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3rd Edition

Textbook of BIOCHEMISTRY

for Dental Students



DM Vasudevan
Sreekumari S
Kannan Vaidyanathan

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Textbook of
BIOCHEMISTRY
for Dental Students

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Third Edition

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Textbook of Biochemistry for Dental Students

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With humility and reverence,
this book is dedicated
at the lotus feet of the Holy Mother
Sri Mata Amritanandamayi Devi

"Today's world needs people who express goodness in their words and deeds. If such noble role models set the example for their fellow beings, the darkness prevailing in today's society will be dispelled, and the light of peace and non-violence will once again illumine this earth. Let us work together towards this goal."

– *Mata Amritanandamayi Devi*

Preface to the Third Edition

We are glad to present this third edition of the *Textbook of Biochemistry for Dental Students*. Many medical colleges and universities in India have accepted the first and second editions of this book as one of the standard textbooks. With humility, we may state that the medical community of India has warmly received the previous editions of this book. In retrospect, it gives immense satisfaction to note that this book served the students and faculty for the past few years. This book is prepared according to the syllabi (modified in 2007) prepared by the Dental Council of India. We have also consulted the syllabi of dental courses of various universities.

In this third edition, we have deleted some extra details shown in the second edition. At the same time, we have added a few more contents in different chapters, as it is necessary to keep the pace of the advance of science. Many figures and tables have been improved. In this third edition, important points are made into bold letters, so that students can grasp the subject easily. This book now gives the "must know points". Those who are interested to get more facts may consult our *Textbook of Biochemistry for Medical Students*, now in 8th edition. A question bank, given at the end of the book, contains essay questions and short note questions, which are compiled from the last 10 years' question papers of various universities. These questions will be ideal for students for last-minute preparation for examinations.

The help and assistance rendered by our students in preparing this book is enormous. Web pictures, without copyright protection, were used in some figures. The remarkable success of the book was due to the active support of the publishers.

This is to record our appreciation for the cooperation extended by Shri Jitendar P Vij (Group Chairman), Mr Ankit Vij (Group President) of M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India, and their associates.

We hope that this third edition will be more student friendly and more attractive to the teachers. Now this is in your hands to judge.

"End of all knowledge must be building up of character"—*Gandhiji*.

DM Vasudevan
Sreekumari S
Kannan Vaidyanathan

Preface to the First Edition

The medical community of India has warmly received the *Textbook of Biochemistry for Medical Students* edited by us. It was originally published in 1995, and now running into the 4th edition. We were getting persistent requests from teachers of dental courses that a concise version of the textbook is necessary for catering their needs. In order to satisfy this continued demand, we are now presenting this *Textbook of Biochemistry for Dental Students*.

This book is prepared after consulting the syllabi of dental courses of various universities. As there are slight variations on the emphasis given by different universities, we have tried to incorporate all the facts prescribed in all those curricula. So, some students may find some chapters unnecessary for their immediate purpose. But a general textbook of this nature has to satisfy the needs of all students.

The important points are made into bold letters, so that students can grasp the subject easily. Moreover, the facts are given in point-wise (with numbers), so as to aid easy memorization. There are two sets of undergraduate students; majority wants only pass the examination. For them, the essentials of biochemistry are given in ordinary font. A small group of students aspire for distinction; for them, a bit more advanced knowledge is given in small prints. Students just before the examination can skip the tiny letters, but these areas will also become important later in their pursuit of knowledge. Those who are interested to get more facts may consult our *Textbook of Biochemistry for Medical Students*.

A question bank, given at the end of the book, contains essay questions, short notes and viva voce type questions. These questions are compiled from the last 10 years' question papers of various universities. These questions will be ideal for students for last-minute preparation for examinations.

The first author desires more interaction with faculty and students who are using this textbook. All are welcome to communicate at his e-mail address <dmvasudevan@aims.amrita.edu>. We extend our thanks for the enormous help rendered by Dr Ananth N Rao and Dr R Krishnaprasad in preparation of this book. We also thank Shri Jitendar P Vij, Chairman and Managing Director, M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi and his associates, for their active support in making this publication possible.

A textbook will be matured only by successive revisions. We hope that this first edition will be friendly to the students and be attractive to the teachers. Now this is in your hands to judge.

"End of all knowledge must be building up of character."—*Gandhiji*

DM Vasudevan
Sreekumari S

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CHAPTER 1

Subcellular Organelles and Cell Membranes

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Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Nucleus
- Endoplasmic reticulum
- Golgi apparatus
- Lysosomes
- Mitochondria
- Plasma membrane
- Transport mechanisms
- Simple and facilitated diffusion
- Ion channels
- Active transport
- Uniport, symport and antiport

INTRODUCTION

Biochemistry is the language of biology. The tools for research in all the branches of medical science are mainly biochemical in nature. The study of biochemistry is essential to understand basic functions of the body. How the food that we eat is digested, absorbed, and used to make ingredients of the body? How does the body derive energy for the normal day-to-day work? How are the various metabolic processes interrelated? What is the function of genes? Answer for such basic questions can only be derived by a systematic study of biochemistry.

Modern day medical practice is highly dependent on the laboratory analysis of body fluids, especially the blood. The disease manifestations are reflected in the composition of blood and other tissues. The study of biochemistry is necessary to give the scientific basis for disease and is useful for intelligent treatment of patients.

The practice of medicine is both an art and a science. The word "doctor" is derived from the Latin root, "docere", which means "to teach". The word chemistry is derived from the Greek word "chemi" (the black land), the ancient name of Egypt, when science was called the "black art". Indian medical science, even from ancient times, had identified the metabolic and genetic basis of diseases. Charaka, the great master of Indian Medicine, in his treatise (circa 400 BC) observed that *madhumeha* (diabetes mellitus) is produced by the alterations in the metabolisms of carbohydrates and fats; the statement still holds good.

The term "Biochemistry" was coined by Neuberg in 1903 from Greek words, bios (= life) and chymos (= juice). Some of the important milestones in the development of science of biochemistry are given in Table 1.1. Biochemistry is the most rapidly developing subject in medicine. Thanks to the advent of DNA-recombination technology, genes can now be transferred from one person to another, so that many of the genetically determined diseases are now amenable to gene therapy.

BIOMOLECULES

More than 99% of the human body is composed of 6 elements, i.e. oxygen, carbon, hydrogen, nitrogen, calcium and phosphorus. Human body is composed of about 60% water, 15% proteins, 15% lipids, 2% carbohydrates and 8% minerals.

In living organisms, biomolecules are ordered into a hierarchy of increasing molecular complexity. These biomolecules are covalently linked to each other to form **macromolecules** of the cell, e.g. glucose to glycogen, amino acids to proteins, etc. Major complex biomolecules are proteins, polysaccharides, lipids and nucleic acids. The macromolecules associate with each other to form

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Table 1.1: Important milestones in the history of Biochemistry

Scientists	*Year*	*Landmark discoveries*
Rouell	1773	Isolated urea from urine
Lavoisier	1785	Oxidation of food stuffs
Wohler	1828	Synthesis of urea
Louis Pasteur	1860	Fermentation process
Edward Buchner	1897	Extracted the enzymes
Fiske & Subbarao	1929	Isolated ATP from muscle
Lohmann	1932	Creatine phosphate
Hans Krebs	1937	Citric acid cycle
Avery & Macleod	1944	DNA is genetic material
Watson & Crick	1953	Structure of DNA
Nirenberg &Matthai	1961	Genetic code in mRNA
Holley	1963	Sequenced gene for tRNA
Khorana	1965	Synthesised the gene
Paul Berg	1972	Recombinant DNA technology
Kary Mullis	1985	Polymerase chain reaction
	2003	Human gene mapping

supramolecular systems, e.g. ribosomes, lipoproteins. Finally at the highest level of organization in the hierarchy of cell structure, various supramolecular complexes are further assembled into cell organelle. In prokaryotes (bacteria; Greek word "pro" = before; karyon = nucleus), these macromolecules are seen in a homogeneous matrix; but in eukaryotic cells (higher organisms; Greek word "eu" = true), the cytoplasm contains various subcellular organelles. Comparison of prokaryotes and eukaryotes are shown in Table 1.2.

SUBCELLULAR ORGANELLES

When the cell membrane is disrupted, the organized particles inside the cell are externalized. These are called subcellular organelles. They are described below.

Nucleus

It is the most prominent organelle of the cell. All cells in the body contain nucleus, except mature RBCs in circulation. In some cells, nucleus occupies most of the available space, e.g. small lymphocytes and spermatozoa. (Fig.1.1)

Nucleus is surrounded by two membranes: the inner one is called perinuclear membrane with numerous pores. The outer membrane is continuous with membrane of endoplasmic reticulum. Nucleus contains the DNA, the chemical basis of genes which governs all the functions of the cell. DNA molecules are complexed with proteins to form **chromosomes**. DNA replication and RNA synthesis (transcription) are taking place inside the nucleus. In some cells, a portion of the nucleus may be seen as lighter shaded area; this is called **nucleolus.** This is the area for RNA processing and ribosome synthesis. The nucleolus is very prominent in cells actively synthesizing proteins.

Table 1.2: Comparison between prokaryotic cells and eukaryotic cells

	Prokaryotic cell	*Eukaryotic cell*
Size	Small	Large; 1000 to 10,000 times
Cell wall	Rigid	Membrane of lipid bilayer
Nucleus	Not defined	Well defined
Organelles including mitochondria and lysosomes	Nil	Several

Endoplasmic Reticulum (ER)

It is a network of interconnecting membranes enclosing channels or cisternae, that are continuous from perinuclear envelope to outer plasma membrane. Under electron microscope, the reticular arrangements will have railway track appearance. (Fig.1.1). This will be very prominent in cells actively synthesizing proteins, e.g. immunoglobulin secreting plasma cells. The proteins, glycoproteins and lipoproteins are synthesized in the ER. Moreover, enzyme present in ER, cytochrome P-450 will detoxify various drugs.

Golgi Apparatus

It may be considered as the converging area of ER. While moving through ER, carbohydrate groups are successively added to the nascent proteins. (Fig.1.1). These glycoproteins finally reach the Golgi area. The carbohydrate chains are further added in the Golgi prior to secretion. Main function of Golgi apparatus is protein sorting, packaging and secretion.

Lysosomes

Solid wastes of a township are usually decomposed in incinerators. Inside a cell, such a process is taking place within the lysosomes. They are bags of enzymes. (Fig.1.1). Lysosomes contain enzymes that hydrolyse polysaccharides, lipids, proteins, and nucleic acids. Endocytic vesicles and phagosomes are fused with lysosome (primary) to form the **secondary lysosome** or digestive vacuole. Foreign particles are progressively digested inside these vacuoles.

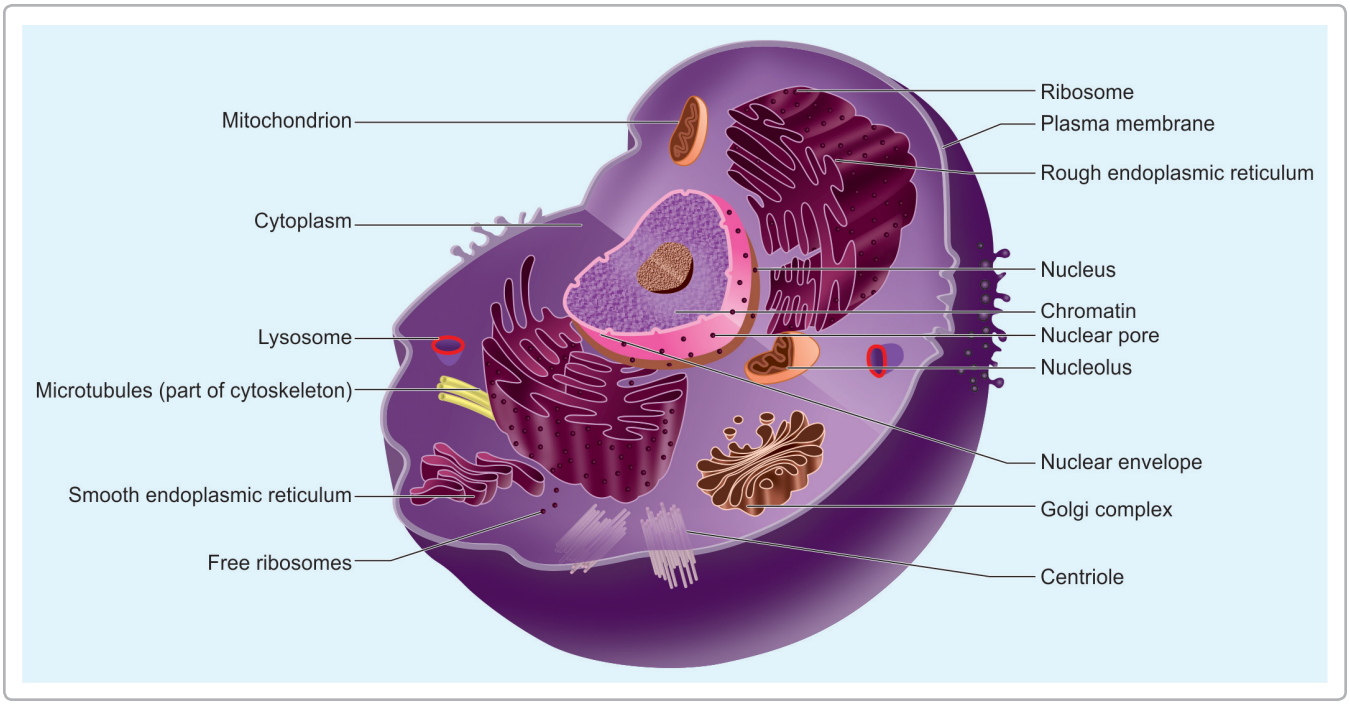


Figure 1.1: A typical cell

Mitochondria

They are spherical, oval or rod-like bodies, about 0.5–1 micrometers in diameter and up to 7 micrometers in length. (Fig.1.1). Erythrocytes do not contain mitochondria. The tail of spermatozoa is fully packed with mitochondria. Mitochondria are the **powerhouse** of the cell, where energy released from oxidation of food stuffs is trapped as chemical energy in the form of ATP (See Chapter 13).

Mitochondria have two membranes. The inner membrane convolutes into folds or cristae. The mitochondrial membrane contains the enzymes of **electron transport chain.** The fluid matrix contains the enzymes of citric acid cycle. Mitochondria also contain **specific DNA** which encodes information for synthesis for certain mitochondrial proteins. The division of mitochondria is under the command of mitochondrial DNA. **Antibiotics** inhibiting bacterial protein synthesis do not affect cellular processes, but do inhibit mitochondrial protein biosynthesis. Functions of organelles are shown in Tables 1.3 and 1.4.

Plasma Membrane

The plasma membrane separates the cell from the external environment. The membranes also separate different parts of the cell from one another, so that cellular activities are compartmentalized. It has highly selective

Table 1.3: Metabolic functions of subcellular organelles

Nucleus	DNA replication, transcription
Endoplasmic reticulum	Biosynthesis of proteins, glycoproteins, lipoproteins, drug metabolism
Golgi body	Maturation of synthesized proteins
Lysosome	Degradation of proteins, carbohydrates, lipids and nucleotides
Mitochondria	Electron transport chain, ATP generation, TCA cycle, beta oxidation of fatty acids, ketone body production
Cytosol	Protein synthesis, glycolysis, glycogen metabolism, transaminations, fatty acid synthesis

Table 1.4: Comparison of Cell with a Factory

Plasma membrane	Fence with gates; gates open when message is received
Nucleus	Manager's office
Endoplasmic reticulum	Conveyer belt of production units
Golgi apparatus	Packing units
Lysosomes	Incinerators
Vacuoles	Lorries carrying finished products
Mitochondria	Power generating units

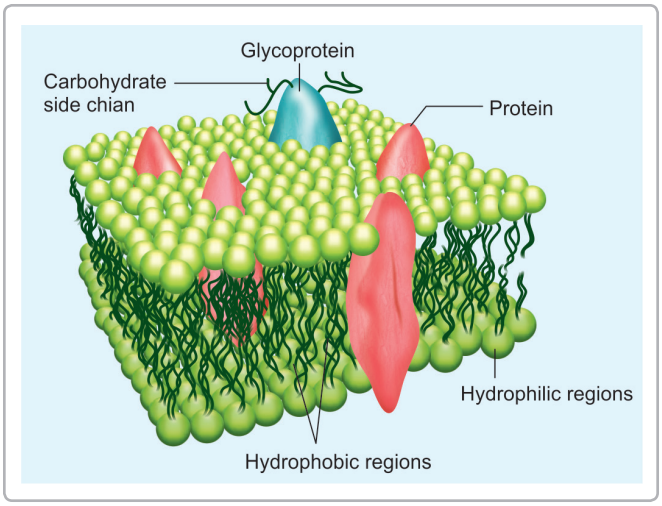


Figure 1.2: Fluid mosaic model of membrane

permeability properties so that the entry and exit of compounds are regulated. The membrane is very active metabolically.

Membranes are mainly made up of lipids, proteins and small amount of carbohydrates. The contents of these compounds vary according to the nature of the membrane. The carbohydrates are present as glycoproteins and glycolipids. Phospholipids are the most common lipids present and they are amphipathic in nature. Cell membranes contain cholesterol also.

Fluid Mosaic Model: Membranes are made up of **lipid bilayer**. The phospholipids are arranged in bilayers with the polar head groups oriented towards the extracellular side and the cytoplasmic side with a hydrophobic core (Fig. 1.2). Each leaflet is 25 Å thick. The total thickness is about 50 to 80 Å.

The lipid bilayer shows free lateral movement of its components, hence the membrane is said to be **fluid in nature**.

Membrane Proteins: The **peripheral proteins** exist on the surfaces of the bilayer. They are attached by ionic and polar bonds to polar heads of the lipids. The **integral membrane proteins** are deeply embedded in the bilayer. Some of the integral membrane proteins span the whole bilayer and they are called **transmembrane proteins**. They can serve as **receptors** (for hormones, growth factors, neurotransmitters), tissue specific antigens, ion channels, membrane based enzymes, etc.

TRANSPORT MECHANISMS

The permeability of substances across cell membranes is dependent on their solubility in lipids and not on their molecular size. Water soluble compounds are generally impermeable and require carrier mediated transport. Transport mechanisms are classified into:

- Passive transport
  - Simple diffusion
  - Facilitated diffusion
- Active transport
- **Ion channels** allow passage of molecules in accordance with the concentration gradient
- **Pumps** can drive molecules against the gradient using energy.

Simple Diffusion

Solutes and gases enter into the cells passively. They are driven by the concentration gradient. The rate of entry is proportional to the solubility of that solute in the hydrophobic core of the membrane. Simple diffusion occurs from higher to lower concentration. This does not require any energy. But, it is a very slow process.

Facilitated Diffusion

This is a **carrier mediated process** (Fig.1.3)**.** Important features of facilitated diffusion are: Structurally similar solutes can competitively inhibit the entry of the solutes. This mechanism does not require energy but the rate of transport is more rapid than diffusion process. It is dependent on concentration gradient. **Hormones** regulate the number of carrier molecules. Example of facilitated transport of glucose across membrane is by **glucose transporters**.

Ion Channels

Membranes have special devices called ion channels for quick transport of electrolytes such as Ca^{++}, K^{+}, Na^{+} and Cl^{--}. These are selective ion conductive pores. Ion channels are specialized protein molecules that span the membranes. **Cation conductive channels** generally remain closed but in response to stimulus, they open allowing rapid flux of ions down the gradient. This may be compared to opening of the gate of a cinema house, when people rush to enter in. Hence, this regulation is named as "gated" (Fig.1.3). Based on the nature of stimuli that trigger the opening of the gate, they are classified into "voltage gated" or "ligand gated" ion channels. Voltage gated channels are opened by membrane depolarization. Ligand gated channels are opened by binding of effectors.

Amelogenin

It is a protein present in enamel of teeth has hydrophobic residues on the outside. It acts as a calcium channel, which helps in mineralization (See Chapter 8).

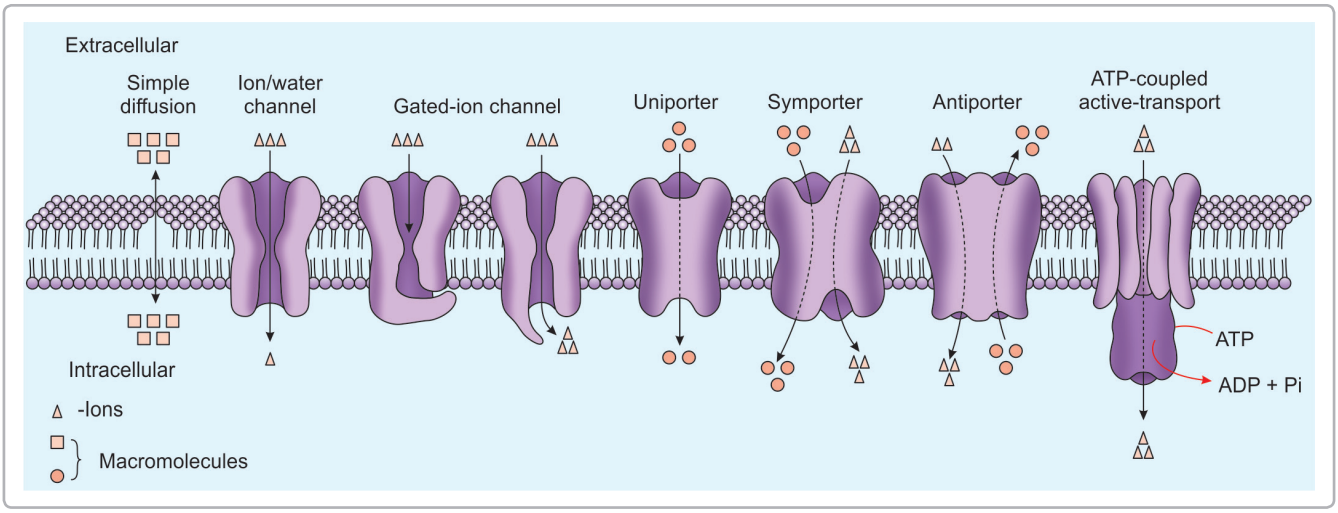


Figure 1.3: Transport mechanisms

Active Transport

The salient features of active transport are: (1) This form of transport **requires energy**. About 40% of the total energy expenditure in a cell is used for the active transport system. (Fig. 1.3). (2) It requires specialized integral proteins called **transporters**. The transporters are susceptible to inhibition by specific organic or inorganic compounds.

Cell has low intracellular sodium; but concentration of potassium inside the cell is very high. This is maintained by the sodium–potassium activated ATPase, generally called as **sodium pump**. The hydrolysis of one molecule of ATP can result in expulsion of 3 Na^+ ions and influx of 2 K^+ ions. The ion transport and ATP hydrolysis are tightly coupled.

Clinical Applications: Cardiotonic drug **digoxin** inhibits the sodium–potassium pump. This leads to an increase in Na^+ level inside the cell and extrusion of Ca^+ from the myocardial cell. This would enhance the contractility of the cardiac muscle and so improve the function of the heart.

Calcium Pump: The ATP dependent calcium pump also functions to regulate muscle contraction. A specialized membrane system called sarcoplasmic reticulum is found in skeletal muscles which regulates the Ca^{++} concentration around muscle fibers. In resting muscle, the concentration of Ca^{++} around muscle fibers is low. But stimulation by a nerve impulse results in a sudden release of large amounts of Ca^{++}. This would trigger muscle contraction. The function of calcium pump is to remove cytosolic calcium and maintain low cytosolic concentration, so that muscle can receive the next signal. For each ATP hydrolyzed, 2Ca^{++} ions are transported.

Uniport, Symport and Antiport

Transport systems are classified as uniport, symport and antiport systems.

Uniport system (Fig.1.3) carries single solute across the membrane, e.g. glucose transporter in most of the cells. Calcium pump is another example. If the transfer of one molecule depends on simultaneous or sequential transfer of another molecule, it is called **co-transport** system. The active transport may be coupled with energy indirectly. Here, movement of the substance against a concentration gradient is coupled with movement of a second substance down the concentration gradient; the second molecule being already concentrated within the cell by an energy requiring process. The cotransport system may either be a symport or an antiport. In **symport** (Fig.1.3), the transporter carries two solutes in the same direction across the membrane, e.g. sodium dependent glucose transport. The **antiport** system (Fig.1.3) carries two solutes or ions in opposite direction, e.g. sodium pump or chloride-bicarbonate exchange in RBC (Chapter 14). Features of transport modalities are summarized in Table 1.5.

Table 1.5. Summary of transport mechanisms

Type	*Carrier*	*Energy required*	*Examples*
Simple diffusion	Nil	Nil	Water
Facilitated diffusion	Yes	Nil	Glucose to RBCs
Primary active	Yes	Directly	Sodium pump
Secondary active	Yes	Indirect	Glucose to intestine
Ion channels	Yes	No	Sodium channel

ENDOCYTOSIS

Endocytosis is the mechanism by which cells internalize extracellular macromolecules, to form an endocytic vesicle. This requires energy in the form of ATP as well as calcium ions in the extracellular fluid. Cytoplasmic contractile elements take part in this movement. In general, plasma membrane is invaginated, enclosing the matter. This forms the **endocytic vesicle**. The endocytosis may be phagocytosis or receptor mediated endocytosis.

Receptor Mediated Endocytosis

Low density lipoprotein (LDL) binds to the LDL receptor and the complex is later internalized. The cytoplasmic side of these vesicles are coated with filaments; mainly composed of Clathrin. These are called **Clathrin coated pits**. Several hormones are also taken up by the cells by receptor-mediated mechanism. Many viruses get attached to their specific receptors on the cell membranes. Examples are influenza virus, hepatitis B virus, poliovirus and HIV.

Phagocytosis

The term is derived from the Greek word "phagein" which means to eat. It is the engulfment of large particles such as bacteria by **macrophages and granulocytes.** They extend pseudopodia and surround the particles to form **phagosomes**. Phagosomes later fuse with lysosomes to form phagolysosomes, inside which the particles are digested. An active macrophage can ingest 25% of their volume per hour.

A QUICK LOOK

1. Marker enzymes are present only in particular organelle and used to identify these organelles during cell fractionation.
2. Nucleus is the storehouse of genetic information containing DNA organized into 23 pairs of chromosomes. All cells in the body contain nucleus except mature erythrocytes.
3. Endoplasmic reticulum, a network of interconnecting membranes is the site of protein synthesis (Rough endoplasmic reticulum).
4. Smooth endoplasmic reticulum is the site for complex lipid and carbohydrate synthesis and detoxification of drugs.
5. Nascent proteins synthesized in ER are modified in Golgi bodies and then exported to specific destinations in the cell. Golgi apparatus is primarily involved in glycosylation, protein sorting, packaging and secretion.
6. Lysosomes are bags of hydrolytic enzymes responsible for autophagy, postmortem autolysis and phagocytosis.
7. Mitochondria, the powerhouse of the cell has its own DNA encoding mitochondrial proteins and a role in triggering apoptosis.
8. Plasma membrane is mainly made up of phospholipid bilayers, interspersed with proteins and carbohydrate residues attached to proteins and lipids.
9. Cholesterol is also a component of animal cell membranes.
10. Phospholipid bilayers are oriented in such a way that there is a hydrophobic core with hydrophilic interior (cytoplasmic side) and outer layer (extracellular side).
11. Transmembrane proteins serve as receptors, tissue specific antigens, ion-channels, etc.
12. Membrane structure is described as the fluid mosaic model.
13. Short chain fatty acids and unsaturated fatty acids present in phospholipids will increase the fluidity of the membrane.
14. Cholesterol modulates the fluidity of membranes.
15. Lipophilic compounds can easily pass through the lipid membrane whereas hydrophilic molecules may require channels or pores.
16. Transport of molecules across the plasma membrane may be energy dependent (active) or energy independent (passive).
17. Active transport involves expenditure of energy and occurs against a concentration gradient.
18. Na^+/K^+–ATPase (Sodium pump) is an example of Active transport. Cardiotonic drugs like Digoxin and Ouabain competitively inhibit K^+ ion binding.
19. Ion channels that transport Na^+, K^+, Ca^{++} and Cl^- are transmembrane proteins. They are highly selective. They may be voltage gated or ligand gated.
20. Glucose transporters are examples of uniport transport by facilitated diffusion.
21. Sodium-dependent glucose transport in intestine and renal tubules are examples of symport systems.
22. Sodium-potassium-ATPase is an active transport system which causes efflux of sodium and influx of potassium with ATP hydrolysis.

CHAPTER 2

Amino Acids and Proteins

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Classification of amino acids based on structure
- Based on side chain character
- Based on metabolic fate
- Based on nutritional requirements
- Isoelectric point
- Reactions due to carboxyl and amino groups
- Peptide bond formation
- Primary, secondary, tertiary and quaternary structure of proteins
- Precipitation reactions of proteins
- Classification of proteins

The word protein is derived from Greek word, "proteios" which means primary. As the name shows, the proteins are of paramount importance for biological systems. Out of the total dry body weight, 3/4ths are made up of proteins. Proteins are used for the body building; all the major structural and functional aspects of the body are carried out by protein molecules. Abnormality in protein structure will lead to molecular diseases with profound alterations in metabolic functions.

Proteins contain carbon, hydrogen, oxygen and nitrogen as the major components while sulfur and phosphorus are minor constituents. **Nitrogen** is characteristic of proteins. *On an average, the nitrogen content of ordinary proteins is 16% by weight.*

All proteins are polymers of amino acids. Commonly occurring amino acids are 20 in number. Most of the amino acids (except proline) are **alpha amino acids,** which means that the amino group is attached to the same carbon atom to which the carboxyl group is attached (Fig. 2.1).

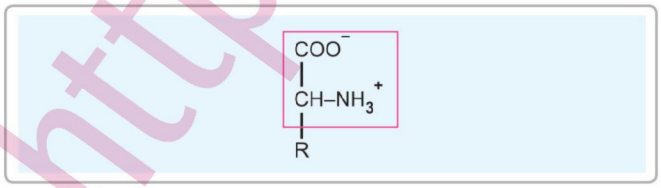


Figure 2.1: General structure

CLASSIFICATION OF AMINO ACIDS

1. Based on Structure

A. Aliphatic amino acids

- Simple amino acids:
  1. Glycine,
  2. Alanine (Fig. 2.2)
- Branched chain amino acids:
  1. Valine
  2. Leucine
  3. Isoleucine (Fig. 2.3)
- Hydroxy amino acids:
  6. Serine,
  7. Threonine (Fig. 2.4)
- Sulfur containing:

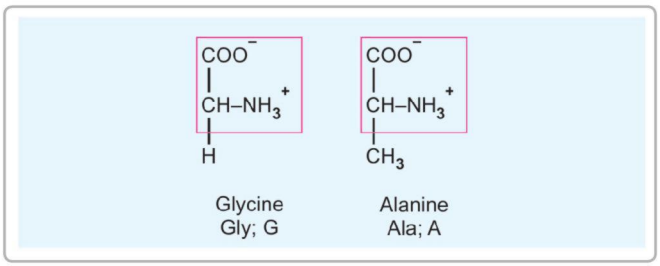


Figure 2.2: Simple amino acids

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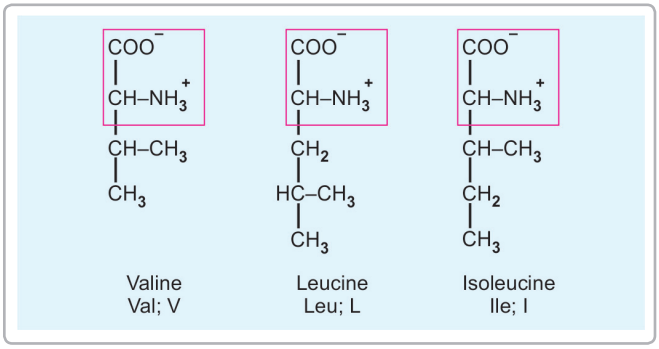


Figure 2.3: Branched amino acids

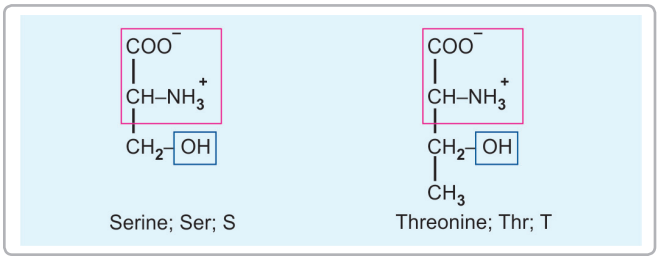


Figure 2.4: Hydroxy amino acids

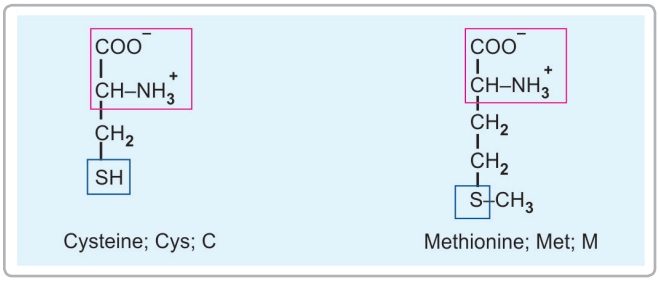


Figure 2.5: Sulfur containing amino acids

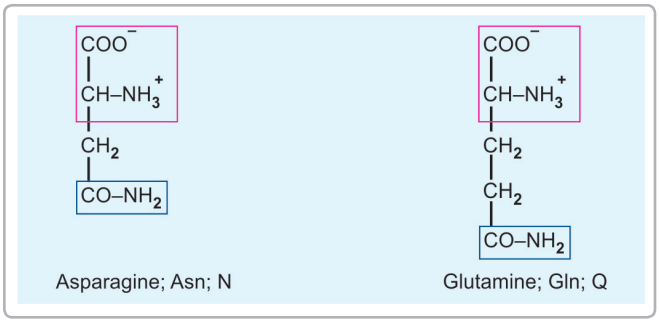


Figure 2.6: Amino acids with amide groups

1. Cysteine
2. Methionine (Fig. 2.5)

- Having amide group:
  1. Asparagine
  2. Glutamine (Fig. 2.6)
- **Mono amino dicarboxylic acids** (Fig. 2.7):
  1. Aspartic acid,
  2. Glutamic acid

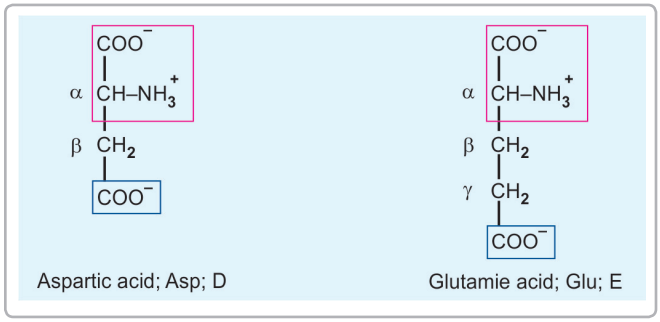


Figure 2.7: Dicarboxylic amino acids

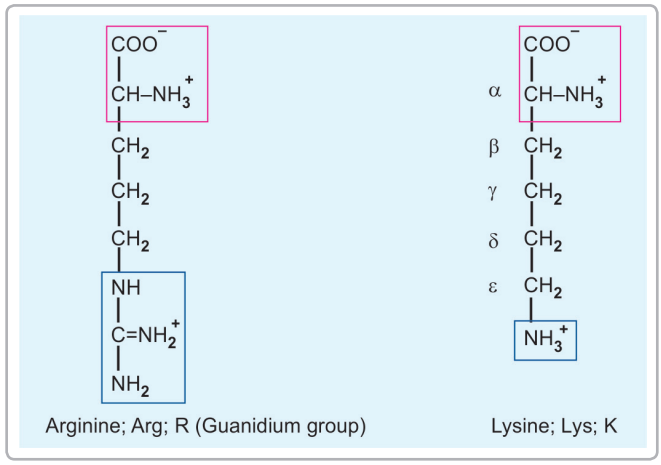


Figure 2.8: Dibasic amino acids

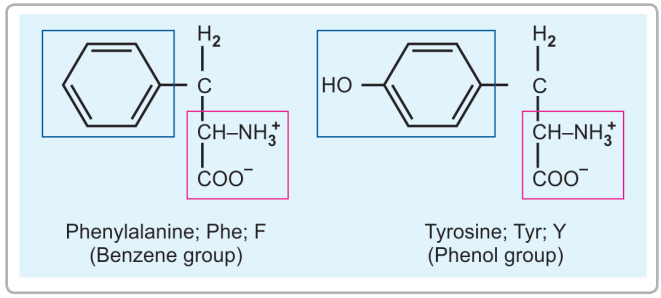


Figure 2.9: Aromatic amino acids

- **Dibasic monocarboxylic acids:**
  1. Lysine
  2. Arginine (Fig. 2.8)

B. Aromatic amino acids:

1. Phenylalanine,
2. Tyrosine (Fig. 2.9)

C. Heterocyclic amino acids:

1. Tryptophan (Fig.2.10)
2. Histidine (Fig. 2.11)

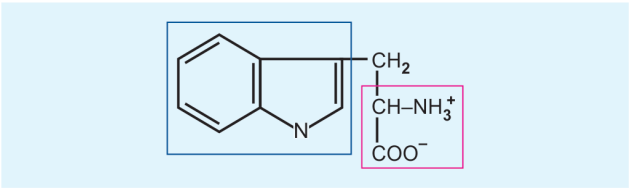


Figure 2.10: Tryptophan (Trp) (W) with indole group

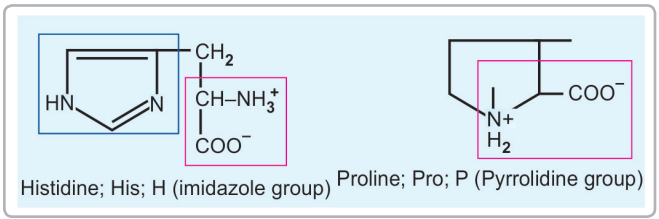


Figure 2.11: Histidine and Proline

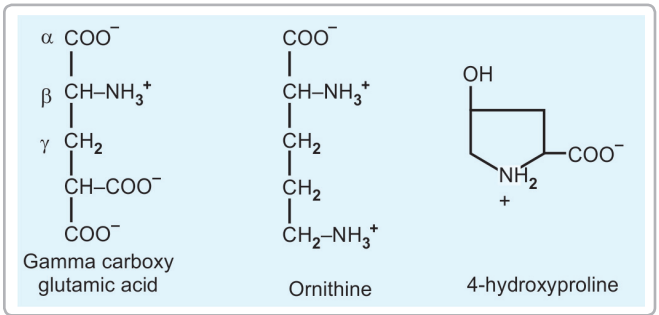


Figure 2.12: Some derived amino acids

D. Imino acid:

1. Proline (Fig. 2.11).

E. Derived amino acids:

a. Derived amino acids found in proteins: After the synthesis of proteins, some of the amino acids are modified, e.g. hydroxy proline (Fig. 2.12) and hydroxy lysine are important components of collagen. Gamma carboxylation of glutamic acid residues of proteins is impor-tant for clotting process (Fig. 2.12).

b. Derived amino acids not seen in proteins: (Non-protein amino acids): Some derived amino acids are seen free in cells, e.g. Ornithine (Chapter 11), Citrulline, Homocysteine. These are produced during the metabolism of amino acids.

Each amino acid will have three-letter and one letter abbreviations which are shown in Figures 2.2 to 2.11, as well as in Table 2.1.

Table 2.1: Common amino acids

Name of amino acid	*Special group present*	*3-letter abbreviation*	*1-letter abbreviation*
Glycine		Gly	G
Alanine		Ala	A
Valine		Val	V
Leucine		Leu	L
Isoleucine		Ile	I
Serine	Hydroxyl	Ser	S
Threonine	Hydroxyl	Thr	T
Cysteine	Sulfhydryl	Cys	C
Methionine	Thioether	Met	M
Asparagine	Amide	Asn	N
Glutamine	Amide	Gln	Q
Aspartic acid	Beta-carboxyl	Asp	D
Glutamic acid	Gamma-carboxyl	Glu	E
Lysine	Epsilon-amino	Lys	K
Arginine	Guanidinium	Arg	R
Phenylalanine	Benzene	Phe	F
Tyrosine	Phenol	Tyr	Y
Tryptophan	Indole	Trp	W
Histidine	Imidazole	His	H
Proline (imino acid)	Pyrrolidine	Pro	P

Special Groups in Amino Acids

Arginine contains guanidinium group (-NH-CNH--NH_2); Phenyl alanine contains benzene group; Tyrosine (phenol); Tryptophan (Indole group); Histidine (imidazole); and Proline (pyrrolidine) (Table 2.1). Proline has a secondary amino group, and hence it is an **imino acid**.

2. Classification Based on Side Chain

A. Amino acids having nonpolar side chains: These include alanine, valine, leucine, isoleucine, me-thionine, proline, phenylalanine and tryptophan. These groups are hydrophobic (water repellant) and lipophilic.

B. Amino acids having uncharged or non-ionic polar side chains:
Glycine, serine, threonine, cysteine, tyrosine, glutamine and asparagine belong to this group. These amino acids are hydrophilic in nature.

C. Amino acids having charged or ionic polar side chains:

They are hydrophilic in nature.

a. Acidic amino acids: They have a negative charge on the R group: Aspartic acid and glutamic acid. (Tyrosine is mildly acidic).

b. Basic amino acids: They have a positive charge on the R group: Lysine, arginine and histidine.

3. Classification Based on Metabolic Fate

A. Purely ketogenic:
Leucine is purely ketogenic because it will enter into the metabolic pathway of ketogenesis.

B. Ketogenic and glucogenic:
Lysine, isoleucine, phenylalanine, tyrosine and tryptophan are partially ketogenic and partially glucogenic. During metabolism, part of the carbon skeleton of these amino acids will enter the fatty acid metabolic pathway and the other part into glucose pathway.

C. Purely glucogenic:
All the remaining 14 amino acids are purely glucogenic as they enter only into the glucogenic pathway.

4. Classification Based on Nutritional Requirement

A. Essential or indispensable:
The amino acids may further be classified according to their essentiality for growth. Thus, **isoleucine, leucine, threonine, lysine, methionine, phenylalanine, tryptophan, and valine** are essential amino acids. Their carbon skeleton cannot be synthesized by human beings and so preformed amino acids are to be taken in food for normal growth. One tip to remember these names is given in Box 2.1.

B. Partially essential or semi-essential:
Histidine and arginine are semi-indispensable amino acids. Growing children require them in food. But they are not essential for the adult individual.

C. Non-essential or dispensable:
The remaining 10 amino acids are non-essential. However, they are also required for the normal protein synthesis. All body proteins do contain all the non-essential amino acids. But their carbon skeleton **can be synthesized** by metabolic pathways and therefore their absence in the food will not adversely affect the growth.

BOX 2.1: Memory aid for essential amino acids

"**A**ny **H**elp **I**n **L**earning **T**hese **L**ittle **M**olecules **P**roves **T**ruly **V**aluable"
This stands for
Arginine, **H**istidine, **I**soleucine, **L**eucine, **T**hreonine, **L**ysine, **M**ethionine, **P**henylalanine, **T**ryptophan and **V**aline in that order.
Arginine and histidine are semi-essential amino acids; while others are essential

Naming of Carbon Atoms

Carbon atoms in amino acids in sequence are named with letters of Greek alphabets, starting from the carbon atom to which carboxyl group is attached. (Fig. 2.7).

PROPERTIES OF AMINO ACIDS

Isoelectric Point

Amino acids can exist as **ampholytes** or **zwitterions** (German word "zwitter" = hybrid) in solution, depending on the pH of the medium. The pH at which the molecule carries no net charge is known as *isoelectric point or isoelectric pH* (pI). In acidic solution, they are cationic in form and in alkaline solution they behave as anions (Fig. 2.13). At isoelectric point, the amino acid will **carry no net charge;** all the groups are ionized but the charges will cancel each other. Therefore at isoelectric point, there is *no mobility in an electrical field*. Solubility and buffering capacity will be minimum at isoelectric pH.

When 50% of molecules are cations, that pH is called pK1 (with respect to COOH). When 50% of molecules are anions, that pH is called pK2 (with respect to NH_2). For monoamino monocarboxylic amino acids;

$$pI = \frac{pK1 + pK2}{2};$$

$$\text{e.g. pI of glycine} = \frac{2.4 + 9.8}{2} = 6.1$$

The buffering action is maximum in and around pK1 or at pK2 and minimum at pI. The pK value of imidazolium group of **Histidine** is 6.1, and therefore effective as a buffer

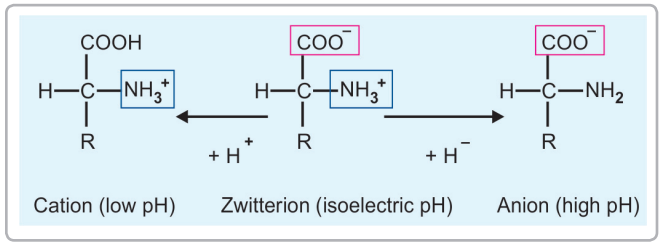


Figure 2.13: Ionic forms of amino acids

at the physiological pH of 7.4. *The buffering capacity of plasma proteins and hemoglobin is mainly due to histidine residue.*

Optical Activity

Amino acids having an asymmetric carbon atom exhibit optical activity. **Asymmetry** arises *when 4 different groups are attached to the same carbon atom* (Fig. 2.14). Glycine is the simplest amino acid and has no asymmetric carbon atom and therefore shows no optical activity. All others are optically active. These mirror image forms produced with reference to the alpha carbon atom, are called D and L isomers (Fig. 2.14). The L-amino acids occur in nature and are therefore called **natural amino acids.** D-amino acids are seen in small amounts in microorganisms and as constituents of certain antibiotics such as gramicidin and bacterial cell walls.

Reactions due to Carboxyl Group

Decarboxylation

The amino acids will undergo alpha decarboxylation to form the corresponding amine (Fig. 2.15). Thus, some important amines are produced from amino acids. For example,

i. Histidine → Histamine + CO_2
ii. Tyrosine → Tyramine + CO_2
iii. Tryptophan → Tryptamine + CO_2
iv. Glutamic acid → Gamma amino butyric acid (GABA) + CO_2

Amide Formation

The -COOH group of dicarboxylic amino acids (other than alpha carboxyl) can combine with ammonia to form the corresponding amide (Fig. 2.16). For example,

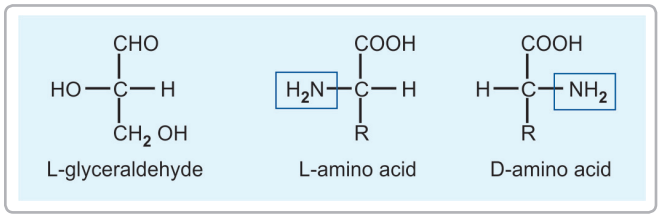


Figure 2.14: L and D amino acids

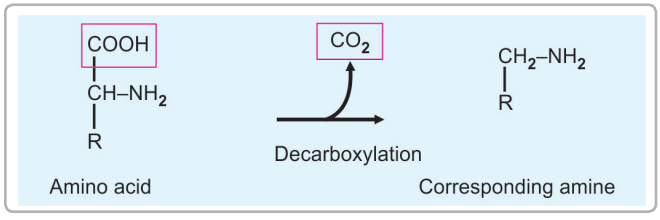


Figure 2.15: Decarboxylation of amino acid

Aspartic acid + NH3 → Asparagine
Glutamic acid + NH3 → Glutamine

These amides are also components of protein structure. The amide group of glutamine serves as the source of nitrogen for nucleic acid synthesis.

Reactions due to Amino Group

Transamination

The alpha amino group of amino acid can be transferred to alpha keto acid to form the corresponding new amino acid and alpha keto acid (Fig. 2.17). This is an important reaction in the body for the interconversion of amino acids and for **synthesis of non-essential amino acids**.

Oxidative Deamination

The alpha amino group is removed from the amino acid to form the corresponding keto acid and ammonia (Fig. 2.18). In the body, **Glutamic acid** is the most common amino acid to undergo oxidative deamination.

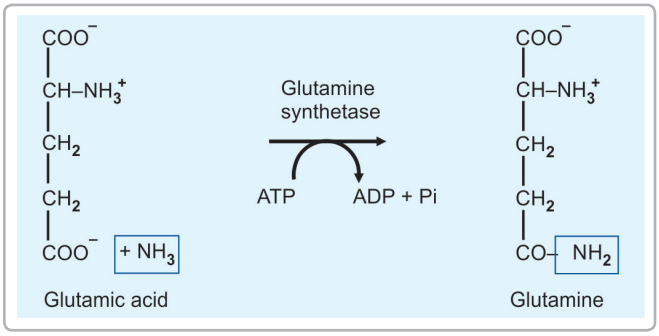


Figure 2.16: Formation of glutamine

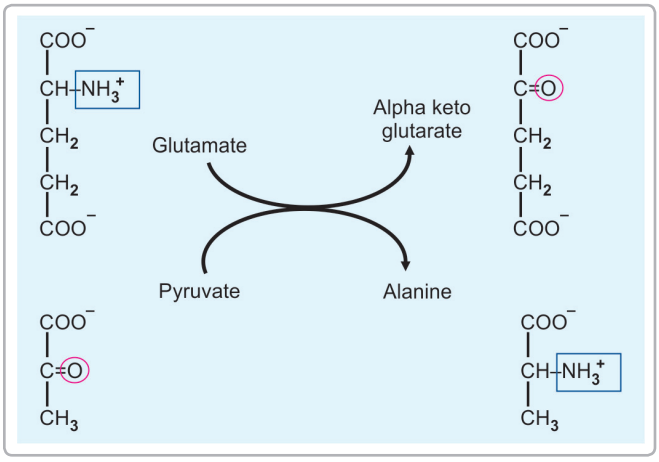


Figure 2.17: Transamination reaction

Reactions due to Side Chains

Ester Formation by OH Group

The hydroxy amino acids can form esters with phosphoric acid. In this manner, the **Serine and Threonine** residues of proteins are involved in the formation of phosphoproteins. Similarly, these hydroxyl groups can form O-glycosidic bonds with carbohydrate residues to form glycoproteins.

Reaction of the Amide Group

The amide groups of **Glutamine and Asparagine** can form N-glycosidic bonds with carbohydrate residues to form glycoproteins.

Reactions of SH Group

Cysteine has a sulfhydryl (SH) group and it can form a disulfide (S-S) bond with another cysteine residue (Fig. 2.19). The two cysteine residues can connect two polypeptide chains by the formation of **interchain disulfide bonds** or links (Fig. 2.21). The dimer formed by two cysteine residues is called **Dicysteine (cystine)**. They also take part in redox reactions, metal ion complexation and form thioethers.

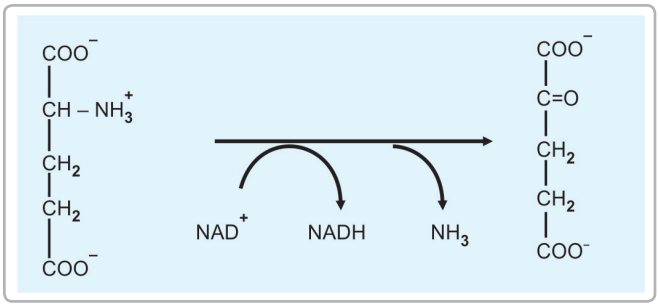


Figure 2.18: Oxidative deamination

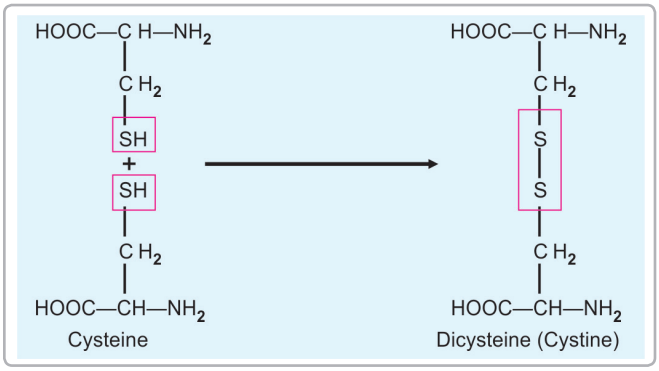


Figure 2.19: Formation of disulfide bridges

Special Functions of Amino Acids

Gamma amino butyric acid (GABA, a derivative of glutamic acid) and dopamine (derived from tyrosine) are neurotransmitters. **Histamine** (synthesized from histidine) is the mediator of allergic reactions. **Thyroxine** (from tyrosine) is an important thyroid hormone.

Color Reactions of Amino Acids and Proteins

Table 2.2 gives the important color reactions answered by specific amino acids. Proteins containing those amino acids will also give the corresponding color reactions.

Peptide Bond

Alpha carboxyl group of one amino acid reacts with **alpha amino group** of another amino acid to form a peptide bond or CO-NH bridge (Fig. 2.20). Proteins are made by polymerization of amino acids through peptide bonds. Two amino acids are combined to form a **dipeptide.** Three amino acids form a **tripeptide.** Four will make a **tetrapeptide.** A few amino acids together will make an **oligopeptide.** Combination of 10 to 50 amino acids is called as a **polypeptide.** Big polypeptide chains containing more than 50 amino acids are called **proteins**.

STRUCTURE OF PROTEINS (ORGANIZATION OF PROTEINS)

Proteins have different levels of structural organization; primary, secondary, tertiary and quaternary.

Table 2.2: Color reactions of amino acids

Reaction	*Answered byspecific group*
1. Ninhydrin	Alpha amino group
2. Biuret reaction	Peptide bonds
3. Xanthoproteic test	Benzene ring (Phe, Tyr, Trp)
4. Millon's test	Phenol (Tyrosine)
5. Aldehyde test	Indole (Tryptophan)
6. Sakaguchi's test	Guanidium (Arginine)
7. Sulfur test	Sulfhydryl (Cysteine)
8. Nitroprusside test	Sulfhydryl (Cysteine)
9. Pauly's test	Imidazole (Histidine)

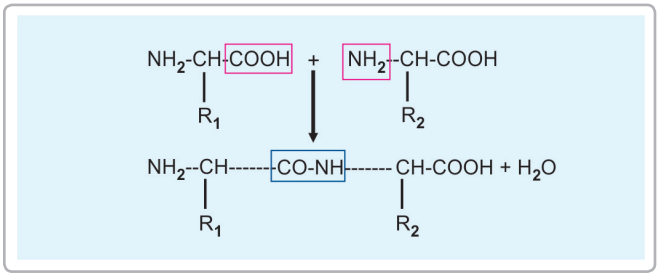


Figure 2.20: Peptide bond formation

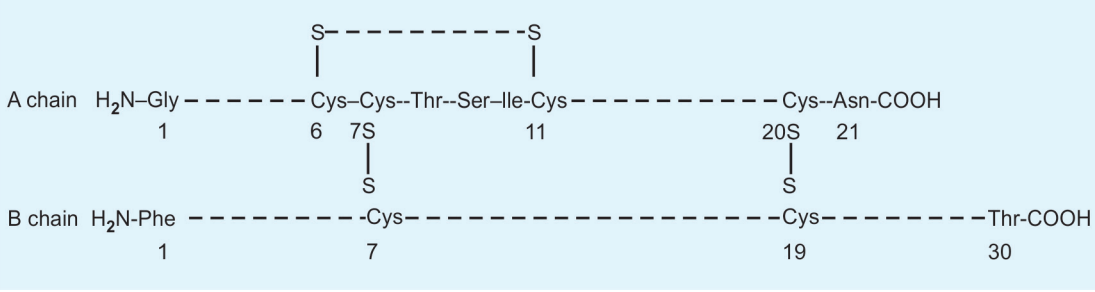


Figure 2.21: Primary structure of human insulin

Primary Structure; sequence

Primary structure denotes the number and sequence of amino acids in the protein. The higher levels of organization are decided by the primary structure. Each polypeptide chain has a unique amino acid sequence decided by the genes.

The following example may be taken to have a clear idea of the term "sequence".

Gly - Ala - Val

Gly - Val - Ala

Both the tripeptides shown above contain the same amino acids; but their sequence is altered. When the sequence is changed, the polypeptide is also different.

The *primary structure is maintained by the covalent bonds of the peptide linkages* (Fig. 2.20).

Numbering of Amino Acids in Proteins

In a polypeptide chain, at one end there will be one free alpha amino group. This end is called the **amino terminal (N-terminal) end** and the amino acid contributing the alpha-amino group is named as the **first amino acid**. The other end of the polypeptide chain is the **carboxy terminal end (C-terminal)**, where there is a free alpha carboxyl group which is contributed by the **last amino acid**. All other alpha amino and alpha carboxyl groups are involved in peptide bond formation (Figs 2.20 and 2.21). Usually, the N-terminal amino acid is written on the left hand side when the sequence of the protein is denoted. In nature, the biosynthesis of the protein also starts from the amino terminal end.

Take an example of a tripeptide: Peptide bonds formed by combination carboxyl group of glycine with amino group of alanine, and further combination of carboxyl group of alanine with amino group of valine. This tripeptide is called glycyl-alanyl-valine and abbreviated as

NH_2-Gly-Ala-Val-COOH

or Gly-Ala-Val

or GAV

Primary Structure of Insulin

As an example of the primary structure of a protein, that of insulin is shown in Fig. 2.21. This was originally described by Sanger in 1955 who received the Nobel prize in 1958. Insulin has **two polypeptide chains;** the A chain (Glycine chain) with 21 amino acids and B (Phenyl alanine) chain with 30 amino acids. They are held together by a pair of **disulfide bonds**. (Fig. 2.21). Primary structure determines biological activity. If primary structure is altered, protein function may be lost.

Primary Structure Determines Activity

A protein with a specific primary structure, when put in solution, will automatically form its natural three dimensional shape. So the higher levels of organization are dependent on the primary structure. Even a single amino acid change (**mutation**) in the linear sequence may have profound biological effects on the function. For example, **sickle cell anemia** due to HbS, where the 6th amino acid in the beta chain, the normal glutamic acid is replaced by valine.

Secondary Structure of Proteins

The term "secondary structure" denotes the configurational relationship between residues which are about 3–4 amino acids apart in the linear sequence. Some portions of the protein exhibit regularly repeating types of secondary structure, e.g. alpha helix, beta pleated sheet, collagen helix. Secondary and tertiary levels of protein structure are preserved by **noncovalent forces** or bonds like hydrogen bonds, electrostatic bonds, hydrophobic interactions and van der Waals forces.

Alpha helix

The alpha helix is a **spiral structure** (Fig. 2.22). The polypeptide bonds form the back-bone and the side chains of amino acids extend outward. The structure is stabilized

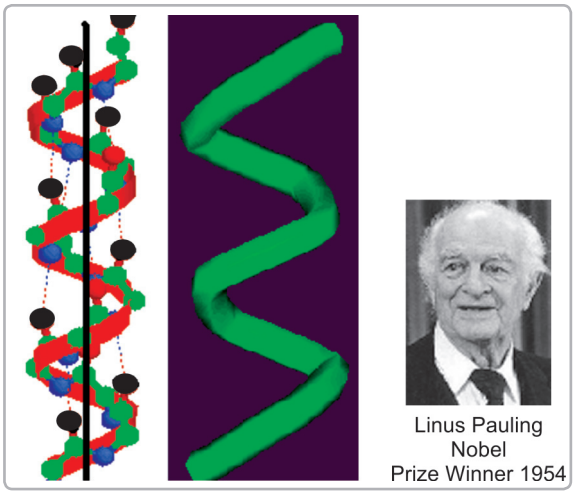


Figure 2.22: Alpha helix of proteins. Vertical line is the axis of helix. Each turn is formed by 3.6 amino acid residues. The distance between each amino acid (translation) is 1.5 Å

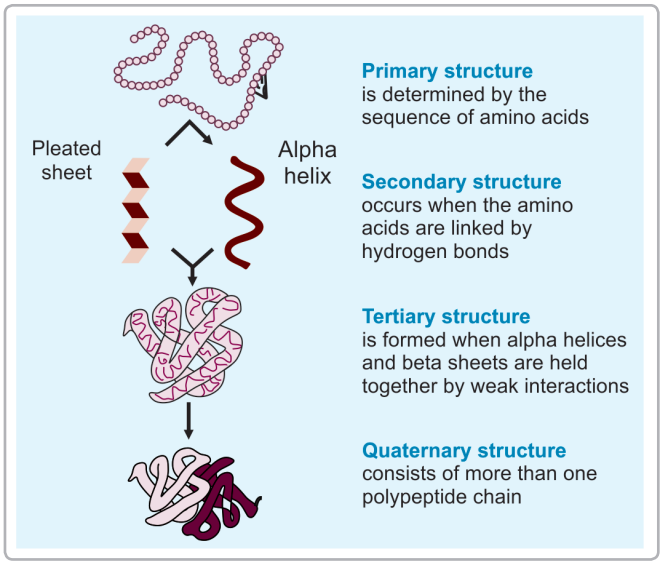


Figure 2.24: Levels of organizations of proteins

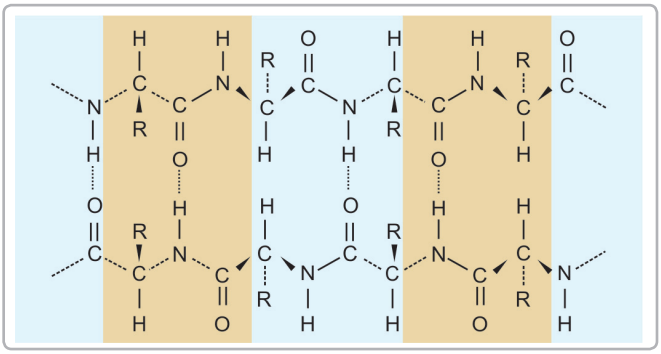


Figure 2.23: Structure of beta-pleated sheet

BOX 2.2: Definitions of levels of organization

1. Primary structure of protein means the order of amino acids in the polypeptide chain and the location of disulfide bonds, if any
2. Secondary structure is the steric relationship of amino acids, close to each other
3. Tertiary structure denotes the overall arrangement and inter-relationship of the various regions, or domains of a single polypeptide chain
4. Quaternary structure results when the proteins consists of two or more polypeptide chains are held together by non-covalent forces

by hydrogen bonds between NH and C = O groups of the main chain. Each turn is formed by 3.6 residues. The distance between each amino acid residue (translation) is 1.5 Å. The alpha-helix is generally **right handed**. In proteins like hemoglobin and myoglobin, the alpha-helix is abundant. The alpha-helix is the most common and stable conformation for a polypeptide chain.

Beta-pleated sheet

The polypeptide chains in beta-pleated sheet is almost fully extended (Fig. 2.23). It is stabilized by hydrogen bonds between NH and C = O groups of neighboring polypeptide segments. Beta-pleated sheet is the major structural motif in proteins like silk Fibroin and carbonic anhydrase.

Tertiary Structure

The secondary and tertiary structures cannot be demarcated by a definite rule and these levels overlap each other. Secondary structure defines the organization at immediate vicinity of amino acids. The tertiary structure denotes three dimensional structure of the whole protein. The tertiary structure is maintained by hydrophobic bonds, electrostatic bonds and van der Waals forces.

Quaternary Structure

Certain polypeptides will **aggregate** to form one functional protein. This is referred to as the quaternary structure (Fig. 2.24). A summary is shown in Box 2.2. The protein will lose its function when the subunits are dissociated.

Depending on the number of monomers, the protein may be termed as dimer (2), tetramer (4), etc. Each polypeptide chain is termed as **subunit** or **monomer.** For example, 2 alpha-chains and 2 beta-chains form the **hemoglobin** molecule; 2 heavy chains and 2 light chains combine to form one molecule of **immunoglobulin** G.

Structure-Function Relationship

The three dimensional structural conformation provides and maintains the functional characteristics. The three dimensional structure, in turn, is dependent on the primary structure. So, any difference in the primary structure may produce a protein which cannot serve its function. To illustrate the structure–function relationship, the following three proteins are considered.

Hemoglobin

It is the transporter of oxygen is a tetrameric protein (a_2b_2), with each monomer having a heme unit (see Chapter 14). Binding of oxygen to one heme facilitates oxygen binding by other subunits. Binding of H^+ and CO_2 promotes release of O_2 from hemoglobin. Even a single amino acid substitution alters the structure and thereby the function. For example, in sickle cell anemia (HbS), the 6th amino acid in the beta chain is altered from normal glutamic acid to abnormal valine. This leads to profound clinical manifestations.

Collagen

It is the most abundant protein in mammals and is the main fibrous component of skin, bone, tendon, cartilage and teeth. Collagen forms a superhelical cable where the *3 polypeptide chains are wound around* (see Chapter 21). In collagen, every third residue is a glycine. The only amino acid that can fit into the triple stranded helix is glycine. The **quarter staggered triple helical structure** of collagen is responsible for its tensile strength.

Keratin

Keratin is the protein present in hair and nails. It is a fibrous protein having a specialized secondary structure called coiled coil .The protein is rich in cysteine and the properties of the keratin present in different tissues is due to the differences in the number and position of disulfide bonds.

PHYSICAL PROPERTIES OF PROTEINS

Protein solutions exhibit colloidal properties and therefore scatter light and exert **osmotic pressure**. Osmotic pressure of plasma proteins is clinically important (Chapter 12). **Molecular weights** of some of the proteins are: Insulin (5,700); hemoglobin (68,000); albumin (69,000); immunoglobulins 1,50,000. **Shape** of the proteins also vary. Thus, insulin is globular, albumin is oval in shape, while fibrinogen molecule is elongated. Bigger and elongated molecules will increase the viscosity of the solution.

ISOELECTRIC PH (PI) OF PROTEINS

The isoelectric pH of amino acids has been described earlier in this chapter. The amino acid composition will determine the isoelectric pH (pI) of protein. All the ionisable groups present in the protein will influence the pI of the protein. At the isoelectric point, the number of anions and cations present on the protein molecule will be equal and the **net charge is zero** (See Fig. 2.13). At the pI value, the proteins **will not migrate** in an electrical field; solubility, buffering capacity and viscosity will be minimum and **precipitation** will be maximum. On the acidic side of pI, the proteins are cations and on alkaline side, they are anions in nature. The pI of pepsin is 1.1; casein 4.6; human albumin 4.7; human globulins 6.4; human hemoglobin 6.7; Myoglobin 7; and Lysozyme 11.

PRECIPITATION REACTIONS

The stability of proteins in solution will depend mainly on the charge and hydration. Polar groups of the proteins (-NH_2, COOH, OH groups) tend to attract water molecules around them to produce a shell of hydration. *Any factor which neutralizes the charge or removes water of hydration will therefore cause precipitation of proteins.* The following common procedures are used for protein precipitation:

Salting Out

When a neutral salt such as **ammonium sulfate** or sodium sulfate is added to protein solution, the shell of hydration is removed and the protein is precipitated. This is called salting out.

Isoelectric Precipitation

All proteins are least soluble at their isoelectric pH. Some proteins are precipitated immediately when adjusted to their isoelectric pH. The best example is **Casein** which forms a flocculent precipitate at pH 4.6 and redissolves in highly acidic or alkaline solutions. When milk is curdled, the casein forms the white curd, because lactic acid produced by the fermentation process lowers the pH to the isoelectric point of casein.

Precipitation by Organic Solvents

When an organic solvent is added to the protein solution, water molecules available for proteins are reduced, and

precipitation occurs. Organic solvents reduce the dielectric constant of the medium which also favors protein precipitation. Hence ether and **alcohol** are powerful protein precipitating agents. This may explain the disinfectant effect of alcohol.

Precipitation by Heavy Metal Ions

Proteins can also be precipitated by heavy metal ions like copper, mercury, lead, etc. that are positively charged. So they are toxic when consumed.

Anionic or Alkaloidal Reagents

Precipitation of proteins by negatively charged anionic reagents like sulfosalicylic acid, picric acid, etc. form the basis of estimation of proteins in urine and CSF. In acidic medium proteins are cations, which then complex with negatively charged ions to form protein-picrate, etc. **Tanning** in leather processing is based on the protein precipitating effect of tannic acid.

DENATURATION OF PROTEINS

Brief heating, urea, salicylate, X-ray, ultraviolet rays, high pressure, vigorous shaking and such other physicochemical agents produce non-specific alterations in secondary, tertiary and quaternary structures of protein molecules. This is called denaturation. **Primary structure is not altered** during the process of denaturation (Fig. 2.25). It generally decreases the solubility, increases precipitability and often causes **loss of biological activity**. Native proteins are often resistant to proteolytic enzymes, but denatured proteins will have more exposed sites for enzyme action. Since cooking leads to denaturation of proteins, cooked foods are more easily digested.

HEAT COAGULATION

When heated at isoelectric point, some proteins will denature irreversibly to produce thick floating conglomerates called coagulum. This process is called heat coagulation. **Albumin** is easily coagulated, and globulins to a lesser extent. Some proteins when heated, though denatured, are still soluble, they may be precipitated by bringing to isoelectric pH. This is the basis of "heat and acetic acid test", very commonly employed to detect the presence of albumin in urine (Chapter 30).

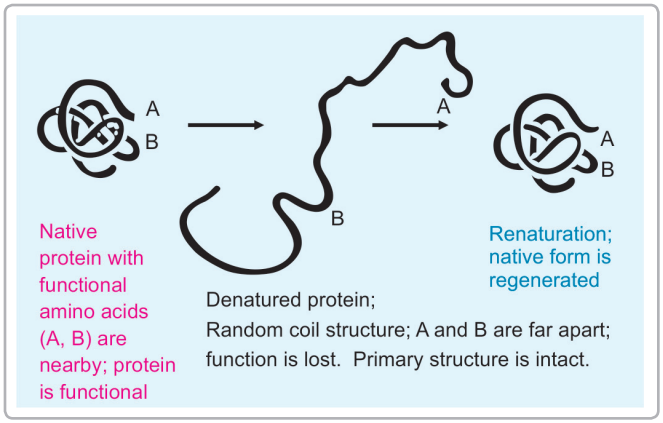


Figure 2.25: Denaturation of protein

CLASSIFICATION OF PROTEINS

1. Classification based on Functions

- Catalytic proteins, e.g. enzymes
- Structural proteins, e.g. collagen, elastin, keratin
- Contractile proteins, e.g. myosin, actin, flagellar proteins
- Transport proteins, e.g. hemoglobin, myoglobin, albumin, transferrin
- Regulatory proteins or hormones, e.g. ACTH, insulin, growth hormone
- Genetic proteins, e.g. histones
- Protective proteins, e.g. immunoglobulins, clotting factors.

2. Classification based on Solubility

Proteins may be divided into three major groups—*simple*, *conjugated* and *derived*.

Simple Proteins

According to definition, they contain only amino acids. But they also contain very small quantity of carbohydrates.

- **Albumins:** They are soluble in water and coagulated by heat. Human serum albumin has a molecular weight of 69,000.
- **Globulins:** These are insoluble in pure water, but soluble in dilute salt solutions. They are also coagulated by heat. Examples are egg globulin and serum globulins.
- **Protamines:** They contain large number of arginine and lysine residues, and so are strongly basic.
- **Scleroproteins:** They form supporting tissues. Examples are collagen of bone, cartilage and tendon; keratin of hair, horn, nail and hoof.

Conjugated Proteins

They are combinations of protein with a non-protein part, called **prosthetic group** (Table 2.3). Conjugated proteins may be classified as follows:

- **Glycoproteins:** These are proteins combined with carbohydrates.

Table 2.3. Examples of conjugated proteins

Conjugated protein	*Protein part*	*Prosthetic group*
Hemoglobin	Globin	Heme
Nucleoprotein	Histones	DNA
Rhodopsin	Opsin	11-cis-retinal
Ferritin	Apoferritin	Iron
Ceruloplasmin	Apoceruloplasmin	Copper

- **Lipoproteins:** These are proteins loosely combined with lipid components. They occur in blood and on cell membranes. Serum lipoproteins are described in Chapter 11.
- **Nucleoproteins:** These are proteins attached to nucleic acids, e.g. histones. The DNA carries negative charges, which combines with positively charged proteins.
- **Chromoproteins:** These are proteins with colored prosthetic groups. Hemoglobin (Heme, red); Flavoproteins (Riboflavin, yellow), Visual purple (Vitamin A, purple) are some examples of chromoproteins.
- **Phosphoproteins:** These contain phosphorus. Casein of milk and vitellin of egg yolk are examples. The phosphoric acid is added to the hydroxyl groups of serine and threonine residues of proteins.
- **Metalloproteins:** They contain metal ions. Examples are hemoglobin (Iron), cytochrome (Iron), tyrosinase (Copper) and carbonic anhydrase (Zinc).

Derived Proteins

They are degradation products of native proteins.

3. Classification based on Nutritional value

Nutritionally rich proteins

They are also called as complete proteins or **first class proteins**. They contain all the essential amino acids in the required proportion. On supplying, these proteins in the diet, the young individuals will grow satisfactorily. A good example is **casein** of milk.

Incomplete proteins

They lack one essential amino acid. They cannot promote body growth in young individuals; but may be able to sustain the body weight in adults. Proteins from **pulses are deficient in methionine**, while proteins of cereals lack in lysine. If both of them are combined in the diet, good growth could be obtained.

Poor proteins

They lack in many essential amino acids and a diet based on these proteins will not even sustain the original body weight. **Zein** from corn lacks tryptophan and lysine.

Related Topics

Plasma proteins are described in Chapter 12; RIA and ELISA tests are described in Chapter 28.

A QUICK LOOK

1. Most amino acids in the body are alpha amino acids.
2. Amino acids can be classified based on their (i) Structure; (ii) Side chain characters; (iii) Metabolic fate; (iv) Nutritional requirements.
3. All proteins are made of 20 common amino acids linked by peptide bonds.
4. Amino acids may be classified based on the nature of side chains into aliphatic, aromatic and imino acids.
5. Aliphatic amino acids may be simple, branched chain, hydroxyl, sulfur containing and those with amide groups.
6. Acidic amino acids have more than one COOH group and basic amino acids have more than one amino group.
7. Derived amino acids may be found in proteins, or they may be non-protein amino acids formed as metabolic intermediates.
8. A functional classification based on the polar or nonpolar and ionized and non-ionized nature of side chains is more useful.
9. Based on the metabolic fate, amino acids may be glucogenic, ketogenic or both.
10. Depending on nutritional requirements they may be essential or nonessential.
11. In solution, amino acids exist as 'Zwitterions' or 'Ampholytes' at their characteristic Isoelectric pH (pI).
12. Isoelectric pH is the pH at which amino acids have no net charge, no mobility in electric field and are less soluble.
13. Each amino acid has a specific isoelectric pH or pI depending on the pK value of the ionizable groups.
14. Amino acids possessing an asymmetric carbon atom have optical activity and all naturally occurring amino acids are L amino acids.
15. Glycine has no asymmetric carbon atom and therefore has no optical activity.
16. Decarboxylation and amide formation are reactions involving alpha COOH group.
17. Transamination, oxidative deamination and formation of carbamino compounds are reactions where amino group takes part.
18. Peptide bonds are formed between the alpha amino group of one amino acid and the alpha carboxyl group of the next with elimination of a water molecule.
19. Polymers of amino acids linked by peptide bonds are called polypeptides.
20. Peptide bonds are covalent bonds between C = O and NH2 groups having a transconfiguration.
21. Depending on the number of amino acids, oligopeptides (10), polypeptides (10–50) and proteins (>50) are formed.

22. Nitrogen content of ordinary proteins is on the average 16% by weight.
23. Protein structure can be defined and studied at four levels viz. primary, secondary, tertiary and quaternary.
24. Proteins have the primary level of structure which denotes the linear sequence of amino acids linked by peptide bonds.
25. The primary sequence is genetically determined and is unique and fixed for each protein produced by a particular species of organism.
26. Primary structure determines the biological activity of the protein. Alterations lead to loss of functional capacity, e.g. Sickle cell hemoglobin (HbS).
27. The N terminal amino acid is the 1st amino acid having a free alpha NH2 group and the C terminal amino acid, (the last amino acid) has a free alpha COOH group.
28. A protein having more than one polypeptide chain, like insulin, has interchain disulfide bonds to hold the chains together. Cysteine forms disulfide linkages between two polypeptide chains in oligomeric proteins.
29. Secondary and tertiary levels of structure are maintained by noncovalent bonds.
30. The noncovalent bonds maintaining the higher levels of structure are hydrogen bonds, ionic bonds (electrostatic bonds), hydrophobic interactions and van der Waals forces between the side chains of amino acids.
31. The two major types of secondary structure are alpha helix and beta pleated sheet structure found in fibrous proteins. A beta-pleated sheet may further be parallel or anti-parallel.
32. Tertiary structure of a protein is the most thermodynamically stable configuration.
33. Quaternary structure is present only in certain proteins having more than one polypeptide chain, e.g. Hemoglobin.
34. The function of a protein is dependent on subunit interaction.
35. Chemical properties of the proteins depend on the colloidal nature of the particles in solution and the nature of the side chains. This explains the precipitation reactions and color reactions of proteins.
36. Solubility of a protein is dependent on the ionic concentration of the medium. Hence, proteins may be 'salted in' or 'salted out'.
37. Denaturation of protein results in loss of biological activity but not the primary structure. Denaturation may be reversible.
38. Proteins can be classified based on (i) Functions; (ii) Composition; (iii) Shape; and (iv) Nutritional value.
39. Methods of protein estimation include colorimetry (Biuret and Lowry's method) and enzyme linked immunosorbent assay (ELISA).

CHAPTER 3

Enzymology

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Classification of enzymes
- Coenzymes
- Fischer's template theory
- Koshland's induced fit theory
- Michaelis constant, Km value, Vmax
- Factors influencing enzyme activity
- Inhibition, competitive, noncompetitive
- Allosteric inhibition
- Isoenzymes
- Lactate dehydrogenase and creatine kinase
- Alkaline phosphatase and acid phosphatase

Once upon a time, there was a rich merchant. In his last will and testament, he put aside his 17 white horses to his 3 sons to be shared thus; 1/2 for the 1st son, 1/3 for the 2nd son and 1/9 for the 3rd son. After his death, the sons started to quarrel, as the division could not produce whole number. Then their brother-in-law told them that they should include his black horse also for the sharing purpose. Thus now they had 17 + 1 = 18 horses, and so division was possible; 1st son got one-half or 9 horses; 2nd son got 6 and 3rd son had 2 horses. Now all the 17 white horses were correctly divided among the sons. The remaining black horse was taken back by the brother-in-law. **Catalysts** are similar to this black horse. The reaction, although theoretically probable, becomes practically possible only with the help of catalysts. They enter into the reaction, but come out of the reaction without any change. Catalysts are substances which accelerate the rate of chemical reactions, but do not change the equilibrium.

Enzymes are biocatalysts. Life is possible due to the coordination of numerous metabolic reactions inside the cells. Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long time; but inside the body, with the help of enzymes, proteolysis takes place within a short time at body temperature. Lack of enzymes will lead to block in metabolic pathways causing **inborn errors of metabolism**.

The substance upon which an enzyme acts, is called the **substrate**. The enzyme will convert the substrate into the **product** or products.

All enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins. They are heat labile, soluble in water, precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid) and contain 16% weight as nitrogen.

CLASSIFICATION OF ENZYMES

Early workers gave whimsical names such as Pepsin, Trypsin, Chymotrypsin, etc., some of which are still used. Later, enzymes are named by adding the suffix "-ase" to the substrate. Thus, enzyme lactase acts on the substrate lactose. These are known as the **trivial names** of the enzymes. But there may be more than one enzyme acting on the same substrate.

IUBMB system of nomenclature of enzymes

International Union of Biochemistry and Molecular Biology (IUBMB) in 1964, (modified in 1972 and 1978), suggested the nomenclature of enzymes. It is complex and cumbersome; but unambiguous.

As per this system, the *name starts with EC (enzyme class) followed by 4 digits.* The first digit represents the

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class; second digit stands for the subclass; third digit is the sub-sub class or subgroup; and the 4th digit gives the number of the particular enzyme in the list. The enzymes are *grouped into 6 major classes* (Table 3.1). For example, Class 1 is called oxidoreductases.

COENZYMES

Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the **prosthetic group.** The protein part of the enzyme is then named the **apoenzyme**, the prosthetic group the **coenzyme**; and these two portions combined together is called the **holoenzyme**. The coenzyme is essential for the biological activity of the enzyme. A coenzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction. One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of enzyme. Coenzymes may be divided into **a)** Those taking part in reactions catalyzed by *oxidoreductases* by donating or accepting hydrogen atoms or electrons. **b)** Those coenzymes taking part in reactions transferring groups other than hydrogen.

First Group of Coenzymes

In the first group, the change occurring in the substrate is counter-balanced by the coenzymes. Therefore, such co-enzymes may be considered as cosubstrates or secondary substrates. In the example shown in Fig. 3.1, the substrate lactate is oxidized, and simultaneously the coenzyme (cosubstrate) is reduced. If the reaction is reversed, the opposite effect will take place. Other coenzymes engaged in oxidoreductase reactions are NADP, FAD and FMN.

Nicotinamide Adenine Dinucleotide (NAD^+)

This is a coenzyme synthesized from nicotinamide, a member of vitamin B complex. The structure of NAD^+ could

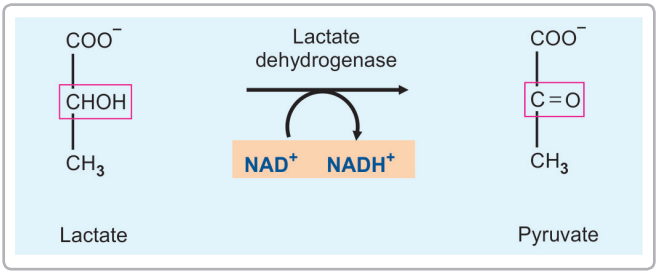


Figure 3.1: Reaction of lactate dehydrogenase

be written as Nicotinamide-Ribose-P-P-Ribose-Adenine (Fig. 16.3). The reversible reaction of lactate to pyruvate is catalyzed by the enzyme lactate dehydrogenase, but the actual transfer of hydrogen is taking place on the coenzyme, NAD^+ (Fig. 3.1). Two hydrogen atoms are removed from lactate, out of which one hydrogen and two electrons are accepted by the NAD^+ to form NADH, and the remaining H^+ is released into the surrounding medium. The hydrogen is accepted by the **nicotinamide** group.

Second Group of Coenzymes

Those coenzymes taking part in *reactions transferring groups other than hydrogen*, may be considered as the second category. A particular group or radical is transferred from the substrate to another substrate or from the coenzyme to the substrate. Here also coenzymes may be considered as cosubstrates. Most of them belong to vitamin B complex group. A few such examples are given in Table 3.2.

Adenosine Triphosphate (ATP)

Fiske and Subba Rao first isolated ATP from muscle in 1929 and showed the importance of ATP in muscle contraction. ATP is considered to be the **energy currency** in the body. During the oxidation of food stuffs, energy is released, a part of which is stored as **chemical energy** in the form of ATP. The structure of ATP is discribed in Figure 3.2.

Table 3.1: Classification of enzymes

Class	*Name*	*Function*	*Example*
Class 1	Oxidoreductases	Transfer of hydrogen	Alcohol dehydrogenase
Class 2	Transferases	Transfer of groups other than hydrogen	Subclass: Kinase, transfer of phosphoryl group from ATP, e.g. hexokinase
Class 3	Hydrolases	Cleave bond; adds water	acetyl choline esterase
Class 4	Lyases	Cleave without adding water	Aldolase. (Subclass: Hydratase; add water to double bond)
Class 5	Isomerases	Intramolecular transfers	This class includes racemases and epimerases. For example, triose phosphate isomerase.
Class 6	Ligases	ATP dependent condensation of two molecules	Acetyl-CoA carboxylase

Table 3.2: Examples of coenzymes	
Co-enzyme	*Group transferred*
Thiamine pyrophosphate (TPP)	Hydroxyethyl
Pyridoxal phosphate (PLP)	Amino group
Biotin	Carbon dioxide
Coenzyme-A (Co-A)	Acyl groups
Tetrahydrofolate (FH4)	One carbon groups
Adenosine triphosphate (ATP)	Phosphate

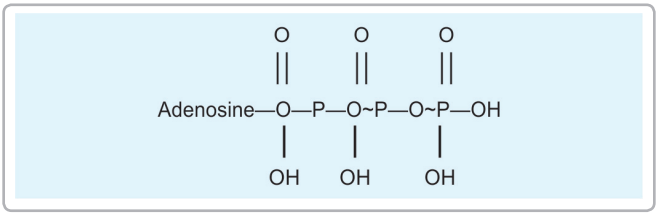


Figure 3.2: Adenosine triphosphate (ATP)

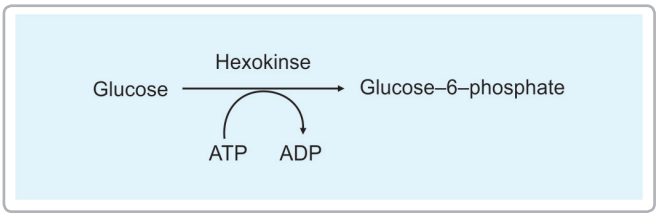


Figure 3.3: Hexokinase reaction

In the ATP molecule, the second and third phosphate bonds are '**high energy**' bonds. The endergonic reactions are carried out with the help of energy released from the hydrolysis of ATP (Fig. 3.3).

Salient features of Coenzymes

In general, the protein part of the enzyme gives the necessary three dimensional infrastructure for chemical reaction; but the group is transferred from or accepted by the coenzyme. Coenzymes are heat stable. They are low-molecular weight substances. The coenzymes combine loosely with the enzyme molecules and so, the coenzyme can be separated easily by **dialysis**. When the reaction is completed, the coenzyme is released from the apoenzyme, and goes to some other reaction site (see Figure 5.7).

Metalloenzymes

These are enzymes which require certain metal ions for their activity. Some examples are given in Table 3.3.

Table 3.3: Metalloenzymes	
Metal	*Enzyme containing the metal*
Zinc	Carbonic anhydrase, carboxy peptidase, alkaline phosphatase
Magnesium	Hexokinase, phosphofructokinase, enolase
Manganese	Hexokinase, enolase
Copper	Tyrosinase, cytochrome oxidase, superoxide dismutase
Iron	Cytochrome oxidase, xanthine oxidase
Calcium	Lecithinase, lipase
Molybdenum	Xanthine oxidase

In certain cases, e.g. copper in tyrosinase, the metal is tightly bound with the enzyme. In other cases, when metal ion is removed from the enzyme, the activity of the enzyme will be minimal; but when the metal ion is added, the activity is enhanced. They are called **ion-activated enzymes,** e.g. chloride ions will activate salivary amylase.

MODE OF ACTION OF ENZYMES

There are a few theories explaining the *mechanism of action of enzymes*.

1. Lowering of Activation Energy

Substrates are remaining in an energy trough, and are to be placed at a higher energy level, whereupon spontaneous degradation can occur. Suppose, we want to make a fire; even if we keep a flame, the wood will not burn initially; we have to add kerosene or paper for initial burning. Similarly, the activation energy is to be initially supplied. **Activation energy** is defined as the energy required to convert all molecules in one mole of a reacting substance from the ground state to the transition state. Enzymes reduce the magnitude of this activation energy. This can be compared to making a tunnel in a mountain, so that the barrier could be lowered (Fig. 3.4). For example, activation energy for hydrolysis of sucrose by H^+ is 26,000 cal/mol, while the activation energy is only 9,000 cal/mol when hydrolyzed by sucrase.

2. Michaelis-Menten Theory

Lenor Michaelis and Maud Menten (1913) put forward the **Enzyme–Substrate complex theory**. The enzyme (E) combines with the substrate (S), to form an

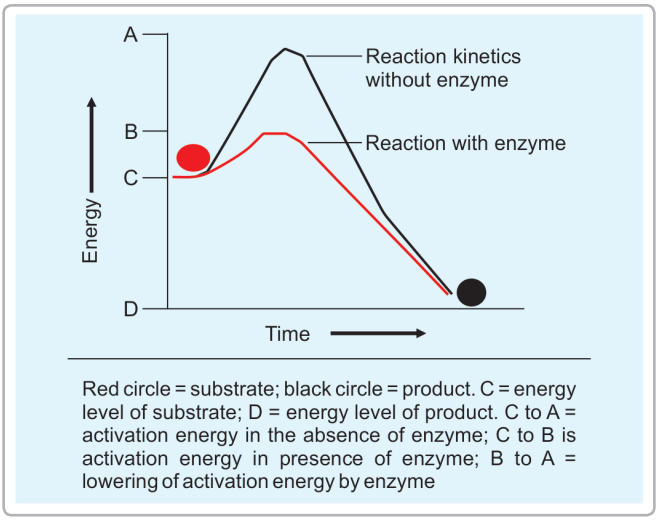


Figure 3.4: Lowering of activation energy by enzymes

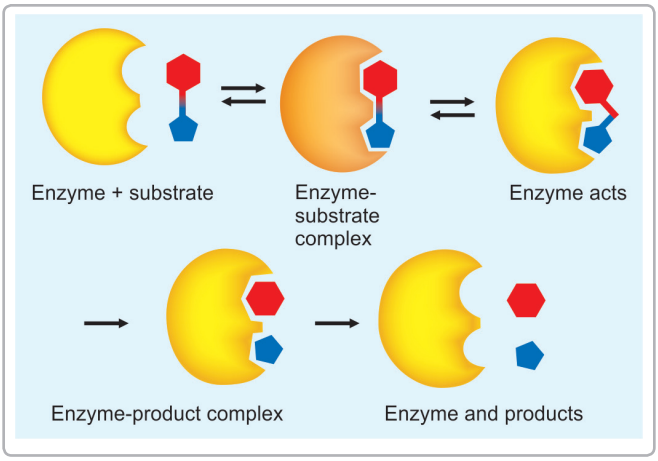


Figure 3.5: Enzyme substrate complex

enzymesubstrate (ES) complex, which immediately breaks down to the enzyme and the product (P). (Fig. 3.5).

E + S → E–S Complex → E + P

3. Fischer's Template Theory

It states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus *enzyme and substrate fit each other*. (Fig. 3.6). The explanation is that substrate fits on the enzyme, similar to **lock and key**. The key will fit only to its own lock. Koshland proposed induced fit theory which explained among other things covalent inhibition.

Koshland's Induced Fit Theory

The *substrate induces conformational changes in the enzyme*, such that precise orientation of catalytic groups is effected.

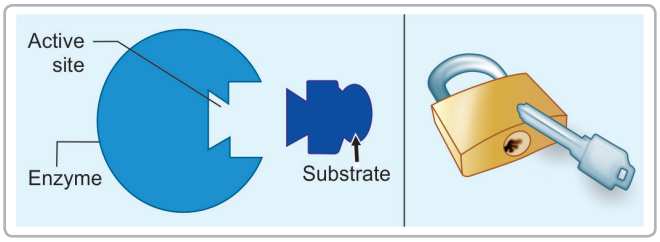


Figure 3.6: Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory)

ACTIVE SITE OR ACTIVE CENTRE

That area of the enzyme where catalysis occurs is referred to as active site or active center. For example, Serine is the important amino acid at the catalytic site of Trypsin. Salient features of the active sites of the enzymes are:

Although all parts are required for keeping the exact three dimensional structure of the enzyme, the reaction is taking place at the active site. The active site occupies only a small portion of the whole enzyme. Generally active site is situated in a crevice or cleft of the enzyme molecule. The amino acids or groups that directly participate in making or breaking the bonds (present at the active site) are called catalytic residues or catalytic groups. To the active site, the specific substrate is bound. The binding of substrate to active site depends on the alignment of specific groups or atoms at active site. During the binding, these groups may realign themselves to provide the unique conformational orientation so as to promote exact fitting of substrate to the active site. Proteolytic enzymes having a serine residue at the active center are called **serine proteases**, e.g. trypsin, chymotrypsin and coagulation factors.

THERMODYNAMICS

From the standpoint of energy, the enzymatic reactions are divided into 3 types:

1. Exergonic or exothermic reaction

Here energy is released from the reaction, and therefore reaction essentially goes to completion, e.g. **urease** enzyme:

$$\text{Urea} \rightarrow \text{ammonia} + CO_2 + \text{energy}$$

At equilibrium of this reaction, the substrate will be only 0.5% and product will be 99.5%. Such reactions are generally irreversible.

2. Isothermic reaction

When energy exchange is negligible, and the reaction is easily reversible, e.g.

Glycogen + Pi → Glucose-1-phosphate

At equilibrium of this reaction, 77% glycogen will be unutilized and 23% glucose-1-phosphate will be formed.

3. Endergonic or endothermic reaction

Energy is consumed and external energy is to be supplied for these reactions. In the body, this is usually accomplished by coupling the endergonic reaction with an exergonic reaction, e.g. **hexokinase reaction**

Glucose + ATP → Glucose-6-Phosphate + ADP

ENZYME KINETICS

The reaction rate or velocity is proportional to the concentration of reacting molecules.

$$A + B \longrightarrow C + D$$

At equilibrium, forward reaction and backward reaction are equal, so that

$$A + B \underset{K2}{\overset{K1}{\rightleftharpoons}} C + D$$

At equilibrium, forward and backward reactions are equal. Equilibrium is a dynamic state. Even though no net change in concentrations of substrate and product occurs, molecules are always interconverted. Enzyme makes it quicker to reach the equilibrium. Catalysts increase the rate of reaction, but do not alter the equilibrium.

FACTORS INFLUENCING ENZYME ACTIVITY

1. Enzyme Concentration

Velocity of reaction is increased proportionately with the concentration of enzyme, when substrate concentration is unlimited (Fig. 3.7). Hence this property is made use of in determining the level of particular enzyme in plasma, serum or tissues.

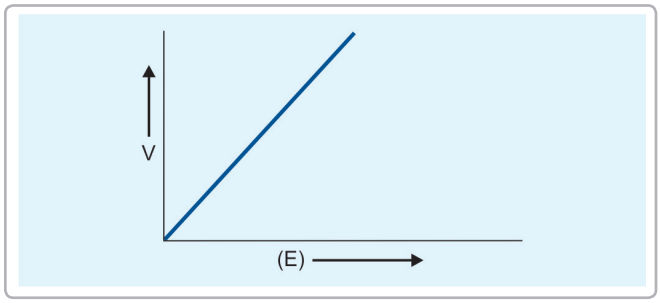


Figure 3.7: Effect of enzyme concentration

2. Substrate Concentration

If the velocity is plotted against the substrate concentration, a typical curve (Fig. 3.8) will be obtained. As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the curve flattens afterwards. *The maximum velocity thus obtained is called* Vmax.

Michaelis Constant

According to Michaelis theory, the formation of enzyme–substrate complex is a reversible reaction, while the breakdown of the complex to enzyme + product is irreversible.

$$E + S \xrightarrow{K1} E - S \underset{K2}{\overset{K3}{\rightleftharpoons}} E + P$$

If concentration of substrate is increased, the forward reaction K1 is increased, and so K3 as well as total velocity is correspondingly enhanced. The three different constants may be made into one equation,

$$Km = \frac{K2 + K3}{K1}$$

This Km is called as **Michaelis Constant**. It is further shown that

$$v = \tfrac{1}{2}\ Vmax$$

In the Fig. 3.10, 50% velocity in Y axis is extrapolated to the corresponding point in X-axis, which gives the numerical value of Km.

Definition of Km

Substrate concentration (expressed in moles/L) **at half-maximal velocity** is the Km value. It denotes that 50% of *enzyme molecules are bound with substrate* molecules at that particular substrate concentration. Km is **independent of enzyme concentration**. If enzyme concentration is doubled, the Vmax will be double.

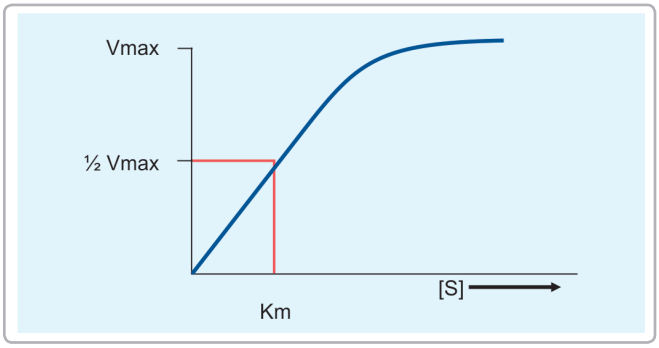


Figure 3.8: Effect of substrate concentration (substrate saturation curve)

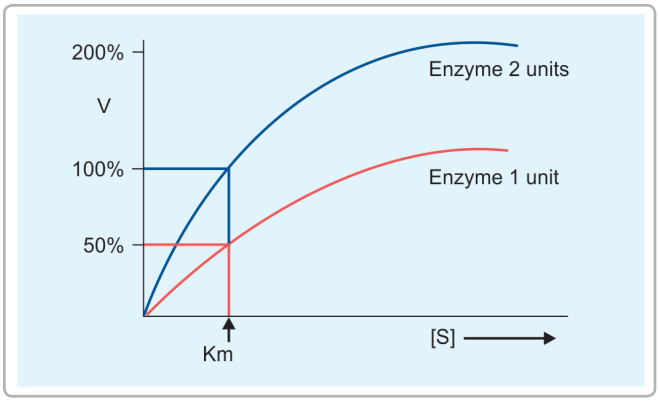


Figure 3.9: Effect of enzyme concentration on Km

But the ½ Vmax (Km) will remain exactly same. (Fig. 3.9). **Km is the signature of the enzyme:** Km value is thus a constant for an enzyme. It is the *characteristic feature of a particular enzyme* for a specific substrate. **Km denotes the affinity of enzyme to substrate:** The *lesser the numerical value of Km, the affinity of the enzyme for the substrate is more.* To cite an example, Km of **glucokinase** is 10 mmol/L and that of **hexokinase** is 0.05 mmol/L. Therefore, hexokinase has more affinity for glucose than glucokinase.

3. Effect of Concentration of Products

In a reversible reaction, S <--------> P, when equilibrium is reached, as per the law of mass action, the reaction rate is slowed down. So when product concentration is increased, the reaction is slowed, stopped or even reversed. In inborn errors of metabolism, one enzyme of a metabolic pathway is blocked. For example,

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D$$

If E3 enzyme is absent, C will accumulate, which in turn, will inhibit E2. Consequently, in course of time, the whole pathway is blocked.

4. Effect of Temperature

The velocity of enzyme reaction increases when temperature of the medium is increased; reaches a maximum and then falls (Bell-shaped curve). The temperature at which maximum amount of the substrate is converted to the product per unit time is called the optimum temperature (Fig. 3.10). As temperature is increased, more molecules get activation energy, or molecules are at increased rate of motion. So their collision probabilities are increased and so the reaction velocity is enhanced. But when temperature is more than 50°C, heat denaturation and consequent loss of tertiary structure of protein occurs. So activity of the enzyme is decreased. Most human enzymes have the optimum temperature around 37°C.

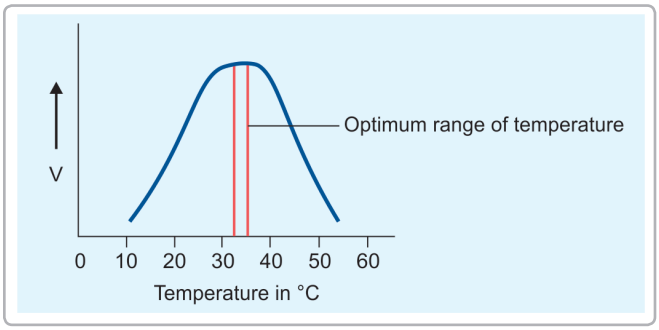


Figure 3.10: Effect of temperature on velocity

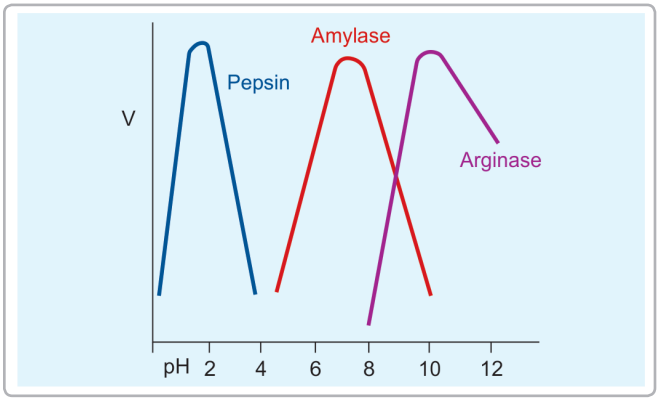


Figure 3.11: Effect of pH on enzyme velocity

5. Effect of pH

Each enzyme has an optimum pH, on both sides of which the velocity will be drastically reduced. The graph will show a bell-shaped curve (Fig. 3.11). The pH decides the charge on the amino acid residues at the active site. Usually enzymes have the optimum pH between 6 and 8. Some important exceptions are Pepsin (with optimum pH 1–2); alkaline phosphatase (optimum pH 9–10) and acid phosphatase (4–5).

ENZYME ACTIVATION

In presence of certain **metallic ions**, some enzymes show higher activity. Thus, chloride ions will activate salivary amylase and calcium will activate lipases. Another type of activation is the conversion of an inactive **proenzyme** or **zymogen** to the active enzyme. Thus by splitting a single peptide bond, and removal of a small polypeptide from **trypsinogen**, the active trypsin is formed. This results in

unmasking of the active center. Similarly trypsin activates **chymotrypsinogen**. All the gastrointestinal enzymes are synthesized in the form of proenzymes, and only after secretion into the alimentary canal, they are activated. This prevents autolysis of cellular structural proteins.

Covalent Modification

The process of altering the activity of enzymes by adding or removing groups (breaking or making covalent bonds) is called covalent modification. Two typical examples are partial proteolysis and addition and removal of phosphate groups. Activation of zymogens to the functional enzyme is by partial proteolysis, that exposes the active site to which substrate can bind. All digestive enzymes are secreted as zymogens that are further activated, e.g. trypsinogen to trypsin, pepsinogen to pepsin. Covalent modification by phosphorylation and dephosphorylation is brought about by the action of hormones. Binding of hormones to G protein coupled receptors leads to activation of a cyclic AMP mediated cascade activation pathway. (For details refer Chapter 31). Protein kinases are activated that add phosphate groups to enzyme proteins. Some enzymes become active on phosphorylation, e.g. Glycogen phosphorylase. The removal of the phosphate group by specific protein phosphatases, activates some enzymes, e.g. Glycogen synthase. Opposing metabolic pathways are thus reciprocally regulated so that futile reaction cycles will not take place.

ENZYME INHIBITION

All the reactions in the body are appropriately controlled. Control of the whole pathway is achieved by inhibition of such **key enzymes** or regulatory enzymes.

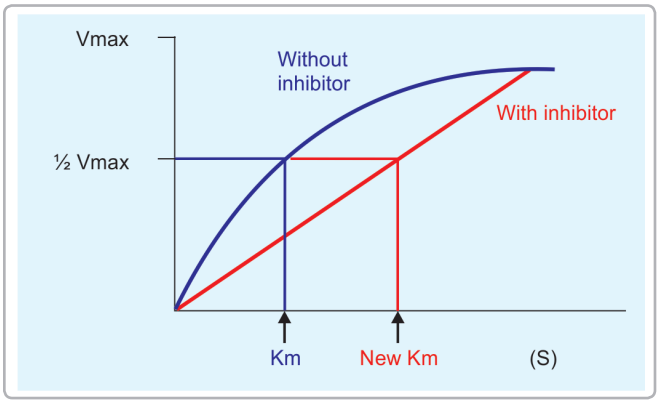


Figure 3.12: Substrate saturation curve in presence and absence of competitive inhibitor

1. Competitive Inhibition

The *inhibitor molecules are competing with the normal substrate molecules for attacking with the active site of the enzyme.*

$$E + S \longrightarrow E\text{-}S \longrightarrow E + P$$
$$E + I \longrightarrow E\text{-}I$$

Since E-I (enzyme–inhibitor complex) can react only to reform the enzyme and inhibitor, the number of enzyme molecules available for E-S formation is reduced. Suppose 100 molecules of substrate and 100 molecules of inhibitor are competing for 100 molecules of the enzyme. So, half of enzyme molecules are trapped by the inhibitor and only half the molecules are available for catalysis to form the product. Since effective concentration of enzyme is reduced, the reaction **velocity is decreased**. (Fig. 3.12).

In competitive inhibition, the inhibitor will be a **structural analogue** of the substrate. There will be similarity in three dimensional structure between substrate (S) and inhibitor (I). For example, the succinate dehydrogenase reaction is inhibited by malonate, which are structural analogues of succinate. (Fig. 3.13). Competitive inhibition is usually **reversible.** *Excess substrate abolishes the inhibition.* If substrate concentration is enormously high when compared to inhibitor, then the inhibition is reversed (Fig. 3.12). From the graphs, it is seen that in the case of competitive inhibition, the **Km is increased** in presence of competitive inhibitor *but Vmax is not changed.* Thus competitive inhibitor apparently increases the Km.

Clinical Significance

Pharmacological action of many drugs may be explained by the principle of competitive inhibition. A few important examples are given below: **Sulphonamides** are commonly employed antibacterial agents. (Fig. 3.14). Bacteria synthesize folic acid by combining PABA with pteroylglutamic acid. Bacterial wall is impermeable to folic acid. Sulfa drugs, being structural analogues of PABA,

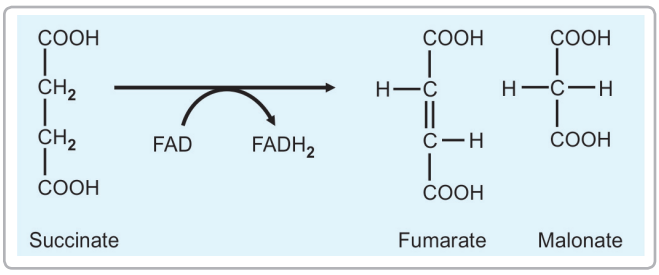


Figure 3.13: Malonate inhibits succinate dehydrogenase

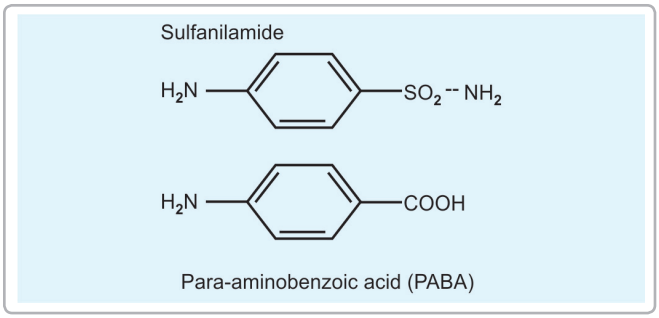


Figure 3.14: Competitive inhibition

will inhibit the folic acid synthesis in bacteria, and they die. The drug is nontoxic to human cells, because human beings cannot synthesize folic acid. Preformed folic acid is essential for man. **Methotrexate** (4-amino-N^{10}-methyl folic acid) is a structural analogue of folic acid, and so can competitively inhibit folate reductase enzyme. This is essential for DNA synthesis and cell division. Therefore, methotrexate is used as an anticancer drug.

2. Noncompetitive Inhibition

A variety of **poisons**, such as iodoacetate, heavy metal ions (silver, mercury) and oxidizing agents act as irreversible noncompetitive inhibitors. The inhibitor usually binds to a different domain on the enzyme, other than the substrate binding site. Since these i*nhibitors have no structural resemblance to the substrate*, an increase in the substrate concentration generally does not relieve this inhibition. (Fig. 3.15). **Cyanide** inhibits cytochrome oxidase. **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis. The inhibitor combines with the enzymes and the reaction becomes **irreversible**. The velocity (Vmax) is reduced. But **Km value is not changed**, because the remaining enzyme molecules have the same affinity for the substrate. *Increasing the substrate concentration will abolish the competitive inhibition, but will not abolish non-competitive inhibition.* A comparison of the two types of inhibitions is shown in Table 3.4.

3. Allosteric Regulation

Allosteric enzyme has one catalytic site where the substrate binds and another **separate allosteric site** where the modifier binds (*allo* = other) (Fig. 3.17). In most cases, the substrate saturation curve is sigmoid in shape (Fig. 3.16). Most allosteric enzymes are made up of subunits, e.g.

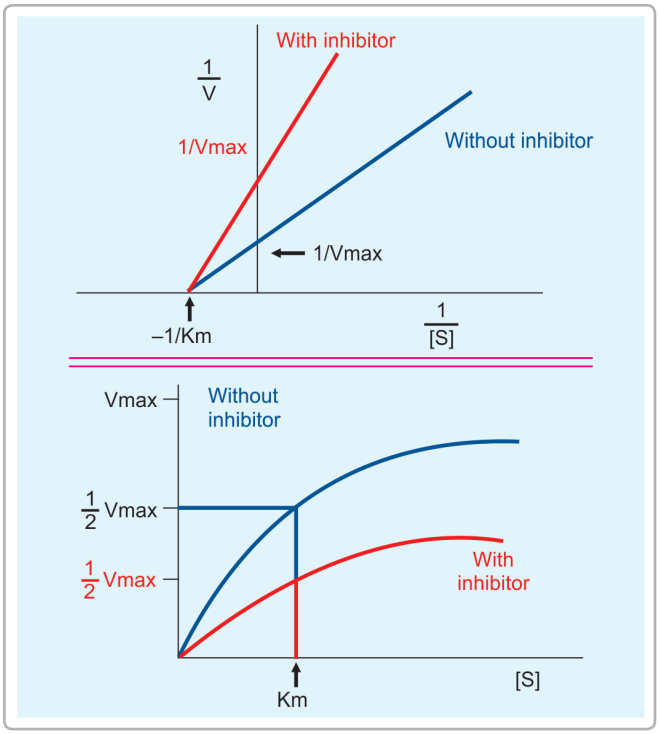


Figure 3.15: Noncompetitive inhibition

Table 3.4: Comparison of two types of inhibition

	Competitive inhibition	*Non-competitive inhibition*
Structure of inhibitor	Substrate analogue	Unrelated molecule
Inhibition is	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
Km	Increased	No change
Vmax	No change	Decreased
Significance	Drug action	Toxicological

Table 3.5: Examples of allosteric enzymes

Enzyme	*Allosteric inhibitor*	*Allosteric activator*	*Chapter*
ALA synthase	Heme	--	14
Aspartate transcarbamoylase	CTP	ATP	23
HMGCoA-reductase	Cholesterol	--	10
Phosphofructokinase	ATP, citrate	AMP, F-2,6-P	5

Aspartate transcarbamoylase has 6 subunits and pyruvate kinase has 4 subunits. A selected list of allosteric enzymes are shown in Table 3.5.

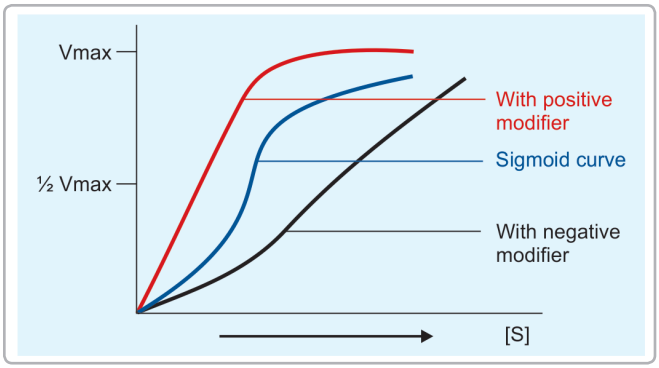


Figure 3.16: Allosteric inhibition

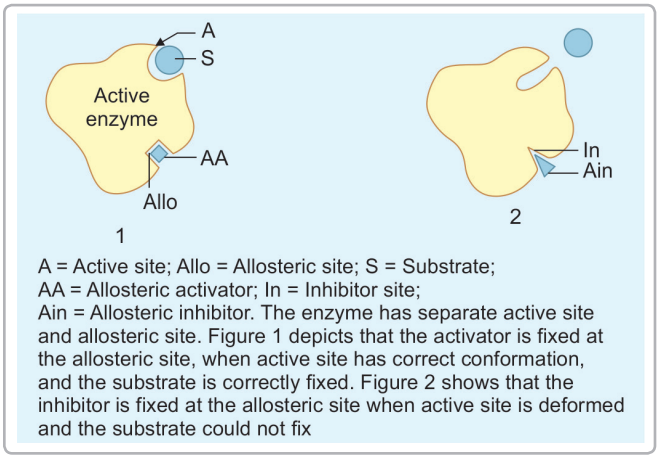


Figure 3.17: Action of allosteric enzymes

Key Enzymes

Allosteric enzymes are utilized by the body for regulating metabolic pathways. Such a **regulatory enzyme** in a particular pathway is called the **key enzyme** or **rate limiting** enzyme. The flow of the whole pathway is constrained as if there is a bottle neck at the level of the key enzyme. For example, the glycolytic pathway is regulated by Phosphofructokinase which catalyses the phosphorylation of fructose -6-phosphat to fructose 1,6 bisphosphate.

Fructose -6-phosphate + ATP ⟶ Fructose 1,6 bisphosphate + ADP

The reaction is allosterically inhibited by ATP and activated by AMP. The glycolytic pathway operates to produce ATP. So when ATP level in the cell is high, High energy charge, the pathway slows down and is activated when energy charge is low as indicated by a high AMP level.

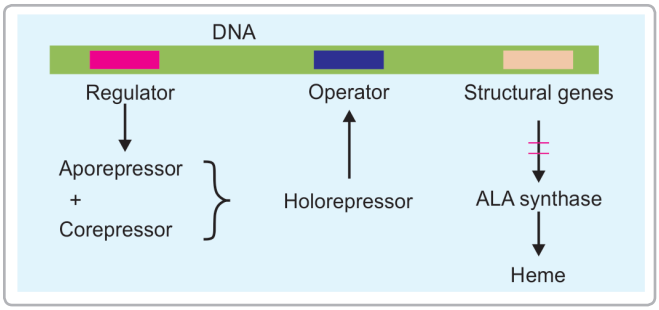


Figure 3.18: Repression of ALA synthase

Induction

Induction is effected through the process of derepression. The *inducer will relieve the repression on the operator site* and will remove the block on the biosynthesis of the enzyme molecules. Classical example is the induction of lactose-utilizing enzymes in the bacteria when the media contains lactose in the absence of glucose. In human beings, ALA synthase is induced by barbiturates.

Repression

Even though both inhibition and repression reduce the enzyme velocity, the mechanisms are different. In the case of **inhibition,** the inhibitor acts on the enzyme directly; and the number of enzyme molecules is not changed by the inhibitor. On the contrary, the **repression** acts at the gene level; and the number of enzyme molecules is reduced in the presence of repressor molecule. The key enzyme of heme synthesis, **ALA synthase** is autoregulated by the heme by means of repression. (Fig. 3.18). The structural gene is transcribed and later translated to produce the enzyme molecules (For details, refer Chapter 26).

ISOENZYMES

They are physically distinct forms of the same enzyme activity. Multiple molecular forms of an enzyme are described as isoenzymes or isozymes. If we take a few rupee coins and examine them carefully, there will be minor variations of ridges on the rims and number of dots below the year. In the market, all these coins have the same face value; but to an experienced numismatist, these variations will explain from which mint it was produced. Similarly, different molecular forms of the same enzyme synthesized from various tissues are called isoenzymes. For example, lactate dehydrogenase has 5 forms. The study of isoenzymes is useful to understand diseases of different organs.

CLINICAL ENZYMOLOGY

Plasma contains many **functional enzymes,** which are actively secreted into plasma. For example, enzymes of blood coagulation. On the other hand, there are a few **nonfunctional enzymes** in plasma, which are coming out from cells of various tissues due to normal wear and tear. Their normal levels in blood are very low; but are drastically increased during cell death (necrosis) or disease. Therefore assays of these enzymes are very useful in diagnosis of diseases.

Lactate Dehydrogenase (LDH)

Isoenzymes of LDH

LDH enzyme is a tetramer with 4 subunits. But the subunit may be either H (heart) or M (muscle) polypeptide chains. These two are the products of 2 different genes. So 5 combinations of H and M chains are possible; H4, H3M, H2M2, M3H and M4 varieties, forming **5 isoenzymes.** All these 5 forms are seen in all persons. M4 form is seen in skeletal muscles; while H4 form is seen in heart. Normally, LDH-2 (H3M1) concentration in blood is greater than LDH-1 (H4); but this pattern is reversed in myocardial infarction; this is called **flipped pattern.** The isoenzymes are usually separated by cellulose acetate **electrophoresis**. In myocardial infarction, LDH activity is increased. Within a few hours after the heart attack, the enzyme level starts to increase, reaches a peak on the 5th day, and reaches normal levels by 10–12 days.

