been stated that acetate is used together with cysteine in the formation of mercapturic acids. However, a range of compounds with an amino group undergo conjugation with acetic acid alone as acetyl-CoA in a reaction catalyzed by a **acetyl-transferase.**
- The drugs that undergo acetylation prior to their excretion are:
  - Sulfanilamide (sulfa drug)
  - Isoniazid (antituberculosis drug)
  - Para-aminobenzoic acid (PABA).
- Acetylation is represented by:

$$\text{Acetyl-CoA} + \underset{\text{(Xenobiotic)}}{X} \xrightarrow{\text{Acetyl transferase}} \text{Acetyl-}X + \text{CoA}$$

Conjugation with Sulfate (Sulfation)

- The sulfur donor for detoxification reactions is ***phosphoadenosyl phosphosulfate (PAPS).*** This compound is called **active sulfate**.
- Sulphotransferase transfers sulfate from PAPS to the alcoholic OH of the phenol, cresol, indole, skatole, steroids or to the NH_2 of aliphatic and aromatic amines to form various **etheral sulfate.**
- The general reaction can be represented as follows:

$$\underset{\text{(Xenobiotic)}}{\text{PAPS + XOH}} \xrightarrow{\text{Sulphotransferase}} \underset{\text{(Sulphated Xenobiotic)}}{XOSO_3^-}$$

Conjugation by Methylation

A few xenobiotics are subjected to methylation for their excretion. The methyl donor is ***S-adenosylmethionine (SAM),*** active form of methionine. The reaction is catalyzed by methyltransferase. For example:

- Pyridine, nicotinic acid, nicotinamide, thyroxine
- Estrogenes
- Epinephrine and norepinephrine are methylated before their excretion.

Conjugation with Thiosulfate

- Cyanides, highly toxic compounds are conjugated with thiosulfate and converted to relatively nontoxic **thiocyanate (-SCN)**.
- Small quantities of cyanide formed during the course of normal metabolism is converted to thiocyanate by an enzyme called ***rhodanase (thiosulfate-sulfur transferase).***

$$\text{HCN} + \underset{\text{Thiosulphate}}{Na_2S_2O_3} \xrightarrow{\text{Rhodanase}} NaCNS + NAHSO_3$$

Conjugation with British Antilewisite (BAL)

- Certain heavy metals like arsenic, cadmium, gold and mercury are detoxified by British antilewisite (BAL) which is 2, 3 ***mercapto propanol.***
- Heavy metals impair the functioning of the enzymes by binding with -SH group of the enzymes, BAL acts as an antidote, it has an high affinity for these metal ions and removes these ions from the enzyme.

SUMMARY

- Xenobiotics are chemical compounds which are foreign to the body, such as food additives, chemical carcinogens, environmental pollutants, drugs, etc.
- The term detoxification is now being replaced by the more appropriate biotransformation.
- Xenobiotics is metabolized into two phases. In phase I reactions oxidation, reduction and hydrolysis are involved. In phase II, compounds produced in phase I are converted to polar metabolites by conjugation with various conjugating agents.
- The major reaction of phase I, is hydroxylation catalyzed by monoxygenases, also called cytochrome P_{450} or mixed function oxidases (MFOs), acting on many exogenous and endogenous substrates.
- The overall purpose of the two phases of metabolism of xenobiotics is to increase their water solubility (polarity) and to facilitate their excretion from the body in urine or bile.

EXERCISE

Multiple Choice Questions (MCQs)

1. Toxic cyanides are conjugated with:
a) Cysteine b) Active sulfate
c) Glucuronic acid d) Thiosulfate

2. All of the following are detoxifying agents, *except:*
a) Glycine b) Glutathione
c) Glucuronic acid d) Glycogen

3. All of the following are true for cytochrome P_{450}, *except:*
a) Is also called mixed function oxidase
b) Uses NADPH
c) Associated with smooth endoplasmic reticulum
d) Is an allosteric enzyme

4. Biotransformation by oxidation is catalyzed by:
a) Cytochrome c
b) Cytochrome aa_3
c) Cytochrome b
d) Cytochrome P_{450}

5. All of the following compounds are detoxified by sulfation, *except:*
a) Indole b) Phenol
c) Cresol d) Benzoic acid

Correct Answers for MCQs

1-d 2-d 3-d 4-d
5-d

CHAPTER

29

Cancer

INTRODUCTION

The term cancer applies to a group of diseases in which cells grow abnormally. It may be defined as ***"malignant neoplasm."*** Neoplasm means new growth. Neoplasia is a general term given to diseases that cause abnormal growth of cells.

A mass of tissue formed as a result of abnormal excessive, uncoordinated, autonomous and purposeless proliferation of cells is called ***tumor.*** The branch of science dealing with the study of neoplasm or tumor is called oncology (oncos = tumor, logos = study). Tumors may be 'benign' (that is, it does not invade or spread to distant sites in the body and does not destroy the tissue in which it originates, i.e. a non-cancerous tumor) or "malignant" (that is, it invades and destroys the tissue in which it originates and can spread to other sites in the body via the blood stream and lymphatic systems). The term used for all malignant tumors is ***cancer.***

CHARACTERISTICS OF CANCER CELLS

Cancer cells are characterized by three properties:

1. Unrestricted growth
2. Invasion of local tissues (direct spread)
3. Spread or metastasis (distant spread) to other parts of the body (meta = transformation, stasis = residence).

- Once a cell is transformed into a tumor or cancer cell, it shows few ***morphological*** and ***biochemical*** changes from normal cells.
- Morphological changes affect **Shape, Motility** and **Growth** of the cell.
  - Transformed cells often have a much rounder shape than normal cells.
  - Due to loss of contact inhibition of movement, transformed cells grow over one another, while normal cells stop moving when they come into contact with each other.
  - Due to loss of contact inhibition of growth transformed cells often form multilayers instead of a monolayer usually form by normal cells.
- Biochemical changes which occur in cancer cell are:
  - Increased synthesis of RNA and DNA.
  - Increased activity of **ribonucleotide reductase** required for the formation of deoxyribonucleotides from ribonucleotides.
  - Increased rates of aerobic and anaerobic **glycolysis.** Thus, more pyruvate is produced than can be metabolized. This in turn results in excessive production of lactate and ***lactic acidosis*** results.
  - Synthesis of certain fetal proteins, e.g. carcino embryonic antigen.
  - Inappropriate synthesis of certain growth factors and hormones.

– Alterations of the cell surface due to changes in the composition of glycoproteins or glycosphingolipids.

Types of Cancer

Cancers are classified according to the tissue and cell types from which they arise, e.g.

- Carcinomas: Cancer arises from epithelial tissues
- Sarcomas: Cancer arises from mesenchymal tissues
- Leukemia: Cancer of blood forming organs
- Lymphoma: Cancer of lymph node
- Hepatomas: Carcinoma of hepatocytes.

CARCINOGENESIS AND CARCINOGENS

Carcinogenesis means formation of cancer. Agents which can induce cancer are called ***carcinogens.*** Carcinogens are a variety of external agents which are divided into three groups:

1. Chemical carcinogens
2. Physical carcinogens
3. Biologic carcinogens.

Chemical Carcinogens (Table 29.1)

Depending upon the mode of action of carcinogenic chemicals, they are divided into two groups:

1. ***Initiators*** of carcinogenesis
2. ***Promoters*** of carcinogenesis.

Initiators of carcinogenesis

These are the chemical carcinogens which can initiate the process of abnormal new growth of cells. These are further classified into two subgroups:

a. ***Indirect*** acting carcinogens
b. ***Direct*** acting carcinogens.

Indirect acting carcinogens (Procarcinogens)

Indirect acting carcinogens require prior metabolism to become carcinogenic. One or more enzyme catalyzed reactions convert procarcinogens to active carcinogens. This is called metabolic activation of procarcinogens. Examples of procarcinogens are: **(Table 29.1).**

- **Aromatic hydrocarbons,** e.g. Benzo [a] pyrene, Tobacco smoke, industrial and atmospheric pollutants.
- **Aromatic amines,** e.g. Benzidine, β-naphthylamine, azo dyes used in rubber industries.
- **Naturally occurring products,** e.g. Aflatoxin B_1.
- **Inorganic compounds,** e.g. Vinyl chloride, Asbestos, metals like nickel, lead, chromium, etc.
- **Nitrosamine compounds,** e.g. Dimethylnitrosamine, diethylnitrosamine found in whisky, new car interiors, tobacco smoke.

Direct acting carcinogens

These do not require metabolic activation. These include mainly various anticancer drugs, e.g. cyclophosphamide, nitrosourea, acetyl imidazole, etc.

Promoters of carcinogenesis

Certain chemical substances are not carcinogenic but they help the initiated cell to proliferate further are called promoters of carcinogenesis **(Table 29.1).** For example, phenols, phenobarbital, artificial sweetners like saccharine and cyclamates.

Table 29.1: Important chemical carcinogens

Class	*Examples*
Anticancer drugs	Cyclophosphamide, Nitrosourea, Acetyl imidazole
Aromatic hydrocarbons	Benzo[a]pyrene, Tobacco, smoke, smoked animal foods, industrial and atmospheric pollutants
Aromatic amines	β-Naphthylamine, Benzidine, Azo dyes used in rubber industries
Naturally occuring compounds	Aflatoxin B_1
Inorganic compounds	Vinyl chloride, Asbestos, metals like Nickel, Lead, Chromium, Cobalt, etc.
Nitrosamine compounds	Dimethylnitrosamine, diethylnitrosamine
Miscellaneous compounds	Phenols, Hormones (estrogen), Phenobarbital, Artificial sweetners like saccharine and cyclamates

Action of chemical carcinogens (Figure 29.1)

Direct or indirect acting carcinogens are usually **electrophiles,** i.e. they are deficient in electrons (free radicals). These free radical carcinogens can covalently bind to purines, pyrimidines and phosphodiester bonds of DNA causing unrepairable damage. These unrepaired damage generate mutations in DNA and mutation in DNA may lead to cancer.

Physical Carcinogens

- Physical carcinogenic agent is radiant energy both **ultraviolet light** and **ionizing radiation,** i.e. **X-rays, α, β** and **γ-rays.**
- These rays damage DNA which is the basic mechanism of carcinogenicity with radiant energy.
- The main source of UV radiation is the sunlight, others are UV lamps, welder's arcs etc. In humans, excessive exposure of UV rays can cause various forms of skin cancers.
- Ionizing radiation of all kinds like X-rays, α, β and γ-rays, radioactive isotopes, protons, and neutrons can cause cancer.

Mode of action of radiation

- Ultraviolet light and ionizing radiation differ in their mode of action.
- UV rays damage the DNA by formation of **pyrimidine dimmers** in DNA or by formation of **apurinic** or **apyrimidine** sites in DNA.
- While ionizing radiations cause the formation of highly reactive **free radicals,** that can interact with DNA leading to molecular damage.

Biologic Carcinogens

- Biologic carcinogens are chiefly ***viruses, parasites*** and ***bacteria.*** The role of viruses in the causation of cancer is more significant.
- Oncogenic (carcinogenic) viruses contain either DNA or RNA as their genome. The two types of carcinogenic viruses are:
  1. DNA oncogenic viruses
  2. RNA oncogenic viruses.

DNA oncogenic viruses

DNA oncogenic viruses are classified into five subgroups. These are:

a. Papoviruses
b. Herpes viruses
c. Adenoviruses
d. Pox viruses
e. Hepadna viruses.

RNA oncogenic viruses

The RNA viruses use RNA as the genome. RNA oncogenic viruses are **retroviruses** they contain the enzyme ***reverse transcriptase***. All retroviruses are not oncogenic. The examples of RNA oncogenic viruses are:

- Rous Sarcoma virus
- Leukemia sarcoma virus
- Mouse mammary tumor virus etc.

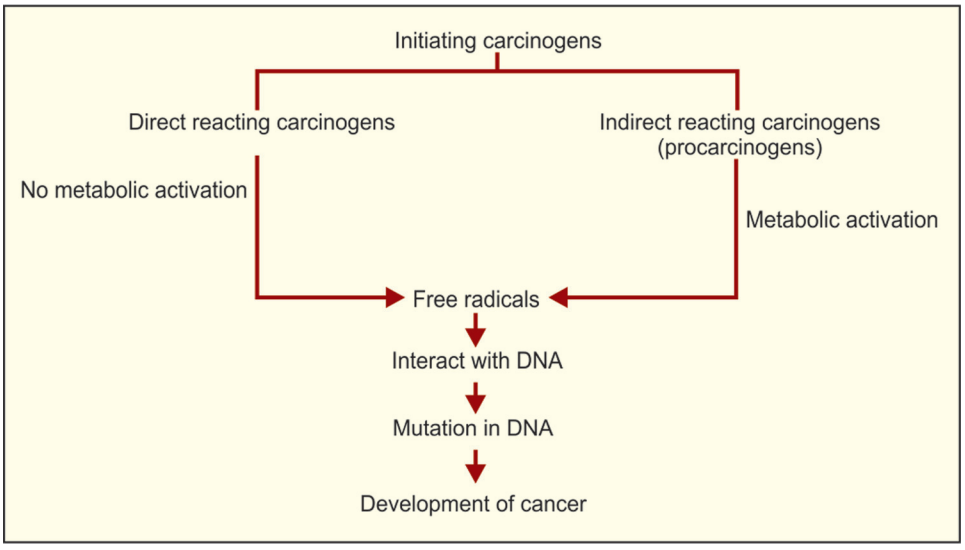


Figure 29.1: Action of chemical carcinogens

Mechanism of viral carcinogenesis

DNA and RNA oncogenic viruses differ in their mode of action.

Mode of action of DNA oncogenic virus

The DNA virus infects the host cell. Then, DNA virus binds tightly to host cell DNA and causes alterations in gene expression of host cell DNA and thus causes cancer by altering the types of protein made in cell. Viral oncoproteins bind to tumor supressors and inactivate them.

Mode of action of RNA oncogenic virus

The RNA viruses use RNA as the genome. The RNA gets copied by **reverse transcriptase** to produce single strand of viral DNA. Single strand of viral DNA is then copied to form another strand of complementary DNA, resulting in double stranded ***viral DNA*** or ***provirus***. The provirus is then integrated into the DNA of the host cell genome and may transform the cell into cancer cell.

PROTO-ONCOGENES AND ONCOGENES

- Proto-oncogenes are normal genes which stimulate cell division.
- Cellular proto-oncogenes code for a number of proteins, e.g. growth factors, receptors, transcription factors and other proteins involved in cell proliferation.
- *When proto-oncogenes get mutated they become* **oncogenes.**
- Oncogenes are genes capable of causing cancer. In the cancer cell, their normal proto-oncogenes are permanently changed to oncogenes and the balance between factors stimulating and the factors inhibiting cell growth is permanently lost resulting in increased proliferation.
- When oncogenes are expressed, they produce mutated protiens, e.g. growth factors, receptors, transcription factors and other proteins involved in cell proliferation.

Thus, various factors that cause cancer may all act through their effects on proto-oncogene. Radiation, chemical carcinogens and viruses may cause mutations in the proto-oncogene.

TUMOR SUPPRESSOR GENES

Tumor suppressor genes are normal genes, which **inhibit cell proliferation** and which normally suppresses tumor formation. These tumor suppressor genes, sometimes called **recessive *oncogenes*** or **anti-oncogenes.** Inactivation by mutation of tumor suppressor gene can cause some types of tumor.

Two of the most widely studied tumor suppressor genes are **retinoblastoma (RB) gene** and ***P*^{53}** gene**.**

- The retinoblastoma (RB) gene was the first tumor supressor gene discovered. Retinoblastoma is a rare tumor in children. The RB gene codes for nuclear phosphoprotein.
- p^{53} gene codes for a nuclear protein. Mutations of p^{53} gene which are found in at least 30% of the all cancers.

Apoptosis

Apoptosis (Greek ***"dropping off"*** **or** ***falling off***) is the normal and programmed destruction of cells during embryogenesis, development and adult life. Disruption of apoptosis can promote inappropriate cell survival and the development of cancer.

TUMOR MARKERS

Tumor marker is a biological substance synthesized and released by cancer cells and found in an increased amount in the blood, other body fluids or tissues which may suggest the presence of a type of cancer. Different types of tumor marker are **(Table 29.2)**:

Oncofetal Oncogenes

- Oncofetal antigens are proteins produced during fetal life. These proteins are present in high concentrations in the sera of fetuses and decreases to low level or disappear after birth.
- These proteins reappear in cancer patients because certain genes are reactivated as the result of the malignant transformation of cells, e.g. **α-feto-protein (AFP)** and **carcinoembryonic antigen (CEA)**.

Hormones

- Several hormones that are used as tumor markers are:
  - Calcitonin
  - Growth hormone
  - Parathyroid hormone
  - Prolactin
  - Human chorionic gonadotropin (HCG).

Carbohydrate Markers

- Carbohydrate markers are high molecular weight glycoproteins. They usually are abbreviated CA for carbohydrate antigen. These are:
  - CA 125
  - CA 549

Proteins

Some of the protein markers are:
- β-microglobulin
- C-peptide
- Ferritin
- Immunoglobulin (Bence Jones protein)
- Prostate specific antigen (PSA).

Enzymes

Several enzymes that are used as tumor markers are:
- Amylase
- Alkaline phosphatase
- Prostatic acid phosphatase
- Lactate dehydrogenase.

Clinical Applications of Tumor Markers

Tumor markers can be used in a number of ways including:
- Differential diagnosis in symptomatic individuals
- Clinical staging of cancer

Contd.
- Prognosis of disease
- Evaluation of success of treatment
- Detection of recurrence of cancer
- Monitoring of response to therapy.

CANCER AND DIET

- Increased fat and animal protein intake in the diet may increase the risk of cancer.
- This diet may favour bacterial flora capable of increasing the conversion of acid and neutral sterols to carcinogens.
- The feces produced from high beef diets also contain carcinogens such as **nitrosamide**.
- A **high fiber diet** is associated with reduced incidence of cancer of colon.
- Intake of food high in **antioxidant,** (e.g. β-carotene, vitamin E, vitamin C, trace element selenium) eicosapentenoic acid and also certain compounds found in the cruciferous vegetables, broccoli, cabbage, and cauliflower have protective effect against some forms of cancer.

Table 29.2: Tumor markers commonly used in clinical practice

Tumor markers	*Associated cancer or tumor*
Oncofetal antigens	
α-Fetoprotein (AFP)	Hepatocellular, germ cell
Carcinoembryonic antigens (CEA)	Colorectal, gastrointestinal, pancreatic, lung
Hormones	
Calcitonin	Thyroid
Growth hormone	Pituitary adenoma, renal, lung
Parathyroid hormone	Liver, renal, breast, lung
Prolactin	Pituitary adenoma, renal, lung
Human chorionic gonadotropin (HCG)	Teratoma of testes, choriocarcinoma
Carbohydrate markers	
CA 125	Ovarian, endometrial
CA 549	Breast, ovarian
Proteins	
β-microglobulin	Multiple myeloma, B-Cell lymphoma,
C-peptide	Insulinoma
Ferritin	Liver, lung, breast, leukemia
Immunoglobulin (Bence Jones protein)	Multiple myeloma
Prostatic specific antigen (PSA)	Prostate
Enzymes	
Amylase	Pancreatic
Alkaline phosphatase	Bone, liver, leukemia, sarcoma
Prostatic acid phosphatase	Prostate
Lactate dehydrogenase	Liver, lymphomas, leukemia

SUMMARY

- The term cancer (malignant neoplasm) applies to a group of diseases in which cells grow abnormally.
- Malignant cancer cells are characterized by loss of growth control, invasiveness and metastasis. Benign tumor cells have lost growth control but do not metastasize.
- Carcinogenesis means induction of cancer and the agents which can induce cancer are called carcinogens.
- Carcinogens are physical, chemical and biologic agents, that can cause cancer by damaging or altering DNA.
- Proto-oncogenes are normal genes which stimulate cell division. Activation of these genes to oncogenes (genes capable of causing cancer) is achieved by mutation.
- Tumor suppressor genes or anti-oncogenes are normal genes which inhibit cell division. Mutation of these genes cause cancer.
- Certain enzymes, hormones and antigens are released by tumor cells into the blood and used as tumor markers in diagnosis and for monitoring, treatment and recurrence of disease.
- Certain dietary constituent such as fats are promoters of some cancers, while intake of food high in antioxidant nutrients are protective in some forms of cancer.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Activation of proto-oncogenes to oncogenes occur by following mechanisms, *except:***
   a) Promoter insertion
   b) Chromosomal translocation
   c) Gene amplification
   d) Post-translational modification
2. **All of the following statements regarding proto-oncogene are true, *except:***
   a) They are present in normal cells
   b) They are present only in cancer cells
   c) They code for growth factors, receptors and other proteins involved in cell proliferation
   d) When they get mutated, they become oncogenes.
3. **Which of the following statements regarding tumor suppressor genes are true?**
   a) Normal genes
   b) Antioncogenes
   c) Mutation of this gene causes tumor formation
   d) All of the above
4. **All of the following are the characteristics of cancer cells, *except:***
   a) Diminished or unrestrained control of growth
   b) Invasion of focal tissues
   c) Function normal
   d) Spread or metastasis
5. **Retinoblastoma (RB) gene is a:**
   a) Proto-oncogene
   b) Oncogene
   c) Carcinogene
   d) Antioncogene
6. **Genes capable of causing cancer are, *except:***
   a) Mutagenes
   b) Oncogenes
   c) Carcinogenes
   d) Antioncogenes
7. **Which one of the following is not a tumor marker?**
   a) Alpha-fetoprotein
   b) Carcino embryonic antigen
   c) Prostatic acid phosphatase
   d) Parathromone
8. **Tumor suppressor genes are sometimes called:**
   a) Antioncogenes
   b) Proto-oncogenes
   c) Oncogenes
   d) Proximate carcinogenes

Correct Answers for MCQs

1-d	2-b	3-d	4-c
5-d	6-d	7-d	8-a

CHAPTER

30

Environmental Biochemistry

INTRODUCTION

The term environment implies all the external factors, living and nonliving, material and nonmaterial with which man is in constant interaction. This includes air, water, food, soil, etc. The modern concept of environment includes not only the water, air, food and soil, but also social and economic conditions under which we live.

CLASSIFICATION OF ENVIRONMENT

For convenience, the environment has been divided into four groups, which are closely related to each other **(Figure 30.1)**. These are:

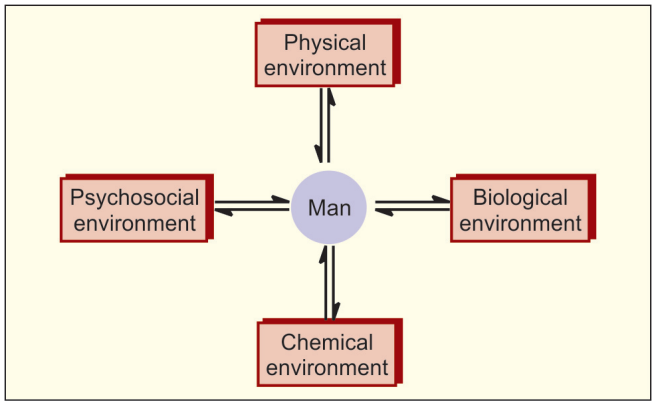


Figure 30.1: Different groups of environment related to man

1. Physical environment
2. Chemical environment
3. Biological environment
4. Psychosocial environment.

Physical Environment

The term physical environment is applied to nonliving things and physical factors, with which man is in constant contact. For example, water, air, soil, heat, noise, climate, radiation, etc.

Chemical Environment

It includes chemical factors affecting health. For example, food pollutants, natural toxins, xenobiotics, etc.

Biological Environment

The biological environment is of living things which surrounds man. For example, viruses, bacteria and other microbial agents, insects, rodents, animals and plants.

Psychosocial Environment

It includes psychosocial factors affecting personal health, health care and community well-being. For example, culture, customs, habits, occupation, income, religion, etc.

ENVIRONMENTAL BIOCHEMISTRY

Changes in environment are tolerated within normal limits and the organism tends to adapt itself to the altered situation. Environmental biochemistry involves environmental pollution, as well as the response of the body to the various factors like temperature, cold, etc.

The metabolic response to environmental factors and the limits of metabolic changes leading to adaptation, constitute environmental biochemistry.

ENVIRONMENTAL POLLUTION

Pollution is defined as the addition of certain substances into the environment (air, water, food and soil) resulting in undesirable and unwanted changes in the environment that are harmful to human beings. The pollutants may be organic, inorganic, biological or physical factors.

We shall discuss water and air pollution and metabolic responses or adaptation to an altered environmental temperature.

Water Pollution

Water intended for human consumption should be free from:

- Harmful chemical substances
- Pathogenic organisms
- Color and odor.

Water is said to be polluted or contaminated when it does not fulfill the above criteria. Pure uncontaminated water does not occur in nature. It contains impurities of various kinds—natural and manmade.

The Natural Impurities

Natural impurities are not essentially dangerous. The natural impurities picked up during rainfall include:

- Dissolved gases, e.g. nitrogen, carbon dioxide, hydrogen, sulfide, etc.
- Dissolved minerals, e.g. salts of calcium, magnesium, sodium, etc.

Manmade Impurities or Pollutants

Manmade impurities which cause water pollution are due to:

- Human activity
- Urbanization
- Industrialization
- Agricultural and domestic wastes.

Water pollutants resulting from these factors could be ***organic, inorganic*** or ***physical substances.***

Organic Water Pollutants

- Sewage contains decomposible organic matter. As a result, microbial population increases and oxygen gets depleted. Normal ***dissolved oxygen (DO)*** content of water is **4–6 ppm.** Decrease in DO content of water indicates pollution due to organic matter.
- Organic wastes are measured by **biochemical oxygen demand (BOD)** or **chemical oxygen demand (COD)**.
- Agricultural pollutants include fertilizers and pesticides run off from agricultural lands. Pesticides that are added to water are:
  - Chlorinated hydrocarbons and their derivatives
  - Herbicides
  - Soil insecticides, etc.

Inorganic Water Pollutants

Industrial wastes contain toxic agents and include:

- Detergent solvents
- Cyanides
- Heavy metals (arsenic, cadmium, lead and mercury)
- Minerals (chromium, fluoride, selenium)
- Organic acids
- Nitrogenous substances
- Bleaching agents
- Dyes
- Pigments
- Sulfides
- Ammonia, etc.

Physical Water Pollutants

Physical water pollutants include radioactive substances generated in:

- Nuclear power plant
- Medical, industrial and research field
- Nuclear weapon.

Effect of Water Pollutants on Human Health

Human health may be affected adversely by the ingestion of polluted water and lead to certain diseases or metabolic impairment. These water related disorders may be classified as follows:

- Biological water borne diseases
- Chemical water borne disorders.

Table 30.1: Biological water-borne diseases caused by infective pathogens and aquatic hosts

	Organism	*Disease*
By infective pathogens	Virus	Viral hepatitis A, Poliomyelitis, Rotavirus diarrhea in infants
	Bacteria	Typhoid, paratyphoid fever, Bacillary dysentery, *E.coli* diarrhea, Cholera
	Protozoa	amebiasis, Giardiasis
	Helminthis	Roundworm, Threadworm
	Leptospira	Weil's disease
By an aquatic hosts	Snail	Schistosomiasis
	Cyclops	Guineaworm, fish tapeworm

Biological water borne diseases

Biological water borne diseases caused by presence of infective pathogenic organisms and due to presence of aquatic hosts are listed in **Table 30.1**.

Chemical water borne disorders

Nitrates: This is a rare occurrence, occur when surface of the water from farmland treated with fertilizer, gains access to the water supply. In infants high nitrate content water is associated with **methemoglobinemia,** that leads to ***cynosis*.**

Fluoride: Mottling of the dental enamel (dental fluorosis). High levels of fluoride cause mottling of the dental enamel. The presence of fluoride at about 1 mg/liter in drinking water has protective effect against dental caries. Excess fluoride causes inhibition of a enzyme enolase in glycolysis.

Cyanide: It causes anoxia by chelating ferric ions in the cytochrome-aa_3 complex within mitochondria, thereby inhibits mitochondrial respiratory chain. Chronic toxicity of cyanide affects functioning of thyroid and nervous system.

Lead: Lead is toxic to both central and peripheral nervous system. Lead inhibits δ-aminolevulinic acid (ALA) dehydratase, δ-ALA synthase, and ferrochelatase enzymes involved in synthesis of porphyrin and heme and leads to anemia.

Arsenic: Arsenic inhibits the sulphydryl groups of enzymes and interferes with cellular metabolism.

- It competes with phosphate for reaction with ADP, resulting in the formation of low energy products.
- It may also cause intravascular hemolysis leading to hemoglobinemia and hemoglobinuria.
- The toxic effects of arsenic lead to dizziness, cramps and paralysis leading to death.

Mercury: Hg being lipid soluble, once it is absorbed it crosses the blood brain barrier and placenta, which is retained by the kidney and brain for many years and causes neurological disorders or death.

Cadmium: Cadmium accumulates primarily in the kidneys. It acts through displacing zinc from the enzymes requiring zinc as a catalytic or structural component. It is a potent uncoupler of oxidative phosphorylation.

Air Pollution

Air is a mechanical mixture of gases. The normal composition of air by volume is approximately as follows:

- Nitrogen: 78.1%
- Oxygen: 20.93%
- Carbon dioxide: 0.03%
- Argon, neon and traces of krypton, xenon, and helium.
- Air also contains water vapor, traces of ammonia and suspended matter such as dust, bacteria, spores, and vegetable debris.

Under ordinary conditions, the composition of outdoor air is remarkably kept constant by certain natural self-cleaning mechanisms such as wind, sunlight, rain and plants.

Definition of Air Pollution

Air pollution is the presence, in the surrounding atmosphere, of excess concentration of certain substances, generated mostly by the activities of man and animal, such as, gases, mixture of gases and particulate matter, which interfere with human health, safety or comfort or which are injurious to vegetation and animals.

Air Pollutants

More than 100 substances are known which pollute air. Air pollutants may be in the form of solids, liquids (vapors) or gases. ***The combination of smoke and fog is called "smog."***

Air pollution is one of the present day health problems throughout the world. About 1.3 billion urban residents worldwide are exposed to air pollution level above recommended limits, which can affect health and social and economic state. Some important pollutants are given below:

- Carbon monoxide
- Carbon dioxide
- Sulfur dioxide
- Photochemical oxidants
- Lead
- Hydrogen sulfide
- Polynuclear aromatic hydrocarbons (PAH)
- Cadmium
- Chlorofluorocarbons (CFCs) and halons (ozone depleting substances)
- Particulate matter.

Causes of Air Pollution

Air is polluted by:

- Respiration of men and animals
- Combustion of coal, gas, oil, etc.
- Decomposition of organic matter
- Trade, traffic and manufacturing process which give off dust, fumes, vapors and gases.

Sources of Air Pollution

The main sources of air pollution are:

- Industries
- Automobiles
- Domestic sources
- Miscellaneous sources.

Industries

Industries discharge into the atmosphere their wastes from chimneys at high temperature and high speed. Combustion of fuel to generate heat and power produces smoke, sulfur dioxide, nitrogen oxides, and fly ash into the atmosphere.

- Petrochemical industries generate hydrogen fluoride, hydrochloric acid and organic halides.
- Many industries discharge carbon dioxide, carbon-monoxide, hydrogen sulfide, sulfur dioxide and ozone.

Automobiles

Motor vehicles emit hydrocarbons, lead, carbon monoxide, nitrogen oxides and particulate matter. In the presence of strong sunlight, certain hydrocarbons and oxides of nitrogen are converted in the atmosphere into photochemical oxidants, which are oxidizing agents.

Domestic sources

Domestic combustion of coal, wood or oil is a source of smoke, sulfur dioxide and nitrogen oxides.

Miscellaneous sources

These include tobacco smoke, burning refuse, incinerators, pesticide spraying, natural sources, e.g. windborne dust, fungi, molds, and bacteria.

Effect of Air Pollution on Human Health

Air pollution mainly affects respiratory system. The effects may be ***immediate*** or ***delayed.***

- Immediate effects result in acute bronchitis. If the air pollution is intense it may result even in death by suffocation.
- The delayed effects result in chronic bronchitis, lung cancer, bronchial asthma, emphysema and respiratory allergies.

Effect of Air Pollution on Social and Economic State

These include destruction of plant and animal life, corrosion of metals, damage of building, cost of cleaning and maintenance and repairs. It also reduces visibility in towns and damages soil and clothing.

Acid Rain

Acid rain results from the reaction of **SO_2** and **NO_2** with water vapor in the air to form nitric acid (HNO_3) and sulfuric acid (H_2SO_4) respectively, which are soluble in water and pollute rivers, streams and soils. They also decrease the fertility of the soils.

Adverse Effect of Some Air Pollutants

Carbon monoxide

Carbon monoxide has high affinity for hemoglobin than oxygen. Carbon monoxide combines with hemoglobin to form carboxy hemoglobin and impairs the functioning of hemoglobin and leads to hypoxia with decreased mental performance.

Carbon dioxide

Carbon dioxide is a natural constituent of the air. This is not commonly regarded as an air pollutant. Excess CO_2 traps heat, **("Greenhouse" effect)** and causes increase in earth's temperature, leading to ***"global warming."*** This in turn leads to melting of polar, icecaps, with increased levels of seas all over the world and low lying towns and cities at the risk of flooding.

Sulfur dioxide

It damages the lung tissue by destroying lung surfactant, dipalmitoyl lecithin, leading to respiratory disorders. Prolonged exposure results in bronchitis and lung cancer. It also causes spotting and burning of leaves, destruction of crops and retarded growth of plants.

Photochemical oxidants (Nitrogen dioxide and hydrocarbons)

Nitrogen dioxide and hydrocarbons, in the presence of sunlight, form complex secondary products, which are oxidizing agents.

They lead to eye and lung irritation. Prolonged exposure causes asthma. They also damage vegetation.

Lead

Discussed earlier in this chapter.

Hydrogen sulfide

Hydrogen sulfide has an unpleasant odor, and can cause conjunctival irritation and show neurological and mental symptoms.

Polynuclear aromatic hydrocarbons (PAHs)

PAHs are a large group of organic compounds. These are carcinogenic substances. They are formed by incomplete combustion of organic materials. There are about 500 PAH in the air. The best known is ***Benzo[a]pyrine (BaP)*** present in the smoke of cigarette. It can cause skin and lung cancer.

Cadmium

It is produced in the steel industries, waste incineration, volcanic action and zinc production. Tobacco contains cadmium and smoking contributes to the uptake of cadmium (Hazards discussed earlier in this chapter).

Chlorofluorocarbons (CFCs) and Halons (ozone depleting substances)

Chlorofluorocarbons (CFCs) are used in refrigerators, air conditioners, perfumes, room fresheners, foam and cushion industries. Halons are used as fire extinguishers. Both CFCs and halons, on leaking into the air, get photodissociated when they reach the upper atmosphere (stratosphere) and the chlorine atoms are released. These chlorine atoms destroy the ozone layer and hence CFCs and halons are known as **"ozone depleting substances".** Ozone is a protective gas which shields the earth from the harmful ultraviolet (UV) rays of the sun, which cause skin disorders.