Creatine Kinase (CK)

CK value in serum is increased in **myocardial infarction.** The CK level starts to rise **within 3 hours** of infarction. Therefore CK estimation is very useful to detect early cases, where ECG changes may be ambiguous. The CK level is not increased in hemolysis or in congestive cardiac failure; and therefore CK has an advantage over LDH.

CK and Muscle Diseases

The level of CK in serum is very much elevated in **muscular dystrophies**. The level is very high in the early phases of the disease. CK level is highly elevated in crush injury, fracture and acute cerebrovascular accidents. Estimation of total CK is employed in muscular dystrophies and MB isoenzyme is estimated in myocardial infarction.

Isoenzymes of CK

CK is a dimer. The subunits are called B for brain and M for muscle. Therefore, three isoenzymes are seen in circulation. MM (CK3) is originating from skeletal muscles. MB (CK2) is from heart and BB (CK1) is from brain. Hence, the *detection of MB-isoenzyme is important in myocardial infarction.*

ALANINE AMINOTRANSFERASE (ALT)

In old literature, it was called as serum glutamate pyruvate transaminase (SGPT). The enzyme needs pyridoxal phosphate as coenzyme. Details of the reaction are shown in Fig. 2.17. **Normal serum** level of ALT for male is <45 U/L and for female is <35 U/L. Very high values (100 to 1000 U/L) are seen in **acute hepatitis,** either toxic or viral in origin. Both ALT and AST levels are increased in liver disease, but ALT >> AST. Rise in ALT levels may be noticed several days before clinical signs such as jaundice are manifested. Moderate increase (25 to 100 U/L) may be seen in chronic liver diseases such as cirrhosis, and malignancy in liver.

Alkaline Phosphatase (ALP)

ALP is a nonspecific enzyme which hydrolyses aliphatic, aromatic or heterocyclic compounds. The pH optimum for the enzyme reaction is between 9 and 10. It is produced by **osteoblasts** of bone, and is associated with the calcification process. **Normal serum** value of ALP is 40–125 U/L. In children, the upper level of normal value may be more, because of the increased osteoblastic activity in children. **Moderate increase** (2–3 times) in ALP level is seen in **hepatic diseases** such as infective hepatitis, alcoholic hepatitis or hepatocellular carcinoma. **Very high levels of ALP** (10–12 times of upper limit) may be noticed in **extrahepatic obstruction** (obstructive jaundice) caused by gallstones or by pressure on bile duct by carcinoma of head of pancreas. **Drastically high levels of ALP** (10–25 times of upper limit) are also seen in **bone diseases** where osteoblastic activity is enhanced such as Paget's disease (osteitis deformans), rickets, osteomalacia, osteoblastoma, metastatic carcinoma of bone and hyperparathyroidism.

Acid Phosphatase (ACP)

It hydrolyses phosphoric acid ester at pH between 4 and 6. ACP is secreted by prostate cells, RBC, platelets and WBC.

Table 3.6: Enzyme patterns (Enzyme profiles) in diseases	
I Hepatic diseases	Marked increase in parenchymal liver diseases
1. Alanine amino transferase (ALT)	Marked increase in obstructive liver disease
2. Alkaline phosphatase (ALP)	
II. Myocardial infarction	
1. Creatine kinase (CK-MB)	First enzyme to rise following infarction, CK-MB isoenzyme is specific
2. Aspartate aminotransferase (AST)	Rises after the rise in CK and returns to normal in 4–5 days
3. Lactate dehydrogenase (LDH)	Last enzyme to rise. LDH-1 becomes more than 2 (Flipped pattern)
III. Muscle diseases	
1. Creatine kinase (CK-MM)	Marked increase in muscle diseases. CK-MM fraction is elevated
IV. Bone diseases	
1. Alkaline phosphatase (ALP)	Marked elevation in osteoblastic bone activity as in rickets. Heat labile bone isoenzyme is elevated. Also in Paget's disease
V. Prostate cancer	
1. Prostate specific antigen (PSA)	Marker for prostate cancer. Mild increase in benign prostate enlargement
2. Acid phosphatase (ACP)	Marker for prostate cancer. Metastatic bone disease especially from a primary from prostate. Inhibited by L tartrate

Table 3.7: Therapeutic use of enzymes	
Enzyme	*Therapeutic application*
Asparaginase	Acute lymphoid leukemia
Streptokinase	To lyse intravascular clot
Urokinase	do
Hyaluronidase	Enhances local anesthetics
Pancreatin	Pancreatic insufficiency
Papain	Anti-inflammatory

The prostate isoenzyme is inactivated by **tartaric acid**. ACP total value is increased in **prostate cancer** and highly elevated in metastatic bone disease especially from a primary from prostate. In these conditions, the tartrate labile isoenzyme is elevated. This assay is very helpful in follow up of treatment of prostate cancers. ACP is therefore an important **tumor marker** (For details refer Chapter 29). Alterations of enzymes in various diseases are shown in Table 3.6. Examples of therapeutic uses of enzymes are shown in Table 3.7.

A QUICK LOOK

1. Enzymes are biocatalysts that are essential for biochemical reactions to proceed in the human body.
2. Biological activity of enzymes is dependent on the structural conformation of the enzyme protein.
3. Enzymes can be classified into (i) Oxidoreductases (e.g. alcohol dehydrogenase) (ii) Transferases (e.g. hexokinase) (iii) Hydrolases (e.g. acetylcholine esterase) (iv) Lyases (e.g. Aldolase) (v) Isomerases (e.g. triose phosphate isomerase) (vi) Ligases (e.g. acetyl-CoA carboxylase).
4. Enzymes requiring the presence of a certain metal ion for their activity are called metalloenzymes. Examples are zinc in carbonic anhydrase, Iron in catalase and peroxidase, Calcium in lipase, etc.
5. Apoenzyme (protein part) combines with coenzyme (prosthetic group) to form the functional holoenzyme.
6. Coenzymes may take part in reactions as cosubstrates, but are regenerated.
7. Some vitamin coenzymes are derivatives of nucleotide phosphates, e.g. NAD+, FAD. Deficiency of coenzymes can affect the rate of enzymatic reactions.
8. Area of an enzyme where the catalysis occurs is called the 'active site'.
9. Enzymes catalyze reactions by lowering the activation energy, but does not change the equilibrium constant.
10. Michaelis-Menten theory states that an enzyme (E) combines with a substrate (S) to form an enzyme substrate (E-S) complex, which breaks down to give product (P).
11. Fischer's lock and key hypothesis puts forward a rigid structure for the active site where substrate binds.
12. Koshland's induced fit theory proposes a conformational change in active site to allow binding of regulatory molecules.
13. Enzyme activity is influenced by enzyme concentration, substrate concentration, pH, temperature and presence of inhibitors. Velocity is directly proportional to the concentration of enzymes.
14. Velocity at saturating concentration of substrate is called Maximum velocity or Vmax.
15. Km value (Michaelis-Menten Constant), the substrate concentration at half maximum velocity is a constant for each enzyme–substrate pair.
16. Km value is characteristic of a given enzyme. No two enzymes can have the same Km value. It denotes the affinity of the enzyme to its substrate. Lesser the Km, greater the affinity and vice versa.
17. Km value indicates the affinity of enzyme for substrate; higher the affinity, lower the Km.
18. Velocity is maximum for each enzyme at an optimum pH and temperature.
19. Inhibition may be reversible or irreversible.
20. Enzyme inhibition can be competitive or noncompetitive or uncompetitive. Competitive inhibition is usually reversible.

21. Competitive inhibitor is a structural analogue of substrate that binds to the catalytic site.
22. The Competitive inhibitor increases the Km and its effect can be reversed by increasing the substrate concentration.
23. Many drugs are competitive inhibitors of specific enzymes, e.g. Folic acid synthesis is inhibited by sulphonamides since they are structurally similar to PABA.
24. Actions of drugs such as sulfonamides, methotrexate, dicoumarol and isoniazid are based on the principle of competitive inhibition.
25. Noncompetitive inhibitor binds to a site other than the catalytic site and reduces the Vmax.
26. Noncompetitive inhibition is irreversible and can be caused by toxins or poisons.
27. Allosteric enzymes can be regulated by the binding of positive or negative modifiers to the allosteric site, thus affecting substrate binding to active catalytic site.
28. Suicide inhibition is an irreversible inhibition. The inhibitor makes use of the natural reaction of the enzyme for inhibition, e.g. ornithine decarboxylase.
29. Covalent modification by reversible protein phosphorylation or zymogen activation is a common mechanism of short-term regulation. Rate of synthesis of enzyme proteins may be induced or repressed for long-term regulation.
30. Isoenzymes are physically distinct forms of the same enzyme activity. They may be products of the same gene or different genes.

CHAPTER 4

Carbohydrates–I: Chemistry

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Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Nomenclature and classification of sugars
- Stereoisomers
- Glucose, mannose and galactose
- Fructose
- Reactions of monosaccharides
- Glycosides
- Amino sugars, deoxy sugars, pentoses
- Sucrose, lactose and maltose
- Starch, glycogen and cellulose
- Heteroglycans, mucopolysaccharides
- Absorption of glucose; glucose transporters

FUNCTIONS OF CARBOHYDRATES

1. Carbohydrates are the main sources of **energy** in the body. Brain cells and RBCs are almost wholly dependent on carbohydrates as the energy source. Energy production from carbohydrates will be 4 kcal/g.
2. Storage form of energy (starch and glycogen).
3. Excess carbohydrate is converted to fat.
4. Glycoproteins and glycolipids are components of cell membranes and receptors.
5. Structural unit of many organisms: Cellulose of plants; exoskeleton of insects, cell wall of microorganisms, mucopolysaccharides as ground substance in higher organisms.
6. The general molecular formula of carbohydrates is $C_n(H_2O)_n$. For example, glucose has the molecular formula $C_6H_{12}O_6$. Carbohydrates are polyhydroxy aldehydes or ketones or compounds which yield these on hydrolysis (Fig. 4.1).

NOMENCLATURE

Molecules having only one actual or potential sugar group are called **monosaccharides** (Greek, mono = one); they cannot be further hydrolyzed into smaller units. When two monosaccharides are combined together with elimination of a water molecule, it is called a **disaccharide** (e.g. $C_{12}H_{22}O_{11}$). Trisaccharides contain three sugar groups. Further addition of sugar groups will correspondingly produce tetrasaccharides, pentasaccharides and so on, commonly known as **oligosaccharides** (Greek, oligo = a few). When more than 10 sugar units are combined, they are generally named as **polysaccharides** (Greek, poly = many). Polysaccharides having only one type of monosaccharide units are called *homopolysaccharides* and those having different monosaccharide units are called *heteropolysaccharides*.

Sugars having aldehyde group are called **aldoses** and sugars with keto group are **ketoses**. Depending on the number of carbon atoms, the monosaccharides are named as triose (C3), tetrose (C4), pentose (C5), hexose (C6), heptose (C7) and so on. Commonly occurring monosaccharides are given in Table 4.1. Hexoses of physiological importance are shown in Table 4.2.



Figure 4.1: Keto group and aldehyde group

Table 4.1: Common monosaccharides

No. of carbon atoms	*Generic name*	*Aldoses (with aldehyde group)*	*Ketoses (with keto group)*
3	Triose	Ex: Glyceraldehyde	Ex: Dihydroxyacetone
4	Tetrose	Erythrose	Erythrulose
5	Pentose	Arabinose	--
5	Pentose	Xylose	Xylulose
5	Pentose	Ribose	Ribulose
6	Hexose	Glucose	Fructose
6	Hexose	Galactose	--
6	Hexose	Mannose	--

Table 4.2: Hexoses of physiological importance

Sugar	*Importance*
D-Glucose	Blood sugar. Main source of energy in body
D-Fructose	Constituent of sucrose, the common sugar
D-Galactose	Constituent of lactose, glycolipids and glycoproteins
D-Mannose	Constituent of globulins, mucoproteins and glycoproteins

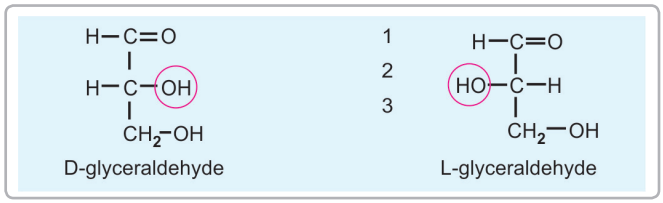


Figure 4.2: Stereoisomers

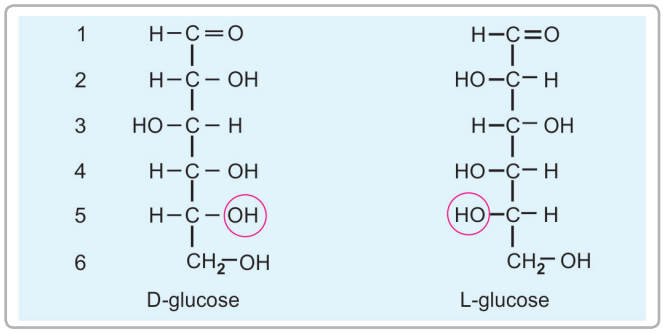


Figure 4.3: Penultimate (reference) carbon atom

STEREOISOMERS

Compounds having same structural formula, but differ in spatial configuration are known as stereoisomers. While writing the molecular formula of monosaccharides, the spatial arrangements of H and OH groups are important, since they contain asymmetric carbon atoms. The reference molecule is glyceraldehyde which has a single asymmetric carbon atom (Fig 4.2).

D and L isomerism of glucose

All monosaccharides can be considered as molecules derived from glyceraldehyde. Depending on the configuration of H and OH around the reference carbon atom, the two mirror forms are designated as D and L forms (Fig. 4.2). In the case of monosaccharides having more than 3 carbon atoms, the penultimate carbon atom (C5 in the case of glucose) is considered as the **reference carbon atom** (Fig. 4.3). It may be noted that in D and L varieties, the groups in 2nd, 3rd, 4th and 5th carbon atoms are totally reversed, so as to produce the mirror images. Since enzymes are stereospecific, only D sugars are metabolized by the human body. All **naturally occurring sugars are D sugars.**

Diastereoisomers of Glucose

Configurational changes with regard to C2, C3 and C4 will produce eight different diastereoisomers for aldohexoses. Out of these, glucose, mannose and galactose are shown in Fig. 4.4. Others are not seen in human body. With reference to C5, all of them will have D and L forms. Hence the molecular formula of hexose ($C_6H_{12}O_6$) represents 16 different monosaccharides, due to spatial arrangement of constituent groups.

Glucose, Mannose and Galactose

They are the common aldohexoses. **Glucose** is the sugar in human blood. It is the major source of energy. (Fig. 4.4). **Mannose** is a constituent of many glycoproteins. Mannose was isolated from plant mannans; hence the name. (Fig. 4.4). **Galactose** is a constituent of lactose (milk sugar) and glycoproteins.

Epimerism of Aldoses

When sugars are different from one another, *only in configuration with regard to a single carbon atom* (other than the reference carbon atom), they are called epimers.

For example, glucose and mannose are an epimeric pair which differ only with respect to C2. Similarly, galactose is the 4th epimer of glucose. (Fig. 4.4). Galactose and mannose are not epimers but diastereoisomers.

Anomerism of Sugars

This is explained by the fact that D-glucose has two *anomers,* **alpha and beta varieties.** These anomers are produced by the spatial configuration with *reference to the first carbon atom in aldoses and second carbon atom in ketoses.* Hence, these carbon atoms are known as **anomeric carbon atoms**. The anomeric forms of glucose are shown in Figure 4.5. The difference in alpha and beta anomeric forms is dependent on the 1st carbon atom only. In the previous section 16 stereoisomers of glucose are described. Each of them will have 2 anomers; and hence there are a total of 32 isomers for glucose.

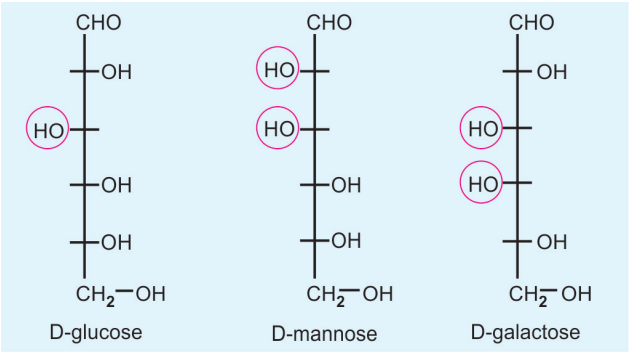


Figure 4.4: Epimers of D-glucose

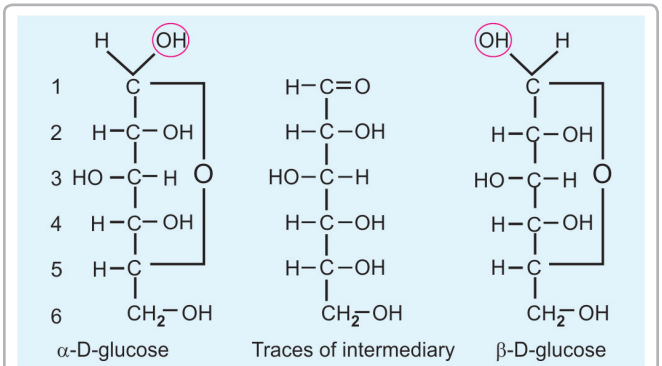


Figure 4.5: Anomers of D-glucose

Three Representations of Glucose Structure

1. The 1st carbon aldehyde group is condensed with the hydroxyl group of the 5th carbon to form a ring. (Fig. 4.6). The open chain projection formula and the ring structure of glucose were proposed by Emil Fischer (Nobel Prize, 1902).
2. Later, it was shown that the glucose is existing in biological systems, not as a rectangle, but as a pyranose ring. This was established by Sir Walter Haworth (Nobel prize, 1937) (Fig. 4.6).
3. Monosaccharides have three forms of structure, open chain, closed hemiacetal and the Haworth's ring structure. Each successive form adds more details (Fig. 4.6).

Fructose is a Ketohexose

In fructose, the keto group is on the 2nd carbon atom. Thus, second carbon atom is the anomeric carbon atom. Fructose has 4 isomers. Each of them has D and L forms with regard to 5th carbon atom. Fructose has the same molecular formula as glucose, but differs in structural formula. So glucose and fructose are functional group (**aldose-ketose**) isomers. D fructose is levorotatory. Only D variety is seen in biological systems. Fructose remains

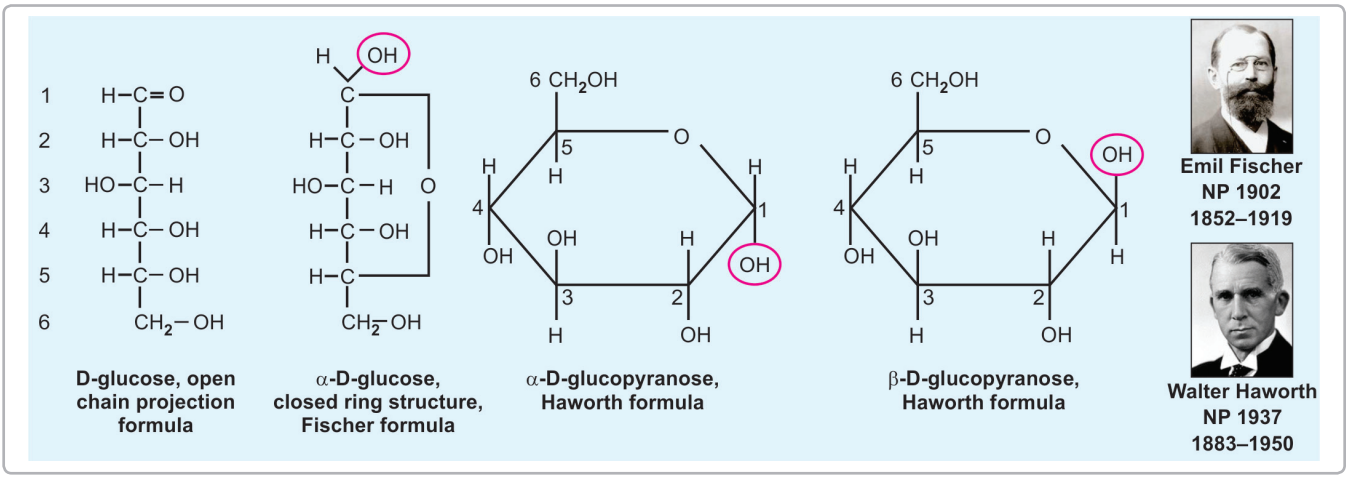


Figure 4.6: Comparison of different representations of D-glucose.

predominantly as furanose ring structure (Fig. 4.7). Fructose is a major constituent of honey.

REACTIONS OF MONOSACCHARIDES

All the sugars showing mutarotation, will show reduction property and will form osazone with phenylhydrazine.

1. Enediol Formation

In mild alkaline solutions, carbohydrates containing a free sugar group (aldehyde or keto) will tautomerize to form **enediols**, where two hydroxyl groups are attached to the double-bonded carbon. This property forms the basis of the reduction tests for sugars. (Fig. 4.8.)

2. Benedict's Reaction

Benedict's reagent is very commonly employed to detect the presence of glucose in urine (glucosuria) and is a standard laboratory test employed in diabetes mellitus. Benedict's reagent contains sodium carbonate, copper sulfate and sodium citrate. In the alkaline medium provided by sodium carbonate, the copper remains as cupric hydroxide. Sodium citrate acts as a stabilizing agent to prevent precipitation of cupric hydroxide. In alkaline medium, sugars form enediol, cupric ions are reduced, correspondingly sugar is oxidized. **Glucose is a reducing sugar** (Fig. 4.9). In practice, 0.5 mL of urine and 5 mL of Benedict's reagent are boiled for 2 minutes. If sugar is present, copper is reduced to produce green, yellow, orange or red precipitate, depending on the concentration of sugar. Therefore, this can be used as a semiquantitative test. Any sugar with free aldehyde/keto group will reduce the Benedict's reagent. Therefore, this is not specific for glucose. Reducing substances in urine are described refer Chapter 6.

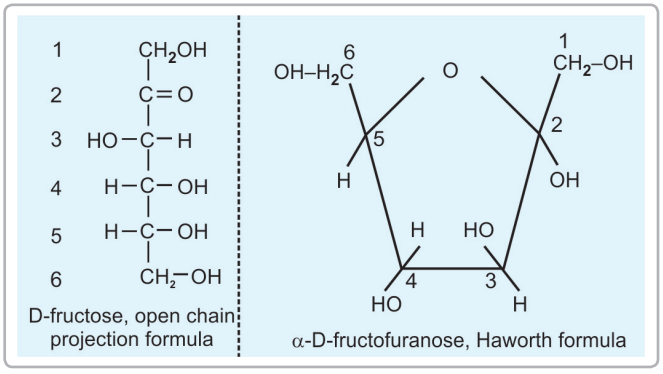


Figure 4.7: Different representations of D-fructose

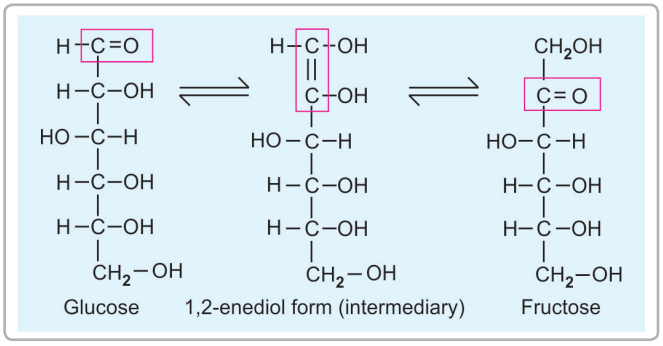


Figure 4.8: Interconversion of sugars

3. Osazone Formation

All reducing sugars will form osazones with excess of phenylhydrazine when kept at boiling temperature. Each sugar will have characteristic crystal form of osazones. *The differences in glucose, fructose and mannose are dependent on the first and second carbon atoms, and when the osazone is formed these differences are masked.* Hence, these 3 sugars will produce the same needle, shaped crystals arranged like sheaves of corn or a broom (Fig. 4.10). Osazones may be used to differentiate sugars in biological fluids like urine.

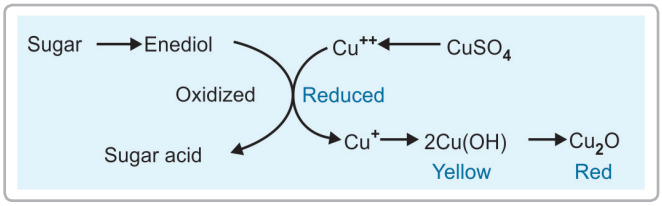


Figure 4.9: Benedict's test, principle

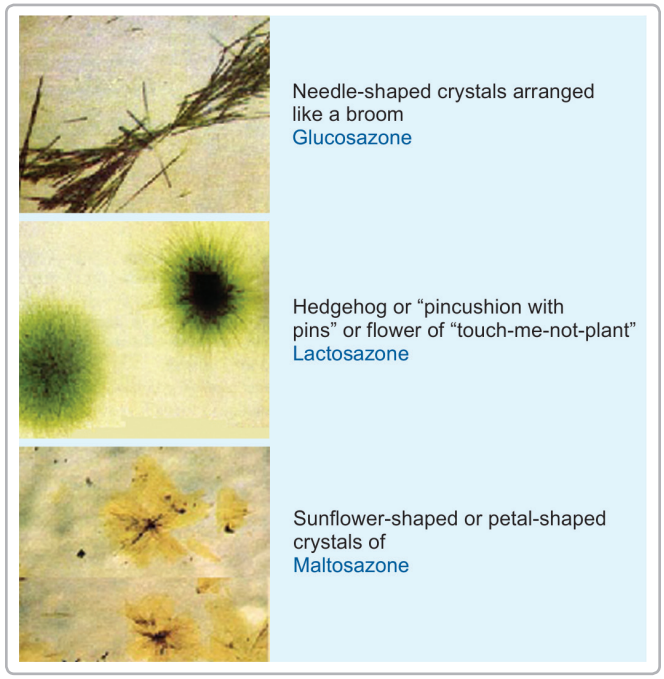


Figure 4.10: Shape of osazones under microscope

4. Reduction to Form Alcohols

Under specific conditions reduction of aldose yields corresponding alcohol. Thus, glucose is reduced to sorbitol (Fig. 4.11). But ketose forms two alcohols, because of appearance of a new asymmetric carbon atom in this process. Fructose is reduced to mannitol and sorbitol. Similarly galactose is reduced to dulcitol and ribose to ribitol. The osmotic effect of **sorbitol and dulcitol** produces changes in tissues when they accumulate in abnormal amounts, e.g. cataract of lens.

5. Oxidation of Sugars

Under mild oxidation conditions (hypobromous acid), the aldehyde group is oxidized to carboxyl group to produce **aldonic** acid. Thus, glucose is oxidized to **gluconic acid.** Under special conditions, the last carbon becomes COOH group to produce **uronic acid.** Thus glucose is oxidized **glucuronic acid.** It is used in the body for conjugation with insoluble molecules to make them soluble in water (For detail refer Chapter 19) and also for synthesis of heteropolysaccharides. Under strong oxidation conditions (nitric acid and heat), the first and last carbon atoms are both oxidised to form dicarboxylic acids, known as saccharic acids. Glucose is thus oxidized to **glucosaccharic acid.**

6. Furfural Derivatives

Monosaccharides when treated with concentrated sulfuric acid undergo dehydration with the removal of 3 molecules of water. The furfural derivative can condense with phenolic compounds to give colored products. This forms the basis of **Molisch test**, general test for carbohydrates.

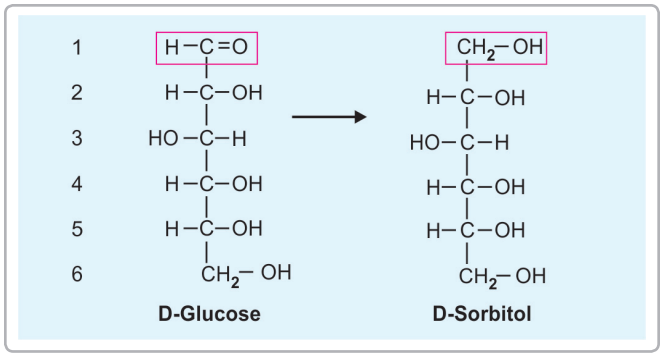


Figure 4.11: Reduction of sugar to alcohol

7. Glycosides

When the hemiacetal group is condensed with an alcohol or phenol group, it is called a glycoside (Fig. 4.12). Glycosides do not reduce Benedict's reagent, because the sugar group is masked. Some glycosides of medical importance are given in Table 4.3**.** Glycosidic bonds may be alpha or beta depending on the configuration of the sugar contributing the 1st carbon atom for the formation of the glycosidic bonds. Hence both alpha and beta glycosidic bonds may be formed. For example, starch has glucose linked in alpha-1,4-glycosidic bonds, whereas cellulose has glucose units linked in beta glycosidic bonds. Hence, human enzymes can digest only starch.

8. Formation of Esters

Sugar phosphates are of great biological importance. Metabolism of sugars inside the body starts with phosphorylation. Glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate and fructose-1-6-phosphate are important intermediaries of glucose metabolism (Fig. 4.13).

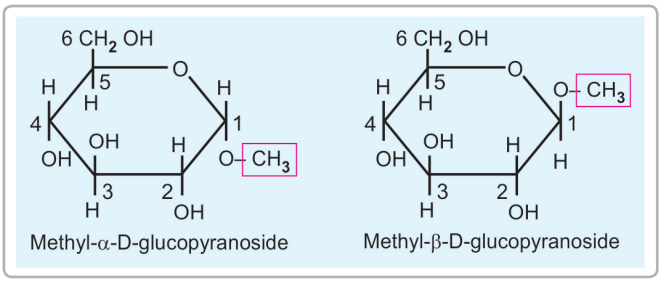


Figure 4.12: Glycosides

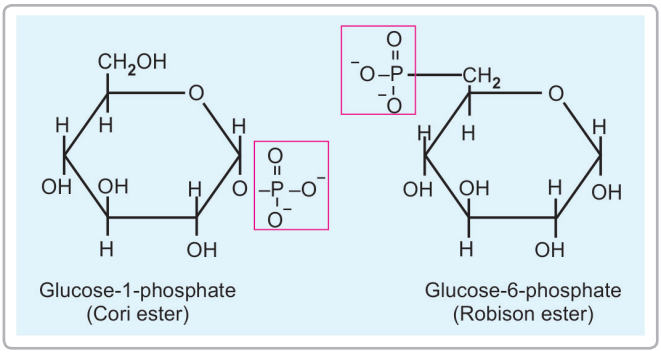


Figure 4.13: Phosphorylated sugars

Table 4.3: Glycosides

Sugar	*+ Aglycon*	*= Glycoside*	*Source*	*Importance*
Glucose	phloretin	Phlorizin	Rose bark	Renal damage
Galactose, xylose	digitogenin	Digitonin	Leaves of foxglove	Cardiac stimulant
Glucose	indoxyl	Plant indican	Leaves of indigofera	Stain

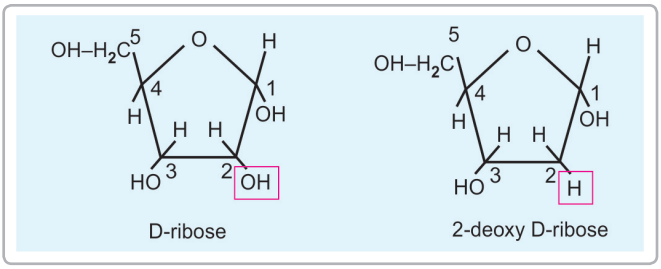


Figure 4.14: Sugars of nucleic acids

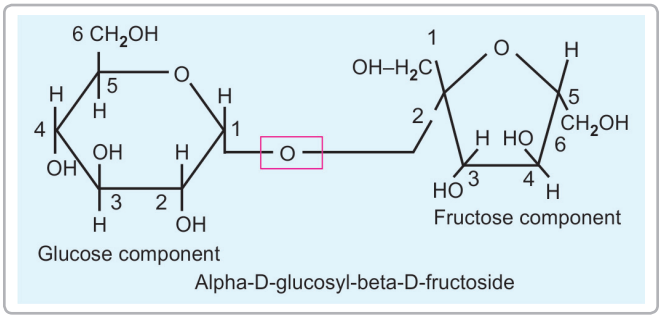


Figure 4.15: Structure of sucrose (1–2 linkage)

9. Amino Sugars

Amino groups may be substituted for hydroxy groups of sugars to give rise to amino sugars. Generally, the amino group is added to the second carbon atom of hexoses. Amino sugars will not show reducing property. They will not produce osazones. **Glucosamine** is seen in hyaluronic acid, heparin and blood group substances. **Galactosamine** is present in chondroitin of cartilage, bone and tendons. **Mannosamine** is a constituent of mucopolysaccharides. The amino group in the sugar may be further acetylated to produce N-acetylated sugars such as N-acetyl-glucosamine (GluNac), N-acetyl-galactosamine (GalNac), etc. which are important constituents of glycoproteins, mucopolysaccharides and cell membrane antigens.

10. Deoxy Sugars

Oxygen of the hydroxyl group may be removed to form deoxy sugars. Deoxy sugars will not reduce and will not form osazones. **Deoxyribose** is an important part of nucleic acid (Fig. 4.14). L-fucose is present in blood group antigens and many other glycoproteins.

11. Pentoses

They are sugars containing 5 carbon atoms. **Ribose** is a constituent of RNA while deoxyribose is seen in DNA (Fig. 4.14). Ribose is also seen in coenzymes such as ATP and NAD. **Arabinose and Xylose** are other pentoses seen in cell membranes.

DISACCHARIDES

When two monosaccharides are combined together by glycosidic linkage, a disaccharide is formed. The important disaccharides are sucrose, maltose, isomaltose and lactose.

1. Sucrose

It is the sweetening agent known as cane sugar. It is present in sugarcane and various fruits. Hydrolysis of sucrose will produce one molecule of **glucose** and one molecule of **fructose**. The enzyme producing hydrolysis of sucrose is called **sucrase**. Sucrose is **not a reducing sugar**; and it will not form osazone. This is because the linkage involves first carbon of glucose and second carbon of fructose, and free sugar groups are not available (Fig. 4.15). When sucrose is hydrolyzed, the products have reducing action. A sugar solution which is originally nonreducing, but becomes reducing after hydrolysis, is inferred as sucrose (**specific sucrose test**).

2. Lactose

It is the sugar present in milk. It is a **reducing disaccharide** and forms osazone which resembles "pincushion with pins" or "hedgehog" or flower of "touch-me-not" plant (see Fig. 4.10). It can be hydrolyzed by lactase to yield glucose and galactose. Beta glycosidic linkage is present in lactose. The structure is given in Figure 4.16. In lactose, the galactose residue is attached to the glucose through beta-1,4 glycosidic linkage. Lactose and lactate should not be confused (Box 4.1).

3. Maltose

It is another **reducing disaccharide**. It forms petal-shaped crystals of maltose-osazone (see Fig. 4.10). Maltose contains two glucose residues with alpha-1,4 linkage. (Fig. 4.17). Maltose is a product of the hydrolysis of starch. Salient features of important sugars are shown in Box 4.2.

BOX 4.1: Lactose and lactate are different

- Lactose is the milk sugar; a disaccharide made of galactose and glucose
- Lactate or lactic acid is a product of anaerobic metabolism of glucose

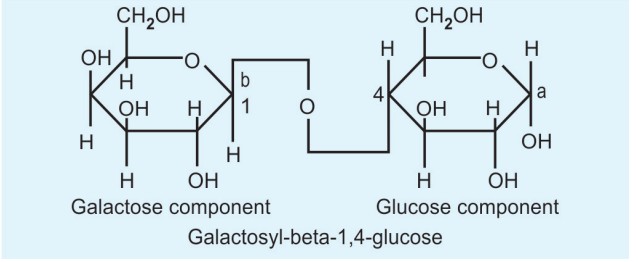


Figure 4.16: Lactose (Beta-1-4 linkage)

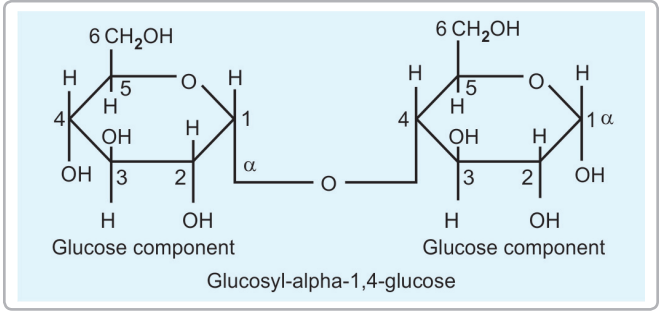


Figure 4.17: Structure of maltose (alpha-1-4 linkage).

BOX 4.2: Salient features of important sugars

Monosaccharides

Glucose	Aldohexose
Galactose	4th epimer of glucose
Mannose	2nd epimer of glucose
Fructose	Ketohexose

Disaccharides

Glucose + Galactose	= Lactose (reducing)
Glucose +Glucose	= Maltose (reducing)
Glucose +Fructose	= Sucrose (nonreducing)

POLYSACCHARIDES

These are polymerized products of many monosaccharide units. They may be **homoglycans** composed of single kind of monosaccharides, e.g. starch, glycogen and cellulose. **Heteroglycans** are composed of two or more different monosaccharides.

1. Starch

It is the reserve carbohydrate of **plant kingdom** and present abundantly in potatoes, tapioca, rice, wheat and other food grains. When starch is treated with boiling water, 10–20% is solubilized; this part is called amylose. The insoluble part is called amylopectin.

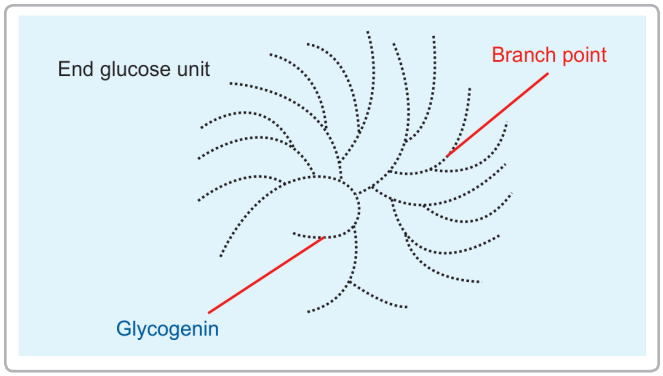


Figure 4.18: Branched glycogen molecule

Hydrolysis of starch: Starch will form a blue colored complex with iodine; this color disappears on heating and reappears when cooled. This is a sensitive test for starch. Starch is nonreducing because the free sugar groups are negligible in number. When starch is hydrolyzed by mild acid, smaller and smaller fragments are produced. Finally **maltose** units are produced.

Action of alpha-amylases on Starch: Salivary amylase and pancreatic amylase are alpha-amylases, which act at random on alpha-1,4 glycosidic bonds to split starch into smaller units (dextrins), and finally to **alpha-maltose**.

2. Glycogen

It is the **reserve carbohydrate in animals**. It is stored in liver and muscle. About 5% of weight of liver is made up by glycogen. Glycogen is composed of glucose units joined by alpha-1,4 and alpha-1,6 glycosidic linkages. (Fig. 4.18). Molecular weight of glycogen is about 5 million Daltons. *Glycogen is more branched and more compact than amylopectin*. The branching points are made by alpha-1, 6 linkages.

3. Cellulose

It is the chief carbohydrate in plants. Cellulose constitutes 99% of cotton, 50% of wood and 40% of straw, and is the most abundant organic material in nature. It is made up of glucose units combined with cellobiose bridges or **beta -1,4 linkages.** It has a straight line structure, with no branching points.

4. Inulin

It is clinically used to find renal clearance value and glomerular filtration rate (For details refer Chapter 30). Inulin and Insulin are different (Box 4.3).

BOX 4.3: Inulin and insulin are different

- Inulin is a polysaccharide (carbohydrate) made up of fructose units. It is used for renal function studies
- Insulin is a polypeptide (protein) hormone, with wide ranging actions on carbohydrate and lipid metabolism

HETEROGLYCANS

These are polysaccharides containing more than one type of sugar residues. A few examples are given below:

1. Agar

It is prepared from sea weeds and contains galactose, glucose and other sugars. Agar is used as a supporting medium for electrophoresis and immunoelectrophoresis. **Agarose** is made up of galactose combined with anhydrogalactose units.

2. Mucopolysaccharides

Mucopolysaccharides or glycosaminoglycans (GAG) are carbohydrates containing uronic acid and amino sugars. Because of the presence of charged groups, they attract water molecules and so they produce viscous solutions. Some examples of mucopolysaccharides are given below:

Hyaluronic Acid

It is present in connective tissues, tendons, synovial fluid and vitreous humor. It contains glucosamine and glucuronic acid.

Heparin

It is an **anticoagulant** widely used when taking blood in vitro for clinical studies. It is also used in vivo in suspected thromboembolic conditions to prevent intravascular coagulation. It contains sulfated glucosamine.

GLYCOPROTEINS AND MUCOPROTEINS

When the carbohydrate chains are attached to a polypeptide chain, it is called a **proteoglycan**. If the carbohydrate content is less than 10%, it is generally named as a **glycoprotein.** If the carbohydrate content is more than 10%, it is a **mucoprotein**. They are seen in almost all tissues and cell membranes. The oligosaccharide chains of glycoproteins are composed of varying numbers of the following carbohydrate residue: Glucose (Glu); mannose (Man); galactose (Gal); N-acetyl glucosamine (GluNAc); N-acetyl galactosamine (GalNAc), etc.

DIGESTION OF CARBOHYDRATES

Cooking helps in breaking of glycosidic linkages in polysaccharides and thus makes the digestion process easier. In the diet, carbohydrates are available as polysaccharides (starch, glycogen), and to a minor extent, as disaccharides (sucrose and lactose). These complex carbohydrates are hydrolyzed to monosaccharide units in the gastrointestinal tract. This process of digestion starts in mouth by the salivary alpha-amylase. However, the time available for digestion in the mouth is limited. The gastric hydrochloric acid will inhibit the action of salivary amylase. In the pancreatic juice another **alpha-amylase** is available which will hydrolyze the alpha-1,4 glycosidic linkages randomly, so as to produce smaller subunits like maltose, isomaltose, dextrins and oligosaccharides. The intestinal juice (succus entericus) and brush border of intestinal cells contain enzymes, which will hydrolyze disaccharides into component monosaccharides. These enzymes are **sucrase, maltase, isomaltase** and **lactase**. The monosaccharides are then absorbed.

Lactose Intolerance

This is a comparatively common condition produced by the deficiency of lactase. This enzyme hydrolyses lactose to glucose and galactose. Lactase is present in the brush border of enterocytes. In this condition, lactose accumulates in the gut. It is acted upon by bacteria to produce organic acids. These take up water into bowels by osmotic effect. Irritant diarrhea is thus produced. Lactase is an inducible enzyme. If milk is withdrawn temporarily, the diarrhea will be limited. Curd is also an effective treatment, because the lactobacilli present in curd contains the enzyme lactase. Lactase activity is high during infancy and it decreases to adult levels by 5–7 years of age. Majority of adult population had hypolactasia and may get secondary lactose intolerance if a milk based diet is consumed.

ABSORPTION OF CARBOHYDRATES

Only monosaccharides are absorbed by the intestine. Minute quantities of disaccharides that may be absorbed, are immediately eliminated through kidneys. Absorption rate of galactose is more than glucose; while fructose is absorbed at a lesser rate than glucose. Glucose is absorbed with the help of specific **transporters.** These are transmembrane proteins spanning the width of the membrane.

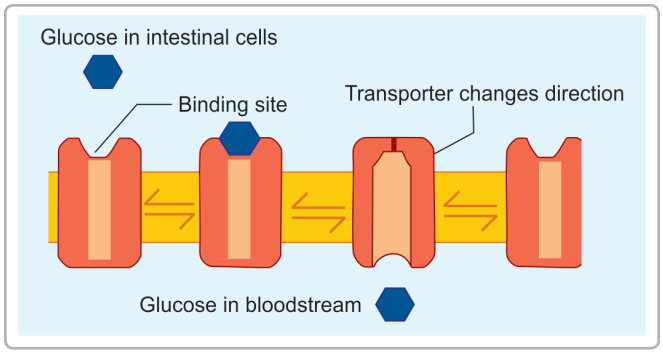


Figure 4.19: Glucose absorption (Glu2)

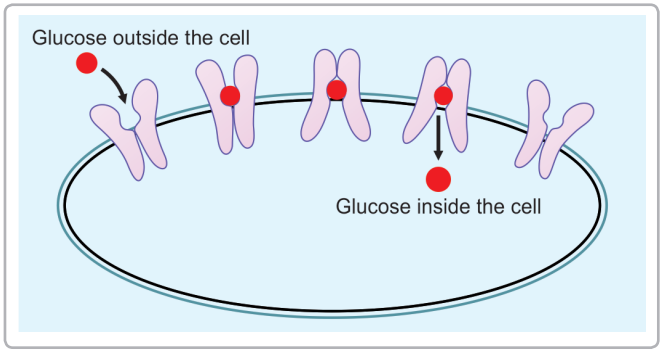


Figure 4.20: GluT4; glucose transport in cells

1. Cotransport from Lumen to Intestinal Cell

Absorption from intestinal lumen into intestinal cell is by cotransport mechanism (secondary active transport) (For details refer Chapter 1). This mechanism is also called **Sodium Dependent Glucose Transporter (SGluT)**.

Glucose is transported from a lower concentration to a higher concentration. This is coupled with the movement of sodium from a higher concentration to lower concentration. Energy is needed indirectly.

2. Release of Glucose into Blood

The same intestinal epithelial cells have a different transport mechanism on the membrane facing capillaries (Fig. 4.19). Intestinal cells release glucose into blood stream by the carrier mechanism called **Glucose Transporter Type 2 (GluT2)**. This transporter is not dependent on sodium, but it is a **uniport, facilitated diffusion** system.

3. GluT2 in Other Tissues

In other tissues, GluT2 is involved in absorption of glucose from blood stream. GluT2 is present not only in intestinal epithelial cells, but also in liver cells, beta cells of pancreas and kidney.

Table 4.4: Glucose transporters

Transporter	*Present in*	*Properties*
GluT1	RBC, brain, kidney	Glucose uptake in most of cells
GluT2	Serosal surface of intestinal cells, beta cell pancreas	Glucose uptake in liver; glucose sensor in beta cells
GluT3	Neurons, brain	Glucose into brain
GluT4	Skeletal, heart muscle, adipose tissue	Insulin mediated glucose uptake

4. GluT4 in Muscle and Adipose Tissue

GluT4 is the major glucose transporter in skeletal muscle, heart muscle and adipocytes (Fig. 4.20). The GluT4 is under control of **insulin**. Insulin increases the number of transporter molecules, and thus glucose uptake is enhanced. In Type 2 **diabetes mellitus** (For details refer Chapter 6), insulin resistance is seen in muscle and fat cells. In diabetes, entry of glucose into muscle is only half of normal cells. This is because membrane bound GluT4 is reduced in insulin deficiency, due to defective recycling. Different glucose transporters are shown in Table 4.4.

A QUICK LOOK

1. Carbohydrates are polyhydroxy aldehydes or ketones or compounds, which yield them on hydrolysis. Simplest carbohydrates are monosaccharides which may be trioses (3C), tetroses (4C), pentoses (5C) and hexoses (6C).
2. Carbohydrates are classified into monosaccharides, disaccharides and polysaccharides, based on the number of sugar/saccharide units they possess. Disaccharides have 2 monosaccharide units, oligosaccharides around 10, and polysaccharides more than 10. They could also be classified as aldoses and ketoses based on the functional group they possess.
3. Common examples of monosaccharides include Glucose, Fructose, Galactose, and Mannose.
4. Common examples of disaccharides are Sucrose, Lactose and Maltose.
5. Monosaccharides exhibit stereoisomerism, optical isomerism, anomerism and pyranose-furanose isomerism.
6. All carbohydrates are considered to be derived from glyceraldehyde by successive addition of carbons. The penultimate carbon atom is thus the reference carbon atom for naming mirror images.
7. Stereoisomerism is the property of monosaccharides, due to the difference in orientation of H and OH around the reference carbon atom. The stereoisomers are prefixed as 'D' or 'L'. D sugars are naturally occurring and human body can metabolize only D sugars.

8. A carbon atom bound by four different groups on all its valencies is referred to as an asymmetric carbon. When two sugars differ from each other in the configuration around one carbon atom (other than the reference carbon), they are diastereoisomers.
9. A pair of monosaccharides which differ from each other in the configuration around a single carbon atom are called epimers. Anomers of monosaccharides are produced by the spatial configuration with reference to the first carbon atom in aldoses and the second carbon atom in ketoses.
10. Two anomers of glucose are alpha-D glucose and beta-D glucose. Mutarotation is the result of anomerism.
11. Optical isomerism is based on the rotation of plane polarized light by a pure solution of the sugar. The prefix 'd' or (+) and 'l' or (–) is used to indicate dextro and levorotatory compounds respectively.
12. All reducing sugars form characteristic osazone crystals. Glucose and fructose form needle-shaped crystals, maltose forms sunflower-shaped crystals and lactose forms hedgehog-shaped crystals.
13. Amino sugars form important components of mucopolysaccharides. For example, Galactosamine, Glucosamine.
14. Sucrose is formed from glucose and fructose linked by 1, 2 glycosidic linkage.
15. Lactose is formed from galactose and glucose linked by beta 1,4 glycosidic linkage.
16. Maltose is formed from two glucose molecules linked by alpha 1,4 glycosidic linkage.
17. Starch is made of two components; straight chain amylose and branched amylopectin. The linkages are alpha-1,6 type at branch points while alpha-1,4 linkages form the straight chain.
18. Action of amylase on starch yields limit dextrins.
19. Mucopolysaccharides or Glycosaminoglycans (GAGs) such as Hyaluronic acid, Chondroitin sulfate, Keratan sulfate, Dermatan sulfate are associated with connective tissue.
20. Keratan sulfate is the only GAG that does not contain uronic acid.
21. When the carbohydrate chains are attached to a polypeptide chain it is called a proteoglycan.

CHAPTER 5

Carbohydrates–II: Major Metabolic Pathways of Glucose

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Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Glycolysis pathway
- Regulation of glycolysis
- Cori's cycle
- Pyruvate as a junction point
- Gluconeogenesis
- Glycogenolysis; degradation of glycogen
- Glycogenesis; glycogen synthesis
- Regulation of glycogen; Cyclic AMP
- Glycogen storage diseases

GLUCOSE METABOLISM

Importance of Glucose

1. Glucose is the preferred source of energy for most of the body tissues. Brain cells derive energy mainly from glucose.
2. When glucose metabolism is deranged, life-threatening conditions may occur. A minimum amount of glucose is always required for normal functioning.
3. **Normal fasting plasma glucose level is 70 to 110 mg/dL.** After a heavy carbohydrate meal, in a normal person, this level is below 150 mg/dL.

GLYCOLYSIS (EMBDEN-MEYERHOF PATHWAY)

Importance of the Pathway

Glycolysis is derived from the Greek word, glykys = sweet; and lysis = splitting. In this pathway, glucose is converted to **pyruvate** (aerobic condition) or **lactate** (anaerobic condition), along with production of a small quantity of energy. (see Fig. 5.3). All the reaction steps take place in the cytoplasm. It is the only pathway that is taking place in all the cells of the body. Glycolysis is the only source of energy in erythrocytes. In strenuous exercise, when muscle tissue lacks enough oxygen, *anaerobic glycolysis forms the major source of energy for muscles*. The glycolytic pathway may be considered as the preliminary step before complete oxidation. The glycolytic pathway also provides carbon skeletons for synthesis of certain non-essential amino acids as well as glycerol part of fat. Most of the reactions of the glycolytic pathway are reversible, which are also used for gluconeogenesis.

Step 0: Glucose Entry into Cells

Glucose transporter-4 (GluT4) transports glucose from extracellular fluid to muscle cells and adipocytes. This translocase is under the influence of **insulin**. So, in diabetes mellitus, insulin deficiency hinders the entry of glucose into the peripheral cells. But GluT2 is the transporter in liver cells; it is not under the control of insulin.

Step 1 of Glycolysis

The metabolic fates of Glucose-6-phosphate are shown in Fig. 5.1. Glucose is activated by phosphorylation to glucose-6-phosphate (Fig. 5.2). The enzyme is **Hexokinase** (HK), which splits the ATP into ADP, and the Pi is added on to the glucose. The energy released by the hydrolysis of ATP is utilized for the forward reaction.

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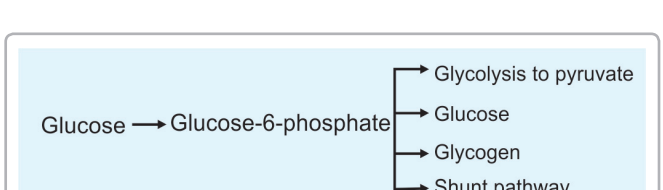


Figure 5.1: Fate of Glucose-6-phosphate

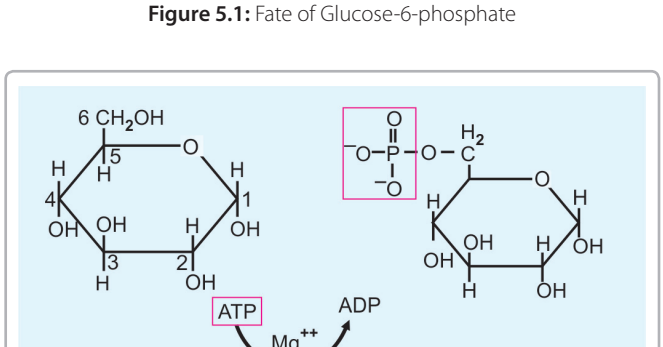


Figure 5.2: Step 1 of glycolysis; irreversible step

Hexokinase and glucokinase may be considered as isoenzymes. Glucokinase is under the influence of insulin; but hexokinase is not. The kinase reaction is **irreversible**; the same enzyme cannot produce glucose. But this irreversibility is circumvented by another enzyme glucose-6-phosphatase (see gluconeogenesis). Hexokinase is a key glycolytic enzyme, while glucose-6-phosphatase is a key gluconeogenic enzyme.

Step 2 of Glycolysis

Glucose-6-phosphate is isomerized to fructose-6-phosphate by an isomerase. This is readily reversible. (Fig. 5.3)

Step 3 of Glycolysis

Fructose-6-phosphate is further phosphorylated to fructose-1,6-bisphosphate (Box. 5.1). The enzyme is **phosphofructokinase** (PFK). It needs ATP. PFK is an allosteric and inducible enzyme. It is an important key enzyme of this pathway. This reaction is an **irreversible** step in glycolysis. However, this difficulty is circumvented by another enzyme, fructose-1,6-bisphosphatase (see gluconeogenesis).

Step 4 of glycolysis

The fructose-1,6-bisphosphate is cleaved into two halves; one molecule of glyceraldehyde-3-phosphate and another molecule of dihydroxyacetone phosphate (Fig. 5.4). The enzyme is called **aldolase**. This reaction is reversible.

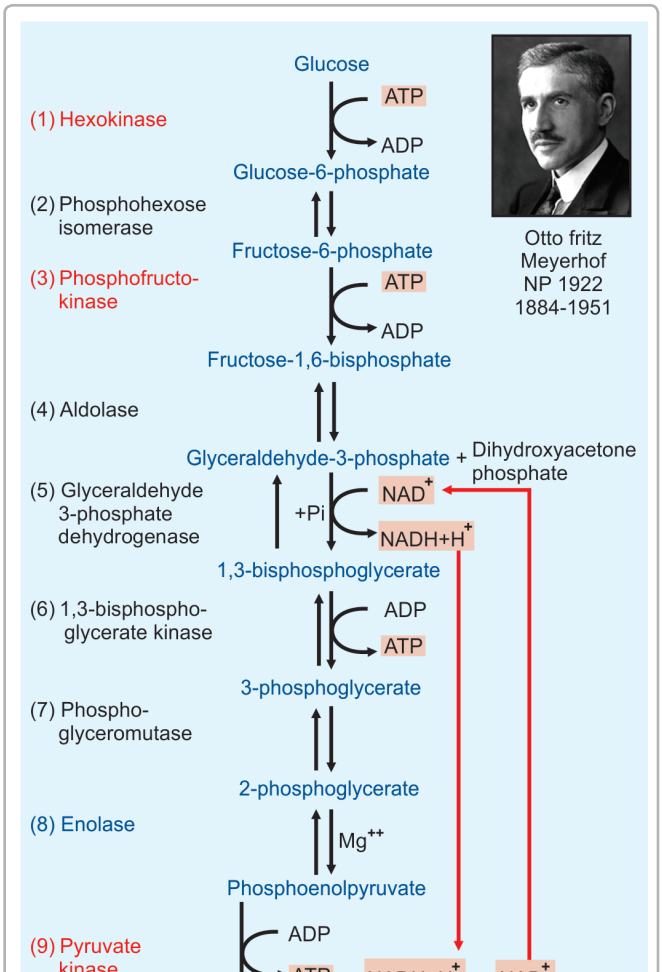


Figure 5.3: Summary of glycolysis (Embden-Meyerhof pathway). Steps 1, 3 and 9 are key enzymes; these reactions are irreversible. Steps 5, 6 and 9 produce energy and step 5 generates NADH

BOX 5.1: Diphosphate and bisphosphate are different

- When two phosphate groups are linked together and then attached to a parent compound, it is called diphosphate, e.g. adenosine-di-phosphate
- But when phosphoric acid groups are present at two different sites of the compound, it is named as bisphosphate, e.g. fructose-1,6-bisphosphate

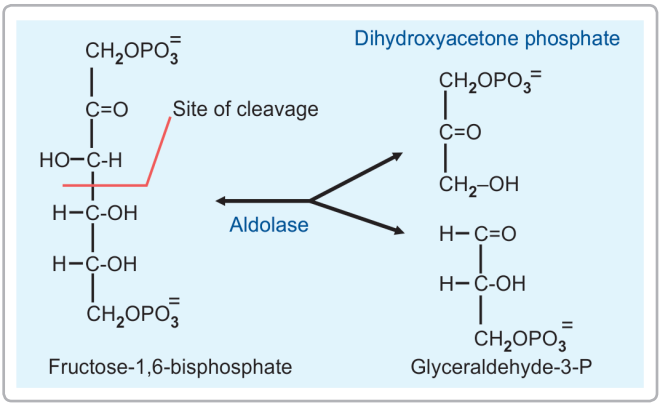


Figure 5.4: Step 4 of glycolysis; reversible

Step 4-A of Glycolysis

Dihydroxy acetone phosphate is isomerized to glyceraldehyde-3-phosphate by the enzyme phosphotriose **isomerase**. Thus net result is that glucose is now cleaved into 2 molecules of glyceraldehyde-3-phosphate. For synthesis of neutral fat, glycerol is required which is derived from glucose through dihydroxy acetone phosphate.

Step 5 of glycolysis

In this step, Glyceraldehyde-3-phosphate is dehydrogenated and simultaneously phosphorylated to 1,3-bisphospho glycerate (1,3-BPG) with the help of NAD^+ (See Fig. 5.3). The enzyme is **glyceraldehyde-3-phosphate dehydrogenase**. This is a reversible reaction. The product contains a high energy bond.

Step 6 of Glycolysis

The energy of 1,3-BPG is trapped to synthesize one ATP molecule with the help of a kinase. (See Fig. 5.3). This is an example of **substrate level phosphorylation**, where energy is trapped directly from the substrate, without the help of the complicated electron transport chain reactions (When energy is trapped by oxidation of reducing equivalents such as NADH, it is called *oxidative phosphorylation)*. The reaction is reversible with the help of the same enzyme systems.

Step 7 of Glycolysis

3-phosphoglycerate is mutated to 2-phosphoglycerate by shifting the phosphate group from 3rd to 2nd carbon atom. This is a readily reversible reaction. (See Fig. 5.3).

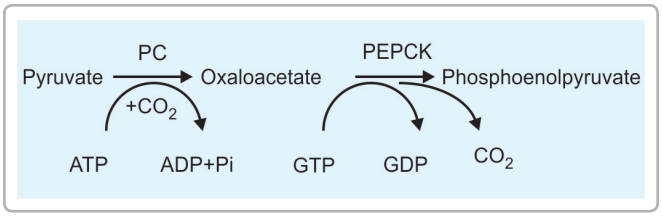


Figure 5.5: Reversal of Step 9 needs two enzymes

Step 8 of Glycolysis

2-phosphoglycerate is converted to phosphoenol pyruvate (PEP) by the enzyme **enolase** by the removal of a water molecule. (See Fig. 5.3). The reaction is reversible. Enolase requires Mg^{++}, and by removing these ions, **fluoride** will irreversibly inhibit this enzyme. By inhibiting enolase, fluoride will stop the whole glycolysis. So fluoride is added to blood; otherwise sugar is oxidized by the blood cells, so that when blood sugar is estimated after some time, false low values are obtained.

Step 9 of Glycolysis

Phosphoenolpyruvate is dephosphorylated to pyruvate. The high energy content of PEP is trapped into ATP by the pyruvate kinase reaction. This is again an example of **substrate level phosphorylation** (See Fig. 5.3). The pyruvate kinase also is a *key glycolytic enzyme*. The pyruvate kinase step is **irreversible**. The reversal, however, can be attained in the body with the help of two enzymes and hydrolysis of 2 ATP molecules (see gluconeogenesis) (Fig. 5.5). While pyruvate kinase is a key glycolytic enzyme, the pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) are key gluconeogenic enzymes.

Step 10 of Glycolysis

In anaerobic condition, pyruvate is reduced to lactate by lactate dehydrogenase (LDH). (Fig. 5.6). (Anaerobiasis is a Greek term; an=not; aer=air; bios=life). When oxygen is available, the main metabolic fate of pyruvate is dehydrogenation to produce acetyl-CoA; which then enters into citric acid cycle. However citric acid cycle can be operated only when there is plenty of oxygen. In the actively contracting muscles, there is comparative lack of oxygen. In such anaerobic condition, the major pathway of pyruvate is thus blocked. LDH has 4 subunits and 5 isoenzymes. The cardiac isoenzyme of LDH has special importance to detect myocardial infarcts (For details refer Chapter 3).

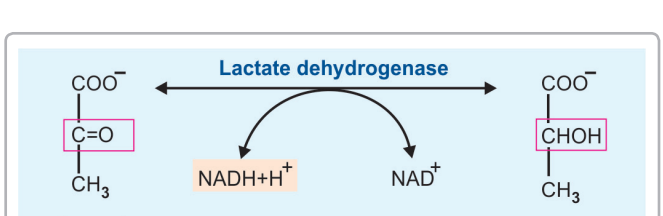


Figure 5.6: Step 10; LDH reaction; reversible

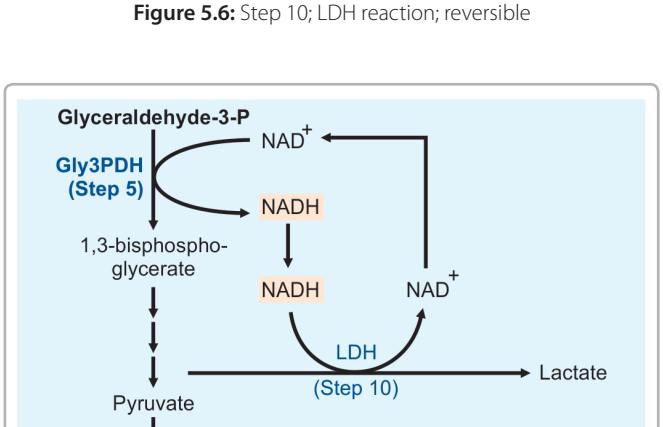


Figure 5.7: Lactate formation is necessary for reconversion of NADH to NAD^+ During anaerobiosis.

Steps 5 and 10 are Coupled

In anaerobiosis, glycolysis is the only major source of energy. In 5th step, for each molecule of glucose entering in the pathway, two molecules of NAD^+ are reduced to NADH. The availability of coenzymes inside a cell is limited. Therefore this step becomes a bottleneck in the whole reaction sequence. (Fig. 5.7). When there is lack of oxygen, the cell has to couple some other reaction in which NAD^+ is regenerated in the cytoplasm itself. Hence pyruvate is reduced to lactate; the NAD^+ thus generated is reutilized for uninterrupted operation of the 5th step.

A summary of glycolysis pathway is shown in Fig. 5.3. In aerobic conditions, the pyruvate enters the citric acid cycle for complete oxidation. The end product of anaerobic glycolysis is lactate which enters the Cori's cycle (Fig. 5.8).

Energy Yield from Glycolysis

1. **During anaerobic (oxygen deficient) condition,** when one molecule of glucose is converted to 2 molecules of lactate, there is a net yield of 2 molecules of ATP. On the whole, 4 molecules of ATP are synthesized by the 2 substrate level phosphorylations (steps 6 and 9). But 2 molecules of ATP are used in the steps 1 and 3, hence the **net yield is only 2 ATP**. (Table 5.1). The whole reaction is summarized as
   Glucose 2 Pi + 2 ADP --------à 2 Lactate + 2 ATP
2. **When oxygen is in plenty,** the two NADH molecules, generated in the glyceraldehyde-3-phosphate dehydrogenase reaction (step 5), can enter the mitochondrial electron transport chain for complete oxidation (For detail refer Chapter 14). As each NADH provides 2.5 ATPs, this reaction generates 2.5 x 2 = 5 ATPs. Thus when oxygen is available, the net gain of energy from the glycolytic pathway is **7 ATPs** (Table 5.2). Hence the ATP yield from glycolysis is different in anaerobic and aerobic conditions (compare Tables 5.1 and 5.2).
3. **Complete oxidation of glucose:** Pyruvate is later oxidatively decarboxylated to acetyl-CoA (see below),

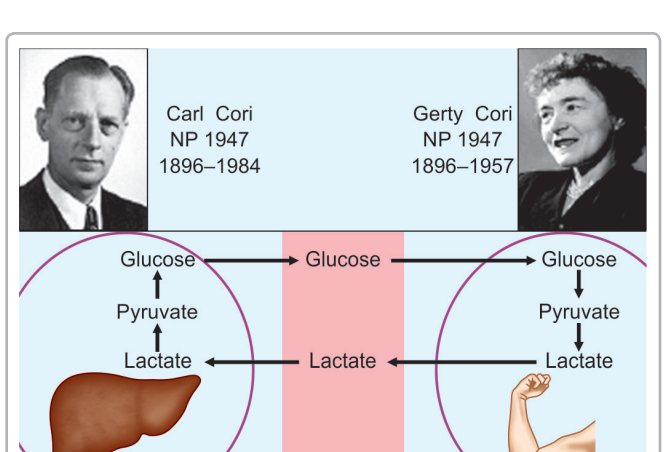


Figure 5.8: Cori's cycle. Contracting muscle has lack of oxygen. So pyruvate is reduced to lactate. This can be reconverted to glucose in liver where oxygen is available

Table 5.1: Energy yield (number of ATP generated) per molecule of glucose in the glycolytic pathway, under anaerobic conditions (Oxygen deficiency)

Step	*Enzyme*	*Source*	*No. of ATPs gained per glucose mol*
1	Hexokinase	--	Minus 1
3	Phosphofructokinase	--	Minus 1
6	1,3-bisphosphoglycerate kinase	ATP	1 x 2 = 2
9	Pyruvate kinase	ATP	1 x 2 = 2
		Total	4 minus 2 = 2

which enters into the citric acid cycle (For detail refer Chapter 14). Complete oxidation of glucose through glycolysis plus citric acid cycle will yield a **net 32 ATPs** (Table 5.3). All the above calculations assume that each NADH generates 2.5 ATPs.

Note: In previous edition of the textbook, calculations were made assuming that in the electron transport chain, NADH produces 3 ATPs and FADH generates 2 ATPs. This will amount to a net generation of 38 ATP per glucose molecule. Recent experiments show that these old values are overestimates. (For detail refer Chapter 13).

Table 5.2: Energy yield (number of ATP generated) per molecule of glucose in the glycolytic pathway, under aerobic conditions (oxygen is available).

Step	*Enzyme*	*Source*	*No. of ATPs gained per glucose mol*
1	Hexokinase	—	– 1
3	Phosphofructokinase	—	– 1
5	Glyceraldehyde-3-phosphate dehydrogenase	NADH	2.5 x 2 = 5
6	l,3-bisphosphoglycerate kinase	ATP	1 x 2 = 2
9	Pyruvate kinase	ATP	1 x 2 = 2
		Total =	9 - 2 = 7

CORI'S CYCLE OR LACTIC ACID CYCLE

In an actively contracting muscle, only about 8% of the pyruvate is utilized by the citric acid cycle, and the remaining molecules are therefore reduced to lactate. The lactic acid thus generated should not be allowed to accumulate in the muscle tissues. The muscle cramps, often associated with strenuous muscular exercise, are thought to be due to lactate accumulation. This lactate diffuses into the blood. During exercise, blood lactate level is increased appreciably. Lactate then reaches liver, where it is oxidized to pyruvate. It is then taken up through gluconeogenesis pathway, and becomes glucose which can enter into blood and then taken to muscle. This cycle is called Cori's cycle, by which the **lactate is efficiently reutilized by the body** (See Fig. 5.8). Carl Cori and Gerty Cori were awarded Nobel prize in 1947.

Regulation of Glycolysis

1. Hormones

Insulin favors glycolysis by activating key glycolytic enzymes (glucokinase, phosphofructokinase and pyruvate kinase). Glucagon and glucocorticoids inhibit glycolysis. **Glucocorticoids** inhibit glycolysis and favors gluconeogenesis (Table 5.4).

Table 5.3: Energy yield (number of ATP generated) per molecule of glucose when it is completely oxidized through glycolysis plus citric acid cycle, under aerobic conditions

Pathway	*Step*	*Enzyme*	*Source*	*No of ATPs gained per glucose*
Glycolysis	1	Hexokinase	--	Minus 1
Glycolysis	3	Phosphofructokinase	--	Minus 1
Glycolysis	5	Glyceraldehyde-3-P DH	NADH	2.5 x 2 = 5
Glycolysis	6	1,3-BPG kinase	ATP	1 x 2 = 2
Glycolysis	9	Pyruvate kinase	ATP	1 x 2 = 2
Pyruvate to Acetyl-CoA		Pyruvate dehydrogenase	NADH	2.5 x 2 = 5
TCA cycle	3	Isocitrate DH	NADH	2.5 x 2 = 5
TCA cycle	4	alpha ketoglutarate DH	NADH	2.5 x 2 = 5
TCA cycle	5	Succinate thiokinase	GTP	l x 2 = 2
TCA cycle	6	Succinate DH	FADH2	1.5 x 2 = 3
TCA cycle	8	Malate DH	NADH	2.5 x 2 = 5
		Net generation in glycolytic pathway		9 minus 2 = 7
		Generation in pyruvate dehydrogenase reaction		5
		Generation in citric acid cycle		20
		Net generation of ATP from one glucose mol		32

Table 5.4: Regulatory enzymes of glycolysis

Enzyme	*Activation*	*Inhibition*
Hexokinase	–	Glucose-6-Phosphate
Glucokinase	Insulin	Glucagon
Phosphofructokinase	Insulin, AMP	Glucagon, ATP
Pyruvate kinase	Insulin, F1,6-BP	Glucagon, ATP

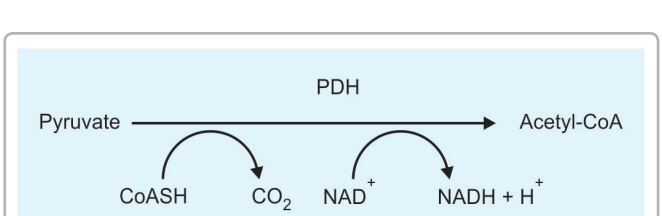


Figure 5.9: Pyruvate dehydrogenase reaction

2. Phosphofructokinase (PFK)

It is the most important **rate-limiting** enzyme for glycolysis pathway. PFK (step 3) is an allosterically regulated enzyme. ATP is the most important allosteric inhibitor. Yet another allosteric inhibitor of PFK is citrate. AMP acts as an allosteric activator.

3. Pyruvate Kinase

Pyruvate kinase catalyzes an irreversible step (step 9). It is a regulatory enzyme of glycolysis. The enzyme is inhibited by ATP. Insulin increases its activity where as glucagon inhibits.

A summary of regulatory enzymes of glycolysis is given in Table 5.4.

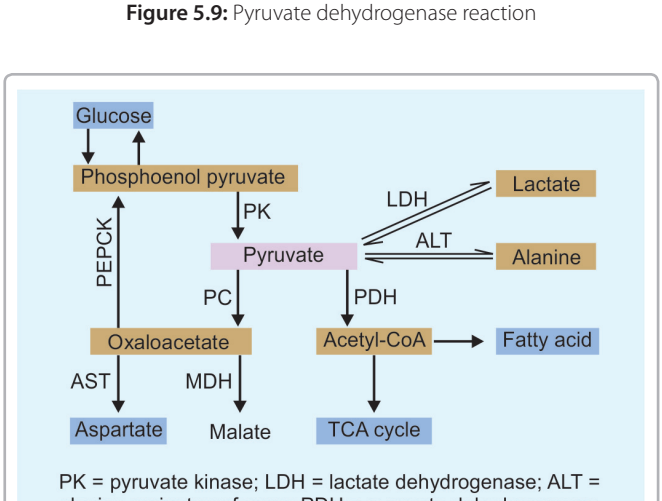


Figure 5.10: Pyruvate as a metabolic junction point

METABOLIC FATE OF PYRUVATE

Pyruvate Dehydrogenase Complex

Under aerobic conditions, pyruvate is converted to acetyl CoA which may be oxidized in TCA cycle to yield ATP. Pyruvate is **oxidatively decarboxylated** to acetyl-CoA by pyruvate dehydrogenase (PDH). (Fig. 5.9). It is an enzyme complex containing thiamine pyrophosphate (TPP), lipoamide, Coenzyme A (CoA), FAD and NAD. All the above cofactors, except lipoamide, are B complex group of substances. The NADH thus generated, then enters the electron transport chain to produce 2.5 ATP molecules. PDH enzyme requires thiamine pyrophosphate (TPP); this explains the serious afflictions in **beriberi** due to thiamine deficiency (For details refer Chapter 16). TPP deficiency in alcoholism causes pyruvate accumulation in tissues. **Completely irreversible Process:** The oxidative decarboxylation of pyruvate to acetyl CoA is a completely irreversible process. There are no pathways available in the body to circumvent this step. Glucose through this step is converted to acetyl-CoA from which fatty acids can be synthesized. But the backward reaction is not possible, and so **there is no net synthesis of glucose from fat.**

Pyruvate as a Junction Point

Pyruvate occupies an important junction between various metabolic pathways. It may be decarboxylated to acetyl-CoA which enters the TCA cycle, or may be utilized for fatty acid synthesis. Pyruvate may be carboxylated to oxaloacetate which is used for gluconeogenesis. These pathways are summarized in Figure 5.10. Pyruvate dehydrogenase step is the committed step towards oxidation of glucose.

GLUCONEOGENESIS

It is the process by which new glucose is synthesized from noncarbohydrate precursors like lactate and glucogenic amino acids. It occurs mainly in the liver. Gluconeogenesis involves several enzymes of glycolysis, but it is not a reversal of glycolysis. The irreversible steps in glycolysis are circumvented by four enzymes which are designated as the key enzymes of gluconeogenesis (Table 5.5).

Table 5.5. Key enzymes	
Irreversible steps in glycolysis	Corresponding key gluconeogenic enzymes
Pyruvate kinase (Step 9)	Pyruvate carboxylase; Phosphoenol pyruvate carboxy kinase
Phosphofructokinase (Step 3)	Fructose-1,6-bisphosphatase
Hexokinase (Step 1)	Glucose-6-phosphatase

1. Pyruvate Carboxylase Reaction

In the first reaction, carboxylation of pyruvate to oxaloacetate is catalysed by the enzyme, pyruvate carboxylase. (See Fig. 5.5). It contains **biotin** which acts as a carrier of active CO_2. The reaction requires ATP. This enzyme is activated by acetyl-CoA.

2. Phosphoenolpyruvate Carboxykinase

This enzyme converts oxaloacetate to phosphoenol pyruvate by losing a molecule of CO_2 and adding a high energy phosphate. GTP or ITP donates the phosphate (See Fig. 5.5). The net effect of these two reactions is the conversion of pyruvate to phosphoenol pyruvate, thus circumventing the irreversible step in glycolysis catalyzed by pyruvate kinase (step 9 of glycolysis).

3. Reversal of Glycolysis

The phosphoenol pyruvate undergoes further reactions catalyzed by the glycolytic enzymes which are all freely reversible to form fructose-1,6-bisphosphate (see glycolysis steps 8,7,6,5 and 4).

4. Fructose-1,6-bisphosphatase

Fructose 1,6-bis-phosphate is then acted upon by fructose 1,6-bisphosphatase to form fructose-6-phosphate. This will bypass the step of PFK reaction (see step 3 of glycolysis).

$$\text{Fructose-1,6-bisphosphate} \xrightarrow{\text{Fructose-1,6-bisphosphatase}} \text{Fructose-6-phosphate} + \text{Pi}$$

Then fructose-6-phosphate is isomerized to glucose-6-phosphate by the freely reversible reaction catalysed by hexose phosphate isomerase (second step in glycolysis).

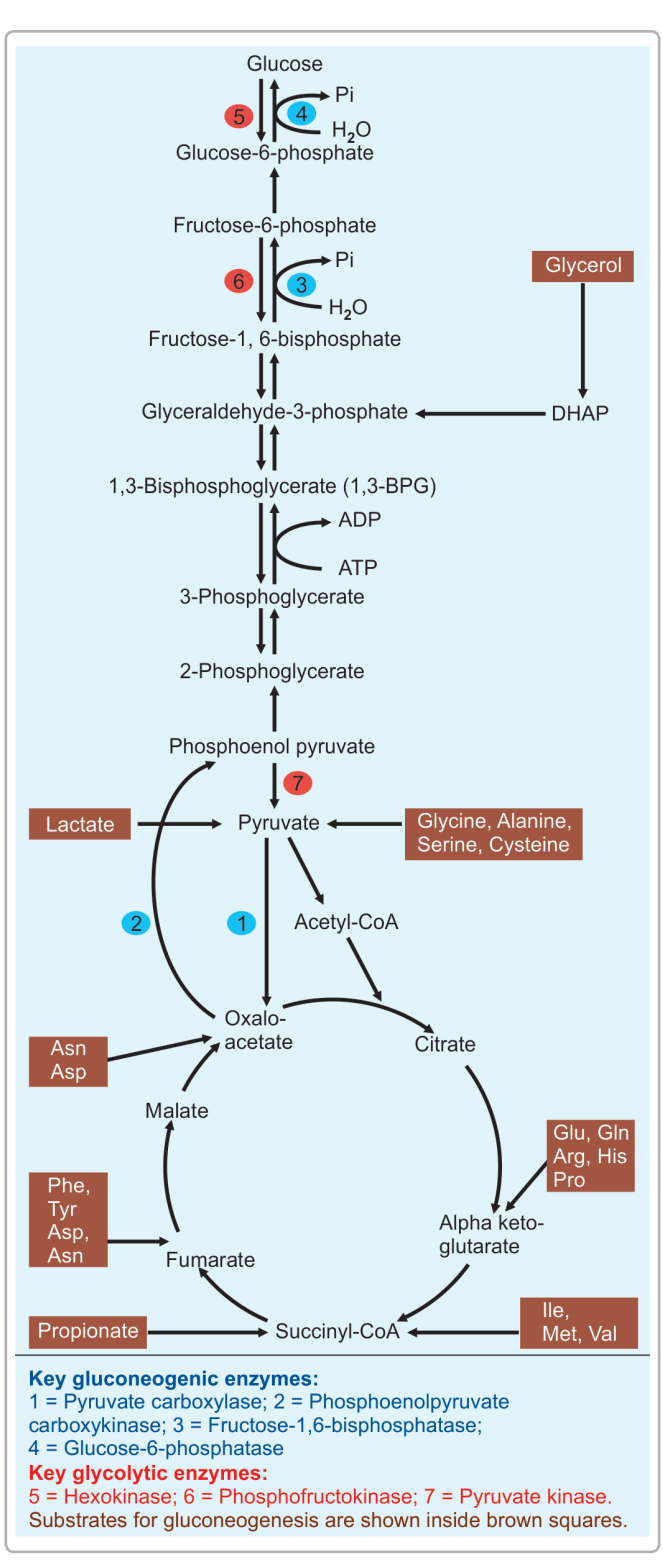


Figure 5.11: Glucogenic pathway

5. Glucose-6-phosphatase

The glucose 6-phosphate is hydrolyzed to free glucose by glucose-6-phosphatase.

Glucose-6-phosphate ⟶ Glucose

Glucose-6-phosphatase is **active in liver**. It is **absent in muscle**. *Therefore only liver can replenish blood sugar through gluconeogenesis.*

See Fig. 5.11 for the summary.

Energy Requirement for Gluconeogenesis

The reactions catalyzed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase and phosphoglycerate kinase require one ATP each; so 3 ATPs are used by 1 pyruvate residue to produce one-half molecule of glucose; or 6 ATPs are required to generate one glucose molecule.

$$\text{Glucose} \underset{\text{Gluconeogenesis (utilizes 6 ATP)}}{\overset{\text{Glycolysis (generates 2 ATP)}}{\rightleftharpoons}} \text{Lactate}$$

Substrates for Gluconeogenesis

Lactate and glucogenic amino acids are the most important substrates for gluconeogenesis.

1. Lactate

One of the important substrates for gluconeogenesis is lactate. The lactate formed in the muscle or RBC is transported to the liver. In the liver cell, the lactate dehydrogenase converts lactate to pyruvate (Fig. 5.12). The pyruvate is then converted to oxaloacetate, which is channeled to glucose (Fig. 5.11). Generation of lactic acid in muscle and its utilization through Cori's cycle is described previously (Fig. 5.8).

2. Glucogenic amino acids

The glucogenic amino acids are transaminated to TCA cycle intermediates so that they form oxaloacetate or pyruvate. **Alanine** released from the muscle is the major substrate for gluconeogenesis (Fig. 5.13). The entry points of other glucogenic amino acids are shown in Fig. 5.11. **Glucose-Alanine Cycle:** Alanine is transported to liver, transaminated to pyruvate and converted to glucose. This glucose may again enter the glycolytic pathway to form pyruvate, which in turn, can be transaminated to alanine. This glucose-alanine cycle is of primary importance in conditions of starvation (Fig. 5.14). Thus net transfer of alanine from muscle to liver and corresponding transfer of glucose (energy) from liver to muscle is affected. A summary of gluconeogenesis pathway is shown in Fig. 5.11. Other sources are glycerol and propionyl-CoA.

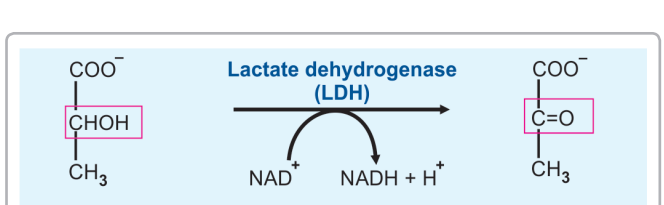


Figure 5.12: Reversal of step 10 of glycolysis

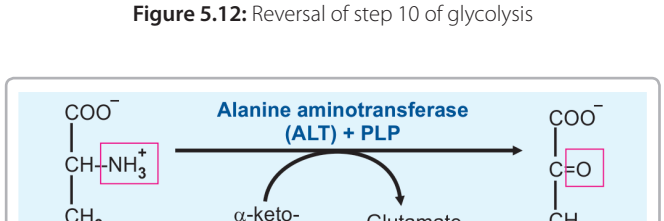


Figure 5.13: Transamination of alanine

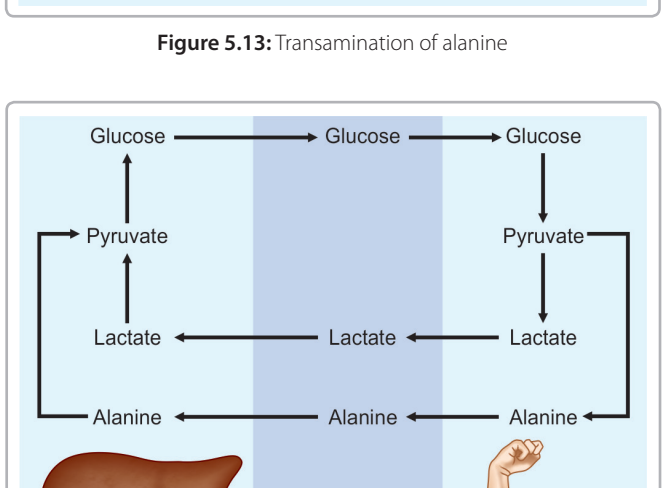


Figure 5.14: Cori's cycle and alanine cycle

Regulation of Gluconeogenesis

Gluconeogenesis and glycolysis are always reciprocally regulated so that one pathway is relatively inactive when the other is active. If not, it would result in a futile cycle or substrate cycle with dissipation of energy.

1. Hormonal Regulation of Gluconeogenesis

Glucagon increases gluconeogenesis. **Glucocorticoids** increase gluconeogenesis. Glucocorticoids induce the synthesis of hepatic amino transferases thereby providing substrate for gluconeogenesis. The high glucagon also favors induction of synthesis of gluconeogenic enzymes (PEPCK, Fructose-1,6-bisphosphatase and glucose-6-phosphatase). At the same time, synthesis of glycolytic

Table 5.6: Regulatory enzymes of gluconeogenesis (compare with Table 5.4)

Enzyme	*Activation*	*Inhibition*
Pyruvate carboxylase	Cortisol, glucagon	Insulin, ADP
Phosphoenolpyruvate carboxykinase	do	Insulin
Fructose-1,6-bisphosphatase	do	F-1,6-BP, AMP
G-6-phosphatase	do	Insulin

enzymes HK, PFK and PK are depressed. **Insulin** inhibits the process (Fig. 5.15). A summary of regulatory enzymes of gluconeogenesis is shown in Table 5.6, which may be compared with key enzymes of glycolysis in Table 5.4.

2. Physiological Significance

The major metabolic significance of gluconeogenesis is *maintenance of blood glucose level especially under conditions of starvation*. The brain has a minimum obligatory requirement of 120 grams of glucose per day. Under conditions of starvation this is provided by gluconeogenesis. The body stores of glycogen are depleted within the first 12–18 hours of fasting. On prolonged starvation, the gluconeogenesis is speeded up.

GLYCOGEN METABOLISM

Functions of Glycogen

1. Glycogen is the storage form of carbohydrates in the human body. The major sites of storage are liver and muscle. The major function of **liver glycogen** is to provide glucose during starvation.
2. When blood glucose level lowers, liver glycogen is broken down and helps to maintain blood glucose level.
3. The function of muscle glycogen is to act as reserve fuel for muscle contraction.
4. After taking food, blood sugar tends to rise, which causes glycogen deposition in liver. About 5 hours after taking food, the blood sugar tends to fall. But, glycogen is lysed to glucose so that the energy needs are met.
5. After about 18 hours fasting, most of the liver glycogen is depleted, when depot fats are hydrolyzed and energy requirement is met by fatty acid oxidation.

Degradation of Glycogen (Glycogenolysis)

1. Glycogen Phosphorylase

The enzyme glycogen phosphorylase removes glucose units one at a time from the nonreducing end of the

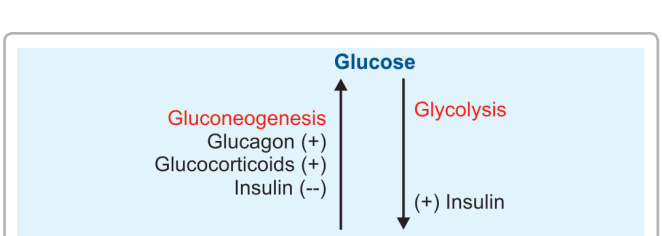


Figure 5.15: Hormonal regulation of gluconeogenesis

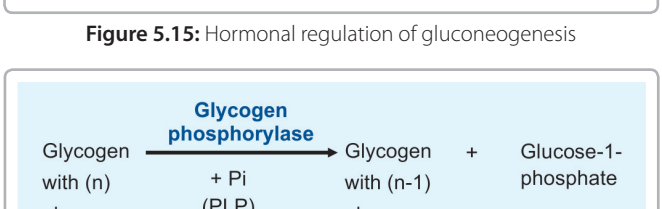


Figure 5.16: Reaction of glycogen phosphorylase

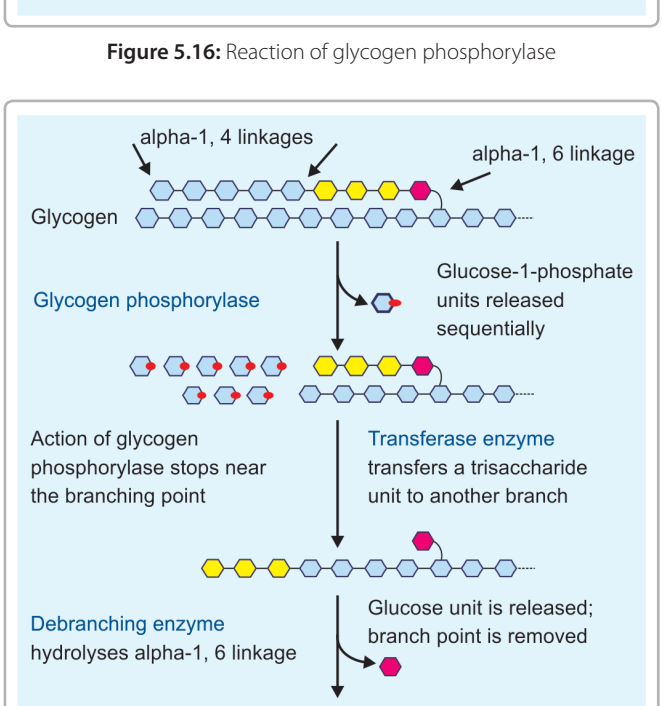


Figure 5.17: Glycogenolysis

glycogen molecule. The product is glucose-1-phosphate (Fig. 5.16). Phosphorylase sequentially attacks alpha-1,4 glycosidic linkages, till it reaches a branch point (Fig. 5.17). It cannot attack the 1,6 linkage at branch point.

2. Debranching Needs Two Enzymes

With the help of **glucan transferase** and **debranching enzyme** (alpha-1,6 glucosidase), the branching point is

also hydrolyzed. This glucose residue is released as **free glucose**. With the removal of branch, phosphorylase enzyme can now proceed with its action.

3. Phosphoglucomutase

Phosphorylase reaction produces glucose-1-phosphate while debrancher enzyme releases glucose. The glucose-1-phosphate is converted to glucose-6-phosphate by phosphoglucomutase.

4. Glucose-6-phosphatase in Liver

Next, hepatic glucose-6-phosphatase hydrolyses glucose-6-phosphate to glucose. The product of hepatic glycogenolysis is free glucose, which is released to the blood stream.

5. Muscle Lacks Glucose-6-phosphatase

But muscle will not release glucose to the blood stream, because muscle tissue does not contain the glucose-6-phosphatase. The energy yield from one glucose residue derived from glycogen is 3 ATP molecules, because no ATP is required for initial phosphorylation of glucose (step 1 of glycolysis). If glycolysis starts from free glucose only 2 ATPs are produced.

Glycogen Synthesis (Glycogenesis)

The glycogen synthesis occurs by a pathway distinctly different from the reversal of glycogen breakdown.

1. Activation of Glucose

UDP glucose is formed from glucose-1-phosphate and UTP (uridine triphosphate) by the catalytic activity of UDP-glucose **pyrophosphorylase**.

$$\text{Glucose-1-phosphate} + \text{UTP} \xrightarrow{\text{UDP-glucose pyrophosphorylase}} \text{UDP-glucose} + \text{PPi}$$

2. Glycogen Synthase

In the next step, activated glucose units are sequentially added by the enzyme glycogen synthase. The glucose unit from UDP-glucose is transferred to a glycogen primer molecule.

$$\text{Glycogen primer(n)} + \text{UDP-glucose} \longrightarrow \text{Glycogen (n+1)} + \text{UDP}$$

The glucose unit is added to the nonreducing (outer) end of the glycogen to form an alpha-1,4 glycosidic linkage and UDP is liberated.

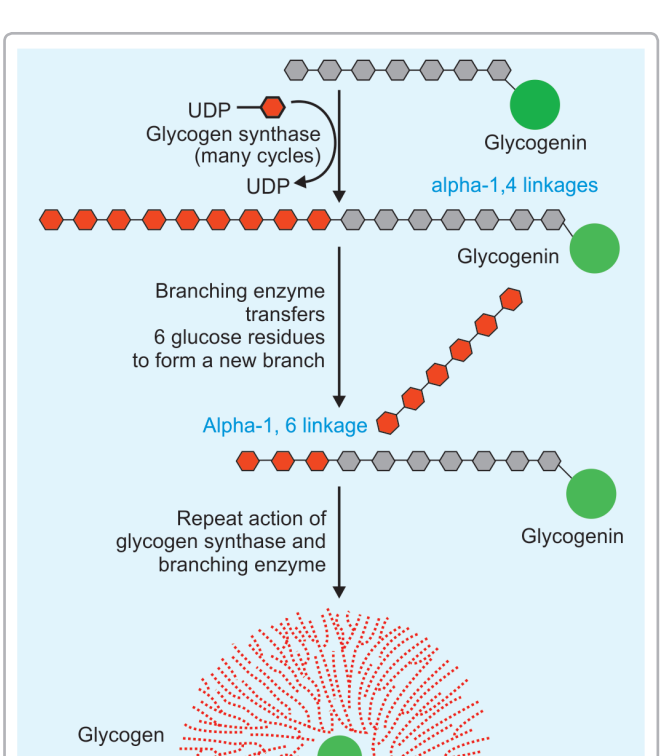


Figure 5.18: Formation of branches in glycogen

3. Brancher Enzyme

A branching enzyme is needed to create the alpha-1,6 linkages. (Fig. 5.18). To this newly created branch, further glucose units can be added in alpha-1,4 linkage by glycogen synthase. Branching makes the molecule more globular.

Regulation of Glycogen Metabolism

The synthesis and degradation pathways are reciprocally regulated to prevent futile cycles. *The phosphorylated form of glycogen phosphorylase is active whereas glycogen synthase becomes relatively inactive on phosphorylation.* The covalent modification of these enzymes is brought by hormones that act through a second messenger, cyclic AMP (cAMP). The mechanism is shown in Fig. 5.19.

Generation of Cyclic AMP (cAMP)

Both liver and muscle phosphorylases are activated by a **cyclic AMP mediated activation cascade** triggered by the hormonal signal. The hormones epinephrine and

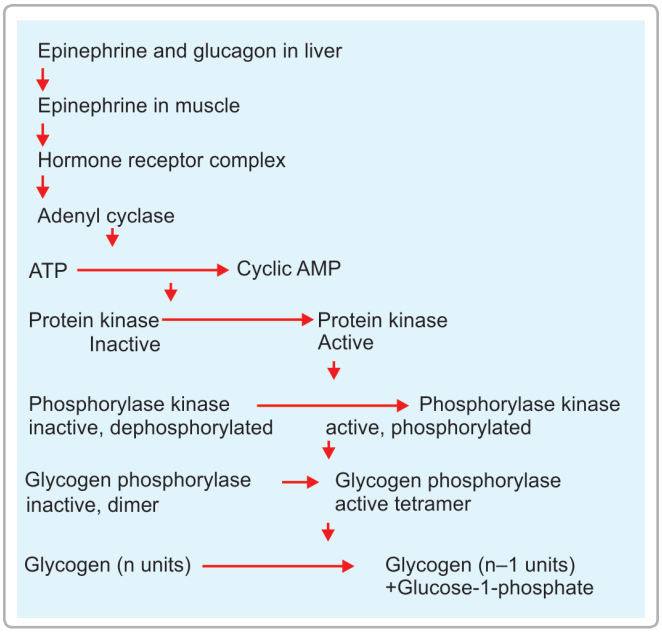


Figure 5.19: Cyclic AMP mediated degradation of glycogen

glucagon can activate liver glycogen phosphorylase but glucagon has no effect on the muscle. When the hormone binds to a specific receptor on the plasma membrane, the enzyme adenyl cyclase that converts ATP to cAMP is activated. When level of cyclic AMP rises, it will activate a protein kinase (Fig. 5.19).

Regulation

The key enzyme for glycogenolysis is **phosphorylase**, which is activated by **glucagon** and adrenaline, under the stimulus of hypoglycemia. The key enzyme for glycogen synthesis is **glycogen synthase**, the activity of which is decreased by adrenaline but is enhanced by **insulin**, under the stimulus of hyperglycemia.

GLYCOGEN STORAGE DISEASES

These are inborn errors of metabolism; the word is coined by Garrod in 1908.

Glycogen Storage Disease Type-I

It is also called **Von Gierke's Disease.** Most common type of glycogen storage disease is type I. Incidence is 1 in 100,000 live births. **Glucose-6-phosphatase** is deficient. **Fasting hypoglycemia that does not respond to stimulation by adrenaline**. The glucose cannot be released from liver during overnight fasting (Table 5.7). Hyperlipidemia, **lactic acidosis** and **ketosis** are seen. Glycogen gets deposited in liver. Massive liver enlargement may lead to **cirrhosis**. Children usually die in early childhood. Treatment is to give small quantity of food at frequent intervals.

Table 5.7: Glycogen storage diseases

Disease	*Deficient*	*Salient features*
Type I Von Gierke's disease	Glucose-6-phosphatase	Fasting hypoglycemia; Hepatomegaly
Type II generalized glycogenosis; Pompe's disease	Lysosomal maltase	Liver, heart and muscle affected; death before 2 years
Type III Limit dextrinosis Cori's disease	Debranching enzyme	Highly branched dextrin accumulates; Hepatomegaly
Type IV Amylopectinosis Anderson's disease	Brancing enzyme	Glycogen with little branches; hepatomegaly
Type V McArdle's disease	Muscle phosphorylase	Exercise intolerance
Type VI Hers disease	Liver phosphorylase	Hypoglycemia
Type VII Tarui's disease	Muscle PFK	Accumulation of glycogen in muscles
Type VIII	Liver phosphorylase kinase	
Type IX Lewis disease	Glycogen synthase	

Glycogen storage diseases (type II to IX) are shown in Table 5.7. They are very rare, incidence being 1 in 1 million births.

A QUICK LOOK

1. Deficiency of Lactase results in lactose intolerance.
2. Insulin dependent GluT4 has been implicated in Type 2 diabetes mellitus.
3. Glycolysis occurs both in aerobic and anaerobic conditions. Anaerobic glycolysis is the major source of energy for muscles, when the muscle tissue lacks oxygen.
4. Phosphofructokinase (PFK) is the regulatory or rate limiting enzyme of glycolysis. It is an allosteric and inducible enzyme. AMP is allosteric activator, while citrate and ATP are allosteric inhibitors.
5. The reaction catalyzed by 1,3-bisphosphoglycerate kinase and pyruvate kinase are examples of substrate level phosphorylation.
6. Energy generating steps of glycolysis are catalyzed by the enzymes glyceraldehyde-3-phosphate dehydrogenase: (NADH); 1,3-bisphosphoglycerate kinase (ATP) and pyruvate kinase (ATP).
7. Cori's cycle ensures efficient reutilization of lactate produced in the muscle.

8. Energy yield per molecule of glucose in the glycolytic pathway under anaerobic conditions is 2 ATPs.
9. Under anaerobic condition pyruvate is reduced to lactate by lactate dehydrogenase. Under aerobic conditions, it is oxidatively decarboxylated to acetyl-CoA by the enzyme complex Pyruvate dehydrogenase (PDH).
10. The PDH enzyme complex requires 5 cofactors for its activity viz. NAD, FAD, TPP, Lipoamide and CoA.
11. The PDH reaction is a totally irreversible reaction. Hence, there is no net synthesis of glucose from fat.
12. Key enzymes of gluconeogenesis are; pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase.
13. Major substrates for gluconeogenesis are lactate and glucogenic amino acids.
14. Glycogen is the storage polysaccharide of the body. It is stored mainly in the liver and muscle.
15. Glycogen phosphorylase is activated by glucagon and adrenaline, while glycogen synthase is activated by insulin.
16. Glycogen storage diseases (GSD) are Inborn errors of metabolism. Type 1 is called von Gierke's disease.
17. The HMP shunt pathway, also known as pentose phosphate pathway (PPP) generates NADPH required for reductive cytoplasmic biosynthesis of biomolecules such as steroids, fatty acids and cholesterol.
18. NADPH generated as a result of HMP pathway is essential to maintain transparency of the eye lens, to prevent methemoglobinemia and to maintain erythrocyte membrane integrity.
19. The pathway also provides pentose sugars (ribose and deoxyribose) for nucleic acid synthesis.
20. GPD deficiency is a common clinical condition, transmitted as X linked recessive trait. Ingestion of fava beans (favism) and anti-malarials such as primaquine precipitates the manifestations.

CHAPTER 6

Carbohydrates–III: Regulation of Blood Glucose: Diabetes Mellitus

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Normal plasma glucose level
- Factors maintaining blood glucose
- Oral glucose tolerance test (OGTT)
- Diagnostic criteria for Diabetes Mellitus
- Reducing substances in urine
- Metabolic derangements in Diabetes
- Glycated hemoglobin

REGULATION OF BLOOD GLUCOSE

The maintenance of glucose level in blood within narrow limits is a very finely and efficiently regulated system. This is important, because it is essential to have continuous supply of glucose to the brain. Brain has an obligatory requirement for glucose.

Factors Maintaining Blood Sugar

1. The plasma glucose level at an instant depends on the balance between glucose entering and leaving the extra cellular fluid. Hormones will make this balance possible (Figs. 6.1 and 6.3).
2. The major factors which cause **entry of glucose** into blood are:
   - Absorption from intestines
   - Glycogenolysis (breakdown of glycogen)
   - Gluconeogenesis
   - Hyperglycemic hormones (glucagon, steroids)
3. Factors leading to **depletion of glucose** in blood are:
   - Utilisation by tissues for energy
   - Glycogen synthesis
   - Conversion of glucose into fat (lipogenesis)
   - Hypoglycemic hormone (insulin)

Post-prandial Regulation

Following a meal, glucose is absorbed from the intestine and enters the blood. The rise in the blood

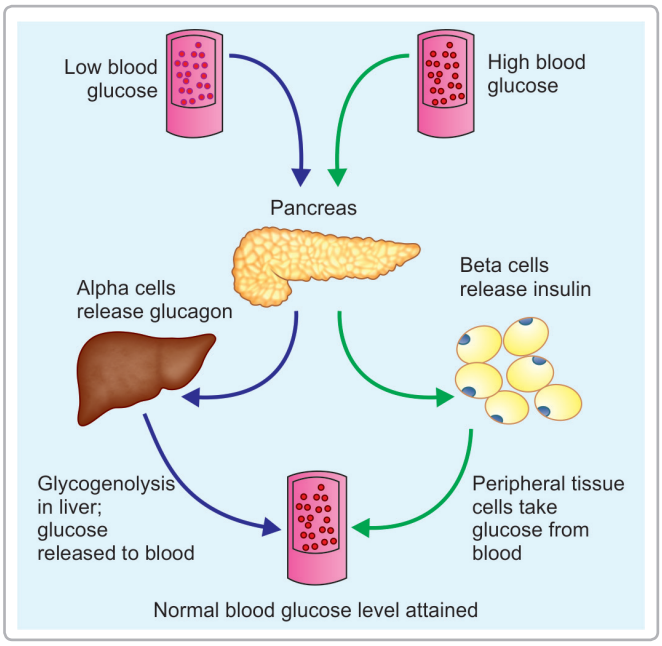


Figure 6.1: Homeostasis of blood glucose

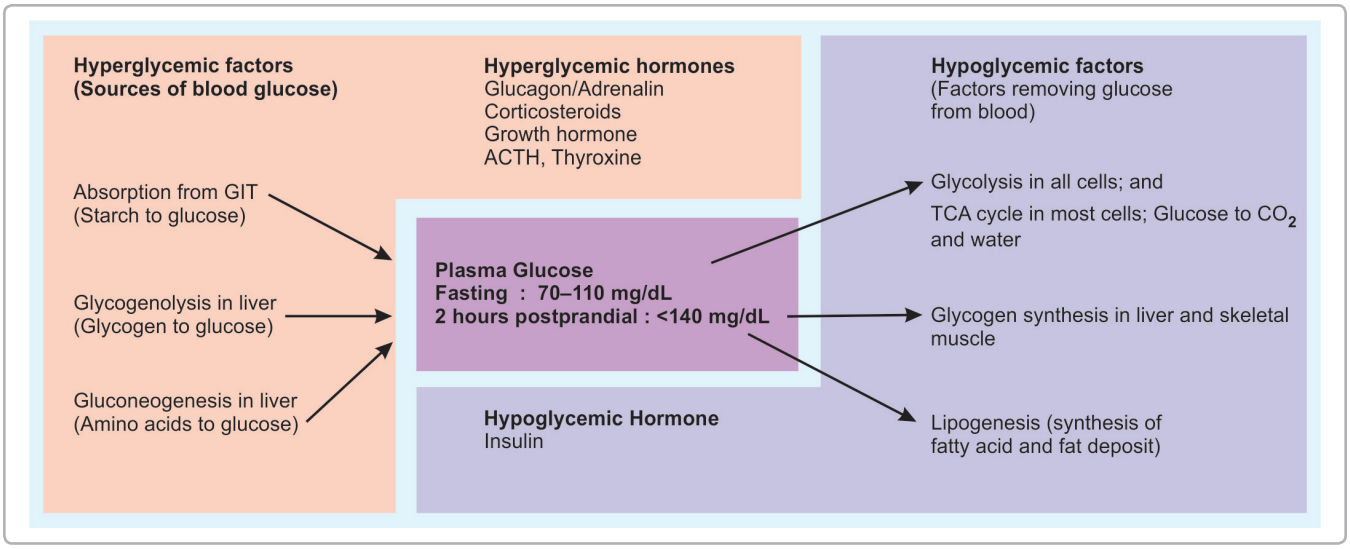
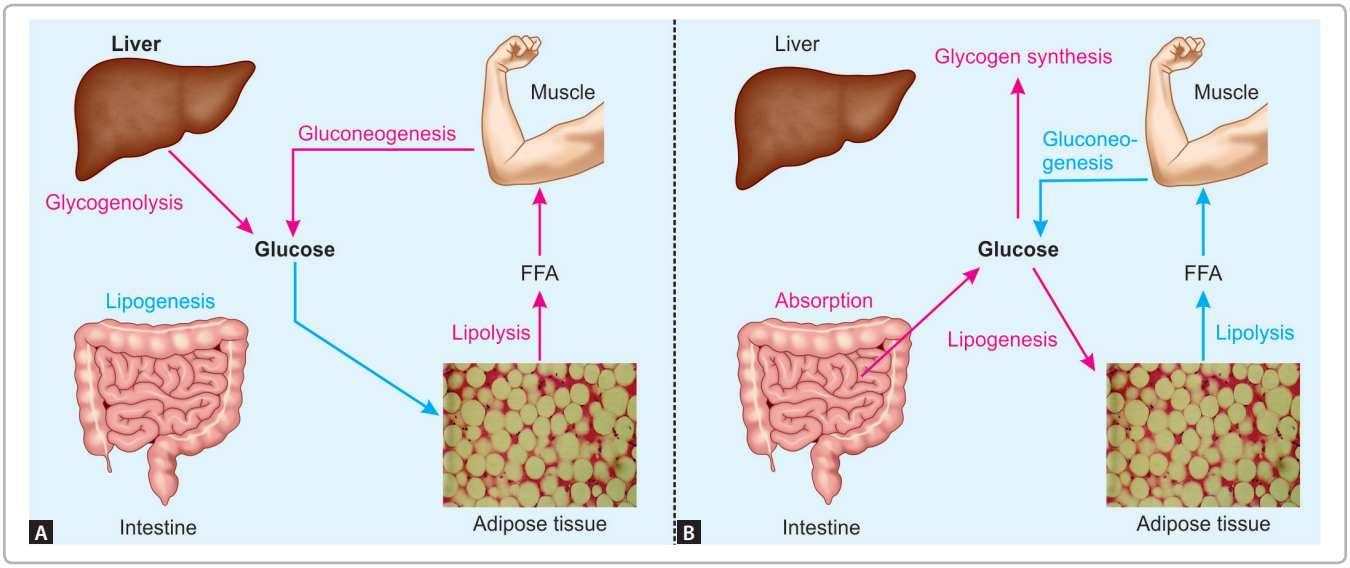


Figure 6.2: Overview of regulation of blood glucose



Figures 6.3A and B: Blood glucose regulation (A) In fasting state, blood glucose level is maintained by glycogenolysis and gluconeogenesis; further, adipose tissue releases free fatty acids as alternate source of energy; (B) In postprandial state, glucose level is high; then blood glucose level is lowered by tissue oxidation, glycogen synthesis and lipogenesis

glucose level stimulates the secretion of **insulin** by beta cells of islets of Langerhans of pancreas. The uptake of glucose by most extrahepatic tissues, except brain is dependent on insulin. Moreover, insulin helps in the storage of glucose as glycogen or its conversion to fat (Fig. 6.2A).

Regulation in Fasting State

Normally, 2 to 2½ hours after a meal, the blood glucose level falls to near fasting levels. It may go down further; but this is prevented by processes that contribute glucose to the blood. For the next 3 hours, hepatic **glycogenolysis** will take care of the blood sugar level. Thereafter, **gluconeogenesis** will take charge of the situation. (Fig. 6.3B). Liver is the major organ that supplies the glucose for maintaining blood glucose level (Fig. 6.1). **Hormones** like glucagon, epinephrine, glucocorticoids, growth hormone, ACTH and thyroxine will keep the blood glucose level from falling. They are referred to as anti-insulin hormones or hyperglycemic hormones.

NORMAL PLASMA GLUCOSE LEVEL

In normal persons, **fasting** plasma glucose value is **70–110 mg/dL**. Fasting state means, glucose is estimated after an overnight fast of 12 hours (post-absorptive state). Following a meal, in a normal person the glucose level does not usually rise above 140 mg/dL due to prompt secretion of insulin. When blood glucose level is within normal limits, it is referred to as **normoglycemia.** When values are above the normal range, it is known as **hyperglycemia**. When the value falls below 50 mg/dL, it is called **hypoglycemia**. (Greek, hyper =above; hypo = below). Hyperglycemia is harmful; while hypoglycemia may be fatal.

Sugar in Urine

Normally glucose is not excreted in urine. But if blood sugar is **more than 180 mg/dL,** urine contains glucose. The blood level of glucose above which glucose is excreted is called **renal threshold**.

Effects of hormones on glucose level in blood

- Insulin (hypoglycemic hormone)
  - Lowers blood glucose
  - Favors glycogen synthesis
  - Promotes glycolysis
  - Inhibits gluconeogenesis
- Glucagon (hyperglycemic hormone)
  - Increases blood glucose
  - Promotes glycogenolysis
  - Enhances gluconeogenesis
  - Depresses glycogen synthesis
  - Inhibits glycolysis.
- Cortisol (hyperglycemic hormone)
  - Increases blood sugar level
  - Increases gluconeogenesis
  - Releases amino acids from the muscle
- Adrenaline or epinephrine (hyperglycemic)
  - Increases blood sugar level
  - Promotes glycogenolysis
  - Increases gluconeogenesis
  - Favours uptake of amino acids
- Growth Hormone (hyperglycemic)
  - Increases blood sugar level
  - Decreases glycolysis
  - Mobilizes fatty acids from adipose tissue

Determination of Glucose in Body Fluids

Estimation of glucose is the commonest analysis done in clinical laboratories. The blood is collected using an anticoagulant (potassium oxalate) and an inhibitor of glycolysis (sodium fluoride).

Fluoride inhibits the enzyme, enolase, and so glycolysis on the whole is inhibited. If fluoride is not added, cells will utilize glucose and false low values may be obtained. Capillary blood from finger tips may also be used for glucose estimation by strip method.

Enzymatic Method

This is highly specific, giving 'true glucose' values (fasting **70–110 mg/dL**). Present day autoanalyzers can use only the enzymatic methods.

Glucose oxidase (GOD) method is the one most widely used. Glucose oxidase is very specific; it will act only on glucose. This enzyme converts glucose to gluconic acid and hydrogen peroxide. The H_2O_2 in turn is split into H_2O and nascent oxygen by the peroxidase. The oxygen oxidizes a colorless chromogenic substrate to a colored one; the color intensity is directly proportional to concentration of glucose.

Effect of food on Glucose Level

Blood sugar analyzed at any time of the day, without any prior preparations, is called **random blood sugar**. Sugar estimated in the early morning, before taking any breakfast (12 hours fasting) is called **fasting blood sugar.** The test done about 2 hours after a good meal is called **postprandial blood sugar** (Latin = after food). The ability of a person to metabolize a given load of glucose is referred to as **glucose tolerance**.

ORAL GLUCOSE TOLERANCE TEST (OGTT)

The patient is instructed to have good carbohydrate diet for 3 days prior to the test. Patient should not take food after 8 PM the previous night. Should not take any breakfast. This is to ensure 12 hours fasting. At about 8 am, a sample of blood is collected in the fasting state. Urine sample is also obtained. This is denoted as the "0" hour sample. **Glucose Load Dose:** The dose is **75 g** anhydrous glucose (82.5 g of glucose monohydrate) in 250–300 mL of water. When the test is done in children, the glucose dose is adjusted as 1.75 g/kg body weight.

Sample Collection: In the classical procedure, the blood and urine samples are collected at 1/2 an hour intervals for the next 2½ hours. (Total six samples, including 0-hour sample). Glucose is estimated in all

the blood samples. Urine samples are tested for glucose qualitatively. Figure 6.4 represents the graph, when plasma glucose values are plotted on the vertical axis against the time of collection on the horizontal axis. But the present WHO recommendation is to collect only the **fasting and 2-hour** post-glucose load samples of blood and urine. This is sometimes called **mini-GTT.** (Total 2 samples only). This is sufficient to get a correct assessment of the patient.

Normal Values and Interpretations

In a normal person, **fasting plasma sugar is 70–110 mg/dL**. The present day tendency is to view values above 100 mg/mL as suspicious. Following the glucose load, the level rises and reaches a peak within 1 hour and then comes down to normal fasting levels by 2 to 2½ hours. This is due to the secretion of insulin in response to the elevation in blood glucose. None of the urine sample shows any evidence of glucose (Table 6.1 and Fig. 6.4).

Diagnostic Criteria for Diabetes Mellitus

- **If the fasting plasma sugar is more than 126 mg/dL,** on more than one occasion (Table 6.1).
- Or, if 2-hours post-glucose load value of OGTT is more than 200 mg/dL (even at one occasion).
- If the random plasma sugar level is more than 200 mg/dL, on more than one occasion. Diagnosis should not be based on a single random test alone; it should be repeated.

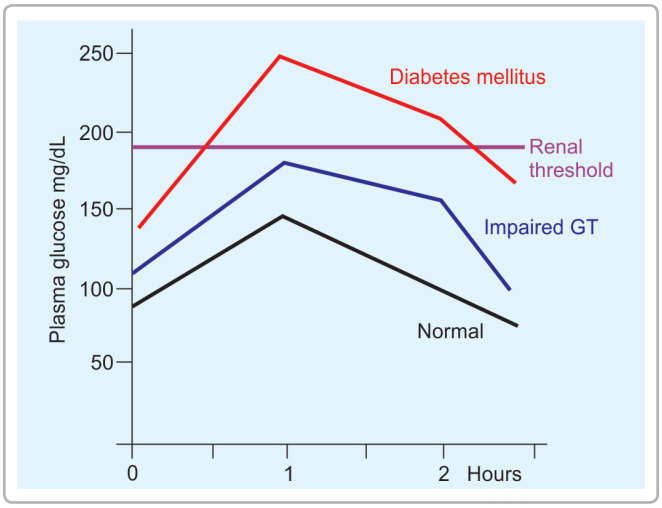


Figure 6.4: Oral glucose tolerance test (OGTT)

Causes for Abnormal GTT Curve

1. Impaired Glucose Tolerance (IGT)

Here blood sugar values are above the normal level, but below the diabetic levels (see Table 6.1).