Particulate matter

It is an airborne mixture of organic and inorganic substances, emitted from a number of sources, may be natural, e.g. dust storms or manmade, e.g. power plants and industrial processes, vehicular traffic, domestic coal burning and industrial incinerators.

Various pulmonary abnormalities occur due to inhalation of:

- Dust particles (pneumoconiosis)
- Silica dust (silicosis)
- Asbestos (asbestosis)
- Cotton dust (byssinosis)
- Sugarcane dust (bagassosis).

METABOLIC RESPONSES OR ADAPTATIONS TO AN ALTERED ENVIRONMENTAL TEMPERATURE

The environmental temperature may be –20°C or + 50°C. In both cases living tissues can function optimally only within a very narrow range of temperature. Therefore, accurate regulation is a great boon. The organism tends to adapt itself to an altered temperature.

HEAT STRESS

Heat stress is the burden or load of heat that must be dissipated if the body is to remain in thermal equilibrium. The factors which influence heat stress are:

- Metabolic rate
- Air temperature
- Humidity
- Air movement
- Radiant temperature.

The amount of heat gained by the body must be equalled by the amount of heat lost from it.

Thermoregulatory Responses

The temperature control system uses three important mechanisms to reduce body heat when body temperature becomes too great.

Vasodilation

The skin blood vessels become intensely dilated and increase the blood flow. Increased blood flow rises the temperature of skin. Thus, full vasodilation can increase the rate of heat transfer to the skin as much as eight-fold.

Sweating

If the environmental temperature exceeds the body temperature, sweating is the major thermoregulatory response.

Decrease in heat production

Mechanisms that cause excess heat production, such as shivering and chemical thermogenesis are inhibited.

Effects of Heat Stress

Heat stroke

This is a condition of failure of the temperature regulatory mechanism when the body temperature rises beyond a critical temperature 105°–108° F.

Symptoms

- The symptoms include dizziness, abdominal distress, sometimes with vomiting, sometimes delirium and eventually unconsciousness if the body temperature is not decreased.
- Because of high temperature, there is heat denaturation of the proteins of the brain, cellular damage of vital organs.
- In fact, even a few minutes of very high body temperature can sometimes be fatal.

Treatment

The treatment consists of reducing the body temperature by immersing the patient in a tub of ice cold water. If that is not possible, the body should be covered with wet ice cold towels and sheets. Cooling should be continued till the body temperature falls below 102°F.

Heat exhaustion

Unlike heat stroke, heat exhaustion is not because of failure of thermoregulation. It is caused by imbalance or inadequate replacement of water and salts lost in perspiration due to thermal stress. Heat exhaustion occurs after several days of exposure to a high temperature. Body temperature may be normal or moderately elevated.

The symptoms are dizziness, weakness and fatigue due to circulatory distress.

Heat cramps

This is a painful condition of limb muscles due to spasmodic contractions of the skeletal muscles. Heat cramps are primarily due to water and salt depletion and can therefore be treated with water and sodium chloride.

Heat syncope

The person standing in the sun becomes pale, his blood pressure falls and he collapses suddenly. Due to dilatation of blood vessels, blood is pooled in lower limbs, with the result that the amount of blood returning to the heart is reduced, which in turn, is responsible for lowering of blood pressure and lack of blood supply to the brain.

The treatment is the patient should be made to lie in the shade with the head slightly down.

COLD STRESS

The ability to survive cold stress lies in the capacity of the body to produce heat. When the body is too cold, the temperature control system operates exactly opposite procedures that occur in heat stress.

Themoregulatory Responses

Skin vasoconstriction

It happens throughout the body.

Piloerection

Piloerection means hairs "standing on end." This upright projection of the hairs allows them to entrap a thick layer of insulatory air, next to the skin, so that transfer of heat of the surroundings is greatly depressed. This is not important in human being, but in lower animals.

Increase in heat production

Heat production by the metabolic systems is increased by promoting:

- Shivering thermogenesis
- Nonshivering or chemical thermogenesis.

Shivering Thermogenesis

This occurs during short periods of exposure of cold. Skeletal muscles play a key role in the production of heat by a process of shivering.

- The shivering center located in the hypothalamus becomes activated when the body temperature falls.
- It then, transmits signals that cause shivering.
- These signals increase the tone of the skeletal muscles throughout the body with production of heat.
- During maximum shivering body heat production can rise to four-to-five times the normal heat production.
- During this phase, the muscle stores of glycogen are used and heat is produced by hydrolysis of ATP.

Nonshivering or Chemical Thermogenesis

This occurs during prolonged exposure to cold. Non-shivering mechanisms involve chemical thermogenesis.

- Cold stimulates the release of norepinephrine, epinephrine and thyroxine.

- Increased levels of these hormones increase the rate of cellular metabolism throughout the body with the increased heat output.
- Norepinephrine and epinephrine can cause an immediate increase in rate of cellular metabolism; but thyroxine requires several weeks of exposure of the body to cold.

The effect of chemical thermogenesis is partially due to the ability of these hormones to uncouple oxidative phosphorylation, and excess food stuffs are oxidized to release energy in the form of heat. This ***type of chemical thermogenesis is directly proportional to the amount of brown fat.*** In adult human beings, who have almost no brown fat, heat production by this chemical thermogenesis is not more than 10–15%. However, in infants who do have a small amount of brown fat, heat production can increase 100%.

Metabolic Adaptations Occur during Chemical Thermogenesis

Various metabolic adaptations, which occur during chemical thermogenesis are:

- Increased food intake.
- The body tries to use all components of the diet for combustion, because thermoregulation is more important than energy balance during cold exposure.
- In addition to carbohydrates and fat, proteins are also utilized as a source of calories.
- The flow of carbohydrate through glycolysis is enhanced by way of activation of enzymes.
- Gluconeogenesis is increased with increased utilization of noncarbohydrate sources of energy.
- Increased activity of citric acid cycle enzymes and electron transport chain components occurs.
- A lipase is activated which releases fatty acids for oxidation. The total free fatty acids in serum come back to normal on acclimatization due to increased oxidation for heat production.
- As a response of cold stress transamination reactions are activated, which stimulate the catabolism of amino acids and carbon skeleton can be used for oxidation.
- Increased arginase facilitates formation and excretion of urea.

Effect of Cold Stress

Hypothermia

It is due to universal lowering of the body temperature as a result of acute exposure to severe cold.

Symptoms: Numbness, loss of sensation, muscular weakness, sleepiness, coma and death.

- If a person is exposed to cold, there is peripheral vasoconstriction resulting in decreased blood supply to the tissues.
- This causes a decrease in oxygen supply to the tissue and accumulation of metabolites, resulting in tissue necrosis.
- Tissue damage occurs because of freezing of intracellular protoplasm and extracellular fluid.

Local cold injury, when it occurs below freezing temperature, is known as **"frostbite,"** i.e. tissues freeze with formation of ice crystals between cells. **"Trench foot"** or **immersion** is an example of local cold injury above freezing temperatures.

SUMMARY

- The term environment implies all the external factors, living and nonliving, material and nonmaterial, which surround man and with which he is in constant interaction.
- The environments have been divided into four groups, physical, chemical, biological and psychosocial environment.
- Environmental biochemistry involves environmental pollution as well as the metabolic responses to environmental factors and the limits of metabolic changes leading to adaptation.
- Environmental pollution is defined as the addition of certain substances into environment resulting in undesirable and unwanted changes in the environment that are harmful to humans.
- Pure uncontaminated water does not occur in nature. It contains natural and man-made impurities, which are due to human activity, urbanization, industrialization, agricultural and domestic wastes.
- Air is polluted by respiration, combustion of coal, gas, oil, etc. decomposition of organic matter, trade, traffic and manufacturing processes which give off dust, fumes, vapors and gases.
- The combination of smoke and fog is called "smog".
- Human health affected by ingestion of polluted water and air leads to certain diseases or metabolic impairment.
- Heat stress is the burden or load of heat that must be dissipated if the body is to remain in thermal equilibrium.
- Thermoregulatory responses of body to reduce body heat are vasodilation, sweating, and decrease in heat production.

- Heat stroke is a condition of failure of the temperature regulatory mechanism.
- When the body is too cold, the temperature control system operates a procedure, which is exactly opposite of the procedures that occur in heat stress, e.g. vasoconstriction, piloerection and increase in heat production by shivering and nonshivering mechanisms.
- Nonshivering mechanisms involve chemical thermogenesis induced by norepinephrine, epinephrine and thyroxine. Various metabolic adaptations occur during chemical thermogenesis.
- Hypothermia is due to universal lowering of the body temperature as a result of acute exposure to severe cold.

EXERCISE

Multiple Choice Questions (MCQs)

1. **All of the following are air pollutants, *except:***
   a) CO_2 b) CO
   c) SO_2 d) H_2S
2. **All of the following are effects of heat stress, *except:***
   a) Heat stroke b) Heat syncope
   c) Heat exhaustion d) Heat coagulation
3. **Greenhouse effect is due to:**
   a) CO_2 b) CO
   c) SO_2 d) O_2
4. **One of the following air pollutants is responsible for acid rain:**
   a) H_2S b) CO
   c) CO_2 d) SO_2
5. **Normal dissolved oxygen (DO) content of water is:**
   a) 10–14 ppm b) 4–6 ppm
   c) 8–10 ppm d) 20–25 ppm
6. **Organic wastes of water are measured by:**
   a) Biochemical or chemical oxygen demand
   b) Centrifugation
   c) Chromatography
   d) Electrophoresis
7. **Ozone depleting substances are:**
   a) Photochemical oxidants
   b) Polynuclear aromatic hydrocarbons (PAH)
   c) Chlorofluorocarbons (CFCs) and halons
   d) Cadmium and lead
8. **Failure of temperature regulatory mechanism causes:**
   a) Heat stroke b) Heat cramps
   c) Heat exhaustion d) Heat syncope
9. **Which of the following for acid rain is not true?**
   a) It results from air pollutants SO_2 and NO_2
   b) It forms nitric acid and sulfuric acid
   c) It decreases fertility of the soil
   d) It results from water pollutants, nitrogen and hydrogen sulfide gas

Correct Answers for MCQs

1-a	2-d	3-a	4-d
5-b	6-a	7-c	8-a
9-d			

CHAPTER

31

Biomedical Waste Management

INTRODUCTION

Health care activities like immunization, diagnostic test, medical treatments and laboratory examinations protect and restore health and save lives. At the same time, however, health services may generate large quantity of wastes and by-products that need to be handled safely and disposed of properly.

The hospital waste, in addition to the risk for patients and personnel who handle these wastes, poses a threat to public health and environment. Proper management of biomedical waste is essential to maintain hygienic, aesthetics, cleanliness and control of environmental pollution.

Historical Background

Public concern about medical waste dates back to early 1980's when large quantities of syringes and needles were found on the beaches of the East coast and in Florida, USA. The public hue and cry due to scare regarding the spread of infectious diseases from this waste led to the first legislation on biomedical waste management in USA. Later, other countries adopted similar legislation to manage their biomedical waste effectively.

The government of India notified the National Bio-Medical Waste (Management and Handling) Rules in July 1998. All the health care facilities in the country are covered under these rules, making it mandatory for such health facilities to manage their waste.

BIOMEDICAL WASTE

Biomedical waste is the waste that is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biologicals. It includes:

- Hospital waste/health care waste includes all the waste generated by health care establishments, research facilities and laboratories including minor or scattered source, such as care taken at home (insulin injection).
- About 75 to 90% of the waste produced by health care providers is non-hazardous **general waste** comparable to domestic waste.
- About 20% of the waste is considered hazardous and/or infectious. If segregation does not take place, all the waste produced should be considered as infectious as it is mixed.

CLASSIFICATION OF HAZARDOUS WASTE

WHO has classified hazardous waste in the following categories:

1. **Infectious waste:** (Suspected to contain pathogens), e.g. laboratory culture, waste from isolation.
2. **Pathological waste:** (Containing human tissue or fluids), e.g. body parts, blood and other body fluids, fetuses.
3. **Sharps:** (Sharp material), e.g. needles, infusion sets, scalpels, knives, blades, broken glass.

4. **Pharmaceutical waste:** (Containing pharmaceuticals), e.g. expired drugs, contaminated bottles, boxes.
5. **Genotoxic waste:** Waste containing cytostatic drugs (drugs used for the treatment of cancer) genotoxic chemicals.
6. **Chemical waste:** (Substance containing chemical substance), e.g. laboratory reagents, film developer, expired disinfectants, solvents.
7. **Waste with heavy metals:** Batteries, broken thermometer.
8. **Pressurized containers:** Gas cylinders, gas cartridges, aerosol cans.
9. **Radioactive material:** (Substances containg radioactive substances), e.g. unused liquid from radiotherapy, contaminated glassware, urine, excreta from patient treated with unsealed radio nucleotides.

Different Locations of Biomedical Waste Generation

Different locations or points of biomedical waste generation are:

- Hospitals: Government/private
- Nursing homes
- Dental clinics
- Dispensaries
- Primary health care centers
- Vaccinating centers
- Laboratories: Clinical/research
- Medical research and training centers
- Blood banks and collection centers
- Mortuaries
- Animal house
- Slaughter houses
- Biotechnology institutions/production unit.

TYPES OF HAZARDS

Exposure to hazardous health care waste can result into:

1. Infection
2. Genotoxicity and cytotoxicity
3. Chemical toxicity
4. Radioactivity hazards
5. Physical injuries
6. Public sensitivity

Infection

The infectious agents can enter in the body through a puncture, abrasion or cut in the skin through mucous membranes by inhalation and ingestion. Commonest infections, which can result from mishandling of hospital/health care wastes are gastroenteric through feces and/or vomit (*Salmonella*, *Shigella spp*, *Vibrio cholerae*, Helminths; Hepatitis A), respiratory through inhaled secretions; saliva (*Mycobacterium tuberculosis*; measles virus; *Streptococcus pneumoniae*), ocular infections through eye secretions (Herpes virus), genital infections (*Neisseria gonnorrheae*; herpes virus), skin infection through pus (*Streptococcus spp*), meningitis through cerebrospinal fluid (*Neisseria meningitidis*), AIDS through blood and sexual secretions (HIV), hemorrhagic fevers through body fluids (Junin, Lassa, Ebola and Marburg viruses), Septicemia and bacteremia through blood (*Staphylococcus aureus*, *Enterococcus*, *Enterobacter*, *Klebsiella* and *Streptococcus*) and Viral Hepatitis B and C through blood and body fluids (hepatitis B and C viruses).

Genotoxicity and Cytotoxicity

Many cytotoxic drugs are extremely irritant and have harmful local effects after direct contact with skin and eyes (alkylating agents; Intercalating agents; vinca alkaloids and derivatives and epipodophyllotoxins). Many neoplastic drugs are carcinogenic and mutagenic; secondary neoplasia is known to be associated with chemotherapy.

Chemical Toxicity

Many of chemicals and pharmaceutical drugs used in health care establishments are hazardous (e.g. toxic, genotoxic, corrosive, flammable, reactive, explosive and shock-sensitive). They may cause intoxication by acute or chronic exposure, injuries including burns, poisoning.

Radioactivity Hazards

The radioactive waste exposure may cause headache, dizziness, vomiting, genotoxicity and tissue damage.

Physical Injuries

May result from sharps, chemicals and explosive agents.

Public Sensitivity

The general public is very sensitive about visual impact of the anatomical waste, recognizable body parts including fetuses, if handled improperly.

BIOMEDICAL WASTE MANAGEMENT PROCESS

- To control the indiscriminate disposal of hospital waste/biomedical waste, the ministry of Environment

and Forest, Government of India, has issued a notification on Biomedical Waste Management under the Environment Protection Act.

- In accordance with these rules, (Rule - 4) it is the duty of every "occupier" (a person who has the control over the institution and/or its premises) generating biomedical waste, to take all steps to ensure that waste generated is handled without any adverse effect to the human health and the environment.
- Every occupier generating the biomedical waste needs to install an appropriate facility in the premises or set up a common facility to ensure requisite treatment of waste in accordance with schedule-I **(Table 31.1).**
- There are various types of biomedical waste. These are categories as mentioned in schedule I **(Table 31.1).** Each type has to be disposed according to its characteristics.

Table 31.1: Categories of biomedical waste

Option	*Waste category*	*Disposal*
Category No. 1	**Human Anatomical Waste** (Human tissues, organs, body parts)	Incineration[@]/deep burial*
Category No. 2	**Animal Waste** (Animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses)	Incineratio[@]/deep burial*
Category No. 3	**Microbiology & Biotechnology Waste** (Wastes from laboratory cultures, stocks or micro-organisms live or vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishes and devices used for transfer of cultures)	Local autoclaving/microwaving/ incineration[@]
Category No. 4	**Waste Sharps** (Needles, syringes, scalpels, blade, glass, etc. that may cause puncture and cuts. This includes both used and unused sharps)	disinfection (chemical treatment [@@]/ autoclaving/microwaving and mutilation/ shredding [# #]
Category No. 5	**Discarded Medicines and Cytotoxic Drugs** (Waste comprising outdated, contaminated discarded medicines)	incineration@/destruction and drugs disposal in secured landfills
Category No. 6	**Soiled Waste** (Items contaminated with blood and body fluids including cotton, dressings, soiled plaster casts, lines, bedding, other material contaminated with blood)	incineration[@] autoclaving/microwaving
Category No. 7	**Solid Waste** (Waste generated from disposal items other than the sharps such a tubings, catheters, intravenous sets, etc.)	disinfection by chemical treatment [@@] autoclaving/microwaving and mutilation/ shredding [# #]
Category No. 8	**Liquid Waste** (Waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities)	disinfection by chemical treatment [@@] and discharge into drains
Category No, 9	**Incineration Ash** (Ash from incineration of any biomedical waste)	disposal in municipal landfill
Category No. 10	**Chemical Waste** (Chemicals used in production of biologicals, chemicals used in disinfection, as insecticides, etc)	chemical treatment[@@] and discharge into drains for liquids and secured landfill for solids

Note:
@ There will be no chemical pretreatment before incineration. Chlorinated plastics shall not be incinerated.
* Deep burial shall be an option available only in towns with population less than five lakhs and in rural areas.
@@ Chemical treatment using at least 1% hypochlorite solution or any other equivalent chemical reagent. It must be ensured that chemical treatment ensures disinfection.
# # Multilation/shredding must be such so as to prevent unauthorized reuse.

Various Steps in Biomedical Waste Management

Various vital steps for safe and scientific management of biomedical waste, are:

- Segregation
- Storage
- Transportation
- Treatment
- Disposal.

Importance of Segregation

As mentioned earlier, only about 20% of the biomedical waste is infectious. If segregation does not take place, all the waste produced should be considered as infectious as it is mixed.

- Segregation of the biomedical waste is the first step in waste management.
- Segregation at source of different types of biomedical wastes and their appropriate storage and/or disinfections, sterilization, etc. would ensure that infectious wastes do not get mixed with non-infectious wastes as this would infect the entire waste.
- Segregation at source makes:
  - It is easier to prevent spread of infection,
  - Makes it easier to choose among the options of disposal
  - Can reduce the load on the incineration and prevent injuries.

How are wastes to be segregated ?

- Infected waste can be segregated into:
  - Dressings
  - Organs and organ parts, placenta, associated body fluids (blood, pus, pleural, or peritoneal fluids, etc.)
  - Broken glassware
  - Intact glassware
  - Needles, razor, blades, scalpel blades, nails, pins, bones, etc.
  - Microbiology waste, non-reusable specimen containers
  - General waste.
- The rules have laid down certain directions regarding segregation and storage to ensure safe and hygienic handling of infectious and non-infectious waste. Among these:
  - No biomedical waste shall be mixed with other wastes
  - Biomedical waste be segregated into container/bags at the point of generation
  - These containers/bags are to be made of different materials and have different **color coding** signifying the different kinds of wastes **(Table 31.2)**

Table 31.2: Categories, color coding and types of container recommended for segregation of biomedical waste by regulations of the Ministry of Environment and Forest, Government of India

Color coding	*Types of container*	*Waste category*	*Treatment as per schedule–I*
Yellow	Plastic bag	Cat–1 Human anatomical waste Cat–2 Animal waste Cat–3 Microbiological waste Cat–6 Soiled waste	Incineration/deep burial
Red	Disinfected container/ plastic bags	Cat–3 Microbiological waste Cat–6 Soilds waste Cat–7 Solid waste	Autoclaving/microwaving/ chemical treatment
Blue/white/ translucent	Plastic bag/puncture proof containers	Cat–4 Waste sharps Cat–7 Plastic disposable tubings, etc.	Autoclaving/microwaving chemical treatment and distraction/shredding
Black	Do	Cat–5 Discarded medicines Cat–9 Incineration ash Cat–10 (Solid) Chemical waste	Disposal in secured landfill

Note:

1. Color coding of waste categories with multiple treatment options as defined in Schedule I, shall be selected depending on the treatment option chosen, which shall be as specified in Schedule I.
2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics.
3. Categories 8 and 10 (liquid) do not require containers/bags.
4. Category 3, if disinfected locally, need not be in containers/bags.

- Segregation is mandatory prior to storage, transportation, treatment and disposal.

Storage of Biomedical Waste after Segregation

- Clinical and general wastes should be segregated at source and placed in **color coded plastic bags** and **containers** of definite specifications prior to collection and disposal **(Table 31.2).**
- The biomedical rules have recommended different color codes for waste containers in which different types of wastes need to be stored **(Table 31.2).**
- The container should comprise an inner plastic bag of varied color depending on the type of waste.
  - It should be of a minimum gauge of 55 micron (if of low density) or 25 micron (if of high density)
  - Leak proof and puncture proof
  - Should match the chosen outer container.
- The outer container is a plastic bin with handles and of a size which will depend on the amount of waste-generated.
- The inner polythene bag should fit into the container with one-fourth of the polythene bag turned over the rim.
- Labeling has been recommended to indicate:
  - The type of waste
  - Site of generation
  - Name of generating hospital or facility.

  This will allow the waste to be traced from the point of generation to the disposal area.
- Bags and containers should be clearly labeled with words **"Biohazard"** and the **Biohazard symbol (Figure 31.1).**

Figure 31.1: The biohazard symbol

- The containers are then to be transported in closed trolleys or wheeled containers that should be designed for easy cleaning and draining.

Transport

- Biomedical waste should be transported within the hospital by means of wheeled trolleys, containers or carts that are not used for any other purpose.
- The trolleys have to be cleaned daily.
- Off site transportation vehicle should be marked with the name and address of carrier.
- Biohazard symbol should be painted.
- Suitable system for securing the load during transport should be ensured.
- Such a vehicle should be easily cleaned with rounded corners.

Care to be taken in storage and transportation

- Untreated biomedical waste shall be transported only in specially designed vehicles.
- No untreated biomedical waste shall be stored beyond a period of 48 hours.
- If for any reasons, it becomes necessary to store the waste beyond such period, permission from the prescribed authority (established by the government of every state and union territory) must be taken and it must be ensured that it does not adversely affect human health and the environment.

Treatment of Biomedical Waste

- If biomedical waste treated in accordance with the following procedures, the waste shall no longer be considered biomedical waste and may be combined and handled with regular solid waste.
- Biomedical waste shall be treated by one of the following methods prior to disposal at a permitted solid waste disposal facility.

Incineration

Incineration is a thermal treatment (high temperature dry oxidation) technology facility that provides complete combustion of waste to render it non-pathogenic.

- Waste types not to be incinerated are:
  - Large amount of reactive chemical wastes
  - Pressurized gas containers
  - Halogenated plastics such as PVC
  - Silver salts and photographic or radiographic wastes
  - Wastes with high mercury or cadmium content such as broken thermometers

- Used batteries
- Lead-lined wooden panels
- Sealed ampules or ampules containing heavy metals.

Wet and dry thermal treatment

- Decontamination by heating with steam under pressure (autoclave) so as to render the biochemical waste non-infectious.
- The process is inappropriate for the treatment of anatomical waste and animal carcasses, and will not efficiently treat chemical and pharmaceutical waste.

Screw-feed technology

- Screw-feed technology is the basis of a non-burn, dry thermal disinfection process in which waste is shredded and heated in a rotating auger.
- This process is suitable for treating infectious waste and sharps but it should not be used to process pathological, cytotoxic or radioactive waste.

Chemical disinfection

- Chemicals such as sodium hypochlorite or chlorine dioxide are added to waste to kill or inactivate the pathogens in it contents, this treatment usually results in disinfection rather than sterilization
- Chemical disinfection is most suitable for treating liquid waste such as blood, urine stools or hospital sewage.

Microwave irradiation

- Most organisms are destroyed by heating the waste at frequency of about 2450 MHz and a wavelength of 12.24 cm by means of microwaves.
- The water contained within the waste is rapidly heated by the microwaves and the infectious components are destroyed by heat.

Land disposal

- There are two types of land disposal:
  - Open dumps landfilling
  - Sanitary landfills.
- Biomedical waste should not be deposited on or around open dumps, because people or animals may come into contact with infectious pathogens.
- **Sanitary landfills:** Sanitary landfills have advantages over open dumps.

Inertization

- Inertization involves mixing biomedical waste with cement and other substances before disposal, in order to minimize the risk of toxic substances contained in the wastes migrating into the surface water or ground water.
- Biomedical waste is mixed in a proportion of 65% pharmaceutical waste 15% lime +15% cement and 5% water.
- A homogeneous mass is formed and cubes are produced on site and then transported to suitable storage site.

Disposal of Biomedical Waste

Biomedical wastes treated in accordance with the regulations shall be properly disposed of at a properly permitted facility.

SUMMARY

- Biomedical waste is the waste that is generated during the diagnosis, treatment or immunization of human beings or animals, or in research activities pertaining thereto, or in the production or testing of biologicals.
- There are various types of biomedical waste. Each type has to be disposed according to its characteristics.
- Various vital steps for safe and scientific management of biomedical waste are: Segregation, storage, transportation, treatment and disposal.
- The biomedical waste needs to be segregated into container/bags at the point of generation in accordance with schedule-II prior to its storage, transportation, treatment and disposal. The container shall be labeled according to schedule-III.
- The biomedical rules have recommended different color codes for waste containers in which different types of wastes need to be stored.
- Untreated biomedical waste shall be transported only in specially designed vehicles. No untreated biomedical waste shall be stored beyond a period of 48 hrs.
- Treated waste shall no longer be considered biomedical waste and may be combined and handled with regular solid waste.

CHAPTER

32

Connective Tissue

INTRODUCTION

As the name implies, connective tissue serves a connecting function. It supports and binds other tissues. Unlike epithelial tissue, connective tissue typically has cells scattered throughout an extracellular matrix. The chief function of connective tissue is to give strength, support and shape to the tissues.

Connective tissue is a system of insoluble protein fibers embedded in a matrix called the ground substance. Connective tissue is widely distributed in the body, the tendons, ligaments, cartilage and matrix of bone.

BASIC COMPONENTS OF CONNECTIVE TISSUE

Connective tissue consists of four major components:

1. Collagen
2. Elastin
3. Proteoglycans
4. Glycoproteins.

COLLAGEN

- Collagen is the main protein of connective tissue. It has great tensile strength and serves to hold cells together.
- Collagen is involved in proper alignment of cells. This, in turn, helps in proliferation and differentiation of cells.

Structure of Collagen

- The basic structural unit of collagen is **tropocollagen,** which consists of three polypeptide chains called **α-chains.** These three polypeptide chains twisted around each other in a **triple helix** forming a rope like structure, which has great tensile strength **(Figure 32.1).**
- The three helically interwind polypeptides are of equal length, each having about 1000 amino acids

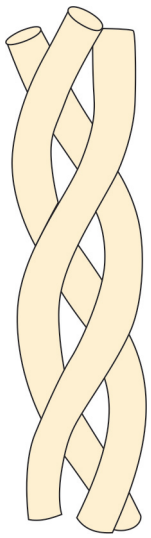


Figure 32.1: Right-handed collagen triple helix formed from three α-chain

residues. The three polypeptide chains are held together by **hydrogen bonds** between chains.

- Multiple types of collagen in human tissues arise from different triple helical combinations of polypeptides. In human tissues, 19 distinct types of collagen have been identified. **Table 32.1** summarizes the most abundant types of collagen found in human tissues.

Table 32.1: Most abundant types of collagen found in human tissues and their distribution

Type of Collagen	*Distribution*
I	Skin, tendon, bone, cornea
II	Articular cartilage, intervertebral disk, vitreous body
III	Fetal skin, cardiovascular system, reticular fibers
IV	Basement membrane
V	Placenta, skin

Structure of α-chain of collagen

- Collagen has an unusual amino acid composition with 33% of the total residues being **glycine (Gly),** 10% **proline (Pro),** 10% **hydroxyproline (Hyp)** and 1% **hydroxylysine (Hyl).**
- Two amino acids that are found in collagen, **hydroxyproline** and **hydroxylysine** are not present in most proteins.
- The primary structure of collagen is unusual in that, collagen has regular arrangement of amino acids in each of the α-chains of the tropocollagen.
- The sequence generally follows the pattern **(Gly-X-Y),** where Gly for glycine and X and Y, for any amino acid residues. Most of the time X is for proline and Y is for hydroxyproline **(Figure 32.2)**.
- Thus, glycine, the smallest amino acid is found in every third position of the polypeptide chain. This is necessary because **glycine is the only amino acid small enough to be accommodated in the limited space available in the central core of the helix (Figure 32.2)**.

Mutation of a single glycine residue in collagen leads to connective tissue disorder which can be lethal, e.g. **osteogenesis imperfecta** (see disorders of connective tissue).

- Collagen is unique in its high content of helix destabilizing amino acids, **proline, hydroxyproline** and **glycine.** These prevent the formation of the usual α-helical and β-pleated structure. Instead, it forms a **triple helical** secondary structure.
- The three α-chains are wound around each other to form a tight, **right handed triple helix**.
- Interchain hydrogen bonding stabilizes the triple helical structure.

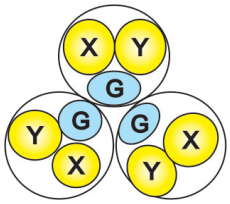


Figure 32.2: Representation of primary structure of α-chain of collagen and cross-section of triple helical structure of collagen, where, G = Glycine, X and Y = Any other amino acid mostly proline and hydroxyproline

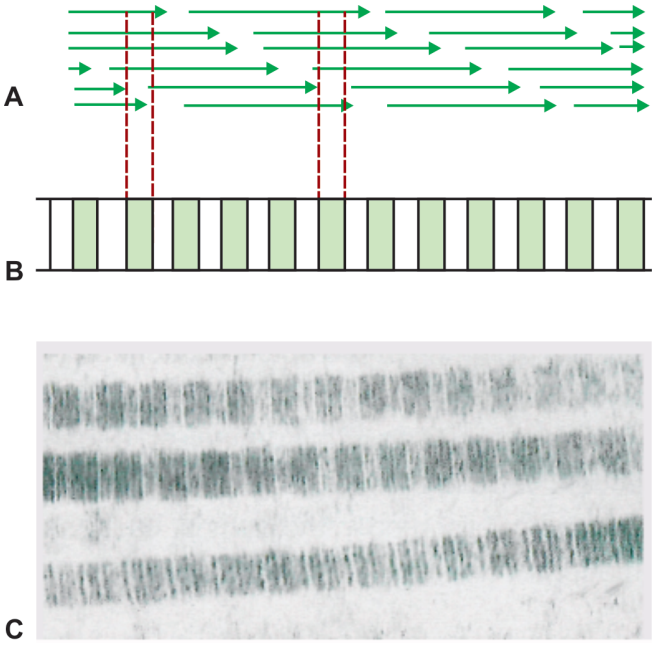
Formation of Collagen Fibrils

- Individual tropocollagen molecules spontaneously laterally aggregate to form fibril. They arrange themselves under physiological conditions into staggered, parallel and overlapping array structures.
- Each tropocollagen molecule overlaps its neighbor by a length approximately three quarters of a molecule. **(Figures 32.3 A to C)**.
- The regularity of gaps and overlaps is responsible for the banded appearance of these fibers in connective tissues.
- These fibrillar array of tropocollagen molecules become connected and subsequently stabilized by intra and intercovalent cross-links through action of **copper requiring enzyme lysyl oxidase.**
- Three types of inter or intramolecular cross-links that stabilize the collagen fibril are **(Figure 32.4):**
  1. Aldol condensation
  2. Schiff base
  3. Lysinonorleucine

  These cross-links are important for the tensil strength of the fibers. Because of the tightness of coiling of triple helix of tropocollagen and its cross-linkages, it has no capacity to stretch.

ELASTIN

- Elastin occurs with collagen in connective tissues. Elastin is a **rubber-like protein,** which can stretch to several times their length and then rapidly return to



Figures 32.3A to C: (A) The quarter staggered, parallel and overlapping array structural arrangement of tropocollagen to form collagen fiber; (B) The regularity of gaps and overlaps generates the banded appearance in the collagen fiber; (C) Electron micrographic banded appearance of fibrillar collagen

their original size and shape when the tension is released.

- Elastin is present in large amounts, particularly in tissues that require these physical properties, e.g. lung, blood vessels and ligaments. Smaller quantities of elastin are also found in skin, ear cartilage and several other tissues.
- Elastin differs from collagen in several properties. **Table 32.2** summarizes the main differences between collagen and elastin.
- In contrast to collagen, there is only one genetic type of elastin.

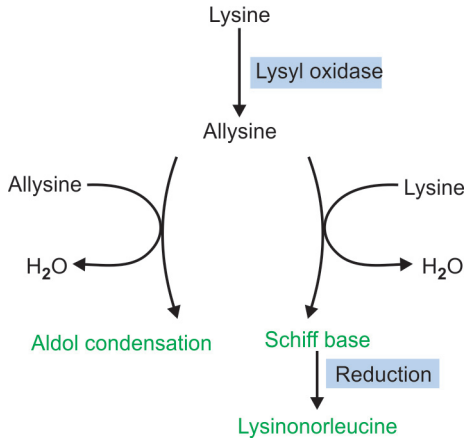


Figure 32.4: Types of cross-links in collagen

Structure of Elastin

- The basic subunit of elastin fibrils is **tropoelastin** which contains about 800 amino acid residues.
- Unlike collagen, elastin does not contain repeat (Gly-X-Y) sequences. Although elastin and collagen contain similarly high amounts of glycine and proline, elastin contains less hydroxyproline and no hydroxylysine.
- Elastin has very high content of **alanine** and other nonpolar aliphatic residue, i.e. **valine, leucine** and **isoleucine.**

Cross-Links of Elastin

- The cross-links in elastin are more complex than those in collagen.
- The major cross-links formed in elastin are the **desmosines,** which is derived from the condensation of three allysine (oxidized form of lysine) residues with lysine.