2. Gestational Diabetes Mellitus (GDM)

This term is used when carbohydrate intolerance is noticed, for the first time, during a pregnancy.

3. Alimentary Glucosuria

Here the fasting and 2-hours values are normal; but an exaggerated rise in blood glucose following the ingestion of glucose is seen (Fig. 6.4).

4. Renal Glucosuria

Normal renal threshold for glucose is 175–180 mg/dL. If blood sugar rises above this, glucose starts to appear in urine.

REDUCING SUBSTANCES IN URINE

The excretion of reducing substances in urine is detected by a positive **Benedict's test**. Such conditions together are sometimes called as "**mellituria**". The substances in urine answering Benedict's test are enumerated in Table 6.2.

Differential Diagnosis of Reducing Substances in Urine

When reducing sugars are excreted in urine, the condition is generally referred to as **glycosuria**.

Table 6.1: The plasma sugar levels in OGTT in normal persons and in diabetic patients

	Normal persons	*Criteria for diagnosing diabetes*
Fasting	<110 mg/dL	>126 mg/dL
1 hour (peak) after glucose	<160 mg/dL	Not prescribed
2 hours after glucose	<140 mg/dL	>200 mg/dL

Table 6.2: Reducing substances in urine

Sugars	*Noncarbohydrates*
Glucose	Homogentisic acid
Fructose	Salicylates
Lactose	Ascorbic acid
Galactose	Glucuronides of drugs
Pentoses	

1. Hyperglycemic Glucosuria

When blood glucose level exceeds the renal threshold (175–180 mg/dL), glucose is excreted in urine. **Diabetes mellitus** is the most common cause.

- Renal glucosuria
- Alimentary glucosuria.

2. Fructosuria

3. Lactosuria

It is the second most common reducing sugar found in urine. It is observed in the urine of normal women during 3rd trimester of pregnancy and lactation. The condition is harmless. In pregnancy, it is important to distinguish lactosuria from glucosuria when gestational diabetes mellitus is suspected.

4. Galactosuria

It is due to deficiency of galactose-1-phosphate uridyl transferase (For details refer Chapter 7).

5. Pentosuria

6. Non-carbohydrate Reducing Compounds

Glucuronides and ascorbic acid

IDENTIFICATION OF REDUCING SUGARS

Benedict's test

About 0.5 mL of urine is boiled with 5 mL Benedict's reagent for 2 minutes (or kept in a boiling water bath for 2 minutes). The formation of a precipitate is observed on cooling.

Any reducing sugar will give a positive Benedict's test. The test is semi-quantitative and the color of the precipitate roughly parallels the concentration of reducing sugar. **Blue** color indicates the absence of sugar in urine. The **green** precipitate means 0.5%; yellow (1%); orange (1.5%) and red indicates 2% or more of sugar (1% means 1g per 100 mL).

DIABETES MILLITUS

The term is derived from the Greek words dia (=through), bainein (=to go) and diabetes literally means pass through. The disease causes loss of weight as if the body mass is passed through the urine. The Greek word, mellitus, means sweet, as it is known to early workers, that the urine of the patient contains sugar. Diabetes mellitus is a disease known from very ancient times. Charaka in his treatise (circa 400 BC) gives a very elaborate clinical description of *madhumeha* (= sweet urine). He had the vision that carbohydrate and fat metabolisms are altered in this disease.

Diabetes mellitus is a metabolic disease due to absolute or relative **insulin deficiency**. Diabetes mellitus is a common clinical condition. About 10% of the total population, and about 1/5th of persons above the age of 50, suffer from this disease. It is a major cause for morbidity and mortality.

Criteria for diagnosis of diabetes mellitus are shown in Table 6.1, and under glucose tolerance test. The disease may be classified as follows (WHO recommendation, 1999):

1. Type 1 Diabetes Mellitus

(Formerly known as **insulin-dependent** diabetes mellitus; IDDM).

About 5% of diabetic patients are of type 1. It is due to **decreased insulin production**. Circulating insulin level is very low. These patients are dependent on insulin injections. Onset is usually below 30 years of age, most commonly during adolescence. They are more prone to develop ketosis.

2. Type 2 Diabetes Mellitus

(Formerly known as **noninsulin dependent** diabetes mellitus; NIDDM).

About 95% of the patients belong to this type. The disease is due to the decreased biological response to insulin, otherwise called **insulin resistance**. So there is a relative insulin deficiency. Circulating insulin level is normal or mildly elevated or slightly decreased. Type 2 disease is commonly seen in individuals above 40 years. These patients are less prone to develop ketosis.

Metabolic Derangements in Diabetes

The effects of insulin on carbohydrate, lipid and amino acid metabolisms have been described in Chapter 31. In diabetes mellitus all these effects are reversed (See Fig. 6.5).

1. Derangements in Carbohydrate Metabolism

Insulin deficiency decreases the uptake of glucose by cells. The insulin dependent enzymes are also less active. Net

effect is an **inhibition of glycolysis and stimulation of gluconeogenesis** leading to **hyperglycemia.**

2. Derangements in Lipid Metabolism

Fatty acid breakdown leads to high FFA levels of plasma and consequent **fatty liver**. More acetyl-CoA is now available, which cannot be efficiently oxidized by TCA cycle, because the availability of oxaloacetate is limited. The stimulation of gluconeogenesis is responsible for the depletion of oxaloacetate. The excess of acetyl-CoA therefore, is diverted to ketone bodies, leading to **ketogenesis**. (For details refer Chapter 10). This tendency is more in Type 1 disease.

3. Derangement in Protein Metabolism

Increased breakdown of proteins and amino acids for providing substrates for gluconeogenesis is responsible for muscle wasting.

CLINICAL PRESENTATIONS IN DIABETES MELLITUS

Cardinal Symptoms

When the blood glucose level exceeds the renal threshold glucose is excreted in urine (**glucosuria**). Due to osmotic effect, more water accompanies the glucose (**polyuria**). To compensate for this loss of water, thirst centre is activated, and more water is taken (**polydypsia**). To compensate the loss of glucose and protein, patient will take more food (**polyphagia**). The loss and ineffective utilization of glucose leads to breakdown of fat and protein. This would lead to **loss of weight.** Often the presenting complaint of the patient may be **chronic recurrent infections** such as boils, abscesses, etc. Any person with recurrent infections should be investigated for diabetes. In India, **tuberculosis** is commonly associated with diabetes.

Acute Metabolic Complications

- **Diabetic Ketoacidosis:** Ketone body formation and explanations for ketosis are described in For details refer chapter 9. As oxaloacetate is diverted for gluconeogenesis, the TCA cycle cannot consume all the acetyl-CoA. Hence more acetyl-CoA is converted to ketone bodies. (Fig. 6.5). This leads to accumulation of ketone bodies in blood (**ketonemia**). The presence of ketone bodies in urine (**ketonuria**) is assessed by Rothera's test. The accumulation of acidic ketone bodies lowers the plasma pH. So, metabolic acidosis occurs. The condition is called diabetic **ketoacidosis**. Smell of acetone in the breath is noticed. If not treated promptly and properly, the condition may be fatal. Patient may become unconscious, comatose and die.

Chronic Complications of Diabetes Mellitus

- **Vascular Diseases:** Atherosclerosis in medium sized vessels, **plaque formation** and consequent intravascular thrombosis may take place. If it occurs in cerebral vessels, the result is paralysis. If it is in coronary artery, myocardial infarction results.
- **Complications in eyes:** Early development of **cataract** of lens is due to the increased rate of sorbitol formation, caused by the hyperglycemia. Retinal microvascular abnormalities lead to **retinopathy** and blindness.

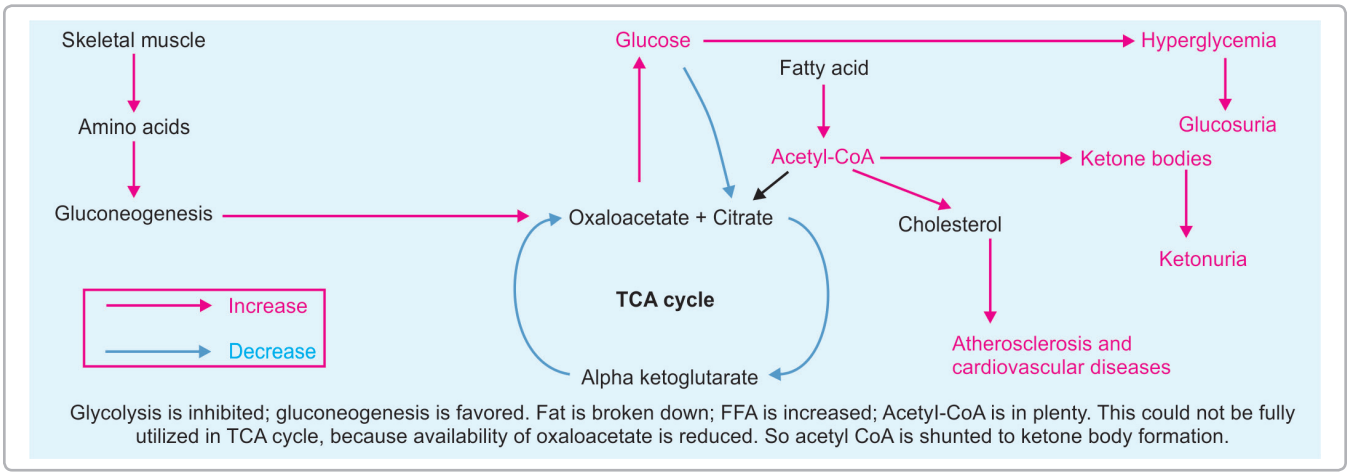


Figure 6.5: Metabolic derangements in diabetes mellitus

- **Neuropathy:** Peripheral neuropathy with paresthesia is very common. Decreased glucose utilization and its diversion to sorbitol in Schwann cells may be cause for neuropathy. Neuropathy may lead to risk of foot ulcers.

LABORATORY INVESTIGATIONS IN DIABETES

1. Blood sugar estimations

Random blood sugar estimation and oral glucose tolerance tests are used for the diagnosis (See Table 6.1). Hyperglycemia and glucosuria will be the hallmarks. For monitoring a diabetic patient, periodic check of blood glucose level (fasting and postprandial) is to be done. Blood glucose level has to be maintained within the normal limits.

2. Glycohemoglobin (HbA1c) estimation

The best index of long-term control of blood glucose level is measurement of glycated hemoglobin or **glycohemoglobin**. When there is hyperglycemia, proteins in the body may undergo glycation. It is a **nonenzymatic** process. When once attached, glucose is not removed from hemoglobin. Therefore, it remains inside the erythrocyte, throughout the life span (120 days) of RBCs. The HbA1 level reveals the mean glucose level over the previous 8–10 weeks. It is unaffected by recent food intake or recent changes in blood sugar levels.

An elevated glycohemoglobin indicates poor control of diabetes mellitus. Value below 6% as normal. Values between 6 and 6.5% indicates impaired glucose tolerance and any value above 6.5% indicates frank diabetes mellitus. In a known diabetic patient who is well controlled, the value should be below 7% and levels above 8% indicate poor control of diabetes mellitus.

Management of Diabetes Mellitus

1. **Diet and exercise:** These are the first line of treatment. Diet control alone will manage more than 50% Type 2 diabetic cases. A diabetic patient is advised to take a balanced diet with high protein content, low calories, devoid of refined sugars and low saturated fat, adequate PUFA, low cholesterol and sufficient quantities of fiber. Vegetables are the major sources of minerals, vitamins and fiber.
2. **Oral hypoglycemic agents:** If the above measures are not sufficient, then oral drugs are tried. They are mainly of two types; sulphonyl urea and biguanides. They are mainly used in Type 2 diabetes.
3. **Insulin injections:** Insulin is the drug of choice in Type 1 disease. It is also used in Type 2 disease, where oral drugs are not sufficient. Insulin and Glucagon are described in Chapter 31.

A QUICK LOOK

1. Major factors that cause increased level of glucose in blood are; absorption from intestines, glycogenolysis and gluconeogenesis. A continuous and adequate supply of glucose is essential for the brain, erythrocytes and renal medulla.
2. Major factors that cause depletion of glucose in blood are; utilization by tissues, glycogenesis and conversion to fat. Blood glucose level varies significantly during the fasting state and in postprandial state (after food).
3. An elevation in blood glucose level stimulates secretion of insulin. Insulin in turn favors uptake of glucose by body cells and glycogen synthesis. Hyperglycemic hormones are glucagon, cortisol, adrenaline and growth hormone. Insulin is a hypoglycemic hormone.
4. The glucose levels are measured in plasma after collecting blood in a tube with oxalate and fluoride. True glucose values are given by enzymatic method (GOP-POD method). Indications for an oral glucose tolerance test (OGTT) are; patient with symptoms suggestive of diabetes mellitus, excess weight gain during pregnancy and to rule out benign glycosuria.
5. Contraindications for an OGTT are; known case of diabetes mellitus, to follow prognosis of diabetes mellitus and performing on acutely ill patients.
6. Conditions that can be assessed by OGTT are impaired glucose tolerance, impaired fasting glycemia, gestational diabetes, alimentary glucosuria and renal glucosuria.
7. Reducing substances in urine other than glucose are fructose, lactose, galactose, pentoses, homogentisic acid, salicylates, glucuronides and ascorbic acid.
8. Insulin has the following biochemical effects: increases uptake of glucose by cells, enhances utilization of glucose, hypoglycemic, antilipolytic, antiketogenic and favors lipogenesis.
9. Insulin acts via a specific insulin receptor present on cells of insulin responsive tissues. This affects a signal transduction pathway, which leads to regulation of gene transcription, DNA synthesis and activation of enzymes.
10. Diabetes mellitus is of two types, Type 1 and Type 2. Type 1 is also known as insulin dependent (IDDM), while the type 2 was previously known as noninsulin dependent diabetes mellitus (NIDDM).
11. Secondary diabetes mellitus can be; manifested in endocrine disorders (Cushing's syndrome, Thyrotoxicosis), drug induced (beta blockers, steroids), seen in pancreatic diseases (chronic pancreatitis).
12. Diabetic ketoacidosis (DKA), lactic acidosis and hypoglycemia are acute metabolic complications of diabetes mellitus.
13. Retinopathy, neuropathy and vascular diseases are chronic complications of diabetes mellitus.

14. Glycated hemoglobin (HbA1c) is used as an index for long-term control of blood glucose level.
15. The fasting plasma glucose (FPG) denotes glucose level after overnight fasting of 8–10 hours. Postprandial glucose (2 hours PPG) is measured 2 hours after taking food.
16. The term random plasma glucose (RPG) is used when blood is collected regardless of the time of the previous meal.
17. Normal fasting blood glucose value is 110 mg/Dl and 2 hours postprandial value is 140 mg/dL. Hyperglycemia refers to elevated glucose levels.
18. A plasma level of glucose below 50 mg/dL is called hyperglycemia.
19. Fasting value between 111 and 126 mg/dL and 2 hours PPG between 140 and 199 mg/dL indicate impaired glucose tolerance.
20. A diagnosis of diabetes mellitus is made when fasting plasma glucose is above 126 mg/dL and 2 hours PPG above 200 mg/dL.
21. Renal threshold for glucose is 180 mg/dL, above which glucose is excreted even at a lower level. In renal glycosuria the glucose tolerance is normal, but glucose is excreted in urine.
22. Insulin is secreted in response to an increase in plasma glucose level.
23. Insulin receptors are located in the plasma membrane with 2-alpha and 2-beta subunits.
24. Binding of insulin to alpha subunits activates the tyrosine kinase activity of beta subunit which will phosphorylate insulin response substrate (IRS). The activated IRS in turn will activate other enzyme system and cascades causing metabolic effects.
25. Insulin has its effects at the level of genes by induction and repression of enzymes as well as covalent modification of enzymes.
26. Insulin recruits GluT4 in cells to the membrane enhancing glucose uptake.
27. Glycolysis and glycogen synthesis are stimulated, thus decreasing glucose level in plasma.
28. Insulin also favors lipogenesis where by excess glucose is converted to fat for storage.
29. Gluconeogenesis and glycogenolysis are inhibited by insulin, favoring fall in plasma glucose level.

CHAPTER 7

Carbohydrates–IV: Minor Metabolic Pathways of Carbohydrates

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Hexose monophosphate shunt pathway
- Glucuronic acid pathway of glucose
- Fructose metabolism
- Galactose metabolism
- Metabolism of alcohol

HEXOSE MONOPHOSPHATE (HMP) SHUNT PATHWAY

Instead of glucose going through the glycolytic pathway, the glucose is shunted through this pathway; so it is known as the **shunt pathway**. In the glycolysis, there are a few bisphosphate intermediates; but in this pathway, there are monophosphates only; hence this is called **hexose monophosphate (HMP) pathway**. The reactions involve the intermediate formation of pentose phosphates; hence this is also called **pentose phosphate pathway**.

About 10% of glucose molecules per day are entering in this pathway. The liver and RBC metabolise about 30% of glucose by this pathway. The major purpose of this pathway is generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and pentose phosphates for nucleotide synthesis (Box 7.1).

Overview of the Shunt Pathway

The HMP shunt pathway has oxidative and nonoxidative phases. During the oxidative phase, glucose-6-phosphate is oxidized with the generation of 2 molecules of NADPH, and one molecule of pentose phosphate, with the liberation of one molecule of CO_2. During the nonoxidative phase, the pentose phosphate is converted to intermediates of glycolysis.

A. Oxidative Phase

Step 1 of HMP pathway

Glucose-6-phosphate is oxidized by $NADP^+$ dependent **Glucose-6-phosphate dehydro-genase** (GPD). 6-phospho-gluconolactone is formed. One molecule of **NADPH** is formed in the reaction (Fig. 7.1). This is the **rate-limiting step**. Regulation is effected on this enzyme.

Step 2 of HMP pathway

The lactone is hydrolyzed by gluconolactone hydrolase to form 6-phosphogluconic acid (Fig. 7.1).

BOX 7.1: Differences between NADH and NADPH

- NADH is used for reducing reactions in catabolic pathways, e.g. pyruvate to lactate. NADH enters the electron transport chain, and ATP is generated
- NADPH is used for reductive biosynthetic reactions, e.g., de novo synthesis of fatty acid, synthesis of cholesterol etc. NADPH is generated mainly by the HMP shunt pathway. NADPH is not entering the electron transport chain; and NADPH will not generate ATP.
- $NADP^+$ differs from NAD^+ in having an additional phosphate group. These two coenzymes are specific for enzymes; they are not interchangeable.

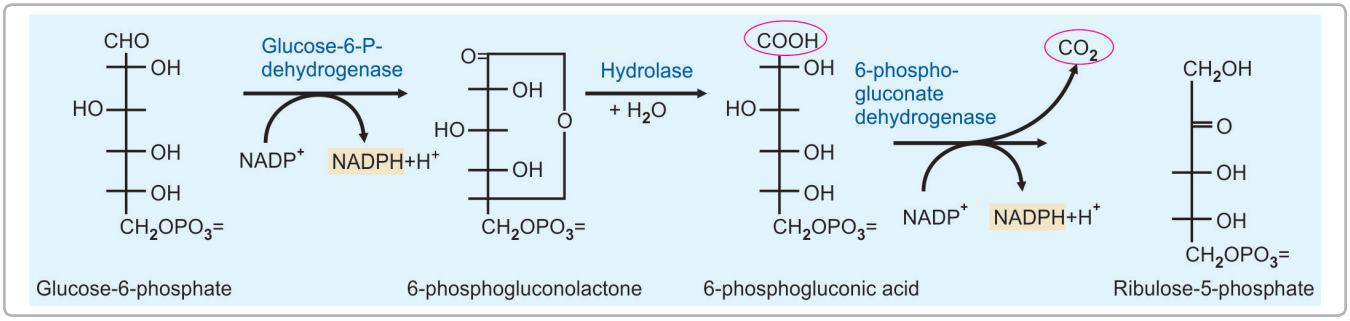


Figure 7.1: Oxidative phase of HMP shunt pathway; steps 1, 2 and 3

Step 3, NADPH is again Generated

This is an oxidative step coupled with decarboxylation. The enzyme is 6-phosphogluconate **dehydrogenase**. Thus ribulose-5-phosphate is formed. (Fig. 7.1). In this step, a second molecule of **NADPH is generated**.

Suppose, 6 molecules of glucose (6 × 6 = 36 carbons) are entering in this pathway. In the oxidative phase, the first carbon atom of all 6 glucose molecules are removed as 6 molecules of CO_2. (This is equivalent to complete oxidation of 1 molecule of glucose). In this process, 12 NADPH are generated.

Non-oxidative Phase

In the non-oxidative phase, the remaining 6 molecules of 5-carbon pentoses (6 × 5 = 30C) are interchanged such a way that 5 molecules of glucose (5 × 6 = 30C) are regenerated.

Regulation of HMP Shunt Pathway

The pathway is mainly regulated by the level of $NADP^+$. The first reaction catalyzed by **GPD is the rate-limiting** step and it is inhibited by NADPH. **Insulin** will induce GPD and therefore will increase the overall pathway.

Significance of the HMP Shunt Pathway

1. Tissues

The oxidative phase of shunt pathway is seen in organs where fatty acid or steroid synthesis is taking place, such as liver, mammary glands, testis, ovary, adipose tissue and adrenal cortex. It has also an important role in RBCs and lens of eye.

2. Generation of Reducing equivalents

The major metabolic role of the pathway is to metabolic role of the pathway is to provide cytoplasmic NADPH for **reductive biosynthesis** of fatty acids, cholesterol and steroids.

3. Erythrocyte membrane

NADPH is required by the RBC to keep the **glutathione** in the reduced state. *NADPH, glutathione and glutathione reductase are required to preserve the integrity of RBC membrane.*

4. Lens of eye

Maximum concentration of NADPH is seen in lens of eye. For preserving the transparency of lens, NADPH is required.

5. Availability of Ribose

Ribose and deoxyribose are required for DNA/RNA synthesis. Ribose is also necessary for nucleotide coenzymes. Reversal of non-oxidative phase is present in all tissues, by which ribose could be made available.

6. What about ATP?

ATP is neither utilized nor produced by the HMP shunt pathway. Cells do not use the shunt pathway for energy production.

7. GPD deficiency

The enzyme GPD may be deficient in some persons. It is the most common enzyme deficiency seen in clinical practice. It is an X-linked condition. It will lead to **drug-induced hemolytic anemia.** The deficiency is manifested only when exposed to certain drugs or toxins, e.g. intake of **antimalarial drugs** like primaquine and ingestion of fava beans (**Favism**). Sulfa drugs and furadantin may also precipitate the hemolysis. This is characterized by jaundice and severe anemia. GPD deficiency is reported from almost all regions of India.

8. Methemoglobinemia

GPD deficient persons will show increased methemoglobin in circulation, even though cyanosis may not be manifested.

GLUCURONIC ACID PATHWAY OF GLUCOSE

The pathway is shown in Figure 7.2.

Importance of the glucuronic acid pathway

It provides **UDP-glucuronic acid,** which is the active form of glucuronic acid. It is used for the following purposes:

1. Conjugation of bilirubin and steroids
2. Conjugation of various drugs. Conjugation will make these substance more water soluble and more easily excretable. Barbiturates, antipyrine and aminopyrine will increase the uronic acid pathway, leading to availability of more glucuronate for conjugation purpose.

Vitamin C in lower animals

The enzyme **L-gulonolactone oxidase is absent in human beings**, primates, guinea pigs and bats. Hence, ascorbic acid cannot be synthesized by these organisms. Hence ascorbic acid became essential in diet for human beings (Fig. 7.2).

Essential Pentosuria

It is one of the members of the Garrod's tetrad. The incidence is 1 in 2,500 births. It is an inborn error of metabolism due to deficiency of enzyme **xylulose reductase**. L-xylulose is excreted in urine and gives a **positive Benedict's test**. **Barbiturates**, aminopyrine, etc. will induce uronic acid pathway and will increase xylulosuria in such patients. This condition does not produce any harm; but it should be differentiated from diabetes mellitus.

FRUCTOSE METABOLISM

Fructose is a ketohexose present in fruits, honey and sucrose. Fructose is promptly metabolized by the liver. Fructose is phosphorylated by **fructokinase,** an enzyme present in liver with a high affinity for fructose (Fig. 7.3). The fructose-1-phosphate is then cleaved by the enzyme, fructose-1-phosphate-aldolase or **aldolase-B**. The products are glyceraldehyde and dihydroxyacetone phosphate (Fig. 7.3). Fructose is mainly metabolized by liver, but free fructose is seen in large quantities in **seminal plasma**. The energy for mobility of spermatozoa is mainly derived from fructose. Fructose is secreted by seminal vesicles.

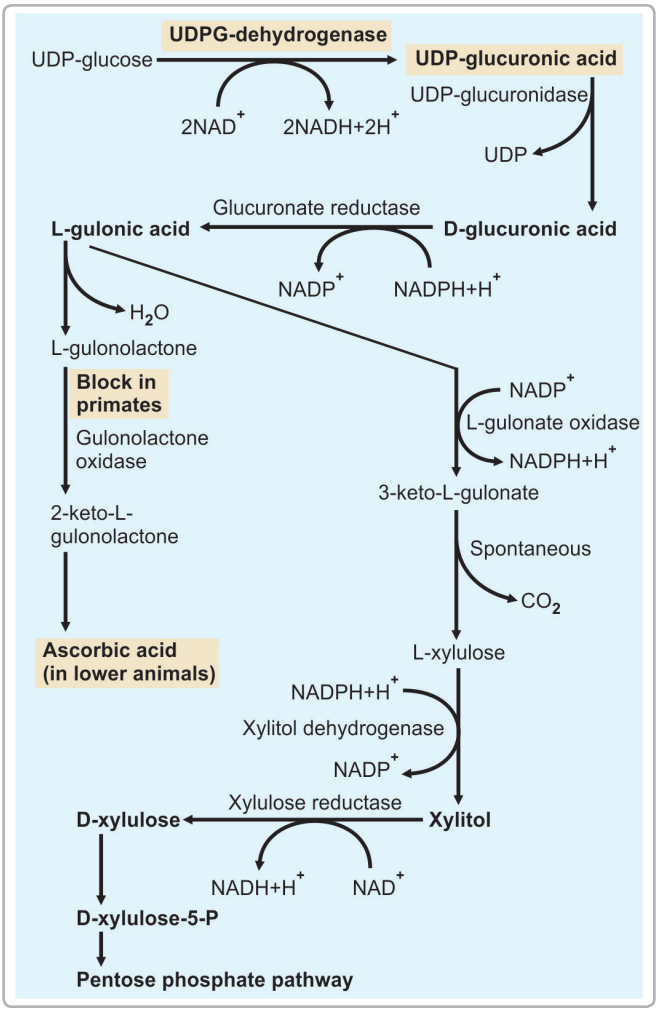


Figure 7.2: Glucuronic acid pathway

HEREDITARY FRUCTOSE INTOLERANCE (HFI)

It is an autosomal **recessive** inborn error of metabolism. Incidence of the disease is 1 in 20,000 births, while 1 in 70 persons are carriers of the abnormal gene. The defect is in **aldolase-B;** hence fructose-1-phosphate cannot be metabolized (Fig. 7.3). This is seen when sucrose (containing fructose) is introduced in the diet of infants, usually around 6 months of age. Accumulation of fructose-1-phosphate will inhibit glycogen phosphorylase. This leads to accumulation of glycogen in liver and associated **hypoglycemia**. Vomiting and loss of appetite are seen.

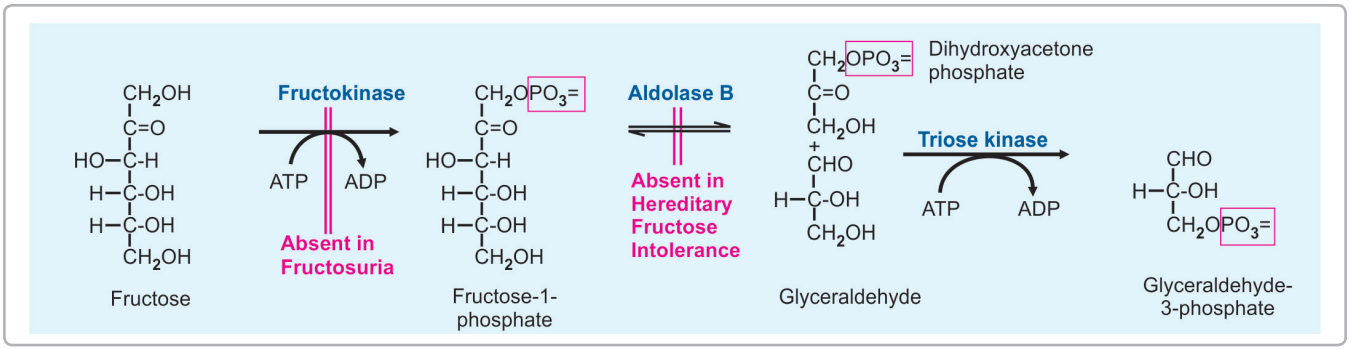


Figure 7.3: Fructose entering glycolysis

The infants often fail to thrive. Hepatomegaly and jaundice may occur. If liver damage progresses, death will occur. Fructose is also excreted in urine when urine gives positive Benedict's test. Withdrawal of fructose from the diet will immediately relieve the symptoms.

Fructosuria

This is a benign metabolic defect due to deficiency of **fructokinase** (Fig. 7.3). There is no abnormality other than excretion of fructose in urine.

GALACTOSE METABOLISM

Galactose is an aldohexose and is the 4th epimer of glucose. Galactose is a constituent of lactose of milk sugar, and is taken in the diet. Galactose is metabolized almost exclusively by the liver. UDP galactose is the active donor of galactose during synthetic reactions. Galactose is necessary for synthesis of lactose, glycosaminoglycans and glycoproteins.

Lactose synthesis: Lactose synthesis is seen in lactating mammary glands.

$$\text{UDP glucose} \xrightarrow{\text{Epimerase}} \text{UDP galactose}$$

$$\text{UDP galactose} + \text{glucose} \xrightarrow{\text{Lactose synthase}} \text{Lactose}$$

Galactose Catabolism

Galactokinase reaction: Galactose is first phosphorylated by galactokinase to galactose-1-phosphate (step 1, Fig. 7.4).

Galactose-1-phosphate uridyltransferase: This is the **rate–limiting** enzyme in the galactose metabolism (Step 2, Fig. 7.4). UDP-galactose may be used as such for synthesis of compounds containing galactose (e.g., lactose)

Epimerase reaction: By this reaction, galactose is channelled to the metabolism of glucose (Step 3, Fig. 7.4). Since the reaction is freely reversible,

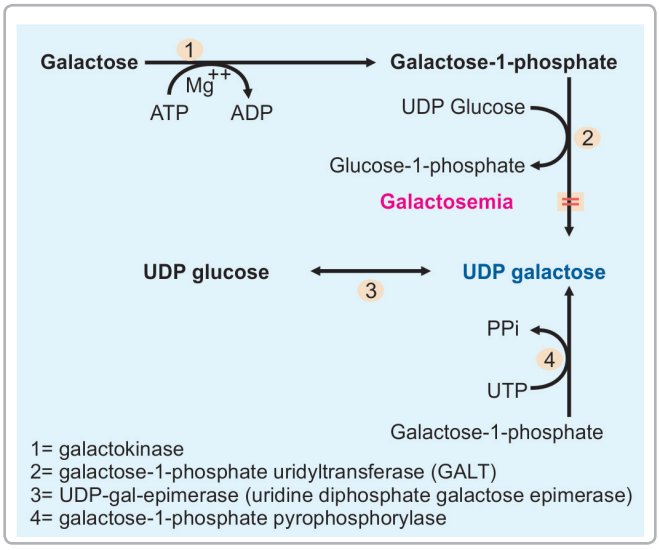


Figure 7.4: Glucuronic acid pathway

even if the dietary supply of galactose is deficient, UDP-glucose can be epimerized to UDP-galactose.

Alternate pathway: The galactose-1-phosphate pyrophosphorylase in liver becomes active only after 4 or 5 years of life. The enzyme will produce UDP-galactose directly which can be epimerised to UDP-glucose (Step 4, Fig. 7.4).

Galactosemia

There is deficiency of enzyme **galactose-1-phosphate uridyltransferase**. It is an inborn error of metabolism. The incidence is 1 in 35,000 births. Herman Kalckar described it in 1958. Due to the block in this enzyme, galactose-1-phosphate will accumulate in liver. This will inhibit galactokinase as well as glycogen phosphorylase. **Hypoglycemia** is the result. Bilirubin uptake is less and bilirubin conjugation is reduced; so **unconjugated bilirubin** level is increased in blood (for bilirubin

For details refer Chapter 14). There is enlargement of liver, and jaundice. Severe **mental retardation is present**. Free galactose accumulates, leading to **galactosemia.** It is partly excreted in urine (**galactosuria**). The accumulation of metabolic produces in the lens results in cataract due to its osmotic effect. This is called **congenital cataract** and is a very characteristic feature of galactosemia. These salient clinical findings are summarized in Fig. 7.5. If lactose is withdrawn from the diet, most of the symptoms recede. But mental retardation, when established, will not improve. Hence early detection is most important.

Treatment: For affected infant **lactose-free diet** is given. Such special diets may be withdrawn after 4 years, when galactose-1-phosphate pyrophosphorylase (step 4, Fig. 7.4) becomes active. However, residual mental retardation cannot be reversed.

METABOLISM OF ALCOHOL

Ethyl alcohol was first isolated in pure form in 1820 by Jean Dumas, who has also noticed the clinical effect of chronic alcoholism. Most of alcohol is absorbed by intestine. About 1% of the ingested alcohol is excreted through the lungs or urine. Major fraction of the alcohol is oxidized in the liver.

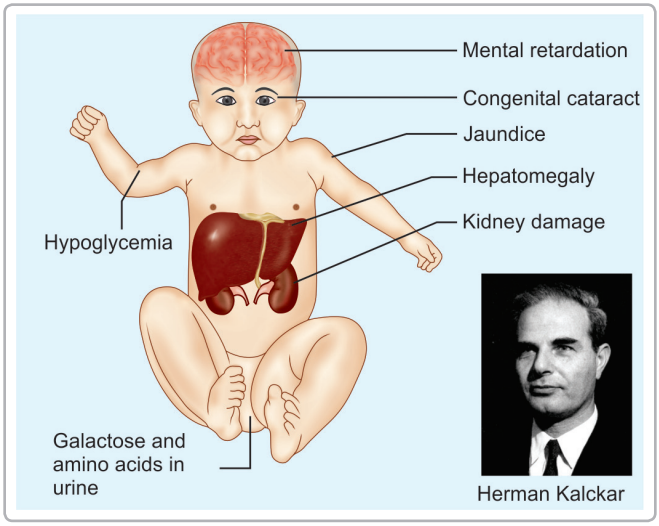


Figure 7.5: Clinical features of galactosemia

1. Alcohol Dehydrogenase (ADH)

It is an NAD^+ dependent **cytoplasmic** enzyme. It oxidizes ethanol to acetaldehyde (Fig. 7.6). In some individuals, the enzyme is mutated. This mutation rate is more in orientals. In such individuals, alcohol metabolism is slower and even small quantity of alcohol may produce symptoms of intoxication.

2. Aldehyde Dehydrogenase

The acetaldehyde is further oxidized to acetate by a **mitochondrial** NAD^+ dependent enzyme (Fig. 7.6). The acetate is then converted to acetyl-CoA pathway. The activity of alcohol dehydrogenase is more than aldehyde dehydrogenase. So acetaldehyde accumulates in liver. **Aldehyde is toxic**, which in excess may lead to cell death. The activity of aldehyde dehydrogenase is less in Indians, when compared to Europeans.

3. Biochemical Alterations in Alcoholism

Both the oxidation steps of alcohol produces NADH. The high NADH level favors conversion of pyruvate to lactate, leading to **lactic acidosis**. Deficiency of pyruvate leads to inadequate formation of oxaloacetate. This results in depression of gluconeogenesis, leading to **hypoglycemia**. Increased level of acetyl-CoA causes increased fatty acid synthesis; but fatty acid is not oxidized. So fat is accumulated in liver, resulting in **fatty liver**. Accumulated toxic effect of acetaldehyde leads to cellular death. This is followed by replacement by fibrous tissue. Fibrosis of liver is called **cirrhosis**. When liver functions are reduced, hepatic coma results. Five percent of all deaths in India are due to liver diseases, for which the most important culprit is alcohol. In India, chronic alcoholism is the most leading cause for morbidity and consequent loss of man hours. *Indians are more prone for alcoholic cirrhosis.* Alcohol causes **CNS depression** by inhibiting excitatory receptors and by potentiating inhibitory neurotransmitter (GABA) receptors. In chronic alcoholics, the brain neurons are lost, neurodegenerative changes set in, and the memory

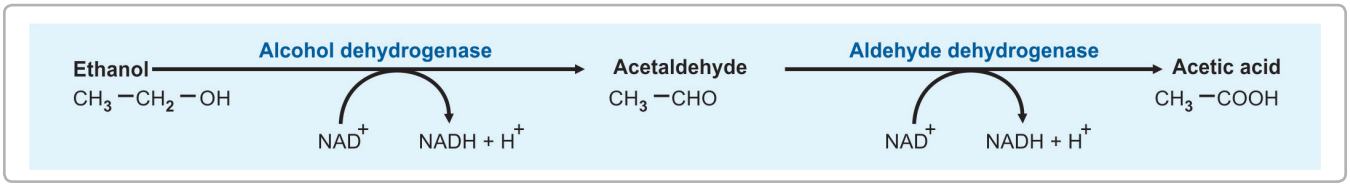


Figure 7.6: Alcohol metabolism

Table 7.1: Inborn errors associated with carbohydrate metabolism (*)

Name	*Defective enzyme*	*Salient features*	*Chapter no.*
Lactose intolerance	Lactase	Milk-induced diarrhea	4
Fructose intolerance	Aldolase B	Hypoglycemia, vomiting, hepatomegaly	7
Fructosuria	Fructokinase	Benign; urine sugar	7
Galactosemia	Gal-1-P-uridyltransferase	Hypoglycemia; hepatomegaly; mental retardation; jaundice; congenital cataract	7
Essential pentosuria	Xylitol dehydrogenase	Benign	7
GPD deficiency	Glucose-6-phosphate dehydrogenase	Drug-induced hemolytic anemia	7

(*) Glycogen storage diseases are important inborn errors associated with carbohydrate metabolism; these shown separately in Table 5.7.

is affected. In alcoholics, combined thiamine deficiency leads to **Wernicke's disease.**

Mucopolysaccharidoses

These are a group of inborn errors of metabolism characterized by excessive **intralysosomal accumulation** of glycosaminoglycans (GAG) in various tissues. They are progressive disorders. Most of these diseases are inherited as autosomal recessive traits.

The clinical manifestations include coarse facial features, thick skin and corneal opacity due to accumulation of GAG. **Mental retardation**, growth deficiency and skeletal dysplasia are also seen due to defective cell function. The inborn errors associated with carbohydrate metabolism, are shown in Table 7.1.

A QUICK LOOK

1. The HMP shunt pathway (Pentose Phosphate pathway) generates NADPH required for reductive biosynthesis of steroids, fatty acids and cholesterol.
2. It also provides pentose sugars (Ribose and Deoxyribose) for nucleic acid synthesis.
3. Glucuronic acid is used for conjugation of bilirubin, steroids and drugs.
4. In lower animals, ascorbic acid (vitamin C) is synthesized by the glucuronic acid pathway; but human beings, could not synthesize vitamin C.
5. Deficiency of aldolase B enzyme leads to an inborn metabolic disorder termed hereditary fructose intolerance (HFI).
6. Deficiency of galactose-1-phosphate uridyltransferase is the cause of galactosemia.
7. Characteristic features of galactosemia are congenital cataract, generalized aminoaciduria, hepatomegaly, jaundice and mental retardation. Lactose free diet forms the main stay of treatment.
8. Metabolism of alcohol involves two enzymes; alcohol dehydrogenase and aldehyde dehydrogenase.
9. Gamma glutamyltransferase (GGT) is used as a clinical marker of alcoholism.
10. Blood group antigens are glycoproteins.
11. Mucopolysaccharidoses (MPS) are a group of inborn metabolic disorders caused due to accumulation of mucopolysaccharides in tissues. Features include mental retardation.

CHAPTER 8

Lipids–I: Chemistry of Fats

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Classification of lipids
- Classification of fatty acids
- Saturated and unsaturated fatty acids
- Neutral fats or tri acylglycerols
- Phospholipids
- Phosphatidylcholine or lecithin
- Sphingomyelin
- Non-phosphorylated lipids
- Prostaglandins

Lipids constitute a heterogeneous group of compounds of biochemical importance. Lipids may be **defined as** compounds which are relatively insoluble in water, but freely soluble in nonpolar organic solvents like benzene, chloroform, ether, hot alcohol, acetone, etc.

Functions of Lipids

- Storage form of energy (triglycerides)
- Structural components of biomembranes (phospholipids and cholesterol)
- Metabolic regulators (steroid hormones and prostaglandins)
- Act as surfactants, detergents and emulsifying agents (amphipathic lipids)
- Act as electric insulators in neurons
- Provide insulation against changes in external temperature (subcutaneous fat)
- Give shape and contour to the body
- Protect internal organs by providing a cushioning effect (pads of fat)
- Help in absorption of fat soluble vitamins (A, D, E and K)
- Improve taste and palatability to food.

Clinical applications

- Excessive fat deposits cause obesity. Truncal obesity is a risk factor for heart attack.
- Abnormality in cholesterol and lipoprotein metabolism leads to atherosclerosis and cardiovascular diseases (For details refer Chapter 10).
- In diabetes mellitus, the metabolisms of fatty acids and lipoproteins are deranged, leading to ketosis (For details refer Chapter 9).

CLASSIFICATION OF LIPIDS

Based on the chemical nature, lipids are classified:

I. Simple Lipids

They are esters of fatty acids with glycerol or other higher alcohols. They are subclassified as:

- Triacylglycerol or triglycerides or neutral fat
- Waxes.

II. Compound Lipids

They are fatty acids esterified with alcohol; but in addition they contain other groups. Depending on these extra groups, they are subclassified as:

Phospholipids, containing phosphoric acid.

- Nitrogen containing glycerophosphatides:
  - Lecithin (phosphatidylcholine)
  - Cephalin (phosphatidylethanolamine)
  - Phosphatidyl serine
- Non-nitrogen glycerophosphatides
  - Phosphatidylinositol
  - Phosphatidylglycerol
  - Diphosphatidylglycerol (cardiolipin)
- Plasmalogens, having long chain alcohol
  - Choline plasmalogen
  - Ethanolamine plasmalogen
- Phospho sphingosides, with sphingosine
  Sphingomyelin.

Non-phosphorylated lipids

- Glycosphingolipids (carbohydrate)
  - Cerebrosides (ceramide monohexosides)
  - Globosides (ceramide oligosaccharides)
  - Gangliosides (having N-acetyl neuraminic acid)
- Sulfolipids or sulfatides
  - Sulfated cerebrosides
  - Sulfated globosides
  - Sulfated gangliosides.

III. Derived Lipids

They are compounds which are derived from lipids or precursors of lipids, e.g. fatty acids, steroids, prostaglandins, leukotrienes, terpenes, dolichols, etc. For details of cholesterol and steroids, (For details refer Chapter 10).

IV. Lipids Complexed to Other Compounds

Proteolipids and lipoproteins. Plasma lipoproteins are described in Chapter 10.

FATTY ACIDS

Fatty acids, are included in the group of derived lipids. It is the most common component of lipids in the body. They are generally found in ester linkage in different classes of lipids. In the human body, free fatty acids are formed only during metabolism.

Fatty acids are **aliphatic carboxylic acids** and have the general structural formula, R — CO—OH, where COOH (carboxylic group) represents the functional group. Depending on the R group (the hydrocarbon chain), the fatty acids may vary.

CLASSIFICATION OF FATTY ACIDS

- Depending on total no. of carbon atoms
  - **Even chain,** having carbon atoms 2,4,6 and similar series. Most of the naturally occurring lipids contain even chain fatty acids.
  - **Odd chain,** having carbon atoms 3, 5, 7, etc. Odd numbered fatty acids are seen in microbial cell walls. They are also present in milk.
- Depending on length of hydrocarbon chain
  - **Short chain** with 2 to 6 carbon atoms
  - **Medium chain** with 8 to 14 carbon atoms
  - **Long chain** with 16 and above, usually up to 24 carbon atoms.
- Depending on nature of hydrocarbon chain
  - **Saturated** fatty acids
  - **Unsaturated** which may be subclassified into Mono-unsaturated (monoenoic) having single double bond or polyunsaturated (polyenoic) with 2 or more double bonds. Table 8.1 gives a list of fatty acids belonging to different groups.

SATURATED FATTY ACIDS

- They have the general structural formula CH3-(CH2) n-COOH. Some of the common saturated fatty acids are noted in Table 8.2.
- They are named by adding the suffix 'anoic' after the hydrocarbon.
- The two carbon acetic acid and 4 carbon butyric acid are important metabolic intermediates.
- C16 palmitic and C18 stearic acids are most abundant in body fat.
- Each animal species will have characteristic pattern of fatty acid composition. Thus human body fat contains 50% oleic acid, 25% palmitic acid, 10% linoleic and 5% stearic acid.
- The carbon atoms of fatty acids are numbered as C1, C2, etc. starting from the COOH group. Or, starting from the methyl end, the carbon atoms may be numbered as omega (ω)-1,2,3, etc.

6	5	4	3	2	1
CH3 —	CH2 —	CH2 —	CH2 —	CH2 —	COOH
ω1	ω2	ω3	ω4	ω5	

UNSATURATED FATTY ACIDS

They are named by adding the suffix 'enoic' after the systematic name. They are similar to saturated fatty acids

Table 8.1. Characteristics of common fatty acids

Common name	*No. of carbon atoms*	*Chemical nature*	*Occurrence*
A. Even chain, Saturated fatty acids			
Acetic	2	Saturated; small chain	Vinegar
Butyric	4	do	Butter
Lauric	12	do	Coconut oil
Myristic	14	do	Coconut oil
Palmitic	**16**	**Saturated; long chain**	**Body fat**
Stearic	**18**	**do**	**do**
Arachidic	20	do	Peanut oil
B. Odd-chain fatty acids			
Propionic	3 Saturated; Odd chain	Metabolism	
C. Even chain, Unsaturated fatty acids			
Palmitoleic	16	Monounsaturated (ω7)	Body fat
Oleic	**18**	**do (ω 9)**	**do**
Erucic	22	do (ω 9)	Mustard oil
Linoleic	**18**	**2 double bonds (ω 6)**	**Vegetable oils**
Linolenic	**18**	**3 double bonds (ω 3)**	**do**
Arachidonic	**20**	**4 double bonds (ω 6)**	**Vegetable oils**

TABLE 8.2: Common saturated fatty acids

Structure	*Common name*
CH_3—COOH	Acetic acid
$CH_3(CH_2)_2$—COOH	Butyric acid
CH_3—$(CH_2)_{14}$—COOH	Palmitic acid
CH_3—$(CH_2)_{16}$—COOH	Stearic acid

in the reaction of the carboxylic group but also show properties due to presence of the double bond.

Unsaturated fatty acids exhibit geometrical isomerism at the double bonds. All the naturally occurring fatty acids have the **cis configuration**.

The **polyunsaturated fatty acids** (PUFA) exist in cis configuration in naturally occurring lipids.

Clinical significance of PUFA

- **Linoleic and Linolenic acids** are poly unsaturated fatty acids. Linoleic acid has 2 double bonds; Linolenic acid has 3 double bonds and arachidonic acid has 4 double bonds.
- **Essential fatty acids** cannot be synthesized by the body and have to be supplied in the diet.
- Unsaturated fatty acids are also designated w3 (omega 3) family (Linolenic acid); ω6 family (Linoleic and Arachidonic acids) and ω9 family (Oleic acid)
- Arachidonic acid is the precursor of **prostaglandins**. Arachidonic acid can be synthesized in the body, if the essential fatty acids are supplied in the diet.
- The pentaenoic acid present in fish oils is of great nutritional importance (w3 unsaturated fatty acid).

PROPERTIES OF FATTY ACIDS

1. Hydrogenation

Unsaturated fatty acids may be converted to the corresponding saturated fatty acids by hydrogenation of the double bond.

$$\text{Linolenic} \xrightarrow{(+)2H} \text{Linoleic} \xrightarrow{(+)2H} \text{Oleic} \xrightarrow{(+)2H} \text{Stearic}$$

Hydrogenation of oils can lead to solidification and saturation, e.g. Vanaspathi.

2. Halogenation

When treated with halogens under mild conditions, the unsaturated fatty acids can take up two halogen atoms, at each double bond to form the halogenated derivative of the fatty acid. For example,

Oleic acid + I_2 → Di-iodo oleic acid

The number of halogen atoms taken up will depend on the number of double bonds and is an index of the degree of unsaturation. (See iodine number, under triglycerides).

3. Melting point

The short and medium chain fatty acids are liquids, whereas long chain fatty acids are solids at 25°C. The solubility in water decreases, while melting and boiling points increase, with increase in chain length.

4. Salt formation

Saturated and unsaturated fatty acids form salts with alkali.

CH3—COOH + NaOH → CH_3—COONa + H_2O

Sodium and potassium salts of long chain fatty acids are called **soaps**. Calcium and magnesium soaps are insoluble. Calcium soaps are used in grease.

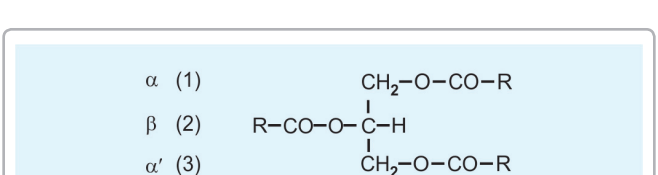


Figure 8.1: Triacylglycerol (TAG) (Triglyceride)

5. Ester formation

Both saturated and unsaturated fatty acids form esters with alcohols, especially with glycerol. Fatty acids can form mono-, di- or tri-esters with alcohol groups of glycerol. Triglycerides or triacylglycerols are also known as **neutral fat** (Fig. 8.1).

Glycerol + fatty acid → Monoacylglycerol
Monoglyceride + fatty acid → Diacylglycerol
Diglyceride + fatty acid →Triglyceride or triacylglycerol

The composition of some of the common oils and fats are given in Table 8.3.

Table 8.3: Composition of oils and fats

Name	*Saturated*	*Mono-unsaturated*	*PUFA*
	fatty acids (%)	fatty acids (%)	(%)
Coconut oil	(*)86	12	2
Groundnut oil	18	46	36
Gingelly oil (Til oil)	13	50	37
Palm oil	42	52	6
Cotton seed oil	26	19	55
Mustard oil (rapeseed)	34	48	18
Safflower oil (Kardi)	9	12	79
Sunflower oil	12	24	64
Butter	75	20	5
Ox (Tallow)	53	42	5
Pig (Lard)	42	46	12
Fish oil	30	13	57

(*) these saturated fatty acids are medium chain fatty acids.

NEUTRAL FATS

Neutral fats are also called as triacylglycerols (TAG) or triglycerides (TG). These are esters of the trihydric alcohol, glycerol with fatty acids (Fig. 8.1).

1. Nomenclature of carbon atoms

The carbon atoms of glycerol are designated as α, β and α' or as 1, 2, 3 as shown in Figure 8.1, where R represents the side chain of fatty acids. Enzymes can distinguish between 1st and 3rd carbon atoms.

2. Mixed Triglycerides

Naturally occurring fats and oils are mixtures of triglycerides. If all the three hydroxyl groups of the glycerol are esterified to the same fatty acid, a **simple** triacylglycerol is formed, e.g. tripalmitin, triolein, etc. A **mixed** triglyceride is formed, when different fatty acids are esterified to the hydroxyl groups of glycerol. When a PUFA is present, it is commonly esterified to the 2nd or beta carbon atom.

3. Physical Properties of Triglycerides

They are hydrophobic and insoluble in water. **Oils are liquids** at 20°C; they are triglycerides which contain a higher proportion of unsaturated fatty acids or short chain triglycerides. Oils are generally of plant origin. **Fats are solids** at room temperature and contain mainly saturated long chain fatty acids. Fats are mainly of animal origin. (Table 8.3).

4. Storage of Energy as Fat

The triacylglycerides are the storage form of lipids in the **adipose tissue**. When stored as TAG, water molecules are repelled and space requirement is minimal. Excess fat in the body leads to obesity.

5. Hydrolysis of Triglycerides

This occurs in the body during digestion of dietary fat and mobilization of TAG from adipose tissue. Triglycerides in the body are hydrolyzed by enzymes, **lipases**. Triacylglycerol is **sequentially hydrolyzed** to diacylglycerol and monoacylglycerol and finally glycerol plus 3 fatty acids (Fig. 8.2).

PHOSPHOLIPIDS

They contain glycerol, fatty acids and a nitrogenous base.

A. Phosphatidates

These are derivatives of phosphatidic acid which is the simplest phospholipid. **Phosphatidic acid** is made up of one glycerol to which two fatty acid residues are esterified to carbon atoms 1 and 2. The 3rd hydroxyl group is esterified to a **phosphoric acid** (Fig. 8.3).

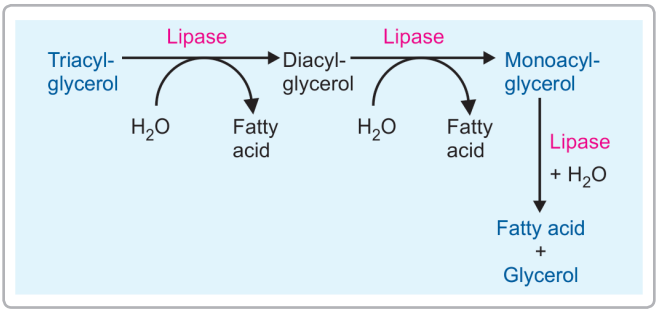


Figure 8.2: Hydrolysis of triglycerides

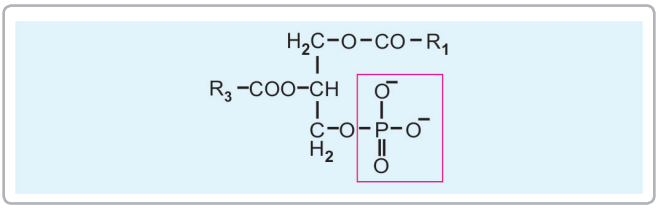


Figure 8.3: L-phosphatidic acid

B. Amphipathic Nature

Phospholipids in general are amphipathic, particularly Lecithin. They have both hydrophobic and hydrophilic portion in their molecule (Fig. 8.4).

D. Biomembranes

The molecules align themselves to form monolayers with the polar heads pointing in one direction and the nonpolar tails in the opposite direction (For details refer Chapter 1). They also act as detergents and emulsifying agents. In vivo, they act as pulmonary surfactants.

1. Phosphatidylcholine or Lecithin

This is a nitrogen containing phospholipid. It contains the glycerol group. The alpha and beta positions are esterified with fatty acids. Usually, the fatty acid attached to the beta-carbon is a **PUFA** molecule (Fig. 8.4). The phosphoric acid is added to the third position, to make it a phosphatidic acid. The phosphate group is esterified to the quaternary nitrogen base, **Choline** (Fig. 8.4).

2. Phosphatidyl ethanolamine or Cephalin

Cephalin differs from lecithin in that the nitrogen base ethanolamine is present instead of choline. Cephalin is also found in biomembranes and possesses amphipathic properties.

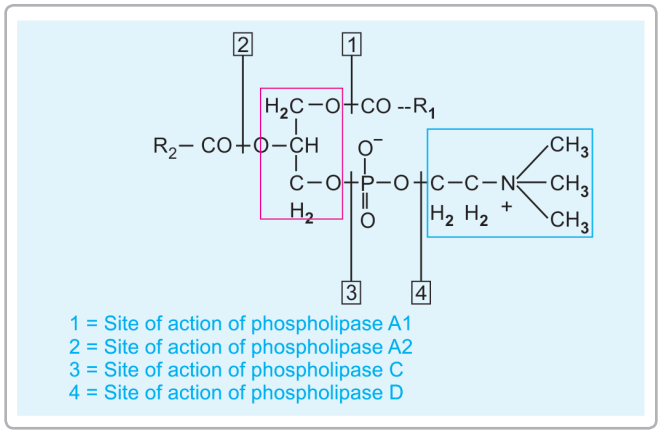


Figure 8.4: Lecithin R1 and R2 are fatty acids. Red square depicts glycerol group. The blue square is choline which shows polar or hydrophilic property

3. Sphingolipids

All sphingolipids have the long aliphatic amino alcohol **sphingosine**, which is attached to a fatty acid in amide linkage to form a **ceramide**. The fatty acid has a chain length varying from C18 to C24.

Phosphosphingosides

They contain phosphoric acid group. A common phosphosphingoside present abundantly in biomembranes, especially of the nervous system, is **sphingomyelin.** It contains ceramide, phosphate and choline. *Sphingomyelins are the only sphingolipid that contain phosphate and have no sugar moiety.* Different fatty acid are attached, usually very long chain fatty acids (22 Carbon, 6 double bonds).

NON-PHOSPHORYLATED LIPIDS

Glycosphingolipids (Glycolipids): They are seen widely in nervous tissues. This group of lipids do not contain phosphoric acid; instead they contain carbohydrates and **ceramide**.

Lipoproteins

In these molecules, the lipid component comprises of triacylglycerol, phospholipids and cholesterol. The protein component has a high proportion of non-polar amino acid residues which can participate in the binding of lipids. They are found in membranes of mitochondria, endoplasmic reticulum and nuclei. Lipoproteins of plasma are described in detail in Chapter 10.

LIPID STORAGE DISEASES OR SPHINGOLIPIDOSES

They form a group of lysosomal storage diseases. The sphingolipids are normally catabolized by a series of bond specific lysosomal hydrolases like alpha and beta glucosidases, galactosidase, neuraminidase, hexosaminidase and arylsulfatase (for sulfate ester hydrolysis). The diseases result from failure of breakdown of a particular sphingolipid due to deficiency of a single enzyme. The children afflicted by these diseases are severely **retarded mentally** and seldom survive for long. Diseases include Niemann Pick's disease, Gaucher's disease and Tay-Sachs disease. Mental retardation, neurological deficit and skeletal abnormalities are common presenting symptoms.

Essential Fatty Acids

Linoleic acid (ω 6, 18C, two double bonds) and **linolenic acid** (w3, 18C, three double bonds) are the only fatty acids which cannot be synthesized in the body. They have to be provided in the food; hence they are essential fatty acids. Arachidonic acid can be formed, if the dietary supply of linoleic acid is sufficient. Normal dietary allowance of PUFA is 2–3% of total calories. Deficiency causes acanthosis, hyperkeratosis and hypercholesterolemia.

Eicosanoids

They are 20 C compounds (Greek, eikosi = twenty), derived from arachidonic acid.

PROSTAGLANDINS (PGs)

PGs were originally isolated from prostate tissue and hence the name. But they are present in almost all tissues. They are the most potent biologically active substances; as low as one nanogram/mL of PG will cause smooth muscle contraction. The diverse physiological roles of prostaglandins confer on them the status of **local hormones**.

Chemical structure: All prostaglandins are considered to be derived from the 20C cyclic saturated fatty acid, prostanoic acid. The five carbon ring is saturated. All naturally occurring PGs have an alpha oriented OH group at C15.

Classification of prostaglandins: According to the attachment of different substituent groups to the ring, PGs are named with capital letters such as A, B, E and F. In the same series, depending on number of double bonds on the side chains they are denoted by a subscript after the capital letter, e.g. PGE1, PGE2, PGE3, etc. **Series 2** have 2 double bonds at 13–14 (trans) and 5–6 (cis). Structure of PGF2 is shown in Figure 8.5.

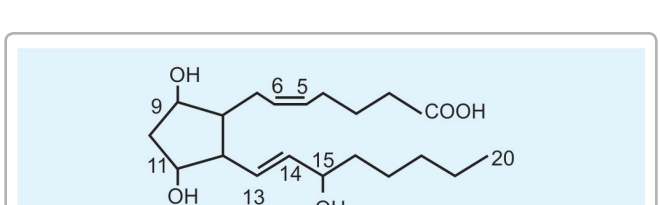


Figure 8.5: Prostaglandin F2

Biosynthesis of Prostaglandins

Prostaglandins are derived from the Polyunsaturated fatty acids. All naturally occurring PGs belong to the 2 series. PGs are not stored as such; the precursor fatty acids are stored in membrane as phospholipids. The arachidonic acid is released by the action of **phospholipase A2** on phospholipids. Synthesis is catalyzed by Prostaglandin synthase, containing two enzymes, **Cyclooxygenase** and peroxidase.

Regulation of Synthesis

The **phospholipase** (PL) is activated by epinephrine. Steroids inhibit PL and prevent release of arachidonic acid from membranes. **Cyclooxygenase** is activated by catecholamines and inhibited by nonsteroid anti-inflammatory drugs (NSAIDs). **Aspirin** acetylates serine in the active site and irreversibly inhibits the cyclooxygenase Platelets cannot regenerate cyclooxygenase and so thromboxane is not formed in platelets. Hence, there is decreased platelet aggregation. Therefore, aspirin is useful in prevention of heart attacks.

Biological Actions

The effects of prostaglandins on different tissues are different and some of these may oppose each other.
Prostaglandins are **local hormones**. In most tissues, PGE increases cAMP (cyclic AMP) level.

Effects on CVS: Prostacyclin or PGI2 is synthesized by the vascular endothelium. Major effect is vasodilatation. It also inhibits platelet aggregation and has a protective effect on vessel wall against deposition of platelets. **Thromboxane** (TXA2) is the main PG produced by

platelets. The major effects are vasoconstriction and platelet aggregation. Prostacyclin and thromboxane are opposing in activity. Prostaglandins increase the contractility and lowers the blood pressure. Hence, it may be used in the treatment of hypertension.

Effects on ovary and uterus: PGF2 stimulates the uterine muscles. Hence, PGF2 may be used for medical termination of pregnancy. Yet another use is in inducing labor and arresting postpartum hemorrhage.

Respiratory tract: PGF is a constrictor of bronchial smooth muscle; but PGE is a potent bronchodilator. PGE is used in aerosols for treating bronchospasm.

Effects on immunity: PGE2 and D2 produce inflammation by increasing capillary permeability. Cortisol and aspirin are strong anti-inflammatory drugs, because they inhibit prostaglandin synthesis.

A QUICK LOOK

1. Lipids may be broadly classified into simple, compound and derived lipids.
2. Compound lipids are phospholipids, sphingolipids, sulfolipids, etc. Compound lipids containing alcohol sphingosine and one or more carbohydrate residues are called glycolipids.
3. Fatty acids are classified based on (i) Number of carbon atoms, (ii) Length of hydrocarbon chain, and (iii) Nature of hydrocarbon chain. Depending on the number of carbon atoms, fatty acids may be even chain or odd chain, which are further subdivided into short chain (2–6C), medium chain (8–14C) and long chain (16–20C).
4. Palmitic and stearic acid are the most abundant saturated fatty acids in the body.
5. Fatty acids may be saturated (no double bonds), mono-unsaturated (one double bond) or polyunsaturated (more than 2 double bonds). Polyunsaturated fatty acids (PUFA) may be essential or nonessential. Essential fatty acids are those, which cannot be synthesized in the human body and have to be supplemented in the diet. As for example, Linoleic acid, Linolenic acid and arachidonic acid.
6. Arachidonic acid is the precursor of prostaglandins.
7. Saponification number is defined as the number of milligrams of KOH required to saponify 1 gram of fat.
8. Iodine number of a fat is defined as the number of grams of iodine taken up by 100 grams of fat. It is directly proportional to the degree of unsaturation.
9. Rancidity refers to the appearance of unpleasant odor and taste to oils and fats. Rancidity can be of two types, Hydrolytic and oxidative.
10. Depending on the position of double bonds from the omega end, fatty acids may be omega 3, omega 6, omega 9.
11. Sodium and potassium salts of fatty acids are called soaps.
12. Fatty acids can form esters with hydroxyl groups of glycerol to form mono-, di- and triacylglycerol.
13. Triacylglycerol or neutral fat are the storage form of energy in adipose tissue.
14. Major fatty acids found in adipose tissue fat are oleic acid, palmitic acid and stearic acid.
15. MUFA and PUFA are commonly esterified to the second carbon (beta carbon) of glycerol.
16. Oils and fats are mixtures of triacylglycerol. Oils are liquids at 25°C and fats are solids.
17. Butter contains short and medium chain fatty acids.
18. Phospholipids may be glycerophosphatides or phosphosphingosides depending on the alcohol present.
19. Simplest glycerophosphatides is phosphatidic acid containing glycerol, 2 molecules of fatty acid and one molecule of phosphoric acid.
20. Phospholipids are amphipathic in nature since they have a polar head and non-polar tail. Amphipathic nature is ideal for the role of phospholipids as components of biomembranes and for micelle formation.
21. Phosphatidic acid may combine with nitrogenous base to form aminophospholipids like phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.
22. Phosphatidyl glycerol or cardiolipin is formed by the esterification of one molecule of glycerol simultaneously to 2 molecules of phosphatidic acid.
23. Phospholipase A2 hydrolyses the ester bond between the second hydroxyl group of glycerol and a PUFA.
24. Phosphosphingosides contain sphingosine as alcohol. Sphingosine esterified to a fatty acid is called ceramide.
25. Sphingomyelin is the only phosphosphingoside which contains choline.
26. Sphingolipids have ceramide attached to carbohydrate residues to form glycolipids like cerebrosides and lactosylceramide.
27. When one molecule of NANA is attached to the ceramide oligosaccharide, it is called ganglioside.
28. Sulfatides are formed when sulfate is esterified to ceramide oligosaccharide.
29. Cholesterol is an animal sterol which is a derived lipid. It is the precursor of all steroids in the body.

CHAPTER 9

Lipids–II: Metabolism of Fatty Acids

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Digestion and absorption of lipids
- Beta oxidation of fatty acids
- Oxidation of odd chain fatty acids
- De novo synthesis of fatty acids
- Synthesis of triglycerides
- Metabolism of adipose tissue
- Fatty liver and lipotropic factors
- Ketogenesis and ketolysis

DIGESTION OF LIPIDS

The major dietary lipids are triacyl glycerol, cholesterol and phospholipids. The average normal Indian diet contains about 20–30 g of lipids per day.

Digestion in Stomach

Digestion in stomach occurs with the help of lingual lipase and gastric lipase. Lingual lipase is more significant in newborn infants.

Digestion in Intestines

Emulsification is a prerequisite for digestion of lipids. The lipids are dispersed into smaller droplets; surface tension is reduced; and surface area of droplets is increased.

Bile Salts are important for digestion of lipids

The bile salts present in the bile (sodium glycocholate and sodium taurocholate) **lower surface tension.** They emulsify the fat droplets in the intestine. The emulsification increases the surface area of the particles for enhanced activity of enzymes.

Lipolytic Enzymes in intestines

Pancreatic lipase with colipase will further hydrolyse the neutral fats. The bile (pH 7.7) entering the duodenum serves to neutralize the acid chyme from the stomach and provides a pH favorable for the action of pancreatic enzymes.

Digestion of Triglycerides

Digestion occurs with the help of pancreatic lipase, isomerase and a colipase. The major end products of the digestion of TAG are 2-MAG, 1-MAG, glycerol and fatty acids (Fig. 9.1). Thus digestion of TAG is partial (incomplete).

ABSORPTION OF LIPIDS

Absorption of Long Chain Fatty Acids

Long chain fatty acids (chain length more than 14 carbons) are absorbed to the lymph and not directly to the blood.. The theory proposed by **Bergstrom** (Nobel Prize, 1982) has the following steps (Fig.9.2).

1. Mixed micelle formation

- The products of digestion, namely 2-monoglycerides, long chain fatty acids, cholesterol, phospholipids and lysophospholipids are incorporated into molecular aggregates to form **mixed micelle**.
- The micelles are spherical particles with a hydrophilic exterior and hydrophobic interior core (Fig. 9.3).

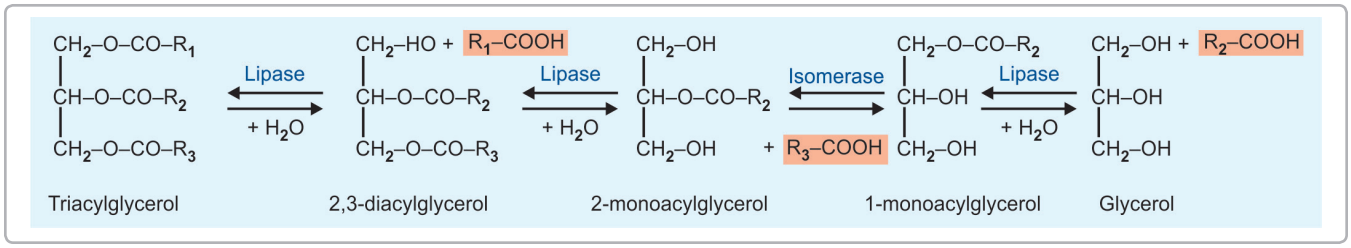


Figure 9.1: Complete hydrolysis of triglyceride. In the intestines, generally fats are only partially hydrolyzed

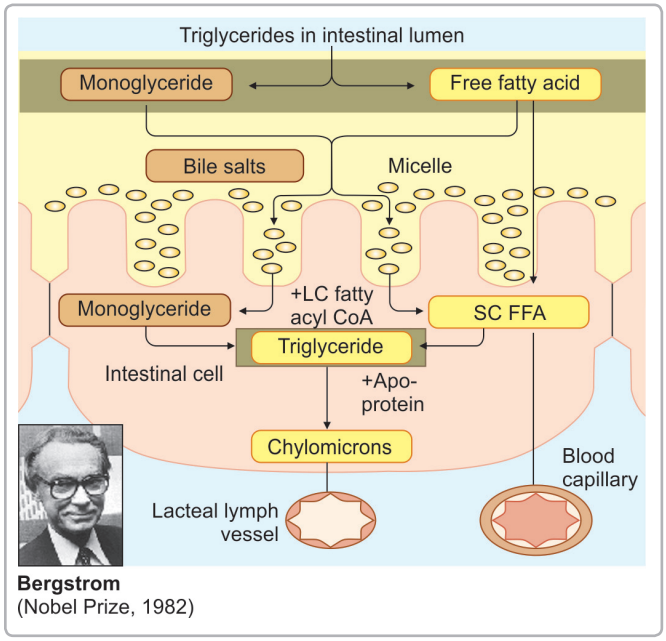


Figure 9.2: Digestion and absorption of triglycerides. LC = long chain; SC FFA = short chain free fatty acid. Triglycerides with long chain fatty acids are absorbed into lymph vessels as chylomicrons. Short chain fatty acids directly enter portal vein (blood vessel)

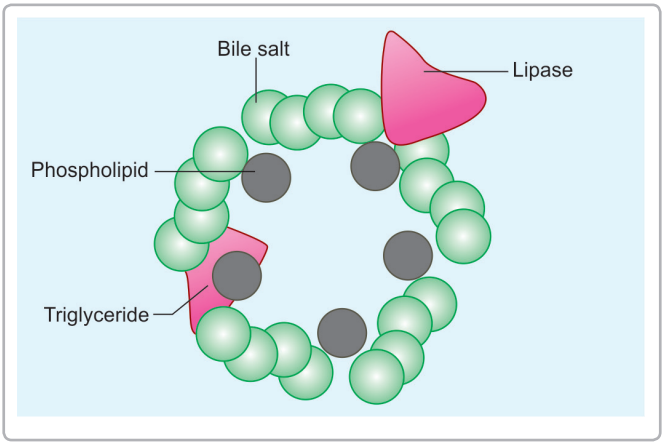


Figure 9.3: Mixed micelle

Due to their detergent action, the **bile salts** help to form micellar aggregates.

- Micellar formation is essential for the absorption of fat soluble vitamins such as vitamin A, D and K.
- This micelles are absorbed at the microvillous surface of the **jejunal mucosa**. Fatty acids, 2-MAG and other digested products passively diffuse into the mucosal cell.

2. Enterohepatic Circulation of Bile Salts

The bile salts are left behind which are mostly reabsorbed from the ileum and returned to the liver to be re-excreted (enterohepatic circulation). About 98% of dietary lipids are normally absorbed.

3. Re-esterification inside the Mucosal Cell

- Once inside the intestinal mucosal cell, the long chain fatty acids are re-esterified to form triglycerides (Fig. 9.2).
- Two fatty acylCoA (activated fatty acids) react with monoacylglycerol (MAG) to form the triglyceride. Majority of molecules take up this MAG pathway (Fig. 9.2).
- Free **glycerol** absorbed from intestinal lumen directly enters into the blood stream. So free glycerol is not available for re-esterification. But the cells can convert glucose to glycerol phosphate, and then add 3 molecules of acyl groups to synthesise TAG.

3. Chylomicrons

The TAG, cholesterol ester and phospholipid molecules along with apoproteins B48, and apo-A are incorporated into chylomicrons (For details refer Chapter 10). The chyle (milky fluid) from the intestinal mucosal cells loaded with chylomicrons are transported through the lacteals into the thoracic duct and then emptied into lymph circulation

(Fig. 9.2). The serum may appear milky after a high fat meal (postprandial lipemia) due to the presence of chylomicrons in circulation. Normally, the lipemia clears within a few hours by the uptake of chylomicrons by tissues.

4. SCFA Absorption is Different

Short chain fatty acids (SCFA) (seen in milk, butter, ghee) and **medium chain** fatty acids (MCFA) (in coconut oil and mother's milk) do not need re-esterification. They can directly enter into blood vessels, then to portal vein, finally to liver where they are immediately utilized for energy. Their absorption is rapid. They are better absorbed than long chain fatty acids.

5. Abnormalities in Absorption of Lipids

- **Defective digestion:** In steatorrhea, daily excretion of fat in feces is more than 6 g per day. (Greek word, "stear", means fat). It is due to chronic diseases of pancreas. In such cases, unsplit fat is seem in feces.
- **Defective absorption:** On the other hand, if the absorption alone is defective, most of the fat in feces may be split fat, i.e. fatty acids and monoglycerides. Defective absorption may be due to **obstruction of bile duct.** This again may be due to gallstones, tumors of head of pancreas, enlarged lymph glands, etc. The result is deficiency of bile salts. In such cases, triglycerides with short chain and medium chain fatty acids (SCT and MCT) are digested and absorbed properly, because they do not require micellerization for absorption. Since milk fat and coconut oil are made up of MCT, they are therapeutically useful in malabsorption syndromes.
- **Chyluria.** There is an abnormal connection between the urinary tract and lymphatic drainage system of the intestine. Urine appears milky due to lipid droplets.

6. Fate of absorbed fat

- The absorbed (exogenous) triglycerides are transported in blood as **chylomicrons**. They are taken up by adipose tissue and liver.
- Liver synthesises endogenous triglycerides. These are transported as **VLDL** (very low density lipoproteins) and are deposited in adipose tissue.
- Triglycerides in adipose tissue are lysed to produce **free fatty acids**. In the blood, they are transported, complexed with albumin.
- Free fatty acids are taken up by the cells, and are then oxidized to get energy.

BETA-OXIDATION OF FATTY ACIDS

This process is known as beta-oxidation, because the oxidation and splitting of **two carbon** units occur at the beta-carbon atom. The oxidation of the hydrocarbon chain occurs by a sequential cleavage of two carbon atoms (Fray Knoop, 1904).

Preparative step for beta-oxidation

Coenzyme A (abbreviated as CoA) is a complex molecule containing beta mercapto ethanolamine (MEA), beta alanine, pantoic acid and ADP. The SH group present in MEA forms thioester bond in acyl-CoA. To emphasize the function of the SH group, the CoA is sometimes written as CoA-SH.

Preparative step 1: Activation of Fatty Acids

Fatty acids are activated to their coenzyme A (CoA) derivative. ATP is hydrolyzed to AMP and PPi and the energy from hydrolysis of PPi drives the reaction forward. Thus **two high energy bonds** are utilized in this reaction. The enzyme is a **thiokinase** or fatty acyl-CoA **synthetase** (step 0, Fig. 9.4). Acetyl group and acyl groups are different (Box 9.1).

Preparative step 2: Role of Carnitine

Fatty acids are activated in the cytoplasm; but the **beta-oxidation is in mitochondria.** So transport of fatty acids through the mitochondrial membrane is essential. The long chain fatty acyl-CoA cannot pass through the inner mitochondrial membrane. Therefore a transporter, carnitine is involved in transfer of fatty acids. **Carnitine** has the following structure:

$$(CH_3)_3\text{–}N^+\text{–}CH_2\text{–}CHOH\text{–}CH_2\text{–}COOH.$$

Preparative step 3: Carnitine Acyltransferase

The enzyme carnitine acyltransferase-I (**CAT-I**) will transfer the fatty acyl group to carnitine to form **acylcarnitine**. The reaction occurs on the cytosolic side

BOX 9.1: Acetyl and acyl groups are different

Acetyl-CoA is the combination of acetate or acetic acid (2 carbon unit) with coenzyme A

Acyl-CoA means acyl group (any fatty acid, C4 to C26 in length) combined with coenzyme A

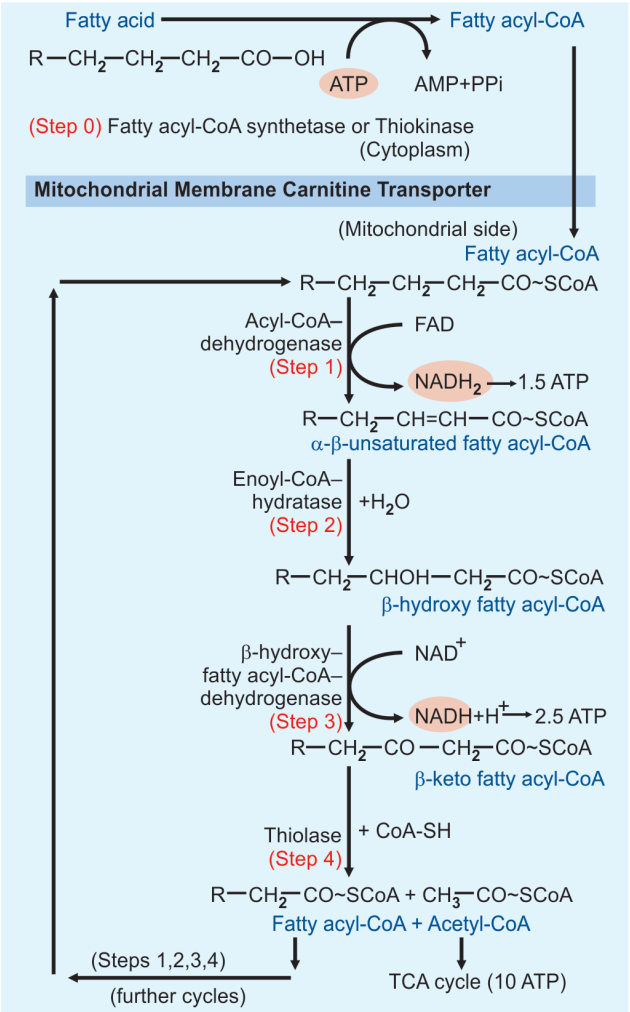


Figure 9.4: Beta oxidation of fatty acids. Important to remember that the first step is FAD dependent and the third step is NAD^+ dependent

of inner mitochondrial membrane. Further, with the help of a translocase, acylcarnitine enters mitochondria. With the help of CAT-II, carnitine is removed, and acyl-CoA (activated acyl group) is now in mitochondria.

Clinical Applications

Medium chain and short chain fatty acids do not require carnitine for transport across the inner mitochondrial membrane. So medium chain and short chain fatty acids are easily oxidized.

Beta-oxidation steps

The next 4 reactions are sequentially repeated for complete oxidation of fatty acids. After one round of four metabolic steps, one acetyl-CoA unit is split off and acyl-CoA with

BOX 9.3: Summary of beta-oxidation

When one molecule of palmitate undergoes beta-oxidation, the net reaction is:

Palmitoyl-CoA + 7FAD + $7NAD^+$ + $7H_2O$ + 7HSCoA → 8 Acetyl-CoA + 7FADH2 + 7NADH + $7H^+$

2 carbon atoms less is generated. This would undergo the same series of reactions again until the fatty acid is completely oxidised. Beta oxidation steps are shown in Fig. 9.4.

Step 1: The fatty acyl-CoA is dehydrogenated with the FAD accepting the hydrogen atoms. **FADH2** when oxidized in electron transport chain will produce **2 ATP** molecules.

Step 2: This step forms a beta-hydroxy fatty acyl-CoA.

Step 3: Another dehydrogenation takes place with the help of **NADH**, which when oxidized in electron transport chain will generate **2.5 ATPs**.

Step 4: One molecule of acetyl-CoA is liberated, leaving behind a fatty acid with 2 carbon atoms less.

Further cycles: The newly formed fatty acyl-CoA will sequentially undergo further cycles of steps 1, 2, 3 and 4 of beta-oxidation until the fatty acid is completely converted to acetyl-CoA (Fig. 9.4). A summary is shown in Box 9.2.

Energetics of beta-oxidation (ATP Yield)

Palmitic acid (16 C) needs 7 cycles of Beta-oxidation, which give rise to 8 molecules of acetyl-CoA (Fig. 9.5). Every molecule of acetyl-CoA when oxidized in the TCA cycle gives 10 molecules of ATP. Each molecule of FADH2 produces 1.5 molecules and each NADH generates 2.5 molecules of ATP when oxidized in the electron transport chain. Hence, the energy yield from one molecule of palmitate may be calculated as

8 acetyl-CoA × 10	=	80	ATP
7 $FADH_2$ × 1.5	=	10.5	ATP
7 NADH × 2.5	=	17.5	ATP
Gross total	=	108	ATP
Net yield	=	**108 minus 2 = 106 ATP**	

(In the initial activation reaction, the equivalent of 2 high energy bonds are utilized). The efficiency of beta-oxidation is about 35%.

Note: In the previous edition of this textbook, calculations were made assuming that NADH produces 3 ATPs and FADH generates 2 ATPs. This will amount to a net

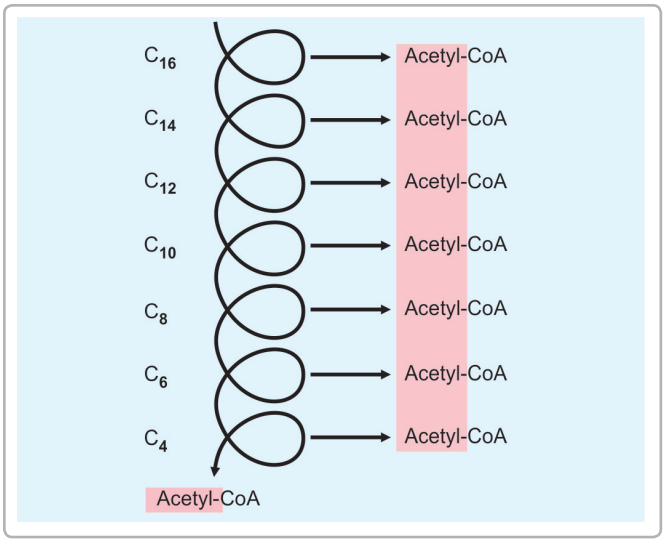


Figure 9.5: Summary of beta-oxidation of palmitic acid (16 C). It undergoes 7 cycles, which give rise to 8 molecules of acetyl-CoA

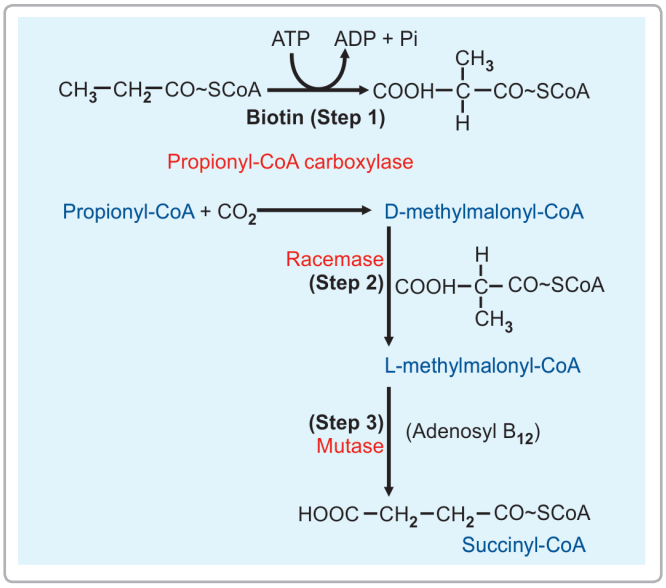


Figure 9.6: Metabolism of propionyl-CoA

generation of 129 ATPs per palmitate molecule. Recent experiments show that these old values are over-estimates, and net generation is only 106 ATPs.

Regulation of Beta-oxidation

The availability of free fatty acid (FFA) regulates the net utilization through beta-oxidation. The level of FFA, in turn, is controlled by glucagon: insulin ratio. Glucagon increases FFA level and insulin has the opposite effect. CAT-I is the regulator of entry of fatty acid into mitochondria. Malonyl-CoA inhibits CAT-I activity. Thus during de novo synthesis of fatty acid, the beta-oxidation is inhibited.

OXIDATION OF ODD CHAIN FATTY ACIDS

The odd chain fatty acids are oxidized exactly in the same manner as even chain fatty acids. However, after successive removal of 2-carbon units, at the end, one 3 carbon unit, **propionyl-CoA** is produced.

Fate of Propionyl-CoA: With the help of carboxylase, racemase and mutase, propionate is converted into succinyl-CoA (Fig. 9.6). Carboxylase is **biotin** dependent while mutase is **vitamin B12** dependent. The succinyl-CoA then enters TCA cycle, finally converted into oxaloacetate, and is used for gluconeogenesis.

Propionate is Gluconeogenic

Ordinary fatty acids are cleaved to acetyl-CoA units which on entering the Krebs cycle are completely oxidized to CO_2,

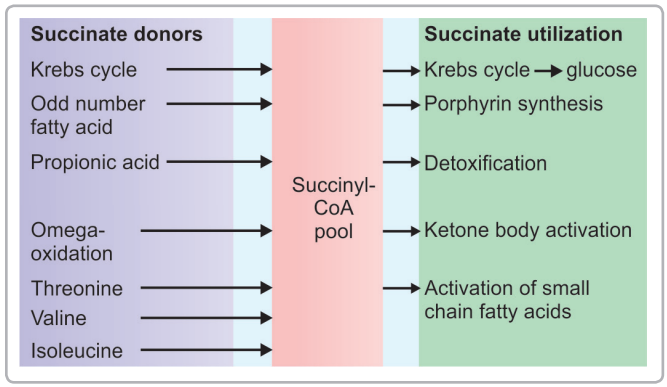


Figure 9.7: Succinyl-CoA pool

and hence as a general rule, **fatty acids cannot be used for gluconeogenesis.** However propionate is entering into the citric acid cycle at a point after the CO_2 elimination steps, so propionate can be channelled to gluconeogenesis. Thus 3 carbon units from **odd carbon fatty acids are gluconeogenic**. **Cow's milk** contains significant quantity of odd chain fatty acids. The succinyl-CoA pool (donors and utilization) is shown in Fig. 9.7. Inborn errors of propionate metabolism include methylmalonic aciduria and other organic acidurias.

DE NOVO SYNTHESIS OF FATTY ACIDS

The process of fatty acid synthesis was studied by Feodor Lynen, who got Nobel prize in 1964. The pathway is referred to as **Lynen's spiral**. It is not a reversal of oxidation.

Table 9.1: Difference in the two pathways		
	Beta-oxidation	*Fatty acid synthesis*
Site	Mitochondria	Cytoplasm
Intermediates	Present as CoA derivatives	Covalently linked to SH group of ACP
Enzymes	Present as independent proteins	Multienzyme complex
Sequential units	2 carbon units split off as acetyl-CoA	2 carbon units added, as 3 carbon malonyl-CoA
Coenzymes	NAD^+ and FAD are reduced	NADPH used as reducing power

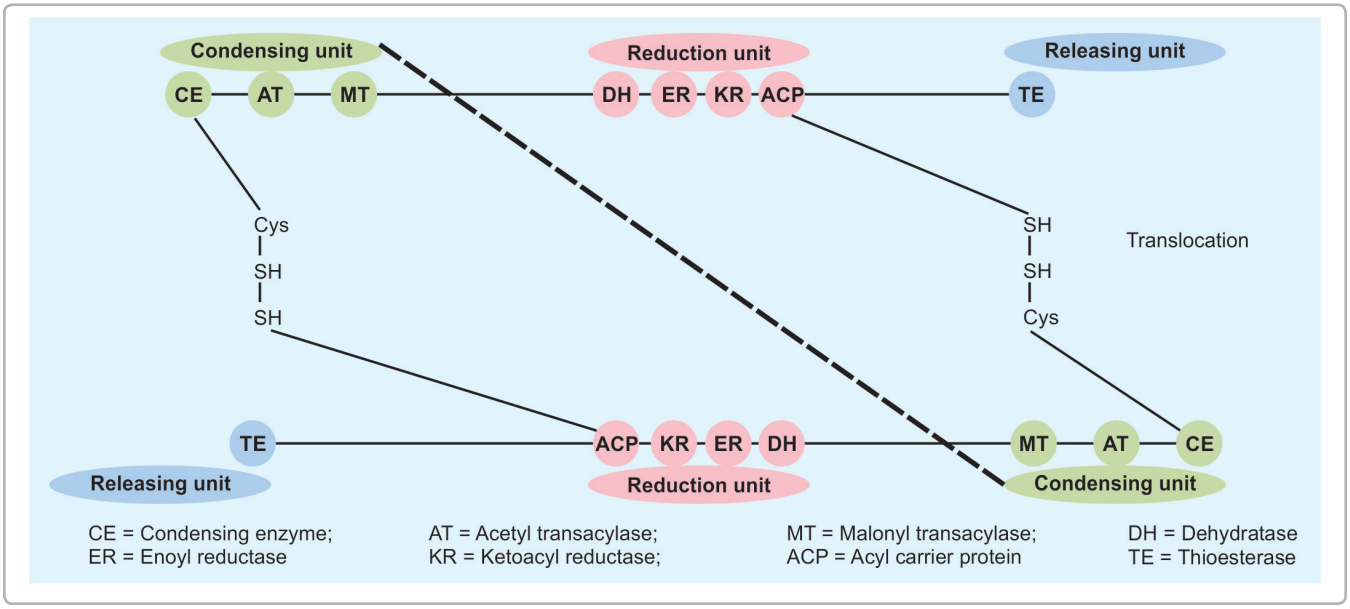


Figure 9.8: Fatty acid synthase complex. Upper and lower units are two monomers of the complex. Dotted line represents functional division

Important differences in synthesis and breakdown of fatty acids are given in Table 9.1.

Fatty acids are synthesized mainly by a *de novo* synthetic pathway operating in the **cytoplasm**. So it is referred to as **extramitochondrial** or cytoplasmic fatty acid synthase system.

The major fatty acid synthesised *de novo* is **palmitic acid**, the 16C saturated fatty acid. The process occurs in liver, adipose tissue, kidney, brain, and mammary glands.

Fatty Acid Synthase (FAS) Complex

This system exists as a **multienzyme complex**. The enzymes form a **dimer** with identical subunits. Each subunit of the complex is organized into 3 **domains** with 7 enzymes (Fig. 9.8). First domain is called condensing unit; 2nd domain is the reduction unit and the 3rd domain is known as the releasing unit.

Step 1: Carboxylation: The first step in the fatty acid synthesis is the carboxylation of acetyl-CoA to form malonyl-CoA. The acetyl-CoA carboxylase is the **rate-limiting enzyme. Biotin**, a member of B complex vitamins, is necessary for this reaction (Step 1 in Fig. 9.9). The enzyme is allosterically regulated, the major effectors being citrate (positive) and palmitoyl-CoA (negative).

Step 2: Units are added: The elongation of the fatty acid occurs by addition of 2 carbon atoms at a time. But the 2-carbon units are added as 3-carbon, **malonyl units**. The whole reaction sequence occurs while the intermediates are bound to ACP (acyl carrier protein). The **acetyl transacylase** (AT in Fig. 9.8) catalyzes the transfer of the acetyl group (2 carbons) to the **condensing enzyme** (CE) (step 2A in Fig. 9.9). One molecule of acetyl-CoA (2 carbon) and one molecule of malonyl-CoA (3 carbon) bind to the multienzyme complex. **Malonyl transacylase** (MT in Fig. 9.8) transfers the malonyl group to the enzyme (step 2B in Fig. 9.9).

Step 3: Condensation: The acetyl (2C) and malonyl (3C) units are condensed by the condensing enzyme, to form acetoacetyl ACP (4C). (step 3 in Fig. 9.9).

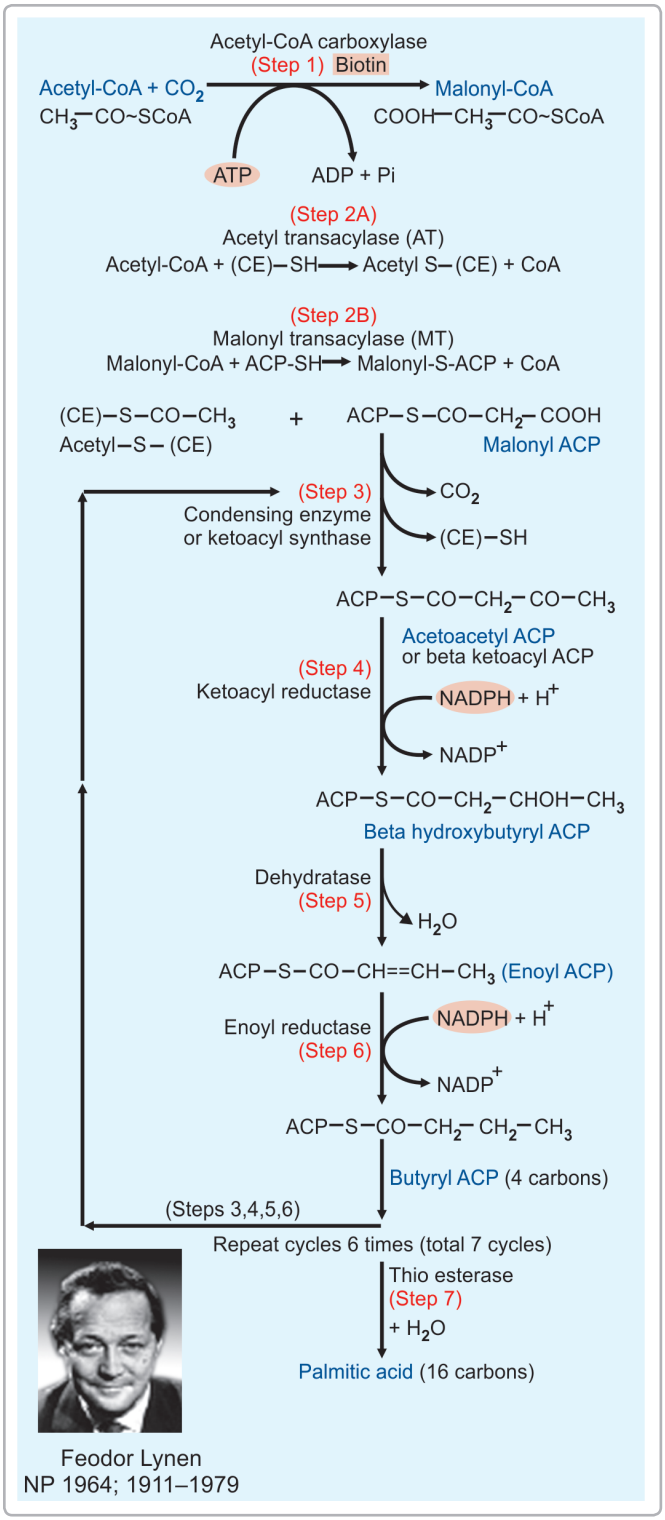


Figure 9.9: De novo Synthesis of Fatty Acid (Lynen cycle). Steps 4 and 6 utilise NADPH

Step 4: Reduction: The acetoacetyl ACP is reduced by **NADPH** dependent reductase (step 4 in Fig. 9.9).

Step 5: Dehydration: It is then dehydrated by a dehydratase (DH) to form unsaturated acyl ACP (step 5 Fig. 9.9).

Step 6: Second reduction: It is again reduced by enoyl reductase (ER) utilizing a 2nd molecule of **NADPH** to form butyryl ACP (step 6 in Fig. 9.9).

Cycling of reactions: The butyryl group (4C) is now transferred to the condensing enzyme. Then condensation, reduction, dehydration and reduction (steps 3,4,5,6) are repeated. The cycles are repeated a total of **seven times,** till the 16-Carbon, palmitic acid is formed.

Step 7: Release: The elongation of the fatty acid occurs by addition of 2 carbon atoms at a time. But the 2-carbon units are added as 3-carbon, malonyl units. The thioesterase activity (TE) releases palmitate from the multienzyme complex (step 7, Fig. 9.9).

The end point is palmitic acid (16 C) in liver and adipose tissue. But in lactating mammary gland, the end products are Capric (10 C) and Lauric (12 C) acids. Mother's milk contains these medium chain fatty acids. Cow's milk contains odd numbered fatty acids.

Summary of de novo Synthesis

The net reaction of de novo synthesis of fatty acid may be summarized as

1 Acetyl-CoA +7 Malonyl-CoA +14NADPH +14H^+ →

1 Palmitate + 7CO_2 +14$NADP^+$ + 8CoA+ 6H_2O

Fatty acid synthesis is not an exact reversal of beta-oxidation. A comparison of these pathways is given in Table 9.1.

Coenzymes of Fatty Acid Synthesis

An important point to remember is that the coenzyme utilized for de novo synthesis is **NADPH.** The sources of NADPH for fatty acid synthesis is mainly **Pentose phosphate pathway** (For details refer Chapter 7). Tissues having active lipogenesis (liver, adipose tissue, lactating mammary gland) have an active HMP shunt pathway also.

Regulation of Fatty Acid Synthesis

Acetyl-CoA carboxylase is the **key enzyme**; citrate activates this enzyme. The citrate level is high only

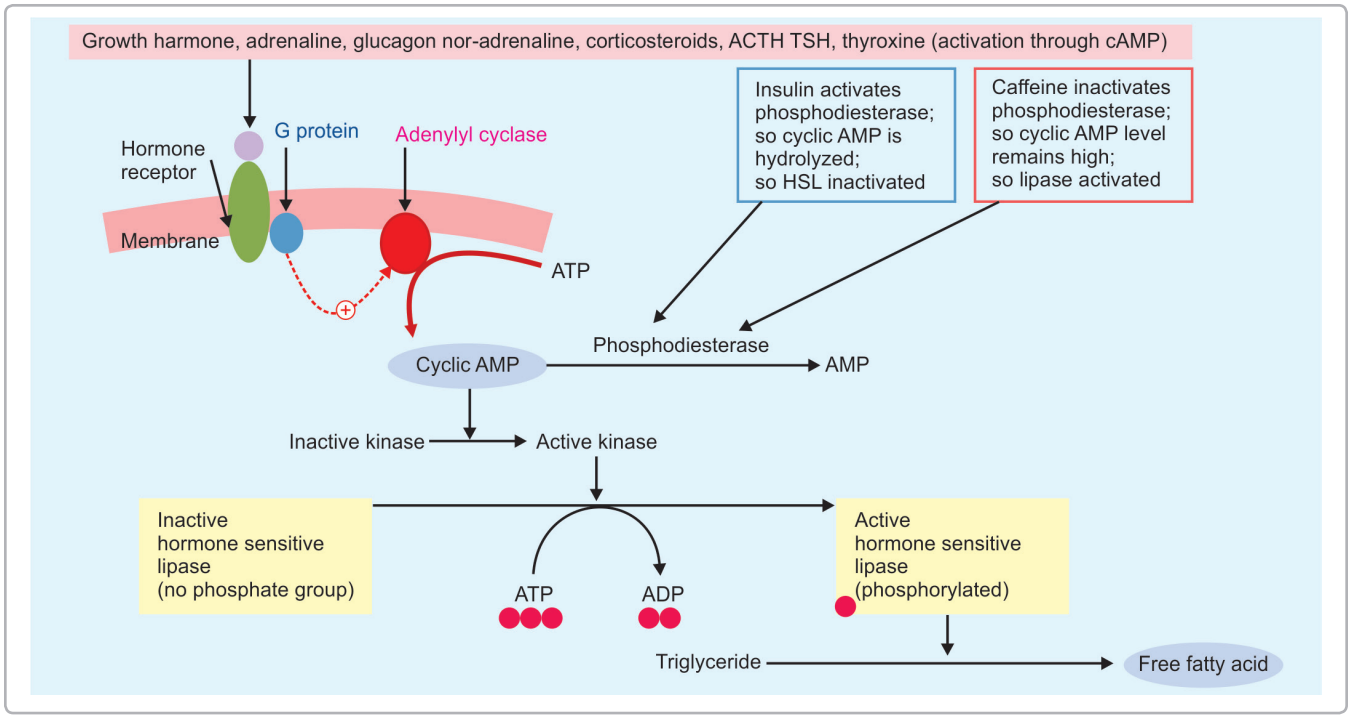


Figure 9.10: Cascade activation of hormone sensitive lipase

when both acetyl-CoA and ATP are abundant. Fatty acid synthesis decreases when glucose level is low. The enzyme is inhibited by palmitoyl-CoA, the end product.

Insulin Favors Lipogenesis. Insulin enhances the uptake of glucose by adipocytes and increases the activity of pyruvate dehydrogenase, acetyl-CoA carboxylase and glycerol phosphate acyltransferase. Insulin also depresses the hormone sensitive lipase. Insulin also causes inhibition of **hormone sensitive lipase**, and so lipolysis is decreased (Fig. 9.10).

Glucagon inhibits lipogenesis by inactivating the acetyl-CoA carboxylase.

SYNTHESIS OF TRIGLYCERIDES (TAG)

Liver and adipose tissue are the major sites of triacylglycerol (TAG) synthesis. The TAG synthesis in adipose tissue is for storage of energy whereas in liver it is mainly secreted as VLDL and is transported.

The TAG is synthesized by esterification of fatty acyl-CoA with either glycerol-3-phosphate or dihydroxyacetone phosphate (DHAP) (Fig. 9.11). The glycerol part of the fat is derived from the metabolism of glucose. DHAP is an intermediate of glycolysis.

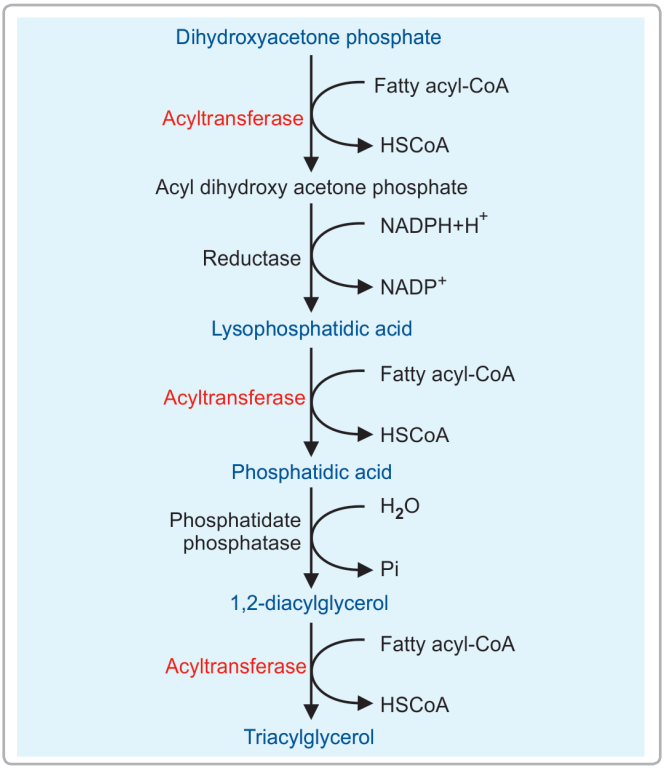


Figure 9.11- Triacyl glycerol synthesis. 1= glycerol-3-phosphate dehydrogenase

In adipose tissue, glycerol kinase is deficient and the major source is DHAP derived from glycolysis. However, in liver glycerol kinase is active.

Obesity

The fat content of the adipose tissue can increase to unlimited amounts, depending on the amount of **excess calories** taken in. This leads to obesity. High levels of plasma insulin level is noticed. But the **insulin receptors are decreased**; and there is peripheral resistance against insulin action. When fat droplets are overloaded, the nucleus of adipose tissue cell is degraded, cell is destroyed, and TAG becomes extracellular. Such TAG cannot be metabolically reutilized and forms the dead bulk in obese individuals.

Role of Liver in Fat Metabolism

- Secretion of bile salts
- Synthesis of fatty acid, triacyl glycerol and phospholipids
- Oxidation of fatty acids
- Production of lipoproteins
- Production of ketone bodies
- Synthesis and excretion of cholesterol.

Liver–Adipose Tissue Axis

Liver produces fatty acid and TAG (triacylglycerol), which is transported as VLDL (very low density lipoprotein) in the blood. The fatty acids from VLDL are taken up by adipose tissue with the help of lipoprotein lipase, and stored as TAG. This neutral fat is hydrolyzed by hormone sensitive lipase (see Fig. 9.10) into NEFA, which in the blood is carried by albumin. The NEFA is utilized by the peripheral tissues, excess of which can be taken up by liver cells. Thus, there is a constant flux of fat molecules from liver to adipose tissue and back (Fig. 9.12).

FATTY LIVER AND LIPOTROPIC FACTORS

Fatty liver refers to the deposition of excess triglycerides in the liver cells. The balance between the factors causing fat deposition in liver versus factors causing removal of fat from liver determines the outcome.

Causes of Fatty Liver

- **Excessive mobilization of fat:** The capacity of liver to take up the fatty acids from blood far exceeds its capacity for excretion as VLDL. So fatty liver can occur in **diabetes mellitus and starvation** due to increased lipolysis in adipose tissue (step 1, Fig. 9.13).
- **Excess calorie intake:** Excess calories, either in the form of carbohydrates or as fats, are deposited as fat. Hence **obesity** may be accompanied by fatty liver (step no. 2 in Fig. 9.13).
- Toxic injury to liver. In **protein calorie malnutrition**, amino acids required to synthesize apoproteins may be lacking. This is due to defective apoprotein synthesis. **Hepatitis B virus** infection reduces the function of hepatic cells (step no. 3 in Fig. 9.13).
- **Alcoholism:** It is the most common cause of fatty liver and cirrhosis in India. The metabolism of alcohol is described in Chapter 7. An increase in factors (1) & (2) or a decrease in factors (3) & (4) will cause excessive accumulation, leading to fatty liver. These pathways are summarized in Figure 9.13.

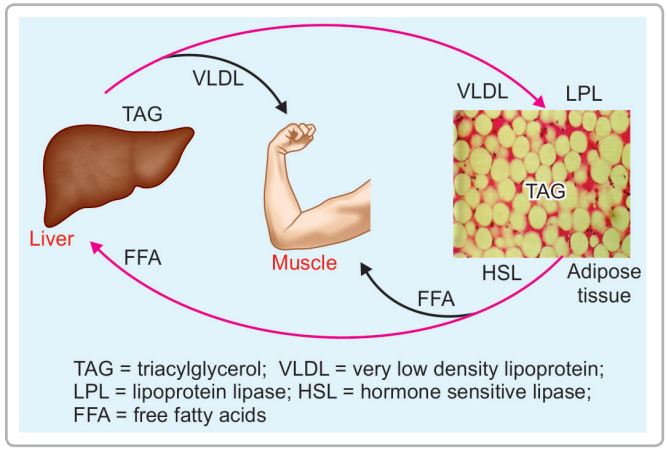


Figure 9.12: Liver adipose tissue axis

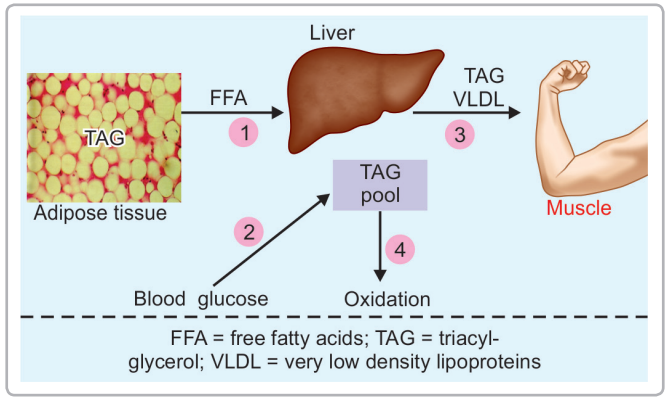


Figure 9.13: Causes of fatty liver

5. Fatty liver progresses to cirrhosis

Fat molecules infiltrate the cytoplasm of the cell (**fatty infiltration**). These are seen as fat droplets, which are merged together so that most of the cytoplasm become laden with fat. By this time, the nucleus is pushed to a side of the cell, nucleus further disintegrated **(karyorrhexis)**, and ultimately the hepatic cell is lysed. As a healing process, fibrous tissue is laid down, causing **fibrosis** of liver, otherwise known as **cirrhosis.** *Liver function tests* (Chapter 30) will show abnormal values.

Lipotropic Factors

They are **required for the normal mobilization** of fat from liver. Therefore deficiency of these factors can result in fatty liver. They can afford protection against the development of fatty liver.

- **Choline**: Feeding of choline has been able to reverse fatty changes in animals.
- **Lecithin and Methionine.** They help in synthesis of apoprotein and choline formation. The deficiency of methyl groups for carnitine synthesis may also hinder fatty acid oxidation.
- **Vitamin E and selenium** give protection due to their anti-oxidant effect.
- **Omega 3 fatty acids** present in marine oils have a protective effect against fatty liver.

METABOLISM OF KETONE BODIES

Carbohydrates are essential for the metabolism of fat or **fat is burned under the fire of carbohydrates**. The acetyl-CoA formed from fatty acids can enter and get oxidized in TCA cycle only when carbohydrates are available.

During **starvation and diabetes mellitus**, the acetyl-CoA takes the alternate fate of formation of ketone bodies.

A. Ketogenesis

Acetoacetate is the **primary ketone body** while beta-hydroxybutyrate and acetone are **secondary ketone** bodies. They are synthesized exclusively by the **liver mitochondria**. The steps involved are shown in Fig. 9.14.

Step 1. Two molecules of acetyl-CoA are condensed to form acetoacetyl-CoA.

Step 2. One more acetyl-CoA is added to form HMG-CoA (betahydroxy beta methylglutaryl-CoA). The enzyme is HMG-CoA synthase. **Mitochondrial HMG-CoA is used for ketogenesis**, while cytosolic fraction is used for cholesterol synthesis.

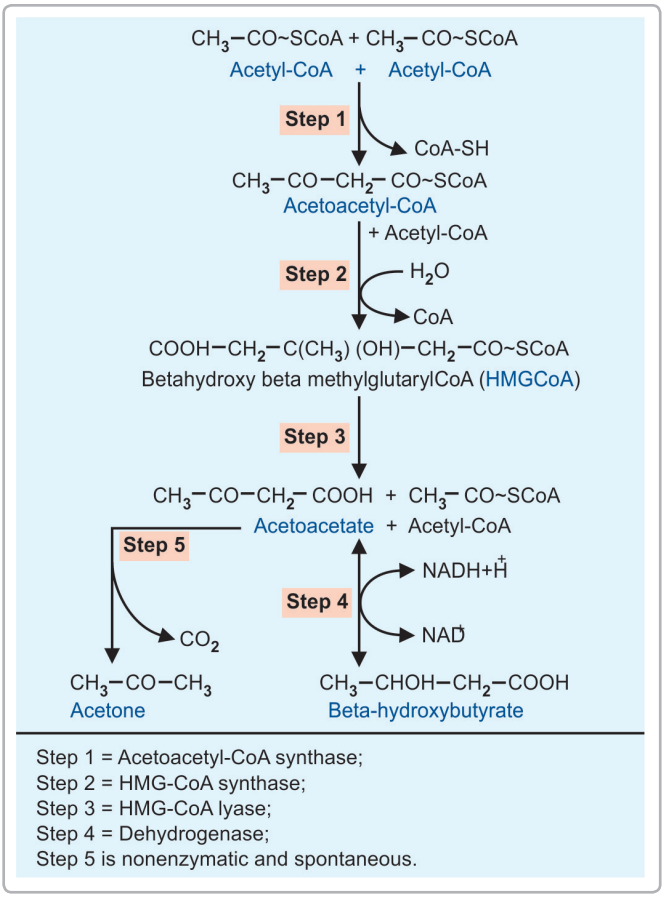


Figure 9.14: Ketone body formation (Ketogenesis)

Step 3. Lysis: Then HMG-CoA is lysed to form acetoacetate.

Step 4. Reduction: Beta-hydroxy butyrate is formed by reduction of acetoacetate.

Step 5. Spontaneous decarboxylation: Acetone is formed by spontaneous (non-enzymatic) decarboxylation (Fig. 9.14).

B. Ketolysis

The ketone bodies are formed in the liver; but they are utilized by **extrahepatic tissues**. The heart muscle and renal cortex prefer the ketone bodies to glucose as fuel. Tissues like skeletal muscle and brain can also utilize the ketone bodies as alternate sources of energy, if glucose is not available. Acetoacetate is activated to acetoacetyl CoA by **thiophorase** enzyme.

$$\text{Acetoacetate} + \text{Succinyl CoA} \xrightarrow{\text{Thiophorase}} \text{Acetoacetyl CoA} + \text{Succinate}$$

Then acetoacetyl-CoA enters the beta-oxidation pathway to produce energy.

KETOSIS

- Normally, the rate of synthesis of ketone bodies by the liver is such that they can be easily metabolized by the extrahepatic tissues. Hence the blood level of ketone bodies is less than 1 mg/dL and only traces are excreted in urine (not detectable by usual tests).
- But when the rate of synthesis exceeds the ability of extrahepatic tissues to utilize them, there will be accumulation of ketone bodies in blood.
- This leads to **ketonemia**, excretion in urine (**ketonuria**) and smell of **acetone** in breath. All these three together constitute the condition known as **ketosis**.

A. Causes for ketosis

- **Diabetes Mellitus:** Untreated diabetes mellitus is the most common cause for ketosis. Even though glucose is in plenty, the **deficiency of insulin** causes accelerated lipolysis and more fatty acids are released into circulation.
- **Starvation:** In starvation, the dietary supply of glucose is decreased. The increased rate of lipolysis is to provide alternate source of fuel. The excess acetyl-CoA is converted to ketone bodies. The high **glucagon** favors ketogenesis. The brain derives 75% of energy from ketone bodies under conditions of fasting.
- **Hyperemesis in early pregnancy**

B. Regulation of Ketogenesis

During starvation and diabetes mellitus, the blood level of **glucagon** is increased. Glucagon (For details refer Chapter 31) inhibits glycolysis, activates gluconeogenesis, activates lipolysis, and stimulates ketogenesis. **Insulin** (For details refer Chapter 31) has the opposite effect; it favors glycolysis, inhibits gluconeogenesis, depresses lipolysis, and decreases ketogenesis. The ketone body formation is regulated at the following 3 levels (Fig. 9.15)

Level 1: Lipolysis

Free fatty acids are the precursors of ketone bodies. So factors regulating the mobilization of fatty acid from adipose tissue will also control ketogenesis (see Fig. 9.11). Insulin inhibits lipolysis, while glucagon favors it.

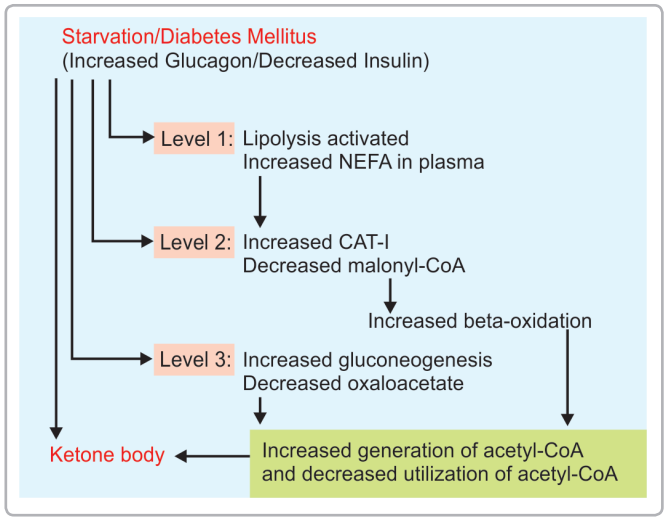


Figure 9.15- Summary of ketosis.