Table 32.2: Difference between collagen and elastin

Collagen	*Elastin*
Many different genetic type	One genetic type
It has no capacity to stretch	It has capacity to stretch and subsequently to recoil
Primary structure has repeating (Gly-X-Y) sequences	Primary structure has no repeating (Gly-X-Y) sequences
Formation of triple helical secondary structure	No formation of triple helix
Presence of hydroxylysine	No hydroxylysine present
Glycosylated hydroxylysine is present	No glycosylated hydroxylysine present
Formation of intramolecular aldol cross-links	Formation of intramolecular desmosine cross-links

- These cross-links permit the elastin to stretch in two dimensions and subsequently recoil during the performance of its physiologic functions.

Destruction of elastin by **elastase** is normally inhibited by **α-trypsin,** the genetic deficiency of which can result in **emphysema.**

Proteoglycans

- Intracellular ground substance of connective tissue contains **proteoglycans** and **glycoproteins** in which fibrous elements of connective tissues are embedded. They connect cell and other connective elements in the extracellular matrix.
- Proteoglycan is a complex formed by polysaccharide called glycosaminoglycans (about 95%) and protein (about 5%).
- Hyaluronic acid, chondroitin sulfate, keratan sulfate, heparan sulfate and heparin are the major glycosaminoglycans, ***(For details please Refer Chatper 2).***

Glycoproteins

- Glycoproteins are proteins to which oligosaccharides are covalently attached.
- The main function of glycoprotein is to facilitate adhesion between various elements of connective tissue.
- Some glycoproteins present in connective tissue are:
  - Fibrillin
  - Fibronectin
  - Lamin
  - Tenascin.

DISORDERS OF CONNECTIVE TISSUE

Mutations in genes that are responsible for production of collagen can lead to number of disorders. Some of them are discussed below.

Osteogenesis Imperfecta

- Osteogenesis imperfecta, also called **brittle bone syndrome.** This is a congenital disorder caused by multiple genetic defects in the synthesis of **type-I collagen.**
- The disorder is characterized by fragile bones, thin skin, abnormal teeth and weak tendons. The sclerae are often abnormally thin and translucent and may appear blue owing to a deficiency of connective tissue.
- Many of these mutations are single base, substitutions that replace glycine in the (Gly-X-Y) repeat sequence by another bulkier amino acid **cysteine,** preventing the correct folding of the collagen chains into a triple helix and their assembly to form collagen fibrils.

Ehlers-Danlos Syndrome

- Ehlers-Danlos syndrome is a group of inherited disorder of connective tissue.
- This is characterized by hyperextensibility of the skin, hypermobility of the joints, fragile skin and laxity in the musculoskeleton.

Epidermolysis Bullosa

Epidermolysis bullosa is a rare heritable disorder due to mutation in gene encoding collagen. It is characterized by skin breaks and blistering of the skin and epithelial tissue.

Marfan's Syndrome

- Marfan's syndrome is an inherited disease of connective tissue. Abraham Lincoln may have had this condition. Marfan's syndrome is relatively rare genetic disorder caused by mutations in genes coding for **fibrillin,** a glycoprotein, which gives stability to the elastic fibers.
- Mutations in fibrillin affects the eyes, the skeletal system and cardiovascular system.
- Marfan's syndrome is characterized by:
  - Ectopia lentis, i.e. dislocation of the lens (due to weakness of the suspensory ligaments).
  - Tall structure, long arms and legs, hyper-extensibility of the joints.

Scurvy

Scurvy is a nutritional disorder, not a genetic disease caused by a deficiency in ascorbic acid (vitamin C).

Symptoms seen in Scurvy

- Abnormal bone development in infants and children
- Osteoporosis
- Loosening of teeth and swollen bleeding gums
- Poor wound healing
- Easy bruising and bleeding due to fragile capillaries.

Vitamin C plays a role in collagen biosynthesis by acting as a cofactor in the hydroxylation reactions of proline and lysine. Without hydroxylation of lysine and proline, the procollagen that is formed, is unstable and degradable.

Lathyrism

- Lathyrism is a dietary disease due to inhibition of **lysyl oxidase,** an enzyme required for the cross-linking of collagen chains.
- Lathyrism is characterized by deformation of spine, demineralization of bone, dislocation of joints and aortic aneurysm.

SUMMARY

- Connective tissue is a system of insoluble protein fibers embedded in a matrix (ground substance).
- Connective tissue is widely distributed in the body, the tendons, ligaments, cartilage and matrix of bone.
- Connective tissue consists of four major components, i.e. collagen, elastin, proteoglycans and glycoproteins.
- Collagen is the main protein of connective tissue. It has great tensile strength and is present in nearly all organs that serve to hold cells together in discrete units.
- The basic structural unit of collagen is **tropocollagen,** which consists of three polypeptide chains called α-chains in a triple helical form.
- Collagen has regular arrangement of amino acids in each of the α-chains of the tropocollagen. The sequence generally follows the pattern (Gly-X-Y).
- Collagen is unique in its high contents of helix destabilizing amino acids, proline, hydroxyproline and glycine. These prevent the formation of the usual α-helical and β-pleated structure, instead it forms a triple helical structure.
- Three types of inter or intramolecular cross-links that stabilize the collagen fibril are, aldol condensation, schiff base and lysinonorleucine.
- Elastin that occurs with connective tissues is a rubber-like protein. In contrast to collagen, there is only one genetic type of elastin.
- The major cross-links formed in elastin are the desmosines.
- Destruction of elastin by elastase is normally inhibited by α-trypsin, the genetic deficiency of which can result in emphysema.
- Intracellular ground substance of connective tissue contains proteoglycans and glycoproteins.
- Mutations in genes that are responsible for production of collagen can lead to a number of disorders, e.g. osteogenesis imperfecta, Ehlers-Danlos syndrome, epidermolysis bullosa and Marfan's syndrome.
- Scurvy and lathyrism are the dietary disorders of connective tissue.

EXERCISE

Multiple choice questions (MCQs)

1. Basic components of connective tissue are, *except:*
a) Collagen b) Elastin
c) Proteoglycans d) Cholesterol

2. Hydroxylation of proline requires which of the following?
a) Vitamic C b) Fe^{2+}
c) α-ketoglutarate d) All of the above

3. Collagen contents of helix destabilizing amino acid is:
a) Glycine b) Serine
c) Alanine d) Threonine

4. Cross-links present in collagen are, *except:*
a) Aldol condensation b) Schiff base
c) Lysinonorleucine d) Desmosine

5. The basic subunit of elastic fibrils is:
a) Troponin b) Tropoelastin
c) Tensin d) Laminin

6. Which of the following is *not* a genetic disorder of collagen?
a) Marfan's syndrome
b) Osteogenesis imperfecta
c) Scurvy
d) Epidermolysis bullosa

7. A deficiency of copper affects the formation of normal collagen by reducing the activity of:
a) Propyl hydroxylase
b) Lysyl oxidase
c) Lysyl hydroxylase
d) Glucosyl transferase

Correct Answers for MCQs

1-d	2-d	3-a	4-d
5-b	6-c	7-b	

CHAPTER

33

Muscle

INTRODUCTION

Muscle is the major biochemical machine that converts chemical (potential) energy into mechanical (kinetic) energy. Muscle tissue is responsible for movement of the body and its parts and for changes in the size and shape of internal organs.

Muscle tissue is characterized by aggregates of specialized, elongated cells arranged in parallel array, whose primary role is contraction.

CLASSIFICATION OF MUSCLE

Muscle is classified on the basis of the appearance of the contractile cells. Two principal types of muscle are:

Striated Muscle

Striated muscles are those in which cells appear striated upon microscopic observation. Striated muscle tissue is further subclassified on the basis of its location as follows:

- Skeletal muscle which is attached to bone.
- Cardiac muscle which is found in the wall of the heart.

Smooth Muscle

Smooth muscle, in which cells are nonstriated.

STRUCTURE OF SKELETAL MUSCLE (FIGURE 33.1)

- The cells of skeletal muscle are cylindrical in shape. Each cell is commonly called **muscle fiber.**
- Each muscle fiber is **multinucleated (syncytical)** cell. Each cell is surrounded by an electrically excitable plasma membrane, the **sarcolemma.**
- Each of the muscle fibers are made of many myofibrils, held together by connective tissue.
- Myofibrils are composed of bundles of myofilaments.
- Myofilaments consist of **thick filament** and **thin filament.**
  - The thick filament consists of protein **myosin.**
  - The thin filament consists of protein **actin, tropomyosin** and **troponin.**
- Myofilaments are the actual contractile elements of striated muscle.
- When myofibril is examined by electron microscope alternating dark A (anisotropic) bands and light I (isotropic) bands can be observed.
- The central region of the A-band termed the **H-band,** is less dense than the rest of the band (in German, Hell means light).
- The I (light) band is bisected by a very dense, narrow **Z-line** or **Z-disks.** The Z-line is made of the protein **α-actinin** and **desmin.**

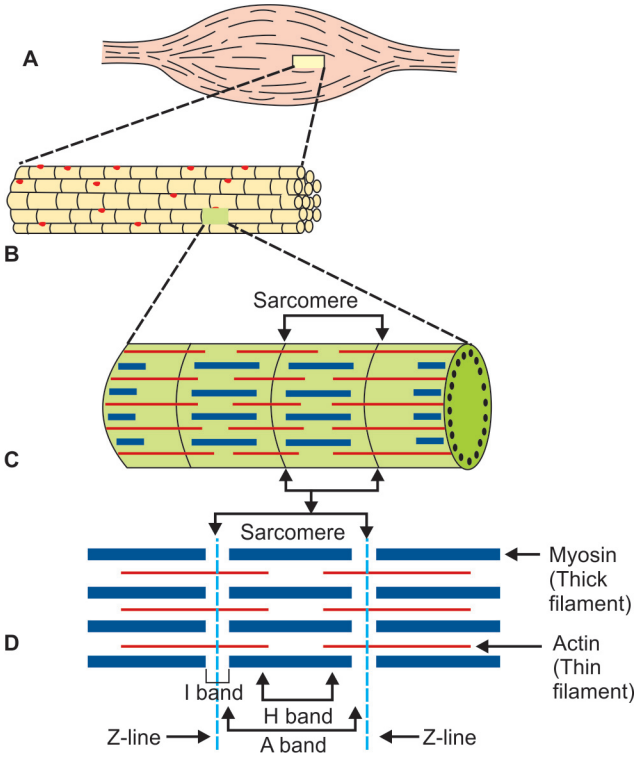


Figure 33.1: Structure of muscle fiber
A: Skeletal muscle;
B: Muscle fiber composed of myofibrils;
C: Myofibril composed of myofilaments,
D: Arrangement of myofilaments in striated muscle

- The portion of the myofibril that lies between two successive Z-line is called **sarcomere,** i.e. sarcomere, is a Z-line to Z-line repeat **(Figure 33.1)** which repeats every 2.3 mm (2300 Å) along the fibril axis.
- **Sarcomere represents the smallest functional unit of myofibril.** It is the basic contractile unit of striated muscle.
- The thick and thin filaments of sarcomere do not change length or width during muscle contraction.
- During muscle contraction, the thick and thin filaments overlap each other. Consequently, the H bands and I bands shorten **(Figure 33.2)**.
- The length of the sarcomere which is 2300 nm in an extended form of myofibril is reduced to 1500 nm in a contracted form.

Protein Composition of Muscle Fibers

Muscle contains following types of proteins:

1. Contractile proteins
   i. Myosin
   ii. Actin.
2. Regulatory proteins
   i. Tropomyosin
   ii. Troponin.
3. Minor or accessory proteins.

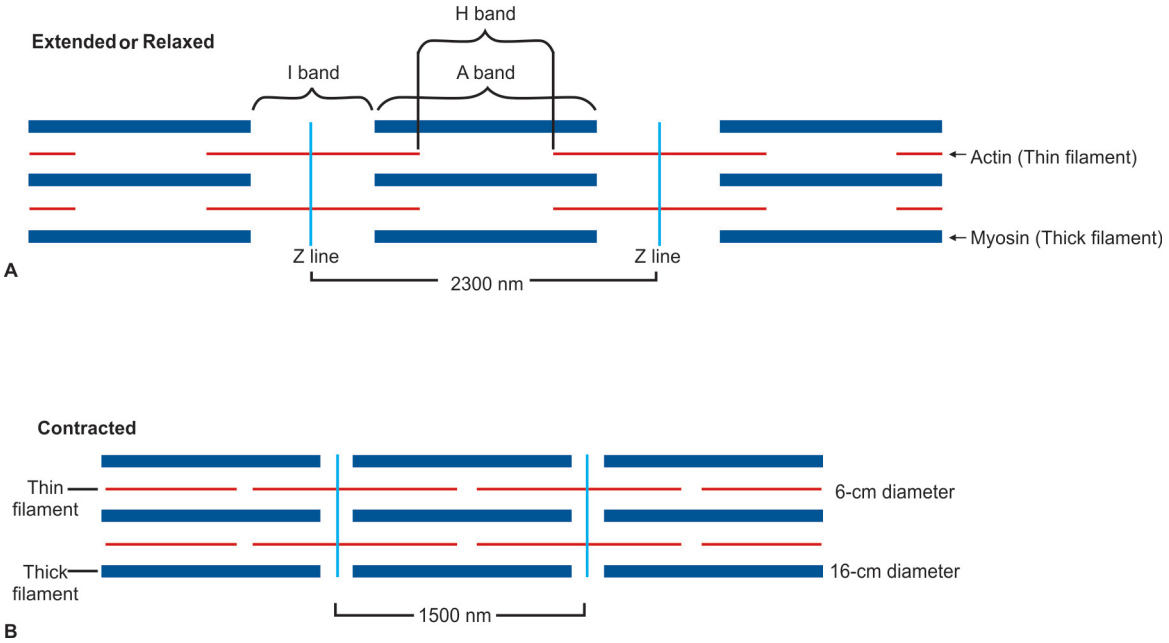


Figure 33.2: Arrangement of muscle filaments
where A: Relaxed or extended position; B: Contracted position

Contractile Protein

Myosin

- Each myosin molecule is composed of six polypeptide chains, two identical **heavy chains** and four **light chains.** Light chains are of two types **(Figures 33.3A and B)**.
- Two identical heavy chains are wound around each other to form a double helix, which is called the **tail of the myosin** molecule.
- The amino terminal end of each of these chains is folded into a globular structure called the **myosin head.** Thus, there are two free heads lying side-by-side at one end of the double helix myosin molecule.
- One each type of light chain is associated with each of the myosin heavy chain heads. These light chains help to control the function of the head during muscle contraction.
- Globular head of myosin has two specific binding sites, one for ATP and one for actin. lt also exhibits ATPase activity which can hydrolyze ATP to ADP and Pi.
- Tails of the myosin molecules bundled together to form the thick filament, while many heads of the myosin molecule hang outward to the sides of the thick filament except H-region, where there are no myosin heads.

Functions of myosin

Myosin has three important functions as follows:

- Constituent of thick filament of muscle fiber.
- The amino terminal globular ends of myosin exhibit **adenosine triphosphatase (ATPase)** activity. It hydrolyzes ATP to ADP + Pi and provides free energy for muscle contraction.
- Myosin interacts with actin and generates the force (power stroke) that moves the thick and thin filament past each other.

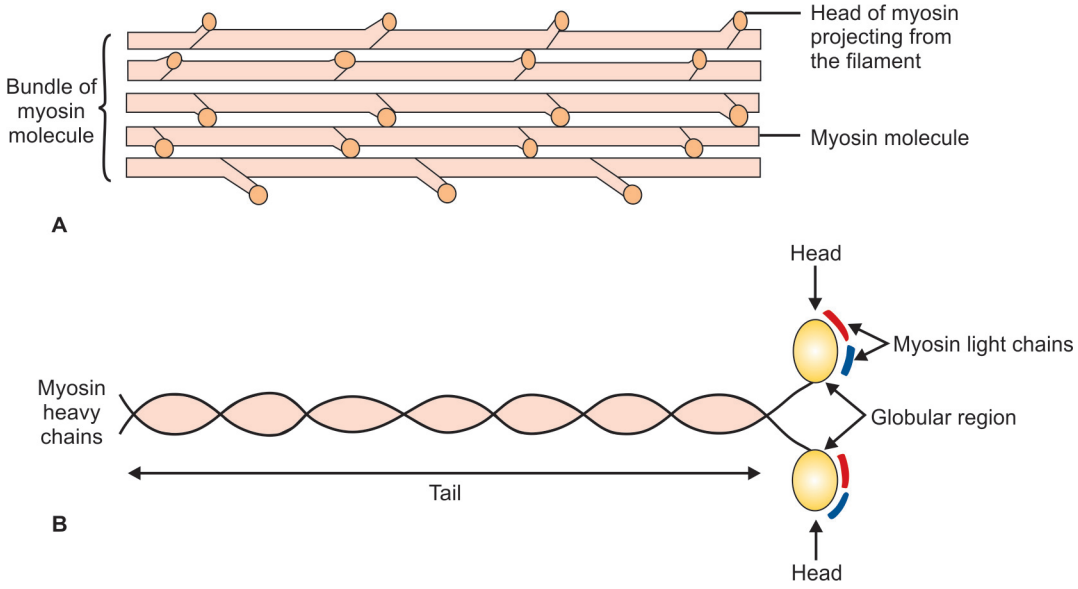
Actin

- Actin is the major constituent of thin filament of muscle fiber.
- Actin is a polymer of globular-shaped subunit called **G-actin**.
- In presence of Mg^{2+} ions, G-actin polymerizes spontaneously into a fibrous or filamentous form called F-actin **(Figure 33.4)**.
- Each G-actin molecule of the thin filament has a binding site for myosin.
- In association with myosin, actin plays a major role in muscle contraction (discussed later).

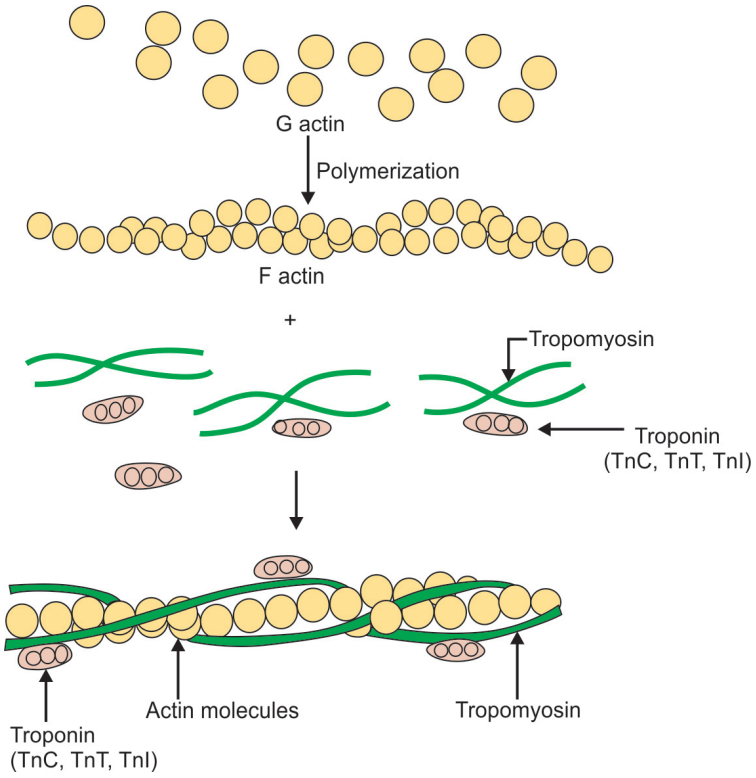
Regulatory Proteins

Tropomyosin

- Tropomyosin is a constituent of thin filaments of the muscle.



Figures 33.3A and B: (A) The thick filament consists of bundles of myosin molecules; (B) Schematic structure of myosin molecule



- Tropomyosin is made up of two polypeptide chains, which is wrapped spirally arround the sides of the F-actin helix **(Figure 33.4)**.
- In the resting state, the tropomyosin molecules lie on top of the active sites of the actin strands, so that attraction cannot occur between actin and myosin filaments to cause contraction.
- Tropomyosin is involved in the contraction process by regulating the attachment of actin and myosin.

Troponin

- Troponin is also a constituent of thin filaments of the muscle.
- Troponin is a complex of three polypeptide chain, **TnC, TnI** and **TnT,** which is attached to tropomyosin **(Figure 33.4)** and each of which plays a specific role in controlling muscle contraction as follows:

  TnC (Troponin C): It binds calcium ions, the essential step in the initiation of muscle contraction.
  TnI (Troponin I): It binds to actin and inhibits actin-myosin attachment.
  TnT (Troponin T): It binds to tropomyosin, anchoring the troponin complex.

Minor or Accessory Proteins of Myofibril

- There are a large number of other proteins that are involved in stabilizing the structure and function of muscle are called minor or accessory proteins. These include
  - α-actinin
  - Cap-Z
  - Titin
  - Nebulin
  - Desmin
  - Dystrophin.

MECHANISM OF MUSCLE CONTRACTION

"Sliding filament model", is the most accepted model of muscle contraction proposed in 1954. According to this mechanism, the thin filaments slide past the thick filaments during contraction so that the total length of the fiber is shortened. Although the length of the sarcomere decreased during muscular contraction, the lengths of the individual thick and thin filaments did not change but the H-zones and I-bands shortened **(Figure 33.2).**

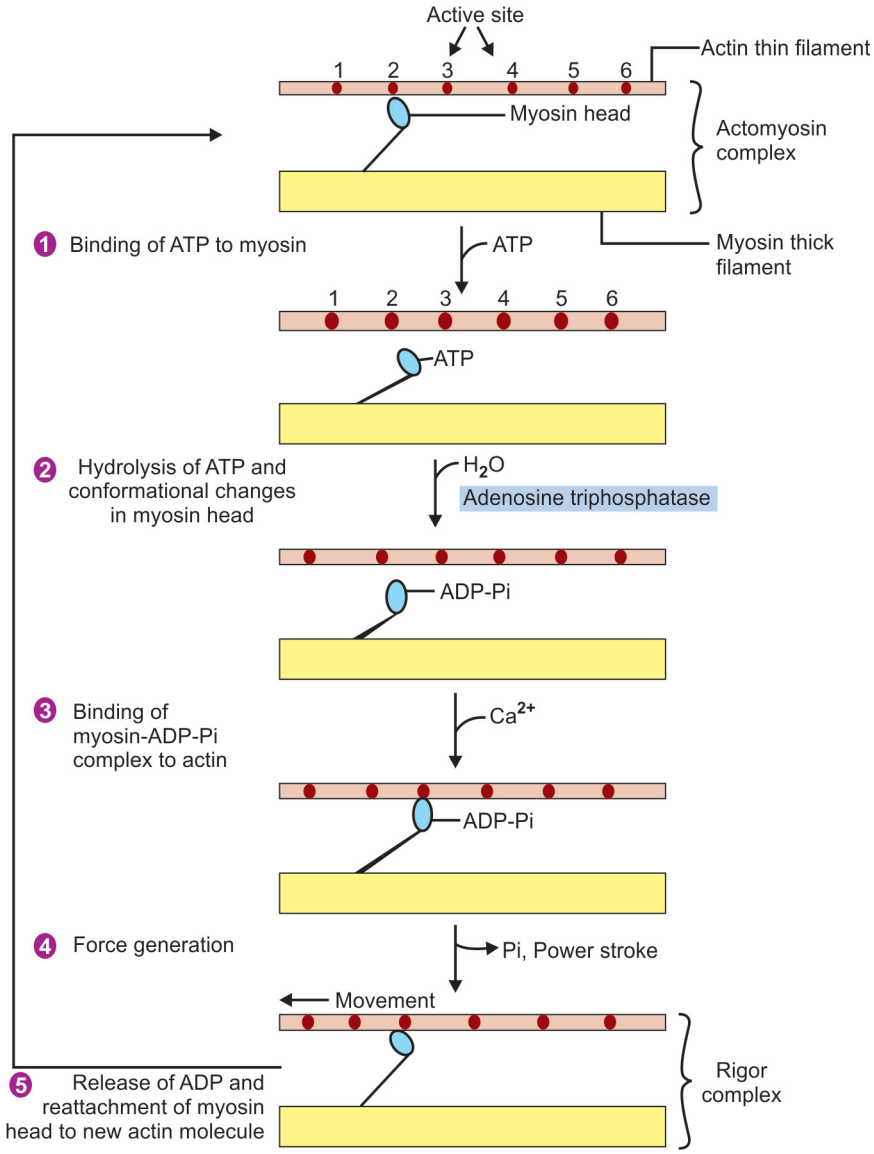


Figure 33.4: Proteins of thin filament

Biochemical Events Occurring During Muscle Contraction

Each contraction cycle consists of five stages as follows **(Figure 33.5):**

1. **Binding of ATP to head of myosin** of thick filaments and detachment of myosin from actin (thin filament).
2. **Conformational changes** in the myosin head as a result of hydrolysis of ATP to ADP+Pi.
3. **Binding of myosin-ADP-Pi** complex to actin.
4. **Force generation** in which myosin head releases inorganic phosphate and power stroke occurs.
5. **Reattachment of myosin head** to the new actin molecule of the thin filament and formation of rigor complex **(Figure 33.5)**.

Stage 1

- In the first stage, ATP binds to the myosin head of the thick filament which is tightly bound to the actin molecule of the thin filament.

- Binding of ATP dissociates actomyosin into actin and myosin.
- Binding of ATP induces conformational changes in the myosin head. This change reduces the affinity of the myosin head for the actin molecule of the thin filament causing the myosin head to detach from the thin filament.

Stage 2

- Adenosine triphosphatase (ATPase) of the myosin hydrolyzes ATP to ADP and Pi and remain bound to myosin head.
- The energy released by the splitting of ATP is stored in the myosin molecule.
- Myosin in the form of **myosin ADP-Pi complex** is now in a high energy state. This is the predominant state at **rest, i.e. relaxed state**.

Stage 3

- Upon muscle stimulation via calcium, the inhibition of actin-myosin attachment imposed by the regulatory proteins (tropomyosin and troponin) is removed and consequently the myosin with bound ADP and Pi attaches to actin.
- It is believed that the angle of myosin head attachment is 90°.

Stage 4

- The actin-myosin reassociation stimulates the release of inorganic phosphate (Pi) from myosin head; which results in increasing the strength of the myosin-actin attachment.
- This tight binding of myosin to actin results in a conformational change in myosin head, tilting the angle of head from 90° to 45°.
- This change in myosin head, causes, the generation of force called the **'Power Stroke'** of muscle contraction which pulls the thin filament a distance about 70°A (10 nm) towards the center the sarcomere.

Stage 5

- The myosin head is again tightly bound with release of ADP to a new actin molecule of the thin filament which is known as **rigor configuration**.
- The actin-myosin complex remains intact until another molecule of ATP binds to the head of myosin molecule.
- With a new ATP, a new cycle may begin and the cycling may continue as long as regulatory calcium and ATP are present.

Rigor Mortis

- At the beginning of the contraction cycle, the myosin head is tightly bound to the actin molecule of the thin filament and ATP is absent. This arrangement is known as **rigor configuration.**
- ATP is needed for the detachment of myosin from the actin during muscle contraction. In case of ATP depletion, the cycle is arrested and actin does not dissociate and relaxation does not occur.
- When actin and myosin are permanently bound in the absence of ATP, the muscle becomes rigid and stiff. The muscular rigidity and stiffening that begins at the moment of death due to lack of ATP is known as **rigor mortis.**

Source of Energy in Muscle Contraction

- The immediate source of energy in muscle for contraction is ATP. ATP can be generated from the following ways:
  - By substrate level phosphorylation of glycolysis using glucose or muscle glycogen
  - By oxidative phosphorylation
  - From creatin phosphate.

MUSCLE DISORDERS

Muscular Dystrophy

- Muscular dystrophies are a group of genetic conditions characterized by progressive muscle weakness and wasting without involvement of nervous system.
- This is an X-linked disorder and caused by mutations in the gene coding for the protein dystrophin.
- The different types of muscular dystrophy affect different sets of muscles and result in different degrees of muscle weakness.
- The commonest and best characterized types are:
  - Duchenne muscular dystrophy (DMD)
  - Becker muscular dystrophy (BMD).
- Duchenne and Becker types of muscular dystrophy primarily affect the skeletal muscles, which are used for movement, and the muscles of the heart.

SUMMARY

- Muscle transduces chemical energy into mechanical energy.
- The sarcomere is the functional unit of muscle.

- Thick filaments contain myosin, thin filaments contain actin, troponin and tropomyosin.
- The sliding filament model is the most accepted model of muscle contraction. According to this mechanism, the thin filaments slide the thick filaments during contraction, so that the total length of the fiber is shortened without changing the lengths of the individual thick and thin filaments.
- The hydrolysis of ATP is used to drive movement of the filaments.
- Ca^{2+} plays a key role in the initiation of muscle contraction by binding to troponin C.
- Muscular dystrophy is due to mutations in the gene, located on the X-chromosome, encoding the protein dystrophin.

EXERCISE

Multiple Choice Questions (MCQs)

1. **The primary components of thin filaments include which of the following?**
   a) Myosin
   b) Tropomyosin and myosin
   c) Actin, tropomyosin and troponin
   d) None of the above

2. **Which of the following does *not* change length during muscle contraction?**
   a) Sarcomere
   b) I band
   c) A band
   d) H-zone

3. **The following proteins are involved in muscle contraction, *except:***
   a) Myosin
   b) Myoglobin
   c) Actin
   d) α-Actinin

4. **Which of the following acts as source of energy in muscle contraction?**
   a) Blood glucose
   b) Oxidative phosphorylation
   c) Creatin phosphate
   d) All of the above

5. **Which of the following is true for sarcomere?**
   a) It is the functional unit of muscle
   b) It consists of thick filament myosin
   c) It consists of thin filament actin
   d) All the above

Correct Answers for MCQs

1-c 2-c 3-b 4-d 5-d

CHAPTER

34

Neurotransmitters

INTRODUCTION

Neurotransmitter is a chemical substance or chemical messenger (within brain) that is secreted by neurons and that allow communication between nerve cells to produce physiological response such as **muscle contraction**.

OVERVIEW OF THE NERVE CELL

- **The neuron:** The neuron is the structural and functional unit of the nervous system **(Figure 34.1).**
- Neurons do not divide, they must last for a lifetime.
- Neurons function by the production, propagation and transfer of nerve impulses.
- The functional components of a neuron consists of:
  - The cell body (perikaryon) having the nucleus (karyon) and those organelles that maintain the cell
  - The processes extending from the cell body which consists of **axon** and **dendrites**.
- Axon, usually the longest process, which transmits impulses away from the cell body to other neurons or effector (target) cells such as muscle cell or gland (secretory cells).
- Dendrites, usually the shorter processes that transmit impulses from the periphery (i.e. from other neuron) towards the cell body.

Synapses

- A synapse is a site of functional contact between neurons that facilitate transmission of impulses from one (presynaptic) neuron to another (postsynaptic) neuron or effector (target) cells such as muscle and gland cells. Thus, a synapse is a site of neuron-to-neuron or neuron-to-effector cell communication.

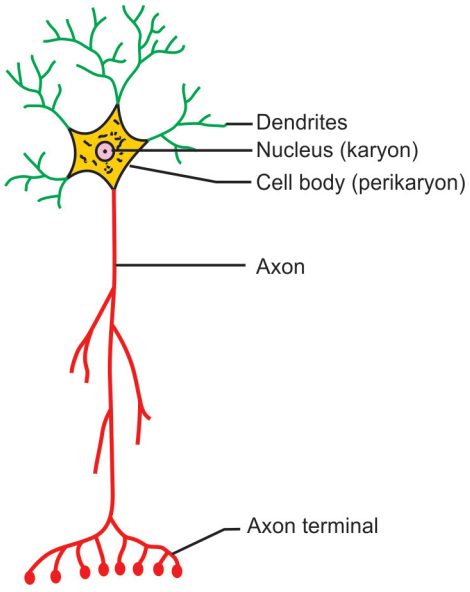


Figure 34.1: Structure of typical neuron

- A typical synapse contains:
  - presynaptic bouton or knob
  - synaptic cleft
  - postsynaptic membrane.

Presynaptic Bouton or Knob

- The end of a neuro process is terminated in a bulb like end called a **bouton** or **presynaptic knob** from which neurotransmitters are released.
- It consists of **synaptic vesicles** in which neurotransmitters are stored **(Figure 34.2)**.

Synaptic Cleft

- There is no actual continuity between one neuron and another, a minute gap exists between them.
- The space that separates one neuron from another neuron or effector (target) cell, which the transmitter must cross is called **synaptic cleft (Figure 34.2)**.

Postsynaptic Membrane

It is a portion of the plasma membrane of the postsynaptic neuron which contains receptor sites with which the neurotransmitter interacts **(Figure 34.2)**.

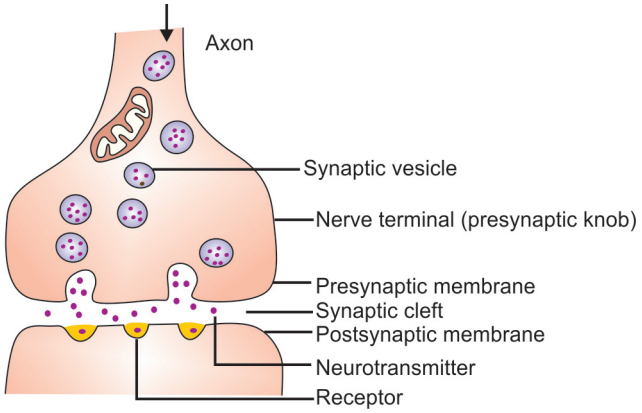


Figure 34.2: Structure of typical synapse

CLASSIFICATION OF NEUROTRANSMITTERS

A classification of neurotransmitters based on chemical composition is given in **Table 34.1**. All are synthesized at the nerve ending and packaged into vesicles there.

MECHANISM OF RELEASE OF NEUROTRANSMITTERS

- Various stimuli (physical, chemical or electrical) activate neurons from their resting state and cause them to produce nerve impulses.
- At rest, a neuron maintains a difference in potential (i.e. voltage) of about –50 to –80 mV between the inside and outside of its surface membrane. Thus the cell surface is polarized in respect to the cell interior.
- When a nerve impulse reaches the bouton, the voltage reversal across the membrane produced by the impulse (called depolarization) causes Ca^{2+} channels to open in the plasma membrane of the bouton.
- The influx of Ca^{2+} from the extracellular space causes the synaptic vesicles to migrate to, and fuse with the presynaptic membrane, thereby releasing the neurotransmitter into the synaptic cleft by exocytosis.
- The neurotransmitter then diffuses across the synaptic cleft and binds to specific receptors on the postsynaptic membrane causing Na^+ channels in that membrane to open and allowing Na^+ to enter the neuron.
- Influx of Na^+ causes local depolarization in the postsynaptic membrane and thereby generating a new action potential or nerve impulse.
- The release of neurotransmitter by the presynaptic bouton can cause either **excitation** or **inhibition** at postsynaptic membrane.