Level 2: Entry of Fatty Acid to Mitochondria

The mobilized fatty acid then enters mitochondria for beta-oxidation. Carnitine acyltransferase I (CAT-I) regulates this entry. Malonyl-CoA is the major regulator of CAT-I activity. In diabetes and starvation, beta oxidation is stimulated.

Level 3: Oxidation of Acetyl CoA

When the above two steps are increased, more acetyl CoA is produced. Normally, acetyl-CoA is completely oxidized in the citric acid cycle. In diabetes and starvation, glucagon insulin ratio is increased, and key gluconeogenic enzymes are activated. Thus **oxaloacetate is diverted for gluconeogenesis**; then citric acid cycle cannot function optimally. Thus, on the one hand, acetyl-CoA is generated in excess, on the other hand, its utilization is reduced. This excess acetyl-CoA is channelled into ketogenic pathway. In both diabetes mellitus and starvation, the oxaloacetate is channelled to gluconeogenesis; so the availability of oxaloacetate is decreased. Hence acetyl-CoA cannot be fully oxidized in the TCA cycle.

C. Diabetes, Starvation, Ketosis, Cholesterol

Acetyl-CoA is the starting point for both ketone bodies and cholesterol. So, in both diabetes mellitus and starvation, ketosis is noticed. (Because in both cases, Insulin is deficient, and acetyl-CoA pool is enlarged). On the other hand, in diabetes, cholesterol synthesis is enhanced; but in starvation, there is no increase in cholesterol. Why?

In starvation, glucagon is increased, which inhibits HMG-CoA reductase, the key enzyme for cholesterol synthesis. So, in starvation, acetyl-CoA pool is increased, which goes for ketone body formation , but not to cholesterol synthesis.

D. Consequences of Ketosis

- **Metabolic acidosis.** Acetoacetate and beta-hydroxy butyrate are acids. When they accumulate, metabolic acidosis results (For details refer Chapter 20).
- **Buffers.** The plasma bicarbonate is used up for buffering of these acids.
- **Kussmaul's respiration.** Patients will have typical acidotic breathing due to compensatory hyperventilation.
- **Smell of acetone** in patient's breath.
- **Coma.** Hypokalemia, dehydration and acidosis are contributing for the lethal effect of ketosis.

E. Diagnosis of Ketosis

The presence of ketosis can be established by the detection of ketone bodies in urine by **Rothera's test.** Supportive evidence may be derived from estimation of serum electrolytes, acid–base parameters, glucose and urea estimation.

Rothera's Test: Saturate 5 mL of urine with solid ammonium sulfate. Add a few drops of freshly prepared sodium nitroprusside followed by 2 mL of liquor ammonia along the sides of the test tube. Development of a purple ring indicates the presence of ketone bodies in urine. Strip tests based on the same principle are also available.

F. Differential Diagnosis

The urine of a patient with **diabetic** ketoacidosis will give positive Benedict's test as well as Rothera's test. But in **starvation** ketosis, Benedict's test is negative, but Rothera's test will be positive.

G. Management of Ketoacidosis

- Treatment is to give insulin and glucose. When glucose and insulin are given intravenously, potassium is trapped within the cells. Hence, the clinician should always monitor the electrolytes.
- Administration of bicarbonate, and maintenance of electrolyte and fluid balance are very important aspects.

A QUICK LOOK

1. Digestion of lipids involves the following enzymes, Lingual lipase, gastric lipase and pancreatic lipase. Pancreatic lipase is the major digestive enzyme and requires bile salts.
2. Lipids are absorbed by emulsification and micelle formation with the help of bile salts.
3. Short and medium chain fatty acids are absorbed directly without re-esterification.
4. Defective absorption of lipids occurs in celiac disease, Crohn's disease.
5. Mammalian tissues oxidize fatty acids primarily by the beta-oxidation pathway which occurs in the mitochondria.
6. Transport of fatty acids (long chain acyl-CoA) through the inner mitochondrial membrane is facilitated by carnitine acyltransferase and translocase.
7. Net yield of ATP from one molecule of palmitic acid is 106 ATP.
8. Oxidation of odd chain fatty acids produces propionyl-CoA, which may be further metabolized by the TCA cycle.
9. Alpha-oxidation and omega-oxidation are two other modes of fatty acid oxidation.
10. De novo synthesis of fatty acids occurs in the cytoplasm with the help of a dimeric multienzyme complex termed fatty acid synthase.
11. Synthesis of fatty acid requires NADPH, while degradation requires NAD and FAD.
12. Insulin favors fatty acid synthesis.
13. The white adipose tissue is concerned with energy storage and the brown adipose tissue is concerned with thermogenesis.
14. Obesity is the result of an increase in the fat content of the adipose tissue. It is associated with insulin resistance.
15. Fatty liver refers to deposition of excess triglycerides in the liver cells. It is facilitated by lipotropic factors such as methionine, choline and lecithin.
16. Acetoacetate is the primary ketone body. Beta hydroxybutyric acid and acetone are secondary ketone bodies.
17. Ketosis is seen in diabetes mellitus and starvation.
18. Rothera's test is commonly used to detect presence of ketone bodies in urine.
19. Acetyl-CoA formed from fatty acids is further oxidized in TCA cycle to generate energy, when availability of oxaloacetate is sufficient.
20. Under conditions of fasting and starvation, the oxaloacetate is channelled to gluconeogenesis. Excess acetyl-CoA is then used for ketogenesis by liver.
21. A similar situation is seen in uncontrolled diabetes mellitus where gluconeogenesis and lipolysis are both enhanced.
22. The excess acetyl-CoA is converted to ketone bodies in hepatic mitochondria.
23. The HMG-CoA formed is cleaved by liver enzyme lyase to the primary ketone body acetoacetate.
24. Acetoacetate may be reduced to beta-hydroxy butyrate or spontaneously decarboxylated to acetone.
25. Ketone bodies are synthesized by liver and metabolized by extrahepatic tissues, mainly cardiac muscle and skeletal muscle.
26. Under conditions of starvation, brain starts metabolizing ketone bodies for energy needs.
27. Since ketone bodies are acids, metabolic acidosis occurs. Excessive accumulation of ketone bodies can be dangerous since it can result in acidosis, dehydration and coma.

CHAPTER 10

Lipids–III: Cholesterol, Lipoproteins and Cardiovascular Diseases

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Structure of cholesterol
- Biosynthesis of cholesterol
- Plasma lipids
- Chylomicrons
- Very low density lipoproteins
- Low density lipoproteins
- High density lipoproteins
- Atherosclerosis and coronary artery disease
- Risk factors for coronary artery disease
- Preventions of atherosclerosis

The word cholesterol is derived from Greek words, chole = bile; steros = solid; ol = alcohol. Cholesterol is widely distributed in the body. Cholesterol is a light yellow crystalline solid. Cholesterol is soluble in chloroform and other fat solvents. Cholesterol is widely distributed in animal tissues

SIGNIFICANCE AND FUNCTIONS OF CHOLESTEROL

- **Heart diseases:** The level of cholesterol in blood is related to the development of **atherosclerosis**. Abnormality of cholesterol metabolism may lead to cardiovascular accidents and heart attacks.
- **Cell membranes:** Cholesterol is a component of membranes and has a modulating effect on the fluid state of the membrane.
- **Nerve conduction:** Cholesterol is a poor conductor of electricity, and is used to insulate nerve fibers.
- **Bile acids and bile salts:** The 24 carbon bile acids are derived from cholesterol. Bile salts are important for fat absorption.
- **Steroid hormones:** Glucocorticaids (21 carbon), androgens (19 carbon) and estrogens (18 carbon) are synthesized from cholesterol.
- **Vitamin D:** It is synthesized from cholesterol.

Structure of Cholesterol

Cholesterol has cyclopentano perhydro phenanthrene ring system. It has A, B, C and D rings. (Fig. 10.1); It has 27 carbon atoms; One hydroxyl group on 3rd carbon atom; Double bond between 5 and 6 carbon atoms and eight carbon side chain.

BIOSYNTHESIS OF CHOLESTEROL

The major sites of synthesis of cholesterol are liver, adrenal cortex, testis, ovaries and intestine. Liver is responsible for 80% of the endogenous cholesterol synthesis. All nucleated

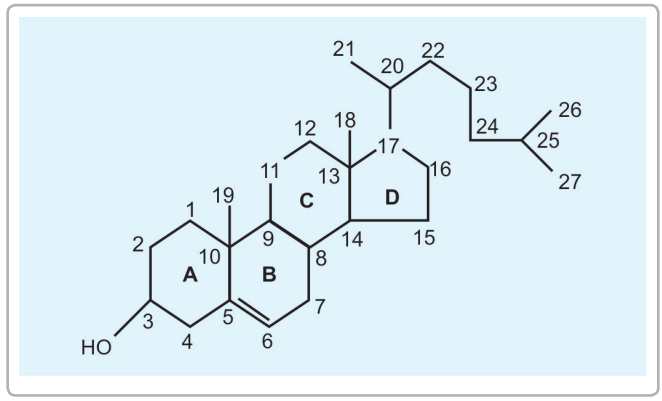


Figure 10.1: Structure of cholesterol

cells can synthesize cholesterol. The biosynthetic pathway was described by Sir John Cornforth and Vladimir Prelog; both of them got Nobel prizes in 1975.

- **Condensation:** Two molecules of acetyl-CoA condense to form acetoacetyl-CoA catalysed by mitochondrial **acetoacetyl-CoA synthase** (Fig. 9.14).
- **Production of HMG-CoA:** A third molecule of acetyl CoA condenses with acetoacetyl-CoA to form beta-hydroxy beta-methyl-glutaryl-CoA (HMG-CoA). The enzyme is **HMG-CoA synthase** (Fig. 9.14). HMG-CoA is present in both cytosol as well as mitochondria of liver. The mitochondrial pool is used for ketogenesis whereas the **cytosolic fraction** is utilized for cholesterol synthesis.
- **Committed Step:** The reduction of HMG-CoA to **mevalonate** is catalyzed by **HMG-CoA reductase**. It is a microsomal (endoplasmic reticulum) enzyme. It uses 2 molecules of NADPH. (Step 3 in Fig. 10.2). Steps 1 and 2 are shared with ketogenic pathway; but step 3 is the first reaction that is unique to the cholesterol biosynthetic pathway. It is the **rate-limiting step.**
- **Step 4: Five carbon unit:** Mevalonate is phosphorylated and then undergoes decarboxylation to give **isopentenyl pyro phosphate**, a 5 carbon unit (Fig. 10.2). Steps 1, 2, 3 and 4 together may be considered as the first phase of the cholesterol synthesis.
- **Step 5:** Six numbers of 5-carbon units are condensed to form **squalene**. In summary
  5C+5C → 10C; 10C+5C → 15C; 15C+15C → 30C
- **Step 6:** A cyclase converts squalene to **lanosterol**. Lanosterol is the first steroid compound synthesized. It is a 30 carbon sterol.
- **Step 7: Cutting to size:** Three extra methyl groups are removed to produce 27 carbon sterol, zymosterol**.** Then the double bond is made 5–6 position, when desmosterol is formed. Finally, the double bond in the side chain (between carbon 24–25) is removed when **cholesterol** is formed (Fig. 10.1). A summary of the whole pathway of cholesterol synthesis is given in Fig. 10.2.

Regulation of Cholesterol Synthesis

Regulation at transcription: The regulatory enzyme is **HMG-CoA reductase.** When sufficient cholesterol is present in the cell, transcription of the gene for HMG-CoA reductase is suppressed, and cellular synthesis of cholesterol is decreased. When cholesterol in diet is low, synthesis is increased (Fig. 10.3).

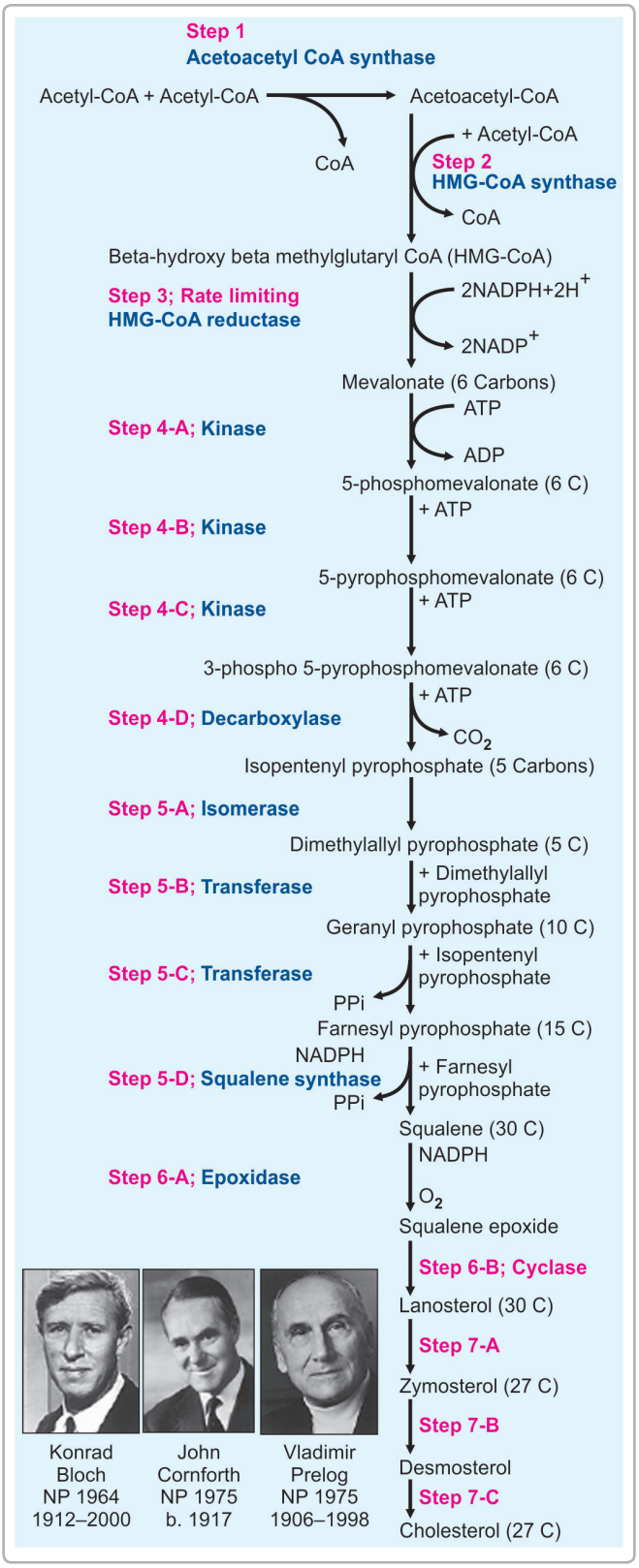


Figure 10.2: Cholesterol biosynthesis

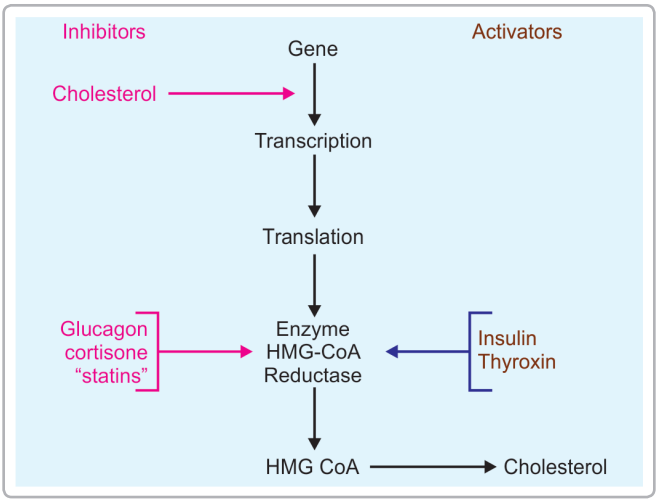


Figure 10.3: Regulation of cholesterol synthesis

Table 10.1: Plasma Lipid Profile (Normal values)

Analyte	*Normal value*
Total plasma lipids	400–600 mg/dL
Total cholesterol	150–200 mg/dL
HDL cholesterol, male	30–60 mg/dL
HDL cholesterol, female	35–75 mg/dL
LDL cholesterol, 30–39 years	80–130 mg/dL
Triglycerides, male	50–150 mg/dL
Triglycerides, female	40–150 mg/dL
Phospholipids	150–200 mg/dL
Free fatty acids (FFA) (NEFA)	10–20 mg/dL

Covalent modification: Short-term regulation is by covalent modification of the enzyme. Cyclic AMP mediated cascade phosphorylates the enzyme which is inactive. Dephosphorylated form is active.

Insulin and thyroxine increase the activity of HMG-CoA reductase (Fig. 10.3).

Cortisol and glucagon decrease its activity (Fig. 10.3).

Drugs: Lovastatin and other "statin" group of drugs are competitive inhibitors of HMG-CoA reductase. So, they are used in clinical practice to reduce cholesterol level in blood.

Excretion of Cholesterol

Average diet contains about 300 mg of cholesterol per day. Body synthesize about 700 mg of cholesterol per day. Out of this total 1000 mg, about 500 mg of cholesterol is excreted through bile. This cholesterol is partly reabsorbed from intestines. Vegetables contain plant sterols which inhibit the re-absorption of cholesterol. The unabsorbed portion is acted upon by intestinal bacteria to form cholestanol and coprostanol. These are excreted (fecal sterols). Another 500 mg of cholesterol is converted to bile acids, which are excreted as bile salts.

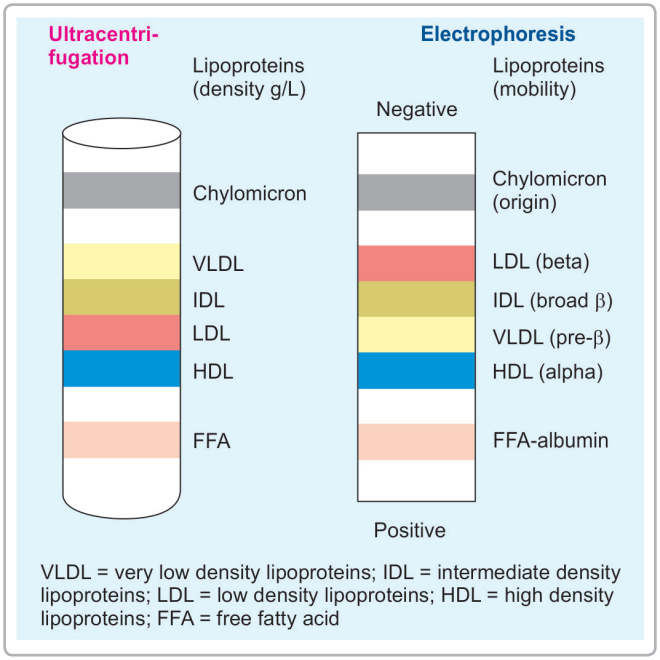


Figure 10.4: Comparison of electrophoretic and ultracentrifuge patterns of lipoproteins

PLASMA LIPIDS

Total plasma lipid is 400–600 mg/dL. Normal values of lipid fractions are shown in Table 10.1. Roughly speaking, one-third is cholesterol; one-third is triglyceride and one-third is phospholipid.

Carriage of Cholesterol in Blood

Cholesterol, being a lipid, is insoluble in water, but blood is a watery medium. So, transport of cholesterol in blood needs special transporters or carriers. Therefore, cholesterol is complexed with proteins to form **lipoproteins.** The protein part of lipoprotein is called **apolipoprotein**. The lipoproteins are usually abbreviated as Lp.

Classification of Lipoproteins

Depending on the density (by ultracentrifuge) or on the electrophoretic mobility, the lipoproteins in plasma are classified into the following varieties (Figs 10.4 and 10.5).

Chylomicrons

- **Very low density lipoproteins** (VLDL) or pre-beta lipoproteins
- **Intermediate density lipoproteins** (IDL) or broad-beta lipoproteins
- **Low density lipoproteins** (LDL) or beta-lipoproteins
- **High density lipoproteins** (HDL) or alpha-lipoproteins.
- **Free fatty acids** (FFA) or non-esterified fatty acids (NEFA) are complexed with albumin. FFA is not generally considered as lipoprotein, because it is loosely bound to the protein.

General Characteristics of Lipoproteins

Their salient characteristics and compositions of lipoproteins are given in Table 10.2. The lipoprotein molecules have a polar periphery made of proteins, polar heads of phospholipids and cholesterol. The inner core consists of the hydrophobic TAGs and tails of phospholipids. The apoproteins also increase the solubility of lipids. Lipoproteins are separated by ultracentrifugation or by electrophoresis (Fig. 10.4).

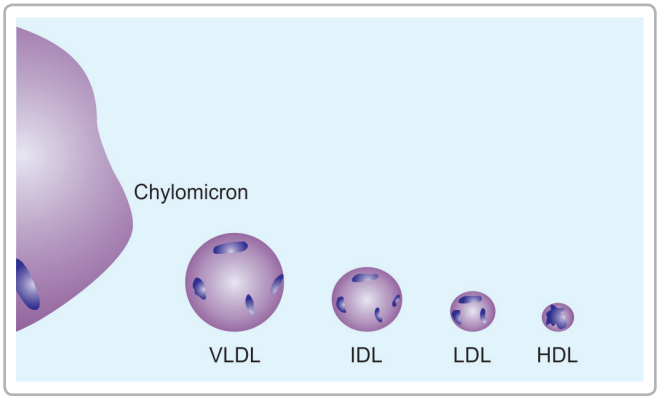


Figure 10.5: Comparison of sizes of lipoproteins

Apolipoproteins

The protein part of lipoprotein is called apolipoprotein (apo-Lp) or apoprotein. All apoproteins are mainly synthesized in liver. Intestinal cells produce small quantities of apo-A. Apart from **solubilizing** the lipid part, the protein components have specific functions.

Apo-A-I. It activates lecithin-cholesterol acyltransferase (LCAT). It is the ligand for HDL receptor. It is antiatherogenic.

Apo-B-100. It is a component of LDL; it attaches with LDL receptor.

Apo-B-48. It is the component of chylomicrons.

1. CHYLOMICRONS

Synthesis: Chylomicrons are formed in the **intestinal mucosal** cells, and secreted into the lacteals of lymphatic system (For details refer Chapter 9). They are **rich in triglyceride** (Fig. 10.6). If lipemic serum is kept overnight in the refrigerator, chylomicrons rise as a creamy layer to the top, leaving the subnatant clear. When the chylomicrons are synthesised by the intestinal mucosa, they contain **apo-B-48** (Fig. 10.6).

Metabolism of Chylomicrons: Main sites of metabolism of chylomicrons are adipose tissue and skeletal muscle (Fig. 10.7). The half-life of chylomicrons in blood is about 1 hour. The enzyme **lipoprotein lipase**

Table 10.2: Characteristics of different classes of lipoproteins

	Chylomicron	*VLDL*	*LDL*	*HDL*	*FFA (*)*
Density g/L	<0.95	0.95–1.006	1.019–1.063	1.063–1.121	1.28–1.3
Diameter (nm)	500	70	25	15	---
Electrophoretic mobility	origin	pre-beta	beta	alpha	albumin
Protein %	2	10	22	50	99
TAG%	80	50	10	10	0
Cholesterol %	10	20	45	20	0
FFA %	0	0	0	0	1
Apoproteins	A,B-48,C-II,E	B-100, C-II,E	B-100	A-I, C, E	Albumin
Transport function	TAG from gut to muscle and adipose	TAG from liver to muscle	Cholesterol from liver to heart	Cholesterol from heart to liver	FFA from adipose tissue to muscle and liver

(*) Free fatty acids are not generally included in the lipoproteins. They are seen in circulation, weakly bound to albumin.

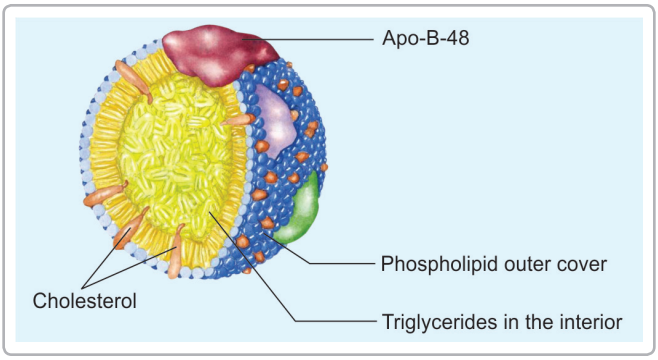


Figure 10.6: Structure of chylomicrons

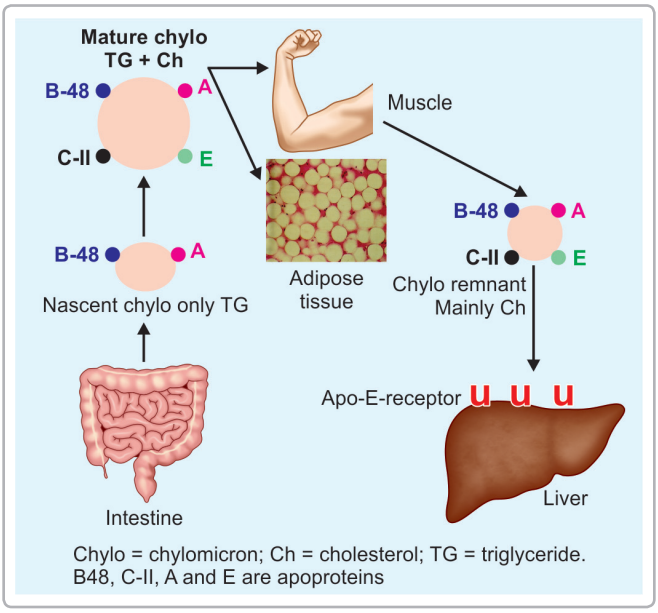


Figure 10.7: Metabolism of chylomicrons

(LpL) is located at the endothelial layer of capillaries of adipose tissue, muscles and heart; but not in liver. Muscle or adipose tissue cells take up the liberated fatty acids.

Insulin increases LpL activity.

Liver Takes Chylomicron Remnants: As the TAG content is progressively decreased, the chylomicrons shrink in size. These remnants containing apo B-48 and apo E are taken up by hepatic cells by receptor mediated endocytosis. Apo E binds the hepatic receptors (Fig. 10.7).

Function of Chylomicrons: They are the transport form of dietary triglycerides **from intestines to the adipose tissue** for storage; and to muscle or heart for their energy needs.

2. Very low density lipoproteins

Synthesis of VLDL: They are synthesized in the liver from glycerol and fatty acids and incorporated into VLDL. **Apo-B-100** is the major lipoprotein present in VLDL.

Metabolism of VLDL: When they reach the peripheral tissues, apo C-II activates LpL which liberates fatty acids that are taken up by adipose tissue and muscle. The remnant is now designated as IDL (intermediate density lipoprotein) and contains less of TAG and more of cholesterol (Table 10.2). The IDL further loses triglyceride, so as to be converted to LDL (low density lipoprotein). This conversion of VLDL to IDL and then to LDL is referred to as **lipoprotein cascade pathway** (Fig. 10.8)

Function: VLDL carries triglycerides **(endogenous triglycerides)** from liver to peripheral tissues for energy needs.

3. Low Density Lipoproteins (LDL)

The LDL molecules are cholesterol rich lipoprotein molecules containing only **apo B-100**. Most of the LDL particles are derived from VLDL.

Metabolism of LDL and LDL receptors

LDL receptors are present on all cells but most abundant in hepatic cells. LDL receptors are located in specialized regions called **clathrin-coated pits** (Fig. 10.9).

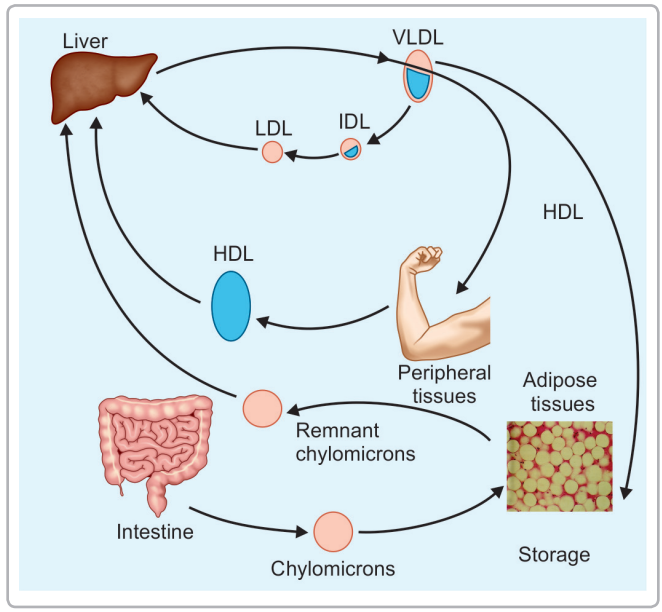


Figure 10.8: Summary of lipoprotein metabolism

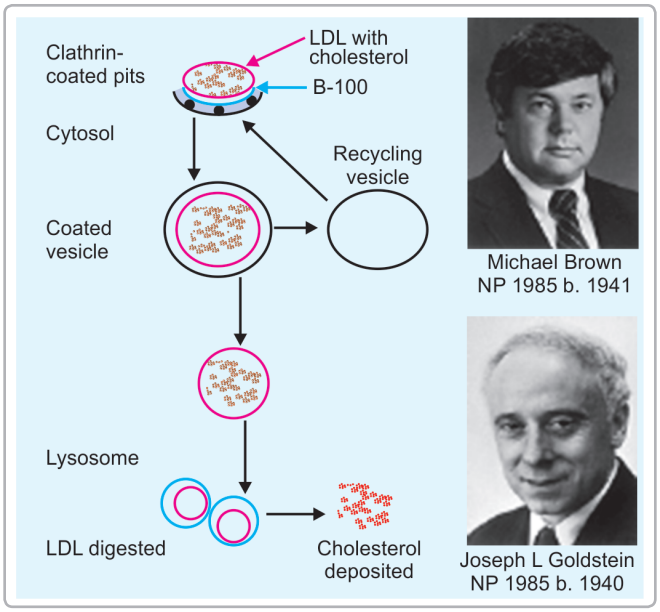


Figure 10.9: Uptake and fate of LDL

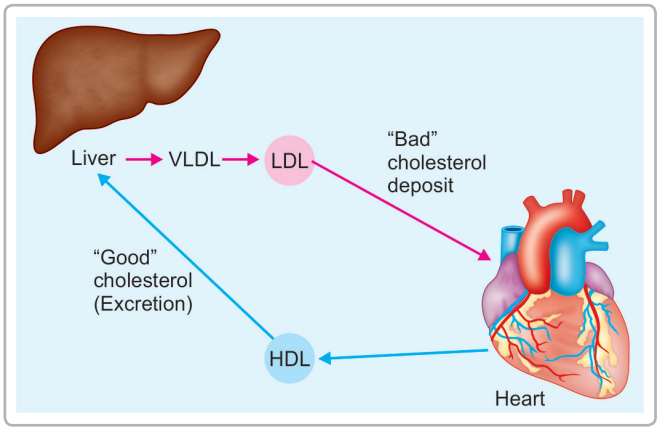


Figure 10.10: Forward and reverse transport of cholesterol

When the apo B-100 binds to the receptor, the receptor-LDL complex is internalized by endocytosis. These vesicles would fuse with lysosomes. The lysosomal enzymes now degrade the apoproteins of the LDL and also hydrolyze the cholesterol esters to free cholesterol. The free receptors can now return to the membrane site to bind further LDL molecules (Fig. 10.9). For their work on LDL receptors, Michael Brown and Joseph Goldstein were awarded Nobel prize in 1985.

Function of LDL

LDL transports cholesterol **from liver to the peripheral tissues**. LDL concentration in blood has positive correlation with incidence of **cardiovascular diseases**. About 75% of the plasma cholesterol is incorporated into the LDL particles.

BOX 10.1: Lp(a) and apo-A are different

Apo-A is a constituent of HDL. This "A" is always written in capital letters. It is seen in all persons. It is antiatherogenic.
Lp(a) is seen only in some persons. When present, it is associated with LDL. This "a" is always written in small letters. It is highly atherogenic and connected with heart attacks in younger age group.

LDL and Clinical Applications

LDL infiltrates through arterial walls, and are taken up by macrophages or scavenger cells. This is the starting event of **atherosclerosis** leading to myocardial infarction (see coronary artery diseases section in this chapter). Since LDL-cholesterol is thus deposited in tissues, the LDL (low density lipoprotein) variety is called "**bad cholesterol**" in common parlance (Fig. 10.10).

Lipoprotein (A)

Lipoprotein (a) or **Lp(a)** should not be confused with apo A (Box 10.1). Lp(a) is very strongly associated with **myocardial infarction**.

4. High Density Lipoprotein (HDL)

Metabolism of HDL

The intestinal cells synthesize components of HDL and release into blood (Fig. 10.11). The free cholesterol derived from peripheral tissue cells are taken up by the HDL. The **apo A-I** of HDL activates **LCAT** (lecithin cholesterol acyl transferase) present in the plasma. The LCAT then binds to the HDL disk. Polyunsaturated fatty acids bind to HDL to form esterified cholesterol. Mature HDL spheres are taken up by liver cells by **apo A-I** mediated receptor mechanism

Function of HDL

HDL is the main transport form of cholesterol from **peripheral tissue to liver**, which is later excreted through bile (Fig. 10.12). This is called **reverse cholesterol transport** by HDL. The only excretory route of cholesterol from the body is the bile. Excretion of cholesterol needs prior esterification with PUFA. Thus, *PUFA will help in lowering of cholesterol in the body,* and so **PUFA is anti-atherogenic**.

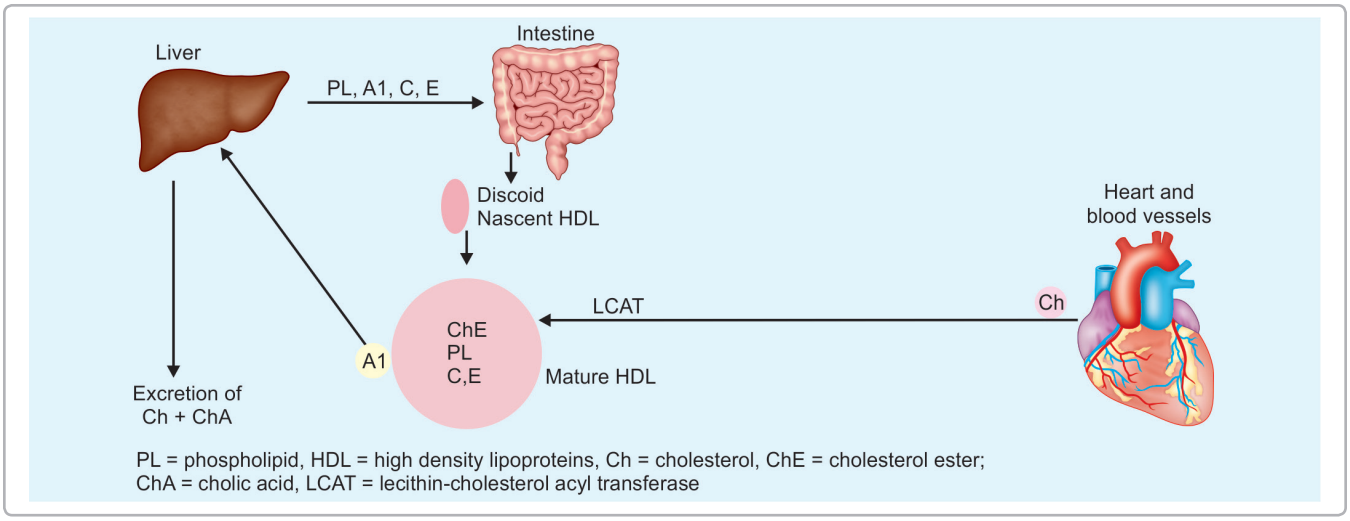


Figure 10.11: HDL metabolism

Clinical Significance of HDL

The level of HDL in serum is inversely related to the incidence of myocardial infarction. As it is "**antiatherogenic**" or "protective" in nature, HDL is known as "**good cholesterol**" in common parlance (Fig. 10.10). HDL level below 35 mg/dL increases the risk, while level above 60 mg/dL completely protects the person from coronary artery diseases.

An overview of the lipoprotein metabolism is shown in Fig. 10.12.

5. Free Fatty Acid (FFA)

It is also known as non-esterified fatty acids (**NEFA**). (Table 10.2). It is complexed with **albumin** in plasma. The FFA is derived from lipolysis of triglyceride stored in adipose tissue by **hormone sensitive lipase** (For details refer Chapter 9). Free fatty acids contain long chain saturated or unsaturated fatty acids. The free fatty acids are either oxidized to supply energy or incorporated into tissue lipids by esterification. The half-life of free fatty acids in plasma is very short.

HYPERLIPIDEMIAS

The most widely accepted **Frederickson's** classification is shown in Table 10.3. The elevation of lipids in plasma leads to the deposition of cholesterol on the arterial walls, leading to **atherosclerosis.** (See under coronary artery diseases). The coronary and cerebral vessels are more commonly affected. Thromboembolic episodes in these vessels lead to **ischemic heart disease** and cerebrovascular accidents.

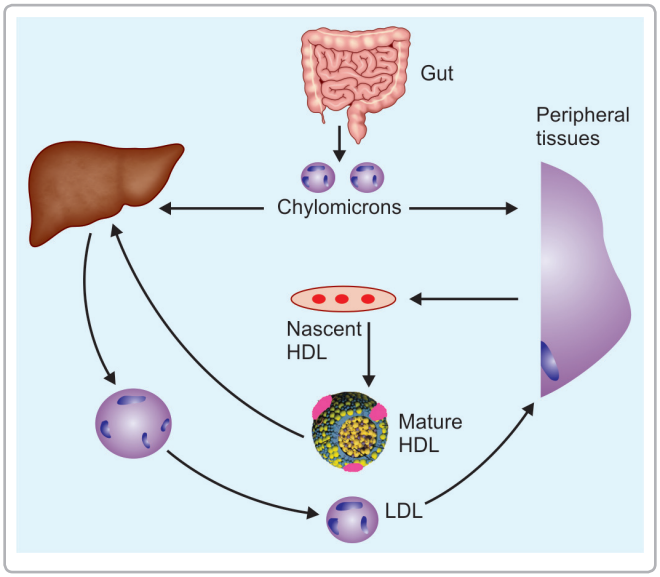


Figure 10.12: Overview of lipoprotein metabolism

The deposition of lipids in subcutaneous tissue leads to **xanthomas**. **Xanthelasma** are lipid deposits under the periorbital skin and contain cholesterol. Deposits of lipids in cornea lead to **corneal arcus**; indicating hypercholesterolemia.

Type IIA (Primary Familial Hypercholesterolemia)

Out of these diseases, the most common condition is Type IIA. The cause is **LDL receptor** defect. There is elevation of LDL. Patients seldom survive the second decade of life due to ischemic heart disease. Other types are shown in Table 10.3. **Secondary hyperlipidemias** are shown in Table 10.4.

Table 10.3: Frederickson's classification of hyperlipoproteinemias (N = Normal; ↑ = Increased)

Type	*Lipoprotein fraction elevated*	*Cholesterol level*	*TAG level*	*Metabolic defect*	*Features*	*Management*
Type I	Chylomicrons deficiency	↑	↑↑	Lipoprotein lipase	Eruptive xanthoma; hepatomegaly; pain abdomen	Restriction of fat intake; MCT supplementation
Type II A	LDL	↑↑	N	LDL receptor defect; Apo B ↑	Atherosclerosis, coronary artery disease	Low cholesterol diet. Give PUFA
Type II B	LDL and VLDL	↑↑	↑	Apo B ↑ Apo CII ↑	Corneal arcus	Do
Type III	Broad beta-VLDL and chylomicrons	↑↑	↑	apo-E; Apo CII ↑	Xanthoma	Reduction of weight, Give PUFA,
Type IV	VLDL	↑	↑↑	Apo CII ↑	Diabetes mellitus, heart disease	Reduction of body weight
Type V	VLDL, chylomicrons	N	↑↑	Secondary to other causes	Ischemic heart diseases	High PUFA intake

Table 10.4: Secondary hyperlipidemias

	Serum cholesterol	*Serum triglyceride*
Diabetes increased	Increased	Increased
Nephrotic syndrome	Increased	Increased
Hypothyroidism	Increased	Increased
Biliary obstruction	Increased	Normal
Pregnancy	Normal	Increased
Alcoholism	Normal	Increased
Oral contraceptives	Normal	Increased

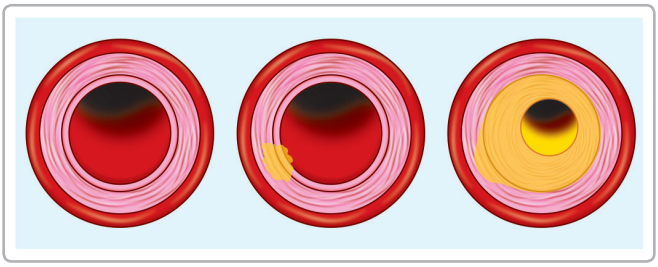


Figure 10.13: Atherosclerosis. Left, cut section of normal artery; middle, early plaque formation; right, advanced plaque formation

ATHEROSCLEROSIS

Coronary artery obstruction and myocardial infarction are the number one killers in the world. In India, 20% deaths are due to coronary artery disease (CAD). It is estimated that by the year 2020, it will account for 33% of all deaths.

Atherosclerosis and LDL

LDL-cholesterol, especially **oxidized** LDL particles are deposited in the subintimal regions of arteries. Aorta, coronary arteries and cerebral vessels are predominantly affected by this process. Plasma LDL is mainly catabolized via apo-B-LDL receptor pathway. But a small part of LDL particles are degraded by non-specific uptake of macrophages. Free-radical-induced **oxidative damage** of LDL will accelerate this process. Later, the macrophages become overloaded with cholesterol, and these are then called **"foam cells"**. These form the hallmark of atherosclerotic plaques.

Progression of atherosclerosis: During early stages of atherosclerosis, the condition is reversible if plasma lipid levels, especially LDL-cholesterol levels are lowered. But when lipid is accumulated, the lesion progresses unchecked and the arterial changes become irreversible. The formation of an atherosclerotic **plaque** leads to narrowing of vessel wall when proliferative changes occur (Fig. 10.13). This fibrous proliferation is due to liberation of various **growth factors** by macrophages and platelets.

Coronary artery disease (CAD): The blood flow through the narrow lumen is more turbulent and there is tendency for clot formation. Finally, a clot is formed which occludes one of the major vessels. **Thrombosis** (coronary, cerebral or peripheral vascular) leads to ischemia of the tissue supplied, due to hindrance to oxygen supply. Finally **infarction** (death of tissue) occurs.

SERUM CHOLESTEROL IS INCREASED IN

- **Coronary artery disease and atherosclerosis**
- **Familial hyperlipoproteinemias:** See Frederickson's classification in Table 10.3.

- **Diabetes mellitus:** Acetyl-CoA pool is increased and more molecules are channelled to cholesterol.
- **Obstructive jaundice:** The excretion of cholesterol through bile is blocked.
- **Hypothyroidism:** The receptors for HDL on liver cells are decreased, and so excretion is not effective.
- **Nephrotic syndrome:** Albumin is lost through urine, globulins are increased as a compensatory mechanism. So, apolipoproteins are increased, and then cholesterol is correspondingly increased.

RISK FACTORS FOR ATHEROSCLEROSIS

1. Serum cholesterol level

In normal persons, cholesterol level varies from 150 to 200 mg/dL. It should be preferably **below 180 mg/dL.** Values around 220 mg/dL will have moderate risk and values above 240 mg/dL will need active treatment. Females have a lower level of cholesterol which affords protection against atherosclerosis.

2. LDL-cholesterol level

Blood levels **under 130 mg/dL** are desirable. Levels between 130 and 159 are borderline; while above 160 mg/dL carry definite risk. Hence LDL is **"bad"** cholesterol.

3. HDL-cholesterol level

HDL level **above 60 mg/dL** protects against heart disease. Hence HDL is **"good"** cholesterol. A level below 40 mg/dL increases the risk of CAD. For every 1 mg/dL drop in HDL, the risk of heart disease rises 3%. If the ratio of total cholesterol/HDL is more than 3.5, it is dangerous. Similarly, LDL : HDL ratio more than 2.5 is also deleterious.

4. Apoprotein levels and ratios

Apo A-I is a measure of HDL-cholesterol (good) and apo B measures LDL-cholesterol (bad). Ratio of **Apo B : A-I** is the most reliable index. The ratio of 0.4 is very good; the ratio 1.4 has the highest risk of cardiovascular accidents.

5. Lp(a)

Lp(a) inhibits fibrinolysis. Levels more than 30 mg/dL increase the risk 3 times; and when increased Lp(a) is associated with increased LDL, the risk is increased 6 times.

6. Cigarette smoke

Nicotine of cigarette will cause lipolysis and thereby increase the acetyl-CoA and cholesterol synthesis. Nicotine also causes transient constriction of coronary and carotid arteries.

7. Hypertension

Systolic blood pressure more than 160 further increases the risk of CAD.

8. Diabetes mellitus

In the absence of insulin, hormone sensitive lipase is activated, more free fatty acids are formed, these are catabolized to produce acetyl-CoA. These cannot be readily utilized, and it is channelled to cholesterol synthesis.

9. Serum triglyceride

Normal level is 50–150 mg/dl. Blood level **more than 150 mg/dL** is injurious to health.

10. Obesity and Sedentary lifestyle

People with "apple type" obesity with a "Ganapathy" belly are more prone to get myocardial infarction.

PREVENTION OF ATHEROSCLEROSIS

The aim is to reduce total cholesterol below 180 mg/dl; to decrease LDL-cholesterol below 130 mg/dL and to keep HDL-cholesterol above 35 mg/dL.

1. Reduce dietary cholesterol

Cholesterol in the diet should be kept less than 200 mg per day. Eggs and meat contain high cholesterol. One egg yolk contains about 500 mg of cholesterol. One double omelet increases the blood cholesterol, 15 mg more than the original level.

2. Vegetable oils and PUFA

Vegetable oils (e.g. sunflower oil) and fish oils contain polyunsaturated fatty acids (PUFA). They are required for the esterification and final excretion of cholesterol. So, PUFA is helpful to reduce cholesterol level in blood. **Omega-3 fatty acids** from fish oils reduce LDL and decrease the risk of CAD.

3. Moderation in fat intake

The accepted standard is that about 20% of total calories may be obtained from fat, out of which about one-third from saturated, another one-third from monounsaturated and the rest one-third from poly unsaturated fatty acids. The recommended daily allowance will be about **20–25 g of oils** and about 2–3 g of PUFA per day for a normal adult.

4. Green leafy vegetables

Due to their **high fiber content,** leafy vegetables will increase the motility of bowels and reduce reabsorption of bile salts. Vegetables also contain plant sterols (**sitosterol**) which decrease the absorption of cholesterol. About 400 g/day of fruit and vegetables are desired.

5. Avoid sucrose and cigarette

Sucrose will raise plasma triglycerides. Sucrose, should be avoided.

6. Exercise

Regular moderate exercise will lower LDL (bad cholesterol) and raise HDL (good cholesterol) levels in blood. It will also reduce obesity.

7. Hypolipidemic drugs

HMG-CoA reductase inhibitors ("statins")**:** Atorvastatin and Simvostatin are popular drugs in this group. They are effective in reducing the cholesterol level and decreasing the incidence of CAD.

A QUICK LOOK

1. Cholesterol has a cyclopentanoperhydrophenanthrene ring with a total of 27 carbon atoms. Acetyl-CoA is the precursor of cholesterol. Cholesterol is a constituent of cell membranes. It is the precursor of all steroid hormones, bile acids and vitamin D3.
2. Cholesterol is synthesized mainly in liver, adrenal cortex and gonads. The rate limiting enzyme of the cholesterol biosynthetic pathway is HMG-CoA reductase.
3. Normal serum cholesterol range is 150–200 mg%.
4. Lipoproteins are of 5 major types; Chylomicrons, VLDL, IDL, LDL and HDL.
5. Chylomicrons contain the apo-B-48. Chylomicrons help in the transfer of triglycerides from the intestine to the muscle and adipose tissue.
6. VLDL helps in the transfer of triglycerides from the liver to the peripheral tissues.
7. LDL carries cholesterol from the liver to the heart, while HDL carries cholesterol from the heart to the liver. LDL contains apo-B-100, while HDL contains apo-A-I
8. LDL is 'bad' cholesterol and HDL is 'good' cholesterol.
9. Higher concentration of Lipoprotein (a) or Lp(a) increases risk of a myocardial infarction.
10. Free fatty acids in plasma are transported bound to albumin, and are taken up by peripheral tissues.
11. Increase in TAG or cholesterol or both in plasma leads to hyperlipidemias, which can lead to early atherosclerosis and coronary artery disease.

CHAPTER 11

Amino Acid Metabolism

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Digestion of proteins; absorption of amino acids
- Transamination and transdeamination
- Urea cycle, Urea cycle disorders, blood urea
- Glycine, creatine and creatinine
- Methionine, transmethylation reactions
- Cysteine, glutathione, homocystinurias
- Phenylalanine, tyrosine metabolism
- Melanin, catechol amines
- Phenylketonuria, alkaptonuria, albinism
- Tryptophan metabolism
- Nicotinic acid, serotonin, melatonin
- Histidine, histamine,
- One carbon metabolism
- Aminoacidurias

The main role of amino acids is in the **synthesis of structural and functional proteins**. The most important function of amino acids is to serve as building blocks for proteins. About 15–20% of energy needs are met by oxidation of carbon skeleton of amino acids. In addition to these amino acids provide several important biologically active molecules like monoamines or contribute carbon and nitrogen atoms for the synthesis of several important compounds.

The non-essential amino acids are either derived from the diet or synthesized in the body. The **essential amino acids are obtained from the diet**. Even if one is deficient, protein synthesis cannot take place. The body amino acid pool is always in a dynamic steady state. In an adult, the rate of synthesis of proteins balances the rate of degradation, so that **nitrogen balance** is maintained (Fig. 11.1).

DIGESTION OF PROTEINS

The dietary proteins are denatured on cooking and therefore more easily digested. All these enzymes are hydrolases in nature. Proteolytic enzymes are secreted as inactive **zymogens** which are converted to their active form in the intestinal lumen. This would prevent autodigestion of the secretory acini. The proteolytic enzymes include:

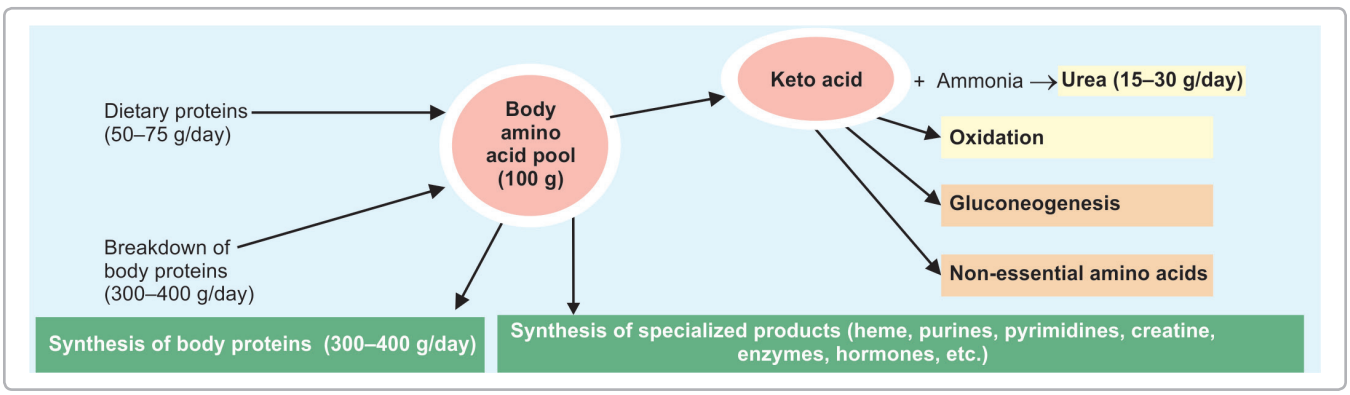


Figure 11.1: Overview of metabolism of amino acids

1. **Endopeptidases.** They act on peptide bonds inside the protein molecule, so that the protein becomes successively smaller and smaller units. This group includes pepsin, trypsin, chymotrypsin, and elastase.
2. **Exopeptidases,** which act at the peptide bond only at the end region of the chain. This group includes **Carboxypeptidase** acting on the peptide bond only at the carboxy terminal end on the chain and
3. **Amino peptidase,** which acts on the peptide bond only at the amino terminal end on the chain.

A. Gastric Digestion of Proteins

In the stomach, hydrochloric acid is secreted. It makes the pH optimum for the action of pepsin and also activates pepsin. The acid also denatures the proteins. But hydrochloric acid at body temperature could not break the peptide bonds. Thus in the stomach, HCl alone will not able to digest proteins; it needs enzymes.

1. Rennin

Rennin otherwise called **Chymosin**, is active in infants and is involved in the curdling of milk. (Box 11.1). It is absent in adults. Milk protein, casein is converted to paracasein by the action of rennin. This denatured protein is easily digested further by pepsin.

2. Pepsin

It is secreted by the chief cells of stomach as inactive **pepsinogen**. The conversion of pepsinogen to pepsin is brought about by the hydrochloric acid. The optimum pH for activity of pepsin is **around 2**. Pepsin is an endopeptidase. By the action of pepsin, proteins are broken into proteoses and peptones.

B. Pancreatic Digestion of Proteins

The optimum pH for the activity of pancreatic enzymes (pH 8) is provided by the alkaline bile and pancreatic juice. The secretion of pancreatic juice is stimulated by the peptide hormones, **Cholecystokinin** and **Pancreozymin.**

BOX 11.1: Rennin and renin are different

- Rennin is the proteolytic enzyme present in gastric juice.
- Renin is proteolytic enzyme, secreted by kidneys. It is involved in the activation of angiotensinogen to angiotensin, a hypertensive agent

Pancreatic juice contains the important endopeptidases, namely—**Trypsin, chymotrypsin, elastase** and **carboxypeptidase.**

1. Trypsin

Trypsinogen is activated by **enterokinase** present on the intestinal microvillus membranes. Once activated, the trypsin activates other enzyme molecules.

Trypsin catalyzes hydrolysis of the bonds formed by carboxyl groups of Arg and Lys.

Acute pancreatitis: Premature activation of trypsinogen inside the pancreas itself will result in the autodigestion of pancreatic cells. The result is acute pancreatitis. It is a life-threatening condition.

2. Chymotrypsin

Trypsin will act on chymotrypsinogen, so that the active site is formed. Thus selective proteolysis produces the catalytic site.

3. Carboxypeptidases

Trypsin and chymotrypsin degrade the proteins into small peptides; these are further hydrolyzed into dipeptides and tripeptides by **carboxypeptidases** present in the pancreatic juice. They are metalloenzymes requiring zinc.

C. Intestinal Digestion of Proteins

Complete digestion of the small peptides to the level of amino acids is brought about by enzymes present in intestinal juice (**succus entericus**). The luminal surface of intestinal epithelial cells contain **Aminopeptidases**, which release the N-terminal amino acids successively.

Absorption of Amino Acids

The absorption of amino acids occurs mainly in the small intestine. It is an energy requiring process. These transport systems are carrier mediated systems. There are **5 different carriers** for different amino acids. Moreover, Glutathione (gamma glutamyl cysteinyl glycine) also plays an important role in the absorption of amino acids.

Clinical Applications

1. The allergy to certain food proteins (milk, fish) is believed to result from absorption of partially digested proteins.
2. Partial gastrectomy, pancreatitis, carcinoma of pancreas and cystic fibrosis may affect the digestion of proteins and absorption of amino acids.

GENERAL METABOLISM OF AMINO ACIDS

These are summarized in Fig. 11.1.

1. Dietary proteins and body proteins are broken down to amino acids. This is called **catabolic** reactions.
2. In **transamination** reaction, amino group of amino acid is removed to produce the carbon skeleton (keto acid). The amino group is excreted as **urea**.
3. The carbon skeleton is used for synthesis of **non-essential** amino acids.
4. It is also used for **gluconeogenesis** or for complete oxidation.
5. Amino acids are used for synthesis of body proteins; this is **anabolic** reaction.

FORMATION OF AMMONIA

The sources and fate of ammonia are shown in Fig. 11.2. The first step in the catabolism of amino acids is to remove the amino group as ammonia. Ammonia is highly toxic especially to the nervous system. Detoxification of ammonia is by conversion to urea and excretion through urine.

A. Transamination

i. Transamination is the exchange of **amino group between one amino acid and another keto acid,** forming a new alpha amino acid.
amino acid 1 + keto acid 2 → amino acid 2 + keto acid 1
ii. As an example, amino group is interchanged between alanine and glutamic acid (Fig. 11.3).
iii. The amino group is accepted by **alpha ketoglutaric acid** so that glutamic acid is formed.
iv. The enzymes catalyzing the reaction as a group are known as **transaminases (aminotransferases)**. These enzymes have **pyridoxal phosphate** as prosthetic group (Fig. 11.3). The reaction is readily reversible.

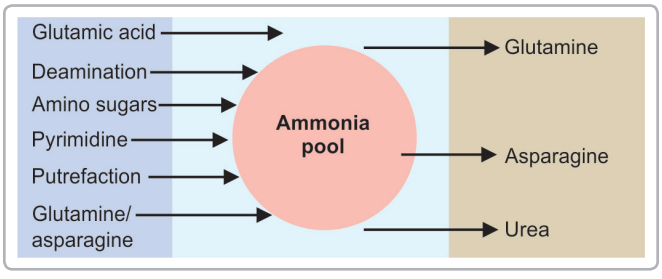


Figure 11.2: Sources and fate of ammonia

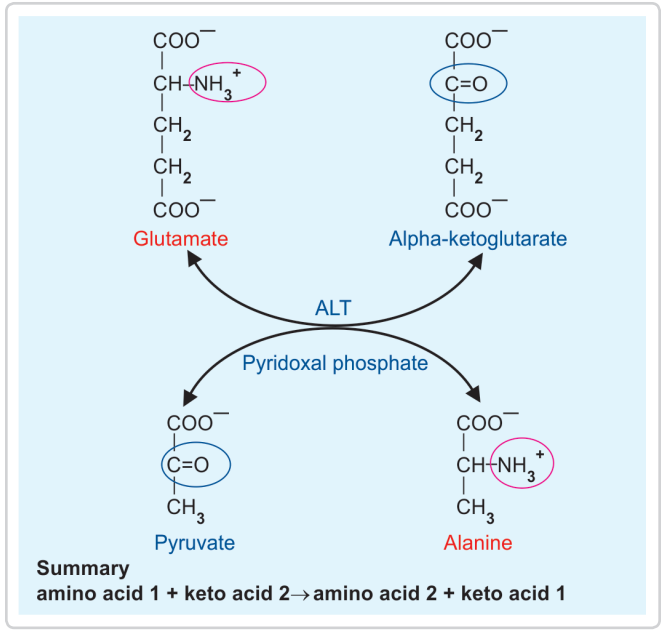


Figure 11.3: Transamination reaction. In this example, enzyme is alanine aminotransferase (ALT) and pyridoxal phosphate is the coenzyme. The reaction is readily reversible

Biological Significance of Transamination

1. First step of catabolism

In this first step, **ammonia** is removed, and then rest of the amino acid is entering into catabolic pathway.

2. Synthesis of non-essential amino acids

By means of transamination, all non-essential amino acids could be synthesized by the body from keto acids available for other sources. For example, **pyruvate** could be transaminated to synthesize **alanine**. Those amino acids, which could not be synthesized in this manner, are therefore essential; they should be available in the food (See Box 2.1 for essential amino acids).

Clinical Significance of Transamination

Aspartate aminotransferase (**AST**) is increased in **myocardial infarction** and Alanine aminotransferase (**ALT**) in **liver** diseases (For details refer Chapter 3).

B. Transdeamination

It means transamination followed by oxidative deamination. All amino acids are first transaminated to

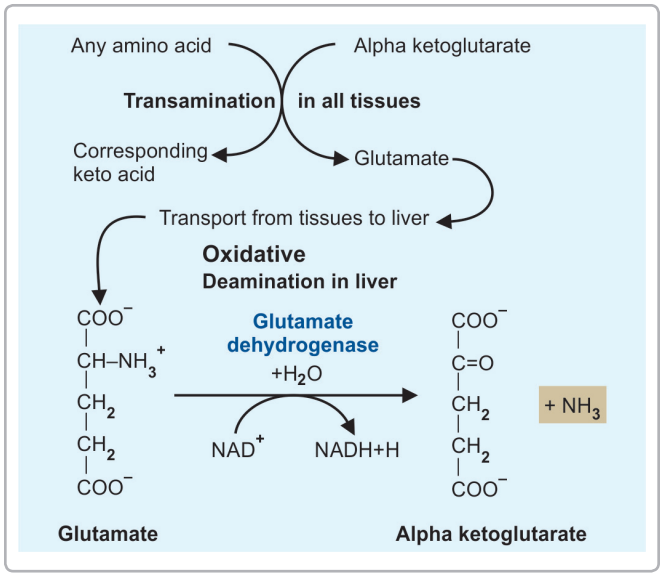


Figure 11.4: Transamination + deamination = trandeamination

glutamate, which is then finally deaminated **glutamate dehydrogenase** reaction is the final reaction which removes the amino group of all amino acids (Fig. 11.4). Thus, the two components of the reaction are physically far away, but physiologically they are coupled. Hence the term transdeamination.

DISPOSAL/DETOXIFICATION OF AMMONIA

1. First line of Defense (Trapping of ammonia)

Even very minute quantity of ammonia may produce toxicity in central nervous system. The intracellular ammonia is immediately trapped by glutamic acid to form glutamine, especially in brain cells (Fig. 11.5). The glutamine is then transported to liver, where the reaction is reversed by the enzyme glutaminase (Fig. 11.5). The ammonia thus generated is immediately detoxified into urea.

2. Final disposal

The ammonia from all over the body thus reaches liver. It is then **detoxified to urea by liver** cells, and then excreted through kidneys. **Urea is the end product of protein metabolism.**

UREA CYCLE

The cycle is known as **Krebs-Henseleit** urea cycle. As ornithine is the first member of the reaction sequences, it is also called as **Ornithine cycle**. The two nitrogen atoms

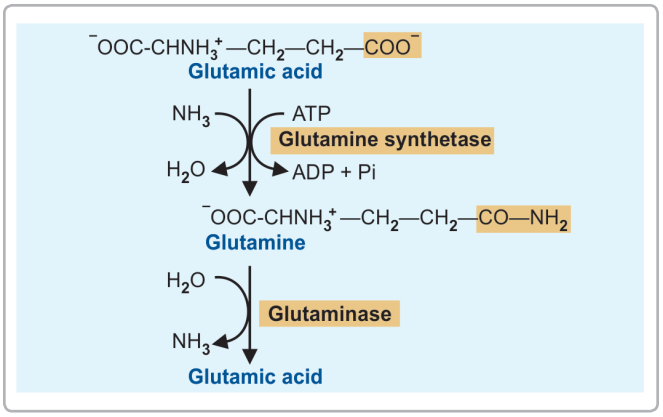


Figure 11.5: Ammonia trapping as glutamine

of urea are derived from two different sources, one from ammonia and the other directly from aspartic acid.

Step 1—Formation of Carbamoyl Phosphate

One molecule of ammonia condenses with CO_2 in the presence of **two molecules of ATP** to form carbamoyl phosphate. The reaction is catalyzed by the mitochondrial enzyme **carbamoyl phosphate synthetase-I (CPS-I).** (Fig. 11.6, Step 1). An entirely different cytoplasmic enzyme, carbamoyl phosphate synthetase-II, (CPS-II) is involved in pyrimidine nucleotide synthesis (For details refer Chapter 23). CPS-I reaction is the **rate-limiting step** in urea formation. It is allosterically regulated.

Step 2—Formation of Citrulline

The carbamoyl group is transferred to the NH2 group of ornithine (Fig. 11.6, step 2). Citrulline is neither present in tissue proteins nor in blood; but it is **present in milk.**

Step 3—Formation of Argininosuccinate

One molecule of aspartic acid is added, which provides the 2nd nitrogen atom of urea. (Fig. 11.6, step 3). This needs hydrolysis of ATP to AMP level, so **two high energy phosphate bonds** are utilized.

Step 4—Formation of Arginine

Argininosuccinate is cleaved to arginine and fumarate (Fig. 12.6, step 4). The 3rd and 4th steps taken together may be summarized as:

Citrulline + aspartate → Arginine + fumarate

Step 5—Formation of Urea

The final reaction of the cycle is the hydrolysis of arginine to urea and ornithine by arginase (Fig. 11.6, step 5).

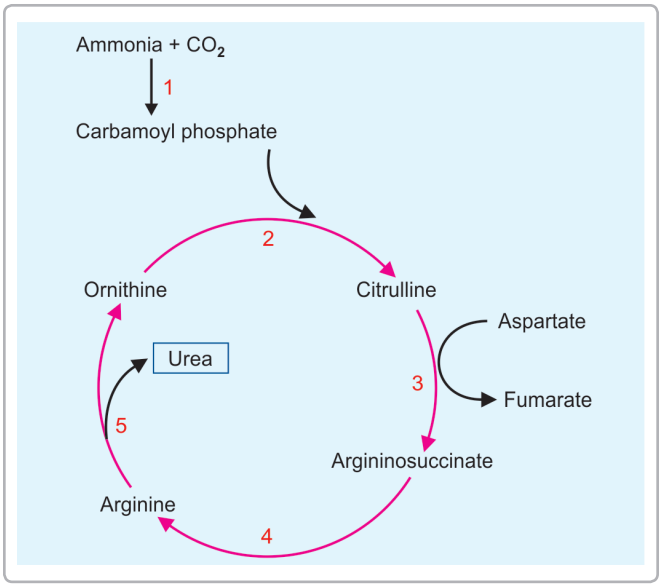


Figure 11.6: Urea cycle. 1= carbamoyl phosphate synthetase. 2= ornithine transcarbamoylase. 3= argininosuccinate synthetase. 4= argininosuccinate lyase. 5= arginase

Ornithine may be considered as a catalyst which enters the reaction and is regenerated.

Regulation of the Urea Cycle

During starvation, the activity of urea cycle enzymes is elevated to meet the increased rate of protein catabolism. The major regulatory step is catalyzed by CPS-I where the positive effector is **N-acetyl glutamate** (NAG).

Disorders of Urea Cycle

Deficiency of any of the urea cycle enzymes would result in **hyperammonemia**. When the block is in one of the earlier steps, the condition is more severe, since ammonia itself accumulates. Deficiency of later enzymes result in the accumulation of other intermediates which are less toxic and hence symptoms are less (Table 11.1).

The accumulation of ammonia in blood (normally less than 50 mg/dL) and body fluids results in toxic symptoms. **Brain is very sensitive to ammonia.** Different clinical disorders are shown in Table 11.1. Child may be put on a low protein diet and frequent small feeds are given.

Since **Citrulline** is present in significant quantities in milk, breast milk is to be avoided in citrullinemia.

Urea Level in Blood and Urine

In clinical practice, blood urea level is taken as an **indicator of renal function**. The normal urea level in plasma is from **20 to 40 mg/dL**. Blood urea level is increased where renal function is inadequate Details of causes of uremia is given Chapter 30. Urinary excretion of urea is 15 to 30 g/day (6-15 g nitrogen/day). Urea constitutes 80% of urinary organic solids.

GLYCINE (GLY) (G)

It is the simplest amino acid. It is non-essential and is glucogenic.

1. Glycine Cleavage System

Glycine undergoes oxidative deamination to form NH3, CO_2 and the one-carbon unit methylene THFA. It needs the coenzymes, NAD, lipoamide, tetrahydrofolic acid and pyridoxal phosphate (Fig. 11.7).

2. Special Metabolic Functions of Glycine

Glycine may be used for the biosynthesis of the following compounds (Fig. 11.8):

Table 11.1: Urea cycle disorders

Diseases	*Enzyme deficit*	*Features*
Hyperammonemia type I	CPS-I	Very high NH3 levels in blood. Autosomal recessive. Mental retardation. Incidence is 1 in 200,000
Hyperammonemia Type II	(OTC) Ornithine transcarbamoylase	Ammonia level high in blood. Increased glutamine in blood, CSF and urine. Orotic aciduria due to channelling of carbamoyl phosphate into pyrimidine synthesis. X-linked
Hyperornithinemia	Defective ornithine transporter protein	Elevated blood level of ammonia and ornithine. Decreased level of urea in blood. Autosomal recessive condition
Citrullinemia	Argininosuccinate synthetase	Autosomal recessive inheritance. High blood levels of ammonia and citrulline. Citrullinura (1–2 g/day)
Argininosuccinic aciduria	Argininosuccinate lyase	Argininosuccinate in blood and urine. Friable brittle tufted hair (Trichorrhexis nodosa). Incidence 3/200,000
Hyperargininemia	Arginase	Arginine increased in blood and CSF. Instead of arginine, cysteine and lysine are lost in urine. Incidence 1 in 100,000

i. Creatine, creatine phosphate and creatinine
ii. Heme
iii. Purine nucleotides
iv. Glutathione
iv. Conjugating agent.

3. Creatine and Creatine Phosphate

Creatine is synthesized from 3 amino acids, glycine, arginine and methionine (Fig. 11.9). Creatine is phosphorylated to **creatine phosphate** (Step 3, Fig. 11.9). The enzyme creatine kinase (CK) is present in **muscle**, brain and liver. The stored creatine phosphate in the muscle serves as an immediate **store of energy in the muscle**. The creatine phosphate is converted to its anhydride, **creatinine** (Step 4, Fig. 11.9). It is a non-enzymatic **spontaneous** reaction. Creatinine is excreted in urine.

Clinical Applications

In **muscular dystrophies**, the serum creatine level and urinary creatinine are increased. Creatine level in blood is an indicator of **renal function** (For details refer Chapter 30). The enzyme CK is clinically important as it is elevated in **myocardial infarction** (For details refer Chapter 30).

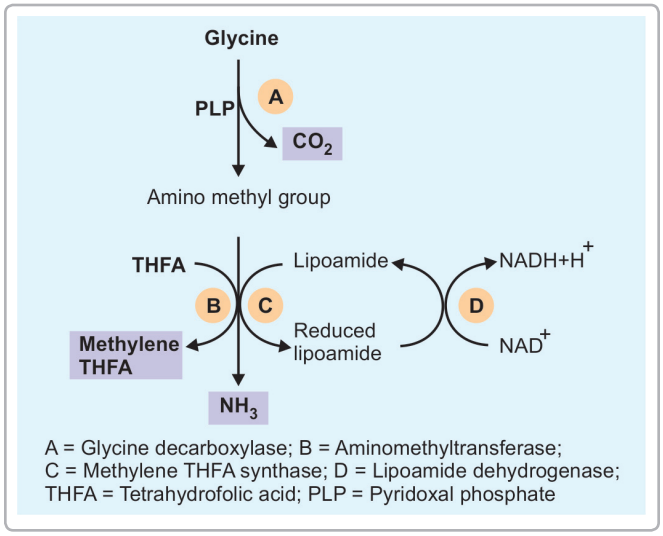


Figure 11.7: Glycine cleavage system

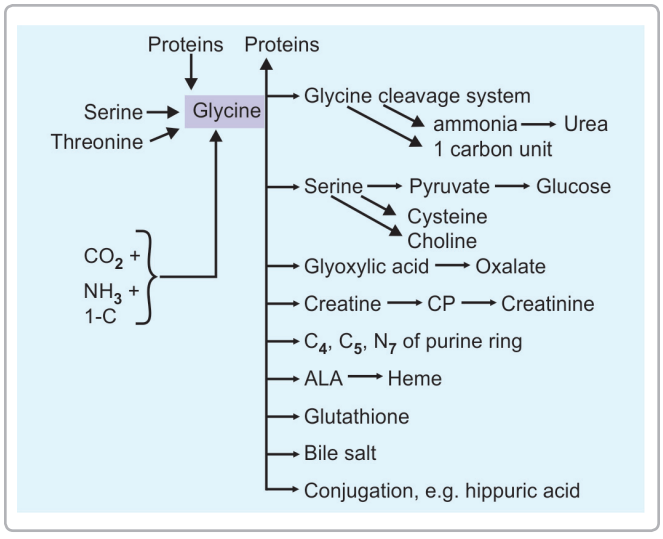


Figure 11.8: Overview of glycine metabolism

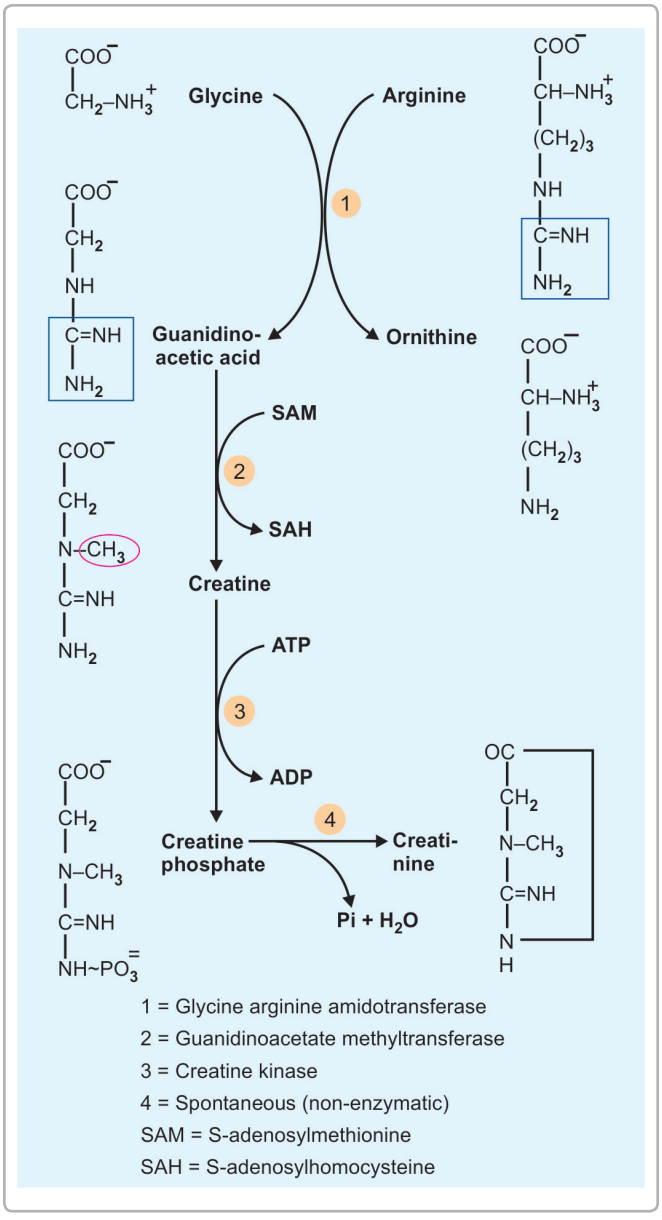


Figure 11.9: Creatine metabolism

4. Glycine as a Conjugating Agent

Bile acids. Glycine is used to conjugate bile acids, to produce bile salts. Glycocholic acid is the main conjugated bile acids. Please see Table 12.5 for a summary of all amino acids.

Methionine (Met) (M)

It is **sulfur containing, essential, glucogenic** amino acid. Methionine is activated to '**active methionine**' or S-adenosyl methionine (SAM). The adenosyl group is transferred to the sulfur atom (Step 1, Fig. 11.10).

Methyl transfer or transmethylation reactions: The methyl group is now labile, and may be transferred easily to other acceptors (Step 2, Fig. 11.10; and Table 11.2). Some important products are **creatine** (Fig. 12.9), **epinephrine** and **melatonin.** These methyl transfer reactions are carried out with the help of **S-adenosylmethionine** (SAM) (Fig. 11.10).

Cysteine synthesis: Finally, the SH group from methionine is transferred to serine to form cysteine. (Fig. 11.10). This is called **transsulfuration** reaction.

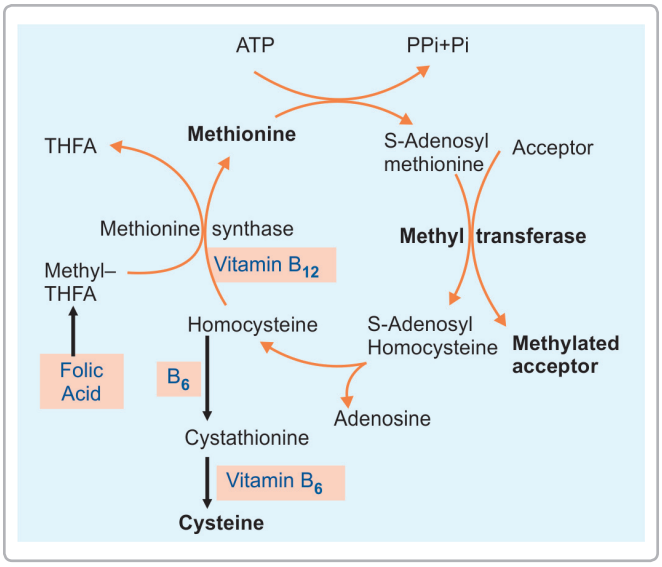


Figure 11.10: Methionine to cysteine conversion.
Note the role played by vitamins.

Functions of Cysteine

Cysteine on decarboxylation gives beta-mercapto ethanolamine. This is used for synthesis of co-**enzyme A** (For details refer Chapter 16). Cysteine is also used for synthesis of Glutathione.

Metabolic Functions of Glutathione

Formation: Glutathione is gamma-glutamyl-cysteinyl glycine. Glutathione is generally abbreviated as GSH, to indicate the reactive SH group.

RBC membrane integrity: Glutathione is present in the RBCs. This is used for inactivation of free radicals formed inside RBC.

Conjugation for detoxification: Glutathione helps to detoxify several compounds such as organo phosphorus compounds, heavy metals, and various drugs. See Table 12.5 for summary of all amino acid metabolisms.

Homocystinuria

It is an autosomal recessive conditions. **Cystathionine Synthase Deficiency** is the cause for homocystinuria. The plasma levels of methionine and homocysteine are elevated. There is increased excretion of homocysteine and methionine in urine. Other manifestations are described in Table 11.3.

Table 11.2: Transmethylation reactions

Methyl acceptor	*Methylated product*
Guanidinoacetic acid	Creatine
Nicotinamide	N-methyl nicotinamide
Norepinephrine	Epinephrine
Epinephrine	Metanephrine
Norepinephrine	Normetanephrine
Ethanolamine	Choline
Acetyl serotonin	Melatonin
Serine	Choline
Histidine	Methylhistidine

Table 11.3: Homocystinuria

Disease	*Manifestation*
Deficiency of enzyme	Cystathionine synthase
Mental retardation	+++
Ectopia lentis	+
Amino acid in urine	Homocysteine
Amino acid increased in blood	Methionine, homocysteine
Nitroprusside test	+++
Supplement	Cysteine, pyridoxine
Restrict	Methionine

PHENYLALANINE (PHE) (F) AND TYROSINE (TYR) (T)

Phenylalanine to Tyrosine

The reaction involves addition of a hydroxyl group to the aromatic ring, by phenylalanine hydroxylase (Fig. 11.11). It needs Nicotinamide adenine dinucleotide phosphate (NADPH), NADH and tetrahydrobiopterin as coenzymes.

Catabolism of Tyrosine (and Phenylalanine)

Tyrosine is partly glucogenic and partly ketogenic. The pathway is described in Fig. 11.12.

Important Specialized Products from Tyrosine

1. Melanin
2. Catecholamines (epinephrine)
3. Thyroxine (For details refer Chapter 31)

1. Synthesis of melanin

Melanin pigment gives the black color to the skin and hair (Greek word Melan means black). The first step is the hydroxylation of tyrosine by **tyrosinase.** It contains **copper**. The product is dihydroxy phenylalanine or DOPA. (Fig. 11.13). When tyrosinase is absent from epidermis, **leukoderma** (white patches) results. **Graying** of hair is also due to the disappearance of melanocytes from the hair root. In **albinism**, tyrosinase is absent in melanocytes all over the body.

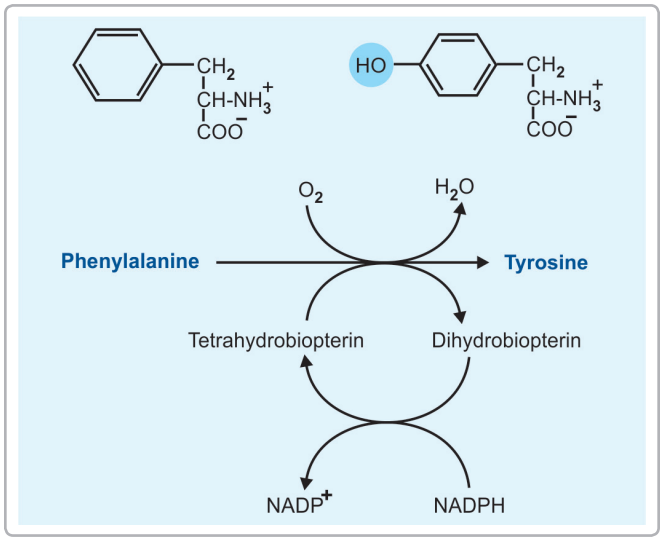


Figure 11.11: Conversion of phenyl alanine to tyrosine

2. Synthesis of Catecholamines

Catecholamines are derived from tyrosine. They include epinephrine, norepinephrine and dopamine. They are produced by the adrenal medulla and sympathetic ganglia. Details are shown in Fig. 11.14.

Tyrosine is first hydroxylated to dihydroxyphenylalanine (**DOPA**) by tyrosine hydroxylase (Fig. 11.14). It is different from tyrosinase involved in melanin synthesis

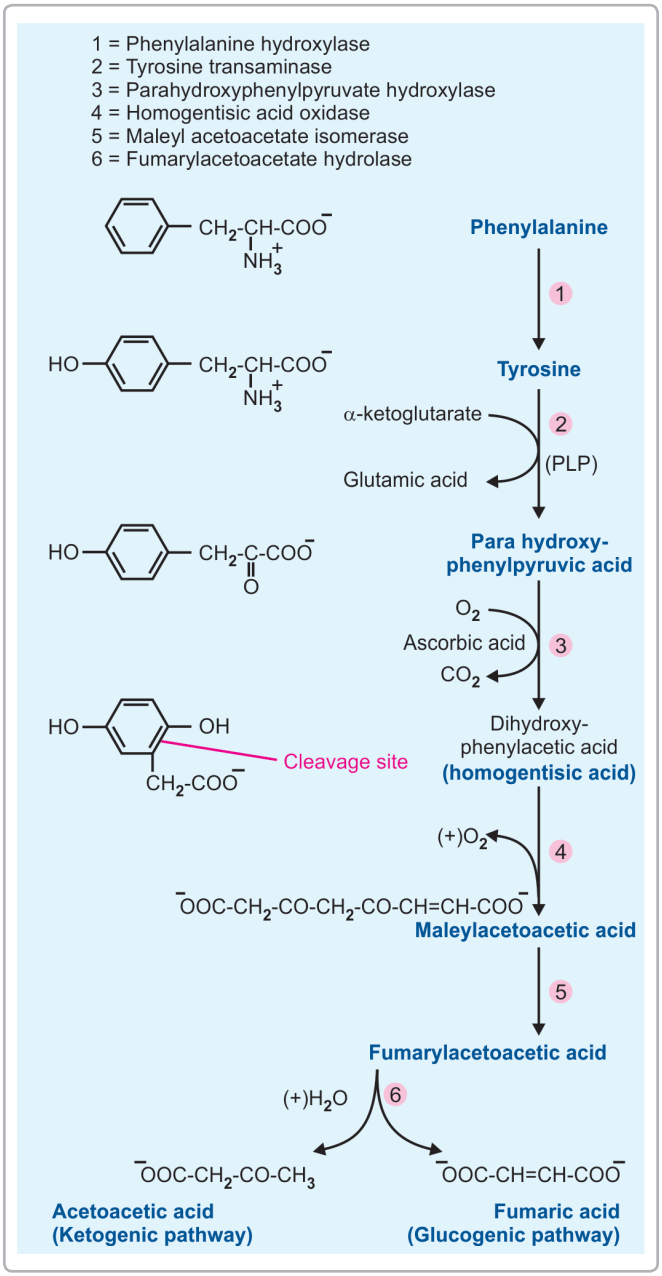


Figure 11.12: Catabolism of phenylalanine and tyrosine

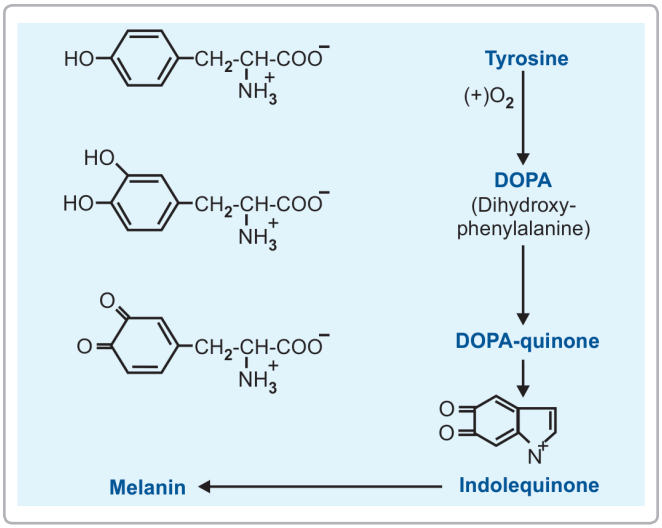


Figure 11.13: Melanine synthesis pathway

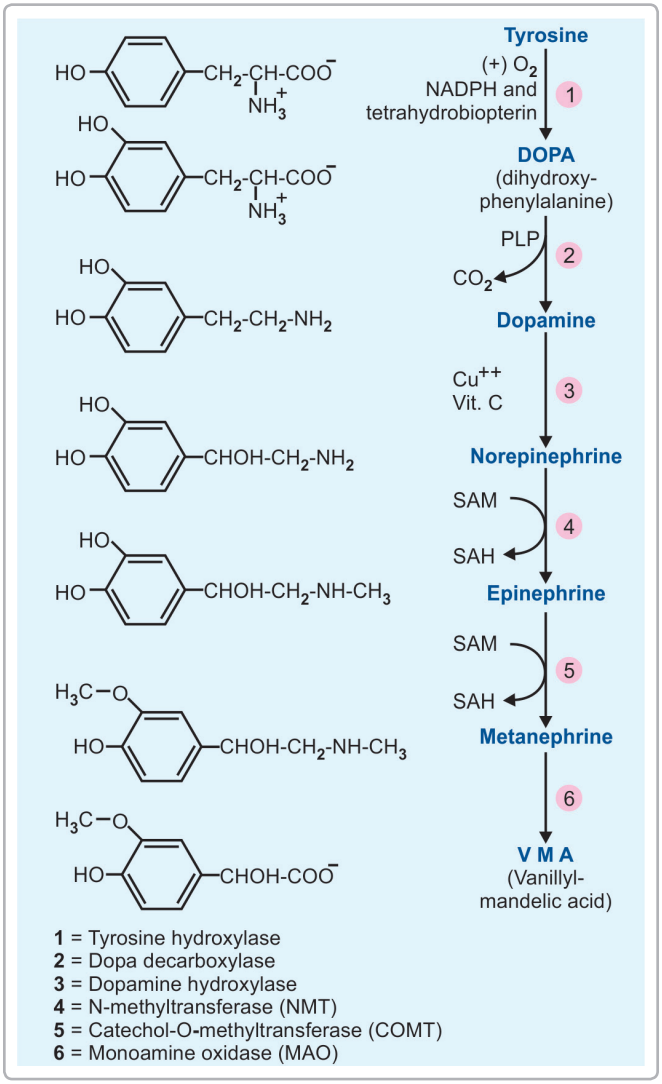


Figure 11.14: Metabolism of catecholamines

which catalyzes a similar reaction. Dopamine is an important neurotransmitter especially in extrapyramidal tract. In **Parkinsonism**, the dopamine content in brain is reduced. Epinephrine and adrenaline are the two names for the same hormone.

Actions of Epinephrine (Adrenaline)

- Increases blood pressure
- Increases rate and force of myocardial contraction
- Relaxation of smooth muscles of bronchi
- Increases glycogenolysis and stimulates lipolysis
- Adrenaline is released from adrenal medulla in response to flight, fight, fright, exercise and hypoglycemia.

Degradation of Adrenaline

Half life of epinephrine is very short, only 2–5 minutes. The major end product is 3-hydroxy-4-methoxymandelic acid or **vanillylmandelic acid** (VMA).

PHENYL KETONURIA (PKU)

Deficiency of **phenylalanine hydroxylase** (see Fig. 11.11) is the cause for this disease. It is a recessive condition. The child is **mentally retarded** with an IQ in the range of 25–50. About 20% inmates of lunatic asylum may have PKU. Agitation, hyperactivity, tremors and **convulsions** are often manifested.

Laboratory Diagnosis

Blood phenylalanine level is drastically increased. **Ferric chloride test:** Phenyl ketone (**phenylpyruvate**), phenyllactate and phenylacetate are excreted in urine. This could be detected by adding a drop of ferric chloride to the urine. A transient blue-green color is a positive test.

Treatment of Phenylketonuria

Early detection is very important. About 5 units of IQ are lost for each 10 week delay in starting the treatment. The treatment is to provide a diet containing **low phenylalanine**. Food based on tapioca (cassava) will have low phenylalanine content. This special diet is to be continued till 5 years of age; by which time brain development is completed. After that the child can have a normal diet.

ALKAPTONURIA

Alkaptonuria is an autosomal **recessive** condition. The metabolic defect is the deficiency of **homogentisate oxidase** (Figs 11.12 and 11.15). This results in excretion of **homogentisic** acid in urine. There is no mental retardation.

Diagnosis : Urine becomes black on standing when it becomes alkaline. The homogentisic acid is oxidized to black colored alkaptone bodies. **Ferric chloride test** will be positive for urine. **Benedict's test** is strongly positive. Therefore alkaptonuria comes under the differential diagnosis of reducing substances in urine.

ALBINISM

Albinism is an autosomal recessive disease. **Tyrosinase** is completely absent, leading to defective synthesis of melanin (Figs 11.13 and 11.15). The skin has low pigmentation, and so skin is sensitive to UV rays. The skin may show presence of nevi and **melanomas**. Hair is also white.

A summary of phenylalanine and tyrosine metabolism is shown in Fig. 11.15. Table 11.5 shows a summary of metabolism of all amino acids.

TRYPTOPHAN (TRP) (W)

Tryptophan is an aromatic, essential amino acid with an indole ring. Tryptophan is both **glucogenic and ketogenic.** (Fig. 11.16). In the major pathway, **kynureninase** is an enzyme dependent on pyridoxal phosphate (Fig. 11.16). Therefore in vitamin **B6 deficiency**, the pathway at this level is blocked. This leads to **niacin deficiency** and manifestations of pellagra.

Nicotinic Acid Pathway of Tryptophan

About **60 mg of tryptophan** will be equivalent to **1 mg of nicotinic acid**. The development of pellagra like symptoms (For details refer Chapter 16) in the maize eating population is due to tryptophan deficiency in maize.

Serotonin

Serotonin (5-hydroxy tryptamine) is an important monoamine. It is mainly produced in the brain, mast cells, platelets and gastrointestinal tract mucosa. The pathway is shown in Fig. 11.17.

Functions of Serotonin

Serotonin is an important neurotransmitter in brain. Serotonin will induce sleep. Serotonin increases gastrointestinal motility. Serotonin is excreted as 5-hydroxy indole acetic acid (**HIAA**) (Fig. 11.17).

Carcinoid Tumors

Serotonin is produced by **argentaffin cells** of the gastrointestinal tract and is necessary for GIT motility. These cells may grow into locally malignant **argentaffinomas**, otherwise known as **carcinoid tumors**. The patient complains of flushing, sweating, intermittent diarrhea and often has fluctuating hypertension. Tryptophan

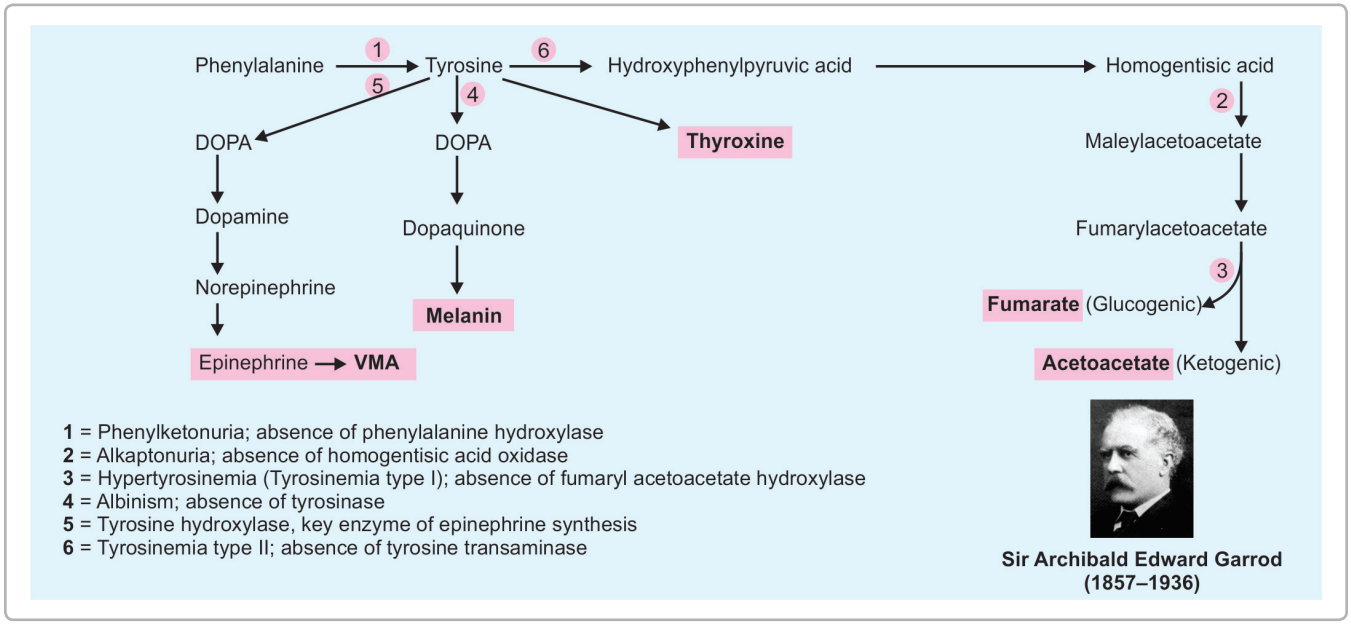


Figure 11.15: Summary of tyrosine metabolism

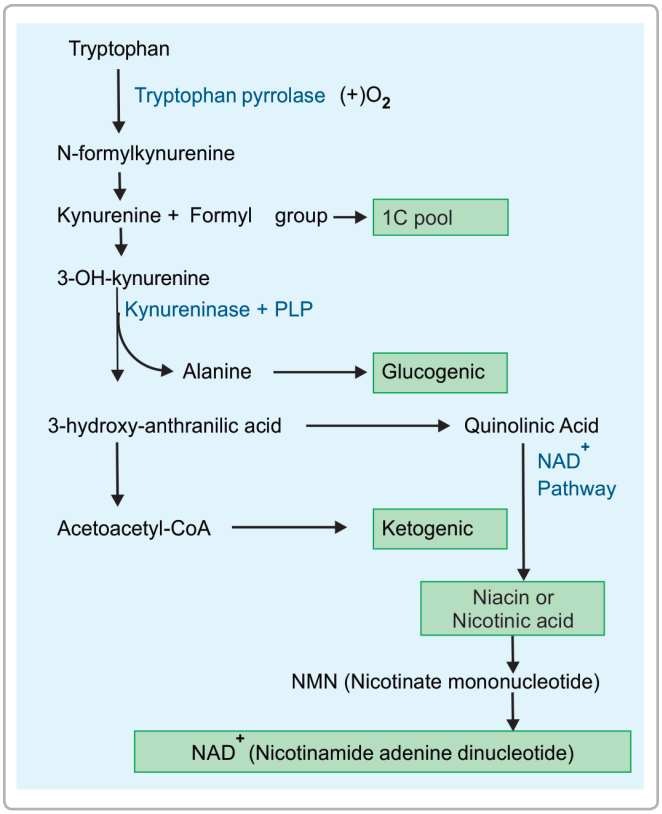


Figure 11.16: Left side, catabolism of tryptophan. Right side, synthesis of niacin from tryptophan

metabolism is diverted to serotonin synthesis. Therefore **niacin deficiency** (pellagra) may also be seen in carcinoid syndrome.

Melatonin

Serotonin is converted to melatonin with the help of S-adenosyl methionine (SAM) (Fig. 11.17). Melanin and melatonin are different. Melanin is the pigment of hair and skin; it is synthesized from tyrosine (see Fig. 11.13) Melatonin is a neurotransmitter synthesized from tryptophan (Fig. 11.17). **Pineal gland** produces melatonin. It is intimately connected with the diurnal variations, sleep wake cycles and the **biological rhythms**.

HISTIDINE (HIS) (H)

Histidine has an imidazole ring. It is a **semi-essential** basic amino acid.

Histamine

Histamine is formed from histidine by decarboxylation, catalyzed by histidine decarboxylase. Smooth muscle contraction, enhanced vascular permeability, increased

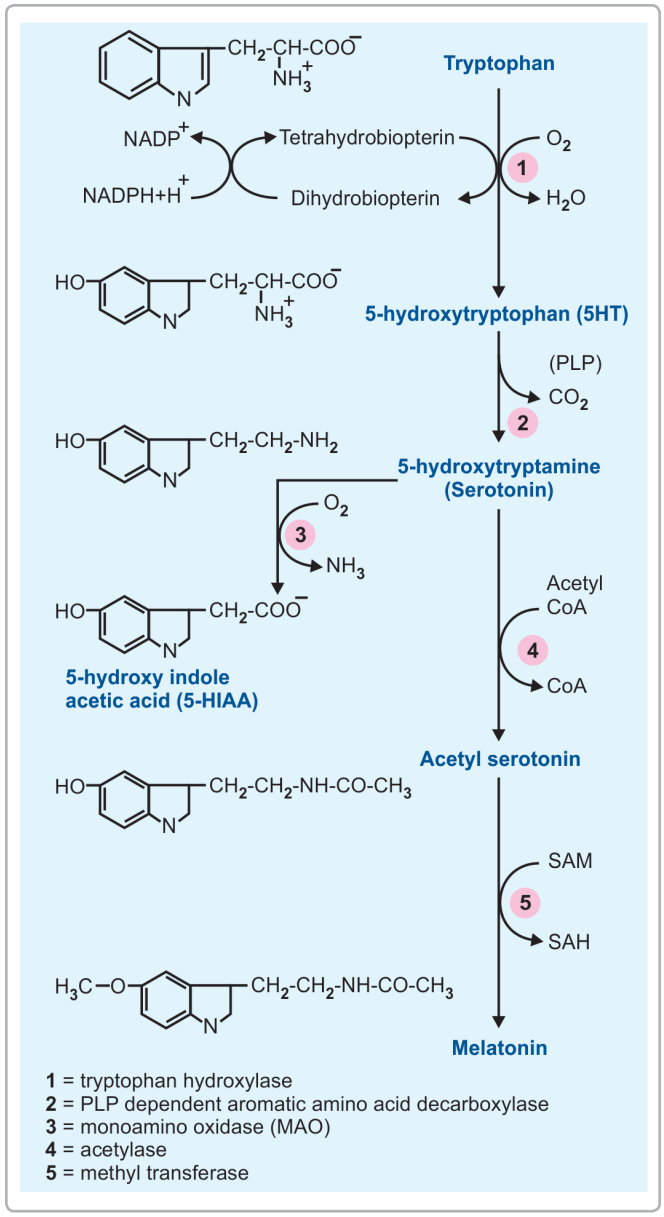


Figure 11.17: Serotonin and melatonin synthesis

acid secretion are the important actions of histamine. So histamine causes fall in blood pressure. Histamine mediates allergy and anaphylaxis.

Antihistamines are drugs which block histamine receptors. They are used to control allergic and anaphylactic reactions as well as peptic ulcer in stomach.

Biogenic Amines

They are generally synthesized by decarboxylation of amino acids. A list of biogenic amines are shown in Table 11.4. They are basic in nature. They have diverse biological functions, which are described in appropriate headings.

Polyamines are **putrescine, spermidine and spermine.** They are aliphatic amines. Several roles are suggested for polyamines, e.g. cell proliferation, synthesis of DNA and RNA, etc. Polyamine concentration is increased in **cancer** tissues. Polyamines are **growth factors** in cell culture systems.

Fate of Carbon skeletons of amino acids

Those amino acids, which give rise to citric acid cycle intermediates can be made into glucose. Hence, those amino acids entering the TCA cycle, or at pyruvic acid level are called **glucogenic** amino acids. This is shown in Fig. 11.18.

On the other hand, those amino acids which produce acetyl-CoA are called **ketogenic** amino acids. Acetyl-CoA entering the TCA cycle, is completely oxidised. Therefore, **there is no net synthesis of glucose from acetyl-CoA**. So, acetyl-CoA is not entering the gluconeogenesis pathway. Acetyl-CoA, however, can give rise to ketone bodies. Thus, **amino acids forming acetyl-CoA are known as ketogenic amino acids**. These amino acids are shown in Figure 11.18.

Table 11.4: Biogenic amines

Substrate	*Decarboxylated product, amine*
DOPA	Dopamine
Tryptophan	Tryptamine
5-OH-tryptophan	Serotonin
Histidine	Histamine
Ornithine	Putrescine

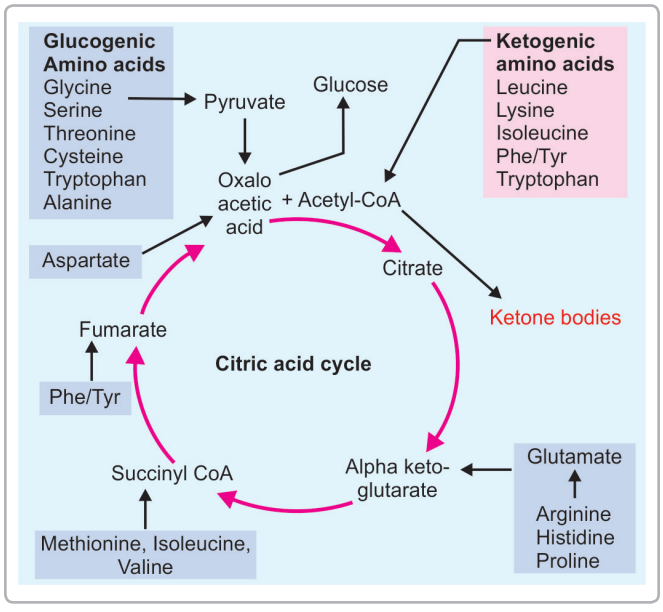


Figure 11.18: Metabolic fates of amino acids

But some amino acids are shown in both the lists. **Phenyl alanine, tyrosine, tryptophan and isoleucine** are both **glucogenic and ketogenic.** This is because, during their metabolism, part of the carbon skeleton will enter the TCA cycle; whereas the other part will generate acetyl CoA (Fig. 11.18). Table 11.5 shows summary of metabolism of amino acids.

ONE-CARBON METABOLISM

One-carbon (1C) groups play a pivotal role in donating carbon atoms for synthesis of different types of compounds. The different one-carbon groups of the **'one- carbon pool'** of the body are

1. **Formyl** group
2. **Formimino** group
3. **Methenyl** group
4. **Hydroxymethyl** group
5. **Methylene** group
6. **Methyl** group (Fig. 11.19).

The one-carbon groups, except methyl group, are carried by tetrahydrofolic acid (THFA). THFA is produced from folic acid (For details refer Chapter 16). Methyl groups are used for transmethylation reactions (see Table 11.2). This is done with the help of S-adenosyl methionine (see under methionine metabolism).

Generation of One-Carbon Groups

The one-carbon groups are contributed to the one-carbon pool by amino acids.

1. **Serine** to glycine (Serine hydroxymethyltransferase reaction) is the primary contributor
2. **Glycine** cleavage system (Fig. 11.7).
3. **Histidine**
4. **Tryptophan** (Fig. 11.16).
5. **Choline** can donate 3 hydroxy methyl groups.

Interconversion of One-Carbon Groups

The different one-carbon groups are interconvertible as shown in Figure 11.19. All one-carbon units are ultimately siphoned into methyl-THFA.

From methyl-THFA, the B12 coenzyme accepts the methyl group to form methyl cobalamin. It then transfers the methyl group to homocysteine to form methionine (Fig. 11.19).

Utilization of One-carbon Groups

The one-carbon units are used for synthesis of the following compounds (Fig. 11.19):

Table 11.5: Summary of Amino acid Metabolism

Amino acid	*Product formed*	*Importance*
Glycine	Heme	Glycine condenses with succinyl-CoA to form heme, which is used for oxygen carriage
	Creatine	Glycine reacts with arginine to form guanidoacetic acid which is then methylated to form creatine. Creatine phosphate is the high energy compound in muscle
	Glutathione	It is gamma glutamyl cysteinyl glycine. It is antioxidant in RBCs
	Purine bases	C4,C5 and N7 of purine ring is contributed by glycine
	One carbon group	Glycine cleavage system provides one carbon groups. 1C groups are used in synthesis of DNA (See Fig. 11.20).
Alanine	Pyruvate	Transamination of alanine to pyruvate is catalyzed by Alanine aminotransferase. Alanine is major glucogenic amino acid
Serine	One carbon group	Conversion of serine to glycine generates one carbon group. It is the major contributor of one carbon groups
	Cysteine	Carbon skeleton of cysteine is from serine.
Methionine	Active methionine	Methionine and ATP forms S-adenosyl methionine, which is the methylating agent for transmethylation
	Cysteine	Degradation of methionine produces cysteine
Cysteine	SH groups in proteins	Keeps active sites of enzymes
	Glutathione	It is gamma-glutamyl-cysteinyl-glycine. It is antioxidant in RBCs
Arginine	Nitric oxide	Nitric oxide synthase acts on arginine releasing NO, which is a potent vasodalator
	Creatine	Glycine + arginine + methionine
Histidine	Histamine	Involved in allergic reactions
	Histidine in proteins	Buffering action
Glutamic acid	Glutamine	Ammonia is added to glutamic acid to form glutamine by glutamine synthetase. Important in ammonia fixation by brain cells
	Alpha-ketoglutaric acid	Member of TCA cycle
	GABA	Inhibitory neurotransmitter
Glutamine	Purines, pyrimidines	Amide group of glutamine contributes N atoms of purines and pyrimidines
	Ammonia	Glutaminase releases ammonia from glutamine in the renal tubular cells. This is important for excreting H ions as ammonium ions
Aspartic acid	Urea	Urea cycle is important for detoxification of ammonia
	Purines, pyrimidines	C and N atoms of aspartate are used for nucleic acids
	Asparagine	Ammonia is added to aspartic acid to form asparagine by asparagine synthetase. Important in ammonia fixation by brain cells
	Oxaloacetate	Transamination of aspartic acid by AST forms oxaloacetate. It is the starting molecule of citric acid cycle
Tyrosine	Epinephrine	Adrenergic activity
	Dopamine	Neurotransmitter in brain
	T4 and T3	Stimulators of metabolism and growth
	Melanin	Sunscreen pigment
Tryptophan	Serotonin	Vasopressor amine; mood regulation
	Melatonin	Acetylated serotonin. Important in sleep wake cycle
	NAD	Coenzyme for dehydrogenases

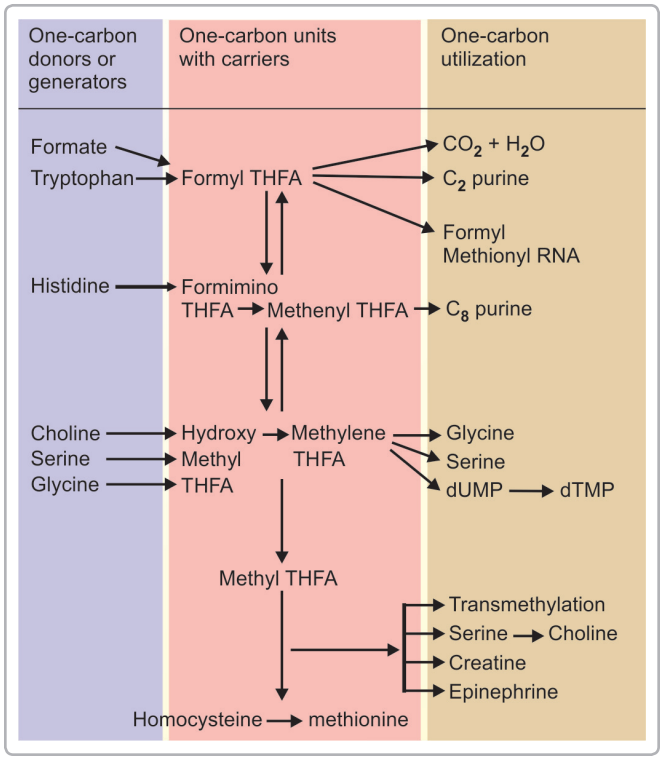


Figure 11.19: One carbon generation and utilization

i. **Carbon atoms 2 and 8 of purine**
ii. **Glycine synthesis** (Fig. 11.7)
iii. **Serine synthesis**
iv. **Choline synthesis**
v. **Transmethylation reactions** including creatine, choline and epinephrine synthesis (see Table 11.2).

Inborn Errors of Metabolism

Sir Archibald Garrod in 1902 reported four diseases, now known as **Garrod's tetrad**. These are Alkaptonuria, albinism, pentosuria and cystinuria. Garrod introduced the term "inborn errors of metabolism" in 1908.

Inborn Errors Associated with Protein Metabolism

- Phenylketonuria (see Fig. 11.15)
- Alkaptonuria (see Fig. 11.15)
- Albinism (see Fig. 11.15)
- Homocystinuria (see Table 11.3)
- Urea cycle defects (see Table 11.1).

Inborn Errors Associated with Carbohydrate Metabolism are:

- Glycogen storage diseases (For details refer Table 5.7)
- Glucose-6-P-dehydrogenase deficiency (For details refer Fig. 7.1)
- Essential pentosuria (For details refer Fig. 7.2)
- Fructose intolerance (For details refer Fig. 7.3)
- Galactosemia (For details refer Figs 7.4 and 7.5)

Amino acid metabolism is summarized in Table 11.5.

A QUICK LOOK

1. Pepsin, trypsin and chymotrypsin are the important protein hydrolyzing enzymes in gastrointestinal tract.
2. Amino acids are transaminated with a keto acid to produce another amino acid.
3. Glutamic acid is deaminated to produce alpha ketoglutaric acid and ammonia.
4. Ammonia in brain is trapped by the glutamic acid to produce glutamine.
5. Ammonia is finally excreted as urea. Urea is synthesized in the urea cycle.
6. Normally urea level in blood is 20–40 mg/dL. It is increased in renal diseases.
7. Glycine is used to synthesize serine, choline, creatine, creatinine, purine ring, heme, glutathione, bile salts.
8. Glycine is also used for conjugation and detoxification reactions.
9. Methionine is activated to S-adenosine methionine, which is used for transmethylation reactions.
10. Methionine and cysteine metabolisms are interconnected.
11. Glutathione is synthesized by using cysteine.
12. Homocystinuria is due to the absence of cystathionine synthase
13. Phenylalanine is converted to tyrosine by phenylalanine hydroxylase.
14. When this enzyme is absent, it leads to phenylketonuria, an inborn error of metabolism. There will be severe mental retardation in this condition.
15. Important specialized products from tyrosine are melanin, epinephrine and thyroxine.
16. Deficiency of homogentisic acid oxidase leads to a condition called alkaptonuria, where homogentisic acid is excreted in urine, leading to black urine.
17. Absence of tyrosinase will lead to albinism.
18. Substances produced from tryptophan are alanine (glucogenic), acetoacetyl-CoA (ketogenic), niacin, NAD^+, serotonin, melatonin.
19. One-carbon (1C) groups play a pivotal role in donating carbon atoms for synthesis of different types of compounds.

CHAPTER 12

Plasma Proteins

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Electrophoresis of plasma proteins
- Albumin, globulins, clinical significance
- Transport proteins in blood
- Acute phase proteins in blood
- Ceruloplasmin
- Clotting factors
- Structure of immunoglobulins
- Immunoglobulins G, M, A, D and E
- Multiple myeloma
- Bence-Jones Proteinuria

Total blood volume is about 4.5 to 5 liters in adult human being.

i. The **defibrinated plasma is called serum,** which lacks coagulation factors including prothrombin and fibrinogen.

ii. **Total protein** content of normal plasma is **6 to 8 g/100 mL.**

iii. The plasma proteins consist of **albumin (3.5 to 5 g/dL), globulins (2.5 to 3.5 g/dL)** and fibrinogen (200 to 400 mg/dL). The albumin: globulin ratio is usually between 1.2:1 to 1.5:1.

iv. Almost all plasma proteins, except immunoglobulins are synthesized in liver.

ELECTROPHORESIS

In clinical laboratory, electrophoresis is employed regularly for separation of serum proteins. The term electrophoresis refers to the **movement of charged particles through an electrolyte when subjected to an electric field**. The details are given in Chapter 28. Abnormal electrophoretic patterns are shown in Figure 12.1. The normal pattern is shown in the upper part of Fig. 12.1. and in Fig. 12.2.

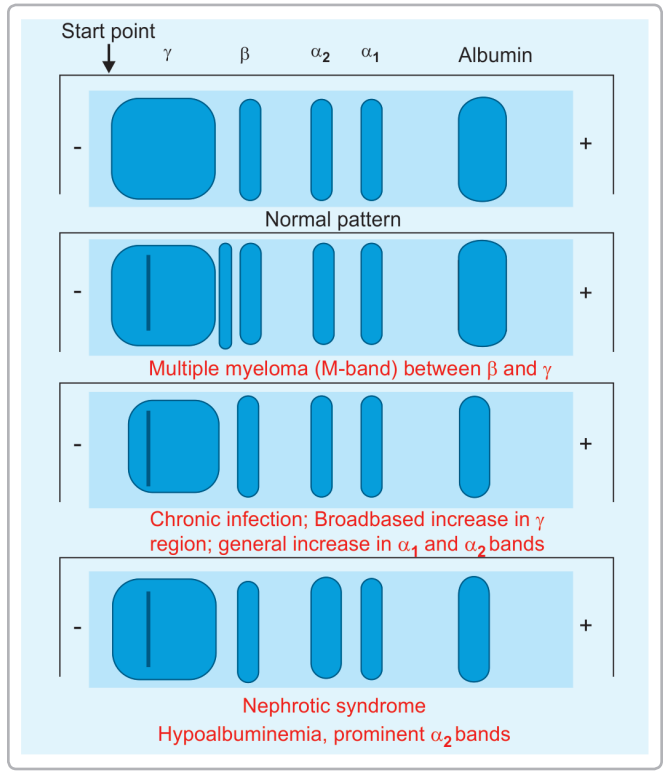


Figure 12.1: Serum electrophoretic patterns

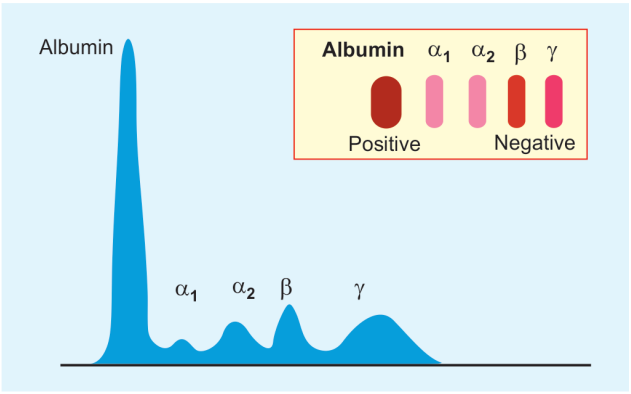


Figure 12.2: Normal electrophoretic pattern

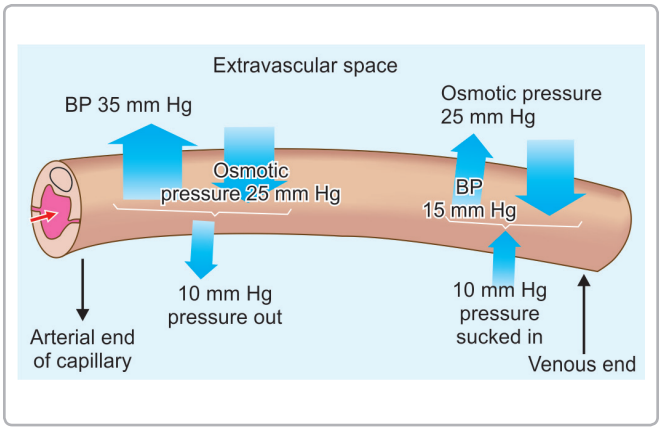


Figure 12.3: Starling's hypothesis

Normal Values and Interpretations

i. In agar gel electrophoresis, normal serum is separated into 5 bands. Albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin, and gamma-globulin.
ii. **Albumin has the maximum and gamma globulin has the minimum mobility** in the electrical field.

Abnormal Patterns in Clinical Diseases

Various abnormalities can be identified in the electrophoretic pattern (see Fig. 12.1).

1. **Chronic infections:** the gamma globulins are increased, but the increase is smooth and wide based.
2. **Multiple Myeloma:** In **paraproteinemias**, a sharp spike is noted and is termed as **M-band**. This is due to monoclonal origin of immunoglobulins in multiple myeloma (see Fig. 12.1).
3. **Nephrotic syndrome:** All proteins except very big molecules are lost through urine, and so alpha-2 fraction (containing macroglobulin) will be very prominent.

ALBUMIN

The name is derived from the white precipitate formed when egg is boiled (Latin, albus = white).

Functions of Albumin

1. Colloid osmotic pressure of plasma

Proteins cannot easily escape out of blood vessels, and therefore, proteins exert the **'effective osmotic pressure'.** It is about 25 mm Hg, and 80% of it is contributed by albumin. The maintenance of blood volume is dependent on this effective osmotic pressure. According to **Starling's hypothesis**, at the capillary end the blood pressure (BP) or hydrostatic pressure expels water out, and effective osmotic pressure (EOP) takes water into the vascular compartment (Fig.12.3). At arterial end of the capillary, BP is 35 mm Hg and EOP is 25 mm; thus water is expelled by a pressure of 10 mm Hg. At the venous end of the capillary, EOP is 25 mm and BP is 15 mm, and therefore water is imbibed with a pressure of 10 mm. Thus the number of water molecules escaping out at arterial side will be exactly equal to those returned at the venous side and therefore blood volume remains the same. If protein concentration in serum is reduced, the EOP is correspondingly decreased. Then return of water into blood vessels is diminished, leading to **accumulation of water in tissues**. This is called **edema**.

2. Transport Function

Albumin is the carrier of various hydrophobic substances in the blood. Being a watery medium, blood cannot solubilize lipid components and lipophilic compounds.

i. **Bilirubin** and **non-esterified fatty acids** are specifically transported by albumin.
ii. **Drugs** (sulfa, aspirin, salicylates, dicoumarol, phenytoin).
iii. **Hormones:** steroid hormones, thyroxine.
iv. **Metals:** calcium, copper and heavy metals are non-specifically carried by albumin.

3. Nutritional function

All tissue cells can take up albumin by pinocytosis. It is then broken down to amino acid level. So albumin may be considered as the transport form of essential amino acids from liver to other tissues.

4. Blood brain barrier

Albumin–fatty acid complex cannot cross blood–brain barrier and hence fatty acids cannot be taken up by brain. The **bilirubin from albumin may be competitively replaced by aspirin** and such other drugs. In newborns, bilirubin is already high, and if such drugs are given, there is a probability that free bilirubin is deposited in brain leading to **kernicterus** and mental retardation.

5. Edema

Hypoalbuminemia will result in **tissue edema** (see Starling's law). **Malnutrition**, where albumin synthesis is depressed (*generalized edema*). **Nephrotic** syndrome, where albumin is lost through urine (*facial edema*). Presence of albumin in urine is called **albuminuria**. It is always pathological. Large quantities (many grams per day) of albumin is lost in urine in nephrotic syndrome. Small quantities are lost in urine in acute nephritis, and other inflammatory conditions of urinary tract. Detection of albumin in urine is done by heat and acetic acid test. **Cirrhosis** of liver (mainly *ascites*). Albumin synthesis is decreased. **Chronic congestive cardiac failure:** Venous congestion will cause increased hydrostatic pressure and decreased return of water into capillaries and so *pitting edema* of feet may result.

Albumin-Globulin Ratio

In hypoalbuminemia, there will be a compensatory increase in globulins which are synthesized by the reticulo-endothelial system. Albumin–globulin ratio (A/G ratio) is thus altered or even reversed. This again leads to edema.

TRANSPORT PROTEINS

Blood is a watery medium; so lipids and lipid soluble substances will not easily mix in the blood. Hence such molecules are carried by specific carrier proteins.

1. **Albumin:** It is an important transport protein, which carries bilirubin, free fatty acids, calcium and drugs (see above).
2. **Pre-albumin or transthyretin:** It is so named because of its faster mobility in electrophoresis than albumin. It is more appropriately named as **Transthyretin** or Thyroxin binding pre-albumin (**TBPA**), because it carries thyroid hormones, thyroxin (T4) and triiodothyronine (T3). Its half life in plasma is only 1 day.
3. **Thyroxine binding globulin (TBG):** It is the specific carrier molecule for thyroxine and triiodothyronine. TBG level is increased in pregnancy; but decreased in nephrotic syndrome
4. **Retinol binding protein (RBP):** It carries vitamin A.
5. **Transcortin or cortisol binding globulin (CBG)** transports cortisol and corticosterone.
6. **Transferrin:** It carries iron in plasma.

ACUTE PHASE PROTEINS

The level of certain proteins in blood may increase 50 to 1000 folds in various inflammatory and neoplastic conditions. Such proteins are acute phase proteins. Important acute phase proteins are described below:

1. C-Reactive Protein (CRP)

It is thus named because it reacts with C-polysaccharide of capsule of pneumococci. It is synthesized in liver. It can stimulate macrophage phagocytosis. When the inflammation has subsided, CRP quickly falls, followed later by ESR (erythrocyte sedimentation rate).

2. Ceruloplasmin and Wilson's Disease

Normal blood level of ceruloplasmin is 25–50 mg/dL. This level is reduced to less than 20 mg/dL in **Wilson's hepatolenticular degeneration.** It is an inherited autosomal recessive condition. Incidence of the disease is 1 in 50,000. Copper is not excreted through bile, and hence copper toxicity. So ceruloplasmin level in blood is decreased. Accumulation in liver leads to hepatocellular degeneration and **cirrhosis**. Deposits in brain basal ganglia leads to **lenticular degeneration** and neurological symptoms.

STRUCTURE OF IMMUNOGLOBULINS

Immunoglobulin is abbreviated as Ig. The terms gamma globulin and immunoglobulin are not synonymous.

Heavy and Light Chains

The structure of lgG molecule is shown in Fig. 12.4. It is made up of 2 heavy (H) chains and 2 light (L) chains, combined through disulfide bridges. In the case of lgG, H chains are composed of 440 amino acids and L chains made up of 214 amino acids. Depending on the heavy chain make up, the immunoglobulins are differentiated into **5 major classes.**

1. **Immunoglobulin-G (lgG)** is made up of heavy chain γ (gamma)
2. **lgM** has μ (mu) heavy chain

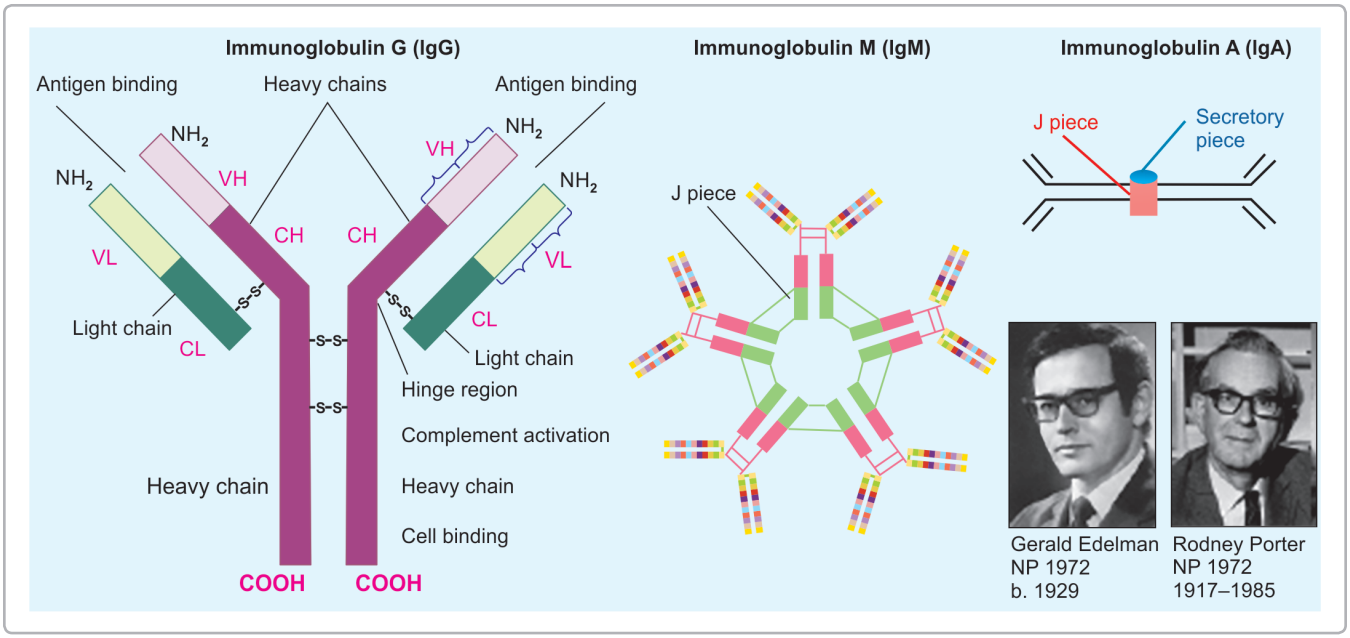


Figure 12.4: Immunoglobulin molecule. HN2 = amino terminal end; COOH = carboxy terminal end. VH = variable heavy region. VL = variable light chain. CH = Constant heavy. CL = Constant light. Chains are connected by disulphide bridges. IgG has one basic unit; IgM has 5 basic units and IgA has 2 basic units

Table 12.1: Characteristics of different immunoglobulin classes

	IgG	*IgA*	*IgM*	*IgD*	*IgE*
Name of heavy chain	γ	α	μ	δ	ε
No. of basic 4-peptide units (2L + 2H)	1	2	5	1	1
Additional unit present	--	S and J	J piece	---	---
Molecular weight (Daltons)	1,46,000	3,85,000	9,70,000	1,85,000	1,90,000
Concentration in normal serum	800–1200 mg / 100 mL	150–300 mg / 100 mL	50–200 mg /100 mL	1–10 mg /100 mL	1.5–4.5 mcg / 100 mL

3. **IgA** has α (alpha) heavy chain
4. **IgD** contains δ (delta)
5. **IgE** heavy chain is called ε (epsilon).

The **light chains** are either κ (kappa) or λ (lambda) in all the classes. For example, IgG may consist of either g2 k2 or g2 l2.

Variable and Constant Regions

Both the heavy and light chains contain relatively variable (V) and constant (C) regions with regard to their amino acid composition. VL and CL are the general terms for these regions on the light chain; while VH and CH specify variable and constant regions on the heavy chain (Fig. 12.4).

At the amino terminal end, about 100 amino acids in light chains and in heavy chains constitute the variable region. Here the amino acid sequence can vary in H and L chains, so that the body could synthesize enormous varieties of different proteins.

Different Classes of Immunoglobulins

1. Immunoglobulin G (IgG)

IgG contains two heavy chains and two light chains; heavy chains being of gamma type (Fig. 12.4 and Table 12.1). It is the antibody seen in **secondary** immune response. It can pass from vascular compartment to interstitial space. It can cross placental barrier, and protects the newborn child from infections.

2. Immunoglobulin M (IgM)

IgM are **macroglobulins** or 19S immunoglobulins. Five subunits, each having 4 peptide chains (total 10 heavy chains and 10 light chains) are joined together by a J-chain polypeptide (Fig. 12.4). It can combine with 5 antigens simultaneously, and so IgM is very effective for agglutinating bacteria. Being a large molecule, it cannot come out of vascular space. IgM are the predominant class of antibodies in **primary** response.

3. Immunoglobulin A (IgA)

IgA usually are dimers (total 4 heavy chains and 4 light chains) (Fig. 12.4). The J chain connects the dimers. They are the **secretory antibodies** seen in seromucous secretions of gastrointestinal tract, nasopharyngeal tract, urogenital tract, tears, saliva, sweat, etc. The dimers are stabilized against proteolytic enzymes by the secretory piece.

4. Immunoglobulin E (IgE)

They mediate allergy, hypersensitivity and **anaphylaxis.** They have the property to fix on mast cells and basophils. When certain antigens such as penicillin are injected a few times, IgE class antibodies are produced which anchor on **mast cells**. **W**hen the same antigen is injected next time, the antigen fixes on cell surface antibodies, causing mast cell degranulation, and release of **histamine** and slow reacting substance. This leads to vasodilatation, hypotension and bronchiolar constriction. This is the basis of penicillin anaphylaxis, hay fever caused by fungus, asthma by pollen and urticaria by absorbed food elements.

PARAPROTEINEMIAS

1. Multiple Myeloma (Plasmacytoma)

When Ig-secreting cells are transformed into malignant cells, one clone alone is enormously proliferated. Thus, Ig molecules of the very same type are produced in large quantities. This is seen in electrophoresis as the myeloma band or **monoclonal band** or M band with a sharp narrow spike (see Fig. 12.1). Multiple myeloma is characterized by paraproteinemia, anemia, lytic bone lesions and proteinuria. Bone marrow examination reveals large number of malignant plasma cells. Bone pain and tenderness are the common presenting complaints. Spontaneous pathological fracture of weight-bearing bones, rib and vertebrae may occur.

2. Bence-Jones Proteinuria

Henry Bence-Jones described it in 1848. This disorder is seen in 20% of patients with multiple myeloma. Monoclonal light chains are excreted in urine. The Bence-Jones proteins have the special property of precipitation when heated between 45°C and 60°C; but re-dissolving at higher than 80°C and lower than 45°C.

3. Hypergammaglobulinemia

This disorder can occur in: Chronic infections, where antibody production is high. Examples are leprosy, tuberculosis, malaria and subacute bacterial endocarditis; Aberrant immune reactions such as rheumatoid arthritis, collagen disorders, glomerulonephritis, and such autoimmune disorders where cryoglobulins may also be present and paraproteinemias such as in multiple myeloma.

A QUICK LOOK

1. Total plasma protein content is 6–8 g/dL of which albumin is 3.5–5 g/dL and the rest is globulin. Almost all plasma proteins are synthesized in the liver except immunoglobulins.
2. On agar gel electrophoresis albumin has maximum mobility while gamma globulin has minimum mobility.
3. In chronic infection, gamma globulins are increased smoothly, while in paraproteinemias, M-band is seen. The alpha-2 fraction is increased in nephrotic syndrome while albumin is decreased in liver cirrhosis, malnutrition, nephrotic syndrome.
4. Albumin contributes to colloid osmotic pressure of plasma, has buffering capacity and is a transport medium for various hydrophobic substances.
5. Hypergammaglobulinemia is seen in conditions of hypoalbuminemia, chronic infection and paraproteinemias.
6. The transport proteins in blood are albumin, prealbumin (transthyretin), RBP, TBG, transcortin and haptoglobin.
7. The levels of certain proteins in blood may increase 50–100-fold in various inflammatory and neoplastic conditions. Such proteins are called acute phase proteins. For example, CRP, ceruloplasmin, haptoglobins, alpha-1 acid glycoprotein, alpha-1 antitrypsin and fibrinogen.
8. Proteins that are decreased in blood during inflammatory response are called negative acute phase proteins. For example, albumin, transthyretin, transferrin.

CHAPTER 13

Tricarboxylic Acid Cycle and Biological Oxidation

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Citric acid cycle, significance
- Integration of metabolism
- Primary, secondary and tertiary metabolism
- Biological oxidation
- High energy compounds
- Organization of electron transport chain
- Chemiosmotic theory
- ATP synthase
- Inhibitors of ATP synthesis

The complete cycle was proposed by Sir Hans Krebs in 1937 (Nobel prize, 1953). The cycle is therefore named after him. Please note that the name is **Krebs cycle (there is no apostrophe).**

Functions of the Citric Acid Cycle

1. It is the final common oxidative pathway that oxidizes acetyl-CoA to CO_2.
2. It is the source of reduced coenzymes that provide the substrate for the respiratory chain.
3. It acts as a link between catabolic and anabolic pathways (amphibolic role).
4. It provides precursors for synthesis of amino acids and nucleotides.
5. Components of the cycle have a direct or indirect controlling effects on key enzymes of other pathways.

REACTIONS OF THE CYCLE

Preparatory Steps

Acetyl-CoA enters the cycle, and is completely oxidized. During this process, energy is trapped. The **sources of acetyl-CoA** are shown in Figure 13.1. **Pyruvate** derived from glycolysis is oxidatively decarboxylated to acetyl-CoA by the **pyruvate dehydrogenase** (Fig. 5.9). This is the link between the triboxy acid (TCA) cycle and glycolysis. All the enzymes of citric acid cycle are located inside the **mitochondria**.

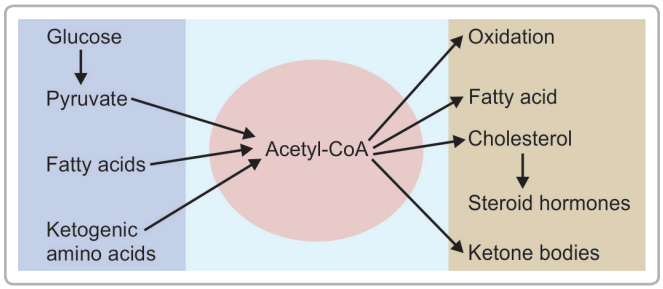


Figure 13.1: Sources and utilization of acetyl-CoA

1st Step: Formation of citric acid

Oxaloacetate (4 carbon) is condensed with **acetyl-CoA** (2 carbon) to form citrate (6 carbon). The enzyme is **citrate synthase** (Step 1, Fig. 13.2).

2nd Step: Formation of isocitrate

Citrate is isomerized to isocitrate by **aconitase** (Step 2, Fig. 13.2). At first, one water molecule is removed from citrate forming **cis aconitate**; a **transient** compound. Immediately, one water molecule is added to aconitate to form isocitrate.

3rd Step: Formation of Alpha Ketoglutarate

This reaction is a two-step process, both catalyzed by the same enzyme, **isocitrate dehydrogenase** (Step 3, Fig. 13.2). Isocitrate is dehydrogenated to form **oxalosuccinate** which undergoes spontaneous decarboxylation to form alpha ketoglutarate. The **NADH generated** in this step is later oxidized in electron transport chain (ETC) to generate **2.5 ATPs**. Isocitrate (6 carbons) undergoes **oxidative decarboxylation** to form alpha ketoglutarate (5 carbons). In this reaction, one molecule of **CO_2 is liberated**.

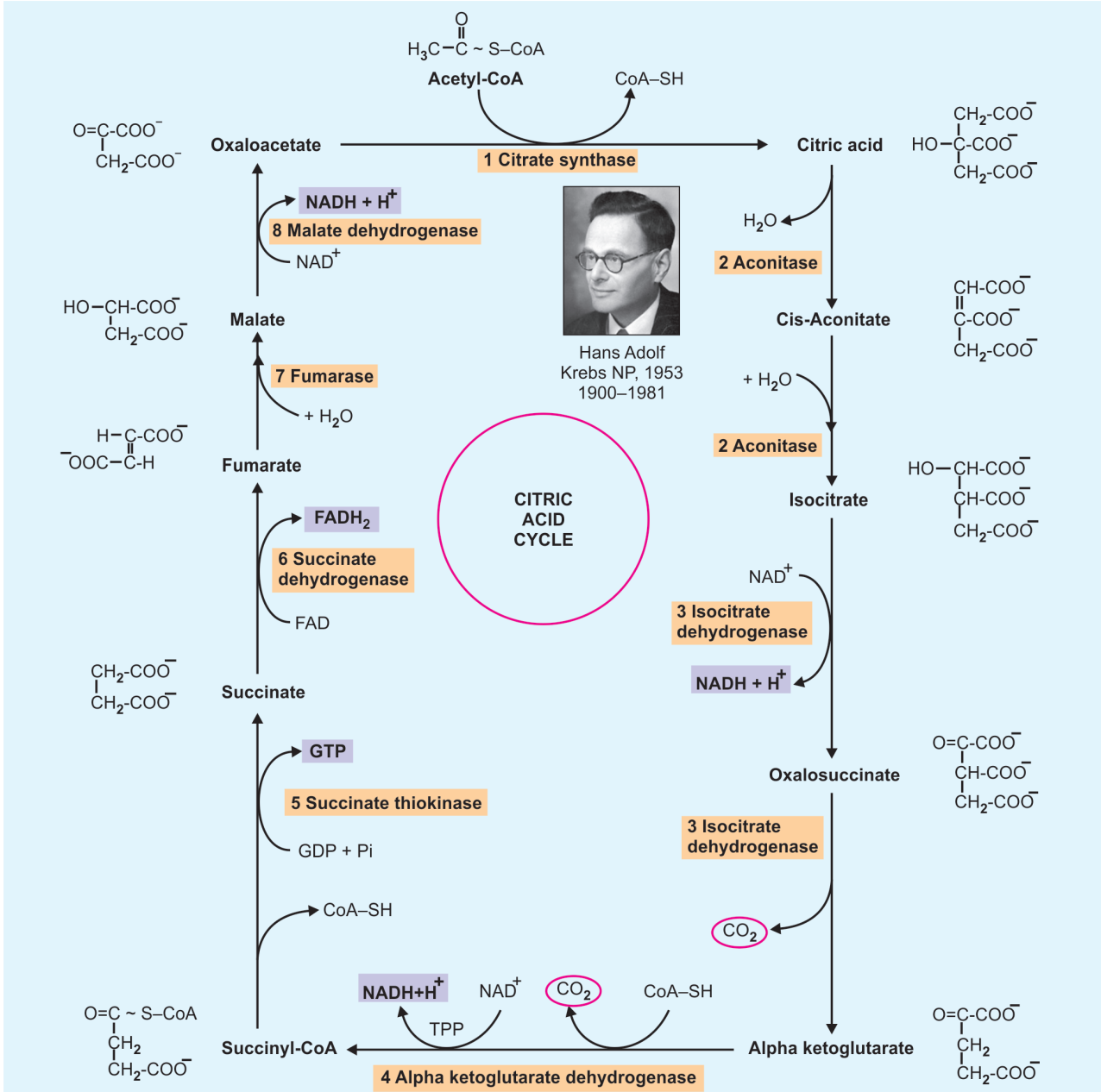


Figure 13.2: Krebs cycle or citric acid cycle or tricarboxylic acid cycle

4th Step: Formation of Succinyl-CoA

Next, alpha ketoglutarate is **oxidatively decarboxylated** to form succinyl-CoA by the enzyme **alpha ketoglutarate dehydrogenase** (Step 4, Fig. 13.2). The **NADH** thus generated enters into ETC to generate **2.5 ATPs**. Another molecule of **CO_2 is removed** in this step. This is the only **irreversible step** in the whole reaction cycle. The enzyme alpha ketoglutarate dehydrogenase is a **multienzyme complex** having 3 enzyme proteins and 5 coenzymes. This is similar to the pyruvate dehydrogenase reaction.

5th Step: Generation of Succinate

The next reaction involves a **substrate level phosphorylation** whereby a high energy phosphate is generated from the energy trapped in the thioester bond of succinyl-CoA. The enzyme is **succinate thiokinase** (step 5, Fig. 13.2). A molecule of GDP is phosphorylated to **GTP** and succinate is formed. The GTP can be converted to ATP by reacting with an ADP molecule:

$$GTP + ADP \rightarrow GDP + ATP$$

6th Step: Formation of Fumarate

Succinate is dehydrogenated to fumarate, an unsaturated dicarboxylic acid, by **succinate dehydrogenase** (step 6, Fig. 13.2). The hydrogen atoms are accepted by FAD. The **$FADH_2$** then enters into ETC to generate **1.5 ATPs**. The enzyme is a flavoprotein. The succinate dehydrogenase is competitively inhibited by malonate (Fig. 13.3).

7th Step: Formation of Malate

The formation of malate from fumarate is catalyzed by **fumarase** (step 7, Fig. 13.2).

8th Step: Regeneration of Oxalo-acetate

Finally malate is oxidized to oxaloacetate by **malate dehydrogenase** (Step 8, Fig. 13.2). The coenzyme is NAD^+. The **NADH** is generated in this step, which enters the electron transport chain, when **2.5 ATPs** are produced. The oxaloacetate can further condense with another acetyl-CoA molecule and the cycle continues (Fig. 13.2). Oxalo acetate may be viewed as a catalyst, which enters

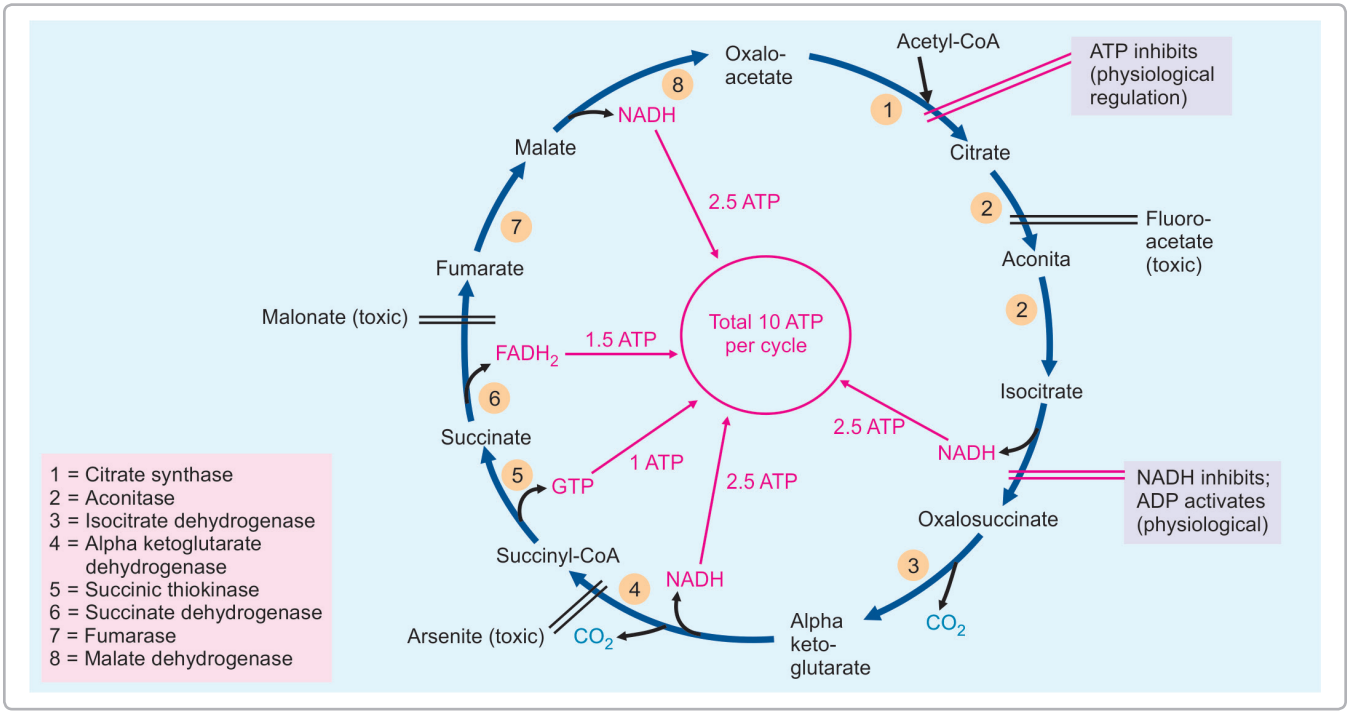


Figure 13.3: Summary of Krebs citric acid cycle. Enzymes are numbered and marked in blue rounds. Steps where energy is trapped are marked in brown color. A total of 10 ATPs are generated during one cycle. Reactions number 3 and 4 are carbon dioxide elimination steps. Physiological regulatory steps are shown as red backgrounds. Step No. 1 (citrate synthase) is physiologically inhibited by ATP. Step No. 3 (ICDH) is inhibited by NADH and activated by ADP

into the reaction, causes complete oxidation of acetyl-CoA and comes out of it without any change.

SIGNIFICANCE OF TCA CYCLE

1. Complete Oxidation of Acetyl-CoA (CO_2 Removal Steps)

During the citric acid cycle, two carbon dioxide molecules are removed in the following reactions:
Step 3, oxalosuccinate to alpha ketoglutarate and **Step 4,** alpha ketoglutarate to succinyl-CoA.

Acetyl-CoA contains 2 carbon atoms. These two carbon atoms are now removed as CO_2 in steps 3 and 4. Net result is that **acetyl-CoA is completely oxidized during one turn of cycle.**

2. ATP Generating Steps in TCA Cycle

Per turn of the cycle, **10 high energy** phosphates are produced. These steps are marked in Figure 13.3 and in Table 13.1.

3. Final Common Oxidative Pathway

Citric acid cycle may be considered as the final common oxidative pathway of all foodstuffs. As shown in Figure 13.6, all the major ingredients of food stuffs are finally oxidized through the TCA cycle.

4. Integration of Major Metabolic Pathways

i. **Carbohydrates** are metabolized through glycolytic pathway to pyruvate, then converted to acetyl-CoA, which enters the citric acid cycle.

Table 13.1: ATP generation steps

Step No.	*Reactions*	*Coenzyme*	*ATP generated*
3	Isocitrate → alpha ketoglutarate	NADH	2.5
4	Alpha ketoglutarate → succinyl-CoA	NADH	2.5
5	SuccinylCoA → Succinate	GTP	1.0
6	Succinate → Fumarate	$FADH_2$	1.5
8	Malate → Oxaloacetate	NADH	2.5
	Total		10

Note: In the first edition of this Textbook, calculations were made assuming that in the electron transport chain, NADH produces 3 ATPs and $FADH_2$ generates 2 ATPs. This will amount to generation of 12 ATPs per acetyl-CoA molecule. Recent experiments show that these old values are overestimates.

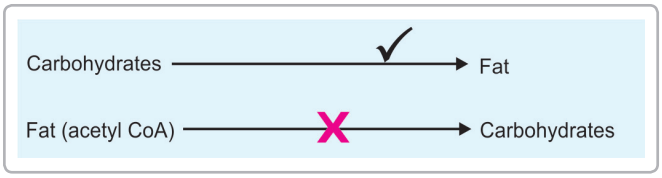


Figure 13.4: Fat cannot be converted to glucose

ii. **Fatty acids** through beta-oxidation, are broken down to acetyl-CoA and then enters this cycle.
iii. **Amino acids** after transamination enter into some or other points in this cycle (Fig. 13.6).
iv. The integration of metabolisms is achieved at junction points by key metabolites (Figs 13.1 and 13.6).

5. Excess carbohydrates are converted as fat

Excess calories are deposited as neutral fat in adipose tissue. The pathway is glucose to pyruvate to acetyl-CoA to fatty acid. However, **fat cannot be converted to glucose** because pyruvate dehydrogenase reaction (pyruvate to acetyl-CoA) is an **absolutely irreversible** step (Fig. 13.4).

6. No Net Synthesis of Carbohydrates from Fat

Acetyl-CoA entering the cycle is completely oxidized to CO_2 by the time circle reaches succinyl-CoA (Fig. 13.2). So, acetyl-CoA is completely broken down in the cycle. Thus acetyl-CoA cannot go for gluconeogenesis. Therefore, there is no net synthesis of carbohydrates from fat (Fig. 13.4).

7. Amphibolic Pathway

All other pathways such as beta-oxidation of fat or glycogen synthesis are either catabolic or anabolic. But TCA cycle is truly amphibolic (catabolic + anabolic). It is also called amphipathic in nature. (Greek word, amphi = both; pathos = feeling). There is a continuous influx (pouring into) (Fig. 13.6) and a continuous efflux (removal) of 4-carbon units from the TCA cycle (Fig. 13.5). In a traffic circle, many roads converge and traffic is followed towards one way. Since various compounds enter into or leave from TCA cycle, it is sometimes called as "**metabolic traffic circle**".

8. Anaplerotic Role of TCA Cycle

The citric acid cycle acts as a source of precursors of biosynthetic pathways, e.g. heme is synthesized from succinyl-CoA and aspartate from oxaloacetate. To counterbalance such losses, and to keep the concentrations of the 4-carbon units in the cell, anaplerotic reactions

are essential. This is called anaplerotic role of TCA cycle (Greek word, ana = up; plerotikos = to fill). Anaplerotic reactions are "**filling up**" reactions or "influx" reactions which supply 4-carbon units to the TCA cycle (Fig. 13.6).

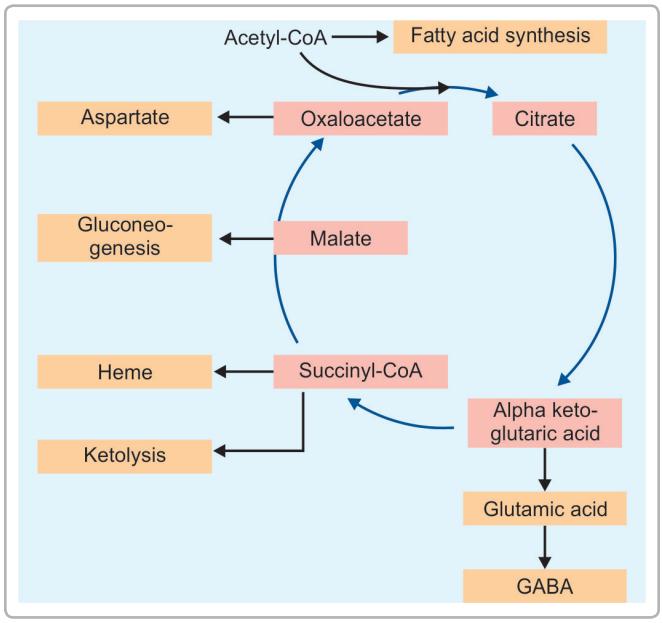


Figure 13.5: Efflux of TCA cycle intermediates

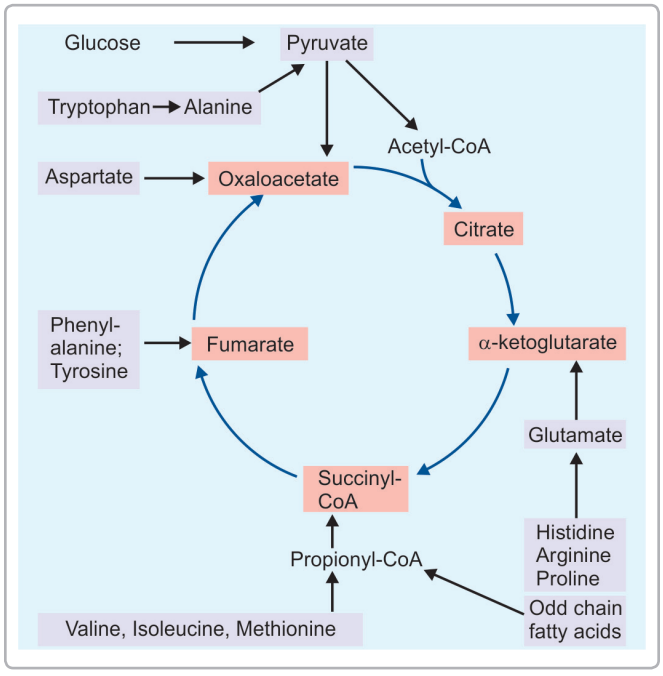


Figure 13.6: Influx of TCA cycle intermediates

REGULATION OF THE CITRIC ACID CYCLE

1. **Citrate:** The formation of citrate from oxaloacetate and acetyl CoA is an important part of control (see step 1, Fig. 13.3). **ATP acts as an allosteric inhibitor of citrate synthase.** Citrate allosterically inhibits PFK, the key enzyme of glycolysis.
2. **Availability and cellular need of ATP:** When the energy charge of the cell is low, as indicated by high level of NAD^+ and FAD, the cycle operates at a faster rate. **The cycle is tightly coupled to the respiratory chain providing ATP.** The Krebs cycle is the largest generator of ATP among metabolic pathways.

Inhibitors of TCA Cycle

The above-said mechanisms are physiological and regulatory in nature. But the following are toxic or poisonous (non-physiological) agents which inhibit the reactions. These are shown in Figure 13.3.

A. Aconitase (citrate to aconitate) is inhibited by fluoroacetate. This is non-competitive inhibition.

B. Alpha ketoglutarate dehydrogenase is inhibited by Arsenite (non-competitive inhibition).

C. Succinate dehydrogenase (succinate to fumarate) is inhibited by malonate; this is competitive inhibition.

Stages of oxidation of foodstuffs

First stage

Digestion in the gastrointestinal tract converts the macromolecules into small units. For example, proteins are digested to amino acids. This is called **primary metabolism** (Fig. 13.7).

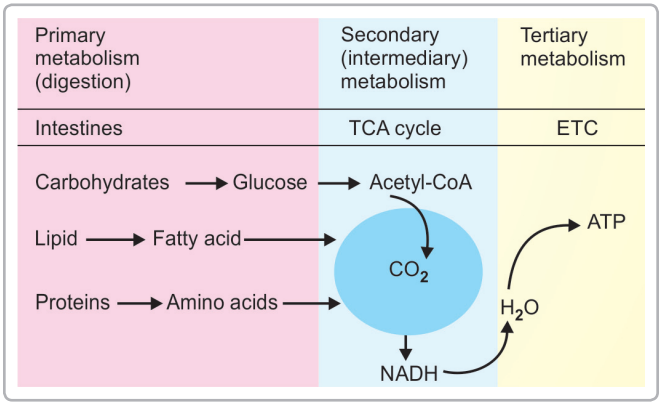


Figure 13.7: Oxidation of food stuffs in three stages

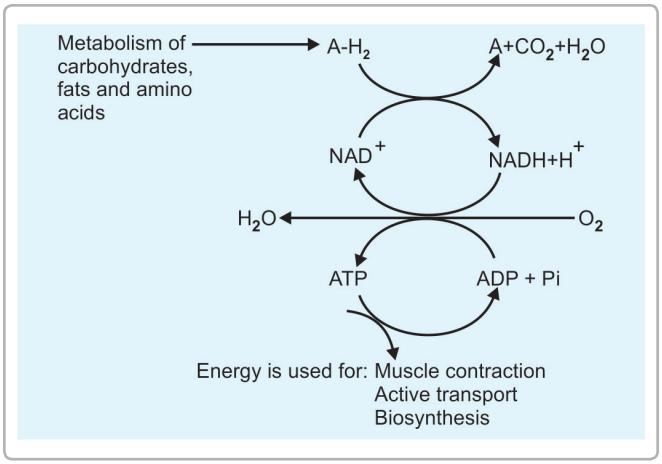


Figure 13.8: ATP generation. Food is catabolized; energy from food is trapped as ATP; it is then used for body anabolism

Second stage

Then these products are absorbed, catabolized to smaller components, and ultimately oxidized to CO_2. The reducing equivalents are mainly generated in the mitochondria by the final common oxidative pathway, citric acid cycle. In this process, NADH or $FADH_2$ are generated. This is called **secondary or intermediary metabolism**.

Third stage

Then these reduced equivalents enter into the **electron transport** chain (ETC), or **Respiratory** chain, where energy is released. This is the **tertiary metabolism** or **Internal respiration** or cellular respiration (Figure 13.7). This energy is then used for body synthetic purpose (Fig. 13.8).

REDOX POTENTIALS

Redox potential of a system is the electron transfer potential E_0'. **Oxidation is defined as the loss of electrons and reduction as the gain in electrons.** When a substance exists both in the reduced state and in the oxidized state, the pair is called a **redox couple**.

Substrate Level Phosphorylation

Here energy from a high energy compound is directly transferred to nucleoside diphosphate to form a triphosphate without the help of electron transport chain, e.g.

- **a.** Bisphospho glycerate kinase (For details refer Chapter 5);
- **b.** Pyruvate kinase (For details refer Chapter 5);
- **c.** Succinate thiokinase (step 5, Fig. 13.2).

Biological Oxidation

The transfer of electrons from the reduced coenzymes through the respiratory chain to oxygen is known as biological oxidation. Energy released during this process is trapped as ATP. This coupling of oxidation with phosphorylation is called **oxidative phosphorylation**. In the body, this oxidation is carried out by successive steps of **dehydrogenations**.

Electron Transport Chain

The electron flow occurs through successive dehydrogenase enzymes, together known as electron transport chain (ETC) (Fig. 13.10). **The electrons are transferred from higher potential to lower potential.**

Energetics of Oxidation Phosphorylation

The free energy change between NAD^+ and water is equal to 53 kcal/mol. This is so great that, if this much energy is released at one stretch, body cannot utilize it. Hence with the help of ETC assembly, the total energy change is released in small increments so that energy can be trapped as **chemical bond energy, ATP** (Fig. 13.8).

NAD^+ linked dehydrogenases

NAD^+ is derived from nicotinic acid, a member of the vitamin B complex. When the NAD^+ accepts the two hydrogen atoms, one of the hydrogen atoms is removed from the substrate as such. The other hydrogen atom is split into one hydrogen ion and one electron. The electron is also accepted by the NAD^+ so as to neutralize the charge on the coenzyme molecule. The remaining hydrogen ion is released into the surrounding medium.

$$H2 \text{ ----------} \rightarrow H + H^+ + e^-$$

$$AH2 + NAD^+ \rightarrow A + NADH + H^+$$

The NAD^+ linked dehydrogenases are

- **i.** Glyceraldehyde-3-phosphate dehydrogenase
- **ii.** Isocitrate dehydrogenase
- **iii.** Glutamate dehydrogenase
- **iv.** Beta hydroxy acyl-CoA dehydrogenase
- **v.** Pyruvate dehydrogenase
- **vi.** Alpha ketoglutarate dehydrogenase.

FAD-linked Dehydrogenases

When FAD is the coenzyme, (unlike NAD^+), both the hydrogen atoms are attached to the flavin ring. Examples:

- **i.** Succinate dehydrogenase (step 6, Fig. 13.2)
- **ii.** Fatty acyl CoA dehydrogenase (For details refer Chapter 9);

Table 13.2: High-energy compounds	
Energy rich compound	*G0´ in kcal / mol*
High energy phosphates	
1. Nucleotides: (ATP, GTP, UTP, UDP-glucose)	– 7.3 kcal
2. Creatine phosphate	–10.5 kcal
3. 1,3-bisphosphoglycerate	–10.1 kcal
4. Phosphoenolpyruvate	–14.8 kcal
5. Inorganicpyrophosphate	– 7.3 kcal
High energy thioesters (Sulfur compounds)	
6. CoA derivatives:	
Acetyl-CoA	– 7.5 kcal
Succinyl-CoA	
Fatty acyl-CoA	
HMG-CoA	
7. S-adenosylmethionine (SAM)	– 7.0 kcal

HIGH ENERGY COMPOUNDS

These compounds when hydrolyzed will release a large quantity of energy. The high energy bond in compounds is usually indicated by a squiggle bond (~). The free energy of hydrolysis of an ordinary bond varies from –1 to –6 kcal/mol. For example, glucose-6-phosphate has a free energy of –3.3 kcal/mol. On the other hand, the free energy of high energy bonds varies from –7 to –15 kcal/mol. High energy compounds are listed in Table 13.2.

Adenosine Triphosphate (ATP)

ATP is the **universal currency of energy** within the living cells. The hydrolysis of ATP to ADP releases **–7.3 kcal/mole**. The energy in the ATP is used to drive all endergonic (biosynthetic) reactions. The energy efficiency of the cell is comparable to any machine so far invented. ATP captures the chemical energy released by the combustion of nutrients and transfers it to synthetic reactions that require energy. More than 90% of ATP are formed through the electron transport chain. Cyrus Fiske and Yellapragada Subba Row discovered ATP in 1929.

ORGANIZATION OF ELECTRON TRANSPORT CHAIN

i. In the Electron transport chain, or respiratory chain, the electrons are transferred from NADH to a chain of electron carriers. The electrons flow from the more electronegative components to the more electropositive components.

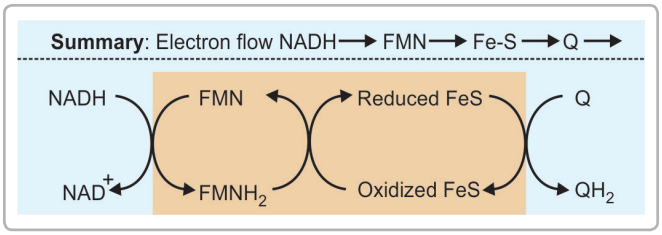


Figure 13.9: Complex I or NADH-CoQ reductase (NADH dehydrogenase ccommplex)

BOX 13.1: Summary of electron flow in ETC

Complex I: NADH → FMN → Fe-S → CoQ →
Complex II: Succinate → FAD → Fe-S → CoQ →
Complex III: CoQ →Fe-S → cyt. b → cyt.c1 →cyt. c
Complex IV: Cyt. c → cyt. a-a3 → O_2

ii. All the components of electron transport chain (ETC) are located in the **inner membrane of mitochondria**. There are four distinct multiprotein complexes.

iii. Complex I is also called NADH-CoQ reductase or NADH dehydrogenase complex (Fig. 13.9). Complex II is also named as succinate-Q-Reductase. Complex III is known as cytochrome reductase. Complex IV is cytochrome oxidase.

iv. These are connected by two mobile carriers, **co-enzyme Q** (CoQ) and **cytochrome c**. CoQ connects complex II and III. Cytochrome c is in between complex III and IV. The arrangement is schematically represented in Figure 13.10. The electron flow is shown in Box 13.1.

Sites of ATP Synthesis

Traditionally, the sites 1, 2 and 3 of ATP synthesis are marked, as shown in Fig. 13.10. However, modern chemiosmotic theory does not support such a precise localization of ATP formation.

Chemiosmotic Theory

The coupling of oxidation with phosphorylation is termed **oxidative phosphorylation**. The transport of electrons from inside to outside of inner mitochondrial membrane is accompanied by the generation of a proton gradient across the membrane. Protons (H^+ ions) accumulate outside the membrane, creating an **electrochemical potential** difference. This proton motive force drives the synthesis of ATP by ATP synthase complex. A summary is shown in Fig. 13.11.

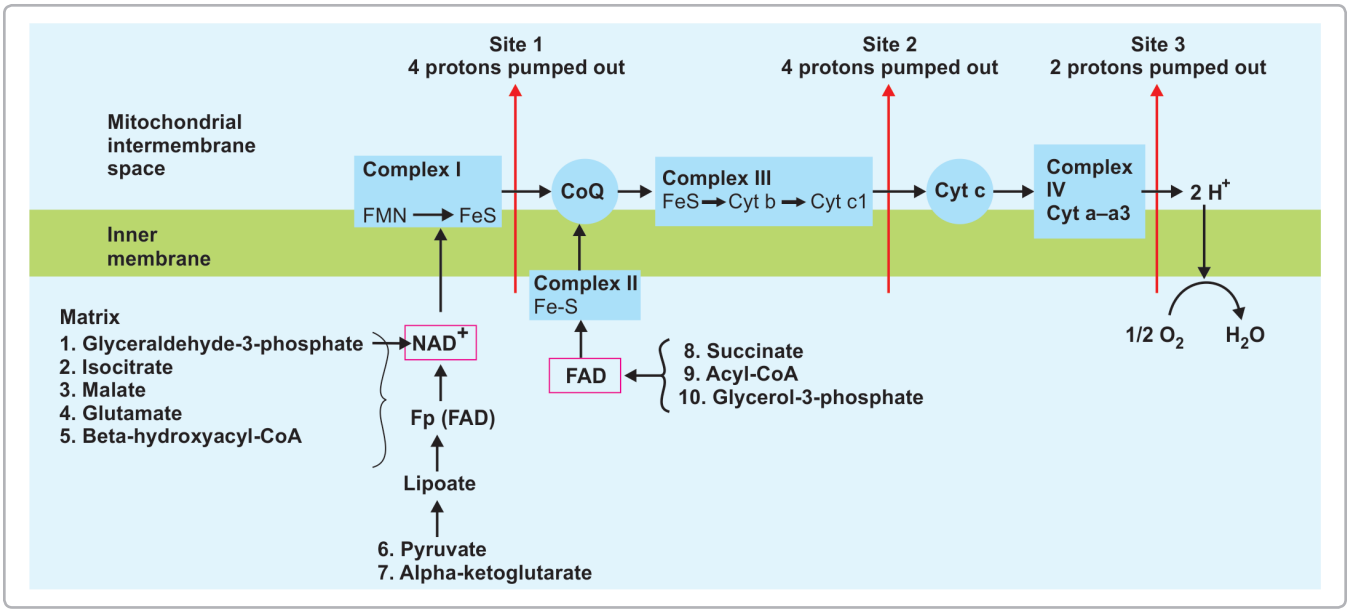


Figure 13.10: Components and sequence of reactions of electron transport chain

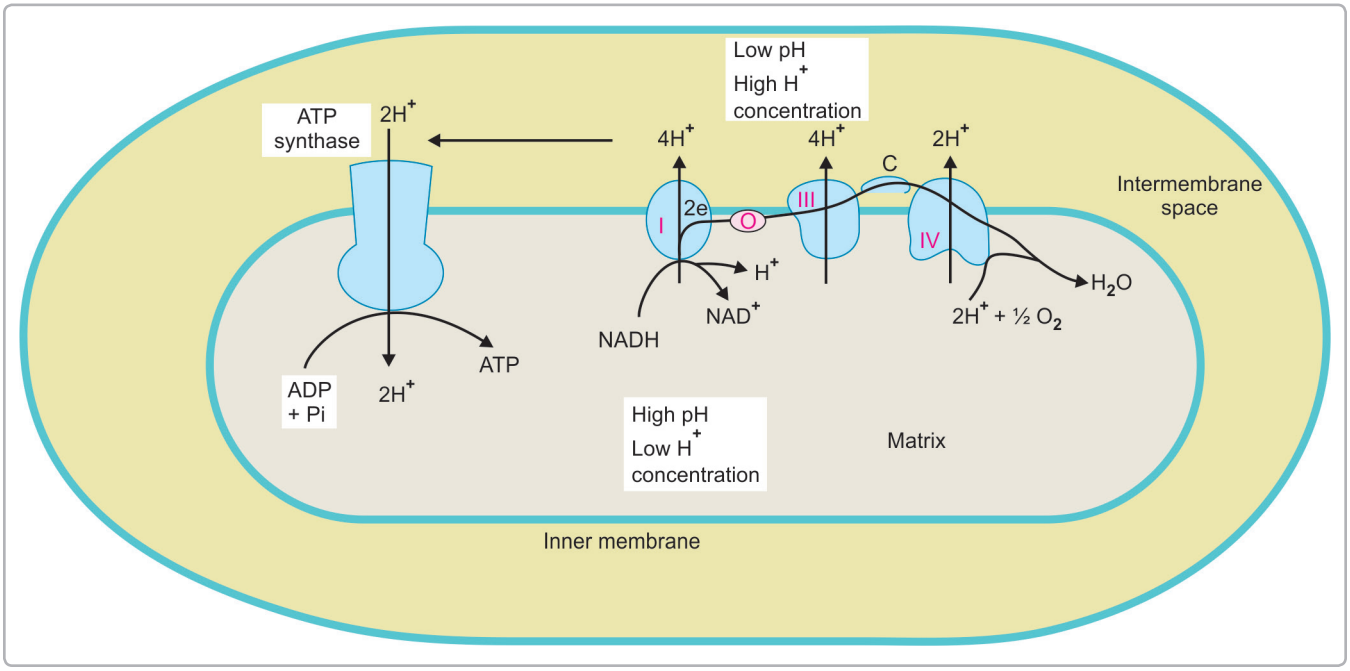


Figure 13.11: Summary of ATP synthesis. One mitochondrion is depicted, with inner and outer membranes. ETC complexes will push hydrogen ions from matrix into the intermembrane space. So, intermediate space has more H^+ (highly acidic) than matrix. So, hydrogen ions tend to leak into matrix through Fo. Then ATPs are synthesized. I, II, III, IV = components of ETC

The proton pumps (complexes I, III and IV) expel H^+ from inside to outside of the inner membrane. So, there is high H^+ concentration outside the inner membrane. This causes H^+ to enter into mitochondria through the channels (Fo); this proton influx causes ATP synthesis by ATP synthase.

ATP Synthase (5th Complex)

It is a protein assembly in the inner mitochondrial membrane. It is sometimes referred to as the **5th Complex** (See left hand structure in Fig. 13.11). It has two units; Fo and F1 units. **Fo** serves as a proton channel, through which protons enter into mitochondria. **F1 Unit** catalyzes the ATP synthesis.

Energetics of ATP Synthesis

When NADH is oxidized, about 2.5 ATP molecules are generated. About 40% energy is trapped, and rest is dissipated as heat.

Inhibitors of ATP Synthesis

i. **Atractyloside** inhibits the translocase whereas **oligomycin** acts through one of the proteins present in the Fo–F1 stalk (Table 13.3).
ii. Ionophores are lipid soluble compounds that increase the permeability of lipid bilayers to certain ions. There are two types of ionophores; mobile ion carries (e.g. **valinomycin**) and channel formers (e.g. **gramicidin**). Valinomycin allows potassium to permeate mitochondria and dissipate the proton gradient.
iii. The toxicity of cyanide is due to its inhibitory effect on the terminal cytochrome which brings cellular respiration to a standstill.

Table 13.3: Compounds which affect electron transport chain and oxidative phosphorylation

1. Site-1 (complex I to Co-Q) specific inhibitors Barbiturates (amobarbital), sedative
2. Site-2 (complex III to cytochrome c) inhibitors BAL (British anti-Lewisite), antidote of war gas
3. Site-3 (complex IV) inhibitors Carbon monoxide, inhibits cellular respiration Cyanide (CN^-)
4. Site between succinate dehydrogenase and Co-Q Malonate, competitive inhibitor of succinate DH
5. Inhibitors of oxidative phosphorylation Atractyloside, inhibits translocase Oligomycin, inhibits flow of protons through Fo Ionophores, e.g. Valinomycin
6. Uncouplers 2,4-dinitrophenol (2,4-DNP)
7. Physiological uncouplers Thyroxine, in high doses

A QUICK LOOK

1. Citric acid cycle is the final common oxidative pathway that oxidizes acetyl-CoA to CO_2. It also acts as a link between catabolic and anabolic pathways (amphibolic role).
2. Citric acid cycle is the source of reduced coenzymes (that form substrate for respiratory chain) as well as precursors for synthesis of proteins and nucleotides (anaplerotic pathway).
3. The sources of acetyl-CoA are pyruvate (from glycolysis) fatty acids (beta-oxidation), and ketogenic amino acids.
4. All enzymes of the cycle are located inside the mitochondria.
5. 3 NADH molecules are generated in the cycle at Steps 3, 4 and 8. One $FADH_2$ is formed at steps and one GTP is formed at step 5.
6. Both the carbon atoms of acetyl-CoA are removed as CO_2 at steps 3 and 4.
7. 10 molecules of ATP are produced per turn of the TCA cycle (1 $FADH_2$ = 1.5 ATP, 3 NADH = (3 × 2.5= 7.5 ATP, 1 GTP = 1 ATP). It is the main generator of ATP among metabolic pathways.
8. Alpha ketoglutarate dehydrogenase is the only irreversible step in the TCA cycle.
9. Oxaloacetate is the true catalyst, which enters and leaves the cycle unchanged.
10. Oxidation of fat (acetyl-CoA) needs help of oxaloacetate whose major source is pyruvate (carbohydrates). In other words, fats are burnt in the fire of carbohydrates.
11. Fat cannot be converted to glucose, as pyruvate to acetyl-CoA is an irreversible step.
12. The TCA cycle is regulated by cellular need of ATP.
13. Deficiency of pyruvate dehydrogenase causes lactic acidosis and neurological disorders.
14. Oxidation of food stuff occurs in 3 stages—primary metabolism where macromolecules are converted to smaller units, secondary metabolism where reducing equivalents are formed and tertiary metabolism where energy is released.
15. Oxidation is loss of electrons and reduction is gain of electrons. A pair that exists in both oxidized and reduced state is a redox couple.
16. In substrate level phosphorylation, energy from high-energy compound is directly transferred to NDP to form NTP without the help of electron transport chain.
17. Transfer of electrons from reduced coenzymes through respiratory chain to O_2 is known as biological oxidation.
18. The energy released is trapped as ATP. This coupling of oxidation with phosphorylation is called Oxidative phosphorylation. All enzymes of biological oxidation are oxidoreductases.
19. Electron flow occurs through successive dehydrogenase enzymes (located in the inner mitochondrial membrane), together known as Electron Transport Chain; the electrons are transferred from higher to lower potential.
20. NADH is impermeable to mitochondrial membrane. Hence, it is transferred via malate-aspartate shuttle in liver, kidney and heart as NADH reducing equivalents and in skeletal muscles as $FADH_2$ through glycerol 3-phosphate shuttle.
21. The ETC has 4 distinct multiprotein complexes— viz; Complex I, II, III and IV connected by two mobile carriers to CoQ and cytochrome c.
22. Inhibitors of oxidative phosphorylation include atractyloside and oligomycin. Cyanide inhibits terminal cytochrome and brings cellular respiration to stand still.

CHAPTER 14

Heme and Hemoglobin

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Structure of heme
- Biosynthesis of heme
- Porphyrias
- Bilirubin metabolism
- Jaundice
- Structure of hemoglobin
- Transport of carbon dioxide
- Fetal hemoglobin, carboxyhemoglobin
- Methemoglobin, hemoglobinopathies
- Sickle cell anemia, thalassemias
- Myoglobin

Red blood cells (RBCs) are biconcave disks, with a diameter of about 7 microns. RBCs live for about 120 days in peripheral circulation. 100 mL blood contains about **14.5 g of Hb**. Mature RBC is non-nucleated; **have no mitochondria and does not contain TCA cycle enzymes.** However, the glycolytic pathway is active which provides energy and 2,3-bisphospho glycerate (2,3-BPG). The HMP shunt pathway provides the nicotinamide adenine dinucleotide phosphate (NADPH). RBC formation in the bone marrow requires amino acids, iron, copper, folic acid, vitamin B12, vitamin C, pyridoxal phosphate, pantothenic acid and hemopoietin.

STRUCTURE OF HEMOGLOBIN

Hemoglobin is a **conjugated protein** having heme as the prosthetic group and the protein, the globin. It is a tetrameric protein with 4 subunits, each subunit having a prosthetic heme group and the globin polypeptide.

The polypeptide chains are usually **two alpha and two beta** chains. Hemoglobin has a molecular weight of about 67,000 Daltons. Each gram of Hb contains **3.4 mg of iron**. Heme is produced by the combination of iron with a porphyrin ring.

Structure of Heme

Heme is usually pronounced as "heem". Heme is a derivative of the porphyrin. **Porphyrins** are cyclic compounds formed by fusion of **4 pyrrole rings** linked by methenyl (=CH-) bridges. Since an atom of iron is present, heme is a **ferroprotoporphyrin**. The pyrrole rings are named as I, II, III, IV and the bridges as alpha, beta, gamma and delta. The possible areas of substitution are denoted as 1 to 8 (Fig. 14.1). When the substituent groups have a symmetrical arrangement (1,3,5,7 and 2,4,6,8) they are called the I series. The III series have an asymmetrical distribution of substituent groups (1,3,5,8, and 2,4,6,7) (Table 14.1).

Type III is the most predominant in biological systems. It is also called series 9, because Fischer, the pioneer in porphyrin chemistry has placed it as the 9th in a series of 15 possible isomers. The usual substitutions are

a. Propionyl ($-CH_2-CH_2-COOH$) group
b. Acetyl ($-CH_2-COOH$) group
c. Methyl ($-CH_3$) group
d. Vinyl ($-CH=CH_2$) group.

The structure of heme is shown in Fig. 14.2.

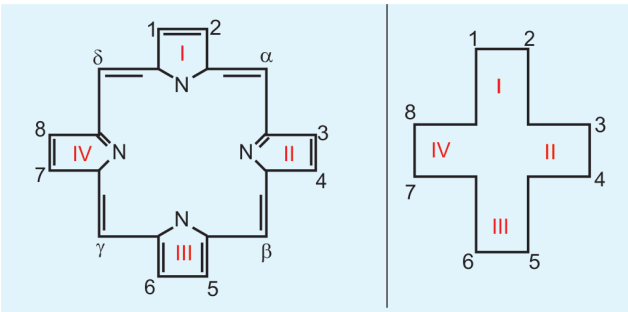


Figure 14.1: Porphyrin ring

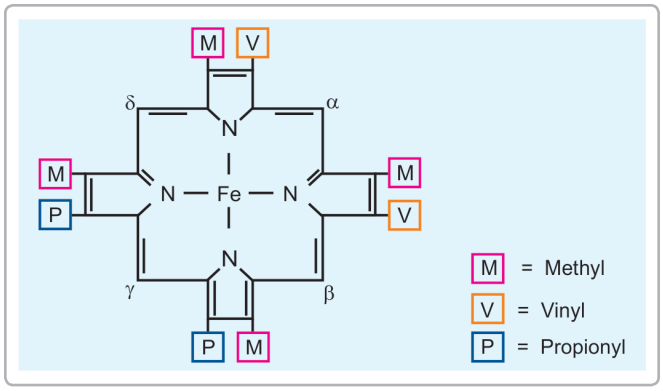


Figure 14.2: Structure of heme

Table 14.1: Porphyrins of biological importance. See also Fig.14.2 for the structure of heme

Name of Porphyrin	*Order of substituents from 1st to 8th positions*
Uroporphyrin I	A,P, A,P, A,P, A,P
Uroporphyrin III	A,P, A,P, A,P, P,A
Coproporphyrin I	M,P, M,P, M,P, M,P
Coproporphyrin III	M,P, M,P, M,P, P,M
Protoporphyrin III	M,V, M,V, M,P, P,M

(A = acetyl; P = propionyl; M = methyl; V = vinyl)

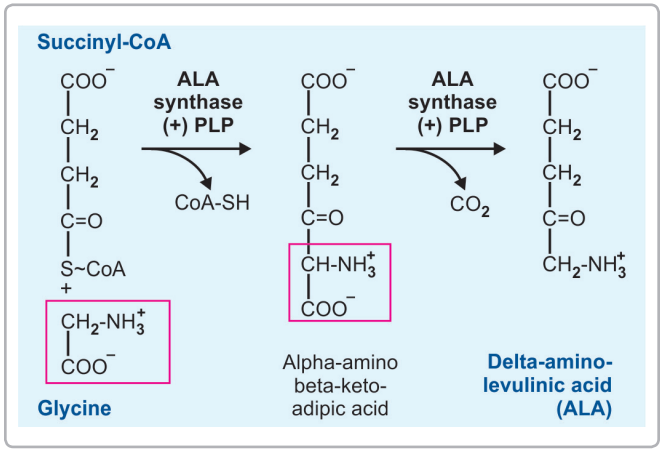


Figure 14.3: Step 1 of heme synthesis

Biosynthesis of Heme

Heme can be synthesized by almost all the tissues in the body. Heme is synthesized in the normoblasts, but not in the matured ones. The pathway is partly cytoplasmic and partly mitochondrial.

ALA synthesis: Succinyl-CoA and glycine are condensed to form alpha aminolevulinic acid (ALA). The enzyme is ALA synthase. It is the **rate-limiting** enzyme. It needs **pyridoxal phosphate** (Fig. 14.3). Hence, anemia may be manifested in pyridoxal deficiency. The enzyme **ALA synthase** is in **mitochondria**.

Remaining steps are summarized in Fig. 14.4.

Iron atom is coordinately linked with 5 nitrogen atoms (4 nitrogen of pyrrole rings of protoporphyrin and 1st nitrogen atom of a histidine residue of globin). The remaining valency of iron atom is satisfied with water or oxygen atom (Fig. 14.5).

Regulation of Heme Synthesis

ALA synthase is regulated by **repression** mechanism. **Heme** inhibits the synthesis of ALA synthase by acting as a co-repressor. Drugs like **barbiturates** induce heme synthesis. The steps catalyzed by ferrochelatase and ALA dehydratase are inhibited by **lead**.

Disorders of Heme Synthesis

Porphyrias are a group of inborn errors of metabolism associated with the biosynthesis of heme. (Greek 'porphyria' means purple). There is increased production and excretion of porphyrins and or their precursors (ALA + PBG). Porphyrias are **not associated with anemia**. Porphyrias may be classified into hepatic and erythropoietic porphyrias. A classical example of hepatic variety is acute intermittent porphyria.

Acute Intermittent Porphyria (AIP)

It is inherited as an autosomal **dominant** trait. **PBG-deaminase** is deficient. This leads to a secondary increase in activity of ALA synthase, since the end-product inhibition is not effective. The levels of **ALA and**

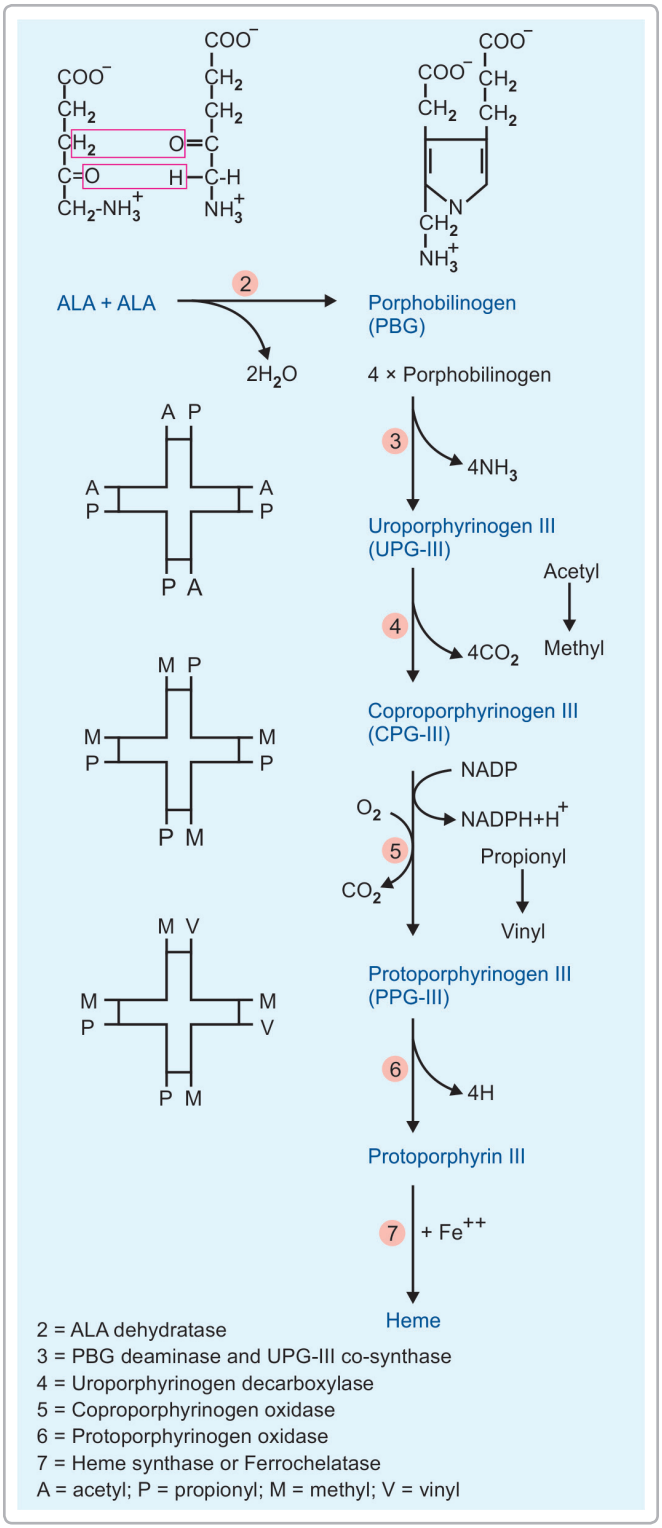


Figure 14.4: Steps of heme synthesis

PBG are elevated in blood and urine. Urine is colorless when voided, but the color is increased on standing due to photo-oxidation of PBG to porphobilin. Symptoms

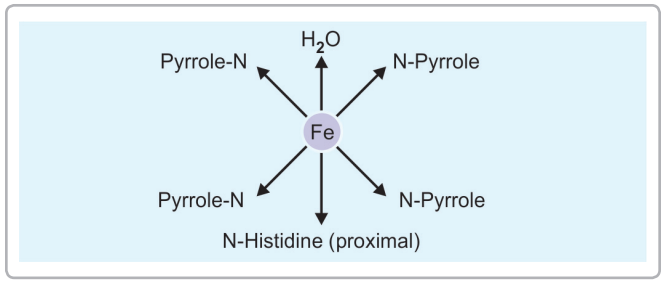


Figure 14.5: In the heme molecule, iron atom is coordinately linked with nitrogen atoms

appear intermittently. Hence, it is at times called the "little imitator". Most commonly, patients present with **acute abdominal pain**. The patients often land up with the surgeon as a case of acute abdomen and on several instances exploratory laparotomies are done. An attack is precipitated by starvation and symptoms are alleviated by a high carbohydrate diet. Drugs like barbiturates, which are known to induce ALA synthase, can precipitate an attack.

Diagnosis of Porphyrias

The presence of porphyrin precursor in urine is detected by Ehrlich's reagent. When urine is observed under ultraviolet light; porphyrins if present, will emit strong red fluorescence.

Acquired Porphyrias

Porphyria can result from **lead poisoning**. Most of the paints contain lead more than the permitted levels. Children suck painted toys; and they get the poison. The toxic effect of lead is due to inhibition of ferrochelatase. So, there is decreased levels of heme with consequent increased activity of ALA synthase.

CATABOLISM OF HEME

1. Generation of Bilirubin

The end-products of heme catabolism are bile pigments. (Box 14.1). The old RBCs breakdown, liberating the hemoglobin. The iron liberated from heme is re-utilised. The porphyrin ring is broken down in reticulo-endothelial (RE) cells of liver, spleen and bone marrow to bile pigments, mainly bilirubin (Fig. 14.6). Approximately 35 mg of bilirubin is formed from 1 g of Hb. About 300 mg of bilirubin is formed every day. **Microsomal heme oxygenase system:** Heme is degraded by a microsomal enzyme system; heme oxygenase. It requires molecular

BOX 14.1: Bile pigments and bile salts

Bile pigments are bilirubin and biliverdin. They are the breakdown products of heme; they are useless excretory products

Bile salts are the sodium salts of bile acids (glycocholate and taurocholate). They are produced from cholesterol; they help in the absorption of fat

Both bile pigments and bile salts are present in the bile

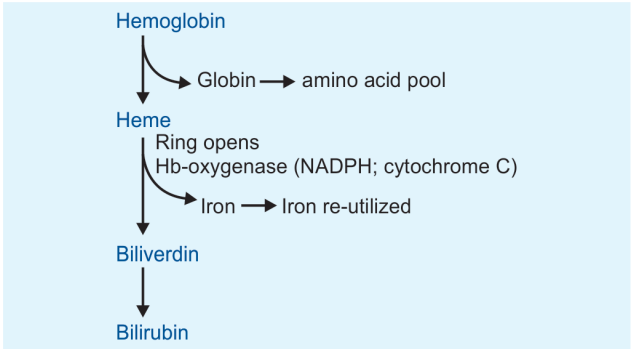


Figure 14.6: Catabolic pathway of hemoglobin

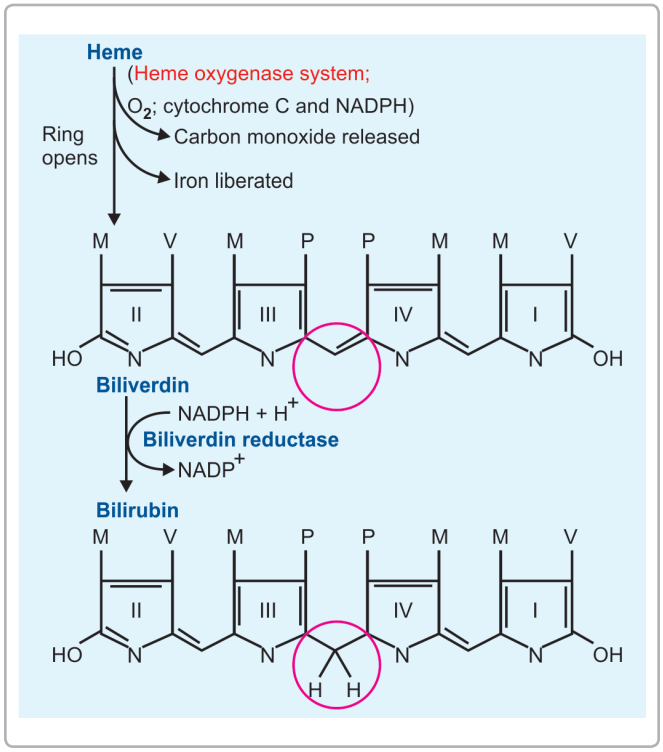


Figure 14.7: Breakdown of heme

oxygen and NADPH. The alpha methenyl bridge is broken, liberating **carbon monoxide** (Fig. 14.7). The linear tetrapyrrole formed is **biliverdin** which is green in color. In mammals, it is further reduced to **bilirubin**, a red-yellow pigment, by an NADPH dependent biliverdin reductase (Fig. 14.7).

2. Transport to Liver

The liver plays the central role in the further disposal of the bilirubin. The bilirubin formed in the reticuloendothelial cells is insoluble in water. The lipophilic bilirubin is therefore transported in plasma bound to **albumin**. Albumin takes bilirubin in loose combination. So when present in excess, bilirubin can easily dissociate from albumin. The binding sites for bilirubin on albumin can be occupied by aspirin, etc. Such drugs can, therefore, displace bilirubin from albumin. Hence, care should be taken while administering such drugs to newborn babies to avoid **kernicterus**.

3. Conjugation in Liver

Liver takes up the bilirubin from the transported complex. Inside the liver cell, the bilirubin is conjugated with glucuronic acid, to make it **water soluble** (Figure 14.8), mainly as bilirubin **diglucuronide**. Drugs like primaquine, chloramphenicol, androgens and pregnanediol may interfere in this conjugation process and may cause jaundice.

4. Excretion of Bilirubin to Bile

The water soluble conjugated bilirubin is excreted into the bile by an active process. This is the **rate-limiting step** in the catabolism of heme. It is induced by phenobarbitone.

5. Fate of Conjugated Bilirubin in Intestine

The conjugated bilirubin reaches the intestine through the bile. Intestinal bacteria **deconjugate** the conjugated bilirubin. This free bilirubin is further reduced to a colorless tetrapyrrole **urobilinogen** (UBG). Further reduction of the vinyl substituent groups of UBG leads to formation of **stercobilinogen** (SBG). The SBG is mostly excreted through feces (250–300 mg/day) (Fig. 14.8).

6. Enterohepatic Circulation

About 20% of the UBG is reabsorbed from the intestine and returned to the liver by portal blood. The UBG is again re-excreted (**enterohepatic circulation**) (Fig. 14.8). Since the UBG is passed through blood, a small fraction is excreted in urine (less than 4 mg/day).

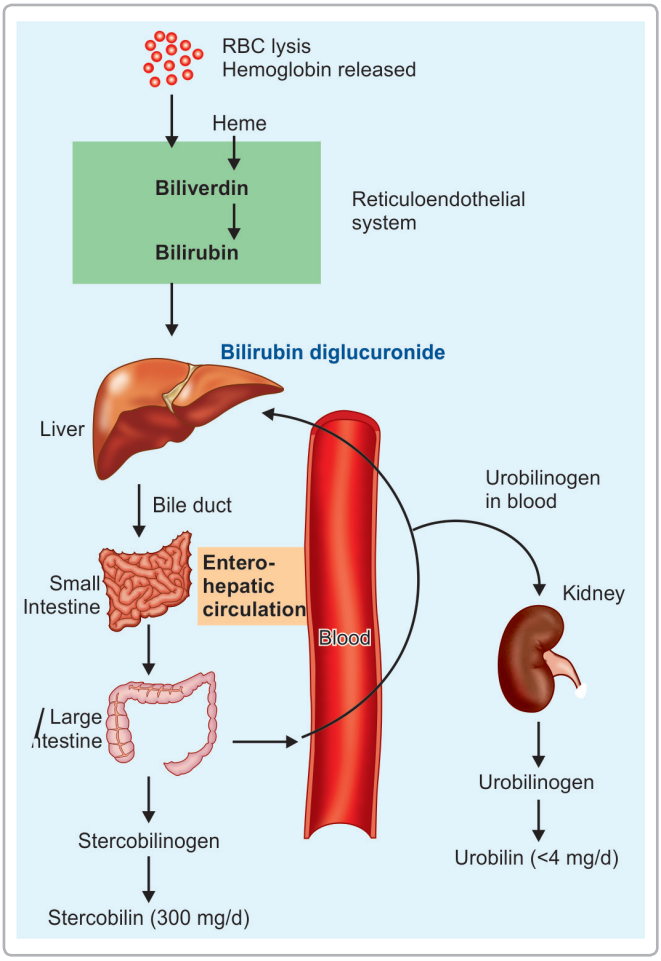


Figure 14.8: Production and excretion of bilirubin

7. Final Excretion

UBG and SBG are both colorless compounds but are oxidized to colored products, urobilin or stercobilin respectively by atmospheric oxidation. Both urobilin and stercobilin are present in urine as well as in feces.

PLASMA BILIRUBIN

Normal plasma bilirubin level ranges from **0.2–0.8 mg/dL**. The unconjugated bilirubin is about 0.2–0.6 mg/dL, while conjugated bilirubin is only 0–0.2 mg/dL. If the plasma bilirubin level exceeds 1 mg/dL, the condition is called **hyperbilirubinemia**. Levels between 1 and 2 mg/dL are indicative of **latent jaundice**. When the bilirubin level exceeds 2 mg/dL, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin and mucous membrane resulting in **jaundice.** Icterus is the Greek term for jaundice. Properties of bilirubin are shown in Table 14.2.

Table 14.2: Properties of conjugated and free bilirubin

	Free bilirubin	*Conjugated bilirubin*
In water	insoluble	soluble
In alcohol	soluble	soluble
Normal plasma level	0.2–0.6 mg/dL	0–0.2 mg/dL
In bile	absent	present
In urine	always absent	normally absent
van den Berg's test	indirect positive	direct positive

Van den Bergh Test for Bilirubin

Bilirubin reacts with *diazo reagent* (diazotized sulfanilic acid) to produce colored azo pigment. At pH 5, the pigment is purple in color. Conjugated bilirubin, being water soluble gives the color immediately; hence called **direct reaction**. Free bilirubin is water insoluble. It has to be extracted first with alcohol, when the reaction becomes positive; hence called **indirect reaction**.

HYPERBILIRUBINEMIAS

Depending on the nature of the bilirubin elevated, the condition may be grouped into conjugated or unconjugated hyperbilirubinemia. Based on the cause it may also be classified into congenital and acquired.

1. Congenital Hyperbilirubinemias

They result from abnormal uptake, conjugation or excretion of bilirubin due to inherited defects.

Crigler Najjar Syndrome

Here the defect is in **conjugation**. In Type 1 (Congenital non-hemolytic jaundice), there is severe deficiency of **UDP glucuronyl transferase**. The disease is often fatal and the children die before the age of 2. Jaundice usually appears within the first 24 hours of life. Unconjugated bilirubin level increases to more than 20 mg/dL, and hence **kernicterus** is resulted.

2. Acquired Hyperbilirubinemias

Physiological Jaundice

It is also called as neonatal hyperbilirubinemia. In all newborn infants after the 2nd day of life, mild jaundice appears. This transient hyperbilirubinemia is due to an accelerated rate of destruction of RBCs and also because of

the immature hepatic system of conjugation of bilirubin. In such cases, bilirubin does not increase above 5 mg/dL. It disappears by the second week of life.

3. Hemolytic Jaundice

Hemolytic Disease of the Newborn

This condition results from incompatibility between maternal and fetal blood groups. Rh positive fetus may produce antibodies in Rh negative mother, leading to Rh incompatibility. When blood level is more than 20 mg/dl, the capacity of albumin to bind bilirubin is exceeded. In young children before the age of 1 year, the blood-brain barrier is not fully matured, and therefore free bilirubin enters the brain (Kernicterus). It is deposited in brain, leading to mental retardation, fits, toxic encephalitis and spasticity.

Hemolytic Diseases of Adults

This condition is seen in increased rate of hemolysis. It usually occurs in adults. The characteristic features are increase in **unconjugated bilirubin** in blood, absence of bilirubinuria and excessive excretion of UBG in urine and SBG in feces (Table 14.3). Common causes are congenital spherocytosis, autoimmune hemolytic anemias and toxins like carbon tetrachloride.

4. Hepatocellular Jaundice

The most common cause is viral hepatitis, caused by hepatitis viruses A, B, C, D or G. Conjugation in liver is decreased and hence **free bilirubin** is increased in circulation. (Table 14.3).

5. Obstructive Jaundice

Conjugated bilirubin is increased in blood, and it is excreted in urine. UBG will be decreased in urine or even absent (Fig. 14.8 and Table 14.3). Since no pigments are entering into the gut, the feces become clay colored. The common causes of obstructive jaundice are:

a. Intrahepatic cholestasis. This may be due to cirrhosis or hepatoma
b. Extrahepatic obstruction. This may be due to stones in the gallbladder or biliary tract; carcinoma of head of pancreas or enlarged lymph glands in the porta hepatitis. More details on different types of jaundice are given in Chapter 30.

STRUCTURE OF HEMOGLOBIN

Normal level of hemoglobin (Hb) in blood in males is **14-16 g/dL** and in females, 13–15 g/dL. Hb is globular in shape. The adult Hb (HbA) has 2 alpha chains and 2 beta chains. Molecular weight of HbA is 67,000 Daltons. Hb F (fetal Hb) is made up of 2 alpha and 2 gamma chains. HbA2 has 2 alpha and 2 delta chains. Normal adult blood contains 97% HbA, about 2% HbA2 and about 1% HbF. Each alpha chain has 141 amino acids. The beta, gamma and delta chains have 146 amino acids. There are 38 histidine residues in Hb molecule; these are important in buffering action. The alpha and beta subunits are connected by relatively weak non-covalent bonds like van der Waals forces, hydrogen bonds and electrostatic forces.

Heme: There are 4 heme residues per Hb molecule, one for each subunit in Hb. The 4 heme groups account for about 4% of the whole mass of Hb. The heme is located in a hydrophobic cleft of globin chain. (Fig. 14.9).

Ferrous iron in hemoglobin: The iron atom of heme occupies the central position of the porphyrin ring. The reduced state is called ferrous (Fe^{++}) and the oxidized state is ferric (Fe^{+++}). In hemoglobin, iron remains in the ferrous state (Box 14.2).

Iron carries oxygen: The oxygen atom directly binds to iron atom, and forms a hydrogen bond with an imidazole nitrogen of the distal histidine. In deoxy-Hb, a water

Table 14.3: Differential diagnosis of jaundice

	Hemolytic jaundice	*Hepatocellular jaundice*	*Obstructive jaundice*
Blood, free bilirubin	Increased	Increased	Normal
Blood, conjugated bilirubin	Normal	Increased	Increased
Blood, ALP	Normal	Increased	Very high
Urine, bile salts	Nil	Nil	Present
Urine, conjugated bilirubin	Nil	Nil	Present
Urine, bilinogen	Increased	Nil	Nil

BOX 14.2: Oxygenation and oxidation

When hemoglobin carries oxygen, the Hb is **oxygenated** The iron atom in Hb is still in the ferrous state

Oxidized hemoglobin is called Met-Hb; then iron is in ferric state and the oxygen carrying capacity is lost

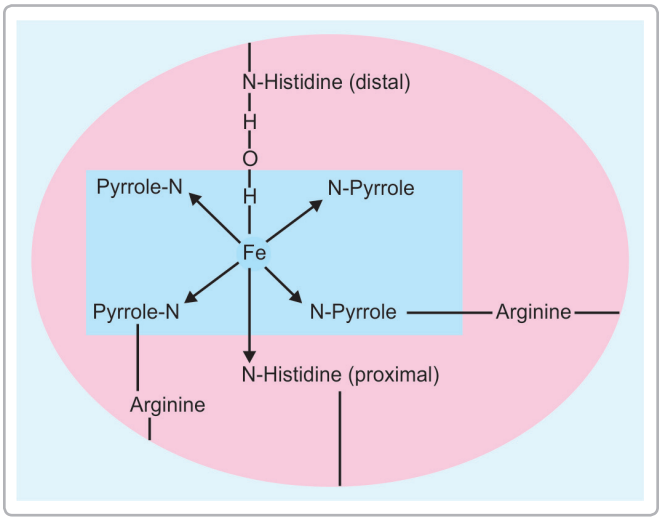


Figure 14.9: Linkage of heme with globin

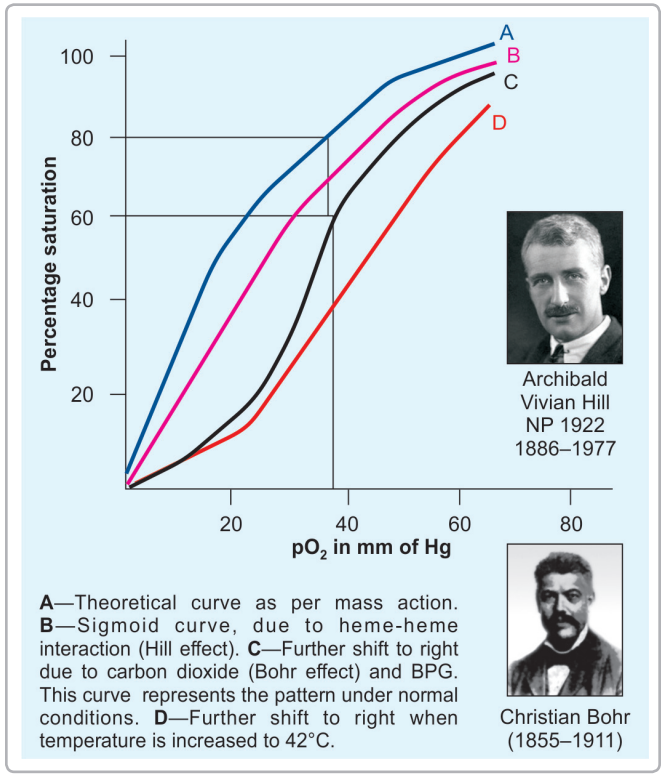


Figure 14.10: Oxygen dissociation curve (ODC)

molecule is present between the iron and distal histidine (Fig. 14.9).

TRANSPORT OF OXYGEN BY HEMOGLOBIN

Hemoglobin has all the requirements of an ideal respiratory pigment (Barcroft):

- It can transport large quantities of oxygen
- It has great solubility
- It can take up and release oxygen at appropriate partial pressures
- It is a powerful buffer.

Oxygen Dissociation Curve (ODC)

The ability of hemoglobin to load and unload oxygen at physiological pO_2 (partial pressure of oxygen) is shown by the oxygen dissociation curve (ODC) (Fig. 14.10). At the oxygen tension in the pulmonary alveoli, the Hb is **97% saturated** with oxygen. Normal blood with 15 g/dL of Hb can carry 20 mL of O2/dL of blood. In the tissue capillaries, Where the pO_2 is only 40 mm of Hg, the Hb is about **60% saturated**. So physiologically, 40% of oxygen is released (Fig. 14.10-C). In tissues, oxygen is liberated from hemoglobin. In lung capillaries, oxygen is taken up by the hemoglobin. Oxygen carriage of hemoglobin is depicted in Fig. 14.11. The following factors will affect the oxygen dissociation curve:

1. Heme-heme Interaction and Cooperativity

The sigmoid shape of the oxygen dissociation curve (ODC) is due to the allosteric effect, or cooperativity. The binding of oxygen to one heme residue increases the affinity of remaining heme residues for oxygen (Fig. 14.10B). This is called **positive** cooperativity.

$$Hb \xrightarrow[(+)O_2]{\text{Affinity 1 time}} HbO_2 \xrightarrow[(+)O_2]{\text{Affinity 2 times}} HbO_4 \xrightarrow[(+)O_2]{\text{Affinity 4 times}} HbO_6 \xrightarrow[(+)O_2]{\text{Affinity 18 times}} HbO_8$$

Thus each successive addition of O_2, increases the affinity of Hb to oxygen synergistically. Similarly, binding of 2,3-BPG at a site other than the oxygen binding site, lowers the affinity for oxygen (Fig. 14.12). When oxygenation occurs the salt bonds are broken successively (Fig. 14.12).

2. Effect of pH and pCO_2

When the pCO_2 is elevated, the H^+ concentration increases and pH falls. In the tissues, the pCO_2 is high and pH is low due to the formation of metabolic acids like lactate. Then the affinity of hemoglobin for O_2 is decreased (the ODC is shifted to the right) and so, more O_2 is released to the tissues (Fig. 14.10-C). In the lungs, the opposite reaction is found, where the pCO_2 is low, pH is high and pO_2 is significantly elevated. More O_2 binds to hemoglobin and the ODC is shifted to the left.

3. The Bohr Effect

The influence of pH and pCO_2 to facilitate oxygenation of Hb in the lungs and deoxygenation at the tissues is known as the Bohr effect binding of CO_2 forces the release of O_2. When the pCO_2 is high, CO_2 diffuses into the red blood cells. The carbonic anhydrase in the red cells favours the formation of carbonic acid (H_2CO_3).

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3 \rightarrow H^+ + HCO_3^-$$

When carbonic acid ionizes, the intracellular pH falls. The affinity of Hb for O_2 is decreased and O_2 is unloaded to the tissues.

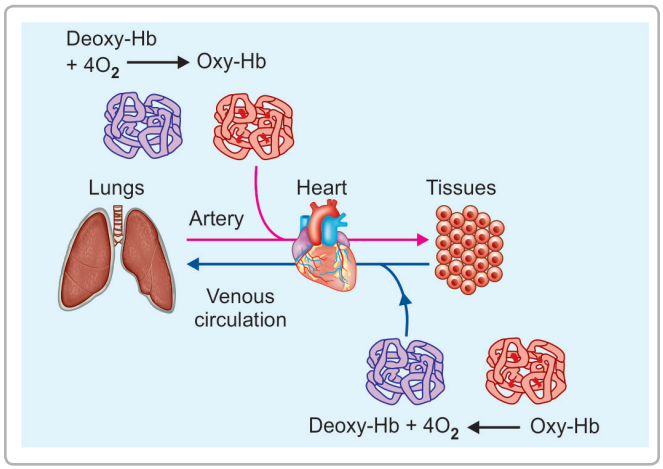


Figure 14.11: In tissues, oxy-Hb releases oxygen

5. Effect of Temperature

Metabolic demand is low when there is relative hypothermia. Shift in ODC to left at low temperature results in release of less O_2 to the tissues. On the other hand, under febrile conditions, the increased needs of O_2 are met by a shift in ODC to right (Fig. 14.10-D).

6. Effect of 2,3-BPG

The 2,3-BPG concentration is higher in young children compared to the elderly. The 2,3-BPG is produced from 1,3-BPG, an intermediate of glycolytic pathway. The 2,3-BPG, preferentially binds to deoxy-Hb. During oxygenation, BPG is released (Fig. 14.12). The high oxygen affinity of fetal blood (HbF) is due to the inability of gamma chains to bind 2,3-BPG.

TRANSPORT OF CARBON DIOXIDE

At rest, about 200 ml of CO_2 is produced per minute in tissues. The CO_2 is carried by the following 3 ways.

1. Dissolved Form

About 10% of CO_2 is transported as dissolved form.

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

The hydrogen ions thus generated, are buffered by the buffer systems of plasma.

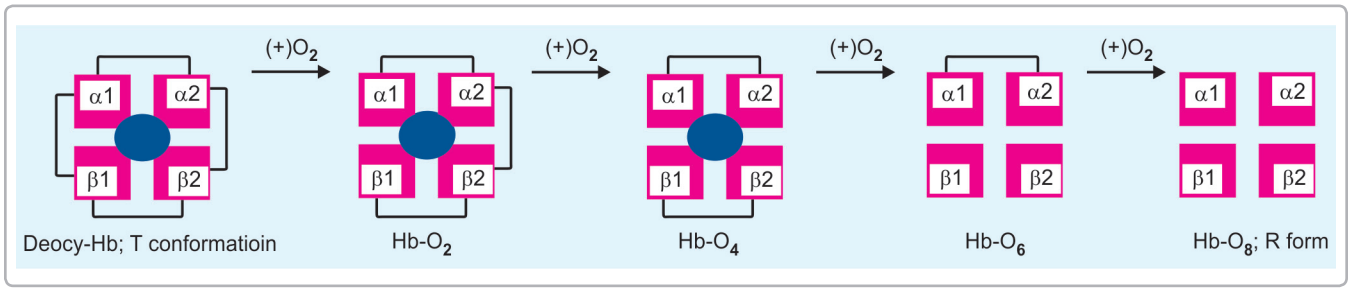


Figure 14.12: Diagramatic representation of the subunit interaction in hemoglobin. Pink rectangles represent Hb monomers. Black connection lines represent salt bridges. As oxygen is added, salt bridges are successively broken and finally 2,3-BPG is expelled. Simultaneously the T (taught) confirmation of deoxy-Hb is changed into R (relaxed) confirmation of oxy-Hb. Blue circle represents 2,3-bisphosphoglycerate (BPG)

2. Isohydric Transport of Carbon Dioxide

Isohydric transport constitutes about 75% of CO_2. It means that there is minimum change in pH during the transport. The H^+ ions are buffered by the deoxy-Hb and this is called the **Haldane effect.**

In tissues: Inside tissues, pCO_2 is high and carbonic acid is formed. It ionizes to H^+ and HCO_3^- inside the RBCs. The H^+ ions are buffered by deoxy-Hb and the HCO_3^- diffuses out into the plasma. Thus, the CO_2 is transported from tissues to lungs, as plasma bicarbonate, without significant lowering of pH. The H^+ are bound by N-terminal NH_2 groups and also by the imidazole groups of **histidine** residues.

Oxy-Hb is more negatively charged than deoxy-Hb: The isoelectric point of oxyhemoglobin is 6.6, while that of deoxy-Hb is 6.8. Thus, oxy Hb is more negatively charged than deoxy Hb. The reaction in tissues may be written as

$$OxyHb = + H^+ \rightarrow HHb^- + O_2$$

Therefore some cation is required to remove the extra negative charge of OxyHb. So H^+ are trapped. 1 millimol of deoxyHb can take up 0.6 mEq of H^+.

In the lungs: In lung capillaries, where the pO_2 is high, oxygenation of hemoglobin occurs. When 4 molecules of O_2 are bound and one molecule of hemoglobin is fully oxygenated, hydrogen ions are released.

$$H\text{–}Hb + 4O_2 \longrightarrow Hb(O_2)_4 + H^+$$

The protons released in the RBC combine with HCO_3^- forming H_2CO_3 which would dissociate to CO_2, that is expelled through pulmonary capillaries.

3. Carriage as Carbamino-Hemoglobin

The rest 15% of CO_2 is carried as carbamino-hemoglobin, without much change in pH. A fraction of CO_2 that enters into the red cell is bound to Hb as a carbamino complex.

$$R\text{–}NH_2 + CO_2 \longrightarrow R\text{–}NH\text{–}COOH$$

The N-terminal amino group (valine) of each globin chain forms carbamino complex with carbon dioxide.

Fetal Hemoglobin (HbF)

HbF has 2 alpha chains and 2 gamma chains. Gamma chain has 146 amino acids. The differences in physico-chemical properties when compared with HbA are: Increased solubility of deoxyHbF, Slower electrophoretic mobility, Increased resistance of HbF to alkali denaturation and decreased interaction with 2,3-BPG. The ODC of fetus and newborn are shifted to left. This increase in O_2 affinity is physiologically advantageous in facilitating transplacental oxygen transport. The major reason is the diminished binding of 2,3-BPG to HbF. When pO_2 is 20 mm Hg, the HbF is 50% saturated. At birth, 80% of Hb is HbF. During the first 6 months of life, it decreases to about 5% of total.

HEMOGLOBIN DERIVATIVES

OxyHb is dark red, deoxyHb is purple, metHb is dark brown, CO-Hb is cherry red and sulfHb is green in color. Normally concentration of deoxyHb is less than 5% of the total Hb. If the level increases **cyanosis** occurs.

1. Carboxy-Hemoglobin (Carbon Monoxy Hb) (CO-Hb)

Hemoglobin binds with carbon monoxide (CO) to form carboxy-Hb. The affinity of CO to Hb is 200 times more than that of oxygen. It is then unsuitable for oxygen transport.
Carbon monoxide poisoning: CO poisoning is a major occupational hazard for workers in mines. Breathing the automobile exhaust in closed space is the commonest cause for CO poisoning. One cigarette liberates 10–20 ml carbon monoxide into the lungs.
Clinical manifestations: Clinical symptoms manifest when carboxy-Hb levels exceed 20%. Symptoms are breathlessness, headache, nausea, vomiting, and pain in chest. At 40–60% saturation, death can result. Administration of O_2 is the treatment.

2A. Methemoglobin (MetHb)

When the ferrous (Fe^{++}) iron is oxidized to ferric (Fe^{+++}) state, metHb is formed. Small quantities of metHb formed in the RBCs are readily reduced back to the ferrous state by metHb reductase enzyme systems, using NADH and NADPH.

2B. Methemoglobinemias

Normal blood has only less than 1% of methemoglobin. An increase in methemoglobin in blood, (methemoglobinemia) is manifested as **cyanosis**. Aniline dye workers have been known to develop methemoglobinemia. Drugs such as acetaminophen, phenacetin, sulfanilamide, amyl nitrite, and sodium nitroprusside may cause methemoglobinemias.

HEMOGLOBIN (GLOBIN CHAIN) VARIANTS HEMOGLOBINOPATHIES

Hundreds of hemoglobin variants leading to hemoglobinopathies have been discovered (Box 14.3). The variants may be either alpha chain variants or beta chain variants.

1. Hemoglobin S (Hb S) (Sickle Cell Hemoglobin)

Of the hemoglobin variants, Hb S constitutes the **most common** variety worldwide. In 1949, Linus Pauling (Nobel prize, 1954) established that a hemoglobin with abnormal electrophoretic mobility is responsible for the sickling disease.

1A. Sickle Cell Disease

The glutamic acid in the **6th position** of beta chain of HbA is changed to valine in Hb S. This single amino acid substitution leads to polymerization of hemoglobin molecules inside RBCs. This causes a distortion of cell into sickle shape (Fig. 14.13). The substitution of hydrophilic glutamic acid by hydrophobic valine causes a localized stickiness on the surface of the molecule (Fig. 14.14). The deoxygenated Hb S may be depicted with a protrusion on one side and a cavity on the other side, so that many molecules can adhere and polymerize. The sickling occurs under deoxygenated state. The sickled cells form small plugs in capillaries. Occlusion of major vessels can lead to infarction in organs like spleen. Death usually occurs in the second decade of life.

1B. Sickle Cell Trait

In heterozygous (AS) condition, 50% of Hb in the RBC is normal. Therefore, the sickle cell trait as such does not produce clinical symptoms. Such persons can have a normal life span. At higher altitudes, **hypoxia** may cause manifestation of the disease. Chronic **lung disorders** may also produce hypoxia-induced sickling in Hb S trait. In the electrophoresis, the abnormal Hb S can be detected along with normal Hb in persons with Hb S trait (Fig. 14.15). **Hb S gives protection against malaria:** The high incidence of the sickle cell gene in population coincides with the area endemic for malaria. Hb S affords protection against *Plasmodium falciparum* infection. Hence, the abnormal gene was found to offer a biologic advantage.

2. Hemoglobin E

It is the **second most prevalent** hemoglobin variant. It is due to the replacement of beta 26 glutamic acid by

BOX 14.3: Hemoglobinopathy and thalassemia

Abnormalities in the primary sequence of globin chains lead to hemoglobinopathies, e.g. Hb S.

Abnormalities in the rate of synthesis would result in thalassemias. In other words, normal hemoglobins in abnormal concentrations result in thalassemias. e.g. beta thalassemia

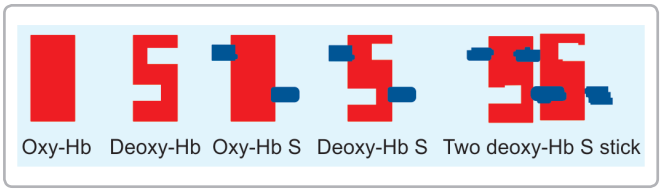


Figure 14.14: Sticky patches on Hb S molecule

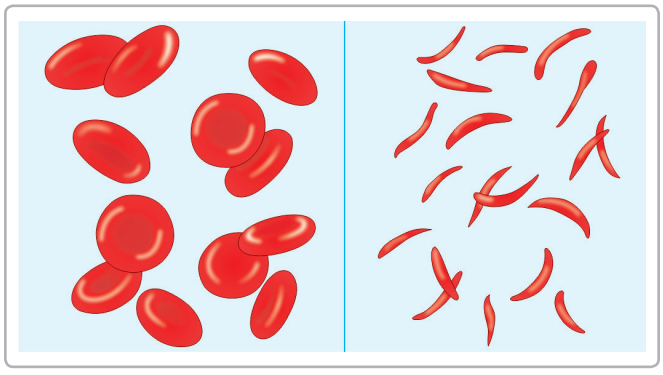


Figure 14.13: Left side normal RBCs; Right side sickle cells

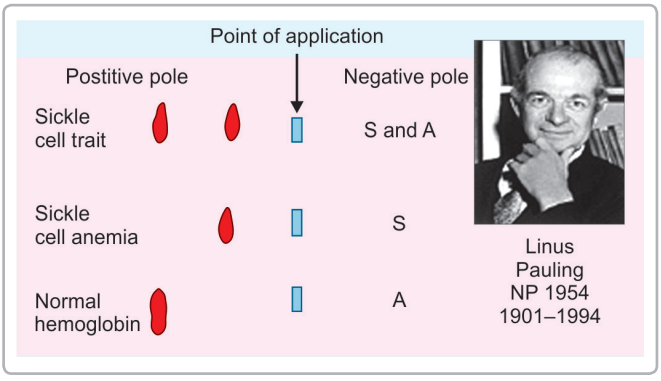


Figure 14.15: Electrophoresis of pH 8.6

Lysine. It is primarily seen in orientals of South-East Asia (Thailand, Myanmar, Bangladesh, etc.). The variant is very prevalent in West Bengal in India. Heterozygotes are completely asymptomatic. HbE has similar mobility as of A2 on electrophoresis.

THALASSEMIAS

The name is derived from the Greek word, "thalassa", which means "sea". Greeks identified this disease present around Mediterranean sea. Thalassemia may be defined as the normal hemoglobins in abnormal proportions (see Box 14.3). Reduction in alpha chain synthesis is called alpha thalassemia, while deficient beta chain synthesis is the beta thalassemia. Alpha thalassemia is rarer because alpha chain deficiency is incompatible with life.

Beta Thalassemia

Beta thalassemia is more common than alpha variety. Beta type is characterized by a decrease or absence of synthesis of beta chains. As a compensation, gamma or delta chain synthesis is increased.

Thalassemia Syndromes

All cases of thalassemias are characterized by **deficit of HbA** synthesis. Hypochromic microcytic anemia is seen. In homozygous state, clinical manifestations are severe, and hence called **Thalassemia major**, e.g. **Cooley's anemia**. In heterozygous conditions, the clinical signs and symptoms are minimal; they are called Thalassemia **minor**.

MYOGLOBIN (MB)

It is seen in muscles. Mb is a **single polypeptide** chain with 152 amino acids. One molecule of Mb can combine with 1 molecule of oxygen. Mb has **higher affinity to oxygen** than that of Hb.

ANEMIAS

In India, anemia is the most common medical problem. Perhaps about 75% of patients attending a primary health center may have signs and symptoms directly or indirectly related to anemia. Anemia results when the Hb concentration in blood is reduced. Normal value for Hb in normal male is 14 to 16 g/dL and in female 13 to 15 g/dL. If the Hb level is below 10 g/dL, it is a severe condition. The most common cause for anemia in India, is **iron deficiency** which is described in Chapter 17.

A QUICK LOOK

1. Hemoglobin—the oxygen transporter is a 67 Kd tetrameric conjugated protein with heme as prosthetic group and globin polypeptide (2α and 2β chains).
2. Heme is a derivative of porphyrin, which is a cyclic compound formed by the fusion of 4 pyrrole rings, linked by methenyl bridges and has an iron atom at its center.
3. Regulation of heme synthesis is by repression of ALA synthase by heme. Glucose prevents ALA synthase induction. Barbiturates induce the enzyme. ALA synthase is also allosterically inhibited by hematin.
4. Porphyrias are the class of metabolic disorders associated with heme synthesis. AIP occurs due to deficiency of PBG deaminase. Congenital erythropoietic porphyria is due to imbalance in activities of uroporphyrinogen I synthase and cosynthase. Acquired porphyrias result from lead poisoning.
5. Normal plasma bilirubin levels range from 0.2–1.0 mg/dL and can be detected by van den Bergh test.
6. Congenital hyperbilirubinemias include, Gilbert's disease where bilirubin uptake is defective, Crigler-Najjar syndrome where conjugation is defective Dubin-Johnson syndrome where defect is in excretion of conjugated bilirubin.
7. Acquired hyperbilirubinemias or jaundice may be physiological jaundice, hemolytic jaundice, hepatocellular jaundice and obstructive jaundice.
8. Hemoglobin (Hb) is a globular protein containing 2 alpha 2 beta (HbA), 2 alpha 2 gamma (HbF), 2 alpha 2 delta (HbA2). Alpha chain has 141 amino acids while beta, gamma and delta have 146 amino acids.
9. The ability of Hb to load and unload O_2 at physiological pO_2 is shown by the O_2 dissociation curve and its sigmoid shape is due to the allosteric effect (cooperativity).
10. The influence of pH and pCO_2 to facilitate oxygenation of Hb in lungs and deoxygenation at the tissue is known as Bohr effect.
11. Entry of Cl^- from plasma to cells to establish neutrality for the HCO_3^- out to the plasma is called chloride shift.
12. HbF moves slower than HbA on electrophoresis, is resistant to alkali denaturation, has decreased interaction with 2,3-BPG.
13. Hb S is sickle cell Hb resulting from a Glu-Val substitution at the 6th position on the beta chain. Hb S gives protection against malaria.
14. Thalassemias are caused due to functional abnormality of alpha or beta chains of Hb. Homozygous states exhibit thalassemia major while heterozygous state exhibit thalassemia minor.
15. Alpha chain gene is on chromosome 16 and beta, (gamma, delta) chain genes on chromosome 11.
16. The factors which affect oxygen binding and release are pO_2, pCO_2, pH and 2,3-BPG concentration in RBCs. Arterial blood is 97% saturated at pO_2 of 100 mm of Hg and can carry about 40 mm of Hg. In venous blood, where pO_2 is about 40 mm of Hg, Hb is only 60% saturated. Net effect is a release of 40% O_2 to tissues.
17. The positive cooperative effect is exhibited by different subunits where by binding of O_2 by one subunit favors binding of O_2 to other subunits. In a similar manner O_2 release also shows a cooperative interaction referred to as heme-heme interaction.

18. Transport of CO_2 by is called isohydric transport, which helps in buffering and the elimination of volatile acid, carbonic acid. In the tissues where pCO_2 is high, CO_2 diffuses into RBCs and combines with H_2O to form carbonic acid catalyzed by carbonic anhydrase. The H^+ formed by dissociation of H_2CO_3, is buffered by HHb, which can take up H^+ when oxygen dissociates.
19. A fraction of CO_2 is transported as carbamino Hb.
20. Fetal hemoglobin has a higher affinity for O_2 than Hb A, since it does not bind 2,3-BPG. This is beneficial in oxygen transport and delivery to tissues in fetal life.
21. The fetal Hb level falls after birth and reaches adult levels by 2 years of age (<2%).
22. In anemic children and children with beta chain defects, Hb F level remains high as a compensatory mechanism.

CHAPTER 15

Fat Soluble Vitamins

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Vitamin A
- Wald's visual cycle
- Deficiency of vitamin A
- Vitamin D
- Deficiency of vitamin D
- Vitamin E
- Vitamin K

Vitamins may be defined as organic compounds occurring in small quantities in different natural foods and necessary for growth and maintenance of good health in human beings and in experimental animals. Vitamins are essential food factors, which are required for the proper utilization of the proximate principles of food like carbohydrates, lipids and proteins. All the vitamins are usually available in an ordinary Indian diet.

The vitamins are mainly classified into two:

1. The fat soluble vitamins are A, D, E and K
2. Water soluble vitamins are B complex and C. The major differences between these two groups of vitamins are given in Table 15.1.

VITAMIN A

Chemistry

Vitamin A is fat soluble. The active form is present only in animal tissues. The **provitamin**, beta-carotene is present in plant tissues. One molecule of **beta-carotene** can theoretically give rise to two molecules of vitamin A. Vitamin A has a **beta-ionone** (cyclohexenyl) ring system. (Fig. 15.1)

Three different compounds with vitamin A activity are **retinol** (vitamin A alcohol), **retinal** (vitamin A aldehyde) and **retinoic acid**. The retinal may be reduced to retinol by retinal reductase. This reaction is readily reversible. Retinal

Table 15.1: Comparison of two types of vitamins

	Fat soluble vitamins	*Water soluble vitamins*
Solubility in fat	Soluble	Not soluble
Water solubility	Not soluble	Soluble
Absorption	Along with lipids requires bile salts	*Absorption simple
Carrier proteins	Present	*No carrier proteins
Storage	Stored in liver	*No storage
Deficiency	Manifests only when stores are depleted	*Manifests rapidly as there is no storage
Toxicity	Hypervitaminosis may result	Unlikely, since excess is excreted
Major vitamins	A, D, E and K	B and C

*Vitamin B12 is an exception

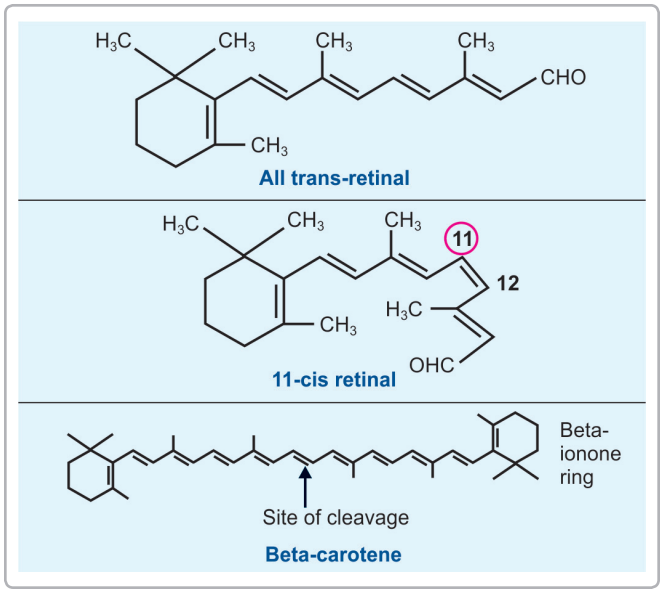


Figure 15.1: Structure of vitamin A

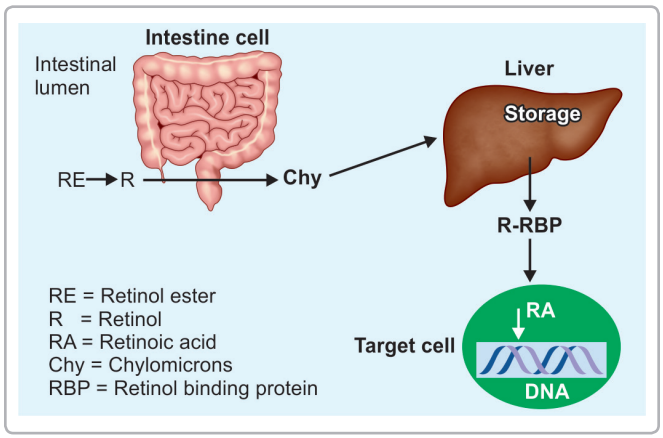


Figure 15.2: Vitamin A metabolism

is oxidized to retinoic acid, which cannot be converted back to the other forms. The side chain contains alternate double bonds, and hence many isomers are possible. The **all-trans** variety of retinal, also called **vitamin A1** is most common (Fig. 15.1). Biologically important compound is **11-cis-retinal**.

Absorption of Vitamin A

The absorption is along with other fats and requires bile salts. In biliary tract obstruction and steatorrhea, vitamin A absorption is reduced. It is carried by chylomicrons and transported to liver. In the liver cells, vitamin is stored as **retinol palmitate** (Fig. 15.2).

Transport from Liver to Tissues

The vitamin A from liver is transported to peripheral tissues as trans-retinol by the **retinol binding protein** or RBP (Fig. 15.2).

Uptake by Tissues

Inside the cytoplasm of cells, vitamin binds to cellular retinoic acid binding protein (CRBP) and finally to hormone responsive elements (HRE) of DNA. Thus genes are activated (Fig. 15.2).

Biochemical Role of Vitamin A

Wald's Visual Cycle

Wald was awarded Nobel prize in 1967, for identifying the role of vitamin A in vision. Rhodopsin is a membrane protein found in the photoreceptor cells of the retina. Rhodopsin is made up of the protein opsin and 11-cis-retinal. When light falls on the retina, the 11-cis-retinal isomerizes to all-trans-retinal (Fig. 15.3).

Generation of nerve impulse: In visual pigments, the 11-cis-retinal locks opsin in its inactive form. The isomerization and photoexcitation leads to generation of the nerve impulse. This is a G-protein coupled reaction. A single photon can excite the rod cell. The photon produces immediate conformational change in rhodopsin and all-trans-retinal is produced. The all-trans-retinal is then released from the opsin protein.

Regeneration of 11-cis-retinal

After dissociation, opsin remains in retina; but trans-retinal enters the blood circulation (Fig. 15.3). The all-trans-retinal is isomerized to 11-cis-retinal in the retina itself in the dark by the enzyme retinal isomerase. The 11-cis-retinal can recombine with opsin to regenerate rhodopsin. The all-trans-retinol is isomerized to 11-cis-retinol and then oxidized to 11-cis-retinal in liver. This is then transported to retina. This completes the Wald's visual cycle (Fig. 15.3).

Dark Adaptation Mechanism

Bright light depletes stores of rhodopsin in rods. Therefore when a person shifts suddenly from bright light to a dimly lit area, there is difficulty in seeing, for example, entering a cinema theater. After a few minutes, rhodopsin is resynthesized and vision is improved. This period is called **dark adaptation time**. It is increased in vitamin A deficiency. Red light bleaches rhodopsin to a lesser extent; so doctors use red glasses, during fluoroscopic X-ray examination of the patients.