Table 34.1: Classification of most common neurotransmitters and their site of synthesis

Class	*Example*	*Derived from*	*Site of synthesis*
Amine	Epinephrine	Tyrosine	Adrenal medulla, some CNS cells
	Norepinephrine	Tyrosine	CNS, sympathetic nerves
	Dopamine	Tyrosine	CNS
	Serotonin (5-Hydroxytryptamine)	Tryptophan	CNS, chromaffin cells
Amino acid derivatives	Histamine	Histidine	Hypothalamus
	GABA	Glutamate	CNS
Miscellaneous	Acetylcholine	Choline	Parasympathetic nerves, CNS

Excitatory Neurotransmitters

Excitatory neurotransmitters like acetylcholine and serotonine open **cation** channels, prompting an influx of Na^+ that causes local depolarization in the postsynaptic membrane. This leads to initiation of an action potential and generation of a nerve impulse.

Inhibitory Neurotransmitters

- Inhibitory neurotransmitters like GABA open anion channels causing Cl^- to enter the cell and hyperpolarize the postsynaptic membrane, making it even more negative so that generation of an action potential (i.e. making membrane depolarized) becomes more difficult and inhibit the production of new impulse.
- Each CNS neuron, in general, receives thousands of synapse, some excitatory and some inhibitory. Whether a given neuron will generate an impulse or not depends on the summation of the excitatory and inhibitory transmitters acting upon its surface at any particular time.

REGULATION OF ACTION OF NEUROTRANSMITTERS

- When neurotransmitters serve their function, they must be removed from the synaptic space.
- The binding of neurotransmitter to receptor appears to be reversible, so that bound transmitter dissociates from its receptors when the local transmitter concentration falls.
- The most common process of removal of the neurotransmitter after its release into the synaptic cleft is by **high affinity reuptake machanism**.
- In high affinity reuptake mechanism neurotransmitters are re-incorporated into the presynaptic vesicles by endocytosis and are available for recycling.
- About 80% of the released neurotransmitters are removed by this mechanism. Remaining 20% of the neurotransmitters are degraded by the enzymes associated with the postsynaptic membrane. For example, acetylcholinesterase (AChE) degrades acetylcholine into acetate and choline, catechol-o-methyltransferase (COMT) and monoamine oxidase (MAO) degrade norepinephrine.
- The degradation or reuptake of neurotransmitters is necessary to limit the duration of stimulation or inhibition of the postsynaptic membrane.

DIFFERENT COMMON NEUROTRANSMITTERS

Acetylcholine

- The first chemical neurotransmitter identified was acetylcholine. It is a neurotransmitter between axons and striated muscle at the neuromuscular junction (contact made by the terminal branches of the axon with muscle).
- Neurons that synthesize and release acetylcholine are termed **cholinergic neurons**.
- Acetylcholine is synthesized in neuronal cytoplasm from choline and acetyl-CoA through the action of choline acetyltransferase **(Figure 34.3)**.
- Acetylcholine is then incorporated into synaptic vesicles and stored therein.

Release and action

- Release of acetylcholine in response to an action potential is Ca^{2+} dependent, as explained above in the mechanism of release of neurotransmitter.
- The released acetylcholine diffuses rapidly across the synaptic cleft to its receptors on the postsynaptic membrane (muscle membrane) causing opening of the Na^+ channels in the receptor that permits a flux of cations across the membrane.
- The consequent entry of Na^+ results in depolarization of the muscle membrane and action potential generated and transmitted along the fiber, resulting in contraction of the muscle.

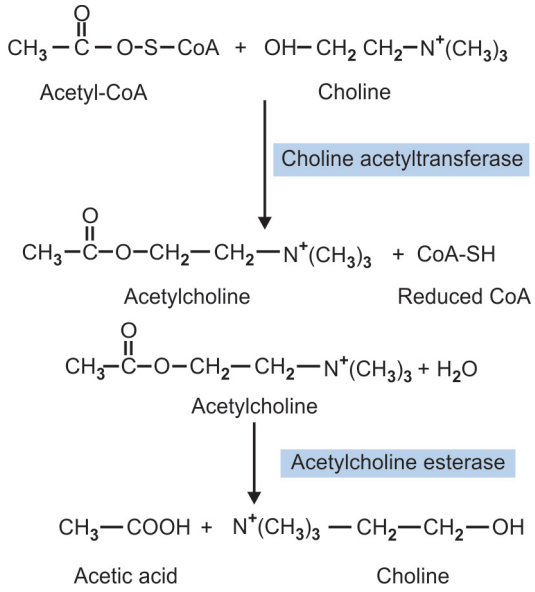


Figure 34.3: Synthesis and hydrolysis of acetylcholine

Catecholamines

- The principal catecholamines are:
  - Norepinephrine (noradrenaline)
  - Epinephrine (adrenaline)
  - Dopamine.

Synthesis

- These neurotransmitters are synthesized from phenylalanine and tyrosine **(Figure 14.19)**.
- Tyrosine is produced in the liver from phenylalanine through the action of phenylalanine hydroxylase.
- Tyrosine is then transported to catecholamine secreting neurons where a series of reactions convert it to dopamine, to norepinephrine and finally to epinephrine.

Dopamine is a major transmitter in nerves that control voluntary movement. Damage to these nerves causes **Parkinson's disease** which is characterized by tremor and difficulties in initiating and controlling movement.

Storage and release

- After synthesis, catecholamines are stored in synaptic vesicles.
- Catecholamines undergo Ca^{2+} dependent release from synaptic vesicles in response to action potentials.
- After action, the catecholamine dissociates from its receptor quickly, causing the duration of the biological response to be brief.

Functions of catecholamines

- Catecholamines exhibit peripheral nervous system excitatory and inhibitory effects as well as respiratory stimulation in the CNS.
- The excitatory effects are exerted upon:
  - Smooth muscle cells of the vessels that supply blood to the skin and mucous membranes.
  - Heart, which lead to an increase in heart rate and force of contraction.
- Inhibitory effects, by contrast, are exerted upon smooth muscle cells in the wall of the gut, the bronchial tree of the lungs, and vessels that supply blood to skeletal muscle.
- In addition to their effects as neurotransmitters, norepinephrine and epinephrine can influence the rate of metabolism by increasing the rate of glycogenolysis and fatty acid mobilization.
- In the periphery, dopamine causes vasodilatation and it is therefore used clinically to stimulate renal blood flow, and is important in the treatment of renal failure.
- Dopamine found in limbic systems of the brain are involved in emotional responses and memory.

Serotonin or 5-Hydroxytryptamine

- Serotonin also called **5-Hydroxytryptamine (5-HT),** is derived from **tryptophan** ***(See Figure 14.23).***
- Neurons that secrete 5-HT are termed serotonergic neurons.

Storage and release

- Serotonin is stored in vesicles in the axon terminals of these neurons.
- Serotonin undergoes Ca^{2+} dependent release from synaptic vesicles.

- The degradation of serotonin results in the formation of 5-hydroxyindoleacetic acid (5-HIAA). This is a useful marker of excess production of serotonin in diseases such as **carcinoid syndrome.**

Functions

- Serotonin is involved with a wide range of functions such as platelet aggregation, and smooth muscle contraction, appetite, mood, hormonal balance, sleep/wake cycles, alertness, sexual behavior and temperature control.

GABA (Gamma Aminobutyric Acid)

GABA is an amino acid derivative and is the most abundant inhibitor in the brian. It balances the brain by inhibiting overexcitation.

Synthesis

- GABA is synthesized from glutamate **(Figure 34.4)** and stored in vesicles in axon terminals.

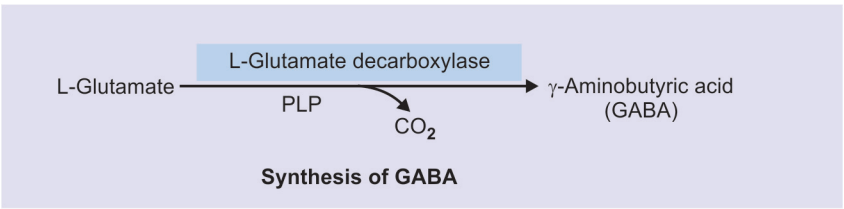


Figure 34.4: Synthesis of GABA

- Glutamate decarboxylase is present in many nerve endings of the brain as well as in the β-cells of the pancreas.

Receptors and action

- Neurons that secrete GABA are called **GABAergic neurons.** GABA exerts its effects by binding to receptors.
- Binding of GABA to receptors increases the Cl^- conductance from outside to inside the neuron creating hyperpolarization and decreases nerve excitability.

Functions of GABA

- GABA contributes to motor control, vision and many other cortical functions. Anxiety is also regulated by GABA. **Some drugs that increase the level of GABA in the brain are used to treat epilepsy** and **Huntington's** disease.
- GABA also stimulates the anterior pituitary, leading to higher levels of human growth hormone (HGH) which contributes significantly to muscle growth and also **prevents the formation of fat cells.**

Histamine

- Histamine is found mainly in the hypothalamus. It is synthesized from histidine by the reaction catalyzed by **histidine decarboxylase,** a pyridoxal phosphate containing enzyme **(Figure 34.5)**.
- Histamine is released from synaptic vesicles by exocytosis and interacts with its receptors.

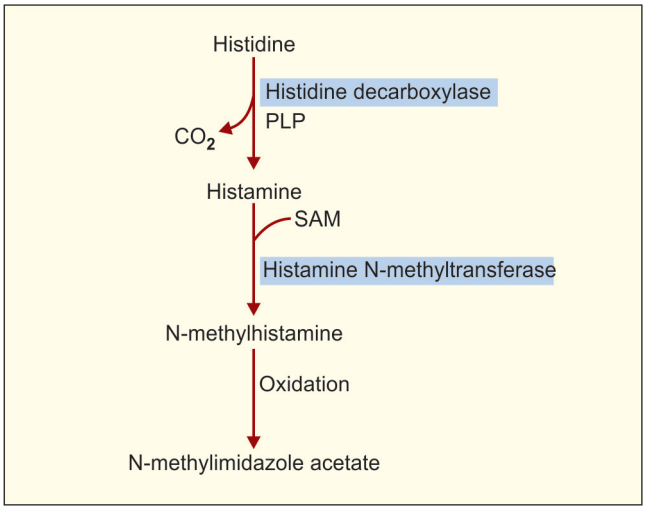


Figure 34.5: Histidine metabolism

- There are three classes of histamine receptors denoted H_1, H_2 and H_3.

Functions

- Histamine has been shown to control the release of pituitary hormones and play a role in sleep-wake cycles and food intake.

Degradation

- After its action, it is inactivated metabolically. In contrast to other neurotransmitters, a high affinity Na^+ dependent transport system does not exist in brain. In brain, histamine undergoes methylation followed by oxidation to methylimidazole acetate **(Figure 34.5)**.

SUMMARY

- Neurotransmitter is a chemical substance or chemical messenger within brain that is secreted by neurons and that allows communication between nerve cells to produce physiological response.
- Neurotransmitters are classified on the basis of their chemical nature as, amines, amino acid derivatives and miscellaneous.
- Neurotransmitters are of two types: excitatory, e.g. acetylcholine, serotonin, etc. and inhibitory, e.g. GABA.
- The degradation or reuptake of neurotransmitters is necessary to limit the duration of stimulation or inhibition of the postsynaptic membrane.
- The first chemical neurotransmitter identified was acetylcholine.
- The principal catecholamine are norepinephrine, epinephrine and dopamine are neurotransmitters, synthesized from tyrosine.
- Serotonin called 5-hydroxytryptamine (5-HT) is derived from tryptophan involved in a wide range of functions.
- GABA is an amino acid derivative which is the most abundant inhibitor in the brain.
- Histamine mainly found in the hypothalamus, synthesized from histidine.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Which of the following is an inhibitory neurotransmitter?**
   a) GABA b) Dopamine
   c) Epinephrine d) Histamine

2. Following are the amine neurotransmitters, *except:*

a) Histamine b) Norepinephrine
c) Dopamine d) Acetylcholine

3. Neurotransmitter serotonin is derived from:

a) Tryptophan b) Tyrosine
c) Histidine d) Glutamine

4. Which of the following neurotransmitters is a catecholamine?

a) Serotonin b) GABA
c) Histamine d) Dopamine

5. Which of the following is not a neurotransmitter?

a) GABA b) Serotonin
c) Histamine d) Serine

Correct Answers for MCQs

1-a 2-d 3-a 4-d
5-d

CHAPTER

35

Laboratory Investigation Techniques

INTRODUCTION

The clinical biochemistry laboratory measures chemical changes in the body for **diagnosis, therapy** and **prognosis of disease.** The primary work of its technologists is the assay of various chemical constituents in blood, urine and other fluids or tissues.

The test accuracy is a prerequisite for the proper interpretation of a laboratory test for which a knowledge of basic principles of laboratory instruments is essential. Accordingly, this chapter summarizes some of the basic principles common to the most biochemistry laboratory methods and essential to good laboratory practice and technique.

CHROMATOGRAPHY

Definition

Chromatography is the process for separation of components in a solution by differences in migration rate as the solution mixture (mobile phase) is passed over or through a stationary phase.

The separation may make use of one or more of the following physicochemical principles, depending upon the particular chromatographic system **(Table 35.1).**

Table 35.1: Physicochemical principle used for chromatographic separations

Type of chromatography	*Physicochemical principle*
Gel filtration	Size and shape
Adsorption chromatography	Adsorption
Partition chromatography	Solubility
Ion exchange chromatography	Ionization

Applications of Chromatography Technique

- The chromatographic technique is used for the separation of amino acids, proteins and carbohydrates.
- Purification of proteins, enzymes, nucleic acids, immunoglobulins and membrane receptors.
- Determination of molecular weight of proteins.
- Analysis of drugs, hormones vitamins and brain amines.
- Qualitative and quantitative analysis of complex mixtures.
- In the clinical laboratory for the identification of sugars, amino acids and drugs in serum or urine.

Classification of Chromatography

- Chromatographic methods are generally classified according to the physical state of the mobile phase

(Figure 35.1). This is further subclassified according to how the stationary phase is contained for a particular chromatographic method.

Paper Chromatography

Principle of paper chromatography

- Paper chromatography is a partition type of chromatography.
- Partition chromatography is a process whereby the solutes of a sample are separated by differences in their relative solubilities between two liquid phases.
- The separation depends on the relative tendencies of the molecule in a mixture to associate more strongly with one or the other phases.
- In paper chromatography, Whatman filter paper No. 3 serves as a solid support to hold the stationary phase.
- Solvent system provides both stationary phase and mobile phase.
- For amino acid chromatography, butanol: acetic acid: water in the proportion of 4:1:5 v/v is used as solvent system.
- **Butanol with a little acetic acid** acts as the mobile phase and **water and acetic** acid forms the stationary phase, which will be held on the paper.

Technique for paper chromatography

- A Whatman filter paper No. 3 of appropriate size is soaked in solvent mixture in a tray, which absorbs aqueous part of solvent mixture.
- The sample which is to be separated is then spotted near the lower edge of filter paper along with known standards with the help of fine capillary.
- Keep the paper in the chamber dipping the end of the paper close to spots inside the solvent as shown in **Figure 35.2.**
- Solvent mixture is then allowed to run almost to the upper edge of paper.
- The paper is then removed, dried and sprayed with appropriate staining solution **(Figure 35.3)**.
- The colored spots seen are identified on the basis of R_f values, which are specific for different substances.
- The ratio of fronts termed R_f, which is given by:

$$R_f \text{ (ratio of fronts)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

- For quantitation, the spots may be cut out and eluted, i.e. dissolves in suitable solvent and intensity of color is measured on photoelectric colorimeter.

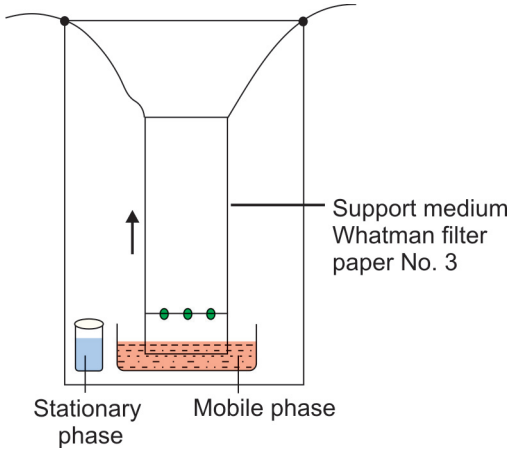


Figure 35.2: Ascending paper chromatography

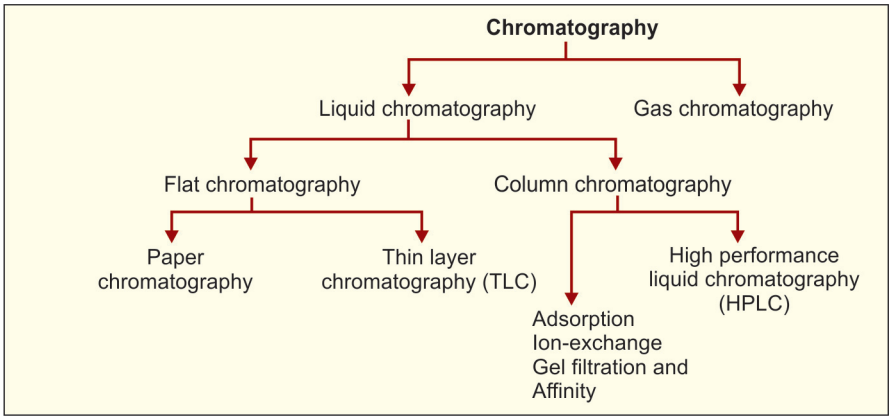


Figure 35.1: Classification of chromatography according to the mobile phase

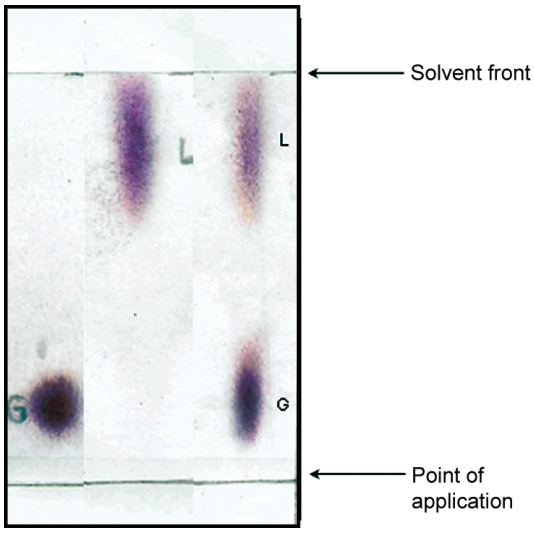


Figure 35.3: Paper chromatogram of amino acids where, G: Glycine, L: Leucine

Application of paper chromatography

Paper chromatography is used for detection of **amino acids, sugars, pigments,** etc.