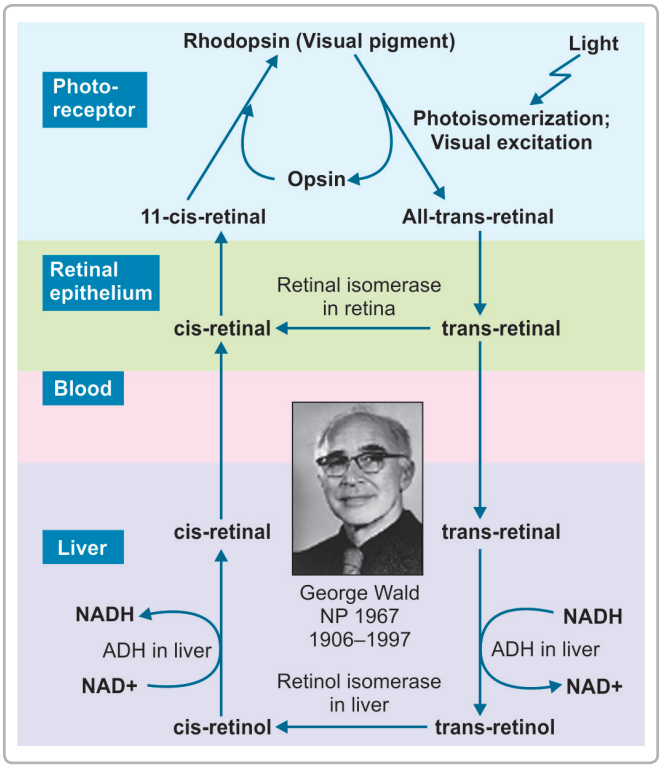


Figure 15.3: Wald's Visual Cycle. Blue color represents reactions in Photoreceptor matrix. Green background represents reactions in retinal pigment epithelium. Red depicts blood. Yellow shows reactions in liver

Rods are for Vision in Dim Light

In the retina, there are two types of photosensitive cells, the rods and the cones. Rods are responsible for perception in dim light. It is made up of 11-cis-retinal + opsin. Deficiency of cis-retinal will lead to increase in dark adaptation time and night blindness.

Cones are for Color Vision

Cones are responsible for vision in bright light as well as color vision. They contain the photosensitive protein, **conopsin** (photopsin). In cone proteins also, 11-cis-retinal is the chromophore. Reduction in number of cones or the cone proteins, will lead to **color blindness**.

Biochemical Functions of Vitamin A

Retinal is the active form required for normal vision. Retinoic acid is implicated in growth and **differentiation** of tissues. Retinol is necessary for normal **reproduction**. In vitamin deficiency, miscarriages are noticed in female rats while atrophy of germinal epithelium and sterility are seen in male rats. **Antioxidant property:** Fresh vegetables containing **carotenoids** were shown to reduce the incidence of cancer.

Deficiency Manifestations of Vitamin A

Night blindness or nyctalopia: Visual acuity is diminished in dim light. The patient cannot read or drive a car in poor light. The dark adaptation time is increased.
Xerophthalmia: The conjunctiva becomes dry, thick and wrinkled. The conjunctiva gets keratinized and loses its normal transparency. Cornea is also keratinized. Infections may supersede.
Bitot's spots: These are seen as greyish-white triangular plaques firmly adherent to the conjunctiva. This is due to increased thickness of conjunctiva in certain areas. All the ocular changes mentioned so far are completely reversible when vitamin is supplemented.
Keratomalacia: When the xerophthalmia persists for a long time, it progresses to keratomalacia (softening of the cornea). Later, corneal opacities develop. Bacterial infection leads to corneal ulceration, and total blindness.
Preventable blindness: The deficiency of vitamin A is the most common cause of blindness in Indian children below the age of 5. One-third of the world's blind population are residing in India. About 40% of blindness is preventable.
Skin and mucous membrane lesions:
Hyperkeratosis of the epithelium occurs. Epithelium is **atrophied**. The alterations in skin may cause increased occurrence of generalized **infections**.

Causes for Vitamin A Deficiency

- Decreased intake
- Obstructive jaundice causing defective absorption
- Chronic nephrosis, where RBP is excreted through urine.

Dietary Sources of Vitamin A

Animal sources include milk, butter, cream, cheese, egg yolk and liver. Fish liver oils (cod liver oil and shark liver oil) are very rich sources of the vitamin. Vegetable sources contain the yellow pigment beta carotene. Carrot contains significant quantity of beta-carotene. (Fig. 15.4). Papaya, mango, pumpkins, green leafy vegetables (spinach, amaranth) are other good sources for vitamin A activity.

Daily Requirements of Vitamin A

The recommended daily allowance (RDA) for

Children = 400 – 600 microgram. Men = 750 – 1000 microgram/day. Women = 750 microgram/day pregnancy = 1000 microgram/day



Figure 15.4: A parady of the old proverb is "One carrot a day will keep the ophthalmologist away"

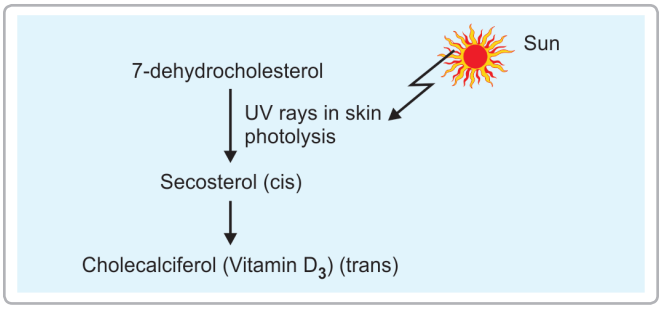


Figure 15.5: Synthesis of vitamin D3

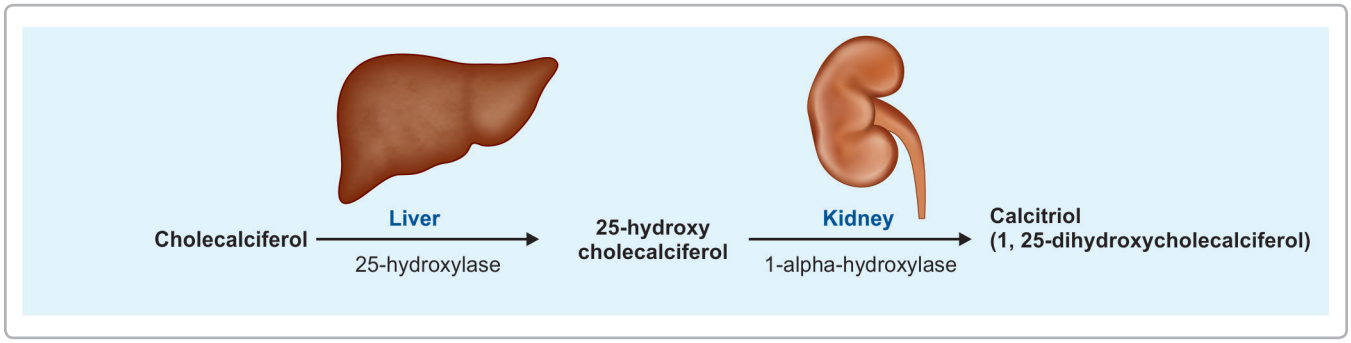


Figure 15.6: Generation of calcitriol

Hypervitaminosis A or Toxicity

Excessive intake can lead to toxicity since the vitamin is stored. It has been reported in children where parents have been overzealous in supplementing the vitamins. Symptoms of toxicity include anorexia, irritability, headache, peeling of skin, drowsiness and vomiting. Enlargement of liver is also seen in children.

BOX 15.1: Calcitriol and calcitonin are different

Calcitriol is the physiologically active form of vitamin D. It increases the blood calcium level

Calcitonin is the peptide hormone released from thyroid gland. It decreases the blood calcium

VITAMIN D (CHOLECALCIFEROL)

Formation of Vitamin D

7-dehydrocholesterol, an intermediate of a minor pathway of cholesterol synthesis, is available in the Malpighian layer of epidermis. In the skin, ultraviolet light breaks the bond, to give rise the provitamin, **secosterol**. The cis double bond between is then isomerized to a trans bond to form vitamin D3 or **cholecalciferol** (Fig. 16.5). So, vitamin D is called the **"sun-shine vitamin".** As sunshine is less in winter months, vitamin deficiency is seen in winter.

Activation of Vitamin D

Vitamin D acts like a **prohormone**. The cholecalciferol is first transported to **liver**, where hydroxylation at **25th position** occurs, to form 25-hydroxycholecalciferol (25-HCC). (Fig. 15.6). 25-HCC is the major storage form. In the **kidney**, it is further hydroxylated at the **1st position**. Thus 1,25-dihydroxy cholecalciferol (**DHCC**) is generated. Since it contains three hydroxyl groups at 1, 3 and 25 positions, it is also called **Calcitriol** (Fig. 15.6). The calcitriol thus formed is the **active form** of vitamin; it acts as a hormone (Box 15.1).

Biochemical Effects of Vitamin D

The sites of action are intestinal mucosal cells, osteoblasts of bones and distal tubular cells of kidney.

Vitamin D and Absorption of Calcium

Calcitriol promotes the absorption of calcium and phosphorus from the intestine. Absorption of calcium needs energy. **Calcitriol** acts like a steroid hormone. It

increases the synthesis of **Calbindin**. Due to the increased availability of calcium binding protein, the absorption of calcium is increased.

Effect of Vitamin D in Bone

Mineralization of the bone is increased by increasing the activity of osteoblasts. Calcitriol stimulates osteoblasts which secrete alkaline phosphatase. Due to this enzyme, the local concentration of phosphate is increased. The ionic product of calcium and phosphorus increases, leading to mineralization.

Effect of Vitamin D in Renal Tubules

Calcitriol increases the reabsorption of calcium and phosphorus by renal tubules, therefore both minerals are conserved. (Parathyroid hormone conserves only calcium).

Deficiency of Vitamin D

The deficiency diseases are **rickets** in children and **osteomalacia** in adults. Hence, vitamin D is known as antirachitic vitamin.

Causes for Vitamin D Deficiency

Nutritional deficiency of vitamin D is the most common cause. This can occur in people who are not exposed to sunlight properly, e.g. inhabitants of northern latitudes, in winter months. Malabsorption of vitamin (obstructive jaundice and steatorrhea). Abnormality of vitamin D activation. Liver and renal diseases may retard the hydroxylation reactions.

Clinical Features of Rickets

Rickets is seen in children. There is insufficient mineralization of bone. Bones become soft and pliable. The bone growth is markedly affected. The classical features of rickets are **bone deformities**. Weight-bearing bones are bent (Fig. 15.7). Continued action of muscles also cause bone malformations. The clinical manifestations include bow legs, knock-knee, rickety rosary, bossing of frontal bones, and pigeon chest.

Clinical Features of Osteomalacia

The term is derived from Greek "osteon" = bone; and "malakia" = softness. The bones are softened due to insufficient mineralization and increased osteoporosis. The abnormalities in **biochemical parameters** are a slightly lower serum calcium, and a low serum phosphate. Serum **alkaline phosphatase** is markedly increased. It may be noted that vitamin D deficiency never produces severe hypocalcemia. Tetany will not be manifested.



Figure 15.7: Bone deformity in rickets

Requirements of Vitamin D

Children = 10 microgram (400 IU)/day; Adults = 5 to 10 microgram (200 IU)/day; Pregnancy, lactation = 10 microgram/day; Senior citizens above the age of 60 = 600 IU per day.

Sources of Vitamin D

Exposure to **sunlight** produces cholecalciferol. Moreover fish liver oil, fish and egg yolk are good sources of the vitamin. Milk contains moderate quantity of the vitamin.

Hypervitaminosis D

Doses above 1500 units per day for very long periods may cause toxicity. Symptoms include weakness, polyuria, intense thirst, and calcification of soft tissues (metastatic calcification), especially in renal tissues.

VITAMIN E

Chemical Nature

They have a chromane ring (tocol) system, with an isoprenoid side chain. There are eight naturally occurring tocopherols. Of these, **alpha tocopherol** has greatest biological activity.

Biochemical Role of Vitamin E

Vitamin E is the **most powerful natural antioxidant** (For details refer Chapter 19). Free radicals are continuously

being generated in living systems. Their prompt inactivation is of great importance. The free radicals would attack bio-membranes. Vitamin E protects RBC from **hemolysis**. Gradual deterioration of **aging** process is due to the cumulative effects of free radicals. Vitamin E prevents early aging. It reduces the risk of myocardial infarction by reducing oxidation of LDL.

Interrelationship with Selenium

Selenium is present in **glutathione peroxidase**; an important enzyme that oxidizes and destroys the free radicals (For details refer Chapter 19). Selenium has been found to decrease the requirement of vitamin E and vice versa. They act synergistically to minimize lipid peroxidation.

Deficiency Manifestations of Vitamin E

In rats, inability to produce healthy ovum and loss of motility of spermatozoa and muscular dystrophy are observed. Human deficiency has not been reported. But in volunteers, vitamin E deficiency has been shown to produce muscular weakness.

Sources of Vitamin E

Vegetable oils are rich sources of vitamin E; e.g. wheat germ oil, sunflower oil, safflower oil, cotton seed oil, etc. Fish liver oils are devoid of vitamin E.

Requirement

Males 10 mg per day; Females 8 mg/day; Pregnancy 10 mg/day; Lactation 12 mg/day.

The requirement increases with higher intake of PUFA. Pharmacological dose is 200 – 400 IU/day.

VITAMIN K

Chemistry of Vitamin K

The letter "K" is the abbreviation of the German word "koagulation vitamin". They are **naphthoquinone** derivatives, with a long isoprenoid side chain. Yet another structurally similar synthetic compound having vitamin K activity is **Menadione.** It is water soluble synthetic vitamin.

Biochemical Role of Vitamin K

Vitamin K is necessary for coagulation such as Factor II (**prothrombin**) and Factor IX (Christmas factor). These factors are synthesized by the liver as inactive zymogens. They undergo gamma carboxylation of glutamic acid residues. These are the binding sites for calcium ions. The **gamma-carboxyglutamic acid** (GCG) synthesis requires vitamin K as a co-factor.

Causes for Deficiency of Vitamin K

In normal adults dietary deficiency seldom occurs since the intestinal bacterial synthesis is sufficient to meet the needs of the body. However deficiency can occur in conditions of **malabsorption** of lipids. This can result from obstructive jaundice. Prolonged **antibiotic** therapy and gastrointestinal infections with diarrhea will destroy the bacterial flora and can also lead to vitamin K deficiency.

Clinical Manifestations of deficiency

Vitamin K deficiency is manifested as **bleeding,** especially internal bleeding. Very minor injuries will go on bleeding, as effective clot formation is lacking. Prolongation of prothrombin time and delayed clotting time are characteristic of vitamin K deficiency. Hemorrhagic disease of the newborn is attributed to vitamin K deficiency. It is often advised that pre-term infants be given prophylactic doses of vitamin K (1 mg Menadione). **Warfarin** and **dicoumarol** will competitively inhibit the gamma-carboxylation system due to structural similarity with vitamin K. Hence, they are widely used as anticoagulants for therapeutic purposes.

Daily Requirements of Vitamin K

Recommended daily allowance is 50–100 microgram/day. This is usually available in a normal diet.

Sources of Vitamin K

Green leafy vegetables are good dietary sources. Even if the diet does not contain the vitamin, intestinal bacterial synthesis will meet the daily requirements, as long as absorption is normal.

A QUICK LOOK

1. Vitamin A is a fat-soluble vitamin whose active form is present only in animal tissues, but provitamin A (beta-carotene) is present in plant tissues.
2. Retinols are polyisoprenoid compounds with vitamin A activity, having the β-ionone ring system.
3. Active forms of the vitamin A include; retinol, retinal, retinoic acid. The two important isomers are all trans-retinal and 11-cis retinal.
4. Vitamin A is transported with the help of retinal binding protein and this retinal–RBP complex has specific receptors in various tissues.

5. Rhodopsin is a membrane protein made up of opsin plus 11-cis-retinal and it is important in the visual cycle.
6. Rods are for dim light vision and cones for color vision.
7. Decrease in number of cones/cone proteins lead to color blindness.
8. Vitamin D is derived from 7 dehydrocholesterol by the action of UV rays.
9. Vitamin D deficiency results in rickets and osteomalacia. Different types of rickets are; vitamin D resistant, and renal rickets.
10. Vitamin E is tocopherol. It is absorbed along with fats with the help of bile salts. It is transported as chylomicrons and stored in adipose tissue.
11. Vitamin E is the most important antioxidant in tissues.
12. Vitamin K is absorbed in intestine along with chylomicrons. They are also synthesized by intestinal flora.
13. Vitamin K is involved in blood coagulation. Vitamin K is required for post-translational modification of coagulation factors.

CHAPTER 16

Water Soluble Vitamins

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Thiamine (Vitamin B1)
- Riboflavin (Vitamin B2) and FAD
- Niacin, NAD^+ and $NADP^+$
- Pyridoxine (Vitamin B6)
- Pantothenic acid and coenzyme A
- Biotin
- Folic acid
- Vitamin B12
- Ascorbic acid (Vitamin C)

Water soluble vitamins are B complex vitamins and vitamin C.

B COMPLEX GROUP OF VITAMINS

These vitamins are chemically not related to one another. They are grouped together because all of them function in the cells as coenzymes.

THIAMINE (VITAMIN B1)

Thiamine is also called as vitamin B1 (Box 16.1).

Sources

Cereals (whole wheat flour and unpolished hand pound rice) are rich sources of thiamine. When the grains are polished, aleurone layer is usually removed. Yeast is also a very good source. Structure of thiamine is shown in Fig. 16.1.

BOX 16.1: Thiamine and thymine are different

THYMINE is the base present in DNA
THIAMINE is the vitamin B1

Physiological Role of Thiamine

Pyruvate dehydrogenase: The coenzyme form is **thiamine pyrophosphate** (TPP). It is used in oxidative decarboxylation of alpha keto acids, e.g. pyruvate decarboxylase, a component of the pyruvate dehydrogenase complex. It catalyzes the breakdown of pyruvate, to acetyl-CoA, and carbon dioxide (see Fig. 5.9).
Alpha ketoglutarate dehydrogenase: An analogous biochemical reaction that requires TPP is the oxidative decarboxylation of **alpha ketoglutarate** to succinyl-CoA and CO_2 (TCA cycle, Fig. 13.2).

Transketolase in the hexose monophosphate shunt pathway of glucose (For details refer Chapter 5).

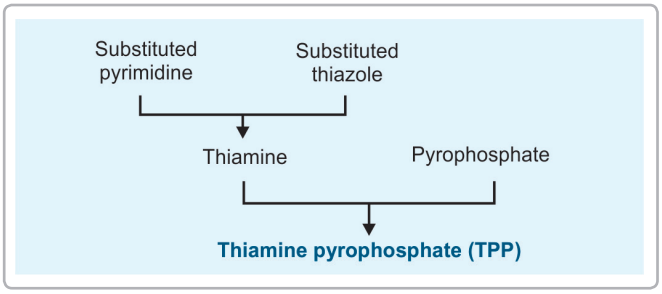


Figure 16.1: Structure of thiamine pyrophosphate

The main role of thiamine (TPP) is in **carbohydrate** metabolism. So, the requirement of thiamine is increased along with higher intake of carbohydrates.

Deficiency Manifestations of Thiamine

Beriberi: Deficiency of thiamine leads to beriberi. The early symptoms are anorexia and weakness.
Wet beriberi: Here cardiovascular manifestations are prominent. Edema of legs, face, and serous cavities are the main features. Death occurs due to heart failure.
Dry beriberi: In this condition, CNS manifestations are the major features. Peripheral neuritis with sensory disturbance leads to complete paralysis.
Wernicke-Korsakoff syndrome: It is also called as **cerebral beriberi**. Clinical features are those of encephalopathy plus psychosis. It is seen only when the nutrition is severely affected.
Polyneuritis: It is common in chronic alcoholics. Alcohol utilization needs large doses of thiamine. Polyneuritis may also be associated with pregnancy and old age.

Recommended Daily Allowance of Thiamine

It depends on calorie intake (0.5 mg/1000 calories). Requirement is 1–1.5 mg/day. Thiamine is useful in the treatment of beriberi, alcoholic polyneuritis, neuritis of pregnancy and neuritis of old age.

RIBOFLAVIN (VITAMIN B2)

Structure of Riboflavin

Structure is shown in Fig. 16.2. Riboflavin is converted to its active coenzyme forms (FMN and FAD) with the help of ATP.

Coenzyme Activity of Riboflavin

Riboflavin exists in tissues bound with enzymes. Enzymes containing riboflavin are called **flavoproteins.** The two coenzymes are **FMN** (flavin mononucleotide) and **FAD** (flavin adenine dinucleotide). The enzyme complex contains molybdenum and iron also. **D**uring the oxidation process, FAD accepts two hydrogen atoms from substrate. In turn, FAD is reduced to $FADH_2$. **FAD-dependent enzymes** are enumerated in Box 16.2. $FADH_2$ when oxidized in the electron transport chain will generate 1.5 ATP molecules.

Riboflavin Deficiency

Causes: Natural deficiency of riboflavin in man is uncommon, because riboflavin is synthesized by the intestinal flora. Riboflavin deficiency usually accompanies other deficiency diseases such as beriberi, and kwashiorkor.
Manifestations: Symptoms are confined to skin and mucous membranes. Glossitis (Greek, glossa = tongue), Magenta colored tongue, Cheilosis (Greek, cheilos = lip), Angular stomatitis (inflammation at the corners of mouth) and circumcorneal vascularization are seen.

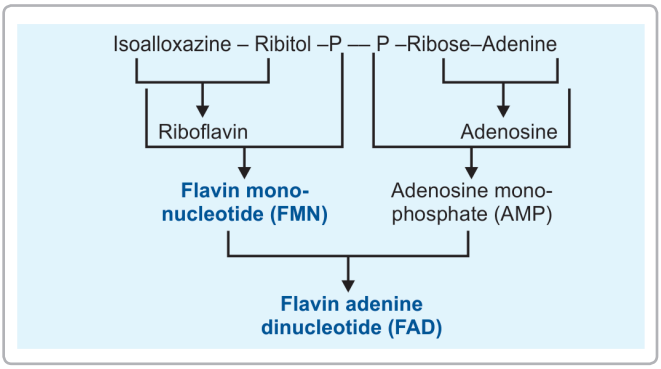


Figure 16.2. Coenzyes FMN and FAD

BOX 16.2: FAD-dependent enzymes

- Succinate to fumarate by succinate dehydrogenase (Fig. 13.2, step 6).
- Acyl-CoA to alpha-beta unsaturated acyl-CoA by acyl-CoA dehydrogenase (Fig. 9.4, step 1)
- Xanthine to uric acid by xanthine oxidase (For details refer Chapter 23).
- Pyruvate to acetyl-CoA by pyruvate dehydrogenase (Fig. 5.9).
- Alpha ketoglutarate to succinyl-CoA by alpha keto-glutarate dehydrogenase (Fig. 13.2).

Dietary Sources of Riboflavin

Rich sources are liver, dried yeast, egg and whole milk. Good sources are fish, whole cereals, legumes and green leafy vegetables.

Daily Requirement

Riboflavin is concerned mainly with energy metabolism and requirement is related to calorie intake. Adults on sedentary work require about 1.5 mg per day. During pregnancy, lactation and old age, additional 0.2 to 0.4 mg/day are required.

NIACIN

Niacin and nicotinic acid are synonyms. It is also called as pellagra preventing factor of Goldberger. The term

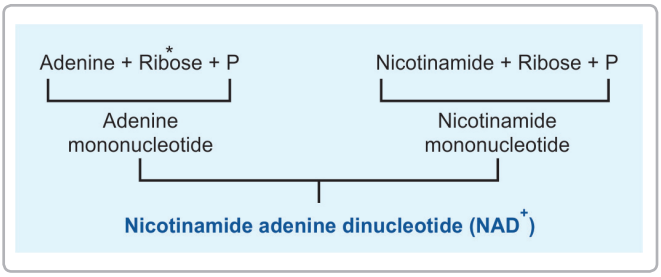


Figure 16.3: Structure of NAD⁺ (In the case of NADP⁺ phosphoric acid residue is attached to the ribose group marked with asterisk)

nicotinic acid should not be confused with nicotine. Nicotinic acid is a vitamin; but, nicotine is the potent poison from tobacco. Niacinamide is the active form of the vitamin, present in tissues.

Chemistry of Niacin

The coenzyme forms are nicotinamide adenine dinucleotide (**NAD⁺**) and nicotinamide adenine dinucleotide phosphate (**NADP⁺**) (Fig. 16.3). The nitrogen atom of niacinamide contains **one positive charge.** The structure is abbreviated as NAD⁺. (The positive sign is always shown). In the case of NADP⁺, one more phosphoric acid is attached to the ribose of the AMP (Fig. 16.3).

One Hydrogen Atom and One Electron

In the oxidized form, nitrogen of the nicotinamide residue has a positive charge. Hence, the oxidized form of coenzyme is usually written as NAD⁺. In the process of reduction, NAD⁺ accepts one hydrogen atom fully. The other hydrogen is ionized. Only the electron is accepted. See the positive sign in the molecule is removed.

$$2H \text{ ----------} \rightarrow H + H^+ + e^-$$

Thus, NAD⁺ accepts one H atom and one e^- (electron), to form NADH. The hydrogen ion (H^+) is released into the surrounding medium. During the oxidation of NADH, the reaction is reversed.

NAD⁺ Dependent Enzymes

They are so many, that an exhaustive listing is not attempted. A few examples are given in Box 16.3.

One NADH molecule is oxidized in the respiratory chain to generate 2.5 ATPs.

NADPH Reactions

Nicotinamide adenine dinucleotide phosphate (NADPH) is not used for ATP synthesis; it is almost exclusively used for the reductive biosynthesis. NADPH generating reactions are shown in Box 16.4. A few examples of NADPH utilising enzymes are shown in Box 16.5.

BOX 16.3: NAD⁺ dependent enzymes

- Lactate dehydrogenase (lactate → pyruvate) (Fig. 5.6)
- Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate → 1, 3-bisphosphoglycerate) (Fig. 5.3)
- Pyruvate dehydrogenase (pyruvate → acetyl-CoA) (Fig. 5.9)
- Alpha-ketoglutarate dehydrogenase (alpha-ketoglutarate → succinyl-CoA (Fig. 13.2)
- Beta-hydroxyacyl-CoA dehydrogenase (beta-hydroxyacyl-CoA → beta-ketoacyl-CoA (Step 3, Fig. 9.4)
- Glutamate dehydrogenase (Glutamate → alphaketoglutarate (Fig. 11.4)

BOX 16.4: NADPH generating reactions

- Glucose-6-phosphate dehydrogenase in the hexose monophosphate shunt pathway (Glucose-6-phosphate → 6-phosphogluconolactone) (Fig. 7.1)
- 6-phosphogluconate dehydrogenase in the shunt pathway (6-phosphogluconate → 3- keto-6-phosphogluconate) (Fig. 7.1).

BOX 16.5: NADPH utilizing reactions

- Ketoacyl-ACP dehydrogenase (Beta-ketoacyl-ACP → beta-hydroxyacyl-ACP) (Step 4, Fig. 9.9)
- Alpha, beta-unsaturated acyl-ACP → acyl ACP (Step 6, Fig. 9.9)
- HMG-CoA reductase (HMG-CoA → mevalonate (Step 3, Fig. 10.2)
- Methemoglobin → hemoglobin
- Folate reductase (Folate → dihydrofolate → tetrahydrofolate) (Fig. 16.7)
- Phenylalanine hydroxylase (Phenylalanine → tyrosine) (Step 1, Fig. 11.11)

Niacin Deficiency

Pellagra: Deficiency of niacin leads to the clinical condition called pellagra. The symptoms are:

Dermatitis: In early stages, bright red erythema occurs, especially in the feet, ankles and face. Increased pigmentation around the neck is known as **Casal's necklace**.

Diarrhea: The diarrhea may be mild or severe with blood and mucus. Nausea and vomiting may also be present.

Dementia: It is frequently seen in chronic cases. Delirium is common in acute pellagra. Irritability, inability to concentrate and poor memory are more common in mild cases.

Niacin is Synthesized from Tryptophan

For details see under tryptophan metabolism (Fig. 11.16). About 60 mg of tryptophan is equivalent to 1 mg of niacin.

Causes for Niacin Deficiency

Dietary deficiency of tryptophan: Pellagra is seen among people whose staple diet is maize (South and Central America). Pellagra is also seen when staple diet is **sorghum** (jowar or guinea corn) as in Central and Western India. Sorghum, contains leucine in high quantities. Leucine inhibits conversion of niacin to NAD^+.

Lack of synthesis of vitamin B6: Kynureninase, an important enzyme in the pathway of tryptophan, is pyridoxal phosphate dependent (Fig. 11.16). So conversion of tryptophan to niacin is not possible in pyridoxal deficiency.

Isoniazid (INH): It is an antituberculous drug, which inhibits pyridoxal phosphate formation. Hence, there is block in conversion of tryptophan to NAD^+.

Carcinoid syndrome: The tumor utilizes major portion of available tryptophan for synthesis of serotonin; so tryptophan is unavailable.

Dietary Sources of Niacin

The richest natural sources of niacin are dried yeast, rice polishing, liver, peanut, whole cereals, legumes, meat and fish. About half of the requirement is met by the conversion of tryptophan to niacin. About 60 mg of tryptophan will yield 1 mg of niacin.

Recommended Daily Allowance (RDA)

Normal requirement is 20 mg/day. During lactation, additional 5 mg are required.

VITAMIN B6

Coenzyme Form

Vitamin B6 is the term applied to a family of 3 related pyridine derivatives; **pyridoxine** (alcohol), **pyridoxal** (aldehyde) and **pyridoxamine** (Fig. 16.4). Active form of pyridoxine is **pyridoxal phosphate (PLP)**. It is synthesized by pyridoxal kinase, utilizing ATP.

Functions of Pyridoxal Phosphate

The pyridoxal phosphate (PLP) acts as coenzyme for many reactions in **amino acid** metabolism (Box 16.6).

Transamination: These reactions are catalyzed by amino transferases (transaminases) which employ PLP as the co-enzyme (Fig. 11.3). For example, alanine aminotransferase Alanine + Alpha-ketoglutarate → Pyruvate + Glutamic acid

The clinical significance of blood levels of transaminases is given in Chapter 3.

Decarboxylation: All decarboxylation reactions of amino acids require PLP as coenzyme. A few examples are given below:

Histidine → histamine, which is the mediator of **allergy** and anaphylaxis (For details refer Chapter 11).

5-hydroxy tryptophan → serotonin (For details refer Chapter 11)

Methionine and cysteine metabolism. For details see Chapter 11.

Homocysteine + Serine → Cystathionine. (Enzyme Cystathionine synthase)

Cystathionine → Homoserine + Cysteine (Enzyme Cystathionase)

Both these reactions require PLP. Hence in vitamin B6 deficiency **homocysteine** in blood is increased.

BOX 16.6: Functions of thiamine and pyridoxine

- Thiamine pyrophosphate is involved with carbohydrate metabolism
- Pyridoxal phosphate is involved in protein metabolism

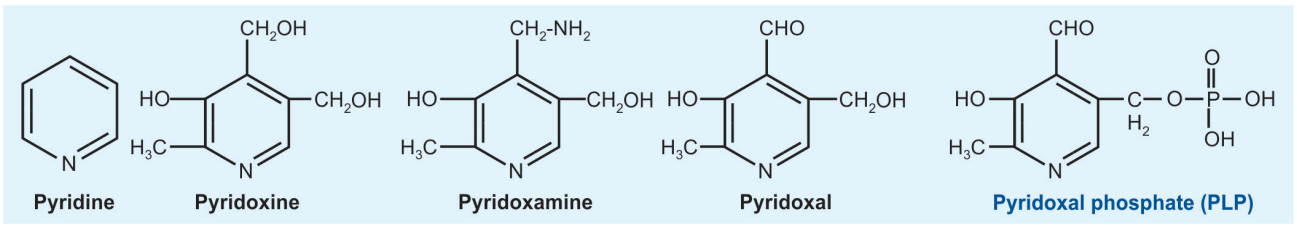


Figure 16.4: Structure of B6 related compounds

Heme synthesis: ALA synthase is a PLP dependent enzyme. This is the rate limiting step in heme biosynthesis (For details refer Chapter 14). So, in B6 deficiency, **anemia** is common.

Production of niacin: Pyridoxal phosphate is required for the synthesis of niacin from tryptophan (one vitamin is necessary for synthesis of another vitamin).

3-hydroxy kynurenine → 3-hydroxy anthranilic acid (Enzyme Kynureninase) (Fig. 11.16).

Kynureninase is a PLP dependent enzyme. Hence in vitamin B6 deficiency niacin production is less. Moreover kynurenine cannot be converted further, which is metabolized to xanthurenic acid and excreted through urine.

Glycogenolysis: Phosphorylase enzyme (glycogen to glucose-1-phosphate) requires PLP.

Deficiency Manifestations of Pyridoxine

Neurological: In vitamin B6 deficiency, PLP dependent enzymes function poorly. So, serotonin, epinephrine, noradrenaline and gamma-aminobutyric acid (GABA) are not produced properly. **Neurological** symptoms are therefore quite common in B6 deficiency. In children, B6 deficiency leads to convulsions due to decreased formation of **GABA**. PLP is involved in the synthesis of sphingolipids; so B6 deficiency leads to demyelination of nerves and consequent **peripheral neuritis**.

Dermatological: Deficiency of B6 will also affect tryptophan metabolism. Since niacin is produced from tryptophan, B6 deficiency in turn leads to niacin deficiency which is manifested as **pellagra**.

Hematological: hypochromic microcytic **anemia** may occur due to the inhibition of heme biosynthesis. Impaired antibody formation is also reported.

Dietary Sources

Rich sources are yeast, rice polishing, wheat germs, cereals, legumes (pulses), oil seeds, egg, milk, meat, fish and green leafy vegetables.

Requirement of B6

Vitamin B6 requirements are related to **protein intake** and not to calorie intake (Box 16.6). Adults need 1 to 2 mg/day. During pregnancy and lactation, the requirement is increased to 2.5 mg/day.

PANTOTHENIC ACID

Structure

The Greek word "pantos" means everywhere. As the name suggests, it is widely distributed in nature. Structure is shown in Fig. 16.5. Pantothenic acid contains beta alanine and pantoic acid. Pantothenic acid and beta mercapto ethanol amine are parts of coenzyme A (CoA).

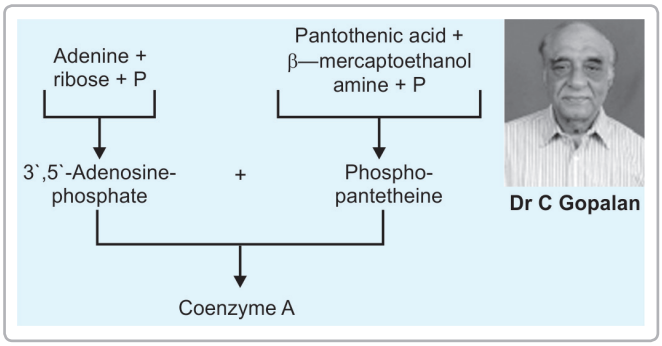


Figure 16.5: Structure of coenzyme A (CoA)

Coenzyme Activity of Pantothenic Acid

The beta mercaptoethanol amine (NH2-CH2-CH2-SH) contains one thiol or **sulfhydryl** (-SH) group. It is the active site where acyl groups are carried. Therefore the coenzyme A is sometimes abbreviated as CoA-SH to denote this active site.

Acetyl-CoA + Choline → Acetylcholine + CoA (enzyme is acetylcholine synthase)

Pyruvate + CoA + NAD^+ → Acetyl-CoA + CO_2 + NADH (enzyme is pyruvate dehydrogenase)

The important CoA derivatives are: Acetyl-CoA, Succinyl-CoA and HMG-CoA. Coenzyme A is an important component of fatty acid synthase complex. The ACP (acyl carrier protein) also contains pantothenic acid.

Deficiency of Pantothenic Acid

Gopalan's **burning feet syndrome** is manifested as paresthesia (burning, lightning pain) in lower extremities, staggering gait due to impaired coordination and sleep disturbances. These deficiency manifestations are rare in human beings. The syndrome is seen during famine, in prison camps, in chronic alcoholics and in some renal dialysis patients.

Sources of Pantothenic Acid

It is widely distributed in plants and animals. Moreover, it is synthesized by the normal bacterial flora in intestines. Therefore, deficiency is very rare. Yeast, liver and eggs are good sources.

Requirement of Pantothenic Acid

RDA is assumed to be about 10 mg/day.

BIOTIN

Biotin has one carboxyl group, which links with a lysine residue in the apoenzyme. Biotin acts as coenzyme for **carboxylation reactions**. Energy required for this reaction is provided by ATP.

Biotin Requiring CO_2 Fixation Reactions

Acetyl-CoA carboxylase: This enzyme adds CO_2 to acetyl CoA to form malonyl-CoA. This is the rate limiting reaction in biosynthesis of fatty acids (Step 1, Fig. 9.9).
Acetyl-CoA + CO_2 + ATP → Malonyl-CoA + ADP + Pi

Propionyl-CoA carboxylase

Propionyl-CoA + CO_2 + ATP → Methylmalonyl-CoA + ADP + Pi (Step 1, Fig. 9.6).

Pyruvate Carboxylase

Pyruvate + CO_2 +ATP*Oxaloacetate +ADP + Pi (Fig. 5.5). This reaction provides the oxaloacetate, which is the catalyst for TCA cycle. Second, it is an important enzyme in the gluconeogenic pathway.

Biotin-Independent Carboxylation Reactions

Carbamoyl phosphate synthetase, which is the stepping stone for urea and pyrimidine synthesis (Step 1, Fig. 11.6).

Biotin Antagonists

Avidin, a protein present in **egg white** has great affinity to biotin. Hence intake of raw (unboiled) egg may cause biotin deficiency. Biotin was originally named as anti-egg-white-injury-factor. One molecule of avidin can combine with four molecules of biotin. It is curious that egg white contains avidin and egg yolk contains biotin.

Requirement of Biotin

About 30 micrograms will meet the daily requirements.

Sources of Biotin

Normal bacterial flora of the gut will provide adequate quantities of biotin. Moreover, it is distributed ubiquitously in plant and animal tissues. Liver, yeast, peanut, soybean, milk, egg yolk are rich sources.

FOLIC ACID

The Latin word folium means leaf of vegetable. Folic acid is abundant in vegetables.

Chemistry of Folic Acid

It is composed of three constituents. The **pteridine** group linked with para-aminobenzoic acid (**PABA**)) is called **pteroic acid**. It is then attached to **glutamic acid** to form pteroylglutamic acid or folic acid (Fig. 16.6).

Coenzyme Functions of Folic Acid

The folic acid is reduced to **tetrahydrofolic acid** (THFA) (Fig. 16.7). This is catalyzed by **NADPH** dependent folate reductase. The THFA is the **carrier of one-carbon** groups. One carbon compound is an organic molecule that contains only a single carbon atom. One carbon metabolism is described in Chapter 11. The one carbon compounds are formyl, formimino, hydroxymethyl and methyl groups (For details refer Chapter 11). These groups can be interchanged. Methyl group in N^5-methyl THFA is used for synthesis of active methionine, which takes part in transmethylation reactions (Fig. 16.8). Such **transmethylation** reactions are required for synthesis of choline, epinephrine, creatine, etc. (Table 11.2).

Causes for Folate Deficiency

Pregnancy: Folate deficiency is commonly seen in pregnancy, where requirement is increased.
Drugs: Anticonvulsant drugs (hydantoin, dilantin, phenytoin, phenobarbitone) will inhibit the intestinal enzyme, so that folate absorption is reduced.

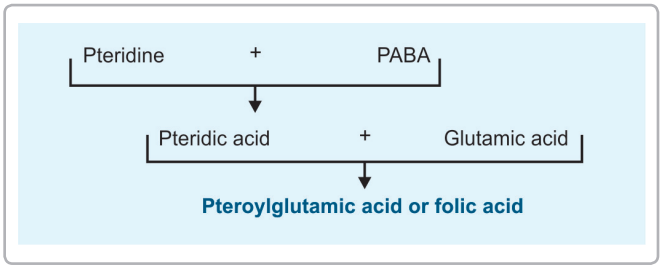


Figure 16.6: Structure of folic acid

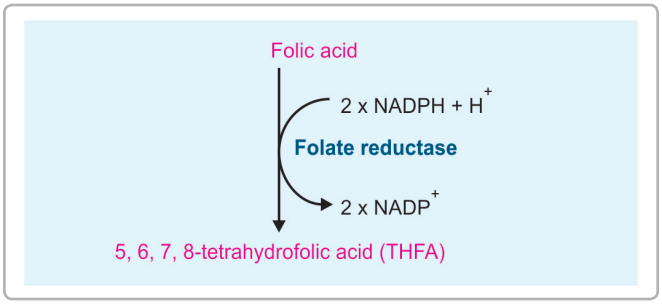


Figure 16.7: Folate reductase

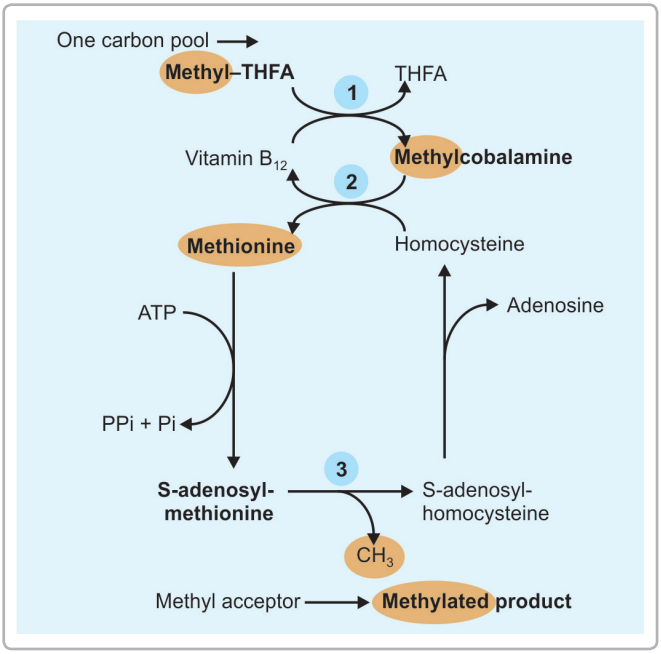


Figure 16.8: Transmethylation reactions. (2)=Homocysteine methyl transferase; (3)=methyltransferase

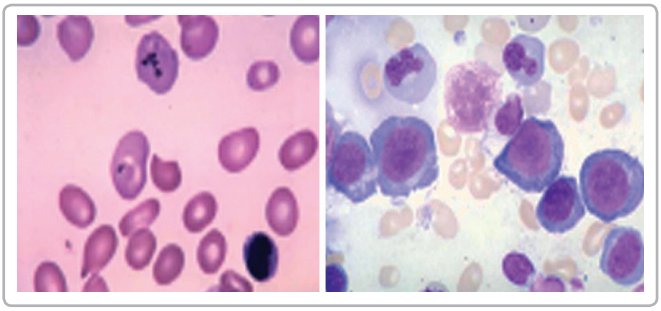


Figure 16.9: (Left) Common manifestation of folic acid deficiency is Macrocytic anemia. (Right) Megalocytic anemia with nucleated RBCs are seen in vitamin B12 deficiency.

Hemolytic anemias: As requirement of folic acid becomes more, deficiency is manifested.
Dietary deficiency: Absence of vegetables in food for prolonged periods may lead to deficiency.

Deficiency Manifestations

Reduced DNA synthesis: In folate deficiency, thymidylate synthase enzyme is inhibited. So dTTP is not available for DNA synthesis. Thus cell division is arrested. Very rapidly dividing cells in bone marrow and intestinal mucosa are therefore most seriously affected.

Macrocytic anemia is the most characteristic feature of folate deficiency. (Fig. 16.9). Asynchrony or dissociation between the maturity of nucleus and cytoplasm is manifested as **immature looking nucleus** and mature eosinophilic cytoplasm in the bone marrow cells. **Reticulocytosis** is often seen. These abnormal RBCs are rapidly destroyed in spleen. This **hemolysis** leads to the reduction of life span of RBC. Reduced generation and increased destruction of RBCs result in anemia. The peripheral blood picture in folate deficiency is described as **macrocytic**.

Sources of Folic Acid

Rich sources of folate are yeast, green leafy vegetables (Fig. 16.9). Moderate sources are cereals, pulses, oil seeds and egg.

Recommended Daily Allowance (RDA)

The requirement of free folate is 200 microgram/day. In pregnancy the requirement is increased to 400 microgram/day and during lactation to 300 microgram/day.

Folate Antagonists

Sulfonamides: They have structural similarity with PABA. Bacteria can synthesize folic acid from the components, pteridine, PABA and glutamate. When sulfonamides are given, microorganisms cannot synthesize folic acid and hence their growth is inhibited. Thus sulfonamides are very good **antibacterial** agents, which do not affect the human cells.

Aminopterin (4-amino folic acid) and amethopterin (**methotrexate**) (4-amino, 10-methyl folic acid) are powerful inhibitors of folate reductase and THFA generation. Thus, these drugs decrease the DNA formation and cell division. They are widely used as **anticancer** drugs, especially for leukemias and choriocarcinomas.

VITAMIN B12

Chemistry

Vitamin B12 is also called as cobalamin, extrinsic factor (EF) of Castle and antipernicious anemia factor. Vitamin B12 is water soluble, heat stable and red in color. The simplified structure of Vitamin B12 is shown in Fig. 16.10. The 6th valency of the cobalt is satisfied by any of the following groups: cyanide, hydroxyl, adenosyl or methyl.

Hydroxycobalamin: When hydroxyl group is attached at the R position, it is called hydroxycobalamin or vitamin B12a. Injectable preparations are in this form.

Adenosylcobalamin (Ado-B12): When taken up by the cells, these groups are removed and deoxyadenosylcobalamin or Ado-B12 is formed. This is the major **storage form**.

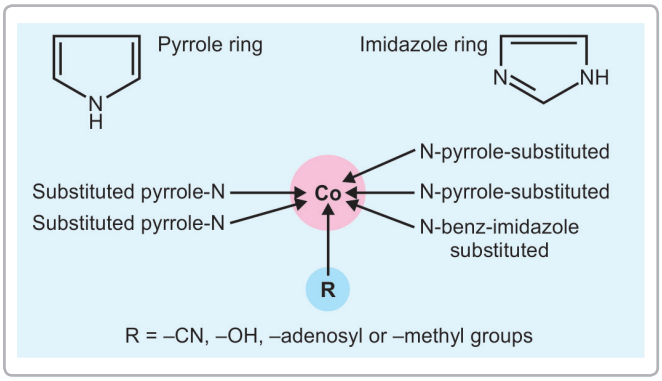


Figure 16.10: Simplified structure of vitamin B12

Methyl cobalamin: When the methyl group replaces adenosyl group, it is known as methyl cobalamin. This is the major form seen in blood circulation. The Ado-B12 and methyl B12 are the **functional co-enzymes**.

Absorption of Vitamin B12

Vitamin B12 combines with the **intrinsic factor** (IF) of Castle. Hence, the B12 is otherwise known as extrinsic factor (EF), that is, the factor derived from external sources. Intrinsic factor is secreted by the gastric parietal cells. This IF-B12 complex is attached with specific receptors on mucosal cells. The whole IF-B12 complex is internalized (Figs 16.11A to D).

Functional Role of B12

Methyl Malonyl CoA Isomerase: During the metabolism of odd chain fatty acids, the propionyl-CoA is carboxylated to Methylmalonyl-CoA. It is then isomerised by methyl malonyl isomerase or mutase (containing Ado–B12) to succinyl-CoA, which enters into citric acid cycle. In B12 deficiency, methylmalonyl-CoA is excreted in urine (**methyl malonic aciduria**).

Homocysteine methyltransferase: Step 2 in Fig. 16.8 is catalyzed by the enzyme methionine synthase or homocysteine methyltransferase. This enzyme needs vitamin B12 (methylcobalamin).

Methylfolate trap and folate deficiency: One carbon compounds may be converted to methyl THFA; but this is an irreversible step. Therefore, the only way for generation of free THFA is step No. 1 in Figure 16.8. When B12 is deficient, this reaction cannot take place. This is called the **methylfolate trap**. This leads to the associated **folic acid scarcity in B12 deficiency**.

Causes of B12 Deficiency

Nutritional: Nutritional vitamin B12 deficiency is common in India.

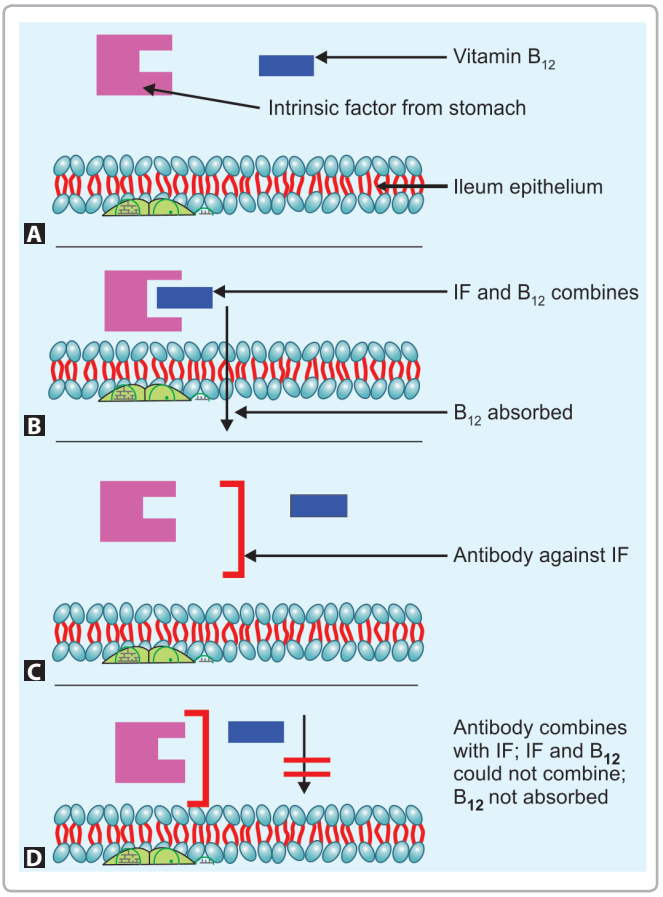


Figure 16.11A to D: (A) Intrinsic factor secreted from stomach reaches intestine; (B) Vitamin B12 absorbed with the help of intrinsic factor; (C) In pernicious anemia, antibody against IF is produced; (D) In presence of antibody, absorption is not taking place

Decrease in absorption: Absorptive surface is reduced by gastrectomy, resection of ileum and malabsorption syndromes.

Addisonian pernicious anemia: It is an autoimmune disease. Antibodies are generated against IF. Thus IF is deficient, leading to defective absorption of B12 (Figs 16.11C and D).

Pregnancy: Increased requirement of vitamin in pregnancy is another common cause for vitamin B12 deficiency in India.

Deficiency Manifestations

Megaloblastic anemia: In the peripheral blood, megaloblasts and immature RBCs are observed. Vitamin B12 deficiency causes simultaneous folate deficiency due to the folate trap. Therefore all the manifestations of folate deficiency are also seen (see under folic acid).

Abnormal homocysteine level: In vitamin B12 deficiency, step No. 2 (see Fig. 16.8) is blocked, so that homocysteine is accumulated, leading to **homocystinuria**.

Subacute combined degeneration: Damage to nervous system is seen in B12 deficiency (but not in folate deficiency). There is **demyelination** affecting cerebral cortex as well as dorsal column and pyramidal tract of spinal cord. Since sensory and motor tracts are affected, it is named as combined degeneration. Symmetrical paresthesia of extremities, alterations of tendon and deep senses and reflexes, unsteadiness in gait, positive **Romberg's** sign (falling when eyes are closed) and positive **Babinski's** sign (extensor plantar reflex).

Requirement of Vitamin B12

Normal daily requirement is 1–2 microgram/day. During pregnancy and lactation, this is increased to 2 microgram/day.

Dietary Sources

Liver is the richest source. Curd is a good source, because *Lactobacillus* can synthesize B12.

ASCORBIC ACID (VITAMIN C)

Chemistry of Vitamin C

It is water soluble and is easily destroyed by heat, alkali and storage. In the process of cooking, 70% of vitamin C is lost. The structural formula of ascorbic acid closely resembles that of carbohydrates (Fig. 16.12). The strong reducing property of vitamin C depends on the double-bonded (enediol) carbons. Only L-ascorbic acid and dehydroascorbic acid have antiscorbutic activity.

Biosynthesis of Ascorbic Acid in Animals

Most animals and plants can synthesize ascorbic acid from glucose. The pathway is described in Fig. 7.2. **Man, higher primates**, guinea pigs and bats are the only species which cannot synthesize ascorbic acid (block in gulonolactone oxidase step).

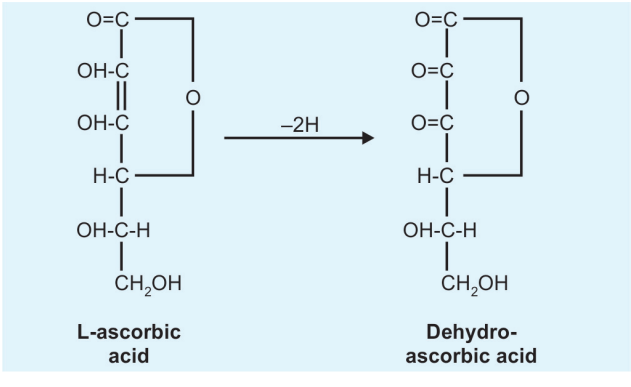


Figure 16.12: Vitamin C; structure and catabolism

Excretion of Ascorbic Acid

The vitamin is excreted in urine. Since vitamin C is a strong reducing agent, the *Benedict's test* will be positive in the urine sample after the vitamin administration.

Biochemical Functions of Vitamin C

Hydroxylation of proline: Ascorbic acid is necessary for the post-translational hydroxylation of proline and lysine residues. Hydroxyproline and hydroxylysine are essential for the formation of cross linkings in **collagen**, which gives the tensile strength of the fibers. This process is absolutely necessary for the normal production of supporting tissues such as osteoid, collagen and intercellular cement substance of capillaries.

Iron metabolism: Ascorbic acid enhances the iron absorption from the intestine (For details refer Chapter 17). Ascorbic acid reduces ferric iron to ferrous state, which is preferentially absorbed.

Hemoglobin metabolism: It is useful for re-conversion of methemoglobin to hemoglobin.

Antioxidant property: As an antioxidant (For details refer Chapter 19), it may prevent cancer formation.

Deficiency Manifestations of Vitamin C

Scurvy: Gross deficiency of vitamin C results in scurvy.

Hemorrhagic tendency: In ascorbic acid deficiency, **collagen is abnormal** and the intercellular cement substance is brittle. So capillaries are fragile, leading to the tendency to bleed even under minor pressure. Subcutaneous hemorrhage may be manifested as **petechiae** in mild deficiency and as **ecchymoses** or even hematoma in severe conditions.

Internal hemorrhage: In severe cases, hemorrhage may occur in the conjunctiva and retina. Internal bleeding may be seen as epistaxis, hematuria or malena.

Oral cavity: In severe cases of scurvy, the gum becomes painful, swollen, and spongy. The pulp is separated from the dentine and finally teeth are lost. Wound healing may be delayed.

Bones: In the bones, the deficiency results in the failure of the osteoblasts to form the intercellular substance, **osteoid**. Without the normal ground substance, the deposition of bone is arrested. The resulting scorbutic **bone is weak** and fractures easily. There may be hemorrhage into joint cavities. Painful swelling of joints may prevent locomotion of the patient.

Anemia: In vitamin C deficiency, microcytic, hypochromic **anemia** is seen.

Dietary Sources of Vitamin C

Rich sources are amla (Indian gooseberry), lime, lemon and green leafy vegetables.

Requirement of Vitamin C

Recommended daily allowance (RDA) is 75 mg/day (equal to 50 mL orange juice). During pregnancy, lactation, and in aged people requirement may be 100 mg/day.

Therapeutic Use of Vitamin C

Vitamin C has been recommended for treatment of ulcer, trauma, and burns.

A QUICK LOOK

1. The coenzyme form of thiamine is thiamine pyrophosphate (TPP).
2. TPP is essential for PDH, transketolase, alpha ketoglutarate dehydrogenase.
3. Deficiency of thiamine leads to beriberi.
4. Coenzyme forms of riboflavin are FMN and FAD.
5. Examples of FAD-dependent enzymes are succinate dehydrogenase and acyl-CoA dehydrogenase.
6. Examples of NAD^+ dependent enzymes are lactate dehydrogenase, glyceraldehyde-3-phosphate Dehydrogenase and pyruvate dehydrogenase.
7. Niacin is synthesized from tryptophan.
8. In Hartnup disease, tryptophan absorption from intestine is limited. This leads to deficiency of tryptophan and consequently of nicotinamide.
9. Active form of pyridoxine is pyridoxal phosphate (PLP).
10. PLP is essential for transamination and decarboxylation reactions of amino acids.
11. ALA synthase in heme biosynthesis is also a PLP dependent enzyme. Hence, anemia is common in B6 deficiency.
12. Isonicotinic acid hydrazide (INH) (isoniazid) used as an antituberculosis drug can produce pyridoxine deficiency.
13. Coenzyme A contains pantothenic acid.
14. Important-CoA derivatives are, acetyl-CoA, succinyl-CoA, HMG-CoA and acyl-CoA.
15. Biotin acts as coenzyme for carboxylation reactions. For example, acetyl-CoA carboxylase, propionyl-CoA carboxylase, pyruvate carboxylase.
16. Avidin, a protein present in egg white has great affinity to biotin. Hence intake of raw (unboiled) egg may cause biotin deficiency.
17. THFA (tetrahydro folic acid) is the carrier of one carbon groups.
18. Macrocytic anemia is the most characteristic feature of folate deficiency.
19. Folate antagonists are sulfonamides, trimethoprim, pyrimethamine, aminopterin and amethopterin.
20. Absorption of vitamin B12 requires the intrinsic factor (IF) of Castle. Transcobalamin-II, a glycoprotein, is the specific carrier of vitamin B12.
21. B12 containing enzymes in the human body are methylmalonyl-CoA isomerase and homocysteine methyltransferase.
22. B12 deficiency leads to pernicious anemia.
23. Man, higher primates, guinea pigs and bats are the only species which cannot synthesize ascorbic acid (vitamin C).
24. Scurvy is characterized by abnormal collagen, ecchymoses, hemorrhage and anemia.

CHAPTER 17

Mineral Metabolism

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Calcium, availability and functions
- Factors regulating blood calcium level
- Calcium, clinical applications
- Phosphorus
- Magnesium
- Sodium, potassium
- Iron, absorption, transport, anemia
- Copper, Ceruloplasmin
- Zinc
- Heavy metal poisons, lead

A few minerals are required for the normal growth and maintenance of the body. If the daily requirement is more than 100 mg, they are called **major elements.** They are listed in Box 17.1.

If the requirement of certain minerals is less than 100 mg/day, they are known as minor elements or microminerals or **trace elements**. They are shown in Box 17.1, in order of their essential nature.

The following minerals are **toxic** and should be avoided: aluminium, lead, cadmium and mercury.

BOX 17.1: Important minerals

Major elements	Trace elements
1. Calcium	1. Iron
2. Magnesium	2. Iodine
3. Phosphorus	3. Copper
4. Sodium	4. Manganese
5. Potassium	5. Zinc
6. Chloride	6. Molybdenum
7. Sulfur	7. Selenium
	8. Fluoride

CALCIUM (CA^{++})

Total calcium in the human body is about 1 to 1.5 kg, 99% of which is seen in bone and 1% in extracellular.

1. Sources of Calcium

Milk is a good source for calcium. Egg, fish and vegetables are medium source for calcium. Cereals (wheat, rice) contain only small amount of calcium. But cereals are the staple diet in India. Therefore, cereals form the major source of calcium in Indian diet.

2. Daily Requirement of Calcium

An adult needs **500 mg** per day and a child about 1200 mg/day. Requirement may be increased to 1500 mg/day during pregnancy and lactation.

3. Absorption of Calcium

Absorption is taking place from the first and second part of **duodenum**. Absorption requires a carrier protein, helped by calcium-dependent ATPase. Factors affecting absorption of calcium are:

Vitamin D: Calcitriol induces the synthesis of the carrier protein **(Calbindin)** in the intestinal epithelial cells, and so facilitates the absorption of calcium (For details refer Chapter 15).
Parathyroid hormone: It increases calcium transport from the intestinal cells.
Acidity: It favors calcium absorption.
Phytic acid: It is present in cereals. It reduces uptake of calcium. Cooking reduces phytate content.
Oxalates: They are present in leafy vegetables, which cause formation of insoluble calcium oxalates; so absorption is reduced.
Phosphate: High phosphate content will cause precipitation as calcium phosphate. The optimum ratio of calcium to phosphorus which allows maximum absorption is 1:2 to 2:1 as present in milk.

Functions of Calcium

Activation of enzymes: Calmodulin is a calcium binding regulatory protein. **Calmodulin** can bind with 4 calcium ions. Calcium binding leads to activation of enzymes. Calmodulin is part of various regulatory **kinases.** Calmodulin dependent enzymes are listed in Box 17.2. Some other enzymes are activated directly by Ca^{++} without the intervention of calmodulin; examples are pancreatic lipase; enzymes of coagulation pathway; and rennin (milk clotting enzyme in stomach).
Muscles: Calcium mediates **excitation and contraction** of muscle fibers. Upon getting the neural signal, calcium is released from sarcoplasmic reticulum. Calcium activates ATPase; increases reaction of actin and myosin and facilitates excitation-contraction coupling. The trigger of muscle contraction is the interaction of calcium with Troponin C. The active transport system utilizing calcium binding protein is called **calsequestrin**. Calcium decreases neuromuscular irritability. Calcium deficiency causes tetany.

Calcium is necessary for transmission of **nerve** impulses through synaptic region.
Secretion of hormones: Calcium mediates secretion of insulin, parathyroid hormone, etc. from the cells.

BOX 17.2: Selected list of enzymes activated by Ca^{++} and mediated by calmodulin

- Adenylyl cyclase
- Ca^{++} dependent protein kinases
- Ca^{++} -Mg^{++} -ATPase
- Glycogen synthase
- Myosin kinase
- Phospholipase C

Second Messenger: Calcium and cyclic AMP are second messengers of different hormones. One example is glucagon (Fig. 5.19).
Coagulation: Calcium is known as factor IV in blood coagulation cascade. Prothrombin contains gamma-carboxy glutamate residues which are chelated by Ca^{++} during the thrombin formation.
Myocardium: Ca^{++} **prolongs systole**. In hypercalcemia, cardiac arrest is seen in systole. This fact should be kept in mind when calcium is administered intravenously. It should be given very slowly.
Bone and teeth: The bulk quantity of calcium is used for bone and teeth formation. Bones also act as reservoir for calcium in the body. Osteoblasts induce bone deposition and osteoclasts produce demineralization.

Calcium in Blood

Normal calcium level in blood is **9–11 mg/dL.** (10 mg/dL of Ca^{++} = 5 mEq/L). **Ionized calcium:** About 5 mg/dL of calcium is in ionized form and is metabolically active. (About 4 mg/dL of calcium is bound to proteins in blood and is nondiffusible).

FACTORS REGULATING BLOOD CALCIUM LEVEL

Vitamin D

The active form of vitamin D is called dihydroxy-cholecalciferol or **calcitriol** (Fig. 15.6). Calcitriol and calcitonin are different (Box 15.1). The calcitriol induces a carrier protein in the intestinal mucosa, which increases the absorption of calcium. Hence blood calcium level tends to be elevated. Vitamin D is acting independently on bone. Vitamin D increases the number and activity of **osteoblasts**, the bone forming cells. Secretion of **alkaline phosphatase** by osteoblasts is increased by vitamin D.

Parathyroid Hormone (PTH)

This hormone is secreted by the four parathyroid glands embedded in the thyroid tissue. The chief cells of the gland secrete the PTH. The mature PTH has 84 amino acids. Storage of PTH is only for about 1 hour. This may be compared with the storage of insulin for several days and thyroxine for several weeks. Control of release of the hormone is by negative feedback by the ionized calcium in serum.

Mechanism of Action of PTH

PTH acts through **cyclic AMP.**
PTH and bones: In the bone, PTH causes demineralization or decalcification. It induces pyrophosphatase in the

osteoclasts. The number of osteoclasts are also increased. Osteoclasts release lactate into surrounding medium which solubilizes calcium. PTH also causes secretion of collagenase from osteoclasts. This causes loss of matrix and bone resorption. As a consequence, mucopolysaccharides and hydroxyproline are excreted in urine.

PTH and kidney: In kidney, PTH causes decreased renal excretion of calcium and increased excretion of phosphates. The action is mainly through increase in **reabsorption of calcium** from kidney tubules.

Calcitonin

It is secreted by the thyroid parafollicular or clear cells. Calcitonin is a single chain polypeptide. It contains about 32 amino acids. Calcitonin secretion is stimulated by serum calcium. Calcitonin level is increased in medullary carcinoma of thyroid and therefore is a **tumor marker**. Calcitonin decreases serum calcium level. It **inhibits resorption of bone**. It decreases the activity of osteoclasts and increases that of osteoblasts. Calcitonin and PTH are directly antagonistic. The PTH and calcitonin together promote the bone growth and remodeling.

Calcitonin, Calcitriol and PTH Act Together

When blood calcium tends to lower, PTH secretion is stimulated and calcitonin is inhibited; bone demineralization leads to entry of more calcium into blood. When blood calcium is increased, PTH is inhibited and calcitonin is secreted, causing more entry of calcium into bone. These effects are summarized in Table 17.1. Bone acts as the major reservoir of calcium.

Phosphorus

There is a **reciprocal** relationship of calcium with phosphorus. The ionic product of calcium and phosphorus in serum is kept as a constant. (In normal adults, calcium = 10 mg/dL × phosphorus 4 mg/dL; so ionic product is 40).

Children

In **children**, the calcium level tends to be near the upper limit. In children, ionic product of calcium and phosphorus in blood is about 50 (instead of 40 in normal adults).

Hypercalcemia

The term denotes that the blood calcium level is more than 11 mg/dL. The major cause is **hyperparathyroidism** (Table 17.1). There is osteoporosis and bone resorption. Pathological fracture of bone may result. In the blood, calcium and alkaline phosphatase levels are increased, while phosphate level is lowered. In urine, calcium is excreted, which may cause inhibition of elimination of chloride. Calcium may be precipitated in urine, leading to recurrent bilateral urinary **calculi**.

Hypocalcemia and Tetany

When serum calcium level is less than 8.8 mg/dL, it is hypocalcemia. If serum calcium level is less than 8.5 mg/dL, there will be mild tremors. If it is lower than 7.5 mg/dL, tetany, a life-threatening condition will result. **Tetany** may be due to accidental surgical removal of parathyroid glands. In tetany, neuromuscular irritability is increased. Main manifestations are carpopedal spasm (Fig. 17.1); laryngismus and stridulus. Laryngeal spasm may lead to death. Serum calcium is lowered with corresponding increase in phosphate level. Urinary excretion of both calcium and phosphate are decreased. Treatment is to give intravenous injection of calcium salts. It should be emphasized that **vitamin D deficiency will not cause tetany**. The vitamin D deficiency causes rickets, where serum calcium level is lowered marginally.

Bone Mineralization and Demineralization

Bone is a specialized connective tissue made up of a matrix with embedded fibers, cells and apatite crystals (Fig. 17.2). See Box 17.3 for the requirement for bone production.

Table 17.1: Comparison of action of three major factors affecting serum calcium

	Vitamin D	*PTH*	*Calcitonin*
Blood calcium	Increased	Drastically increased	Decreased
Main action	Absorption from gut	Demineralization	Opposes demineralization
Calcium absorption from gut	Increased	Increased (indirect)	
Bone resorption	Decreased	Increased	Decreased
Deficiency manifestation	Rickets	Tetany	
Effect of excess	Hypercalcemia^{+}	Hypercalcemia^{++}	Hypocalcemia



Figure 17.1: Carpopedal spasm in tetany

Mineralization of bone It is the process by which inorganic calcium and phosphate are deposited on the organic matrix. The specialized matrix in bone is termed **Osteoid.** In cementum, the matrix is called **Cementoid**. In dentine, the equivalent layer is known as **predentine**. In enamel, there is no equivalent, since the matrix is rapidly calcified. **Osteocalcin** is a unique protein seen in bone.

The **osteoblasts** synthesize and secrete organic matrix, which is then mineralized. **Osteoclasts** are involved in bone resorption. Combined activities of osteoblasts and osteoclasts are important in bone remodeling. The osteoblasts are under the effect of hormones PTH and calcitriol.

Secretion of **alkaline phosphatase** by osteoblasts is increased by vitamin D. The enzyme liberates phosphate from substrates. So the ionic concentration of [calcium x phosphate] is increased to supersaturation level.

Calcium phosphate is deposited as **hydroxy apatite** crystals over the matrix of triple stranded quarter staggered collagen molecules (For details refer Chapter 21). Calcium in the bone is in dynamic equilibrium with serum calcium; hydroxyapatite in trabecular bone acts as a reservoir.

Compact bone	← Trabecular bone	←→ Serum ca
$Ca3(PO4)_2$	$Ca10(PO4)_6(OH)_2$	Ca^{++}
(Total 1 kg)	(about 5 g)	(500 mg)

Osteoporosis

It is the most prevalent metabolic bone disease that is associated with an increased risk for fractures (vertebra, hip and forearm). Women above 50 years of age have a 40% risk for these fractures. The basic abnormality is decrease in bone mass, which attains a peak by the age of 30 and starts declining by 35 to 45 years of age in both men and women. After the age of 45, calcium absorption is reduced and calcium excretion is increased; so, there is net negative balance for calcium. This is reflected in demineralization

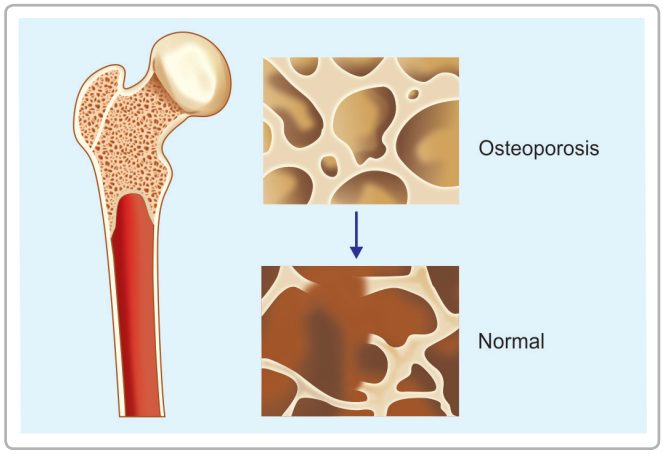


Figure 17.2: Comparison of normal and osteoporotic bone tissue

BOX 17.3: Requirement for bone formation

- Calcium
- Phosphorus
- Vitamin D
- Calcitriol from kidney
- Parathyroid hormone
- Calcitonin
- Vitamin A
- Vitamin C
- Sex steroids
- Amino acids

(Fig. 17.2). After the age of 60, osteoporosis is seen. Then there is reduced bone strength and an increased risk of fractures. Decreased absorption of vitamin D and reduced levels of androgens/estrogens in old age are the causative factors. Treatment is to give calcium with vitamin D.

Paget's Disease

Localized disease of bone characterized by osteoclastic bone resorption followed by disordered replacement of bone. It is common in people above 40 and may affect one or several bones. Familial incidence is also reported. Bone markers are useful in monitoring response to treatment using bisphosphonates.

Markers of Bone Diseases

Serum calcium, serum inorganic phosphorus, serum magnesium and urinary excretion of calcium and phosphorus, total alkaline phosphatase and total acid phosphatase levels. These are the routine tests of bone metabolism

Osteocalcin

It is the major noncollagen protein in human bone. It has 49 amino acids and forming about 1% of total protein in bones. During bone formation, osteocalcin synthesized is released into circulation. In metabolic bone diseases with increased osteoid formation, it is increased.

PHOSPHORUS

Total body phosphate is about 1 kg; 80% of which is seen in bone and teeth and 10% in muscles. Phosphate is mainly an **intracellular** ion.

Functions of Phosphate Ions

- Formation of bone and teeth.
- Production of high energy phosphate. compounds such as ATP, CTP, GTP, creatine phosphate, etc.
- Synthesis of nucleoside coenzymes such as NAD^+ and NADP.
- DNA and RNA synthesis, where phosphodiester linkages form the backbone of the structure.
- Formation of phosphoproteins, e.g. casein.
- Phosphate buffer system in blood. The ratio of $NaHPO_4$: NaH_2PO_4 in blood is 4:1. This maintains the pH of blood at 7.4.

Requirement and Source

Requirement of phosphorus is about 500 mg/day. Milk is a good source, calcitriol increases phosphate absorption.

Serum Level of Phosphorus

Serum level of phosphate is **3–4 mg/dL** in normal adults and is 5–6 mg/dL in children. The phosphate level is regulated by excretion through urine. Renal threshold is 2 mg/dL. Usually 500 mg of phosphate is excreted through urine per day.

SODIUM (Na^+)

Total body sodium is about 4000 mEq. About 50% of it is in bones, 40% in extracellular fluid and 10% in soft tissues. Sodium is the major cation of **extracellular** fluid. Sodium regulates the extracellular fluid volume.

Sodium pump is operating in all the cells, so as to keep sodium extracellular. This mechanism is ATP dependent (For details refer Chapter 1).

Sodium (as sodium bicarbonate) is also important in the regulation of acid-base balance (For details refer Chapter 20).

Normal level of Na^+ in plasma **136-145 mEq/L** and in cells 35 mEq/L.

Sodium excretion is regulated at the distal tubules. Aldosterone increases sodium reabsorption in distal tubules. Antidiuretic hormone (ADH) increases reabsorption of water from tubules.

Edema

In Edema, along with water, sodium content of the body is also increased. When diuretic drugs are administered, they increase sodium excretion. Along with sodium, water is also eliminated. **Sodium restriction** in diet is therefore advised in congestive cardiac failure and in hypertension.

Hypernatremia is seen in

- Cushing's disease
- Prolonged cortisone therapy
- In pregnancy, steroid hormones cause sodium retention in the body.

Causes of Hyponatremia

- Vomiting and diarrhea
- Addison's disease (adrenal insufficiency)
- Renal tubular acidosis (tubular reabsorption of sodium is defective)
- In severe sweating, sodium may be lost considerably, causing muscle cramps and headache.

POTASSIUM (K^+)

Total body potassium is about 3500 mEq, out of which 75% is in skeletal muscle. Potassium is the major **intracellular** cation, and maintains intracellular osmotic pressure.

The depolarization and contraction of heart require potassium. During transmission of nerve impulses, there is sodium influx and potassium efflux; with depolarization. After the nerve transmission, these changes are reversed.

Requirement

Potassium requirement is 3–4 g per day.

Sources

Sources rich in potassium, but deficient in sodium are banana, orange, apple, pineapple, dates, beans, tender coconut water and potato.

Normal Level

Plasma potassium level is **3.5–5 mEq/L**. Excretion of potassium is mainly through urine. Aldosterone and corticosteroids increase the excretion of K^+. On the other hand, K^+ depletion will inhibit aldosterone secretion.

Hypokalemia

This term denotes that plasma potassium level is below 3 mmol/L. It is manifested as muscular weakness, cardiac arrythmias and cardiac arrest. ECG waves are flattened, **T wave is inverted**, ST segment is lowered with AV block. This may be corrected by oral feeding of orange juice. Potassium administration has a beneficial effect in hypertension.

Redistribution of potassium can occur following insulin therapy. For diabetic coma, the standard treatment is to give **glucose and insulin**. This causes entry of glucose and potassium into the cell and hypokalemia may be induced. K^+ should be supplemented in such cases.

Redistribution is also seen in **alkalosis**, where the potassium moves into the cell in exchange for H^+.

Diuretics used for congestive cardiac failure may cause K^+ excretion; hence potassium supplementation is the standard treatment along with diuretics.

Hyperkalemia

Plasma potassium level above 5.5 mmol/L is known as hyperkalemia. Since the normal level of K^+ is kept at a very narrow margin, even minor increase is life-threatening.

In hyperkalemia, there is increased membrane excitability, which leads to ventricular arrhythmia and ventricular fibrillation. Hyperkalemia is characterised by flaccid paralysis, bradycardia and **cardiac arrest**.

ECG shows **elevated T wave**, widening of QRS complex and lengthening of PR interval.

CHLORIDE (Cl^-)

Chloride concentration in plasma is **96–106 mEq/L** .

Excretion of Cl^- is through urine, and is parallel to Na^+. Renal threshold for Cl^- is about 110 mEq/L. Daily excretion of Cl^- is about 5–8 g/day.

Hyperchloremia is seen in

- Cushing's syndrome. Mineralocorticoids cause increased reabsorption from kidney tubules.
- Severe diarrhea leads to loss of bicarbonate and compensatory retention of chloride.
- Renal tubular acidosis.

Causes for Hypochloremia

- Excessive vomiting. HCl is lost, so plasma Cl^- is lowered. There will be compensatory increase in plasma bicarbonate. This is called hypochloremic alkalosis.
- Excessive sweating.
- In Addison's disease, aldosterone is diminished, renal tubular reabsorption of Cl^- is decreased, and more Cl^- is excreted.

IRON (Fe)

Distribution of Iron

Total body iron content is 3 to 5 g; 75% of which is in blood. Iron is present in almost all cells. **Heme containing** proteins are hemoglobin, myoglobin, cytochromes, cytochrome oxidase, catalase. **Non-heme iron containing** proteins are transferrin, ferritin, hemosiderin. Blood contains **14.5 g of Hb per 100 mL.** About 75% of total iron is in hemoglobin, and 5% is in myoglobin and 15% in ferritin. Normal iron kinetics is shown in Fig. 17.3.

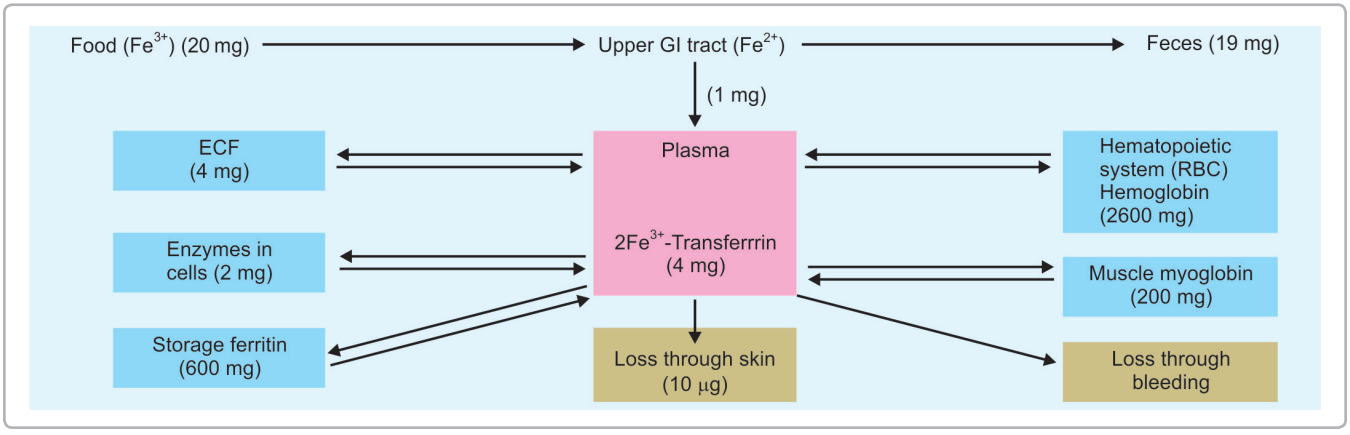


Figure 17.3: Normal iron kinetics

Requirement of Iron (ICMR, 1990)

Daily allowance for iron for an adult Indian is **20 mg** of iron, out of which about 1–2 mg is absorbed. In Western countries, requirement is less (15 mg/day) because the diet does not contain inhibitory substances. Pregnant women need 40 mg/day. Transfer of iron and calcium from mother to fetus occurs mainly in the last trimester of pregnancy. Therefore during this period mother's food should contain surplus quantities of iron and calcium. In the first 3 months of life, iron intake is negligible because milk is a poor source of iron. During this time, child is dependent on the iron reserve received from mother during pregnancy. In premature babies, the transplacental transfer of iron might not have taken place. Hence such babies are at a risk of iron deficiency. After 3 months of life, diet supplementation with cereals is essential for supplying the iron requirement.

Sources of Iron

- **Leafy vegetables** are good sources
- Cereals contains moderate quantity of iron. In a typical Indian diet, the major quantity of iron is received from cereals because of the bulk quantity
- Liver and meat contain moderate quantities
- **Jaggery** is a good source for iron
- Cooking in **iron vessels** will help to get iron
- Milk is a **very poor source** of iron.

Factors Influencing Absorption of Iron

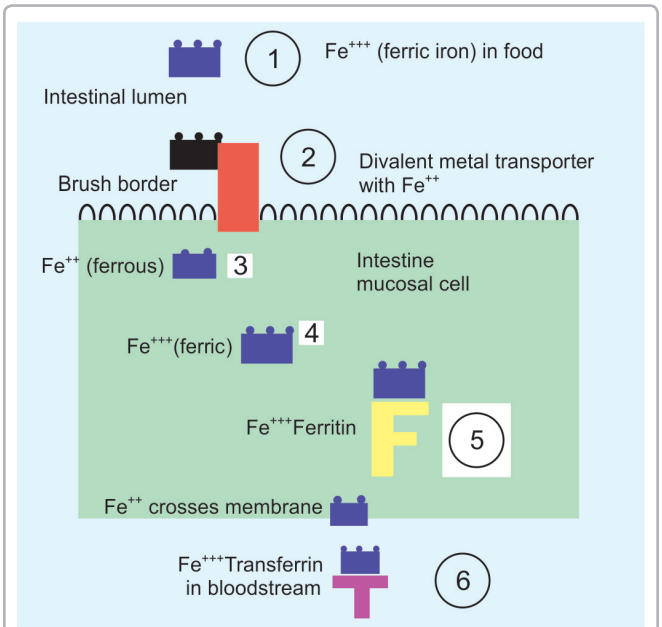
Reduced form of iron: Only Fe^{++} (**ferrous**) form (reduced form) is absorbed. Fe^{+++} (ferric) form is not absorbed.
Ascorbic acid: Ferric ions are reduced with the help of gastric HCl, and ascorbic acid. Therefore these will favor iron absorption.
Interfering substances: Iron absorption is decreased by **phytic acid** (in cereals) and **oxalic acid** (in leafy vegetables) by forming insoluble iron salts. Calcium, copper, lead and phosphates will inhibit iron absorption.

Mucosal Block Theory

Duodenum and jejunum are the sites of absorption. Iron metabolism is unique because homeostasis is maintained by regulation at the **level of absorption** and not by excretion. No other nutrient is regulated in this manner. When iron stores in the body are depleted, absorption is enhanced. When adequate quantity of iron is stored, absorption is decreased. This is referred to as "**mucosal block**" of regulation of absorption of iron. Iron in the intestinal lumen enters the mucosal cell in the **ferrous state**. This is bound to **transferrin** molecule present on the brush border surface of intestinal cell. This is then complexed with a specific **receptor**. The iron-transferrin-receptor is internalized. Iron is taken in by the cells, and receptor molecules are externalized. This receptor mediated uptake is more in iron-deficient state. When iron is in excess, receptors are not produced; this is the basis of "mucosal block". The absorbed iron binds with apoferritin, to form **ferritin.** It is kept temporarily in the mucosal cell. If there is anemia, the iron is further absorbed into the blood stream. Otherwise the iron remaining in the mucosal cell is lost when the cell is desquamated (Fig. 17.4).

Regulation of Absorption

Mucosal regulation: Regulation by mucosal block, as explained above. Absorption of iron needs divalent metal ion transporter and ferroportin. Synthesis of both these proteins is downregulated by **hepcidin**, when body iron reserves are adequate. If there is hypoxia or anemia, the synthesis of hepcidin is reduced; so ferroportin synthesis will increase. **Hepcidin** is produced by liver cells and has bactericidal effect; hence the name. Hepcidin production is increased by high iron stores and also by inflammation.



1 = Fe^{+++} (ferric iron) in food. 2 = Iron is reduced to Fe^{++} (ferrous) state, and attaches to divalent metal transporter on the mucosal surface. 3 = Ferrous iron is internalized. 4 = Iron is oxidized to ferric state. 5 = Ferric iron binds with ferritin for temporary storage. 6 = Ferric iron released, reduced to ferrous state crosses the cell membrane, re-oxidized to ferric state by ceruloplasmin. In the bloodstream, ferric iron is bound with transferrin

Figure 17.4: Absorption of iron from intestine

Iron Transport in Blood

Transport form of iron is **transferrin**. It is a beta-1 globulin. Normal plasma level of transferrin is 250 mg/100 mL. In iron deficiency, this level is increased. Total iron binding capacity (TIBC) in plasma is 400 mg/100 mL; this is provided by the transferrin. One-third of this capacity is saturated with iron. In iron deficiency anemia, TIBC is increased (transferrin level is increased); but serum iron level is reduced. Transferrin takes up iron with the help of ferroxidase. In blood, **ceruloplasmin** is the ferroxidase, which oxidizes ferrous to ferric state.

$$\text{Apo-transferrin} + 2\,Fe^{++} + \tfrac{1}{2}\,O_2 \xrightarrow{\text{Ferroxidase}} \text{Transferrin combined with } 2\,Fe^{+++} + H_2O$$

Storage of Iron

The storage form is **ferritin**. It is seen in intestinal mucosal cells, liver, spleen and bone marrow. In iron deficiency anemia, ferritin content is reduced.

Iron is Conserved

When RBC is lysed, hemoglobin enters into circulation. Being a small molecular weight substance, Hb will be lost through urine. To prevent this loss, Hb is immediately taken up by **haptoglobin** (Hp) (Fig. 17.5). When the globin part is removed from Hb, the heme is produced, and is released into circulation. In order to prevent its excretion through urine, heme is bound with **hemopexin** (Fig. 17.5). Iron is very precious for biological systems. Hence, these elaborate mechanisms are necessary for conservation inside the body.

Excretion of Iron

Iron is a one-way element. That is, very little of it is excreted. *The regulation of homeostasis is done at the absorption level.* Almost no iron is excreted through urine. Feces contains unabsorbed iron as well as iron trapped in the intestinal cells, which are then desquamated. Any type of bleeding will cause loss of iron from the body. Menstrual flow is the major cause for loss of iron in women. All the cells in skin contain iron. The upper layers of skin cells are constantly being lost, and this is another route for iron loss from the body.

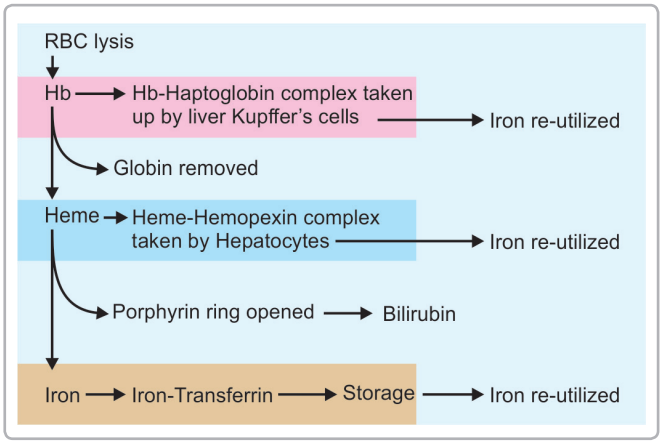


Figure 17.5: Conservation of iron in the body

Iron Deficiency Anemia

It is the most common nutritional deficiency disease. About 30% of world population are anemic. All over India, this is about 70%. Maternal anemia contributes to increase in perinatal mortality. Anemia often leads to irreversible impairment of child's learning ability. In adults, anemia results in impaired work capacity.

Causes for Iron Deficiency

Nutritional deficiency of iron:
Hookworm infection: This may be the most important cause, especially in rural areas, where sanitation is poor.
Repeated pregnancies: About 1 g of iron is lost from the mother during one delivery.
Chronic blood loss: Hemorrhoids (piles), peptic ulcer, uterine hemorrhage.

Manifestations of Iron Deficiency Anemia

Iron deficiency is characterized by **microcytic hypochromic** anemia. Anemia results when hemoglobin level is less than 12 g/dL.

When the level is lower than 10 g, body cells lack oxygen and patient becomes uninterested in surroundings (apathy). All metabolic processes become sluggish. Anemia will lead to impaired attention, irritability, lowered memory and poor scholastic performance. Anemia and apathy go hand in hand.

Treatment of Iron Deficiency

Oral iron supplementation is the treatment of choice. 100 mg of **iron** + 500 microgram of **folic acid** are given to pregnant women, and 20 mg of iron + 100 microgram folic acid to children. Iron tablets are usually given along with **vitamin C,** to convert it into ferrous form, for easy absorption.

Iron Toxicity

Hemosiderosis: Iron excess is called hemosiderosis. Hemosiderin pigments are golden brown granules, seen in spleen and liver. Hemosiderosis occurs in persons receiving repeated blood **transfusions**. Here the regulation at the level of intestine is circumvented leading to iron

overload. Hemophilic children require blood transfusion every 3 months. If whole blood is given every time, by about 20 years of age, the patient will have hemosiderosis. This is the commonest cause for hemosiderosis in India.

Primary Hemosiderosis: It is also called hereditary hemochromatosis. In these cases, iron absorption is increased and transferrin level in serum is elevated. Excess iron deposits are seen.

Hemochromatosis: When total body iron is higher than 25–30 g, hemosiderosis is manifested. In the liver, hemosiderin deposit leads to death of cells and cirrhosis. Pancreatic cell death leads to diabetes. Deposits under the skin cause yellow-brown discoloration, which is called **hemochromatosis**. The triad of cirrhosis, hemochromatosis and diabetes are referred to as **bronze diabetes**.

COPPER (Cu)

Total body copper is about 100 mg. It is seen in muscles, liver, bone marrow, brain, kidney, heart and in hair.

Copper containing enzymes are ceruloplasmin, cytochrome oxidase, cytochrome c, tyrosinase and lysyl oxidase.

Copper **requirement** for an adult is 1.5–3 mg per day. Major dietary sources are cereals, meat, liver, nuts and green leafy vegetables. Milk is very poor in copper content.

Ceruloplasmin: Normal serum level of ceruloplasmin is 25–50 mg/dL. Ceruloplasmin is a blue-colored glycoprotein (Latin "caeruleus" = blue). It is also called **serum ferroxidase**. It promotes oxidation of ferrous ion to ferric form, which is incorporated into transferrin (For details refer Chapter 12).

Functions of Copper

It is necessary for iron absorption and incorporation of iron into hemoglobin (Fig. 17.4). It is a cofactor for vitamin C requiring hydroxylations.

Abnormal Metabolism of Copper

Wilson's disease: Ceruloplasmin level in blood is drastically reduced in Wilson's hepatolenticular degeneration. The incidence of Wilson's disease is 1 in 50,000. Copper is accumulated in cells, leading to copper deposits in liver and brain (For details refer Chapter 12). As zinc decrease copper absorption, zinc is sometimes used therapeutically in Wilson's disease, to reduce copper load in the body.

Copper deficiency anemia: Copper deficiency thus results in microcytic normochromic anemia. If there is added iron deficiency, hypochromic anemia results.

Cardiovascular diseases: Copper is a constituent of **lysyl oxidase**. It makes cross linkages in elastin. In copper deficiency, elastin becomes abnormal, leading to weakening of walls of major blood vessels.

Melanin: Copper is present in tyrosinase which is necessary for melanin formation (For details refer Chapter 11). Copper deficiency thus leads to hypopigmentation and in extreme cases, gray color of hair.

ZINC (Zn)

Total zinc content of body is about 2 g, out of which 60% is in skeletal muscles and 30% in bones.

Rich dietary sources are grains, beans, nuts, cheese and meat. Zinc and copper will competitively inhibit each other's absorption.

More than **300 enzymes** are zinc dependent. Some important ones are carboxypeptidase, carbonic anhydrase, alkaline phosphatase and lactate dehydrogenase. RNA polymerase contains zinc and so it is required for **protein** biosynthesis. **Insulin** when stored in the beta cells of pancreas contains zinc, which stabilizes the hormone molecule.

Zinc Deficiency Manifestations

Poor wound healing, lesions of skin, impaired spermatogenesis, and alopecia are deficiency manifestations of zinc. Zinc deficiency leads to depression, dementia and other psychiatric disorders **Acrodermatitis enteropathica** is a recessive condition where zinc absorption is defective and is characteriaed by acrodermatitis (inflammation around mouth, nose, fingers, etc.), diarrhea, alopecia (loss of hair in discrete areas), and hypogonadism.

Requirement of Zinc

For adults is 10 mg/day; children 10 mg/day; in pregnancy and lactation 15–20 mg/day.

IODINE (I)

Daily requirement of iodine is 150–200 micrograms/day. Its sources are drinking water, fish, cereals, vegetables and iodinated salt. Total body contains 25–30 mg of iodine. All cells do contain iodine; but 80% of the total is stored in the thyroid gland. Iodine level in blood is 5–10 microgram/dl. In most parts of the world, iodine is a scarce component of the soil. Upper regions of mountains generally contain less iodine. Such areas are called **goiterous belts**, e.g. Himalayan region. Commercial source of iodine is seaweeds. The program of iodination of common salt has resulted in increased availability of iodine. Ingredients in foodstuffs, which prevent utilization of iodine are called **goitrogens**. Goitrogens are seen in cassava, maize, millet, bamboo shoots, sweet potatoes and beans. Cabbage and

tapioca contain **thiocyanate**, which inhibits iodine uptake by thyroid. Mustard seed contains **thiourea**, which inhibits iodination of thyroglobulin. The only biological role of iodine is in formation of thyroid hormones, thyroxine (T4) and tri iodo thyronine (T3) (For details refer Chapter 31).

HEAVY METAL POISONS

Lead Poisoning

Lead is the most common environmental poison in India. About 30% of population are already affected by lead poisoning. It is not biodegradable. It is dispersed into air, food, soil and water. **Paint** is the major source for exposure, especially in children, as they bite painted toys. Paint is peeled off as small flakes from walls of living rooms. Increased content of lead is seen in air, water and vegetables in cities and near highways. This is due to the tetraethyllead derived from the **exhaust of vehicles**. Statutory use of lead-free petrol has reduced this type of contamination. **Lead pipes** are important sources for contamination. **Newspapers** and xerox copies contain lead, which is adsorbed to fingertips, and later contaminate foodstuff taken by hands. One pack of **cigarette** contains 15 microgram of lead and chronic smokers have higher blood levels of lead. **Battery repair**, radiator repair, soldering, painting and printing are occupations prone to get lead poisoning.

Lead is a **cumulative poison** and is accumulated in tissues over the years. More than 10 mg/dL in children and more than 25 mg/dL in adults leads to toxic manifestations.

Lead is taken up from environment by enamel and **dentine**. Steady incorporation of lead into dentine makes it a good marker of exposure to lead.

Miscarriage, still birth, and premature birth are reported in lead poisoning of mothers. Developing brains are more susceptible to lead. Permanent **neurological** sequelae, cerebral palsy and optic atrophy may be seen. In children, **mental retardation**, learning disabilities, behavioral problems, hyperexcitability and seizures are seen. **Anemia**, abdominal colic and loss of appetite are very common. Lead inhibits heme synthesis.

If the blood level is more than 70 mg/dL, **acute toxicity** is manifested, as encephalopathy, convulsions, mania, neuropathy, abdominal colic, severe anemia and kidney damage. Discoloration and blue line along the gums are characteristic features of acute lead poisoning.

Preventive measures are: Avoid lead pipes. Adequate iron and calcium will reduce absorption of lead. Plants and grass around the house will absorb lead, and make air cleaner. Lead-free petrol should be used. Strict laws on the lead content of paints should be enacted and implemented.

A summary of requirement and blood level of minerals are shown in Table 17.2.

Table 17.2: Summary of mineral metabolism

	Requirement for adult male/day	*Blood level*
Calcium	500 mg	9–11 mg/dL
Phosphorus	500 mg	3–4 mg/dL
Sodium	5–10 g	136–145 mEq/L
Potassium	3–4 g	3.5–5 mEq/L
Chloride	—	96–106 mEq/L
Iron	20 mg	120 mg/dL (plasma)
Copper	1.5–3 mg	100 microgram/dL
Iodide	150–200 microgram	5–10 microgram/dL
Zinc	15 mg	100 microgram/dL

A QUICK LOOK

1. Factors favorably influencing calcium absorption are —calcitriol, PTH, acid pH, and arginine.
2. Factors decreasing calcium absorption are—phytic acid, high phosphate content and malabsorption syndromes.
3. Calcium activates enzymes, such as protein kinases, glycogen synthase, pyruvate carboxylase, pyruvate dehydrogenase.
4. Calcium mediates contraction of muscle fibers.
5. Calcium mediates secretion of insulin, PTH, calcitonin, ADH and can act as a second messenger for some hormones, e.g. Glucagon.
6. Calcium is known as Factor IV in blood coagulation.
7. Hypercalcemia may occur in hyperparathyroidism, multiple myeloma, bone cancer, and Paget's disease.
8. Hypocalcemia leads to tetany as seen in renal tubular acidosis.
9. Hypernatremia is seen in Cushing's syndrome and after prolonged cortisone therapy. Hyponatremia is seen in Addison's disease.
10. Renal loss of potassium is seen in renal tubular acidosis, tubular necrosis, metabolic alkalosis.
11. Hyperchloremia is seen in dehydration, Cushing's syndrome, respiratory acidosis, renal tubular acidosis.
12. Hypochloremia is seen in excessive vomiting, Addison's disease, and respiratory alkalosis.
13. Iron homeostasis is maintained by regulation of absorption and not by excretion.
14. Transport form of iron transferrin. Storage form is ferritin. Iron deficiency may be caused by hookworm infection, nephrosis, lead toxicity, and leads to microcytic hypochromic anemia. Iron toxicity leads to hemosiderosis.
15. Copper in plasma is bound to ceruloplasmin. It functions as cofactor for Vitamin C mediated hydroxylations.
16. Copper is essential for formation of Hb, deficiency leading to microcytic normochromic anemia.
17. Copper level in blood is lowered in Wilson's hepatocellular degeneration.
18. Menkes Kinky hair syndrome, an X-linked disease is caused due to decreased copper transport in the blood.
19. Zinc is stored in combination with metallothionein. More than 300 enzymes are known to be zinc dependent.

CHAPTER 18

Nutrition

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Calorific value, respiratory quotient
- Basal metabolic rate (BMR)
- Specific dynamic action (SDA)
- Proximate principles
- Nitrogen balance
- Nutritional values of proteins
- Limiting amino acids, supplementation
- Kwashiorkor and marasmus
- Prescription of the diet

Calorific Value

The energy content of food materials is measured in calories. **One calorie** is the heat required to raise the temperature of 1 g of water through 1°C. Since, it is a very small unit, in medical practice, the energy content is usually expressed in **kilocalorie** (kcal) which is equal to 1000 calories.

The calorific value of nutrients (energy yield per unit weight of food) is given in Table 18.1.

Respiratory Quotient (RQ)

Respiratory quotient is defined as the ratio of volume of CO_2 produced to the oxygen consumed.

i) RQ of carbohydrates = 1
ii) RQ of fats = 0.7
iii) RQ of proteins = 0.8.

Table 18.1: Energy yield from nutrients

Nutrient	*Calorific value in kilocalories/g*
Carbohydrates	4
Fats	9
Proteins	4
Alcohol	7

For a mixed diet, it is between 0.7 and 1, often around 0.82–0.85. When the rate of utilization of fat increases in relation to carbohydrates, RQ falls. This happens in diabetes mellitus, when utilization of carbohydrate is reduced. The RQ is lowest when ketolysis is very active.

Energy Requirements of a Normal Person

While calculating the energy requirements, we have to consider the energy required for:

i. Maintenance of basal metabolic rate (BMR)
ii. Specific dynamic action or thermogenic effect of food
iii. Extra energy expenditure for physical activities.

Basal Metabolic Rate (BMR) or Resting Metabolic Rate (RMR)

Definition: The Basal metabolic rate is the energy required by an awake individual during physical, emotional and digestive rest. It is the minimum amount of energy required to maintain life or sustain vital functions like the working of the heart, circulation, brain function, respiration, etc. The metabolic rate during sleep is less than BMR.

Since BMR is affected by body surface area, it is usually expressed in kilocalories per hour/square meter of body surface. Nomograms showing body surface area

from height and weight are also available. The BMR is calculated from oxygen consumption, calorific value and surface area.

Factors Affecting BMR

Age: During the period of active growth, BMR is high. It reaches a maximum by 5 years of age. In old age BMR is lowered.
Sex: Males have a higher BMR than females.
Temperature: BMR increases in cold climate as a compensatory mechanism to maintain body temperature. Eskimos have a higher BMR.
Exercise: The increase in BMR during exercise is due to increased cardiac output. *Starvation* lowers BMR.
Fever: About 12% increase in BMR is noticed per degree centigrade rise in temperature.
Thyroid hormones: Since thyroid hormones have a general stimulant effect on rate of metabolism and heat production, BMR is raised in hyperthyroidism and lowered in hypothyroidism. All other factors (No. i to v) are taken into account in the definition of BMR. Thus, thyroid function determines the changes in BMR.

Normal Value for BMR

For adult men, 34–37 kcal/square meter/hour, and for women, 30–35 kcal/Sq m/hour. For easier calculations, BMR for an adult is fixed as **24 kcal/kg** body weight/day). The values thus obtained are rounded to the nearest whole number.

Specific Dynamic Action (SDA)

This refers to the increased heat production following the intake of food (**thermogenic effect** of food) (regulatory thermogenesis). This is due to the expenditure of energy for digestion and absorption of food. This energy is trapped from previously available energy, so that the actual energy from the food is lesser than that of theoretical calculation. SDA can be considered as the activation energy needed for a chemical reaction. This activation energy is to be supplied initially.

Suppose a person takes 250 g of carbohydrates; this should produce 250 × 4 = 1000 kcal. But before this energy is trapped, about 10% energy (= 100 kcal) is drawn from the reserves of the body. Thus the net generation of energy is only 1000 minus 100 = 900 kcal. If the person wants to get 1000 kcal, he should take food worth 1100 kcal. Thus additional calories, equivalent to SDA has to be added in diet.

The values of SDA are: for proteins 30%, for lipids 15%, and for carbohydrates 5%. This means that out of every 100 g of proteins consumed, the energy available for doing useful work is 30% less than the calculated value. Hence for a mixed diet, an extra 10% calories should be provided to account for the loss of energy as SDA.

Physical Activity

The energy requirements would depend on the occupation, physical activity and lifestyle of the individual. The activity level may be divided into 3 groups—sedentary, moderate and heavy. Additional calories are to be added for each category: For sedentary work, +30% of BMR; for moderate work, +40% of BMR; and for heavy work, +50% of BMR should be added (Table 18.2). The energy requirement of a 55 kg male doing moderate work, may be calculated as shown in Table 18.3.

Requirements of Dietary Nutrients

The recommended daily allowance (RDA) provides extra provisions to prevent the development of deficiency. The RDA has been prescribed for all the essential nutrients as per the stipulations of the WHO and FAO. The Indian Council of Medical Research (ICMR) has suitably modified these for Indian conditions (For details refer appendix III).

Proximate Principles

In the diet proximate principles are carbohydrates, fat and proteins. Moreover, required amounts of minerals

Table 18.2: Energy requirement and occupation

Type of activity	*Occupation*
Light	Office workers, lawyers, accountants, doctors, teachers
Moderate	Students, industry workers, farm workers, housewives without mechanical appliances
Very active	Agricultural workers, miners, unskilled laborers, athletes
Heavy work	Lumber jacks, blacksmiths, and construction workers

Table 18.3: Calculation for energy requirement for a 55 kg person, doing moderate work

For BMR	= 24 x 55 kg	= 1320 kcal
+ For activity	= 40% of BMR	= 528 kcal
Subtotal	= 1320 + 528	= 1848 kcal
+ Need for SDA	= 1848 x 10%	= 184 kcal
Total	= 1848 + 184	= 2032 kcal
Rounded to nearest multiple of 50		= 2050 kcal

and vitamins are also to be provided. Further, additional requirements for growth, pregnancy, lactation and convalescence are to be provided in the food.

IMPORTANCE OF CARBOHYDRATES

The dietary carbohydrates provide a major fraction of the body's energy needs. Ideally carbohydrates may provide about **60–65% of total calories.** In addition to calories, the carbohydrates also provide the fiber.

Dietary Carbohydrates

The major dietary polysaccharide is **starch**. It is digested by amylase to maltose and then hydrolyzed to glucose. On cooking starch is made more soluble and accessible to digestive enzymes. Cereals, pulses and tubers are the major sources of starch in the diet.

Sucrose

- Cane sugar is mainly used as a sweetening agent. In young children high intake of sucrose and sucrose-rich food items predispose to the development of **dental caries**. Sucrose is easily fermented by the bacteria present in dental plaque, which would damage the enamel and lead to caries (tooth decay) (For details refer Chapter 22). Sucrose consumption also results in increased levels of plasma lipids and alterations in blood sugar levels. While prescribing diets for diabetic patients and for weight reduction, sucrose should be strictly avoided.

Dietary Fiber

The unavailable or **indigestible** carbohydrate in the diet is called dietary fiber. These include cellulose, hemicellulose and pectin. All of them are mainly from plant sources. Dietary fiber is necessary to maintain the **normal motility** of gastrointestinal tract. Diet rich in fiber improves bowel motility, prevents constipation, decreases reabsorption of bile acids thus lowering cholesterol level and improves glucose tolerance.

NUTRITIONAL IMPORTANCE OF LIPIDS

Fats provide a concentrated source of energy. In developed countries, the percentage of calories derived from fats may be as high as 40%, but in the developing countries, it is much less, around 10%. A minimum intake of lipids is essential since the requirements of fat soluble vitamins and essential fatty acids are to be met. Fats increase the taste and palatability of food. They are the favored cooking medium. **Visible fat** or fat consumed as such, e.g. butter, ghee, oils. Recommended daily intake of visible fat is 10% of calories or 20 g/day. **Invisible fat** or fat present as part of other food items e.g. egg, fish, meat, cereals, nuts and oil seeds. More than half of essential fatty acid in Indian diet is in the form of invisible fat.

Cholesterol and Heart Diseases

The atherogenic effect of cholesterol and the risk of coronary artery disease in people with hypercholesterolemia are described in Chapter 10. Food items known to be rich in cholesterol (egg yolk, liver, brain, kidney) are to be consumed in limited amounts. Table 18.4 gives a list of food items with their cholesterol content. Vegetables, cereals and pulses do not contain any cholesterol. Further, vegetable sterols will inhibit cholesterol absorption. Saturated fats raise serum cholesterol; while unsaturated fats (vegetable oils and fish oils) lower it. The poly unsaturated fatty acids (PUFA) are present in vegetable oils and fish oils (Table 18.5). They belong to essential fatty acids. The importance of **essential fatty acids** are: They are precursors of prostaglandins and leukotrienes. They are hypocholesterolemic and therefore **antiatherogenic** in effect (For details refer Chapter 10).

The omega-3 fatty acids from fish oils decrease the plasma LDL and thereby decrease the risk of coronary artery disease. The contents of PUFA in oils are given in Table 18.6. High fiber content also reduces serum cholesterol, lowers LDL fraction and raises HDL fraction. Whole cereals, pulses, leafy vegetables and fruits contain good quantity of fiber.

Table 18.4: Cholesterol content of food items

Food item	*Cholesterol content mg/100 g*
Hens egg, whole	300
Egg yolk	1330
Liver	300–600
Brain	2000
Butter	280
Meat and fish	40–200

Table 18.5: Polyunsaturated fatty acids

Name	*Carbon atoms*	*Family omega*	*No. of double bonds*
Linoleic acid	18	ω 6	2
Linolenic acid	18	ω 3	3
Arachidonic	20	ω 6	4

Table 18.6: Fatty acids in oils

Fat or oil	*Saturated (%)*	*Mono-unsaturated (%)*	*Polyunsaturated (%)*
Butter/ghee (*)	75	20	5
Safflower oil	9	12	79
Cotton seed oil	26	19	65
Coconut oil (*)	86	12	2
Ground nut oil	18	46	36

(*) Butter/ghee contains short chain fatty acids and coconut oil contains medium chain fatty acids.

Recommended Daily Intake of Lipids

The ideal fat intake is about 15–20% of total calories, out of which about 25–30% may be PUFA. This will be a total of about 20–25 g of oils and about 3 g of PUFA for a normal person. PUFA should not be more than 30% of total fat. Moreover, the fat content should be such that saturated fatty acid (SFA): mono unsaturated fatty acid (MUFA): polyunsaturated fatty acid (PUFA) may be in 1:1:1 ratio. Further, cholesterol intake should be less than 250 mg/day.

Importance of Proteins

They form the building blocks for body tissues. When enough carbohydrates are present in the diet, the amino acids are not used for yielding energy. This is known as the **protein sparing effect** of carbohydrates. During starvation, amino acids may act as energy sources.

The requirement of protein is shown in Table 18.7. For the synthesis of body proteins, all the essential amino acids should be supplied in adequate quantities at the same time.

Nitrogen Balance

A normal healthy adult is said to be in nitrogen balance (Fig. 18.1), because the dietary intake (I) equals the daily loss through urine (U) feces (F) and skin (S).

$$I = U + F + S$$

When the excretion exceeds intake, it is **negative** nitrogen balance. When the intake exceeds excretion, it is a state of **positive** nitrogen balance.

Factors Affecting Nitrogen Balance

Growth: During the period of active growth, a state of positive nitrogen balance exists. On an average when a person gains 5 kg, about 1 kg proteins (160 g nitrogen) are added to the body.

Hormones: Growth hormone, insulin and androgens promote positive nitrogen balance, while corticosteroids cause a negative nitrogen balance.

Table 18.7: Recommended protein allowances

Infants	2.4 g/kg body wt/day
Children up to 10 years	1.75 g/kg body wt/day
Adult (Men and women)	1 g/kg body wt/day
Pregnancy	2 g/kg body wt/day

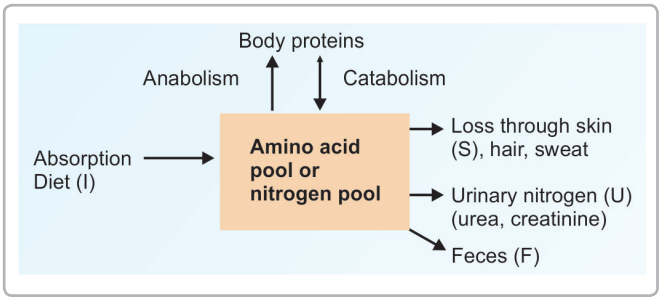


Figure 18.1: Nitrogen balance

Pregnancy: A pregnant woman will be in a state of positive nitrogen balance due to the growth of fetus.

Convalescence: A person convalescing after an illness or surgery will be in positive nitrogen balance, due to active regeneration of tissues.

Acute illness: Negative nitrogen balance is seen in subjects immediately after surgery, trauma and burns.

Protein deficiency: The deficiency of even a single amino acid can cause negative nitrogen balance. Prolonged starvation is another important cause.

Maintenance of Nitrogen Balance

To maintain the nitrogen balance, one has to satisfy the need for nitrogen intake, which are:

1. Obligatory nitrogen loss is 3.5 g of N/day for a 65 kg person. This is equivalent to 22 g of protein.
2. Requirement for protein turnover. The minimum daily requirements to compensate for the above two categories are 0.7 g/kg wt of good quality protein.
3. Protein requirements for growth. This is applicable in the case of infants, children, adolescents, pregnancy, lactation and convalescence. As growth stops, protein requirement also decreases.

Nutritional Values (Nutritional Indices)

The protein content of various food items are shown in appendix IV. The method to assess the nutritional value of a protein, is to give that protein as the only source of nitrogen to an animal, and assess the weight gain (Fig. 18.2).

Limiting Amino Acids

If a particular protein is fed to a young rat as the only source of protein, it fails to grow. This essential amino acid that

is lacking in that protein is said to be the **limiting amino acid**. Limiting amino acid is that which limits the weight gain when a protein is supplied to an animal (Fig. 18.2).

Supplementation

This problem may be overcome by taking a mixture of proteins in the diet. **Mutual supplementation of proteins** is thus achieved. (Table 18.8). For example, pulses are deficient in methionine, but rich in lysine. On the other hand, cereals are deficient in lysine, but rich in methionine. Therefore a combination of pulses plus cereal (e.g. chappathi + dal) will cancel each other's deficiency and become equivalent to first class protein. The supplementation effect of proteins may be seen in weight gain in animals (Fig. 18.3).

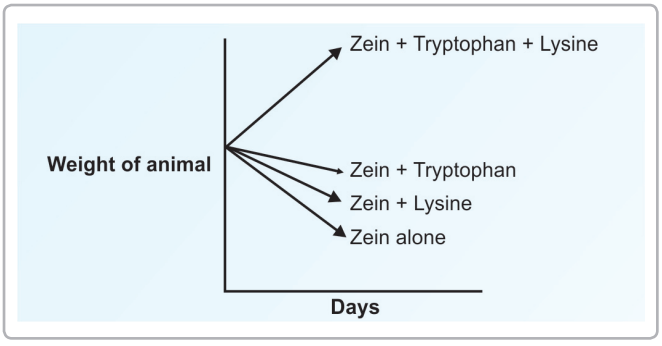


Figure 18.2: Identifying the limiting amino acid

Table 18.8: Limiting amino acids in proteins

Protein	*Limiting amino acid*	*Protein supplemented to cancel the deficiency*
Rice	Lys, Thr	Pulse proteins
Wheat	Lys, Thr	Pulse proteins
Tapioca	Phe, Tyr	Fish proteins
Bengal gram	Cys, Met	Cereals

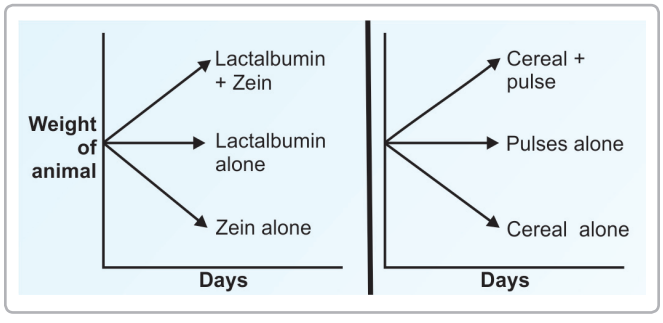


Figure 18.3: Two second class proteins, when combined, are equivalent of the first cclass proteins

PROTEIN–ENERGY MALNUTRITION

It is the most widespread nutritional problem in developing countries. It is predominantly affecting children. The prevalence rate varies from 20-50% in different areas depending on socioeconomic status. At one end of the spectrum of malnutrition is **marasmus** (Greek word, "to waste"), which results from a continued severe deficiency of both dietary energy and proteins (primary calorie inadequacy and secondary protein deficiency). At the other end of the spectrum is **kwashiorkor**, where isolated deficiency of proteins along with adequate calorie intake is seen. Kwashiorkor means "sickness the older child gets, when the next child is born", a term from the local language of Ga tribe of Ghana. Severe malnutrition in early life can lead to permanent and irreversible physical and functional deficits. Severe persistent malnutrition may have deleterious effects on intellectual capacity in later life.

Biochemical Alterations

Table 18.9 gives a comparison between kwashiorkor and marasmus. Most important features are: Metabolic rate is decreased. **Hypoalbuminemia** is seen in kwashiorkor. In marasmus, this may not be so low. **Fatty liver** is seen in some cases of kwashiorkor, but not in marasmus. Fatty liver is due to decreased lipoprotein synthesis. **Glucose tolerance** is often normal, but hypoglycemia may be seen in children with marasmus.

Treatment of Protein Energy Malnutrition

Optimal response is observed with diets providing 150–200 kcal/kg body weight and 3–4 g of protein/kg body weight.

Table 18.9: Comparison between the salient features of kwashiorkor and marasmus

	Marasmus	*Kwashiorkor*
Deficiency of	Calorie	Protein
Cause	Early weaning and repeated infection	Starchy diet after weaning, precipitated by an acute infection
Growth retardation	Marked	Present
Attitude	Irritable	Lethargic
Appetite	Normal	Anorexia
Hair	No characteristic change	Sparse, soft and thin hair
Serum albumin	2 to 3 g/dL	<2 g/dL
Serum cortisol	Increased	Decreased

It is monitored by disappearance of edema, rise in serum albumin level and gain in weight.

OBESITY

Obesity is the most prevalent nutritional disorder in prosperous developed countries. It is a state in which excess fat has accumulated. Obesity can occur only as a result of ingestion of food in excess of the body's needs. The major causes are food habits (intake of calorie rich food in excess amounts) and lack of exercise.

Diseases Related with Obesity

Sensitivity of peripheral tissues to insulin is decreased. The number of **insulin receptors** are decreased in adipose tissue cells. In metabolic syndrome (**Syndrome-X)** insulin resistance, hyperglycemia and obesity are seen. There is increased risk of coronary artery disease and a reduced life span. Calorie-fat-restricted diet may retard aging process and extend the life span.

PRESCRIPTION OF DIET

Recommended daily allowances (RDA) of nutrients are given in detail in appendix III. While prescribing the diet of a person; the following general rules are to be remembered (Box 18.1).

First Step: Calorie Requirement

For a 60 kg sedentary man, the energy requirement is 60 × 30 = 1800 kcal plus additional allowance for specific dynamic action (1800 x 10% = 180). Therefore, the total requirement is roughly 2000 kcal. The recommended dietary intake for a 60 kg sedentary man, based on the above principles is given in Table 19.10. Balanced diet should contain calories from carbohydrate, proteins and fat in the **ratio of 60 : 20 : 20**.

Second Step: Proximate Principles

He requires 60 g proteins. This will give 60 × 4 = 240 kcal of energy. His total requirement is 2000 kcal. Therefore carbohydrates plus fats should produce (2000-240) = 1760 kcal.

As a general rule, about 20% of total calories are supplied by fat. Therefore, fats should supply 1760 × 20% = 350 kcal which is provided by (350/9) = about 35 g of fats. (About 30% of the total fat may be supplied as poly-unsaturated fatty acids).

The rest 1400 kcal are supplied by 350 g of carbohydrates. These calculations are based on the fact that 1 g carbohydrate provides 4 kcal, 1 g fat supplies 9 kcal and 1 g protein gives rise to 4 kcal. Thus, the requirements calculated in Table 18.10 may be rewritten as in Table 18.11.

Third Step: General Composition of Food

The third step is to calculate how these proximate principles are supplied as common foodstuffs. For this exercise, we should familiarize with the nutritive value of foodstuffs. A simplified version is given in Table 18.12. For detailed values see appendix IV.

Mutual Supplementation of Cereals and Pulses

Although protein content of pulses is more than cereals, average Indian diet contains more cereals, and hence proteins are mainly supplied by cereals. But pulses give good quality proteins.

A judicious combination of cereals and pulses provide all the essential amino acids (pulses are deficient in

BOX 18.1: Steps in prescribing a diet

This problem is approached by solving the following questions sequentially

- What is the requirement of the person with regard to calorie and other essential nutrients?
- What is the quantity of proximate principles required?
- Which composition of food will give the above requirement?
- How can a palatable diet that contains these compositions be prescribed?
- The total quantity may be divided into 3 or 4 meals at convenient intervals of time. After surgical procedures (as these procedures disrupt or remove the gingival fiber groups)

Table 18.10: First step in the prescription of diet

Energy required + SDA	*2000 Kcal*
Protein	60 g
Calcium	400 mg
Iron	25 mg

Table 18.11: Prescription of diet; 2nd step

Proteins	*60 g*
Fats	35 g
Carbohydrates	350 g
Calories	2000 kcal
Calcium	400 mg
Iron	25 mg

Table 18.12: Nutritive value of food items

Food	*Protein g/ 100 g of food*	*Fat g / 100 g of food*	*Carbohydrate g /100 g of food*	*Energy kcal/ 100 g of food*
Cereals (Wheat, rice, etc.)	10	1	65	300
Pulses (Bengal gram, etc.)	20	5	55	300
Tubers (Potato, etc.)	1	0	25	100
Green leafy vegetables	2	0	4	20
Fruits (banana, etc.)	2	0	10	50
Nuts and oils seeds	20	50	20	600
Milk and curd	3	4	5	60
Egg	13	13	0	170
Meat	20	3	0	100
Fish	20	10	0	170
Oils and ghee	0	100	0	900

methionine, while cereals lack in lysine). An accepted formula is that the food should contain pulses and cereals in the **ratio of 1:5** to provide good quality proteins.

Fourth Step: Determine the Items of Food

Now we can assemble this knowledge to prepare a diet to suit the requirements (Table 18.13). This will satisfy the requirements regarding protein (60 g) fats (45 g), calories (2000 kcal), calcium (400 mg) and iron (25 mg). See that cereals–pulses ratio is maintained at 5:1. When calories alone are to be increased, as in the case of a person having severe exercise, tubers and roots will serve this purpose.

Table 18.13: A diet for a 60 kg sedentary man

Item	*Quantity for vegetarian*	*Quantity for non-vegetarian*
Cereals	350 g	350g
Pulses	75 g	60 g
Vegetable oil	40 mL	25 mL
Milk	250 mL	150 mL
Leafy vegetables	200 g	200 g
Sugar	25 g	25 g
Fish/meat	---	60 g

A QUICK LOOK

1. One calorie is the heat required to raise the temperature of 1 g of water through 1°C. 1 kcal = 4.2 kJ.
2. Respiratory quotient is defined as the ratio of the volume of CO_2 produced to the O_2 consumed. Carbohydrates, fats, proteins have RQ's of 1, 0.7, and 0.8, respectively.
3. BMR is defined as energy required by an awake individual during physical, emotional and digestive rest. BMR can be measured using Benedict's Roth apparatus.
4. Normal value for men is 34–37 kcal/m^2/hour and for women is 30–35 kcal/m^2/hour.
5. Increased heat production following intake of food is referred to as specific dynamic action. Values of SDA for proteins, lipids and carbohydrates are 30%, 15%, and 5%, respectively.
6. Dietary fibers are essential to maintain normal motility of GI tract, prevent constipation, decrease cholesterol levels and to improve glucose tolerance.
7. Biological value of a protein is the ratio of the amount of nitrogen retained to that absorbed during a specific interval.
8. Marasmus and Kwashiorkor are two conditions of protein energy malnutrition.

CHAPTER 19

Detoxification

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Detoxification mechanisms
- Phase one, two and three reactions
- Oxidation, reduction, hydrolysis
- Glucuronic acid, sulfate, methyl groups
- Reactive oxygen species
- Generation of free radicals
- Damage produced by free radicals
- Free radical scavenger enzyme systems
- Clinical significance
- Antioxidants

Biotransformation is the process whereby a substance is changed from one chemical to another by a chemical reaction within the body. Thereby, toxic xenobiotics and body wastes are converted into less harmful substances and substances that can be excreted from the body. In general, biotransformation reactions generate more polar metabolites, that are readily excreted from the body. The liver plays the most important role in the biotransformation reactions.

The biochemical processes whereby the noxious substances are rendered less harmful and more water soluble, are known as **detoxification**. **Xenobiotics** are compounds which may be accidentally ingested or taken as drugs or compounds produced in the body by bacterial metabolism. (Greek, xenos = strange).

Biotransformation is not exactly synonymous with detoxification, since in many cases, the metabolites are more toxic than the parent substance. This is known as **bioactivation or toxication**. An example is the biotransformation of vinyl chloride to vinyl chloride epoxide, which covalently binds to DNA, a step leading to cancer of the liver. The compounds that are detoxified include:

- Compounds accidentally ingested like preservatives, food additives and adulterants
- Drugs taken for therapeutic purposes
- Compounds produced in the body which are to be eliminated, e.g. bilirubin and steroids.
- Compounds produced by bacterial metabolism, e.g. amines produced by decarboxylation of amino acids:
  Histidine → Histamine
  Tyrosine → Tyramine
  Tryptophan → Tryptamine

Enzyme Systems

The cytochrome P-450 is involved in the biotransformation reactions. They are heme-containing proteins, localized in the endoplasmic reticulum of liver. They are **inducible** enzymes. Some of the isoforms of the enzyme exhibit low catalytic activity. This explains the variation in drug responses among patients.

Phases of Detoxification Processes

Biotransformation reactions are usually classified as phase one and phase two reactions.

Phase one is the alteration of the foreign molecule, so as to add a functional group. Phase 1 reactions result in the formation of compounds with decreased toxicity (**detoxification**). Sometimes this may result in increased toxicity (**entoxification**), e.g. Methanol to formic acid.

The phase 1 reactions include hydroxylation, oxidation, reduction, hydrolysis, dealkylation, epoxidation, etc. Phase 2 reactions are sulfation, acetylation, methylation and conjugation with glucuronic acid, glutathione or glycine.

PHASE ONE REACTIONS

Oxidative Reactions

It may be either aromatic or aliphatic hydroxylation. The reactions also include sulfoxidation, N-oxidation and epoxidation. For example, **toluene** is hydroxylated to benzyl alcohol by mixed function oxidase system (Fig. 19.1). Sometimes both phase 1 and 2 reactions are necessary. The biotransformation of benzene requires both phase one and phase two reactions. Benzene is biotransformed initially to phenol by a phase one reaction (oxidation). Phenol has the functional hydroxyl group that is then conjugated by a phase two reaction (sulfation) to phenyl sulfate (Fig. 19.2). The oxidation and detoxification of **alcohol** is done in liver. Alcohol dehydrogenase (NAD^+ linked enzyme) oxidizes ethyl alcohol to acetaldehyde; and aldehyde dehydrogenase (FAD^+ linked enzyme) oxidizes aldehyde to acid. Benzaldehyde is oxidized to benzoic acid.

Reductive Reactions

Nitro compounds are reduced to their amines, while **aldehydes** or ketones are reduced to alcohols. Examples are:

Nitrobenzene → aniline (aminobenzene)
Picric acid → picramic acid
Para nitrophenol → para aminophenol

Hydrolysis

Hydrolysis is a chemical reaction in which the addition of water splits the toxicant into two fragments or smaller molecules. The hydroxyl group (-OH) is incorporated into one fragment and the hydrogen atom is incorporated into the other. Esters, amines, hydrazines, amides, glycosidic bonds and carbamates are generally biotransformed by hydrolysis. Examples are

Acetanilide → aniline + acetic acid
Aspirin (acetyl salicylic acid) → Salicylic acid + acetic acid

Aspirin is the drug most widely used in clinical practice. It has analgesic, antipyretic and anti-atherogenic activities.
Diisopropyl fluorophosphate (DFP) → Hydrofluoric acid + dialkyl phosphate

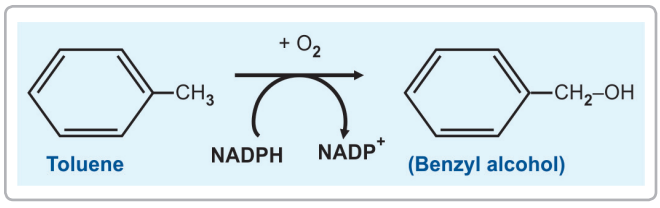


Figure 19.1: Phase one, oxidative reaction.

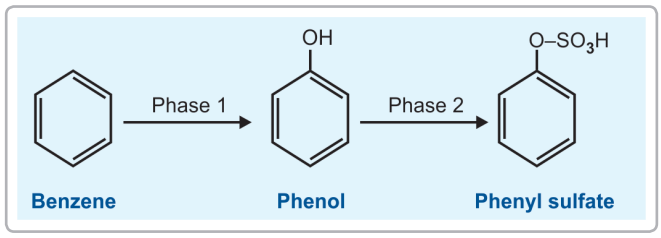


Figure 19.2: Phase one and two reactions combined.

Phase Two Reactions; Conjugations

A xenobiotic that has undergone a phase one reaction is now a new metabolite that contains a reactive chemical group, e.g. hydroxyl (-OH), amino ($-NH_2$), and carboxyl (-COOH). These metabolites must undergo additional biotrans formation as a phase two reaction. Phase two reactions are conjugation reactions, that is, a molecule normally present in the body is added to the reactive site of the phase one metabolite. In most cases, the conjugation will make the compounds nontoxic and easily excretable. Conjugating agents and their active forms are shown in Table 19.1. Glycine and glutamine can also act as conjugating agents.

Glucuronic Acid

Glucuronide conjugation is the most common phase two reactions. **Bilirubin** is a good example for a compound conjugated and excreted as its glucuronide. Glucuronic acid conjugates with hydroxyls (both phenolic and alcoholic), carbonyl, sulfhydryl and amino compounds.

The glucuronic acid is added to xenobiotics by **UDP-glucuronyl transferases**, present in the endoplasmic reticulum.

$$\text{UDP glucuronic acid} \xrightarrow{\text{UDP-glucuronyl-transferase}} \text{R—glucuronide} + \text{R—OH} + \text{UDP}$$

The drug metabolizing systems are induced by the drug, e.g. barbiturates induce glucuronyl transferase and heme synthesis. Conjugation with glucuronic acid is shown in Table 19.2.

Sulfate Conjugation

In general, sulfation decreases the toxicity of xenobiotics. The highly polar sulfate conjugates are readily excreted

Table 19.1: Phase two. Usual conjugating agents

Conjugating agent	*Active form*
Glucuronic acid	UDP-glucuronic acid
Sulfate	PAPS (phosphoadenosine phosphosulfate)
Cysteine	Glutathione
Acetic acid	Acetyl-CoA

Table 19.2: Conjugation with glucuronic acid

Compounds	*Types of bond*	*Products*
Phenol	Glycosidic (Ether)	Phenyl glucuronide
Benzoic acid	Ester	Glucuronic acid monobenzoate
Bilirubin	Ester with propionic acid side chain	Bilirubin glucuronide (For details refer Chapter 14)
Steroids	Ester with OH group	Glucuronide of steroid
Amines	Amide	N-glucuronides

through urine. Often glucuronidation or sulfation can conjugate the same xenobiotics. The sulfate group is transferred from PAPS (phospho adenosine phospho sulfate).

R–OH + PAPS → R–O–SO3 + PAP

Phenol + PAPS → Phenyl sulfate + PAP

Important compounds excreted as their sulfates include steroids and indole compounds.

Cysteine and Glutathione

The cysteine is derived from glutathione, which is the active conjugating agent. Alkyl or aryl halides, epoxides and alkenes are detoxified in this manner.

Acetylation

Conjugation with acetic acid is taking place with drugs like sulfanilamide and isoniazid.

Isoniazid → Acetylated isoniazid

Sulfanilamide → Acetylsulfanilamide

Conjugation with Glycine

Benzoic acid is conjugated with glycine to form **hippuric acid** (benzoyl glycine), which is excreted in urine.

Methylation Reactions

Amino, hydroxy or thiol groups are methylated. **S-adenosyl methionine** (SAM) is the methyl donor and the enzyme is usually O-methyltransferase. For example, catechol-O-methyltransferase converts epinephrine to metanephrine. Transmethylation reactions are given in detail in Chapter 11.

FREE RADICALS

The outermost orbital in an atom or molecule contains two electrons, each spinning in opposite directions. The chemical covalent bond consists of a pair of electrons, each component of the bond donating one electron each.

Definition: A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. Free radical is generally represented by a superscript dot, ($R^{\bullet}$).

Oxidation reactions ensure that molecular oxygen is completely reduced to water. The products of partial reduction of oxygen are highly reactive and produce havoc in the living systems. Hence, they are also called **Reactive oxygen species** or ROS. The following are members of this group:

- Superoxide anion radical (O_2^-)
- Hydroperoxyl radical ($HOO^{\bullet}$)
- Hydrogen peroxide (H_2O_2)
- Hydroxyl radical ($OH^{\bullet}$)
- Lipid peroxide radical ($ROO^{\bullet}$)
- Singlet oxygen ($^1O_2$)
- Nitric oxide ($NO^{\bullet}$)
- Peroxynitrite ($ONOO^{-\bullet}$).

Out of this, hydrogen peroxide and singlet oxygen are not free radicals (they do not have superscript dot). However, because of their extreme reactivity, they are included in the group of reactive oxygen species.

Generation of Free Radicals

They are constantly produced during the normal oxidation of foodstuffs, due to leaks in the electron transport chain in mitochondria. About 1–4% of oxygen taken up in the body is converted as free radicals. N**ADPH oxidase** in the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) produces superoxide anion by a process of respiratory burst during phagocytosis. The superoxide is converted to hydrogen peroxide and then to hypochlorous acid (HClO) with the help of **superoxide dismutase** (SOD) and **myeloperoxidase** (MPO). The superoxide and hypochlorous ions are the final effectors of bactericidal action. Ionizing radiation damages tissues by producing hydroxyl radicals, hydrogen peroxide and superoxide anion.

$$H_2O \xrightarrow{\text{(gamma, UV radiation)}} H' + OH'$$

Cigarette smoke contains high concentrations of various free radicals.

Free Radical Scavenger Enzyme Systems

Superoxide dismutase (SOD) produces hydrogen peroxide (Fig. 19.3).

Glutathione Peroxidase: This H_2O_2 is removed by glutathione peroxidase (POD) (Fig. 19.3). It is a selenium dependent enzyme.

Glutathione reductase: The oxidized glutathione, in turn, is reduced by the glutathione reductase (GR), in presence of NADPH (Fig. 19.3). This NADPH is generated with the help of HMP shunt pathway (For details refer Chapter 7).

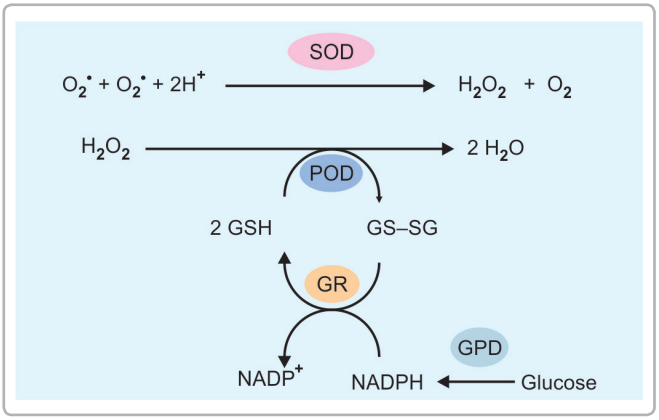


Figure 19.3: Free radical scavenging enzymes. SOD = superoxide dismutase. POD = glutathione peroxidase. GSH = glutathione. GR = glutathione reductase. GPD = glucose-6-phosphate dehydrogenase

Damage Produced by Reactive Oxygen Species

Free radicals are extremely reactive. Their half-life is only a few **milliseconds**. When a free radical reacts with a normal compound, other free radicals are generated. This chain reaction leads to thousands of events. **Peroxidation of PUFA** (polyunsaturated fatty acids) in plasma membrane leads to loss of membrane functions. Oxidation of sulfhydryl containing enzymes, loss of function and fragmentation of **proteins** are noticed. Polysaccharides undergo degradation. **DNA** is damaged by strand breaks. The DNA damage may cause cell death or mutation and carcinogenesis.

Clinical Significance

Free radicals play an important role in chronic inflammatory diseases such as **rheumatoid arthritis**, chronic ulcerative colitis, **chronic glomerulonephritis,** etc.

Moreover, free radicals play an important role in the generation of atherosclerosis and carcinogenesis. Free radicals also play a pivotal role in the aging process, and in the degenerative brain disorders such as Alzheimer's dementia.

Antioxidants

- Apart from the scavenging enzymes described earlier, **Vitamin E** (Alpha tocopherol) (For details refer Chapter 15) acts as the **most effective** naturally occurring **antioxidant** in tissues. Vitamin E is the lipid phase antioxidant.
- **Vitamin C** is the aqueous phase antioxidant.
- **Ceruloplasmin** can act as an antioxidant in extracellular fluid (For details refer Chapter 12).
- **Caffeine** is another effective antioxidant.
- **Vitamin A** and **Beta carotene** are minor antioxidants.

A QUICK LOOK

1. Biotransformation is the process whereby a substance is changed from one chemical to another by a chemical reaction within the body.
2. The biochemical processes whereby the noxious substances are rendered less harmful and more water soluble, are known as detoxification.
3. Xenobiotics are compounds which may be accidentally ingested or taken as drugs or compounds produced in the body by bacterial metabolism.
4. Almost all common drugs are metabolized by the P450 system.
5. Biotransformation reactions are usually classified as phase 1 and phase 2 reactions. Phase 1 is the alteration of the foreign molecule, so as to add a functional group, which can be conjugated in phase 2.
6. The phase 1 reactions include hydroxylation, oxidation, reduction, hydrolysis, dealkylation, epoxidation, etc.
7. Phase 2 reactions are sulfation, acetylation, methylation and conjugation with glucuronic acid, glutathione or glycine.
8. A free radical is a molecule or molecular fragment that has one or more unpaired electrons in its outer orbital.
9. Free radicals are generated in the body during oxidation processes.
10. The stepwise reduction of oxygen to water by electrons in Electron transport chain produces free radicals.
11. These include superoxide, peroxide, hydroxyl and singlet oxygen, together known as reactive oxygen species (ROS). Other free radicals include hydroperoxyl, lipid peroxide, nitric oxide and peroxynitrite radicals.
12. Enzymatic antioxidants are SOD, peroxidase, GSH reductase, catalase and peroxidase most of which are preventive antioxidants as well.
13. Peroxidation of PUFA leads to damage to membranes compromising membrane integrity and function.
14. DNA can also be damaged by free radicals and unless repaired promptly, the change may be perpetuated.
15. Cataract formation and reperfusion injury (after myocardial ischemia) are caused by free radicals.
16. LDL gets oxidized and the modified LDL particle is highly atherogenic and gets deposited beneath the endothelium initiating atheromatous plaque.
17. Degenerative diseases of old age like Alzheimer's, Parkinson's, etc. are due to cumulative effects of free radical injury.
18. The carbon centered radical or lipid peroxide molecule is formed when a free radical reacts with PUFA.
19. Vitamin E is the major biological antioxidant which will directly react with the peroxyl radicals to inactivate them. Other antioxidants in this group are vitamin C, Ceruloplasmin, beta-carotene, etc.

CHAPTER 20

Acid–Base Balance

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Acids and bases, pH, buffers
- Acid-base balance in the body
- Respiratory regulation of pH
- Renal regulation of pH
- Acidosis and alkalosis
- Intake and output of water
- Electrolyte composition of body fluids
- Regulation of sodium and water balance
- Isotonic contraction and hypotonic contraction
- Hypertonic contraction and isotonic expansion

Maintenance of appropriate concentration of hydrogen ion (H^+) is critical to normal cellular function. The acid-base balance or pH of the body fluids is maintained by a closely regulated mechanism.

ACIDS AND BASES

Definition

According to the definition proposed by Bronsted, **acids are substances that are capable of donating protons and bases are those that accept protons.** Acids are proton donors and bases are proton acceptors. For example,

$$HCl \rightarrow H^+ + Cl^-$$
$$HCO^-_3 + H^+ \rightarrow H_2CO_3$$

Weak and Strong Acids

In a solution of HCl, almost all the molecules dissociate and exist as H^+ and Cl^- ions. Hence, the concentration of H^+ is very high and it is a strong acid. But in the case of a weak acid (e.g. acetic acid), it will ionize only partially. So, the number of acid molecules existing in the ionized state is much less, may be only 50%.

Acidity of a Solution and pH

The acidity of a solution is measured by noting the hydrogen ion concentration in the solution. Sorensen expressed the H^+ concentration as the negative of the logarithm (logarithm to the base 10) of hydrogen ion concentration, and is designated as the pH. Therefore,

$$pH = -\log [H^+] = \log \frac{1}{[H^+]}$$

Thus, the **pH value is inversely proportional to the acidity**. Lower the pH, higher the acidity or hydrogen ion concentration while higher the pH, the acidity is lower (Table 20.1). The **pH 7 indicates the neutral pH**.

Table 20.1: Relation between hydrogen ions, hydroxyl ions and pH of aqueous solutions. Ionic product of water = $[H^+][OH^-] = 10^{-14}$

[OH-] mols/liter	[H+] mols/liter	log [H+]	–log [H+] = pH	pOH	Inference
1 x 10^-13	1 x 10^-1	–1	1	13	Strong acid
1 x 10^-10	1 x 10^-4	–4	4	10	Acid
1 x 10^- 7	1 x 10^-7	–7	7	7	Neutral
1 x 10^- 4	1 x 10^-10	–10	10	4	Alkali
1 x10^-1	1 x 10^-13	–13	13	1	Strong alkali

Henderson-Hasselbalch Equation

The relationship between pH, pKa, concentration of acid and conjugate base (or salt) is expressed by the equation,

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

BUFFERS

Definition

Buffers are solutions which can resist changes in pH when acid or alkali is added.

Composition of a Buffer

Buffers are two types:

1. Mixtures of weak acids with their salt with a strong base or
2. Mixtures of weak bases with their salt with a strong acid.

A few examples are given below:

- **i.** $H_2CO_3/NaHCO_3$ (Bicarbonate buffer) (carbonic acid and sodium bicarbonate)
- **ii.** CH_3COOH/CH_3COO Na (Acetate buffer) (acetic acid and sodium acetate)
- **iii.** Na_2HPO_4/NaH_2PO_4 (Phosphate buffer).

Factors Affecting pH of a Buffer

The pH of a buffer solution is determined by two factors:

1. **The value of pK:** The lower the value of pK, the lower is the pH of the solution.
2. **The ratio of salt to acid concentrations:** Actual concentrations of salt and acid in a buffer solution may be varied widely, with no change in pH, so long as the ratio of the concentrations remains the same.

Factors Affecting Buffer Capacity

On the other hand, the buffer capacity is determined by the actual **concentrations of salt and acid** present, as well as by their ratio. Buffering capacity is the number of grams of strong acid or alkali which is necessary for a change in pH of one unit of one liter of buffer solution. The buffering capacity of a buffer is defined as **the ability of the buffer to resist changes in pH when an acid or base is added.**

How do Buffers Act?

Buffer solutions consist of mixtures of a weak acid or base and its salt. To cite an example, when hydrochloric acid is added to the acetate buffer, the salt reacts with the acid forming the weak acid, acetic acid and its salt. Similarly when a base is added, the acid reacts with it forming salt and water. Thus, changes in the pH are minimized.

$$CH_3\text{–}COOH + NaOH \rightarrow CH_3\text{–}COONa + H_2O$$
$$CH_3\text{–}COONa + HCl \rightarrow CH_3\text{–}COOH + NaCl$$

Effective Range of a Buffer

A buffer is most effective when the concentrations of **salt and acid are equal** or when pH = pKa. The effective range of a buffer is **1 pH unit higher or lower** than pKa.

ACID–BASE BALANCE

Normal pH

The **pH of plasma is 7.4**. In normal life, the variation of plasma pH is very small. The pH of plasma is maintained within a **narrow range of 7.38 to 7.42**. The pH of the interstitial fluid is generally 0.5 units below that of the plasma.

Acidosis

If the pH is below 7.38, it is called acidosis. Life is threatened when the pH is lowered below 7.25. Death occurs when pH is below 7.0.

Alkalosis

When the pH is more than 7.42, it is alkalosis. It is very dangerous if pH is increased above 7.55. Death occurs when the pH is above 7.6.

Volatile and Fixed Acids

During the normal metabolism, the acids produced may be **volatile acid** like carbonic acid or nonvolatile (**fixed**) acids like lactate, keto acids, sulfuric acid and phosphoric acid. The carbonic acid, being volatile, is eliminated as CO_2 by the lungs. The fixed acids are buffered and later on the H^+ are excreted by the kidney.

Mechanisms of Regulation of pH

- Buffers of body fluids
- Respiratory system
- Renal excretion. These mechanisms are inter-related Box 20.1.

BOX 20.1: Mechanisms of regulation of pH

First line of defense	:	Blood buffers
Second line of defense	:	Respiratory regulation
Third line of defense	:	Renal regulation

BUFFERS OF THE BODY FLUIDS

Buffers are the first line of defense against acid load. These buffer systems are enumerated in Table 20.2. The buffers are effective as long as the acid load is not excessive, and the alkali reserve is not exhausted. Once the base is utilized in this reaction, it is to be replenished to meet further challenge.

Bicarbonate Buffer System

The most important buffer system in the plasma is the bicarbonate-carbonic acid system ($NaHCO_3/H_2CO_3$). It accounts for 65% of buffering capacity in plasma and 40% of buffering action in the whole body. The base constituent, bicarbonate (HCO_3^-), is regulated by the kidney (**metabolic component**). The acid part, carbonic acid (H_2CO_3), is under respiratory regulation (**respiratory component**). The **normal bicarbonate** level of plasma is **24 mmol/liter**. The ratio of HCO_3^- to H_2CO_3 at pH 7.4 is 20 under normal conditions. This is much higher than the theoretical value of 1 which ensures maximum effectiveness. The Bicarbonate carbonic acid buffer system is the most important for the following reasons:

- Presence of bicarbonate in relatively high concentrations.
- The components are under physiological control, CO_2 by lungs and bicarbonate by kidneys.

Alkali Reserve

Bicarbonate represents the alkali reserve and it has to be sufficiently high to meet the acid load. If it was too low to give a ratio of 1, all the HCO_3^- would have been exhausted within a very short time; and buffering will not be effective. So, under physiological circumstances, the ratio of 20 (a high alkali reserve) ensures high buffering efficiency against acids.

Phosphate Buffer System

It is mainly an **intracellular** buffer. Its concentration in plasma is very low. The pKa value is 6.8. The phosphate buffer system is found to be effective at a wide pH range, because it has more than one ionizable group and the pKa values are different for both. In the body, Na_2HPO_4/NaH_2PO_4 is an effective buffer system, because its pKa value is nearest to physiological pH.

Table 20.2: Buffer systems of the body

	Extracellular fluid	*Intracellular fluid*	*Erythrocyte fluid*
1.	$NaHCO_3$ H_2CO_3 (bicarbonate)	K_2HPO_4 KH_2PO_4 (phosphate)	K+Hb H+Hb (hemoglobin)
2.	Na_2HPO_4 NaH_2PO_4 (phosphate)	K+Protein H+Protein (protein buffer)	K_2HPO_4 KH_2PO_4 (phosphate)
3.	Na+Albumin H+Albumin	$KHCO_3$ H_2CO_3	$KHCO_3$ H_2CO_3

Buffers Act Quickly, But Not Permanently

Buffers can respond immediately to addition of acid or base, but they do not serve to eliminate the acid from the body. They are also unable to replenish the alkali reserve of the body. For the final elimination of acids, the respiratory and renal regulations are very essential.

RESPIRATORY REGULATION OF PH

The Second Line of Defense

This is achieved by changing the pCO_2 (or carbonic acid, the denominator in the equation). The CO_2 diffuses from the cells into the extracellular fluid and reaches the lungs through the blood. When there is a fall in pH of plasma (**acidosis**), the respiratory rate is stimulated resulting in **hyperventilation.** This would eliminate more CO_2, thus lowering the H_2CO_3 level (Box 20.2). When H_2CO_3 level is lowered or pH rises, the respiratory centre is depressed. However, this can not continue for long. The respiratory system responds to any change in pH immediately, but it cannot proceed to completion.

RENAL REGULATION OF PH

Kidneys excrete urine (pH around 6) with a pH lower than that of extracellular fluid (pH = 7.4). This is called **acidification of urine**. The pH of the urine may vary from as low as 4.5 to as high as 9.8, depending on the amount of acid excreted. The major renal mechanisms for regulation of pH are:

- Excretion of H^+ (Fig. 20.1)
- Reabsorption of bicarbonate (Fig. 20.2)
- Excretion of titratable acid (net acid excretion) (Fig. 20.3)
- Excretion of NH_4^+ (ammonium ions) (Fig. 20.4) See also Box 20.2.

Excretion of H^+; Generation of Bicarbonate

This process occurs in the **proximal convoluted tubules.** (Fig. 20.1). The CO_2 combines with water to form carbonic acid, with the help of carbonic anhydrase. The H_2CO_3 then ionizes to H^+ and bicarbonate. The hydrogen ions are secreted into the tubular lumen; in exchange for Na^+ reabsorbed. These Na^+ ions along with HCO_3^- will be reabsorbed into the blood. There is **net excretion of hydrogen ions, and net generation of bicarbonate**. So this mechanism serves to increase the alkali reserve.

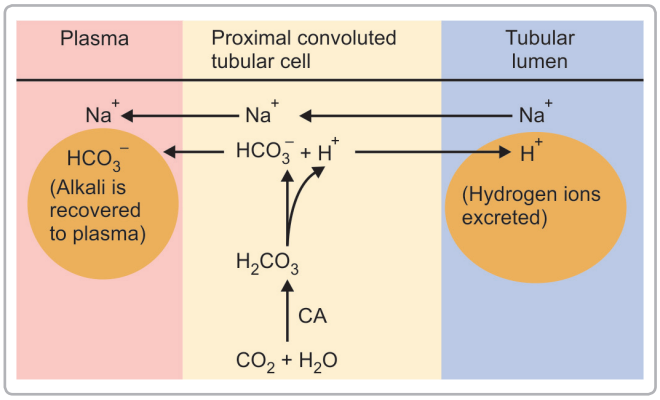


Figure 20.1: Excretion of hydrogen ions in the proximal tubules; *Abbreviation:* CA= Carbonic anhydrase.

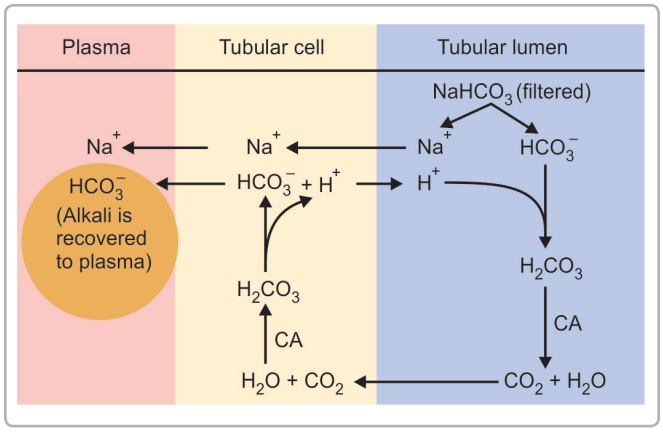


Figure 20.2: Reabsorption of bicarbonate from the tubular fluid. *Abbreviation:* CA= Carbonic anhydrase.

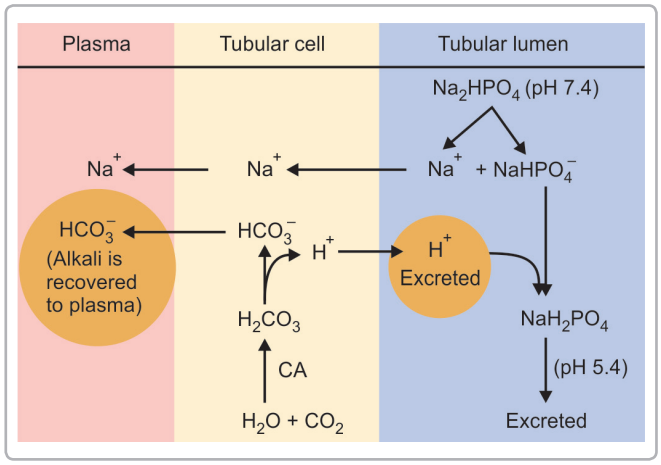


Figure 20.3: Phosphate mechanism in tubules

Reabsorption of Bicarbonate

The bicarbonate is completely reabsorbed. The bicarbonate in the glomerular filtrate combines with hydrogen ions to form carbonic acid. It dissociates to water and carbon dioxide under the influence of carbonic anhydrase. The CO_2 diffuses in, and then combines with water to form carbonic acid. The dissociation of carbonic acid produces hydrogen ions and bicarbonate. The bicarbonate is reabsorbed to replenish the alkali reserve (Fig. 20.2).

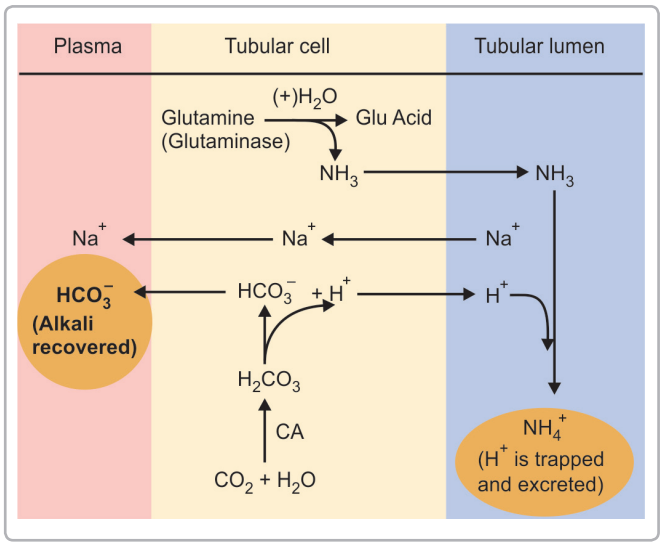


Figure 20.4: Ammonia mechanism

Excretion of H^+ as Titratable Acid

In the distal tubular segments net acid excretion occurs. The hydrogen ions are generated in the tubular cell by a reaction catalyzed by **carbonic anhydrase**. The bicarbonate generated within the cell passes into plasma. The term titratable acidity of urine refers to the number of milliliters of N/10 NaOH required to titrate 1 liter of urine to pH 7.4. This is a **measure of net acid excretion** by the kidney. The major titratable acid present in the urine is sodium acid phosphate. Due to the Na^+ to H^+ exchange occurring at the renal tubular cell border, the Na_2HPO_4 (basic phosphate) is converted to NaH_2PO_4 (acid phosphate). As a result, the pH of tubular fluid falls. The phosphate buffer is considered as the **urinary buffer**. The maximum limit of acidification is pH 4.5 (Fig. 20.3).

Excretion of Ammonium Ions

This would help to excrete H^+ and reabsorb HCO_3^- (Fig. 20.4). This mechanism also helps to trap hydrogen ions in the urine, so that large quantity of acid can be excreted with minor changes in pH. The excretion of ammonia helps in the elimination of hydrogen ions without appreciable change in the pH of the urine. The **Glutaminase** present in the tubular cells can hydrolyze glutamine to ammonia and glutamic acid. The NH_3

BOX 20.2: Summary of buffering against acid load

Stages	Features	Buffer components
Normal	Normal ratio = 20:1	HCO_3^- (N)
	Normal pH = 7.4	H_2CO_3 (N)
First line of defense	Acidosis; H^+ enters blood,	HCO_3^- (↓↓)
Plasma buffer system	bicarbonate is used up	
Second line defense	Hyperventilation	H_2CO_3 (↓)
Respiratory compensation	$H_2CO_3 \rightarrow H_2O + CO_2\uparrow$	
Partially compensated acidosis	Bicarbonate ↓;	HCO_3^- (↓↓)
	pH ↓	H_2CO_3 (↓↓)
Third line of defense renal mechanism	Excretion of H^+;	HCO_3^- (↓↓)
	Reabsorption of bicarbonate;	H_2CO_3 (↓↓)
	Ratio and pH tend to restore	

(ammonia) diffuses into the luminal fluid and combines with H^+ to form NH_4^+(ammonium ion). The glutaminase activity is increased in acidosis. So large quantity of H^+ ions are excreted as NH_4^+ in acidosis. The titratable acidity plus the ammonia content will be a measure of acid excreted from the body.

Relationship of pH with K^+ Ion Balance

In general, acute **acidosis is associated with hyperkalemia** and acute alkalosis with hypokalemia. Sudden hypokalemia may develop during the correction of acidosis. K^+ may go back into the cells, suddenly lowering the plasma K^+. Hence, it is important to maintain the K^+ balance during correction of alkalosis.

Classification of Acid–Base Disturbances

Acidosis (fall in pH)

Where acids accumulate or base is lost, it is **acidosis.**

- Respiratory acidosis: Primary excess of carbonic acid
- Metabolic acidosis: Primary deficiency of bicarbonate

Alkalosis (rise in pH)

A loss of acid or accumulation of base is **alkalosis**

- Respiratory alkalosis: Primary deficiency of carbonic acid
- Metabolic alkalosis: Primary excess of bicarbonate (See Box 21.3).

Compensatory Responses

- Uncompensated
- Partially compensated
- Fully compensated.

BOX 20.3: Calcitriol and calcitonin are different

pCO_2 >45 mm Hg = Respiratory acidosis
pCO_2 <35 mm Hg = Respiratory alkalosis

HCO_3 >33 mmol/L = Metabolic alkalosis
HCO_3 >22 mmol/L = Metabolic acidosis

H+ >45 nmol/L = Acidosis
H+ >35 nmol/L = Alkalosis

Mixed Responses

If the disturbance is pure, it is not difficult to accurately assess the nature of the disturbance (Box 20.3). In mixed disturbances, both HCO_3^- and H_2CO_3 levels are altered; e.g. a primary change in bicarbonate involves an alteration in pCO_2.

Metabolic Acidosis

It is due to a **primary deficit in the bicarbonate**. This may result from an accumulation of acid or depletion of bicarbonate. When there is excess acid production, the bicarbonate is used up for buffering. Depending on the cause, the anion gap is altered.

Metabolic acidosis and Anion Gap (Difference between measured anions and cations) are interrelated. When metabolic acidosis results from accumulation of acid, anion gap is widened.

Metabolic Alkalosis

Primary excess of bicarbonate is the characteristic feature. This results either from the loss of acid or from the gain in base. Loss of acid may result from severe

vomiting or gastric aspiration leading to loss of chloride and acid. Therefore **hypochloremic alkalosis** results. **Hypokalemia** is closely related to metabolic alkalosis. In alkalosis, there is an attempt to conserve hydrogen ions by kidney in exchange for K^+. This potassium loss can lead to hypokalemia. The respiratory center is depressed by the high pH leading to **hypoventilation**.

Respiratory Acidosis

A **primary excess of carbonic acid** is the cardinal feature. It may be acute or chronic. **Acute** respiratory acidosis may result from **bronchopneumonia** or status asthmaticus. Depression of respiratory center due to overdose of sedatives or **narcotics** may also lead to hypercapnia. **Chronic** obstructive lung disease will lead to chronic respiratory acidosis, where the fall in pH will be minimal. The findings in chronic and acute respiratory acidosis are summarized in Box 20.3. The renal compensation occurs, generating more bicarbonate and excreting more H^+.

Respiratory Alkalosis

A **primary deficit of carbonic acid** is described as the respiratory alkalosis. Hyperventilation will result in washing out of CO_2. Hyperventilation can occur in patients on ventilator support, hysteria, raised intracranial pressure and brain stem injury. The pCO_2 is low, pH is high and bicarbonate level increases. But bicarbonate level falls, when compensation occurs.

BOX 20.4: Causes of acid-base disturbances

	Acidosis		Alkalosis
A)	Respiratory acidosis	A)	Respiratory alkalosis
	Pneumonia		High altitude
	Bronchitis, asthma		Hyperventilation
	COPD		Septicemia
	Anesthestic, sedative		
B)	Metabolic acidosis	B)	Metabolic alkalosis
	Diabetic ketosis		Severe vomiting
	Lactic acidosis		Cushing syndrome
	Renal failure		Diuretic therapy
	Diarrhea		

BOX 20.5: Normal serum electrolyte values

pH = 7.4
Bicarbonate = 22–26 mmol/L
Chloride = 96–106 mmol/L
Potassium = 3.5–5 mmol/L
Sodium = 136–145 mmol/L

A summary of common causes of acid-base balance is given in Box 20.4.

Normal Serum Electrolyte Values (Box 20.5)

Students should always remember these values. Upper and lower limits are shown in Box 20.3. The causes of acid–base disturbances are shown in Box 20.4.

ELECTROLYTE AND WATER BALANCE

The maintenance of extracellular fluid volume and pH are closely interrelated. The body water compartments are shown in Box 20.6. Body is composed of about 60–70% water. Osmolality of the intra- and extracellular fluid is the same, but there is marked difference in the solute content.

Intake and Output of Water

During oxidation of food stuffs, 1 g carbohydrate produces 0.6 mL of water, 1 g protein releases 0.4 mL water and 1 g fat generates 1.1 mL of water. Intake of 1000 kcal produces 125 mL water (Table 20.3).

The major factors controlling the intake are thirst and the rate of metabolism. The renal function is the major factor controlling the rate of output. The rate of loss through skin is influenced by the weather.

Osmolality of Extracellular Fluid

Crystalloids and water can easily diffuse across membranes, but an osmotic gradient is provided by the non-diffusible colloidal (protein) particles. The **colloid**

BOX 20.6: The body water compartments

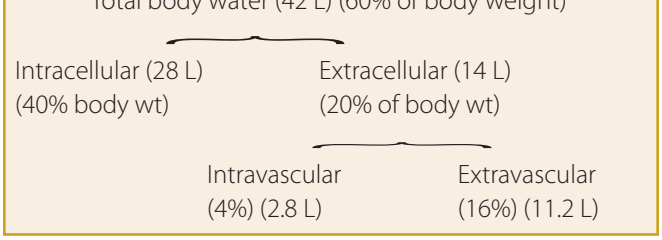


Table 20.3: Water balance in the body

Intake per day		*Output per day*	
Water in food	1250 mL	Urine	1500 mL
Oxidation of food	300 mL	Skin	500 mL
Drinking water	1200 mL	Lungs	700 mL
		Feces	50 mL
	2750 mL		2750 mL

Table 20.4: Electrolyte concentration of body fluid compartments

Solutes	*Plasma (mEq / L)*	*Interstitial fluid (mEq/L)*	*Intracellular fluid (mEq/L)*
Cations:			
Sodium	140	146	12
Potassium	4	5	160
Calcium	5	3	—
Magnesium	1.5	1	34
Anions:			
Chloride	105	117	2
Bicarbonate	24	27	10
Phosphate	2	2	140
Protein	15	7	54

osmotic pressure exerted by **proteins** is the major factor which maintains the intracellular and intravascular fluid compartments. If this gradient is reduced, the fluid will extravasate and accumulate in the interstitial space leading to edema. **Albumin** is mainly responsible in maintaining this osmotic balance (For details refer Chapter 12). The composition of each body fluid compartment is shown in Table 20.4. Since osmolality is dependent on the number of solute particles, the major determinant factor is the **sodium**. Therefore sodium and water balance are dependent on each other and cannot be considered separately. The **osmolality of plasma** varies from 285 to 295 mosm/kg. It is maintained by the kidney which excretes either water or solute as the case may be.

Regulation of Sodium and Water Balance

The major regulatory factors are the hormones (aldosterone, ADH) and the renin angiotensin system.

Aldosterone secreted by the zona glomerulosa of the adrenal cortex regulates the $Na^+ \rightarrow K^+$ exchange and $Na^+ \rightarrow H^+$ exchange at the renal tubules. The net effect is the sodium retention.

Anti-Diuretic Hormone (ADH): When osmolality of the plasma rises, the osmoreceptors of hypothalamus are stimulated, resulting in ADH secretion. ADH will increase the water reabsorption by the renal tubules. Therefore, proportionate amounts of sodium and water are retained to maintain the osmolality.

When osmolality decreases, ADH secretion is inhibited. When ECF volume expands, the aldosterone secretion is cut off.

Table 20.5: Disturbances of fluid volume

Abnormality	*Biochemical features*	*Osmolality*
Expansion of ECF		
Isotonic	Retention of Na^+ and water	Normal
Hypotonic	Water excess	Decrease
Hypertonic	Sodium excess	Increase
Contraction of ECF		
Isotonic	Loss of Na^+ and water	Normal
Hypotonic	Loss of Na^+	Decrease
Hypertonic	Loss of water	Increase

BOX 20.7: Renin and rennin are different

Kindly secretes **Renin;** it is involved in fluid balance and hypetension

Rennin is a proteolytic enzyme seen is gastric juice especially in children.

Renin-Angiotensin System

When there is a fall in ECF volume, renal plasma flow decreases and this would result in the release of renin by the juxtaglomerular cells (Box 20.7). The factors which stimulate renin release are: decreased blood pressure and salt depletion. The inhibitors of renin release are: Increased blood pressure, salt intake and angiotensin-II. Renin is the enzyme acting on the angiotensinogen (an alpha-2 globulin, made in liver).

Autoregulation

Angiotensin-II increases blood pressure by causing vasoconstriction of the arterioles. It stimulates aldosterone production by enhancing conversion of corticosterone to aldosterone. It also inhibits renin release from the juxtaglomerular cells.

Disturbances in Fluid and Electrolyte Balance

Abnormalities in fluid and electrolyte balance can be expressed in terms of tonicity. When the effective osmolality is increased, the body fluid is called **hypertonic** and when osmolality is decreased the body fluid is called **hypotonic**. These abnormalities of extracellular fluid (ECF) are classified in Table 20.5. Clinical effects of **increased** effective osmolality are due to dehydration of cells. A sudden **reduction** of effective osmolality may cause brain cells to swell leading to headache, vomiting and medullary

herniation. **Hypo-osmolality** causes swelling of cells and hyper-osmolality causes cell dehydration. Fatigue and muscle cramps are the common symptoms of electrolyte depletion.

Isotonic contraction of ECF: This results from the loss of fluid that is isotonic with plasma. The most common cause is loss of gastrointestinal fluid, due to small intestinal obstruction.

Hypotonic contraction: There is predominant sodium depletion. The cause is infusion of fluids with low sodium content like dextrose.

Hypertonic contraction: It is predominantly water depletion. The commonest cause is **diarrhea**, where the fluid lost has only half of the sodium concentration of the plasma. Vomiting and excessive sweating can also cause a similar situation.

Isotonic expansion: Water and sodium retention is often manifested as **edema** and occurs secondary to **cardiac failure**. Hemodilution is the characteristic finding. Secondary **hyperaldosteronism** often results from hypo-albuminemia (edema in nephrotic syndrome, protein malnutrition, etc.).

Hypotonic expansion: There is water retention either due to glomerular dysfunction or ADH excess.

Hypertonic expansion: It can occur in cases of Cushing's syndrome. The excess mineralocorticoid would produce sodium retention.

A QUICK LOOK

1. Acids are capable of donating protons.
2. Strong acids dissociate completely.
3. Weak acids ionize incompletely.
4. Acidity of a solution is measured by noting the hydrogen ion concentration.
5. The pH is inversely proportional to acidity.
6. Neutral pH is 7.
7. Buffers are solutions which can resist changes in pH when acid or alkali is added.
8. Buffers can be made by mixtures of weak acids with their salt with a strong base.
9. The pH of buffer is calculated by the Henderson-Hasselbalch equation.
10. Most important buffer system in plasma is the bicarbonate-carbonic acid system.
11. Bicarbonate represents the alkali reserve.
12. Normal bicarbonate level in plasma is 24 mmol/L.
13. Main intracellular buffer is phosphate buffer.
14. First defense against acid entry into blood is by bicarbonate buffer system.
15. Second defense against acid is by respiratory regulation of pH.
16. Third defense system is the renal regulation.
17. Renal regulation has 3 components: 1. Excretion of hydrogen ion; 2 Na^+/H^+ exchange and 3. excretion of ammonium ions.
18. Respiratory acidosis means primary excess of carbonic acid.
19. Metabolic acidosis means primary deficiency of bicarbonate.
20. Respiratory alkalosis is primary deficiency of alkali.
21. Metabolic alkalosis means primary excess of bicarbonate.
22. The major factors controlling the water intake are thirst and the rate of metabolism.
23. The renal function is the major factor controlling the rate of output of water.
24. Albumin is mainly responsible in maintaining the osmotic balance intravascularly.
25. Osmolality of plasma varies from 285 to 295 mOsm/kg. It is maintained by the kidney which excretes either water or solute as the case may be.
26. Regulation of sodium and water balance is by the hormones (aldosterone, ADH) and the renin angiotensin system.

CHAPTER 21

Tissue Proteins

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Collagen, structure and synthesis
- Abnormal collagens
- Elastin
- Keratins
- Muscle proteins
- Myosin, Actin, Troponins

COLLAGEN

The major structural protein found in connective tissue is the collagen. Collagen is a Greek word which means the substance to produce glue. It is the most abundant protein in the body. About 25–30% of the total weight of protein in the body is collagen. It serves to hold together the cells in the tissues. It is the major fibrous element of tissues like bone, teeth, tendons, cartilage and blood vessels.

When a solution of collagen is boiled, the viscosity of the solution decreases, which indicates that the native rod like structure is altered and a protein, with random coil structure results. It is then called **gelatin**.

Structure of Collagen

The **tropocollagen** is made up of three polypeptide chains. There are 6 types of collagen, out of which type I is the most abundant form; it contains 2 chains of alpha-1 and one chain of alpha-2.

Each polypeptide chain of collagen has about 1000 amino acid residues. The amino acid composition of collagen is quite unique. About 33% of the amino acids is **glycine**, i.e. every third residue is glycine. The repetitive amino acid sequence may be represented as Gly - X - Y- Gly - X - Y - ; where X and Y are other amino acids, most commonly **proline, hydroxyproline** and **hydroxylysine** are found in fairly large proportions in collagen. The hydroxylated amino acid residues are of special functional significance.

Synthesis of Collagen

The collagen is synthesized by fibroblasts intracellularly, as a large precursor, called procollagen. It is then secreted. The extracellular procollagen is cleaved by specific peptidases to form tropocollagen.

Hydroxylation of Proline and Lysine

The hydroxylation of proline and lysine residues of collagen is a post-translational modification taking place intracellularly. Prolyl hydroxylase and lysyl hydroxylase enzymes contain ferrous iron at the active site and require a reducing agent like **ascorbic acid**. So, vitamin C deficiency leads to poor hydroxylation. It is the major biochemical defect in scurvy (For details refer Chapter 16).

Triple Stranded Helix

The collagen is a rod-like structure. Each of the 3 polypeptide chains is held in a helical conformation by winding around each other. The resulting superhelical cable is made in a manner that 3.3 amino acid residues make one turn and each turn is separated by 2.9 Å.

The three strands are hydrogen bonded to each other. Glycine, because of its small size can fit into the crowded interior of the collagen triple helix (Fig. 21.1). For the same reason, glycine also produces a shallow groove into which other polypeptide strands are intertwined.

Quarter Staggered Arrangement

The tropocollagen molecules are arranged in a '*quarter staggered array*' to form the collagen fibers (Molecules in each row separated by 400 Å and adjacent rows by 680 Å). The structure repeats after fifth row (Fig. 21.2). Thus, the collagen fiber has **triple stranded, quarter staggered** arrangement. This arrangement helps in mineralisation.

Cross Links in Collagen Fibers

The collagen fibers are strengthened by covalent cross-links between lysine and hydroxylysine residues. The cross links are formed by **lysyl oxidase**. It is a copper containing enzyme, the copper ion being located at its active site. In **copper** deficiency, collagen synthesis is abnormal (For details refer Chapter 17).

The older the collagen, the more the extent of cross linkages. The process continues, especially in **old age**, so that the skin, blood vessels and other tissues become less elastic and more stiff, contributing a great extent to the medical problems of the old people.

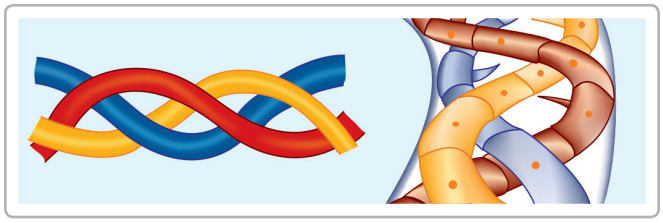


Figure 21.1: Triple stranded collagen fiber

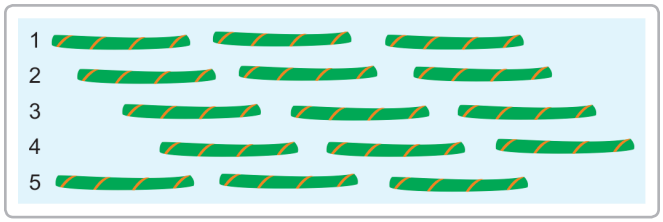


Figure 21.2: Quarter staggered arrangement in collagen fiber; each row moves one fourth length over the last row; the 5th row repeats the position of the first row

Functions of Collagen

- To give support to organs
- To provide alignment of cells, so that cell anchoring is possible. This in turn, helps in proliferation and differentiation of cells
- In blood vessels, if collagen is exposed, platelets adhere and thrombus formation is initiated.

Abnormalities in Collagen

Osteogenesis Imperfecta: It is inherited as a dominant trait. It is the result of a mutation which results in the replacement of a single glycine residue by cysteine. This change disrupts the triple helix near the carboxy terminus, hence the polypeptide becomes excessively glycosylated and hydroxylated. So, unfolding of the helix takes place and fibrillar array cannot be formed. This results in brittle bones leading to multiple fractures and skeletal deformities.

Ehlers Danlos syndrome: It is due to defective collagen formation. It is characterized by loose skin, hypermobile and lax joints.

Deficiency of ascorbic acid: It is characterized by defective hydroxylation of collagen. The collagen formed is weak, leading to fragility of blood vessels, poor wound healing, bleeding gum, etc. (For details refer Chapter 16).

Copper deficiency: Copper deficiency blocks the **lysyl oxidase,** resulting in reduced formation of cross linking. The elastic nature of elastin fibers are due to these different cross links.

Bone structure, bone mineralization, osteoporosis markers of bone diseases are described in Chapter 17. Composition of teeth, dentine and enamel are described in Chapter 22.

Elastin

Elastin is a protein found in connective tissue and is the major component of elastic fibers. The elastic fibers can stretch and then resume their original length. They have high tensile strength. They are found in the ligaments as well as in the walls of the blood vessels, especially large vessels like aorta.

Keratins

Keratins are **fibrous proteins** present in hair, skin and nails, horn, hoof, etc. They mainly have the alpha helical structure. Each fibril has 3 polypeptide chains and each

bundle has about 10–12 fibrils. The matrix has cysteine-rich polypeptide chains which are held together by disulfide bonds. The more the number of disulfide bonds, the harder the keratin is.

MUSCLE PROTEINS

Striated muscle is made up of multinucleated cells bound by plasma membrane called **sarcolemma**. Each muscle cell contains myofibrils about 1 mm in diameter. The myofibrils are immersed in a cytosol that is rich in glycogen, ATP, creatine phosphate and glycolytic enzymes.

The functional unit of a myofibril is a **sarcomere.** The dark A bands and light I bands alternate regularly (Fig. 21.3). The central H zone of A band is lighter, while the dark M line is found in the middle of the H zone. The I band is bisected by a very dense narrow Z line.

These bands are formed by variable combination of thick and thin filaments (Fig. 21.3).

The thick filament is primarily **myosin** and thin filament contains **actin, tropomyosin** and **troponin**. The Z line contains 2 actin molecules and M protein is located in the M line (Fig. 21.3).

Thick and thin filaments slide past each other during the muscle contraction, so that the muscle shortens by as much as a third of its original length. However, the length of the thick and thin filaments do not change during muscle contraction (Fig. 21.3). The mechanism is explained in Fig. 21.4.

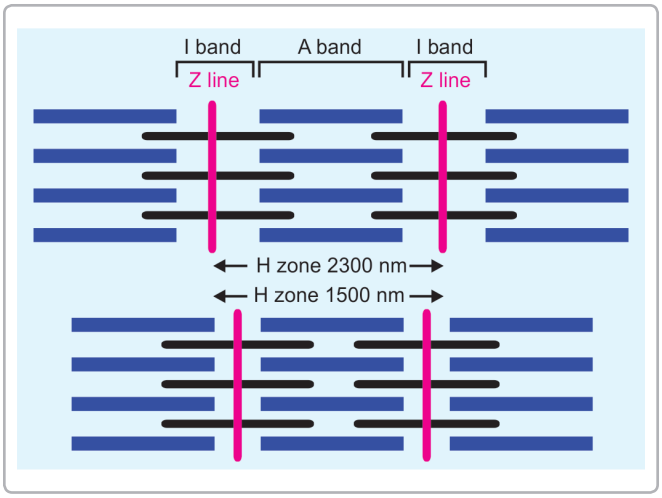


Figure 21.3: Sliding and shortening of actin and myosin. Compare the distance between Z lines in the upper and lower pictures

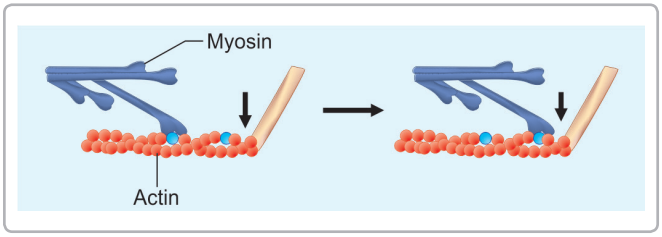


Figure 21.4: During muscle contraction, myosin moves over actin filament

Myosin

Myosin molecules are large (about 540 kD), each with 6 polypeptide chains. Part of the amino acid sequence in the heavy chain is similar to that at the active site of other ATPases.

Actin

It is the major protein of the thin filaments. It is a monomeric protein often referred to as G-actin due to its globular shape. It can polymerize into a fibrous form, called F-actin, which is a helix of actin monomer.

The muscle contraction results from interaction of actin and myosin, to form actomyosin, with energy provided by ATP.

When the two thin filaments that bind the cross bridges of a thick filament are drawn towards each other, the distance between Z lines becomes shorter (Fig. 21.3).

This could result in the process of contraction of muscle fibers. This needs energy from hydrolysis of ATP, effected by the ATPase activity of myosin.

The contractile force is generated by conformational changes, leading to cyclic formation and dissociation of actin and S1 heads of myosin. There is a reversible attachment and detachment of myosin S1 head to actin. This is due to the hinge like movements between the domains of myosin. The action of calcium is brought about by 2 proteins, troponin complex and tropomyosin located in the thin filament.

The troponin complex has 3 different polypeptide chains. Out of this, **troponin-C** (TnC) binds calcium.

Troponin-I (TnI), binds to actin and inhibits binding of actin to myosin. Troponin I is a marker for **myocardial infarction**. Its level in serum is increased within 4 hours of myocardial infarction.

Troponin-T (TnT) binds to tropomyosin. Two isoforms of cardiac TnT, called TnT1 and TnT2 are present in adult human cardiac tissue. Serum levels of TnT2 increases

within 4 hours of myocardial infarction, and remains high for up to 14 days. The TnT2 is 100% sensitive index for myocardial infarction.

The reservoir of high energy phosphate in skeletal muscle is creatine phosphate. The reaction (*Lohman's reaction*) is catalyzed by Creatine Kinase (CK) (For details refer Clinical Enzymology, in Chapter 3).

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{ATP} + \text{Creatine}$$

During muscle contraction, the ATP level remains high as long as creatine phosphate is present. But following contractile activity, the level of ADP and Pi rises. The reduced energy charge of active muscle stimulates glycogen breakdown, glycolysis, TCA cycle and oxidative phosphorylation.

A QUICK LOOK

1. Collagen has a triple helical structure, where the 3 strands are hydrogen bonded to each other and further stabilized by hydrogen bonds between OH groups and water molecules, thus giving high tensile strength to the fiber.
2. Tropocollagen molecules are arranged in a quarter staggered array.
3. The strength of collagen fibers is due to covalent cross links between lysine and hydroxylysine residues. These are formed by lysyl oxidase, which is a copper containing enzyme.
4. Abnormalities of collagen structure are seen in osteogenesis imperfecta, homocystinuria, Marfan's syndrome, ascorbic acid deficiency and copper deficiency.
5. Myosin molecules act as ATPase and binds to actin.
6. Actin is the major protein of the thin filament. Muscle contraction is effected by the sliding of actin over myosin.

CHAPTER 22

Biochemistry of Teeth and Caries

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Functions and composition of saliva
- Alterations in composition in diseases
- Composition of teeth
- Collagen and other proteins in teeth
- Dental caries, plaque
- Microorganisms causing caries
- Sucrose and caries
- Fluoride prevents caries
- Fluorosis

Saliva is the biological fluid, which bathes the oral cavity. It is a complex fluid produced by a number of specialized glands which discharge into the oral cavity. Saliva contains electrolytes and proteins with an osmolality less than or equal to that of plasma. Some amount of cell debris arising from the epithelial cells of the mouth are also constituents of saliva.

The total volume of saliva produced each day in adults is 500 to 1500 mL. Mixed saliva consists of the secretions of submandibular (65%), parotid (20%), and sublingual (5%) glands, the remaining 10% being provided by the numerous small labial, buccal, and palatal glands which line the mouth.

The glandular tissue is comprised of acinar cells, specialized groups of cells arranged as end pieces surrounding a small central lumen that opens into a narrow intercalated duct. Such ducts lead to the striated ducts, that in turn drain into the secretory ducts to form a single main secretory duct which drains into the oral cavity.

Functions of Saliva

- Antibacterial and antifungal action
- Buffering
- Digestion
- Mineralization
- Lubrication
- Many salivary components do multiple jobs. For example, amylase in addition to being an enzyme also inhibits precipitation of calcium salts.

Composition of Saliva

Saliva is not a simple ultrafiltrate of plasma, but rather a complex fluid formed by different mechanisms such as **a)** passive diffusion, **b)** active process against a concentration gradient and **c)** ultrafiltration through pores in the membrane. An active transport mechanism operates for many electrolytes and for some proteins such as IgA. Small molecules can be transported via the ultrafiltration route. Changes in plasma composition or components of diet have little effect on salivary constituents.

The **parotid glands** produce serous secretions only, devoid of mucin. On the other hand, the **submandibular** and sublingual glands secrete both serous and mucinous secretions. The viscosity of the submandibular saliva usually decreases with increasing flow rate since the serous cells have a greater response to stimulation than do the mucin-secreting cells. The sublingual gland contains

predominantly mucin-secreting cells and thus their secretion has a thick, viscous nature. Salivary secretion is stimulated by smell and taste. The regulatory centers are in pons in brain. Salivary secretion is a reflex response controlled by both parasympathetic and sympathetic secretomotor nerves. Stimulation of the sympathetic trunk in the neck or injection of epinephrine causes secretion by the submaxillary but not by the parotid glands. Parasympathomimetic drugs cause high saliva flow.

Inorganic Components

Saliva contains the usual electrolytes of the body fluids, the principal ions being sodium, potassium, chloride and bicarbonate (Table 22.1).

Sodium: The acinar cells of the salivary gland actively pump sodium ions from the blood into the lumen. The resulting osmotic pressure difference between the blood and the fluid in the lumen causes water to flow from the blood into the lumen. Thus, the primary secretion is almost isotonic with plasma. As this initial fluid moves down the ductal system of the salivary gland, an energy-dependent transport process reabsorbs sodium and chloride.

Potassium and bicarbonate ions are actively secreted into saliva. However, the ductal membranes are relatively impermeable to water, so the resulting saliva becomes increasingly hypotonic as it moves down the ductal system. The cells lining the ducts have a limited capacity to pump the sodium out of saliva. As the saliva flow rate increases so does the sodium ion concentration. Potassium, chloride, and bicarbonate ion concentrations also show a marked dependence upon flow rate.

Saliva pH can range from 6.2 to 7.6, with the higher pH exhibited upon increased secretion. Bicarbonate level is significant because of its buffering capacity.

Calcium and phosphate concentrations are directly related to acid dissolution of tooth enamel and to precipitation of calculus.

Minute quantities of thiocyanate ions present in saliva will have antibacterial activity. Fluoride ions have a protective role against caries (see below).

Infants have a higher concentration of calcium, magnesium and chloride ions; but lower phosphate ions, when compared to adult levels.

Table: 22.1: Characteristics of mixed saliva

Volume	:	500–1500 mL/day
Rate of flow	:	0.1–0.25 mL/min
pH	:	5.6–7.2 (mean 6.5)
Water content	:	97–99.5 %
Total protein	:	0.1–0.6 g/dL
Mucin	:	0.27 g/d–
Glucose	:	10–20 mg/d–
Potassium	:	10–40 mmol/L
Sodium	:	2–50 mmol/L
Calcium	:	1–2.5 mmol/L
Magnesium	:	0.2–06 mmol/L
Phosphate	:	2–22 mmol/L
Chloride	:	5–50 mmol/L
Total lipid	:	20 mg/dL
Cholesterol	:	7.5 mg/dL

Organic Components

The total **protein** concentration in saliva is very little and is less than 1% of that in plasma. Important proteins of saliva include, Mucin, Statherins, Histatins, Lysozyme, Proline rich proteins (PRPs), Carbonic anhydrase, Lingual Lipase, Amylase, Peroxidase, Lactoferrin and Immunoglobulin A (IgA). Major carbohydrate in saliva is **glucose** (10–20 mg/dL). Almost all the organic compounds of plasma, such as hormones, immunoglobulins and enzymes may be detected in saliva in trace amounts.

Blood clotting factors, amino acids, urea and lipids are also present in saliva.

Mucins

They constitute the major proteins of the saliva. The salivary Mucins exist in two forms; MG1 and MG2. Both are glycoproteins. They contain negatively charged groups, such as sialic acid and sulfate. They are hydrophilic and trap water resulting in high elasticity and stickiness (Box 22.1). Mucin forms a protective coat around both hard and soft tissues and also lubricates them. The **oligosaccharide** residues bind to bacterial proteins preventing them from adhering to soft tissue and enamel. These oligosaccharides also mimic those found on mucosal cell surface and inhibit bacterial adhesions.

Drugs

Drugs circulating in plasma may pass through the capillary wall, the basement membrane and the membrane of the glandular epithelial cells, and then finally discharged into the salivary duct. The rate-determining step for this

BOX 22.1: Functions of mucin

Tissue Coating
- Protective coating around hard and soft tissues
- Primary role in formation of acquired pellicle
- Concentrates antimicrobial molecules at mucosal interface

Lubrication
- Align themselves with direction of flow
- Increases lubricating qualities (film strength)
- Film strength determines how effectively opposed moving surfaces are kept apart

Aggregation of bacterial cells
- Bacterial adhesion to mucins may result in surface attachment
- Mucin-coated bacteria may be unable to attach to surface

transportation is the passage of the drug through the lipophilic layer of the epithelial membrane. According to physicochemical principles, the drug must be lipophilic for such a passage to occur.

Salivary Enzymes

The main enzymes present in saliva are the amylase, lingual lipase, carbonic anhydrase and peroxidases. Saliva supplies **enzymes** for digestion. These enzymes and other proteins, including saliva-specific glycoproteins, are synthesized by the acinar cells.

Amylase

The major salivary enzyme is alpha amylase. The amylase acts on carbohydrates. It cleaves the alpha-1,4-glycosidic bonds of starch. The products are small quantities of maltose (disaccharide) and smaller sized polysaccharides (For details refer Chapter 4). The optimum pH of salivary amylase is 6. However, its action is short lived as the food passes into stomach and the enzyme becomes inactive at the highly acidic pH of the gastric lumen. The parotid gland secretes most of the amylase. When there is any obstruction to the salivary ducts or inflammation of the glands (as in mumps), the salivary amylase passes into the blood and elevates the level of serum amylase. Amylase also shows weak antibacterial properties as well as buffering property.

Other Enzymes

Lingual lipase acts on triglycerides and is important in the digestion of milk fat in infants. **Carbonic anhydrase** is responsible for the buffering action of saliva. **Peroxidases** assist in the bactericidal function. **Lysozyme** in saliva has antimicrobial action. The bactericidal effect is by breaking down the muramic acid present in bacterial cell walls.

Other Proteins

Immunoglobulin A (IgA) is the antibodies present in body secretions. It may be effective against cariogenic bacteria. IgA levels are found to be low in some persons with dental caries. **Lactoferrin** chelates the iron and in the absence of iron, the metabolic processes in bacteria are inhibited. Saliva also contains a group of histidine rich proteins with antifungal activity. **Statherins** are proteins that keep the supersaturated calcium phosphate in the ductal saliva from crystallizing. The supersaturated calcium phosphate is necessary for the maintenance of enamel integrity. Statherins bind calcium and prevent precipitation of calcium phosphate. So the probability of formation of dental calculus is reduced. The Statherins also help in lubrication. The **Proline Rich Protein** (PRP) contains a large number of proline residues (40% or more). PRP in saliva are mainly secreted by parotid glands. They also reduce precipitation of calcium phosphate. PRPs also help in the formation of the enamel pellicle. This reduces the bacterial attacks and slows down the loss of calcium and phosphate ions from the teeth.

Alterations in Composition in Diseases

Alterations in composition of saliva may indicate diseases of the salivary glands. Sodium and chloride are elevated while phosphate concentrations are lowered in **sialadenitis** (inflammation of salivary gland). Similar picture is seen in **Sjogren's syndrome**, which is a connective tissue disease characterized by decreased secretions from salivary and lacrimal glands and causes dry mouth (xerostomia). **Radiation** to oral cavity (for treatment of oral cancer) will decrease the flow rate of saliva. Such saliva secreted has high sodium chloride, calcium and magnesium levels. Systemic diseases like Cystic fibrosis, Addison's disease, Cushing's syndrome, hyperparathyroidism and generalized immunological disorders can affect salivary flow rate and composition. Presence of leukocyte esterase activity is an index of infection in the salivary glands. HIV (human immunodeficiency virus) causing AIDS (acquired immunodeficiency syndrome) is secreted through saliva; but it is not proved that HIV is transmitted through saliva.

Salivary Biomarkers

Salivary biomarkers are useful for monitoring oral and systemic diseases. These biomarkers help monitor health status and onset of disease as well as response and outcome of treatment. Advantages of salivary testing include it is non-invasive, ease of use and inexpensive, less contamination and easy collection of saliva.

Biomarkers like immunoglobulins, enzymes like salivary esterase, lysozyme, peroxidase, chitinase, acid phosphatase and arginase are implicated in periodontal diseases. Various salivary proteins like mucins, lactoferrin, histatin, fibronectin and cystatin, amino acids, platelet activating factor, growth factors like transforming growth factor alpha and beta, platelet derived growth factor, epidermal growth factor, hepatocyte growth factor and vascular endothelial growth factor, epithelial keratin, hormones like cortisol, calcium, inflammatory cells and volatile sulfur compounds have also been implicated in periodontal diseases.

Other markers include proinflammatory cytokines, matrix metalloproteinases (MMPs), and bone biomarkers like telopeptide, alkaline phosphatase, osteocalcin, as well as stress markers like C-reactive protein. Serum biomarkers have also been detected in saliva including markers of oxidative stress. Markers have also been detected in gingival crevicular fluid. Salivary biomarkers have been increasingly used in systemic diseases like autoimmune diseases, cardiovascular diseases, diabetes, HIV and oral cancer, and in caries.

Saliva Substitutes

Saliva substitutes are available in various formulations, e.g. lozenges, sprays, mouth rinses, gels, oils, chewing gums and toothpastes. But no single product could adequately reproduce the properties of the natural saliva. The saliva substitute products usually contain preservatives. Therefore, their uses are limited only to oral cavity and they are not recommended to be swallowed. Saliva swallowing is critical for food ingestion since the flow of saliva through oropharyngeal isthmus stimulates swallowing process and taste perception in oropharyngeal area. An ideal saliva substitute should be inexpensive, edible, hydrating, easy-to-swallow but retainable in the mouth.

The purpose of oral lubricants is to alleviate the oral discomfort associated with xerostomia. Oral lubricants substitute the need to frequently sip on water, to alleviate oral discomfort and to moisten the oral mucosa. Water is the most commonly used home remedy for the management of discomfort associated with xerostomia. In mild cases of xerostomia, frequent sipping of water, along with avoidance of certain foods and chemicals, such as alcohol, caffeine and sodium lauryl sulfate (SLS) may alleviate xerostomia to an acceptable level. Olive oil is another home remedy used to alleviate the symptoms of xerostomia, which not only has lubricating properties, but has also demonstrated. Milk has also been suggested in the literature as a readily available oral lubricant, which not only provides lubrication to the oral tissues, but may also aid in buffering acids and promoting remineralization of dental hard tissues due to the high calcium and phosphate content, anti-inflammatory and antimicrobial characteristics.

Commercial oral lubricants use chemicals of high viscosity to mimic the physical properties of natural saliva and one of the most frequently used is carboxymethylcellulose (CMC), a water soluble polymer used commonly in pharmaceuticals as a suspension matrix. Due to the water soluble nature of CMC, duration of action tends to be limited and frequent reapplication of the lubricant is required to maintain moisture. Dry Mouth Gel utilizes a CMC base in its range of pH neutral flavored gels for the alleviation of symptoms associated with xerostomia.

COMPOSITION OF TEETH

Connective tissue originates from the **mesoderm**. Bone, dentine and cementum are mesodermal in origin. But dental enamel originates from the **ectoderm**. During the formation of teeth, there is a close association of inorganic (mineral) crystal material, and organic fibrous (polymer) structures, both components playing a structural role in the tooth.

Inorganic Components

The inorganic calcium is deposited along with phosphate as **apatite**, which is the major form of **calcium** in all the tooth tissues. Hydroxy-apatite has the empirical formula, $Ca_{10}(PO_4)_6(OH)_2$. A small proportion of other crystalline forms of calcium phosphate may also exist in teeth. Amorphous (non-crystalline) calcium phosphate may be found in the dentine. The **phosphate** ions constitute the major component of the ions present in the crystal. The phosphate ions are spherical and they are closely packed in hexagonal shape. Such hexagonal close pack

(hcp) phosphate ions stack over many layers, such that the center of the phosphate ion in every alternate layer is directly above the center of phosphate ion in the first layer.

This arrangement results in **octahedral channels** running through the crystal structure. Two-thirds of these channels are occupied by calcium ions; thus representing two-fifths of the total number of calcium ions. The remaining third of the channels are occupied by negative fluorine ions. This is called **fluoroapatite**.

Trace Elements

In human enamel, trace elements such as iron, zinc, copper, and manganese are found. Iron and zinc accumulate near the surface of the tooth, i.e. in the outer layers of enamel.

Organic Components

Collagen

It is the major protein component of calcifying tissues like bone, dentine and cementum. The predominant organic component of teeth is collagen, an insoluble protein present in the dentine and basal plate of the mature tooth. Collagen is the most abundant protein present in vertebrates. Collagen has a very specialised structural role (For details refer Chapter 21). Each polypeptide chain of collagen has about 1000 amino acid residues. About 30% of the amino acids are **glycine**. That is, every third residue is glycine. Proline and lysine constitute another 30%. Proline and lysine residues are later hydroxylated to form **hydroxyproline** and hydroxylysine, which are further glycosylated. The collagen fibers are further strengthened by covalent cross links between lysine and hydroxylysine residues of opposite fibers. About 90% of the organic matrix is type I collagen (containing two alpha-1 and one alpha-2 chains) which is organized to form the **right handed triple helix**. The collagen supercoils align in a parallel, staggered, arrangement. This is called "*triple stranded quarter staggered arrangement*". Several such microfibrils compile to form a collagen fiber. The gaps in the staggered arrangement may provide nucleation sites for apatite mineralization. The structural proteins and apatite of teeth need to be synthesized in an integrated way. In teeth the collagen fibrils are suited to the roles of supporting three-dimensional stress, and of orienting and supporting apatite crystals.

Other Proteins in Teeth

In addition to collagen, the extracellular matrix also contains **glycoproteins** (GP) and glycosaminoglycans (GAG). (GP is short polymer with irregular sequences. GAGs are long polymers of proteins and carbohydrates, with regular sequences). These proteins are associated with the dentine and basal plate. Specialized such proteins seen in bone are **Osteonectin** (GP) and **Osteocalcin** (GAG). Cementum contains the GPs **Osteopontin** and **Amelogenin**. Dentine contains the GP **Osteonectin**, and another highly phosphorylated phosphoprotein called **Phosphoryn**. Enamel contains **Amelogenin** and **Enamelin**. In bone and cementum, the matrix is termed osteoid and **cementoid**. The matrix is added as a layer over the surfaces of bone and cementum, where new deposits of calcified material are laid down. In dentine, the equivalent layer is called **predentine**. However in enamel, there is no equivalent and the matrix is rapidly and highly calcified. The concentrations of inorganic salts increase from 37 to 95% as enamel matures.

Proteins of Dentine

The extracellular matrix proteins of bone and dentine are similar consisting of type 1 collagen, acidic glycoproteins and proteoglycans. Collagen forms the lattice for mineralization, but non-collagen proteins control initiation and growth of crystals. Three major proteins found specifically in dentine but absent in bone are

1. Dentine phosphoryn
2. Dentine matrix protein
3. Dentine sialoprotein.

These proteins play an important role in control of mineralization.

Proteins of Enamel

Amelogenin is a low molecular weight extracellular matrix protein. It constitutes about 90% of all enamel protein. It has hydrophobic residues on the outside. The 27 amino acid portion of amelogenin functions as a calcium channel. Phosphorylation of a serine residue of the protein opens the calcium channel, through which calcium ions zoom through and are funnelled to the mineralization front. The calcium ions are used for the formation of calcium hydroxy apatite crystals. It has a modulating effect

on initiation and growth of hydroxy apatite crystals during enamel mineralization. It also influences the development of cementum.

Mutation of amelogenin gene leads to **Amelogenesis imperfecta** which is an inherited condition characterized by abnormal enamel formation in quantity, growth, maturation and crystallization, amelogenesis imperfecta. The genes are present on X and Y chromosomes, designated AMELX and AMELY. Both genes are transcriptionally active but the sequence differs. The other proteins found in enamel are **ameloblastin**, **enamelin** and **tuftelin**.

Mineralization

Mineralisation is a process by which inorganic calcium and phosphate are deposited on the organic matrix. **Osteoblasts** synthesize and secrete organic matrix, which is then mineralized. **Osteoclasts** are involved in bone resorption. **Alkaline phosphatase** is the key enzyme in the process of mineralization. The enzyme liberates phosphate from substrates, so that ionic concentration (of calcium x phosphate) is increased to supersaturation level, leading to deposition of apatite.

DENTAL CARIES

Caries is a Latin term, meaning "decay". There is local destruction of tooth tissues with demineralization. Alternative terms are dental cavities or tooth decay. In the pits and fissures of premolar and molar teeth, bacterial fermentation of residual food leads to acid production. While tooth decay has been known from prehistoric times, it is only after the introduction of Sucrose into the diet during historic times, caries has become a public health problem.

The initial step in the development of caries is the formation of the **plaque**. Normal bacterial flora of mouth converts all food particles, especially sugar and starch, into acids. Bacteria, acid, food debris, and saliva combine in the mouth to form a sticky substance called 'plaque' that adheres to the teeth.

It is mainly seen on the grooved chewing surfaces of molars, just above the gum line on all teeth, and at the edges of fillings. Plaque is thus a **biofilm** containing micro-organisms seen on the surface of teeth. If the plaque is not regularly removed by brushing, it may be calcified, leading to the formation of **Tartar** or **Calculus.** Impaired salivary secretion hastens plaque growth.

Microbiological Organisms cause Dental Caries

The development of caries lesion requires the presence of the bacteria ***Streptococcus mutans***. This is generally seen in the oral mucosa and in dental plaque. Normally, the saliva that bathes the oral cavity keeps the growth of microorganisms under check. When there is a decrease in saliva flow, the pH of the plaque drops, allowing the acid tolerant bacteria like *S. mutans* to proliferate. *S. mutans* forms dextrans and causes a sticky plaque, trapping bacteria, calcium and phosphate ions.

Streptococcus mutans

S. mutans metabolizes sucrose in a remarkably diverse fashion that is not matched by any other known plaque organism. In this process, the enzyme **invertase** splits sucrose into its component glucose and fructose molecules, which are then converted to lactic acid by the glycolytic pathway. **Glucosyltransferases**, split sucrose but transfer the glucose moiety to glucose polymers known as **glucans**, **dextrans** and **mutans**. *S. mutans* also has enzymes that split sucrose and transfer the fructose moiety to a fructose polymer known as a **fructan**. Several such complex glucans are produced differing in their core linkages, branching properties and molecular weights. Other plaque bacteria can use sucrose to synthesize one or more of these polymers, with the exception of mutan. Only *S. mutans* can form all of them.

Sucrose and Caries

The supragingival plaque flora derives its nutrients from various sources that include diet, saliva, sloughed epithelial cells, dead microbes, and gingival crevice fluid or exudate. All sources, except the foods in the diet provide only small amounts of nutrients. Dietary components are normally high-molecular-weight polymers (starch and proteins). They have a minimal effect on plaque growth.

Sucrose is a low-molecular-weight disaccharide that can be rapidly metabolized by the plaque flora. Sucrose fermentation produces lactic acid with consequent drop in the pH, to 5.0 or lower, at the point of interface between plaque and enamel. When sucrose is ingested during meals, sufficient saliva is secreted to buffer the plaque pH and decay does not occur. In fact, studies show that sucrose consumed daily at meals for two years was not associated with an increase in dental decay.

However, when the same or lesser amounts of sucrose were ingested between meals, subjects developed caries at

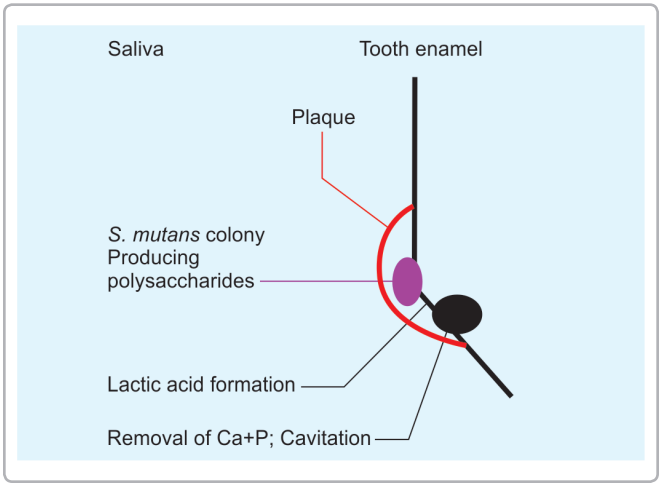


Figure 22.1: Bacterial action produces cavity

the rate of about three to four tooth surfaces per year. When the plaque pH value falls below 5.0, the salivary buffers are overwhelmed. As lactic acid accumulates, the enamel begins to dissolve, releasing calcium and phosphate ions from sites beneath the surface enamel (Fig. 22.1).

Normally, the saliva replenishes these minerals, but if the flux from the enamel is more, repair does not occur and cavitation results. Thus, sucrose consumption per se does not cause decay, but the **frequent ingestion of sucrose** by prolonging the time period by which the plaque is acidic, is cariogenic.

Pathology of Caries

The glucans enable *S. mutans* to adhere tenaciously to the tooth surface and to accumulate on these surfaces, thereby causing decay in the underlying surface. These polymers form a sticky matrix over the tooth surface, allowing the adhesion of bacteria. Decay on smooth surfaces seems to depend on the retentive polymers formed by *S. mutans*, whereas in sites where retention is provided by the anatomy of the teeth (pits, fissures, and contact points between teeth), these polymers are not as important. When the earliest carious lesion is detected, only *S. mutans* are seen in significant levels and proportions (Fig. 22.1). *S. mutans* was found to be more active than the other bacteria at pH 5.0, and thus, it is probably most active at the very pH at which the teeth begin to demineralize.

S. mutans, although scarce in the initial inoculum (fewer than 0.1% of the initial colonizers), is selected, if the average pH value in the site is not well buffered by saliva. Frequent ingestion of sucrose-containing products predisposes toward lower pH values and thus selects for *S. mutans*. When the pH remains in the vicinity of 5.0–5.5, tooth mineral is solubilized, thereby buffering the plaque and maintaining an environment suitable for growth of *S. mutans*.

Eventually, enough mineral is lost so that a **cavitation** occurs in the enamel, and if this enlarges so that it extends into the dentin, a semiclosed system is formed in which the pH value drops below 5.0 (Fig. 22.1). Under these acidic conditions, growth of **lactobacilli** is favored, and these organisms succeed as the predominant flora in the carious lesion. Lactobacilli are the most potent acid-producing plaque bacteria, but these organisms only predominate by the time the carious lesion has extended into the dentin. The acid will corrode the minerals of enamel below the plaque. The initial lesion is a painless white spot caused by decalcification of enamel followed by cavitation and a brownish discoloration. The acid later removes dentine to expose the pulp, leading to inflammation and toothache. When left untreated, tooth decay can result in death of the internal structures of the tooth and ultimate loss of the tooth.

Prevention of Caries

Ideally, oral hygiene is the best way to prevent caries. This consists of proper brushing at least twice a day and regular dental examination and cleaning, every 6 months. However, frequent eating also increases the chances of developing caries, since it keeps the plaque pH low for longer periods. Hence, the importance of proper cleaning and removing food debris after consumption of food. High molecular weight starch and proteins are not well utilized by the bacteria. So, milk, fresh fruits and vegetables are not cariogenic. Dietary factors that protect teeth against caries are fluoride and sugar free salivary stimulants. An important concept about treatment of caries is that the destroyed tooth will not regenerate. The aim of treatment is thus to prevent caries or to arrest the progression of caries.

FLUORIDE

Most of the fluoride ingested is taken up by calcified tissues. Fluoride passes from plasma and extracellular fluid into the calcified tissues. During growth and calcification of these tissues, fluoride is directly incorporated into the new apatite crystals. When growth has ceased, incorporation occurs in areas where bone turnover occurs. Fluoride is also taken up by ion exchange at the bone surface. The

rate of deposition decides the amount of fluoride taken up. When calcium deposition occurs slowly, more fluoride is taken up. Since dentine and cementum are formed at a slow rate during their development, the fluoride distribution is more or less uniform throughout. But these tissues slowly increase in thickness throughout life. So, the surfaces in contact with the interstitial fluid of periodental ligament, or pulp, accumulate higher concentrations of fluoride.

Enamel on the other hand, has a uniform concentration of fluoride throughout its thickness, but shows significant variation on the surface. In younger age, teeth has high concentration of fluoride on enamel surface. But in older age, the surface is removed by attrition, erosion and abrasion. Fluoride accumulation continues up to tooth eruption in enamel. But dentine continues to accumulate fluoride. Enamel of the erupted teeth is exposed to the fluoride ions in the saliva, water and food. As a result of this contact, fluoride content of the surface enamel increases markedly and there is a steep decrease of fluoride content into the deeper layers of enamel. Bone will accumulate fluoride throughout the life, depending on the fluoride content of drinking water. The content of fluoride in a 5-year-old child, on an average is 250 ppm; but in an 80 year old man, it may be 2000 ppm.

Fluoride is Useful to Prevent Caries

Experimental evidence support the view that an intake of **2–4 microgram** fluoride per day leads to decrease in the incidence of dental caries.

Several possible mechanisms are postulated, which include:

- Effect on hard tissues to modulate mineralization, demineralization and remineralization;
- Effect of cariogenic bacteria by altering their metabolism
- Effect on soft tissues to modify the development of teeth.
- The presence of fluorine in water results in fluorine replacing the hydroxyl groups in the mineralization process of the tooth. This results in the formation of **Fluoroapatite**. Fluoroapatite is more resistant to acid digestion than hydroxyapatite. The rate of dissolution is slow and the tooth has time to get remineralized.

Fluoride ions enter the hydration shell surrounding the apatite crystals and may become incorporated into the crystal surface. Initially a friable layer of calcium fluoride is formed on the enamel surface, which leaves the underlying enamel intact. This fluoride-rich surface layer then undergoes slow exchange with fluoride getting incorporated in the crystal lattice forming fluoroapatite. The fluoroapatite makes the tooth surface more resistant to plaque bacterial attack. Incorporation of fluoride into hydroxy apatite makes it more resistant to demineralization. Crystallization of mineral is more rapid when fluoroapatite is formed and more perfect crystals are formed. On developed tooth, the presence of fluoride forms a film of calcium fluoride on enamel surface and these fluoride ions can exchange with hydroxyl ions to form fluoroapatite. Fluoroapatite is more resistant to acid digestion than hydroxyapatite. The rate of dissolution is slow and the tooth has time to get remineralized.

Fluoride ions are also potent inhibitors of the **Enolase** enzyme of the glycolytic pathway. This results in the inhibition of the glycolytic pathway in bacteria with decreased lactic acid formation. The safe limit of fluorine is about **1 ppm** in water. (ppm = parts per million; 1 ppm = 1 gram of fluoride in million gram of water; this is equal to 1 mg per 1000 mL). Fluoride containing tooth-pastes are now available. Not only fluoride, but many other substances are being accumulated in teeth. Recent studies suggest that **lead** may be taken up by enamel and dentine. Steady incorporation of lead into dentine makes it a candidate biomarker of exposure to lead. Treatment of young children with **tetracyclins** will lead to discoloration of teeth; this could be seen as fluorescence under ultraviolet light.

Fluorosis is More Dangerous than Caries

Fluoride level more than 2 ppm will cause chronic intestinal upset, gastro-enteritis, loss of appetite and loss of weight. Levels more than 5 ppm cause **mottling** of enamel, stratification and discoloration of teeth. A level more than 20 ppm is toxic, leading to alternate areas of osteoporosis and osteosclerosis, with brittle bones. This is called **fluorosis.** Ingested fluoride accumulates in bones. It is a cumulative toxin. In fluorosis, blood concentration of fluoride increases to 50 microgram/100 mL; whereas normal value is 4 microgram/100 mL. **Fluorosis** is characterized by joint defects; especially **genu valgum**. Due to increased breakdown of bone matrix, excretion of hydroxy proline in urine is enhanced.

Incidence of Fluorosis

Nellore, and Prakasam districts of Andhra Pradesh, Nalgonda district of Telangana and Patiala district of

Punjab are badly affected by fluorosis. About 25 million people in India are suffering from fluorosis, spread in 15 states of India. In the vicinity of irrigation dams, the water level in wells will come up, along with salts including fluoride. This has resulted in widespread fluorosis in Punjab, Rajasthan, UP, Delhi, Andhra Pradesh, Karnataka and Tamil Nadu. Water from deep subsoil wells will also contain higher fluoride level.

Certain salts used in *paan supari* also have large content of fluoride.

Fluoride rich sources are sea fish, cheese, tea and jowar.

Fluorosis is highly prevalent in areas where jowar is the staple diet.

Fluorinated toothpaste contains 3,000 ppm of fluoride. Even ordinary toothpaste contains fluoride about 700 ppm.

Prevention of fluorosis

Provide fluoride free water, restriction of intake of jowar, supplementation of vitamin C and avoiding fluoride containing tooth paste.

A QUICK LOOK

1. Total volume of saliva produced each day in an adult is 500-1500 mL.
2. Parotid glands produce serous secretions, while the submandibular and sublingual glands produce both serous and mucous secretions.
3. Major carbohydrate in saliva is glucose (10-20 mg/dL)
4. Major proteins of saliva are mucins MG1 and MG2. Mucin forms protective coating around both hard and soft tissues and lubricates them.
5. Major salivary enzyme is alpha amylase. It cleaves the alpha 1,4 glycosidic bonds of starch. Optimum pH for its activity is 6.0.
6. Proteins with antibacterial activities in saliva are Lysozyme, Immunoglobulin A and Lactoferrin.
7. Calcium binding proteins in saliva are statherins, proline rich proteins (PRP). They reduce calculus formation.
8. Inorganic calcium is deposited along with phosphate as apatite.
9. Alkaline phosphatase is the key enzyme in mineralization of teeth.
10. Caries formation requires the presence of sucrose and the bacteria *Streptococcus mutans*. Fermentation of sucrose by the bacteria produces Lactic acid, which corrodes the enamel of the teeth.
11. Safety limit for fluoride is 1 ppm in water.
12. Fluoride in proportions of 2–4 mg per day decreases the risk of caries. Incorporation of fluoride makes teeth resistant to demineralization, resistant to acid digestion and as an inhibitor of enolase, it blocks glycolysis in the bacteria.
13. Fluoride levels more than 5 ppm causes mottling of enamel. Levels greater than 20 ppm lead to fluorosis, with mottling of enamel and osteoporosis.

CHAPTER 24

DNA, Replication

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Watson-Crick model of DNA structure
- Chromosomes
- Replication of DNA
- DNA polymerase
- Okazaki pieces

Thomas Morgan (1866–1945), the founder of modern genetics, showed that chromosomes contain genes in a sequential manner in Drosophila (Nobel prize, 1933). George Beadle, working with mutant strains of Neurospora suggested "one enzyme one gene" hypothesis in 1941 (Nobel prize, 1958). Edwin Chargaff elicited the base pairing rule of DNA in 1950. X-ray crystallographic studies on DNA by Maurice Wilkins (Nobel prize, 1962) showed the details of structure of DNA. Rosalind Franklin worked out the helical structure of DNA. Based on these data, James Watson and Francis Crick in 1953 deduced the double helical structure of DNA (Nobel prize, 1962).

STRUCTURE OF DNA

Deoxyribonucleic acid (DNA) is composed of four deoxyribonucleotides, i.e. deoxyadenylate (A), deoxyguanylate (G), deoxycytidylate (C), and thymidylate (T).

These units are combined through 3' to 5' **phosphodiester bonds** to polymerize into a long chain. The nucleotide is formed by a combination of base + sugar + phosphoric acid. The 3'-hydroxyl of one sugar is combined to the 5'-hydroxyl of another sugar through a phosphate group (Fig. 24.1).

In this particular example, the thymidine is attached to cytidine and then cytidine to adenosine through phosphodiester linkages (Fig. 24.1).

In the DNA, the base sequence is of paramount importance. The genetic information is encoded in the specific **sequence of bases**; if the base is altered, the information is also altered.

The deoxyribose and phosphodiester linkages are the same in all the repeating nucleotides. Therefore, the message will be conveyed, even if the base sequences alone are mentioned as shown:

5'P--Thymine--Cytosine-Adenine-3'OH

Or, 5'------T--C--A---3'

This would convey all the salient features of the polynucleotide shown in Figure 24.1.

Polarity of DNA molecule

In the case of DNA, the base sequence is always written from the 5' end to the 3' end. This is called the polarity of the DNA chain.

Watson-Crick Model of DNA Structure

The salient features of Watson-Crick model of DNA are given below (Figs 24.2 and 24.3):

1. Right handed double helix

DNA consists of two polydeoxy ribonucleotide chains twisted around one another in a right handed double helix

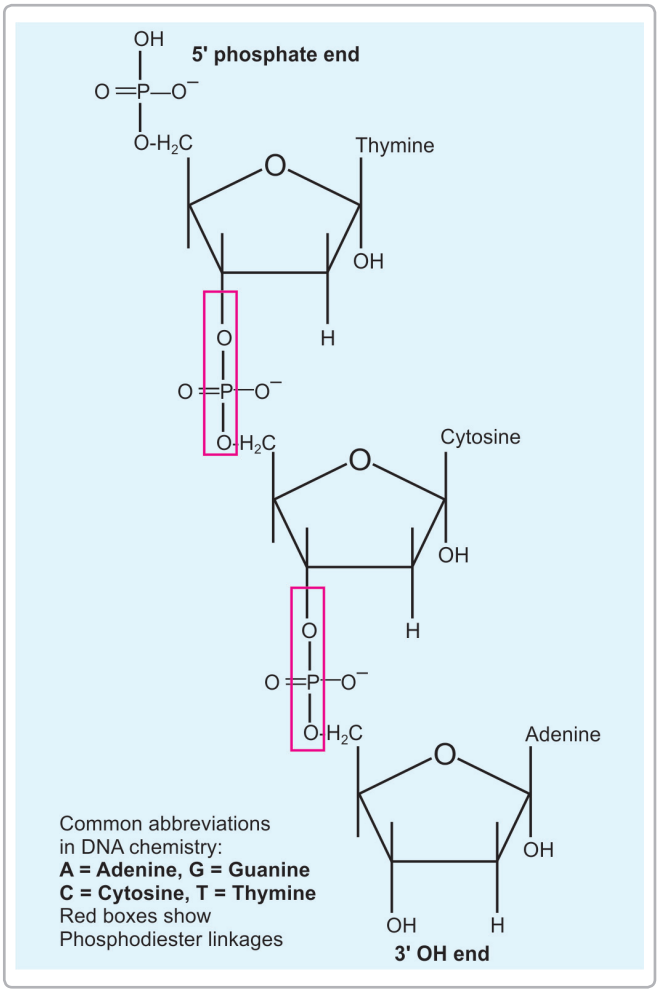


Figure 24.1: Polynucleotide

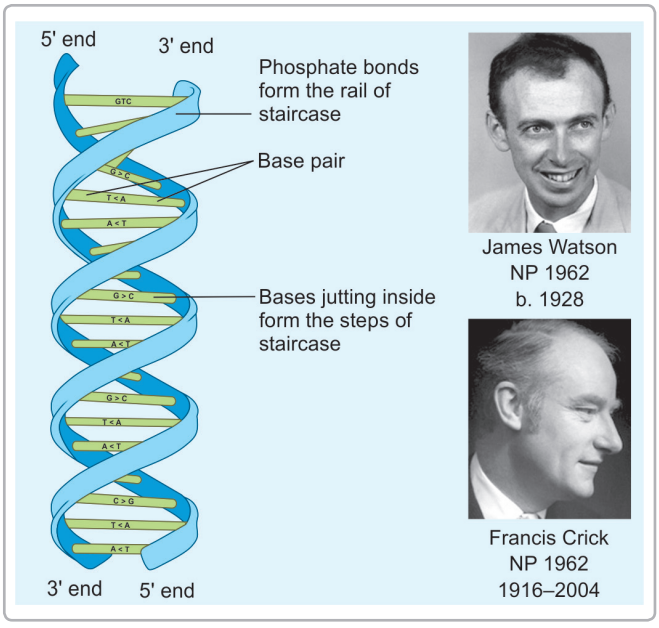


Figure 24.2: Watson-Crick model of double helical structure of DNA. Phosphate bonds form the rail. Bases are jutting inside

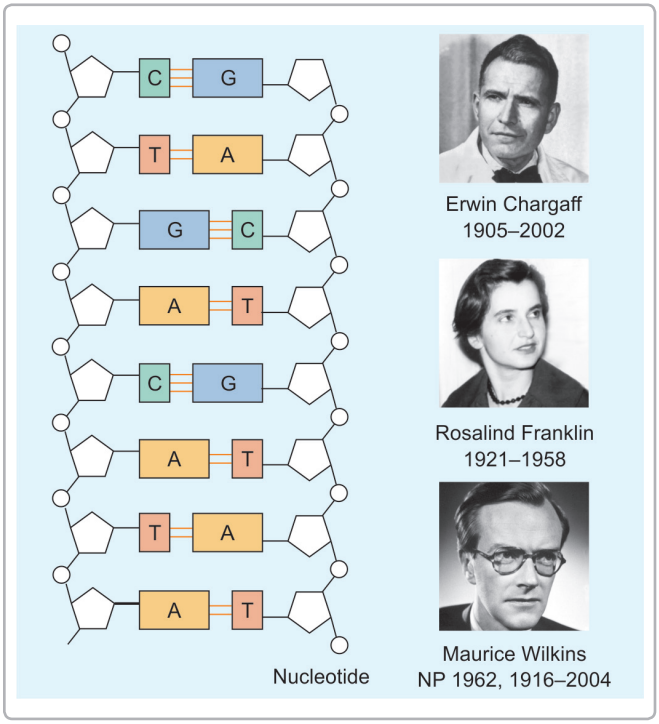


Figure 24.3: Base pairing rule

similar to a spiral stair case. The sugar and phosphate groups comprise the handrail and the bases jutting inside represent the steps of the staircase. The bases are located perpendicular to the helix axis, whereas sugars are nearly at right angles to the axis.

2. The base pairing rule

Always the two strands are **complementary** to each other. So, the adenine of one strand will pair with thymine of the opposite strand, while guanine will pair with cytosine. The base pairing (**A with T; G with C**) is called **Chargaff's rule**, which states that the number of purines is equal to the number of pyrimidines.

3. Hydrogen bonding

The DNA strands are held together mainly by hydrogen bonds between the purine and pyrimidine bases. There are two hydrogen bonds between A and T while there are three hydrogen bonds between C and G.

4. Antiparallel

The two strands in a DNA molecule run antiparallel, which means that one strand runs in the 5' to 3' direction, while the other is in the 3' to 5' direction. This is similar to a road divided into two, each half carrying traffic in the opposite direction (Fig. 24.2).

5. ***The spiral has a pitch of 3.4 nanometers per turn.***

6. ***Within a single turn, 10 base pairs are seen. Thus, adjacent bases are separated by 0.34 nm.***

Other Features

In the DNA, each strand acts as a template for the synthesis of the opposite strand during replication process. The spiral has a pitch of 3.4 nanometers per turn. Within a single turn, 10 base pairs are seen. Thus, adjacent bases are separated by 0.34 nm. The diameter or width of the helix is 1.9 to 2.0 nm. A major groove (1.2 nm) and a minor groove (0.6 nm) wind along the molecule, parallel to the phosphodiester backbone. In these grooves, proteins interact with the exposed bases. DNA is the storehouse of genetic information.

Higher Organization of DNA

In higher organisms, DNA is organized inside the nucleus. Double stranded DNA is first wound over histones; this is called **nucleosomes** (Fig. 24.4). **Chromatin** is a loose term employed for a long stretch of DNA in association with special proteins, called histones. Chromatin is then further and further condensed to form **chromosomes**. (Fig. 24.5). Similarly, the DNA molecule is folded and compressed to 10,000 fold to generate chromosomes.

Nucleosomes

Histones are proteins containing high concentration of basic amino acids. The H1 histone is loosely attached to the DNA (Fig. 24.4). Others are called core histones (Fig. 24.4). The double stranded DNA wraps twice around a histone octamer (Fig. 24.4). This twisted helix forms a spherical particle of 10 nm diameter; called nucleosome. This arrangement also stabilizes DNA.

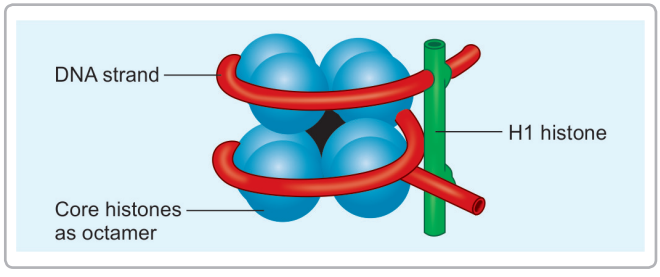


Figure 24.4: DNA wraps twice around histone octamer to form one nucelosome

Further Condensation of DNA

A group of such nucleosomes form the "**DNA fibrils**". About 6 such fibrils are further supercoiled to form 30 nm diameter **chromatin fibers** or chromatin threads. By this time, the DNA is folded to about 50 times. Histones stabilize these fibers. These fibers are further supercoiled and condensed to form chromosomes (Fig. 24.5).

Chromosomes

During metaphase, the DNA can be seen under a microscope, as superpacked chromosomes, where identical sister **chromatids** are connected at the centromere. In humans, there are 23 pairs of chromosomes.

DNA is a Very Long Molecule

Human diploid genome consists of about 7 × 109 base pairs. So when placed end to end it will be about 2 meters long, If one nucleotide is added per second, it will take 250 years to synthesize the whole DNA of a human cell. The length of a DNA molecule is compressed to 8,000 to 10,000 fold to generate the chromosomes.

Introns, Exons, Cistrons

Only about 10% of the human DNA contain genes; the rest are silent areas. The segments of the gene coding for

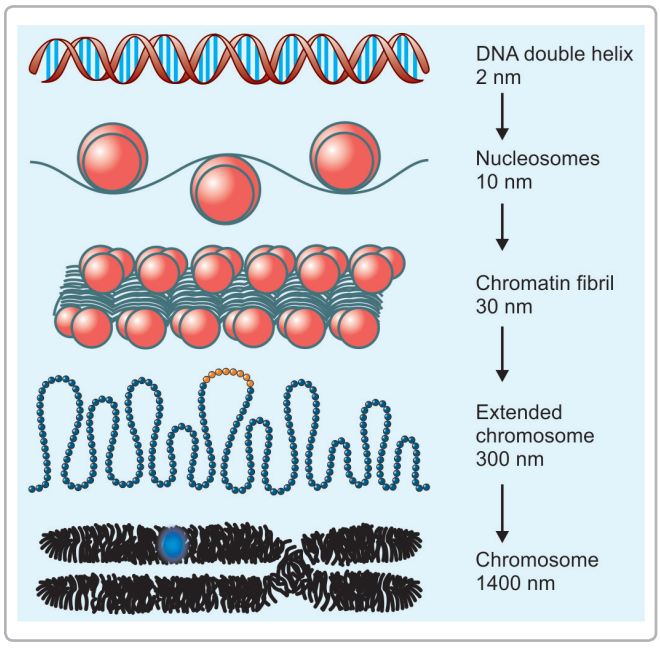


Figure 24.5: DNA condenses repeatedly to form chromosome

proteins are called **exons** (expressed regions). They are interspaced in the DNA with stretches of silent areas, called **introns** (intervening areas). The primary transcripts contain intron sequences; which are later removed to produce mature mRNA. Introns are not translated.

A **Cistron** is the unit of genetic expression. It is the biochemical counterpart of a "gene" of classical genetics. One cistron will code for one polypeptide chain. If a protein contains 4 subunits, these are produced under the direction of 4 cistrons ("one cistron–one polypeptide" concept).

REPEAT SEQUENCES OF DNA

Only about 1–2% of the human DNA contain genes; the rest are silent areas. About 1% of DNA is present inside mitochondria. There are only about 25,000 to 30,000 protein-coding regions in the human DNA. About 90% of DNA is made of noncoding intervening sequences, called **introns**. About 30% of the genome consists of repetitive sequences, 5 to 500 base pairs repeated many times. One such sequence, the **Alu family** is repeated about 5,00,000 times, and accounts for about 5% of total human DNA.

Replication of DNA

During cell division, each daughter cell gets an exact copy of the genetic information of the mother cell. This process of copying the DNA is known as **DNA replication.** In the daughter cell, one strand is derived from the mother cell; while the other strand is newly synthesized. This is called **semi-conservative** type of DNA replication (Fig. 24.6).

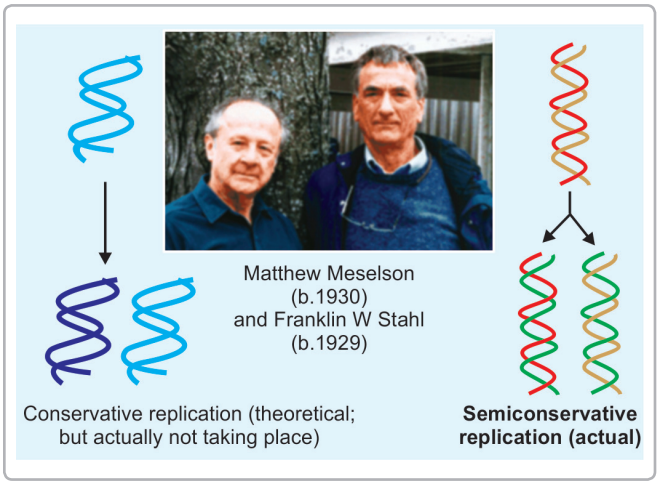


Figure 24.6: Semiconservative replication (A new complementary strand is synthesized over the old template)

Meselson-Stahl Experiment (1958)

Bacteria were grown in a medium containing the heavy isotope of nitrogen 15N, when all the DNA was labeled with heavy nitrogen. These cells were allowed to divide in a medium containing normal nitrogen, 14N. In the first generation, all DNA molecules were half labeled. In the second generation, half labeled and completely unlabeled molecules were present in equal numbers. From this experiment, it was proved that *DNA replication is semiconservative in vivo*.

Steps of Replication

Each strand serves as a **template** or mould, over which a new **complementary** strand is synthesized (Fig. 24.6). The **base pairing** rule is always maintained. The new strand is joined to the old strand by hydrogen bonds between base pairs (A with T and G with C) (Fig. 24.7). Polymerization of the new strand of DNA is taking place from 5' to 3' direction. This means that the template is read in the 3' to 5' direction (Fig. 24.8). So, the 3' end of the last nucleotide is free.

Thus two double strands are produced. One double strand goes to one daughter nuclei, and the other to the second daughter nuclei. But each daughter cell gets only one strand of the parent DNA molecule. Old DNA strand is not degraded, but is conserved for the daughter cell, hence this is semi-conservative synthesis (see Fig. 24.6).

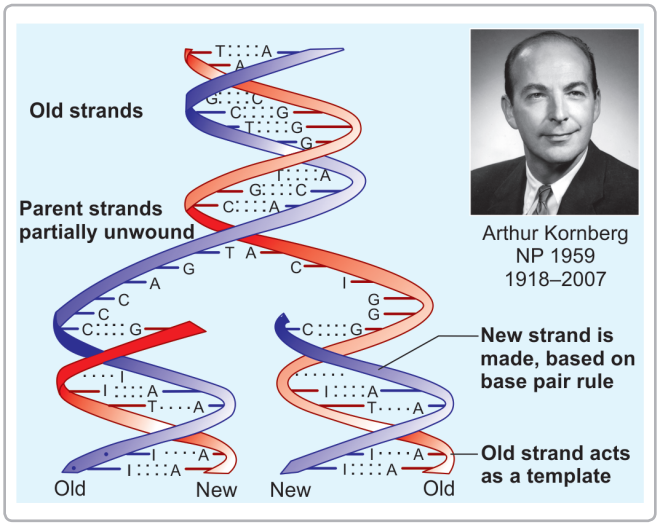


Figure 24.7: Both strands are replicated

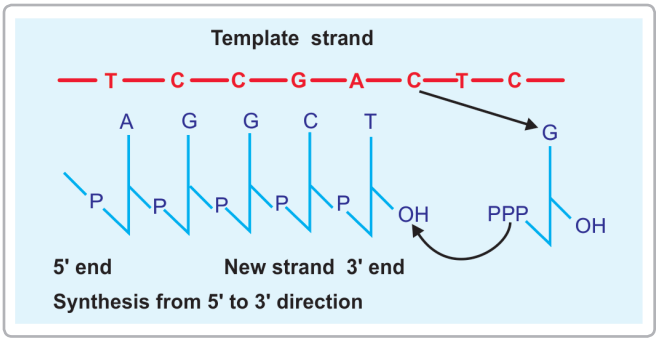


Figure 24.8: New strand is synthesized from 5′ to 3′ direction. Base pairing rule is always maintained

DNA polymerase (DNAP)

This enzyme synthesizes a new complementary strand of DNA, by incorporating dNMP sequentially in 5′ to 3′ direction, making use of single stranded DNA as template. Arthur Kornberg (Nobel prize, 1959) isolated the DNA polymerase I (**Kornberg's enzyme**) from *Escherichia coli*. In mammalian cells (eukaryotic), there are 5 DNAPs, named as α, β, γ, δ, ε.

Bacterial DNA Polymerases

In bacteria, there are 3 DNA polymerases. Arthur Kornberg (Nobel prize, 1959) isolated the DNA polymerase I (**Kornberg's enzyme**) from *Escherichia coli*. It is a repair enzyme. It has both 3′ to 5′ and 5′ to 3′ exonuclease activities. Bacterial **DNA polymerase III** is the main replication enzyme in bacteria.

Mammalian DNA Polymerases

In mammalian cells (eukaryotic), there are 5 DNAPs, named as α, β, γ, δ and ε. Alpha polymerase polymerizes about 100 nucleotides per second. (Bacterial enzyme has 10 times more speed). It is the major enzyme which synthesizes Okazaki fragments. DNAP delta completes lagging strand synthesis and DNAP epsilon is used for leading strand synthesis. DNAP beta is a proofreading and repair enzyme, where as DNAP gamma is concerned with mitochondrial DNA replication.

Initiation of DNA Replication

The DNA replication starts with the recognition of the site of origin of replication. This is done by a **complex**. The complex of enzyme proteins and other factors required for DNA replication is called **Replisome**. Helicases move in both directions, separating the strands in advance of the process of replication. This forms a **replication bubble** (Fig. 24.9).

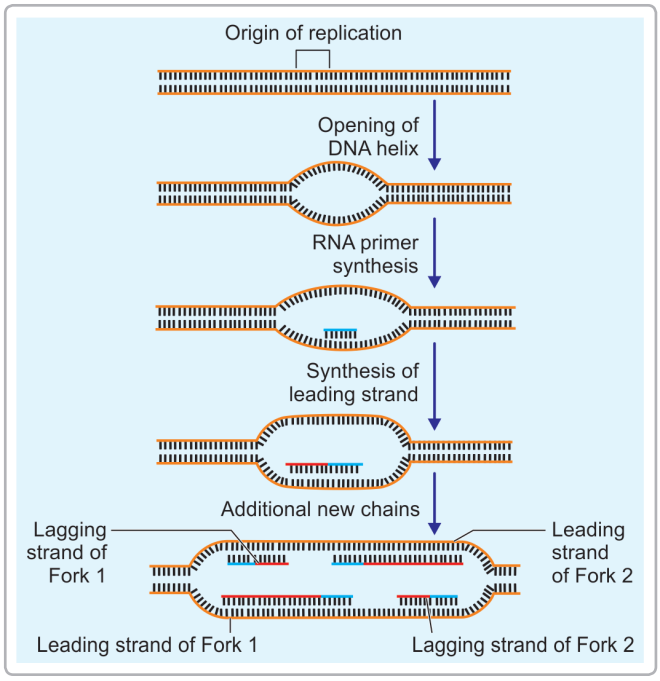


Figure 24.9: Replication bubble (replication fork)

RNA Primer is Required for DNA Synthesis

An RNA primer, about 100–200 nucleotides long, is synthesized by the **RNA primase**. Then the *RNA primer is removed by DNAP,* using exonuclease activity and is replaced with deoxyribonucleotides by DNAP (Fig. 24.11).