Clinical application of paper chromatography

Identification of type of amino aciduria, e.g. cystinuria, phenylketonuria, etc.

High Performance Liquid Chromatography (HPLC)

- HPLC is a highly sensitive and popular technique which can be applied to resolution of different types of compounds and drugs available in very small quantities and can be performed in a short time.
- In HPLC, a high pressure pump is used to force the solvent and sample through a relatively short and narrow-bore, tightly packed column of an adsorbent to give constant flow rate. Pressure of about 1000 to 3000 psi are frequently used in routine separations.
- As the separated components elute from the column, they pass through the detector (UV detector) where they are detected.

Applications

The method is applicable for the separation of carbohydrates, proteins, peptides, amino acids, vitamins, steroids, amines like biogenic amines and the polyamines, neuropeptides, hormones and drugs.

ELECTROPHORESIS

A charged particle placed in an electric field migrates towards the **anode** or **cathode,** depending on the net charge carried by the particle **(Figure 35.4).** Application of electric field to solution of ions makes ions to move.

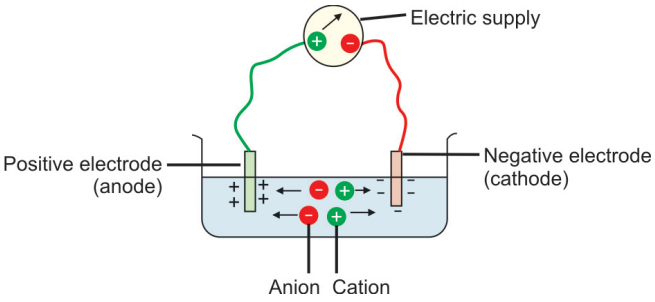


Figure 35.4: Application of electric field to solution of ions makes ions to move

Definition

Electrophoresis is the process of separating the **charged constituents** of a solution by means of an electric current.

Electrophoresis Apparatus (Figure 35.5)

- The electrophoresis apparatus is designed so that the circuit between the two poles is bridged by the support medium holding the sample, and the current flow is partially carried by the components of the sample.
- An electrophoresis chamber consists of two compartments separated from each other by a dividing wall, one side contains the **anode** and the other, the **cathode**.

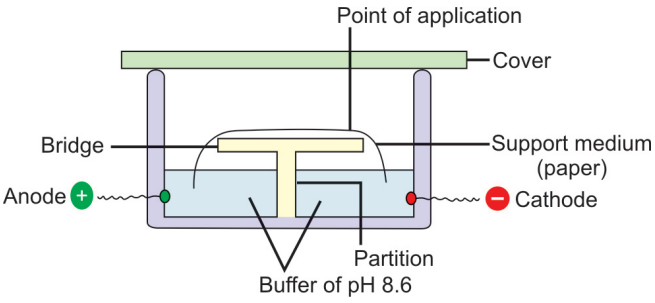


Figure 35.5: Electrophoresis apparatus

- Each side is filled to the same level with a buffer (barbital buffer, pH 8.6) is often used for serum protein separation.
- A "bridge", across the top of the dividing wall holds a membrane or other support material so that each end of it is in contact with the buffer in one of the compartments.
- First membrane is immersed in buffer, blotted and placed in the chamber, and then sample is applied.
- When a voltage is applied to the cell, the current is carried across the porous membrane by the buffer ions.
- At pH 8.6, all the serum proteins carry a net negative charge and tend to migrate towards the anode.
  - **Albumin** carries the largest charge and therefore, moves the **fastest**.
  - The **γ-globulins** have the smallest net charge and move the least distance ***(See Figure 5.1)***.

Classification of Electrophoresis

Depending upon the mode of operation and separation, electrophoresis is classified into various types as shown in **Figure 35.6**.

Clinical Applications of Electrophoresis

- The electrophoresis procedure is used in clinical laboratory for separation of serum proteins, lipoproteins, isoenzymes, hemoglobin and other classes of macromolecules.
- Immuno-electrophoresis is used to determine specific classes of immunoglobulins.
- Western blot technique to identify a specific protein used to confirm the presence of antibodies to human immunodeficiency virus (HIV) is based on electrophoretic principle.
- Southern blot techniques to identify specific nucleic acid sequences (DNA or RNA) used for prenatal diagnosis of inborn errors, diagnosis of viral infections and identification of risk factors for cancer is also based on electrophoretic principle.

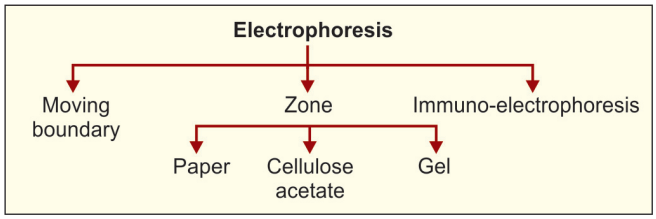


Figure 35.6: Classification of electrophoresis

COLORIMETRY

- The most widely used method for determining the concentration of biochemical compounds is **colorimetry,** which makes use of the following principle.

Principle

- When white light passes through a colored solution, some specific wavelengths of light are absorbed which is related to color intensity. The color intensity will be proportional to the concentration of the chemical responsible for producing the color.

Components of the Colorimeter (Figure 35.7)

- **A lamp (light source):** A lamp provides light in visible region of the spectrum. Usually, tungsten lamp is the source of light.
- **Adjustable slit:** The light emerging from tungston lamp is allowed to pass through a narrow adjustable slit.
- **Condensing lense:** Provides parallel beam of light.
- **Filter:** Filter provides the desired monochromatic light (of single wavelength) by filtering other wavelengths. The color of the filter is complementary to the color of the solution **(Table 35.2).** This allows only appropriate wavelength of light to pass through the colored solution.
- **Cuvette (sample holder):** A special glass tube, which holds the solution to be analyzed in a colorimeter, is called cuvette. Cuvette should have uniform thickness, inner diameter and refractive index. Cuvettes usually have 1 cm light path.
- **Photodetector:** This produces a current in response to the light impinging upon it.
- **Galvanometer readout device**: This measures electric current generated by the photocell to optical density or % transmittance.
- The measurement of color intensity of a colored solution by colorimetry is governed by two laws:
  - Beer's law
  - Lambert's law.

Table 35.2: Complementary colors for selection of filters

Filter	*Color of solution*
Blue	Red
Purple	Green
Yellow	Violet
Orange	Blue green

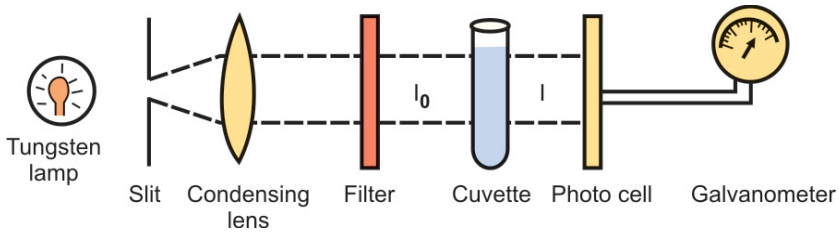


Figure 35.7: Basic components of colorimeter

Beer's Law

- When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as concentration of the light absorbing material increases.
- If **A** is the light absorbed (absorbance) and **C** is the concentration of the solution, then

$$A \propto C$$

Lambert's Law

When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the light path through light absorbing material increases. If L be the length through which light passes, then

$$A \propto L$$

Calculations

Percent concentration of the test =

$$\frac{\text{OD of Test} - \text{OD of Blank}}{\text{OD of Standard} - \text{OD of Blank}} \times \frac{\text{Concentration of std.}}{\text{Volume of test sample}} \times 100$$

Applications of Colorimeter

Colorimetric procedures are widely used in hospital and laboratory for the estimation of various biochemical compounds in various biological samples like blood, plasma, serum, cerebrospinal fluid (CSF), urine and other body fluids.

The biochemical compounds such as glucose, urea, creatinine, uric acid, bilirubin, lipids, total proteins, and enzymes like AST, ALT, ATP, minerals like calcium, phosphorus, etc. are routinely estimated by colorimeter.

FLAME PHOTOMETRY

Flame photometry is a device for measuring the concentration of **alkali metals like Na, K** and **Li** by measuring the intensities of light emitted by the same metals when their solutions are sprayed into a gas flame.

Principle

When sufficient heat energy is supplied by a gas flame, atoms of alkali metals like Na, K and Li become excited, i.e. the electrons at the ground state of such atoms become excited and attain a higher energy state. These electrons while coming back to their original ground state, emit energy in the form of photons of light of characteristic wavelength unique to each alkali metal. This emitted light passes through the suitable filter to isolate it from unwanted light. The emited light then falls on a photocell, generating current which is measured by galvanometer.

Components of the flame photometer (Figure 35.8)

The basic components of the flame photometer are:

An aspirator/nebulizer/automizer

The solution under analysis is sucked in by an aspirator system, consisting of a fine capillary tube, which dips into it. The nebulizer produces a fine spray of droplets of uniform size necessary for constant emission of light. The droplets are then driven to the flame of the gas burner.

Burner and flame

Flame provides heat energy. The heat of the flame evaporates the water and vaporizes the substance which is converted into atomic state. The heat energy also excites the electron in the ground state and moves it into higher energy state.

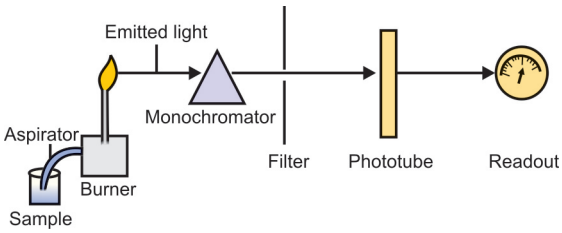


Figure 35.8: Basic components of flame photometer

Monochromator/filter

Monochromatic light is obtained by means of filter. The light of particular wavelength emitted by electrons passes through the suitable filter, e.g.

- For sodium 580 nm (orange), filter is used
- For potassium 766 nm (red), filter is used.

Phototube/detector

A detector is a phototube which converts light energy into electrical energy.

Readout/galvanometer

The electrical energy generated by detector is measured by galvanometer.

Technique

- The solution which is to be analyzed is sprayed as a fine mist of droplets on the flame, which decomposes the compound into atoms.
- The electrons of free atoms absorb heat energy and get excited.
- When they return to ground state they emit characteristic light. This emitted light passes through the suitable filter to isolate it from unwanted light.
- The emitted light then falls on a photocell, generating current which is measured by galvanometer.

Application of flame photometer

- This special technique of emission of flame photometry is widely used in the clinical laboratory to determine the concentrations of sodium and potassium in biological fluids like serum, urine and sweat.
- The serum lithium levels can also be measured in connection with the therapeutic use of lithium salts in the treatment of psychiatric disorders.

IMMUNOCHEMICAL TECHNIQUES (RIA AND ELISA)

- The immunochemical techniques are available to detect or quantitate the **antigen** or **antibody.** They are:
  1. Radioimmunoassay (RIA)
  2. Enzyme-linked immunosorbent assay (ELISA).
- RIA and ELISA are used to measure hormones, drugs, tumor markers, proteins and antigens in biological sample.
- Any immunoassay technique involves the reactions between an antigen and its specific antibody.

Radioimmunoassay (RIA)

Yalow and Berson devised a radioimmunoassay (RIA) for the measurement of the insulin concentration in plasma that was far more sensitive and specific than any method in existence at that time. Now, it is possible to determine by RIA methods the concentration of nearly all of the hormones as well as many drugs, proteins and other compounds.

This immunoassay technique utilizes radioactive isotopes to label antigen. The most commonly used label is ^{125}I, ^{3}H and ^{14}C.

Disadvantages of Radioimmunoassay

The use of radioisotopes as label is associated with the following problems:

- Health hazard due to exposure to radiation.
- Waste disposal is costly and inconvenient.
- Short shelf-life of labeled reagents because of radioactive decay.
- High cost of equipments and reagents.
- Long duration of assay.

Applications of Radioimmunoassay

Variety of compounds of serum or other biological fluids present in very small amount can be estimated, e.g.

- Almost all hormones
- Tumor markers like prostate specific antigen (PSA), Alpha-fetoprotein (AFP), etc.
- Vitamins
- Drugs.

ELISA (Enzyme-Linked Immunosorbent Assay or Enzyme Immunoassay)

ELISA is a **nonisotopic** immunoassay to overcome the disadvantages of using radioactivity, enzymes can be attached to the analyte and used in place of the radioactive antigen.

Technique

The technique of enzyme immunoassay is similar in principle to that of radioimmunoassay except that the antigen is labelled with a stable enzyme **(Table 35.3)** instead of a radioisotope compound.

Table 35.3: Enzyme labels for immunoassays

Enzyme	*Source*	*Specific activity (Units/mg)**
Alkaline phosphatase	Calf intestine	400
β-Galactosidase	*E. coli*	400
Glucose oxidase	*Aspergillus niger*	200
Glucose-6-phosphate dehydrogenase	Leuconstoc mesenteroides	250
Peroxidase	Horseradish	900

* A unit of enzyme activity represents the conversion of 1 μmol of enzyme substrate to product per minute.

Applications of ELISA

- ELISA is used in clinical laboratory to measure:
  - All hormones in the serum
  - Tumor markers in the serum, e.g. PSA, AFP, HCG, CEA, etc.
  - Antibodies in the serum in infectious diseases, e.g. antiviral, antibodies and antibacterial antibodies.
  - Autoantibodies, e.g. anti-DNA, ANA (anti-nuclear antibody).
- ELISA is used in the study of infectious diseases like defection of bacterial toxins, viruses, hepatitis B surface antigens, etc.

Difference between RIA and ELISA

- ELISA is nonisotopic technique
- Lacks radiological hazards of RIA
- Reagents have more shelf-life compared to RIA
- ELISA is cheaper than RIA
- Suitable for use even in small laboratories.

SUMMARY

- Chromatography is a process for separation of components of a mixture based on differential distribution between two immiscible phases—stationary phase and mobile phase.
- The chromatographic technique is used for the separation of amino acids, proteins, carbohydrates, hormones, drugs, vitamins and brain amines. It is also useful for the determination of molecular weight of proteins.
- Electrophoresis is a technique used to separate a mixture of charged particles by migration under the influence of an electric field. Many important molecules like peptides, proteins, nucleotides, nucleo-proteins, etc. possess ionizable groups, due to which they can act as cations or anions at a given pH and migrate towards oppositely charged electrode.
- In colorimetric technique, the concentration of compounds is determined by measuring the intensity of color. The intensity of color is proportional to the concentration of the compound being measured.
- Colorimetry is used for the estimation of various biochemical compounds in various biological samples like blood, plasma, serum, cerebrospinal fluid (CSF), urine and other body fluids.
- The special technique of emission flame photometry is widely used in the clinical laboratory to determine the concentrations of sodium and potassium in biological fluids like serum, urine and sweat. The serum levels of lithium can also be measured in connection with the therapeutic use of lithium salts in the treatment of some psychiatric disorders.
- The immunochemical techniques RIA and ELISA are used to measure hormone drugs, tumor markers, proteins and antigens in biological sample.
- RIA technique utilizes radioactive isotopes to label antigen.
- ELISA is a nonisotopic immunoassay to overcome the disadvantages of using radioactivity, enzymes can be attached to the antigen and used in place of radioactive antigen.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Which of the following chromatographic techniques is based on molecular size?**
   a) Gel filtration chromatography
   b) Ion exchange chromatography
   c) Paper chromatography
   d) Affinity chromatography

2. **Which of the following is used as a label in ELISA?**
   a) Antibody b) Antigen
   c) Enzyme d) Radioisotope

3. **The pH of the buffer used for separation of serum proteins by electrophoresis on agar gel is:**
   a) 10.01 b) 9.6
   c) 5.6 d) 8.6

4. **Which of the following physicochemical principles is used for chromatographic separation?**
   a) Adsorption
   b) Partition
   c) Difference in molecular size
   d) All of the above

Correct Answers for MCQs

1-a 2-c 3-d 4-d

Index

Page numbers with *f* and *t* indicate *figure* and *table*, respectively

B

C

D

E

F

G

H

Q

R

S

T