Elongation of DNA strand

Under the influence of DNA polymerase, nucleotides are sequentially added (Figs 24.8 and 24.11). The newly added nucleotide would now polymerize with another one, forming the next phosphodiester bond. If "A" is present on the template, "T" enters in that place in the newly synthesized DNA strand. The **base pairing rule** is always observed. The DNA polymerase carries out the sequential addition of each nucleotide **complementary** to the one in the template strand (Fig. 24.7).

Discontinuous Synthesis

DNA synthesis is always in the 5′ to 3′ direction in both strands. The strand which is discontinuously synthesized is referred to as the **"lagging strand"** and the one

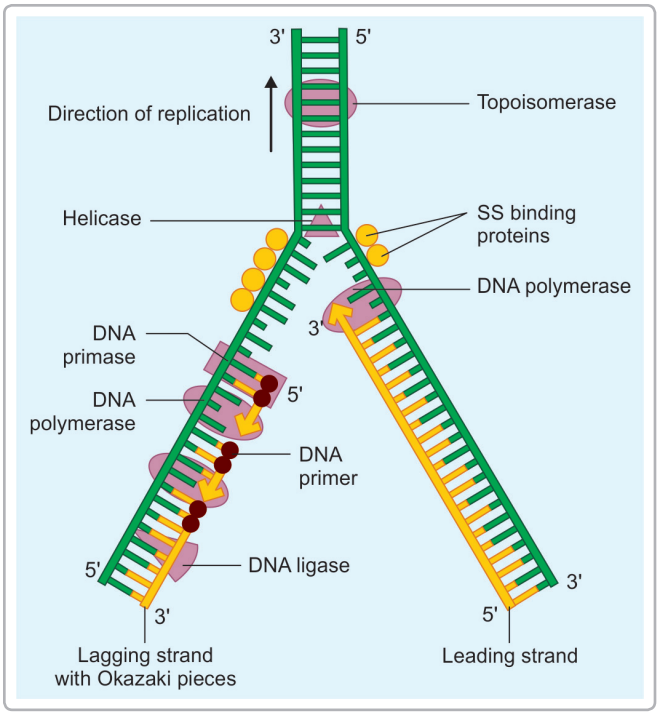


Figure 24.10: Lagging strand and Okazaki pieces

continuously polymerized as the **"leading strand"** (Fig. 24.10). This "*discontinuous DNA synthesis*" produces **replication forks** or replication **bubbles** (Fig. 24.9).

Lagging Strand and Okazaki Pieces

The small DNA molecules attached to its own primer RNA are called **Okazaki** fragments. Several Okazaki pieces are produced. The synthesis along the lagging strand is in 5' to 3' direction. As it moves, the primase synthesizes short RNA primer, to which deoxyribonucleotides are added by DNA polymerase.

Then the *RNA primer is removed by DNAP,* using exonuclease activity and is replaced with deoxyribonucleotides by DNAP. The remaining nick is sealed by the **DNA ligase**. A summary of DNA replication is given in Box 24.1.

Modifications after Replication

DNA methylation at C5 of cytosine catalyzed by DNA methyltransferase is commonly associated with gene

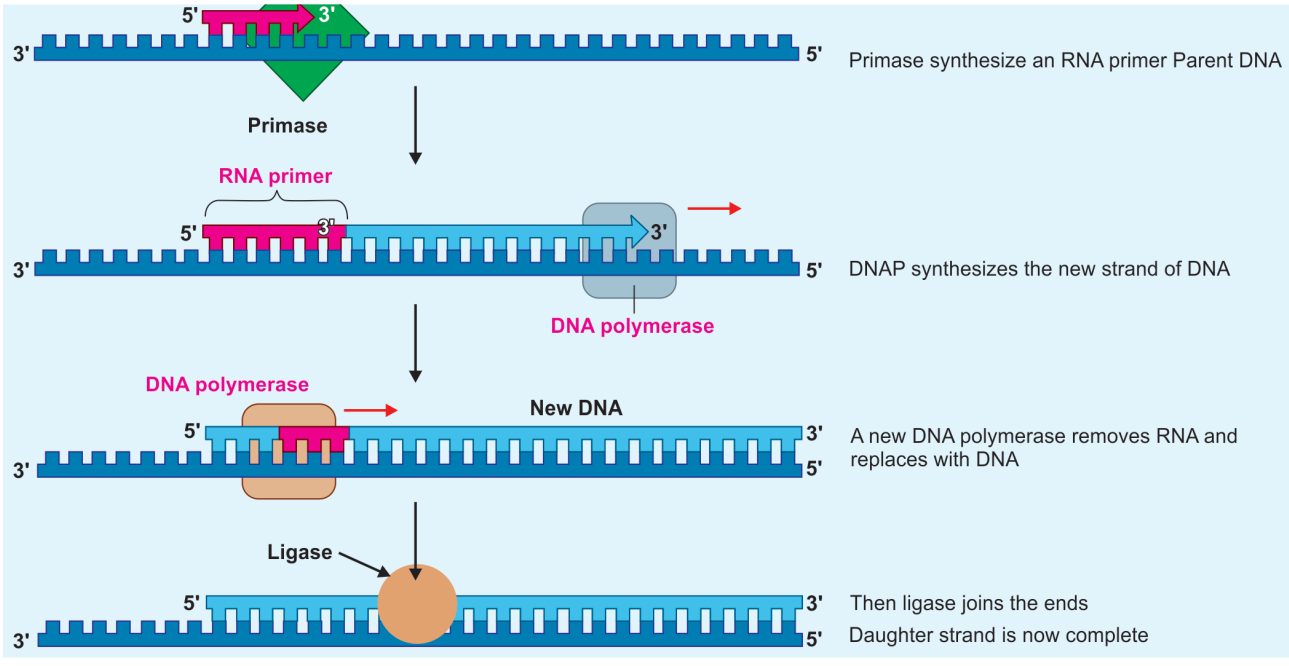


Figure 24.11: RNA primer is needed for the DNA synthesis

BOX 24.1: Summary of DNA replication

1. Unwinding of parental DNA to form a replication fork
2. RNA primer complementary to the DNA template is synthesized by RNA primase
3. DNA synthesis is continuous in the leading strand (towards replication fork) by DNA polymerase
4. DNA synthesis is discontinuous in the lagging strand (away from the fork), as Okazaki fragments
5. In both strands, the synthesis is from 5′ end to 3′ end
6. Then the RNA pieces are removed; the gaps filled by deoxynucleotides and the pieces are ligated by DNA ligase
7. Proofreading is done by the DNA polymerase
8. Finally organized into chromatin.

BOX 24.2: Inhibitors of DNA replication

Drug	Action (inhibition of)
Antibacterial agents	
Ciprofloxacin	Bacterial DNA gyrase
Nalidixic acid	do
Novobiocin	do
Anticancer agents	
Etoposide	Human topoisomerase
Adriamycin	do
Doxorubicin	do
6-mercaptopurine	Human DNA polymerase
5-fluorouracil	do

silencing. One of the effects of ROS (reactive oxygen species, free radicals) is through hypermethylation.

Aberrant methylation is also observed in cellular senescence and may affect age-related diseases, such as type 2 diabetes mellitus.

Inhibitors of DNA Replication

Certain compounds will inhibit bacterial enzymes, but will not affect human cells; such drugs are useful as antibacterial agents.

Some other components will inhibit human enzymes, they will arrest new DNA synthesis, and arrest the cell division. Those drugs are therefore useful as anticancer agents. A list of such drugs are given in Box 24.2.

DNA REPAIR MECHANISMS

The replication process should be carried out with **high fidelity**, otherwise the genetic information is altered. Hence, there should be a foolproof mechanism to correct the mistakes. While printing a page, the typesetter sets the types; an impression is taken, **proofreading** is done to correct mistakes if any, and then the final printing is done. A similar follow-up mechanism operates after DNA synthesis. Nobel prize in 2015 was awarded to Tomas Lindahl, Paul Modrich and Aziz Sancar "for studies of DNA repair". Various physical and chemical agents produce base alterations; these are to be appropriately corrected immediately. There are different types of DNA repair mechanisms; all of them follow the general mechanism outlined above, but details may vary. These mechanisms are briefly described below:

Exonucleolytic Proofreading

The DNA polymerase has 3′ to 5′ exonuclease activity. Hence any mispaired nucleotide added is immediately removed.

Mismatch Repair

The original template DNA contains methylated residues (N6-methyladenine and 5-methylcytosine). The newly synthesized strand will not have methylated bases. So enzymes can recognize the original (correct) DNA strand. The mismatched base is identified and removed along with a few bases around that area. The wrong base is removed by the **endonuclease** activity. A small segment of DNA with correct base sequence is then synthesized by DNA polymerase beta. Then the gap or nick is sealed by DNA ligase.

Nucleotide Excision Repair (NER)

This mechanism repairs damage of DNA and replaces a segment up to 30 nucleotides in length. Pyrimidine dimers caused by UV light, benzopyrene (guanine adducts formed by smoking), other modifications caused by chemical agents, cancer chemotherapy, etc. are thus repaired. Initially two phosphodiester bonds on the damaged strand are hydrolyzed by endonucleolytic cleavage and the activity is referred to as excinuclease. A fragment of DNA, 25–30 nucleotides in length is removed. It is replaced by DNAP delta/epsilon by polymerizing deoxyribonucleotides. The ends are joined by DNA ligase.

Base Excision Repair (BER)

Depurination is a common spontaneous damage occurring in DNA. Deamination of cytosine to uracil may also occur. These abnormal bases are removed and correct bases are added by BER. N-glycosylase recognizes and removes the wrong base. An endonuclease will excise the remaining sugar. The correct base is added by a DNA polymerase and joined by ligase.

Diseases Associated with DNA Repair

Xeroderma pigmentosum: It is derived from the Greek terms xeres = dry and derma = skin. It is an autosomal recessive condition. Defect lies in the NER (**nucleotide excision repair**) mechanism.

Patients with XP have a 1000-fold greater chance of developing skin cancer than do normal persons. Death usually occurs in the second decade of life due to squamous cell carcinoma of skin.

Ataxia telangiectasia: It is a common autosomal recessive disease. Sensitivity to UV, cerebellar ataxia, telangiectasia in eyes and lymphoreticular neoplasms are common. Ataxia telangiectasia mutated (ATM) gene is present in 1% of total population. The disease is manifested in 1:40,000 persons.

Telomere and Telomerase

The replication always takes place from 5' to 3' direction in the new strand. The DNA polymerase enzyme is not able to synthesize the new strand at the 5' end of the new strand. In other words, a small portion (about 300 nucleotides) at the *3' end of the parent strands could not be replicated*. This end piece of the chromosome is called **telomere**. Unless there is some mechanism to replicate telomeres, the length of the chromosomes will go on reducing at each cell division. The stability of the chromosome is thus lost. The shortening of telomere end is prevented by an enzyme **telomerase**. It contains an RNA component, which provides the template for telomeric repeat synthesis. Telomerase acts like a reverse transcriptase. Telomerase recognizes 3' end of telomere, and then a small DNA strand is synthesized. In **old age**, the telomerase activity is lost; leading to chromosome instability and cell death.

As cancer cells have increased and persistent activity of telomerase, the cancer cells become immortal. Elizabeth Blackburn, Carol Greider and Jack Szostak discovered the telomeres and telomerase, all the three were awarded Nobel prize in 2009.

A QUICK LOOK

1. DNA sequence is always written from the 5' end to 3' end. This is called polarity of the DNA chain.
2. Chargaff's rule states that the number of purines is equal to the number of pyrimidines.
3. The two strands run antiparallel to each other.
4. Supercoiling of DNA is mediated by the enzymes topoisomerases and gyrases.
5. DNA is assembled into nucleosomes.
6. Histones are unusually rich in basic amino acids.
7. Transcriptionally active chromatin is called "euchromatin" and stains less densely as compared to "heterochromatin" which is the inactive region of the chromatin.
8. DNA replication in vivo is semiconservative.
9. In mammals, the DNA polymerase is called alpha polymerase. There are five DNA polymerases. (alpha, beta, gamma, delta and epsilon).
10. The DNA polymerase requires a RNA primer synthesized by RNA polymerase for initiation of its activity. This forms the first step in DNA synthesis.
11. DNA synthesis is continuous on the leading strand and discontinuous on the lagging strand. This results in the formation of the replication fork.
12. The small DNA molecules attached to their own RNA primers are called Okazaki fragments.
13. Xeroderma pigmentosum is an autosomal recessive condition caused due to a defective nucleotide excision repair mechanism.
14. In every replication the 3' end of the parent strands cannot be replicated. This end piece of the chromosome is called telomere. This shortening is prevented by telomerases.
15. Telomerases have been implicated in aging process and cancers.

CHAPTER 25

Transcription, Translation

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Ribonucleic acid
- Messenger RNA
- Transcription
- Post-transcriptional processing
- Reverse transcriptase
- Transfer RNA
- Ribosomal RNA
- Genetic code
- Protein biosynthesis
- Post-translational processing
- Inhibitors of protein synthesis

RIBONUCLEIC ACID

Ribonucleic acid (RNA) is also a polymer of purine and pyrimidine nucleotides linked by phosphodiester bonds. However RNA differs from DNA as shown in Table 25.1 and Fig. 25.1.

Cellular RNAs are of five types

- **Messenger RNA (mRNA):** The genetic information present in DNA is transcribed into mRNA, which is the coding RNA. They have a short half-life and are generally degraded quickly.
- **Ribosomal RNA (rRNA):** 28S, 18S and 5S are the major varieties. They are involved in protein biosynthesis and are very stable.
- **Transfer RNA (tRNA):** There are about 60 different species present. They are very stable.
- **Small RNA:** There are about 30 different varieties. They are very stable. **Small Nuclear RNAs** (SnRNAs) are a subgroup of small RNA. They are involved in mRNA splicing.

Table 25.1: Differences between RNA and DNA

No.	*RNA*	*DNA*
1.	Mainly seen in cytoplasm	Mostly inside the nucleus
2.	Usually 100–5000 bases	Millions of base pairs
3.	Generally single stranded	Double stranded
4.	Sugar is ribose	Sugar is deoxyribose
5.	Purines: Adenine, guanine	Adenine, guanine
6.	Pyrimidines: Cytosine, uracil	Cytosine, thymine
7.	Guanine content is not equal to cytosine and adenine is not equal to uracil	Guanine is equal to cytosine and adenine is equal to thymine
8.	Easily destroyed by alkali	Alkali resistant

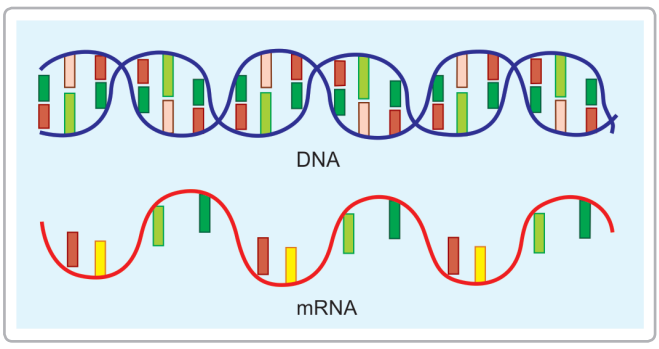


Figure 25.1: DNA is double stranded; while RNA is single stranded

- **MicroRNA (miRNA):** They regulate genetic expression by altering the function of mRNA.

Central Dogma of Molecular Biology

As shown in Figure 25.2, the information available in the DNA is passed to messenger RNA, which is then used for synthesis of a particular protein.

Replication, Transcription and Translation

DNA **replication** is like printing a copy of all the pages of a book. The replication process occurs only at the time of cell division. But **transcription** is taking place all the time. Only certain areas of the DNA are copied (selected regions on the sense strand). This is like taking xerox copy of particular page of the book. So, the genetic information of DNA is transcribed (copied) to the **messenger RNA** (mRNA). During transcription, the message from the DNA is copied in the language of nucleotides (4 letter language). The mRNA then reaches the cytoplasm where it is translated into functional proteins (Fig. 25.2). During **translation,** the nucleotide sequence is translated to the language of amino acid sequence (20 letter language) (Fig. 25.2).

Template and Coding Strands

The **template strand** is transcribed to give rise to mRNA. The template strand has the complementary sequence of mRNA. The opposite strand has the same sequence as the mRNA. As codons are present in mRNA, the DNA strand having the same sequence of mRNA is called **coding strand** (Fig. 25.3). As it is complementary to the template strand, it is also called **antitemplate strand**.

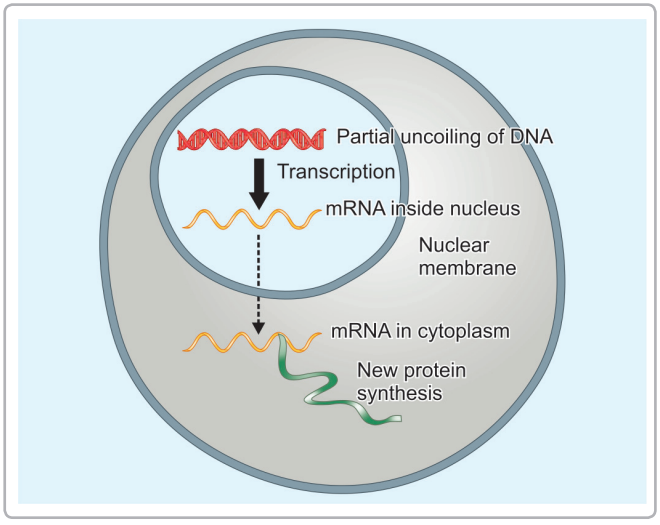


Figure 25.2: Central dogma of molecular biology

Messenger RNA or mRNA

It acts as a messenger of the information in the gene in DNA to the protein synthesizing machinery in cytoplasm. It carries the message to be translated to a protein. The template strand of DNA is transcribed into a single stranded mRNA. This is accomplished by the DNA dependent **RNA polymerase**. The mRNA is a **complementary** copy of the template strand of the DNA (Fig. 25.3). However, thymine is not present in RNA; instead **uracil** will be incorporated.

Promoters

There are certain specific areas on the DNA that act as starting signals for initiation process. The RNAP attaches at the promoter site on the template DNA strand. Such promoters are many. For example, in the case of bacteria, there is a sequence 5'-TATAAT-3'. This is referred to as **TATA box** or Pribnow box. Other regulatory signals for transcription are repressors, inducers and derepressors (For details refer Chapter 26).

TRANSCRIPTION PROCESS

Transcription is catalysed by DNA dependent RNA polymerase (RNAP). Phosphodiester bonds between ribonucleotides are synthesized based on the base sequence of DNA (Fig. 25.4).

Mammalian RNA Polymerases

There are three different DNA dependent RNA polymerases (RNAP) in higher organisms.

- **RNAP type II or B** is the main enzyme synthesizing mRNAs. It is inhibited by alpha **amanitin** (a toxin from the mushroom *Amanita phalloides*).
- RNAP **type I or A** is responsible for synthesis of rRNA (ribosomal); it is not inhibited by amanitin.

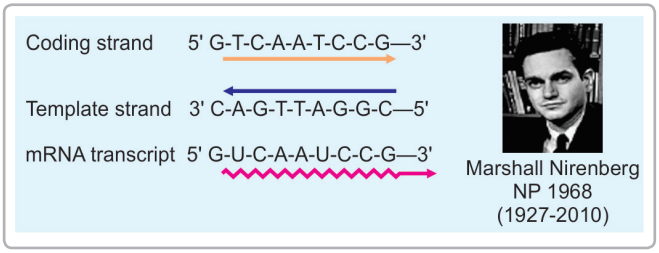


Figure 25.3: Transcription. The mRNA base sequence is complementary to that of the template strand and identical to that of the coding strand. In mRNA, U replaces T

- RNAP **type III or C** is responsible for production of tRNA; it is moderately sensitive to amanitin.
- Bacterial RNA polymerase is different from mammalian RNAP.

1. Initiation of Transcription

The DNA helix partially unwinds, and the RNAP binds with the promoter site on DNA and moves forward (Fig. 25.6). When it reaches the appropriate site on the gene, the first nucleotide of the mRNA attaches to the initiation site on the beta subunit of RNAP. This becomes the 5' end of the mRNA. It will be complementary to the base present in the DNA at that site. The next nucleotide attaches to the RNAP. A phosphodiester bond is formed. Then the enzyme moves to the next base on the template DNA (Fig. 25.5).

Signals for Initiation of Transcription

Promoters: There are certain consensus sequences on DNA which act as start signals which may be located upstream or downstream from the start site. The RNAP attaches at the promoter site on the template DNA strand.

TATA Box and Goldberg-Hogness Box : In the case of bacteria, about 10 bp upstream, there is sequence 5'-TATAAT-3'. This is referred to as *TATA box or Pribnow box*. The TATA box is not on template strand, but on coding strand. In mammals, the exact sequence in TATA box is slightly different (TATAAA) and is known as *Goldberg-Hogness* box. This signal sequence located at –25 to –30 position indicates the start site.

Other regulatory signals: Enhancers increase the rate of transcription and silencers decrease the rate. Other regulatory signals for transcription are hormone response elements (HRE), repressors, inducers and derepressors.

2. Elongation Process

The RNAP moves along the DNA template. New nucleotides are incorporated in the nascent mRNA, one by one, according to the **base pairing rule** (Fig. 25.7). Thus A in DNA is transcribed to U in mRNA; T to A; G to C and C to G. The **synthesis of mRNA is from 5' to 3' end**. That means the reading of template DNA is from 3' to 5' (Figs 25.3 and 25.7). This is analogous to the polarity in DNA synthesis. As the RNAP moves along the DNA template, the **DNA helix unwinds** downstream and winds at the upstream areas. A **transcription bubble** containing RNAP, DNA and nascent RNA is formed (Fig. 25.6).

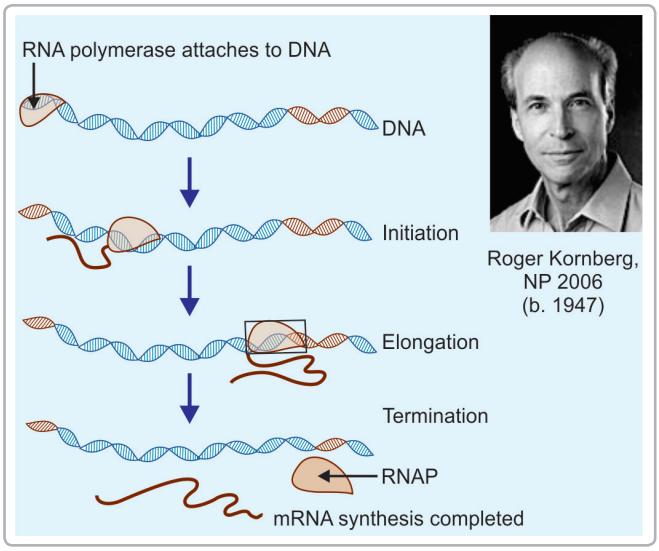


Figure 25.4: Transcription process

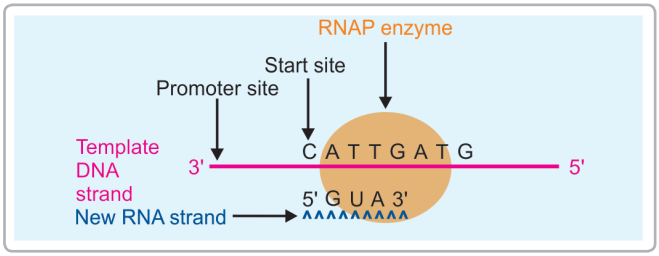


Figure 25.5: Initiation of transcription

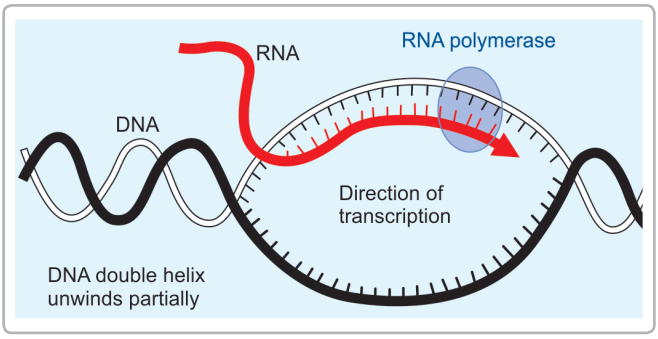


Figure 25.6: DNA unwinds for transcription process

3. Termination of Transcription

The specific signals are recognized by a termination protein, the **Rho factor** (abbreviated with Greek letter, "ρ"). When it attaches to the DNA, the RNAP cannot move further. So, the enzyme dissociates from DNA and consequently newly formed mRNA is released.

4. Post-transcriptional Processing

The mRNA formed and released from the DNA template is known as the **primary transcript**. It is also known as heteronuclear mRNA or hnRNA. In mammalian system, it undergoes extensive editing to become the mature mRNA. Modifications are

- **Poly-A tailing** at 3′ end: The 3′ terminus is polyadenylated in the nucleoplasm (Fig. 25.8). This tail protects mRNA from attack of 3′ exonuclease.
- **5′ capping** by guanosine triphosphate (Fig. 25.8). The cap is useful in recognition of mRNA by the translating machinery.
- **Removal of introns** and splicing (connecting together) of exons. These processings occur mainly in the nucleoplasm.
- The primary transcript contains coding regions (**exons**) interspersed with non-coding regions (**introns**).
- These intron sequences are cleaved and the exons are **spliced** (combined together) to form the mature mRNA molecule. The process which occurs in the nucleus is catalysed by an RNA enzyme (ribozyme) complexed to proteins to form a spliceosome.

Inhibitors of RNA Synthesis

Actinomycin D and mitomycin intercalate with DNA strands, thus blocking transcription. They are used as anticancer drugs.

Rifampicin is widely used in the treatment of tuberculosis and leprosy. Other inhibitors of RNA synthesis are shown in Table 25.2.

Reverse Transcriptase

Generally, the genes are made up of DNA. Usually, DNA dependent RNA polymerase transfers the information from DNA to mRNA. However genetic material of some animal and plant viruses is made up of RNA.

Retrovirus is a subgroup of RNA viruses. The human immunodeficiency virus **(HIV)** causing AIDS is a retrovirus. Here, the RNA acts as a template. Based on this RNA, the enzyme, *RNA dependent DNA polymerase* or *reverse transcriptase* will make a new DNA strand. Temin and Baltimore isolated this enzyme in 1970 and they were awarded the Nobel prize in 1975. From the RNA-DNA hybrid, the RNA part is hydrolyzed by a specific **RNAse-H**. The remaining DNA acts as a template to produce double stranded DNA. Thus genetic information is transferred from RNA to DNA. Some of the **tumor viruses** were also shown to possess reverse transcriptase. The presence of the enzyme may be taken as an indication of a retrovirus infection.

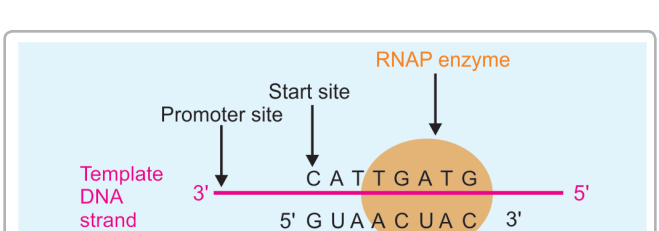


Figure 25.7: Elongation process of transcription

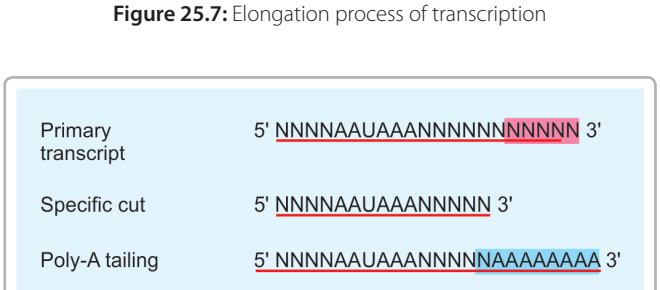


Figure 25.8: Poly-A tail, usually 20–250 nucleotides long at 3′ end. Cap at 5′ terminus. N = any nucleotide

Table 25.2: Inhibitors of RNA synthesis

Inhibitor	*Source*	*Mode of action*
Actinomycin-D	Antibiotic from streptomyces	Insertion between two G-C base pair of DNA
Rifampicin	Synthetic derivative of Rifamycin	Binds to RNA polymerase which is inactivated
Alpha amanitin	Toxin from mushroom	Inactivates RNA polymerase II
3′-deoxy adenosine	Synthetic analogue	Chain termination

Inhibitors of RNA Synthesis

Actinomycin D and **Mitomycin** intercalate with DNA strands, thus blocking transcription. They are used as anticancer drugs. **Rifampicin** is widely used in the treatment of tuberculosis and leprosy (natural antibiotic rifamycin is produced from streptomyces).

PROTEIN BIOSYNTHESIS

The DNA is **transcribed** to mRNA which is **translated** into protein with the help of **ribosomes**. This is summarized in Figure 25.2.

1. Transfer RNA (tRNA) or (sRNA)

Structure of tRNA Molecule

They transfer amino acids from cytoplasm to the ribosomal protein synthesizing machinery; hence the name transfer RNA. They are RNA molecules present in the cytoplasm. Each molecule is only 73–93 nucleotides in length; much shorter than mRNA molecules. Hargobind Khorana chemically synthesized transfer RNA (Nobel prize, 1968). Transfer RNAs show extensive internal base pairing and acquire **clover leaf** like structure (Fig. 25.9). They contain a significant proportion of **unusual bases**. These include dihydrouracil (DHU), pseudouridine (ψ), and hypoxanthine. **Acceptor arm** at 3' end carries the amino acid (Fig. 25.10). The end sequence is **CCA-3'**.

Anticodon arm: At the opposite side of the acceptor arm is the anticodon arm (Fig. 25.9). It recognizes the triplet nucleotide codon present in mRNA. The specificity of tRNA resides in the anticodon site, which has *base sequences complementary to that of mRNA codon.*

The tRNA molecule will show specificity in both aspects; in recognizing the mRNA codon as well as in accepting the specific amino acid coded by that codon. In this way, the tRNA molecules play a pivotal role in translation.

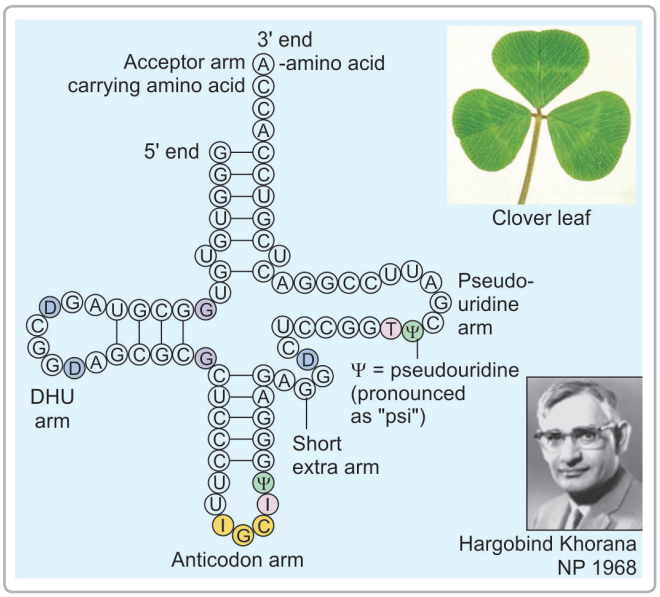


Figure 25.9: Transfer RNA carries amino acid

2. Ribosomal RNA (rRNA)

Ribosomes provide necessary infrastructure for the mRNA, tRNA and amino acids to interact with each other for the translation process. Thus *ribosomal assembly is the protein synthesizing machinery*. The mammalian ribosome has a sedimentation velocity of **80S unit**. It has a larger **60S subunit** and another smaller **40S subunit**. They contain different rRNAs and specific proteins.

Bacteria has 70S ribosomes; with 30S and 50S subunits. So, many antibiotics will inhibit bacterial protein synthesis, but will do no harm to human cells.

3. Genetic Code

A triplet sequence of nucleotides on the mRNA is the codon for each amino acid. Since there are four different bases, they can generate 64 (4^3) different codons or code words. For example, the codon for phenylalanine is UUU. Nirenberg was awarded the Nobel prize in 1968 for deciphering the genetic code. Salient features of genetic code are:

Triplet codons: The codes are on the mRNA. Each codon is a consecutive sequence of three bases on the mRNA, e.g. UUU codes for phenylalanine (Table 25.3).

Non-overlapping: The codons are consecutive. Therefore the starting point is extremely important. The codons are read one after another in a continuous manner, e.g. AUG, CAU, CAU, GCA, etc.

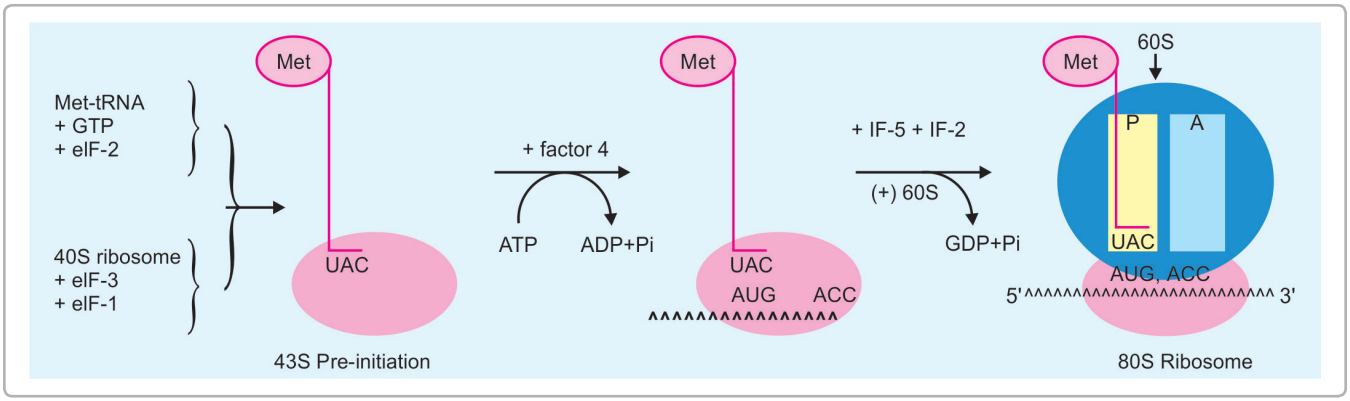


Figure 25.10: Initiation steps; UAC = anticodon on met-tRNA; AUG = start signal; P = peptidyl site; A = amino acyl site

Table 25.3: Triplet codons and corresponding amino acids

First nucleotide 5′ end	*Second nucleotide*				*Third nucleotide 3′ end*
	U	*C*	*A*	*G*	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*Stop*	*Stop*	A
	Leu	Ser	*Stop*	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Val	Ala	Asp	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Giu	Gly	G

Non-punctuated: There is no punctuation between the codons. It is consecutive or continuous.

Degenerate: Table 25.3 shows that 61 codons stand for the 20 amino acids. So **one amino acid has more than one codon**. For example, Serine has 6 codons; while glycine has 4 codons. This is called **degeneracy** of the code.

Unambiguous: Though the codons are degenerate, they are unambiguous; or without any doubtful meaning, i.e. one codon codes for only one amino acid.

Universal: The codons are the same for the same amino acid in all species; the same for "Elephant and *E. coli*". The genetic code has been highly preserved during evolution.

Terminator codons: There are three codons which do not code for any particular amino acid. They are "nonsense codons", more correctly termed as *punctuator codons* or *terminator codons*. They mark "full stop" to the protein synthesis. These three codons are UAA, UAG, and UGA.

Initiator codon: In most of the cases, AUG acts as the initiator codon.

TRANSLATION PROCESS

The translation is a cytoplasmic process. The mRNA is translated from **5′ to 3′ end**. In the polypeptide chain synthesized, the first amino acid is the amino terminal one. The chain growth is from amino terminal to carboxyl terminal. The process of translation can be conveniently divided into the following 5 phases:

1. Activation of Amino Acid

The enzymes **aminoacyl tRNA synthetases** activate the amino acids. The enzyme is highly selective in the recognition of both the amino acid and the transfer RNA acceptor. The CCA 3′ terminus of the acceptor arm carries the amino acid (see Fig. 25.9). Amino acid is first activated with the help of ATP. Then the carboxyl group of the amino acid is esterified with 3′ hydroxyl group of tRNA.

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightarrow{\text{Aminoacyl tRNA synthetase}} \text{Aminoacyl tRNA} + \text{AMP} + \text{PPi}$$

In this reaction, ATP is hydrolyzed to AMP level, and so two high energy phosphate bonds are consumed.

2. Initiation of Protein Synthesis

The first AUG triplet after the marker sequence is identified by the ribosome as the start codon. For the process, **initiation factors** (IF) are required. In eukaryotes, the first amino acid incorporated is methionine (AUG codon).

Formation of initiation complex*:* The met-tRNA (tRNA carrying methionine) and **40S ribosomal** subunit are combined; then mRNA binds to form 48 S initiation complex (Fig. 25.10). .

Formation of 80S ribosomal assembly*:* The 48S initiation complex now binds with **60S** ribosomal unit to form the full assembly of 80S ribosome. This needs hydrolysis of **GTP**. Then all initiation factors are released (see Fig. 25.10).

P and A sites of ribosomal assembly: The whole ribosome contains two receptor sites for tRNA molecules. The "P" site or **peptidyl site** carries the peptidyl-tRNA. It carries the growing peptide chain. The "A" site or **aminoacyl site** carries the new incoming tRNA with the amino acid to be added next. The tRNA-Met is now at the P site.

3. Elongation Process of Translation

Binding of new aminoacyl tRNA: A new aminoacyl tRNA comes to the "A" site. The next codon in mRNA determines the incoming amino acid. **Elongation factor** (EF) and **GTP** are required. The tRNA binds to the "A" site and EF is released (Fig. 25.11).

Peptide bond formation: The alpha amino group of the incoming amino acid in the "A" site forms a peptide bond (CO-NH) with carboxyl group of the peptidyl tRNA occupying the "P" site. This reaction is catalyzed by the enzyme **peptidyl transferase,** which is a **ribozyme**

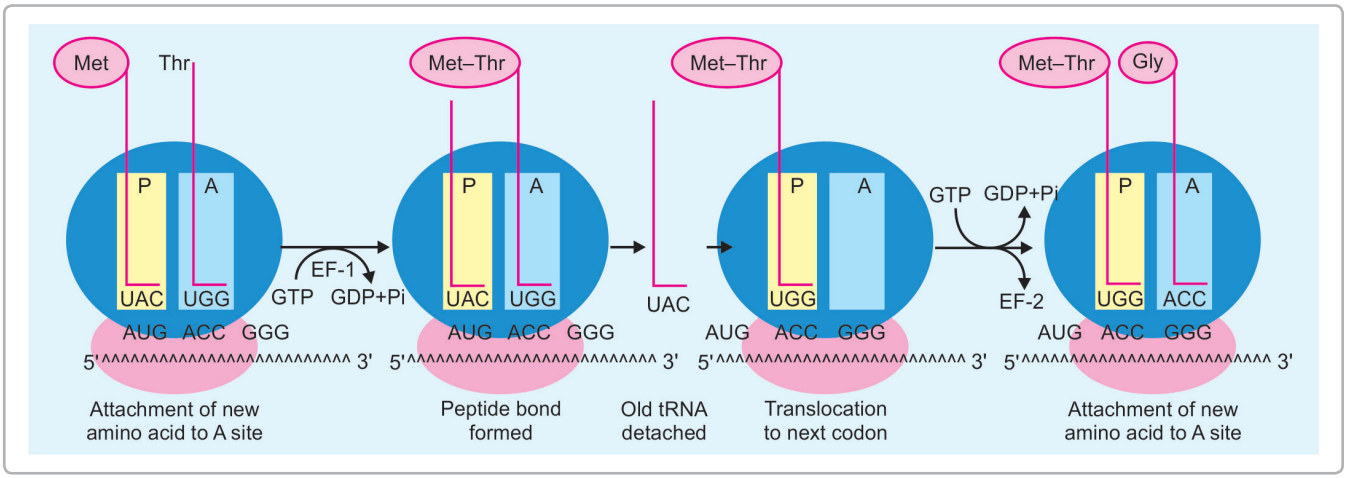


Figure 25.11: Elongation phase; P = peptidyl site; A + amino acyl site

(catalytic RNA). Now the growing peptide chain is occupying the "A" site.

Translocation process: At this time, the tRNA fixed at the "P" site does not carry any amino acid and is therefore released from the ribosome. Then the whole ribosome moves over the mRNA through the distance of one codon (3 bases). The peptidyl tRNA is translocated to the "P" site. (Fig. 25.11).

Now, the "A" site is ready to receive another aminoacyl tRNA bearing the appropriate anticodon. The new aminoacyl tRNA is fixed to the "A" site, by base pairing with the mRNA codon (Fig. 25.11).

The whole process is repeated. Translocation requires **hydrolysis of GTP** to GDP. The elongation reactions (steps 1, 2 and 3 above) are repeated till the polypeptide chain synthesis is completed.

Energy Requirements: For each peptide bond formation, **4 high energy** phosphate bonds are used. Actual peptide bond formation (peptidyltransferase step) does not require any energy, because the amino acids are already activated.

4. Termination Process of Translation

After successive addition of amino acids, ribosome reaches the **terminator codon** sequence (UAA, UAG or UGA) on the mRNA. Since there is no tRNA bearing the corresponding anticodon sequence, the "A" site remains free. The **releasing factor** (RF) enters this site along with hydrolysis of **GTP** to GDP. The RF hydrolyses peptide chain from tRNA at the P site. The completed peptide chain is now released. Finally 80S ribosome **dissociates** into its component units of 60S and 40S.

5. Post-translational Processing

Conversion of pro-insulin to **insulin** by proteolytic cleavage (For details refer Fig. 31.9).

Gamma carboxylation of glutamic acid residues of prothrombin, under the influence of vitamin K (see details refer Chapter 15).

Hydroxylation of proline and lysine in collagen with the help of vitamin C (see details refer Chapter 16).

Glycosylation: Carbohydrates are attached to serine or threonine residues.

Inhibitors of Protein Synthesis

The modern medical practice is heavily dependent on the use of **antibiotics**. They generally act only on bacteria and are nontoxic to human beings. This is because mammalian cells have 80S ribosomes, while bacteria have 70S ribosomes.

Reversible inhibitors in bacteria: These antibiotics are **bacteriostatic. Tetracyclins** bind to the ribosome and so inhibit attachment of aminoacyl tRNA to the A site of ribosomes. **Chloramphenicol** inhibits the peptidyl transferase activity of bacterial ribosomes. **Erythromycin** prevents translocation process.

Irreversible inhibitors in bacteria: These antibiotics are **bactericidal. Streptomycin** causes misreading of mRNA.

Inhibitors of transcription (described in Table 25.2) will also in turn inhibit translation process.

Genomics and Proteomics

Genome means all the DNA contained in an organism or a cell, which includes both the chromosomes within the

nucleus and the DNA in mitochondria. Thus, the genome of an organism is the totality of genes making up its hereditary constitution.

Genomics is the study of the genome and its actions

Proteome is the sum of all proteins expressed by the genome of an organism, thus involving the identification of the proteins in the body and determination of their role in physiological and pathological functions. While the genome remains largely unchanged, the proteins of a particular cell change dramatically as genes are turned on and off in response to the environment.

Proteomics: It directly addresses the protein complement of the genome. The study of all proteins by a cell type or an organism is called 'proteomics.'

A QUICK LOOK

1. Template strand of the DNA is transcribed to mRNA.
2. RNAP type II or B is the enzyme synthesizing RNA in mammals. It is susceptible to amanitin.
3. A transcription bubble contains RNA polymerase, DNA and nascent RNA.
4. Termination of transcription can be Rho factor dependent or independent.
5. Post-transcriptional processing of the primary RNA transcript includes RNAse-P tailing at 3'end, capping at 5'end, methylation and intron splicing.
6. Ribozymes are enzymes made up of RNA. Examples are RNAse-P, peptidyl transferase and spliceosomes.
7. Reverse transcriptases are RNA dependent DNA polymerases. They synthesize a DNA strand using RNA as their template. Retroviruses, such as HIV and tumor viruses, possess this enzyme.
8. DNA replicates before cell division, so that each daughter DNA molecule gets an exact copy of the parent cell.
9. The genetic information present in the mRNA is transcribed to mRNA and then translated into the amino acid sequence of the polypeptide chain, as the gene product.
10. The strand of DNA which is transcribed is called the templet strand, which is complementary to the mRNA. The anti-template strand has the same sequence as the mRNA transcript, and is called the coding strand.
11. Both rRNA and tRNA are noncoding RNAs, but play crucial roles in translation.
12. The enzyme which brings about transcription is DNA dependent RNA polymerase.
13. Bacterial RNA polymerases are different from mammalian enzymes; so antibiotics which inhibit bacterial RNAP will not affect human beings.
14. The reverse transcription process is catalyzed by RNA-dependent DNA polymerase (RT) which was first reported in tumor viruses.
15. The HIV is the most clinically relevant retrovirus now.
16. The inhibitor of RNA synthesis (in both eukaryotes and prokaryotes) is actinomycin D and mitomycin which intercalate with DNA, blocking transcription.
17. The widely used antituberculous drug rifampicin (Rifamycin) inhibits prokaryotic RNA polymerase.
18. The small interfering RNA (si-RNA) protects the genome from bacteriophages and viruses in lower organisms.
19. In eukaryotic cells, small dsRNA molecules will silence specific genes, and this is a normal regulatory mechanism.
20. Use of synthetic antisense strands (RNA molecules having complementary sequence of cellular mRNA) to manipulate genes are now being used as a therapeutic tools.
21. Transfer RNA (tRNA) or soluble RNA (sRNA) is the adapter molecule between transcription and translation. Each amino acid has a specific tRNA.
22. The triplet sequence on the anticodon arm of the tRNA is complementary to the codon triplet on the mRNA.
23. Six important characteristics of the genetic code are that it is triplet, universal, degenerate, non-overlapping, nonpunctuated and exhibit wobbling.
24. Three terminator codons are UAA, UAG and UGA.
25. Four high energy phosphate bonds are required for the formation of one peptide bond, two for initial activation, one for EF-1 step and one for EF-2 step.
26. Post-translational processing of proteins includes removal of the signal sequences, gamma carboxylations, methylations, acylation, subunit aggregation and phosphorylations.
27. Clinically useful protein synthesis inhibitors are streptomycin, chloramphenicol, tetracyclines, erythromycin.

CHAPTER 26

Gene Expression

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Mutations
- Operon concept
- Repression and derepression

MUTATIONS

An alteration in the genetic material results in a mutation. This may be either gross, so that large areas of chromosome are changed, or may be subtle with a change in one or a few nucleotides. Mutation may be defined as an abrupt spontaneous origin of new character. Statistically, out of every 10^6 cell divisions, one mutation takes place.

1. Classification of Mutations

A point mutation is defined as change in a single nucleotide. This may be subclassified as (a) substitution; (b) deletion and (c) insertion. All of them may lead to missense, nonsense or frameshift effects.

A. Substitution

Replacement of a purine by purine (A to G or G to A) or pyrimidine by pyrimidine (T to C or C to T) is called **Transition mutation**. If a purine is changed to a pyrimidine (e.g. A to C) or a pyrimidine to a purine (e.g. T to G), it is called a **transversion.** The point mutation present in DNA is transcribed and translated, so that the defective gene produces an abnormal protein with change in a single amino acid.

B. Deletion

Deletions may be subclassified into

- **Large gene deletions**, e.g. alpha thalassemia (entire gene) or hemophilia (partial)
- **Deletion of a codon**, e.g. cystic fibrosis (one amino acid, 508th phenyl alanine is missing in the cystic fibrosis transmembrane conductance regulato (CFTR) protein.
- **Deletion of a single base**, which will give rise to frameshift effect.

C. Insertion

Insertions or additions or expansions are subclassified into:

- **Single base** additions, leading to frameshift effect.
- **Trinucleotide** expansions. In Huntington's chorea, CAG trinucleotides are repeated 30 to 300 times. This leads to a polyglutamine repeat in the protein. The severity of the disease is increased as the number of repeats are more.

2. Effects of Mutations

Silent mutation: A point mutation may change the codon for one amino acid to a synonym for the same amino acid. Then the mutation is silent and has no effect on the phenotype. For example, CUA is mutated to CUC; both code for leucine, so this mutation has no effect.

Partially acceptable mutation: In these cases, a functional protein is produced. The function may be altered or deficient. Clinical manifestations also are present, but compatible with life. For example, **HbS** or sickle-cell hemoglobin is produced by a mutation of the beta chain in which the 6th position is changed to valine, instead

of the normal glutamate. Here, the normal codon GAG is changed to GUG. HbS has abnormal electrophoretic mobility, decreased solubility and subnormal function, leading to sickle-cell anemia (For details refer Chapter 14).

Unacceptable Mutation: Single amino acid substitution alters the properties of the protein to such an extent that it becomes nonfunctional and the condition is incompatible with normal life. For example, **HbM** results from histidine to tyrosine substitution (CAU to UAU) of the distal histidine residue of alpha chain. There is met-hemoglobinemia which considerably decreases the oxygen carrying capacity of hemoglobin.

Frameshift Mutation: This is due to addition or deletion of bases. From that point onwards, the reading frame shifts. A "garbled" (completely irrelevant) protein, with altered amino acid sequence is produced. An example is given below:

Normal	mRNA	AUG **U**CU UGC AAA.....
Normal	protein	Met Ser Cys Lys.....
Deleted U	mRNA	AUG CUU GCA AA.....
Garbled	protein	Met Leu Ala .....

In this hypothetical example, deletion of one uracil changes all the triplet codons thereafter. Therefore, a useless protein is produced. Frameshift mutations can also lead to thalassemia, premature chain termination and run-on-polypeptide.

Conditional Mutations: Most of the spontaneous mutations are *conditional*; they are manifested only when circumstances are appropriate. Bacteria acquire resistance, if treated with antibiotics for a long time. This is explained by spontaneous conditional mutations. In the normal circumstances, wild bacilli will grow. In the medium containing antibiotic, the resistant bacilli are selected. In a tuberculous patient, a lung cavity may harbor about 10^{12} bacilli. This may contain about 10^6 mutations, out of which a few could be streptomycin resistant. Therefore if the patient is given streptomycin alone, after sometime, there will be overgrowth of drug resistant bacilli. To avoid this, a combination of streptomycin plus INH (isonicotinic acid hydrazide) is given. So, streptomycin resistant mutants are killed by INH and INH resistant mutants are removed by streptomycin. The statistical probability of a single bacillus acquiring resistance against both streptomycin and INH is negligible.

Beneficial mutations: Although rare, beneficial spontaneous mutations are the basis of evolution. Such beneficial mutants are artificially selected in agriculture. New variants are produced by gamma-irradiation of seeds. The plants from irradiated seeds are selected for useful characters. Normal maize is deficient in tryptophan. Tryptophan-rich maize varieties are now available for cultivation. Microorganisms often have antigenic mutation. These are beneficial to microorganisms (but of course, bad to human beings).

Carcinogenic effect: The mutation may not be lethal, but may alter the regulatory controls. Such a mutation in a somatic cell may result in uncontrolled cell division leading to cancer. Since some of the mutations may be of cancerous type, any substance causing increased mutation can also increase the probability of cancer. Thus, *all mutagens are carcinogens.*

3. Mutagens and Mutagenesis

Any agent which will increase DNA damage or cell proliferation can cause increased rate of mutations also. Such substances are called **mutagens.** X-ray, gamma-ray, UV ray, acridine orange, etc. are well known mutagens. The rate of mutation is proportional to the dose of irradiation.

Site-directed Mutagenesis

Michael Smith (Nobel prize, 1993) described this technique. An oligodeoxyribonucleotide is synthesized, whose sequence is complementary to a part of a known gene. A specific deletion/insertion is produced in the oligo. It is then extended by DNAP. After replication, one strand is normal and the other strand contains the mutation at the specific site. This allows study on the effect of that particular mutation.

CELL CYCLE

The term cell cycle refers to the events occurring during the period between two mitotic divisions. It is divided into G1 (gap-1), S (synthesis), G2 (gap-2), and M (mitosis) phases. The cell division is taking place in **M phase**. It is the shortest phase, lasting about 1 hour. The daughter cells then either enter into Go (undividing or dormant) phase or re-enter the cell cycle when there is necessity for growth and replication. In a normal cell population, most of the cells are in Go phase. General metabolic events are taking place in Go phase.

Interphase is the period between the end of M phase and the beginning of the next mitosis. In **G1 phase**, protein and RNA contents increase.

In the **S phase,** DNA is synthesized, but only once. DNA content doubles, nucleus becomes tetraploid (4n). The entire diploid genome is replicated into a tetraploid genome.

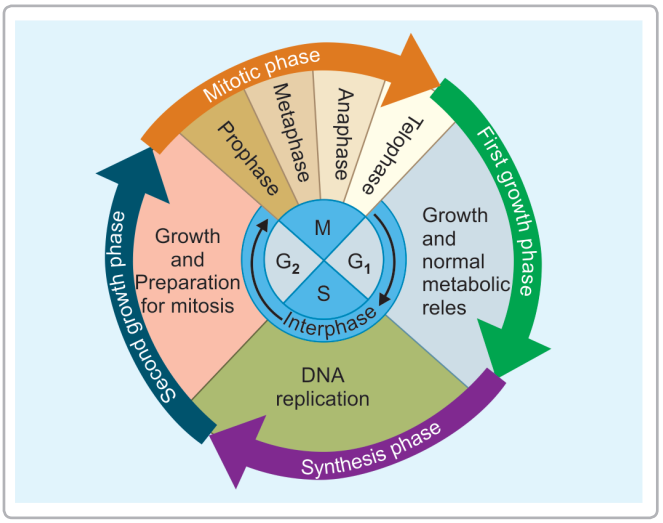


Figure 26.1: Cell cycle phases

In the **G2 phase,** there is cytoplasmic enlargement. DNA repair is also taking place in the G2 phase. Proteins, especially histones are also produced. The total cell cycle is about 20–22 hours duration in mammalian cells (Fig. 26.1).

REGULATION OF GENE EXPRESSION

Synthesis of proteins under the influence of gene is called **gene expression**. All genes of the cell are not expressed at all the time. For example, the insulin gene is expressed only in the beta cells of pancreas; but not in other tissues. In other words, insulin gene is in the state of **repression** in all other cells. Some genes are expressed almost always in all cells. For example, enzymes of glycolysis are synthesized by all cells. Such genes are called **constitutive** genes or housekeeping genes. The same gene may be alternatively switched on or off, as per the need of the metabolism. Such regulation is achieved by induction and repression.

Induction is the increased synthesis of protein or enzyme in response to a certain signal. Such enzymes are said to be inducible, and the signals are called **inducers**. Induction is turning "on" the switch of the gene. **Repression** is turning "off" the gene expression.

Operon Concept of Gene Regulation

Francois Jacob and Jacques Monod put forward the operon concept in 1961, for which they were awarded Nobel prize in 1965. Their theory was based on the observations on lactose metabolism in *Escherichia coli* (bacteria). Cells grown in glucose medium do not contain beta galactosidase (lactase). But when cells are transferred to a medium containing only lactose, then the enzyme level in the cell increases several thousand fold. Thus, lactose metabolism is regulated by an induction or derepression process.

The Lac Operon

Operon is a unit of gene expression which includes structural genes, control elements, regulator/inhibitor gene, promoter and operator areas. In the bacterial cell, the Z gene encodes beta-galactosidase, the enzyme which hydrolyses lactose to galactose and glucose. The Y gene is responsible for production of a permease which transports lactose and galactose into the cell. Since Z and Y code for the structure of the proteins, they are called **structural genes**. These genes are present as continuous segments of DNA (Fig. 26.2). Transcription of these genes start from a common **promoter** (P), located close to the Z gene. The RNA polymerase binds to the promoter and transcribes these 3 structural genes as a single mRNA.

Transcription is Normally Repressed

Transcription of the structural gene is under the control of another **regulator** or the "i" (**inhibitor**) gene. It is far away from the structural genes. Regulatory gene produces a **repressor** molecule. The lac repressor tightly binds to the operator site. The operator site is between the promoter and structural genes (Fig. 26.2-A). When RNAP identifies the promoter sequence and moves towards the structural genes, it is stopped by the hindrance produced by repressor molecule. This is like the action of a zip. If a thread is placed across its way, the zip cannot move further. Similarly, when repressor is attached to the operator, RNAP cannot move further. So structural genes are not transcribed. Thus, when lactose is not available, the lactose utilizing enzymes are not synthesized (Fig. 26.2, left side).

Derepression of Lac Operon

When lactose is introduced into the medium, lactose binds to the repressor protein; one molecule on each subunit (Fig. 26.2, right side). Repressor-lactose complex is inactive, which does not bind to the operator region. So there is no repressor molecule at the operator site. Now, RNAP can transcribe the structural genes, which are then translated (Fig. 26.2, right side). Thus lactose switches the genes "on". Lactose induces the synthesis of lactose utilizing enzymes. Hence lactose is an **inducer** of these genes and the mechanism is said to be **derepression** of the

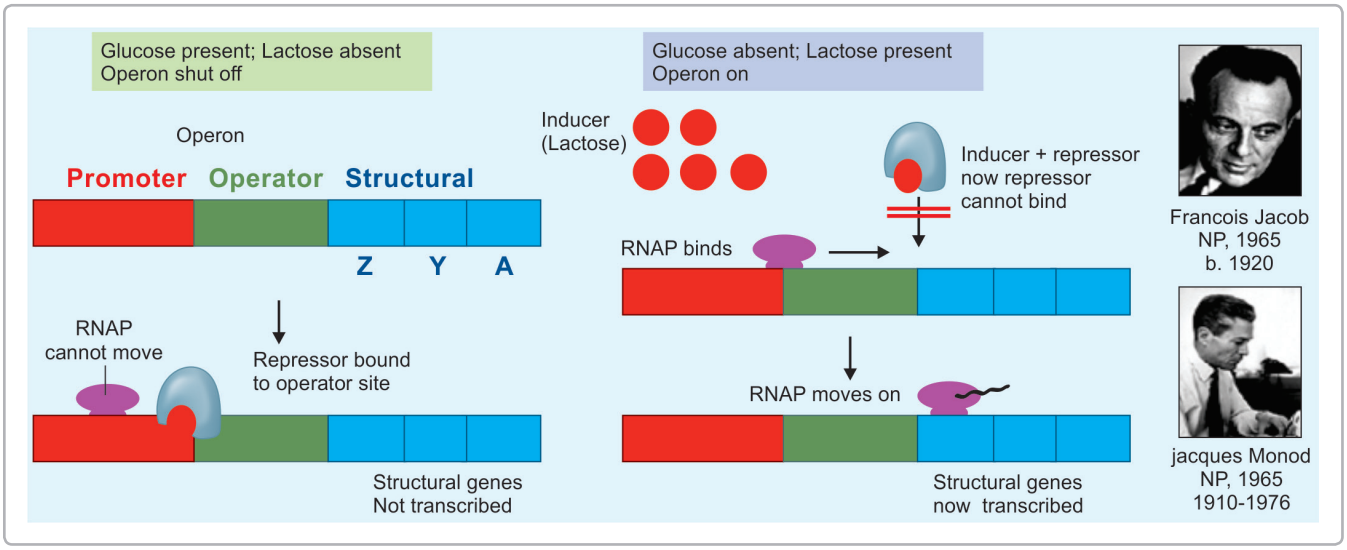


Figure 26.2: Repression of lac operon is shown in the left side. When lactose is absent, repressor molecules fit in the operator site. So RNAP cannot work, and genes are in "off" position. **Induction** or derepression of lac operon is shown in the right side lactose attaches with repressor; so operator site is free; genes are in "on" position; protein is synthesized

gene. Such regulation, where several proteins are regulated as one unit, is termed as **coordinate** gene regulation.

Clinical Applications

Examples of derepression include induction of transaminases by glucocorticoids; and ALA synthase by barbiturates.

Regulation of Genes by Repression

Repression is the mechanism by which the presence of excess product of a pathway shuts off the synthesis of the key enzyme of that pathway. Heme synthesis is an example. It is regulated by repression of **ALA synthase,** the key enzyme of the pathway (For details refer Chapter 14). Transcription of structural gene for ALA synthase is controlled by a regulatory gene. It produces the **aporepressor**, which binds with heme and becomes the active holorepressor. Here, heme acts as the **corepressor**.

The **holorepressor** binds to the operator and stops transcription of the gene. Upstream to the structural genes lies the promoter site, where the RNA-polymerase (RNAP) attaches and starts mRNA synthesis. The operator site is in between promoter and structural genes. So when RNAP reaches operator site, it cannot move further (Fig. 26.3). So enzyme synthesis stops, and heme synthesis slows down.

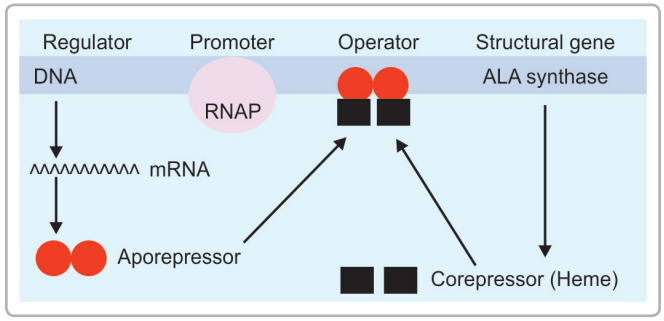


Figure 26.3: Repression by heme on the enzymes responsible for heme synthesis

On the other hand, when heme is not available, corepressor is not available, therefore, repression is not effective and enzyme synthesis starts. Thus, the synthesis of heme is autoregulated by the repression mechanism. Thalassemia is a condition when normal hemoglobins are produced in abnormal ratios (For details refer Chapter 14). When alpha chain synthesis is blocked due to a mutation on the promoter, there is compensatory increase in beta chain synthesis. Instead of the α_2 β_2 combination for normal hemoglobin, an abnormal β_4 combination results (HbH).

Hormone Response Elements (HRE)

Hormones or their second messengers function as inducers. Many hormones, particularly steroid hormones,

elicit physiological response by controlling gene expression. Glucocorticoids attach to a receptor; then the receptor-hormone complex translocates to the nucleus. It finally attaches to the HRE in the DNA. The receptor binds at the enhancer region, which activates the promoter, so that transcription is accelerated.

A QUICK LOOK

1. An alteration in the genetic material results in a mutation.
2. Mutation may be 1) point mutation or 2) affecting large areas of the chromosome.
3. Point mutation may be subclassified as a) substitution; b) deletion and c) insertion.
4. Effects of mutation may be 1) silent; 2) missense but acceptable mutation; 3) missense but partially acceptable mutation; 4) missense but unacceptable mutation; 5) nonsense or terminator codon mutation and 6) frameshift mutation.
5. Manifestations of mutations may be 1) lethal mutations; 2) silent mutations; 3) beneficial mutations and 4) carcinogenic mutations.
6. Any agent which will increase cell proliferation can cause increased rate of mutation; such substances are called mutagens. Most mutagens are carcinogens.
7. Cell cycle is divided into G1, G2, S and M phases.
8. Induction is the phenomenon of increased synthesis of protein or enzyme in response to certain signal.
9. Operon is a unit of gene expression; it includes structural genes, control elements, regulator or inhibitor gene, promoter and operator areas.
10. When lactose is available in the surroundings, lac operon is derepressed; lactose is the inducer of lactose utilizing enzyme.
11. Repression is the mechanism by which the excess product of a pathway shuts off the synthesis of the key enzyme of that pathway; ALA synthase is repressed by heme.

CHAPTER 27

Molecular Biology Techniques

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Recombinant DNA technology
- Restriction endonucleases
- Vectors
- Gene therapy
- Hybridization and blot techniques
- Polymerase chain reaction (PCR)

RECOMBINANT DNA TECHNOLOGY

Biotechnology may be defined as "the method by which a living organism or its parts are used to change or to incorporate a particular character to another living organism".

Genetic recombination is the exchange of information between two DNA segments. This is a common occurrence within the same species. But by artificial means, when a gene of one species is transferred to another living organism, it is called **recombinant DNA technology**. In common parlance, this is known as *genetic engineering*.

Applications of Recombinant Technology

1. Quantitative Preparation of Biomolecules

If molecules are isolated from higher organisms, the availability will be greatly limited. For example, growth hormone. By means of recombinant technology, large scale availability is now assured.

2. Risk of Contamination is Eliminated

It is absolutely essential to make sure that the preparations of vaccines or clotting factors are free from contaminants such as hepatitis B particles. Recombinant DNA technology provides the answer to produce safe antigens for vaccine production.

3. Specific Probes for Diagnosis of Diseases

Specific probes are useful for

- Antenatal diagnosis of genetic diseases
- To identify viral particles or bacterial DNA in suspected blood and tissue samples
- To demonstrate virus integration in transformed cells
- To detect activation of oncogenes in cancer
- To pinpoint the location of a gene in a chromosome
- To identify mutations in genes.

4. Gene Therapy

An important application of recombinant technology is in gene therapy. Normal genes could be introduced into the patient so that genetic diseases can be cured. These techniques are described later.

Restriction Endonucleases (RE)

In order to transfer a gene, it is to be first selectively split from the parent DNA. This is usually achieved by restriction endonucleases which are referred to as "molecular scissors". Certain enzymes of bacteria restrict the entry of phages into host bacteria. Hence, the name restriction endonucleases.

Restriction Sites

Restriction endonucleases have specific recognition sites where they cut the DNA. (Table 27.1). There are more than 800 such enzymes now available commercially. These enzymes recognize specific sequences with **palindrome** arrangement. Palindrome in Greek means "to run backwards". It is similar to a word that reads backwards or forwards similarly, e.g. "madam". These are also called **inverted repeat** sequences, which means the nucleotide sequence in 5' to 3' direction is the same in both strands. The resultant DNA cuts will generally have overlapping or sticky ends (Fig. 27.1).

Restriction Map

The recognition site will be about 4 to 7 nucleotide pairs. If a piece of DNA from a species is made to react with a specific RE, a characteristic array of cut pieces will be produced, this is called a **restriction map**. These fragments can be isolated by electrophoresis. The restriction fragments serve as the "**fingerprint** of the DNA", because the fragments of DNA from one organism will have a characteristic pattern.

Table 27.1: Specificity of restriction enzymes (The arrows show the site or cut by the enzyme)

Enzyme	*Source of enzyme*	*Specific sequence identified by the enzyme*
EcoRI	*Escherichia coli* RY 13	G ↑ AATT C C TTAA ↓ G
Hind III	*Haemophilus influenzae* Rd	A ↑ AGCT T T TCGA ↓ A

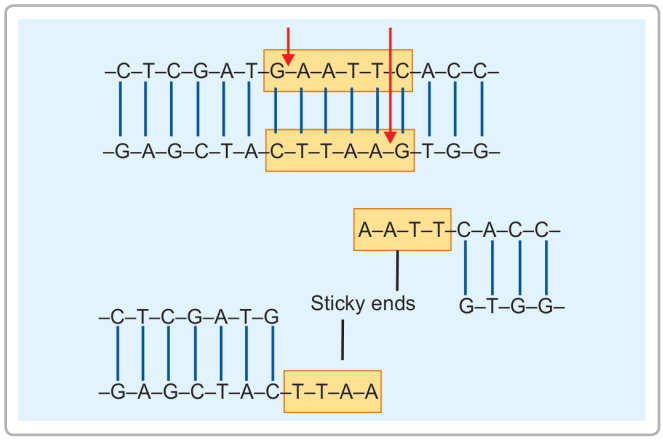


Figure 27.1: EcoRI enzyme cuts the bonds marked with red arrows. This results in the sticky ends

VECTORS

In order to introduce the human gene into bacteria, at first, the gene is transferred into a **carrier**, known as a **vector.** Most commonly used vectors are **plasmids.** Plasmids are circular double-stranded DNA molecules seen inside bacteria. In nature, plasmids confer antibiotic resistance to host bacteria. Plasmids replicate independent of bacterial DNA. Foreign DNA could be incorporated into them by using specific RE.

Procedure of DNA Recombination

1. Preparation of Chimeric DNA Molecules

Chimera is the Greek mythological monster with a lion's head, goat's body and serpent's tail. A vector carrying a foreign DNA is called **Chimeric DNA** or Hybrid DNA or Recombinant DNA. The steps are:

A circular plasmid vector DNA is cut with a specific restriction endonuclease (RE). If EcoRI is used, **sticky ends** will result with TTAA sequence on one DNA strand, and AATT sequence on the other strand (Figs 27.1 and 27.2). The human DNA is also treated with the same RE, so that the same sequences are generated on the sticky ends of the cut piece. Then the vector DNA and

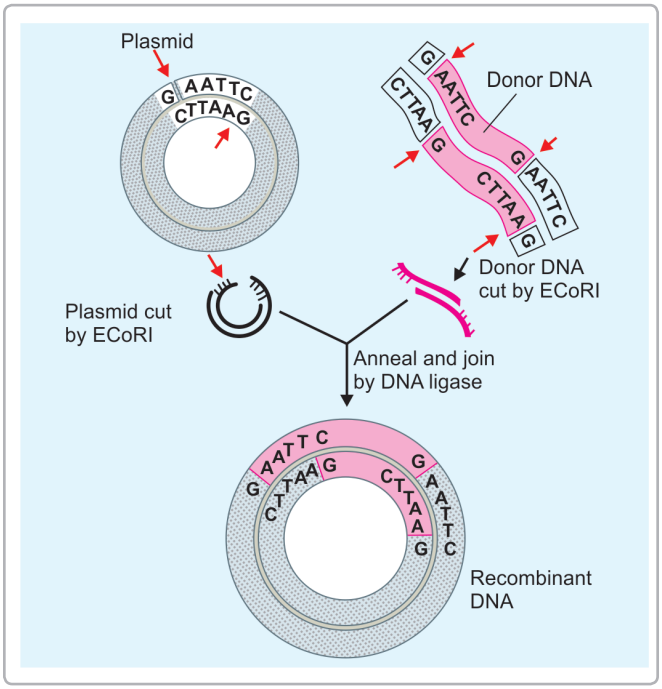


Figure 27.2: Production of chimeric DNA molecule by using EcoRI restriction endonuclease. Red arrows show the site of cut by EcoRI

cut-piece of human DNA are incubated together so that **annealing** takes place. The sticky ends of both vector and human DNA have complementary sequences, and therefore they come into contact with each other. Then **DNA ligase** enzyme is added, which introduces phosphodiester linkages between the vector and the insert molecules. Thus, the chimeric DNA is finally produced.

2. Cloning of Chimeric DNA

The next step is cloning. A clone is a large population of identical bacteria or cells that arise from a common ancestor molecule. Cloning allows the production of a large number of identical DNA molecules. The hybrid molecules are amplified by the cloning technique.

3. Transfection of Vector into the Host

The process by which plasmid is introduced into the host is called *transfection.* Host *E. coli* cells and plasmid vectors are incubated in hypertonic medium containing calcium for a few minutes. Then calcium ion channels are opened, through which the plasmid is imbibed into the host cell. Now the host cells are allowed to grow in agar plates containing growth medium.

4. Selection

Only 5% of bacterial colonies contain the desired vector. Therefore, we have to select the desired colonies. When foreign DNA is inserted, the resistance against chloramphenicol is lost. This insertional inactivation of gene is the marker for hybrid DNA. After the transfection, bacteria are cultured in suitable medium, so that only bacteria with hybrid plasmid will grow.

5. Expression Vectors

To produce the human proteins, *E. coli* carrying the vector with the insert is allowed to grow. The human proteins can be harvested from the bacterial culture. The principle of gene transfer technology is summarized in Figure 27.3.

6. Human Recombinant Proteins

Hundreds of human proteins are now being synthesised by the recombinant technology. Recombinant human **insulin** is now available in market. Other useful products thus produced are **interleukins**, interferons, antihemophilic globulin, hepatitis B surface **antigen** (for vaccination) and **growth hormone**.

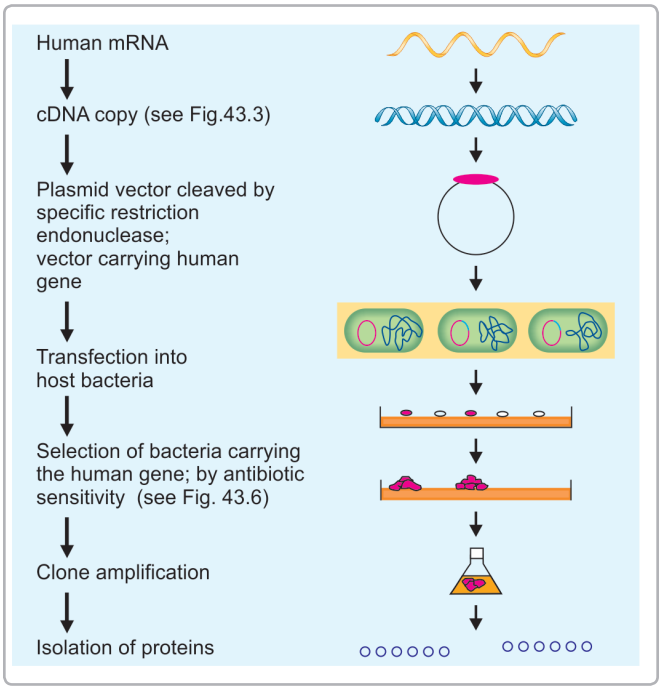


Figure 27.3: DNA recombinant technology

Human Genome Project (HGP)

US National Institutes of Health started this project in 1990. James Watson (co-discoverer of the structure of DNA, Nobel laureate, 1962) was the first head of the project. The project included scientists from 16 centers in USA, Britain, France, Japan and Germany. The ambitious project was to decode the whole human genes and to sequence the whole human DNA. One set of human DNA contains about 3 billion base pairs (one cell contains 2 sets) and about 10,000 genes. The "Book of Human Life" contains "23 Chapters", as the 23 chromosomes.

Human genetic mapping (location of important genes) was completed by 1994. The DNA sequencing was performed later. By December 1998, human Chromosome 5 (about 6% of human genome) was sequenced completely. By November 2002, the total human DNA sequence was announced.

GENE THERAPY

Gene therapy was once considered a fantasy. However, thousands of individuals have already undergone human clinical trials. A great leap in medical science has taken

place on 14th September 1990, when a girl suffering from Adenosine deaminase deficiency (severe Immunodeficiency) was treated by transferring the normal gene for adenosine deaminase.

Gene therapy is intracellular delivery of genes to correct an existing abnormality.

Summary of the Procedure

- Isolate the healthy gene along with the sequence controlling its expression
- Incorporate this gene into a carrier or vector as an expression cassette
- Finally deliver the vector to the target cells.

How the Genes are Introduced?

There are three ways of applying gene carrying vectors:

- **Ex vivo strategy:** Where the patients' cells are cultured in the laboratory, the new genes are infused into the cells; and modified cells are administered back to the patient (Fig. 27.4).
- **In situ strategy:** When the expression cassette is injected to the patient either intravenously or directly to the tissue.
- **In vivo strategy:** Where the vector is administered directly to the cell, e.g. CF (cystic fibrosis) gene to the respiratory tract cells.

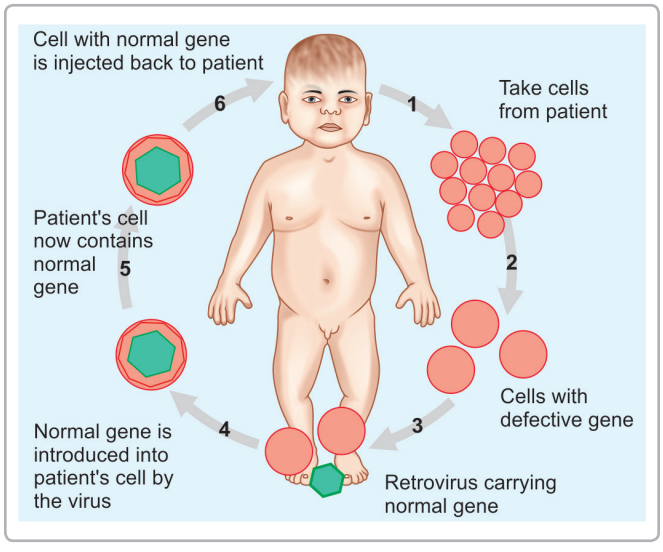


Figure 27.4: Ex vivo gene therapy

The Vectors

Different vector (carrier) systems used for gene delivery are: Retroviruses are the best choice. Retroviruses are **RNA viruses**. Certain genes in the virus are deleted from the retrovirus, rendering it incapable of replication inside human body. Then the human gene is inserted into the virus. The virus enters into the target cell via specific receptor.

In the cytoplasm of the human cells, the **reverse transcriptase** carried by the vector converts the RNA to proviral DNA, which is **integrated** into the target cell DNA. The normal human gene can now express.

5. Accomplishments

Gene therapy is effective in inherited disorders caused by single genes. Several clinical trials have been conducted. Success has already been accomplished by gene therapy in the following disease conditions: a) Severe combined immunodeficiency; b) Duchenne muscular dystrophy; c) Cystic fibrosis, and d) hemophilia.

Obstacles to Success

The potential of gene therapy is enormous. It is now theoretically possible to cure all the genetic diseases. However, it may take several years to get this therapeutic modality available for common clinical use. The following limitations are encountered for gene therapy: (a) Inconsistent results (b) Lack of ideal vector (c) Lack of targeting ability in nonviral vectors (d) Death during the course of gene therapy for OTC (Ornithine transcarbamoylase) deficiency was reported

STEM CELLS

Stem cell research is leading to promising results in treatment of various incurable diseases like coronary artery disease and cancer. Stem cells have the ability to divide for an indefinite period. They can give rise to a variety of specialized cell types. This phenomenon is known as developmental plasticity. Stem cells can be isolated from embryos, umbilical cord as well as any other adult tissue.

Stem cells may be capable of producing all types of cells of the organism (**totipotent**), or able to generate cells of the three germ layers (**pluripotent**), or able to produce only closely related cell types (**multipotent**), or may produce only one cell type (**unipotent**). Stem cells may be **embryonic** (capable to differentiate) or adult type

(limited capacity to differentiate). Active research is going on to utilize stem cells in the treatment of stroke, brain injury, Alzheimer's disease, Parkinsonism, wound healing, myocardial infarction, muscular dystrophy, spinal cord injury, diabetes and cancers.

General belief was that only stem cells are plastic, and the differentiated matured cells will lose their plasticity. But Sir John Gurdon and Shinya Yamanaka have independently discovered that mature cells can be reprogrammed to become pluripotent; they were awarded Nobel Prize in 2012.

HYBRIDIZATION AND BLOT TECHNIQUES

1. Probes

A probe is defined as a single stranded piece of DNA, labeled (either with radioisotope or with nonradioactive label), the nucleotide sequence of which is complementary to the target DNA. The DNA of the specific gene is used for the hybridization techniques. Radioactivity is tagged into the gene.

2. DNA-DNA Hybridization

DNA is denatured by NaOH. The specific probe tagged with radioactivity is added, incubated, washed and autoradiographed (Fig. 27.5).

3. Southern Blot Technique

The method was developed by EM Southern in 1975. This is used to detect a **specific segment of DNA** in the whole genome. It is based on the specific base pairing properties of complementary nucleic acid strands. This technique is also called **DNA hybridization** (Fig. 27.6)

DNA is isolated from the suspected tissue. It is then fragmented by **restriction endonucleases**. The cut pieces are **electrophoresed** on agarose gel. It is then treated with NaOH to denature the DNA, so that the pieces become single-stranded. This is then **blotted** over to a nitrocellulose membrane. The single-stranded DNA is adsorbed on the **nitrocellulose** membrane. An exact replica of the pattern in the gel is reproduced on the membrane (Fig. 27.6). The DNA is then fixed on the membrane by baking at 80°C. There will be many DNA fragments on the membrane, but only one or two pieces contain the specific DNA sequence. The radioactive **virus probe** is placed over the membrane. The probe will detect the complementary nucleotide sequence in the host DNA. So the probe is hybridized on the particular pieces of host DNA. An X-ray plate is placed over the membrane in darkness for a few days. The radiation from the fixed probe will produce its mark on the X-ray plate. This is called **autoradiography** (Fig. 29.6). Abnormal genes such as HbS gene or virus integration can also be identified by this method.

4. Northern Blotting for Identifying RNA

The Northern blot is used to demonstrate **specific RNA**. The total RNA is isolated from the cell, electrophoresed and then blotted on to a membrane. This is then probed with radioactive cDNA. There will be RNA-DNA hybridization. This is used to detect the gene expression in a tissue.

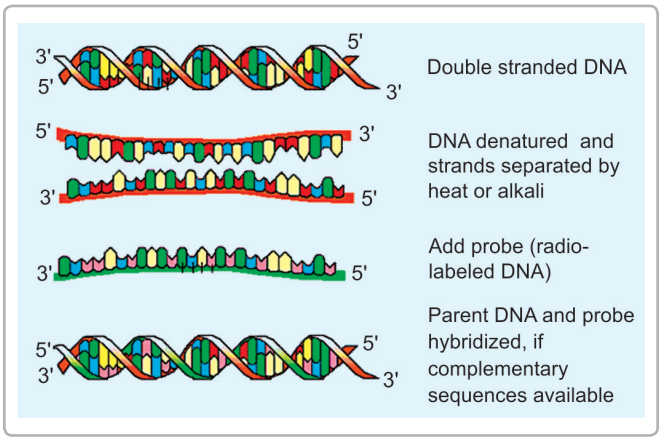


Figure 27.5: DNA-DNA hybridization

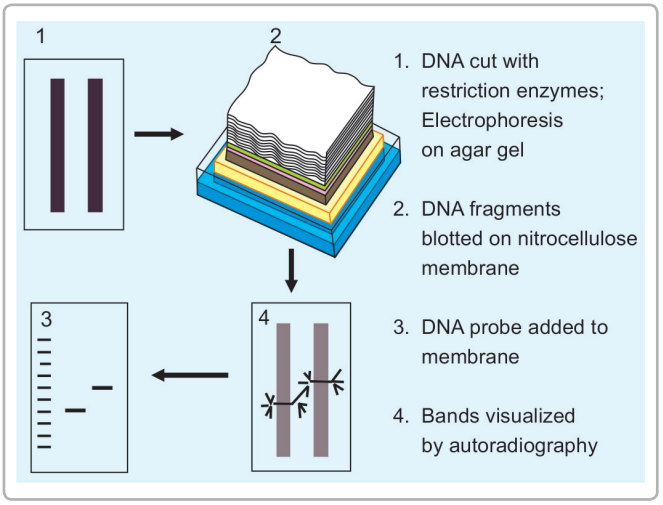


Figure 27.6: Southern blot technique

5. Western Blot Analysis for Proteins

In this technique, proteins (not nucleic acids) are identified. The proteins are isolated from the tissue and electrophoresis is done. The separated proteins are then transferred on to a **nitrocellulose** membrane. After fixation, it is probed with radioactive **antibody** and auto-radiographed. This technique is very useful to identify the specific protein in a tissue, thereby showing the expression of a particular gene.

POLYMERASE CHAIN REACTION (PCR)

Karry Mullis invented this ingenious method in 1989, who was awarded Nobel prize in 1993. PCR is an in vitro DNA amplification procedure in which millions of copies of a particular sequence of DNA can be produced within a few hours. It is like xerox machine for gene copying.

The flanking sequences of the gene of interest should be known. Two DNA primers of about 20–30 nucleotides with complementary sequence of the flanking region can be synthesized. The reaction cycle has the following steps (Fig. 27.7).

- **Step 1: Separation (Denaturation):** DNA strands are separated (**melted**) by heating at 95°C for 15 seconds to 2 minutes.
- **Step 2: Priming (Annealing):** The primers are **annealed** by cooling to 50°C for 0.5 to 2 minutes. The primers hybridize with their complementary single stranded DNA produced in the first step.
- **Step 3: Polymerization:** New DNA strands are synthesized by **Taq polymerase.** This enzyme is derived from bacteria *Thermus aquaticus* that are found in hot springs. Therefore, the enzyme is not denatured at high temperature. The polymerase reaction is allowed to take place at 72°C for 30 seconds in presence of dNTPs (all four deoxyribonucleotide triphosphates). Both strands of DNA are now duplicated.
- The steps of 1, 2 and 3 are **repeated**. In each cycle, the DNA strands are doubled. Thus, 20 cycles provide for 1 million times amplifications. These cycles are generally repeated by automated instrument, called **Tempcycler**.
- After the amplification procedure, DNA hybridization technique or Southern blot analysis with a suitable probe, shows the presence of the DNA in the sample tissue.

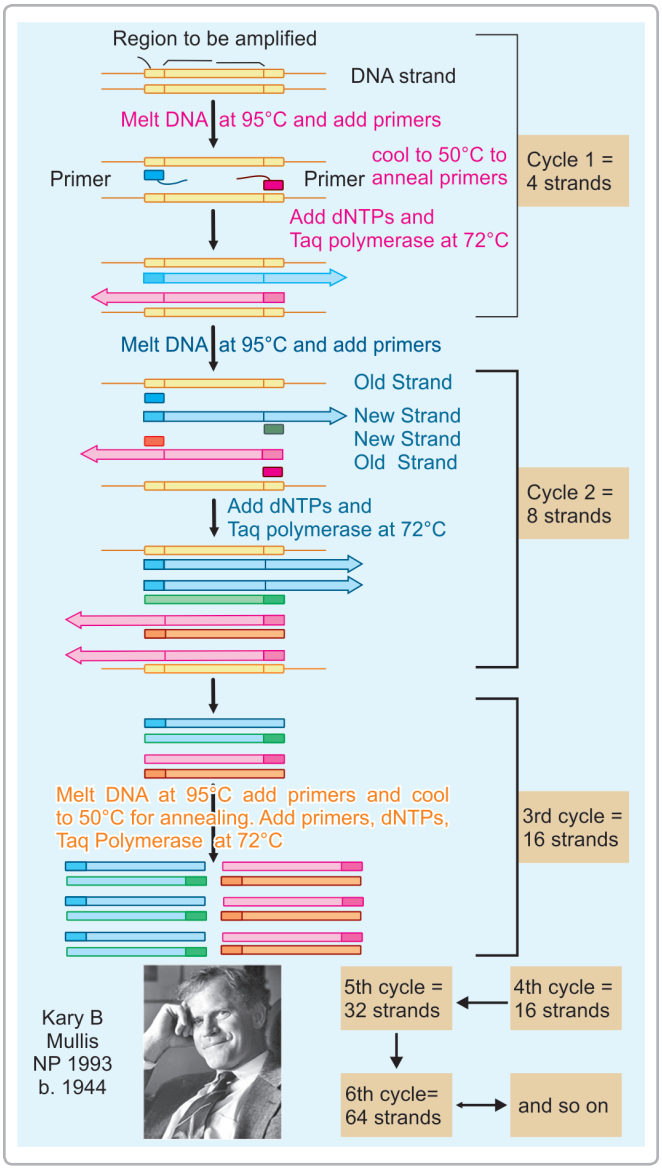


Figure 27.7: Polymerase chain reaction

Clinical Applications of PCR

- **Diagnosis** of bacterial and viral diseases: PCR could detect even one bacillus present in the specimen. This technique is widely used in the diagnosis of bacterial diseases such as tuberculosis and viral infections like Hepatitis C, *Cytomegalovirus* and HIV.
- **Medicolegal** cases: The restriction analysis of DNA from the hair follicle from the crime scene is studied after PCR amplification. This pattern is then compared

with the restriction analysis of DNA samples obtained from various suspects. This is highly useful in forensic medicine to identify the criminal.

- Diagnosis of **genetic disorders**: The PCR technology has been widely used to amplify the gene segments that contain known mutations for diagnosis of inherited diseases such as sickle cell anemia, beta thalassemia, cystic fibrosis, etc.
- PCR is especially useful for **prenatal diagnosis** of inherited diseases, where cells obtained from fetus by amniocentesis are very few.
- **Cancer detection:** PCR is widely used to identify mutations in oncosuppressor genes such as p53, retinoblastoma gene, etc. (For details refer Chapter 29 for oncogenes and oncosuppressor genes).

A QUICK LOOK

1. Genetic recombination involves the exchange of information between two segments of DNA. When a gene of one species is transferred into another under laboratory conditions, the technique is called recombinant DNA technology or genetic engineering.
2. Restriction endonucleases (RE), also known as 'molecular scissors' cut at sequences which are palindromes. Each RE is characterized by a specific 'restriction site'.
3. Plasmids are commonly used vectors. They provide antibiotic resistance to their host bacteria. This property is used as a marker in genetic engineering.
4. A vector carrying a foreign DNA is called 'Chimeric DNA'.
5. The process of introducing a plasmid into a host is called transfection.
6. Gene therapy involves the delivering genes to generate a therapeutic effect by correcting an existing abnormality. Introducing genes involves three ways of applying gene carrying vectors, ex vivo, in situ and in vivo.
7. Retroviruses, adenoviruses and herpes simplex viruses have been used carrier systems in human gene studies.
8. Diseases for which gene therapy has been attempted are severe combined immunodeficiency (SCID), Duchenne muscular dystrophy (DMD), cystic fibrosis, hemophilia.
9. Southern blot technique is used to detect specific segment of DNA in the whole genome.
10. Northern blot is to identify RNA.
11. Western blot analysis is done to identify proteins.

CHAPTER 28

Laboratory Techniques

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Electrophoresis
- Adsorption chromatography
- Partition chromatography
- Enzyme-linked immunosorbent assay (ELISA)
- Colorimeter
- Spectrophotometer

ELECTROPHORESIS

The term refers to the movement of charged particles through an electrolyte when subjected to an electric field. The positively charged particles (cations) move to cathode and negatively charged ones (anions) to anode. Since proteins exist as charged particles, this method is widely used for the separation of proteins in biological fluids. The technique was invented by Tiselius (Nobel Prize 1948).

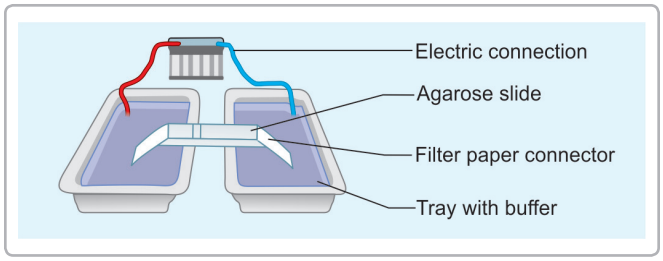


Figure 28.1: Electrophoresis apparatus

Factors Affecting Electrophoresis

The rate of migration (separation of particles) during electrophoresis will depend on the following factors:

- Net charge on the particles (pI of proteins)
- Mass and shape of the particles
- The pH of the medium
- Strength of electrical field
- Properties of the supporting medium.

Electrophoresis Apparatus

The electrophoresis system consists of the electrophoresis tank to hold the buffer. There will be electrodes, and a power pack to supply electricity at constant current and voltage (Fig. 28.1).

When the electrophoresis is carried out, the buffer is chosen in such a way so as to ensure effective separation of the mixture of proteins. e.g. serum proteins are separated at a pH of 8.6 using barbitone buffer. At this pH, all serum proteins will have a net negative charge and will migrate towards the anode.

Cellulose Acetate Membrane

Nowadays the preferred solid support media for horizontal electrophoresis is cellulose acetate membrane strips. The process takes less than one hour and excellent separation without diffusion is achieved. Cellulose acetate strips are widely used for separation and identification of lipoproteins, isoenzymes and hemoglobins.

Agar or Agarose

Both are heterogeneous polysaccharides. They are viscous liquids when hot but solidify to a gel on cooling. The gel is

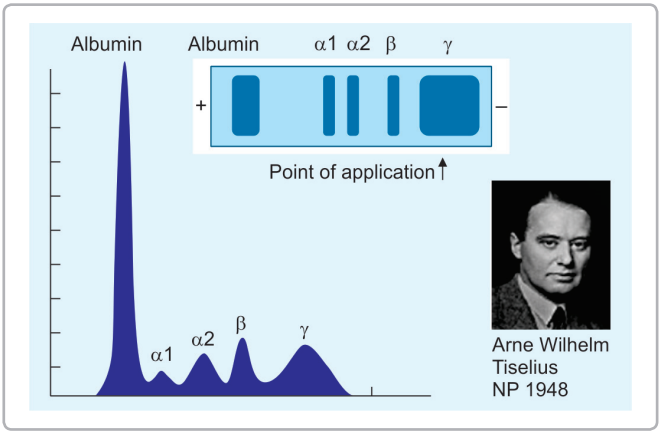


Figure 28.2: Electrophoresis of normal serum sample Arne Tiselius (Nobel prize 1948) is shown in inset

prepared and spread over microscopic slides and allowed to cool. A small sample (few microliters) of serum is applied. The electrophoretic run takes about 90 minutes. Serum proteins are commonly studied by this method.

Visualization of Protein Bands

After the electrophoretic run is completed, the proteins are fixed to the solid support using a fixative such as acetone or methanol. Then it is stained by using dyes (Amido Schwartz or Coomassie Blue). The electrophoretogram can be scanned using a **densitometer** and each band quantitated. The electrophoretic pattern of serum proteins on agar gel are shown in Fig. 28.2. Abnormal patterns are shown in Fig. 12.1.

Immunoelectrophoresis

Here electrophoretic separation is followed by an antigen–antibody reaction. The electrophoresis is carried out first by applying the patient's serum into the wells cut out in the agar or agarose gel. The proteins are now separated. To visualize them, a specific antibody is placed in a trough cut into the gel and incubated. The precipitation arcs are formed where the antigen and antibody molecules are at 1:1 ratio (Fig. 28.3). Serum is fractionated into more than 40 bands. So it is much more sensitive than ordinary electrophoresis.

CHROMATOGRAPHY

The term is derived from the Greek word chroma, meaning color. Nowadays chromatography is used to separate almost all biological substances, including proteins, carbohydrates, lipids and nucleic acids.

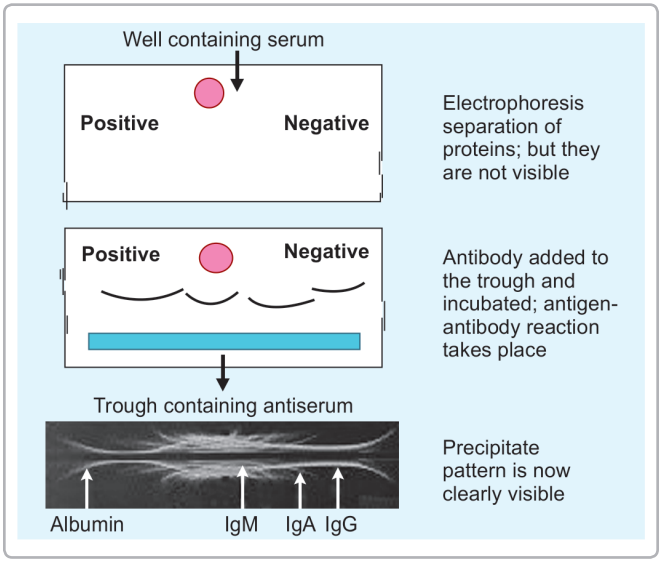


Figure 28.3: Immunoelectrophoresis pattern

Partition Chromatography

This is commonly used for the separation of mixtures of amino acids and peptides. There is a **stationary phase** which may be either solid or liquid over which a liquid or gaseous mobile phase moves. By this process, the components of the mixture to be separated are partitioned between the two phases depending on the **partition coefficient** (solubility) of the particular substances. The redistribution of the substances between the two phases results in separation of the components of the mixture.

Paper Chromatography

The **stationary phase** is water held on a solid support of filter paper (cellulose). The **mobile phase** is a mixture of immiscible solvents which are mixtures of water, a nonpolar solvent and an acid or base, e.g. Butanol-acetic acid-water, Phenol-water-ammonia. A few microliters of the mixture is applied as a small compact spot at one corner of the paper about 1 inch from the edges. The paper is placed in a glass trough containing the solvent which ascends up the solid support medium. The components of the mixture are carried up with the solvent. The components are separated according to their solubility.

Thin Layer Chromatography (TLC)

This is another version of liquid-liquid chromatography. A thin layer of silica gel (Kieselguhr) is spread on a glass plate; biological sample is applied as a small spot; the

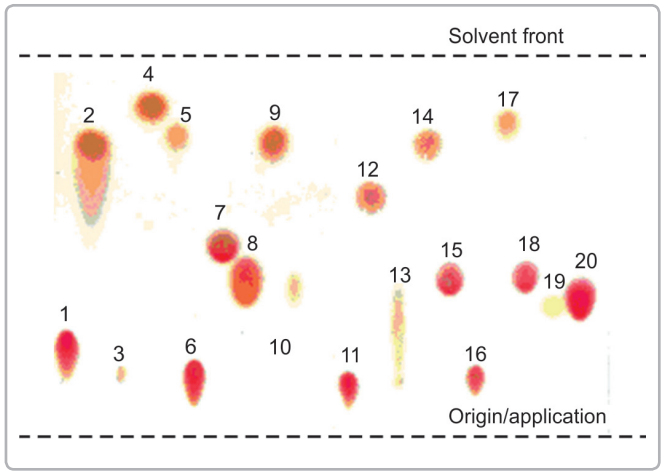


Figure 28.4: TLC separation of amino acids on Silica gel; 1. Arginine; 2. Methionine; 3. Cystine; 4. Leucine; 5. Tyrosine; 6. Lysine; 7. Alanine; 8. Glycine; 9. Phenylalanine; 10. Aspartic acid; 11. Ornithine; 12. Valine; 13. Cysteine; 14. Isoleucine; 15. Threonine; 16. Histidine; 17. Tryptophan; 18. Glutamic acid; 19. Proline; 20. Serine

plate is placed in a trough containing the solvent. The stationary water phase is held on the silica gel and mobile phase of nonpolar solvent moves up. In the case of paper chromatography, it takes 14–16 hours for components to be separated. But in the case of TLC it takes only 3–4 hours. That is a distinct advantage for TLC.

Visualization of Chromatography

After the chromatographic run is over, the paper or plate is dried and sprayed with ninhydrin for amino acids and proteins (Fig. 28.4).

Importance of Rf Value

The Rf value (Rf = ratio of fronts) is the ratio of the distance traveled by the substance (solute) to the distance traveled by the solvent. The Rf value is a constant for a particular solvent system at a given temperature.

ELISA TEST

ELISA is the abbreviation for **enzyme-linked immunosorbent assay**. The ELISA techniques are widely used not only for hormone measurements but also for detecting growth factors, tumor markers, bacterial or viral antigens, antibodies against microbes and any other antigens or antibodies in biological fluids. The disadvantages of RIA are not present in ELISA test.

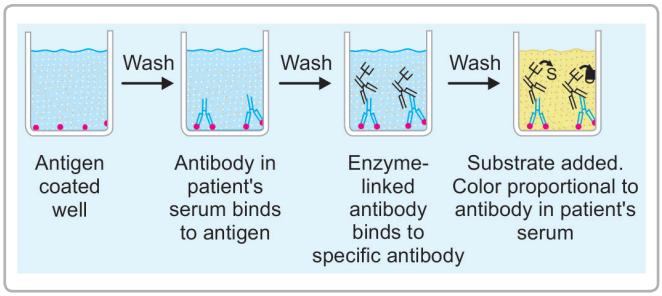


Figure 28.5: Indirect ELISA to detect antibody

Antibody Detection by ELISA

This is useful to detect small quantities of antibodies in the blood. A good example is the test for **detection of HIV antibody**. In patients with AIDS, the human immunodeficiency virus (HIV) produces specific antibody. To detect the HIV antibody, the following method is used.

Antigen from HIV is coated in the wells of a multiwell (microtiter) plate. Patient's serum is added, and incubated. If it contains the antibody, it is fixed.

Next a second antibody (antibody against human immunoglobulin) conjugated with HRP is added. Then colour reagent, containing hydrogen peroxide and diamino benzidine (as described below) is added. If a color develops, it means that the antibody was originally present in the patient's serum (Fig. 28.5). Here also the *color developed is proportional to the antibody concentration*. Therefore from the color intensity, the concentration of the antibody can be calculated.

Antigen Detection by ELISA Method

Specific antibody is fixed to the well of a microtiter plate. The patient's serum is added to the well, and incubated. By this time, if the serum contains the antigen, it is fixed on the antibody. Then, specific antibody tagged with horse radish peroxidase (HRP) is added. Then a color reagent, containing hydrogen peroxide (H_2O_2) and diamino-benzidine (DAB) are added. The reaction is as follows:

$$H_2O_2 \xrightarrow{HRP} H_2O + \text{Nascent Oxygen (O)}$$

$$\downarrow$$

$$\text{Diaminobenzidine (Colorless)} \longrightarrow \text{Oxidized DAB (Brown color)}$$

This is known as "sandwich" ELISA. Development of a brown color indicates that the antigen is originally present in the patient's serum. This is diagrammatically represented in Figure 28.6. *Color developed is proportional*

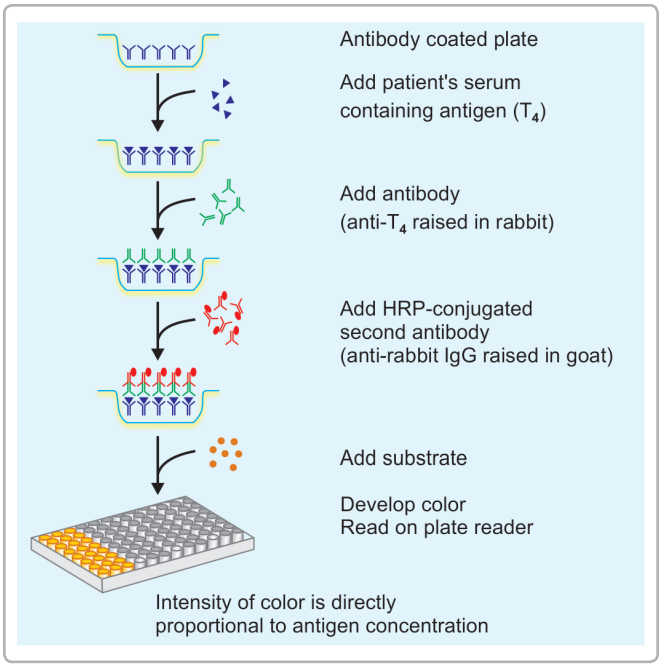


Figure 28.6: Sandwitch ELISA to detect antigen

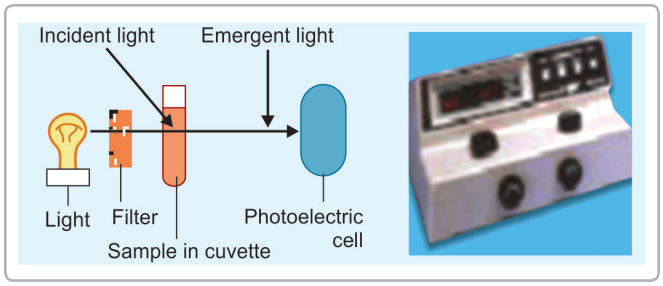


Figure 28.7: Photoelectric colorimeter

Table 28.1: Color of filter and colour of solution are complementary

Color of filter	*Wave length*	*Color of solution*
Violet	420	Brown
Blue	470	Yellowish brown
Green	520	Pink
Yellow	580	Purple
Red	680	Green/blue

to the antigen in the serum. Therefore intensity of the color may be measured, from which the concentration of the antigen is calculated.

COLORIMETER

Colored solutions have the property of absorbing light of definite wavelengths. The amount of light absorbed or transmitted by a colored solution is in accordance with the Beer-Lambert law. As per the **Beer's law**, the intensity of the color is directly proportional to the concentration of the colored particles in the solution. The **Lambert's law** states that the amount of light absorbed by a colored solution depends on the length of the column or the depth of the liquid through which light passes. The **Beer-Lambert law** combines these two laws.

In the colorimeter, the length of the column through which the light passed is kept constant, by using test tubes or cuvettes of the same diameter for both test and standard, so that the only variable is the concentration.

The ratio of intensity of emergent light to intensity of incident light (E/i) is termed as **transmittance** (T). The absorbance is expressed as –log T. The **Optical Density** is calculated as –log T. The plot of the concentration versus transmittance is not linear, but a graph of the concentration against absorbance (OD) is linear.

Most of the clinical chemistry estimations are done by colorimetric methods. A colored derivative of the compound to be measured is prepared and its absorbance or OD is measured using a photoelectric colorimeter. This value is compared with that of a **standard** of known concentration. The basic components of a **photoelectric colorimeter** are shown in Fig. 28.7. The color of filter should be complementary to the color of the solution. Table 28.1 gives the color of filters to be used for the colors of solutions.

In clinical laboratory, serum sample and reagents are mixed and incubated at 37°C for a fixed time, say 10 minutes, to develop the colour optimally. After the incubation period, the OD is ascertained and the concentration of the substances is calculated.

SPECTROPHOTOMETER

A spectrophotometer has all the basic components of photoelectric colorimeter with more sophistication. Wave lengths in the ultraviolet region are also utilized in the spectrophotometer. Light is separated into a continuous spectrum of wavelengths and passed through the solution. [In colorimeter, wavelengths of one color are grouped together.] **Wavelength selection** is achieved by diffraction gratings. The **advantage** of the spectrophotometer over the

colorimeter, is that the former is 1000 times more **sensitive**. Therefore even minute quantities of the substance (very dilute solution) can be assessed in the spectrophotometer.

A QUICK LOOK

1. Electrophoresis refers to the movement of charged particles when subjected to an electrical field.
2. Cellulose acetate electrophoresis is used for separation of lipoproteins and hemoglobins.
3. Agar electrophoresis is used to separate serum proteins.
4. Thin layer chromatography is widely used to separate and identify the amino acids.
5. In enzyme-linked immunosorbent assay (ELISA), horse radish peroxidase (HRP) is tagged to the enzyme.

CHAPTER 29

AIDS, Cancer

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Acquired immunodeficiency syndrome (AIDS)
- Human immunodeficiency virus (HIV)
- Immunology of AIDS
- Mutagens and carcinogens
- Oncogenic viruses
- Oncogenes and oncosuppressor genes
- Oncofetal antigens
- Anticancer drugs
- Tumor markers

AIDS AND HIV

About 36.7 million HIV seropositive individuals are living in the world, which include 1.8 million children. Awareness about the spread and introduction of antiretroviral therapy has brought down the infection rate markedly. Based on the clinical manifestations, the disease was named as **Acquired Immunodeficiency Syndrome** with acronym of AIDS. The isolation of a virus from the lymphocytes of the AIDS patients was done in 1984 independently by Robert Gallo at the National Institute of Health, USA and Montagnier at the Pasteur Institute, Paris. The new virus was named as HIV (Human Immunodeficiency Virus).

The Indian Scene

The first seropositive individuals in India were identified in 1986. By the end of 2005, five million cases have been reported in India. But by the end of 2015, the prevalence rate has decreased to 50%, especially in India.

Transmission

- About 80% of the total patients got the infection as a sexually transmitted disease
- In about 15% of patients, the disease was transmitted through blood. The drug abusers usually use the same needle without any sterilization for intravenous injection. The risk of getting HIV is high in patients who receive blood transfusion many times, e.g. hemophilia patients
- In the rest 5% cases, virus may be transmitted from mother to fetus through placenta. About 30% of infants born to HIV positive mothers may get the infection.

Natural Course of the Disease

1. Window Period

When the virus enters the body, it multiplies in the body cells, but cannot be detected easily. This is called the window period (Fig. 29.1). The viral capsid antigen p24 can be detected in the blood during this time.

2. Seropositive Stage

After a few months, antibodies are seen in circulation. This is called **seropositivity** (Fig. 29.1). During this period, the person is completely normal. However, this person is a **carrier** of the disease, and can transmit the disease to others. About 50% seropositive individuals will go for the 3rd stage of AIDS disease within 10 years. For each AIDS patient, there are 100 seropositive persons in the general population.

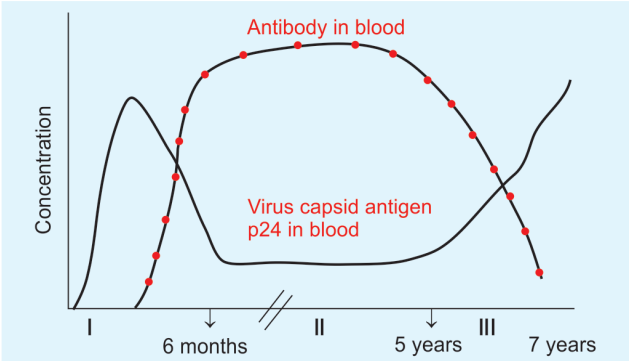


Figure 29.1: Course of HIV infection; I = window period; II = seropositive period; III = AIDS disease. Black line is antigen in blood; Red dots indicate antibody response

3. AIDS Disease

The third stage is when the clinical manifestations start**.** By this time, the immune status of the individual is depressed. Therefore, commensal microbes will start multiplication inside the body. Patient usually succumbs to death within about 2 years after entering this stage.

Pathology of HIV and AIDS

Structure of Virus

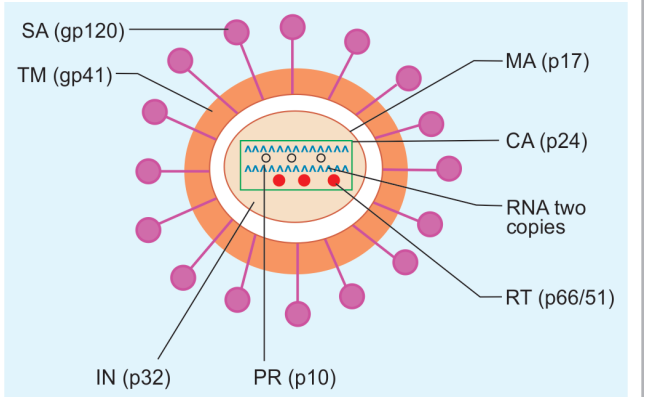
HIV belongs to the **retrovirus** group. They are RNA-containing viruses that replicate with the help of the reverse transcriptase (RT) or RNA dependent DNA polymerase. A schematic representation of the structure of the virus is shown in Figure 29.2. The virus contains two copies of single stranded genomic RNA. The core of the virus contains **reverse transcriptase**.

Virus Entry

The binding of HIV with target cell is through a receptor mechanism. The **gp 120** of the virus envelope will specifically bind with CD4 molecule on the surface of target cells. Thus **CD4 acts as a receptor** for the virus. The CD4 molecules are present on the surface of **T-helper cells** and therefore helper cells receive the maximum attack of HIV. Macrophages and monocytes are also susceptible to HIV entry and propagation. Macrophages act as the reservoir of the virus.

Replication of HIV

After entry, the viral RNA is acted upon by the **reverse transcriptase** (RT). This DNA double strand (proviral DNA) migrates into the nucleus of the host cell. The viral DNA is then integrated into the host cell DNA. The viral genes are transcribed and translated by the host cell mechanisms.



P = protein having molecular weight in kD, e.g. p17 = protein having molecular weight 17,000. gp = glycoprotein with molecular weight in kD, e.g. gp120 = glycoprotein with mol. wt. 120,000. RT = reverse transcriptase; IN = integrase: PR = protease. SA = surface antigen; TM = transmembrane protein; MA = membrane antigen; CA = capsid antigen

Figure 29.2: Structure of HIV

Immunology of AIDS

The basic defect lies in the immunodeficiency. This is because the CD4 (T-helper) lymphocytes are decreased in number. The gp120 surface unit could specifically attach with CD4 molecule present on the surface of T-helper cells. Therefore HIV preferentially enters into the T-helper cells and they are lysed. Since T-helper cells play a pivotal role in the immunological system, their deficiency will lead to suppression of almost all the immunological effectors. Antibody response against a foreign antigen is poor. When all the effector mechanisms of immunity are thus paralyzed, opportunistic pathogens get entry into the body.

Laboratory Diagnosis

The antibodies in the blood are detected by the ELISA test (For details refer Chapter 28). In this test, antibody against only one antigen (**gp 120**) is being tested; so there is probability of false positive results. So, Western blot analysis is done to confirm the ELISA positive cases. In **Western blot** analysis, antibodies against 6 different antigens of the virus are analyzed; so it is confirmatory.

T-helper count is lowered. The normal level is more than 400/cmm. In AIDS patients, the level is always below 300. As the disease progresses, the helper cell count is correspondingly lowered. Nucleic acid testing (NAT) for viral RNA is now taken as the confirmatory test. This has decreased the window period.

Anti-HIV Drugs

Reverse Transcriptase (RT) inhibitors; nucleoside analogues: Dideoxy nucleosides are nucleosides. They inhibit RT of the HIV.

RT inactivators: These agents bind to regions outside the active site of the HIV-RT, and make the enzyme inactive.

Protease inhibitors: They block final assembly and package of HIV particles. Most effective method is HAART(Highly active anti retroviral therapy) which has decreased mortality and morbidity as well as vertical transmission from the mother to child.

Prevention

Vaccines are in experimental stages. Since the major method of transmission is through sexual connection, avoidance of extramarital relationships will stop the spread. All the blood samples should be tested for the presence of HIV antibodies before transfusion. Syringes and needles either properly sterilized or disposable ones are to be used. Boiling for 10 minutes will inactivate the virus. Ordinary autoclaving at 120°C for 20 minutes is effective to sterilize instruments and gloves. Blood spills can be decontaminated by washing with 1% sodium hypochlorite solution, containing 10,000 ppm chlorine. Heat sensitive instruments may be decontaminated by immersing in 2% glutaraldehyde (cidex) for 3 hours.

CANCER

The term "cancer" is derived from Latin word "cancrum" or Greek "karkinoma", that is equivalent to Sanskrit term "karkitakam", which means "crab". The disease is so called because of swollen veins around the area, resemble a crab's limbs. Oncology deals with the etiology, diagnosis, treatment, prevention and research aspects of cancer.

Etiology of Cancer

All cancers are multifactorial in origin. They include genetic, hormonal, metabolic, physical, chemical and environmental factors. Most human cancers are spontaneous. Cancer is the second most common cause for death in developed countries, second only to cardiovascular diseases. When the average life expectancy is less, as in the case of India, the death due to cancer is also low.

Mutagens

Any substance which increases the rate of mutation can also enhance the rate of incidence of cancer. Therefore, all mutagens are carcinogens. Examples are X-ray, gamma-ray, ultraviolet ray. Some human cancers are caused by chemicals. These may be introduced into the body by means of (a) occupation (aniline, asbestos), (b) diet (aflatoxins) or (c) lifestyle (smoking). Chemical carcinogens act cumulatively. Tobacco, food additives, coloring agents and aflatoxins are common carcinogens in our environment.

Aflatoxins

They are a group of chemically related compounds synthesized by the fungi, *Aspergillus flavus*. The mold grows on rice, wheat and groundnut, when kept in damp conditions. The fungi may grow in cattle fodder, which may enter into human body through the cow's milk. Aflatoxins are powerful carcinogens, which produce hepatomas.

Cigarette

Lung cancer is associated with the habit of cigarette smoking. Cigarette contains many carcinogens, the most important group being benzopyrenes. Other important deleterious substances in cigarette smoke are nicotine, carbon monoxide, nitrogen dioxide and carbon soot. The incidence of lung cancer is increased to 15 times more in persons smoking 10 cigarettes per day and 40 times more in those smoking 20 cigarettes per day. Thus, WHO suggested the slogan 'cigarette smoke is injurious to health'. Moreover, non-smoking spouse of a heavy smoker will have 5 times more probability to get lung cancer than a non-smoker. This effect of **'passive smoking'** made the International Union against Cancer (UICC) to change the slogan to 'Your smoking is injurious to our health'. **Oral cancer** is strongly associated with chewing of tobacco. Oral cancer constitutes 20% of all cancers seen in India, whereas it is less than 1% in Western countries.

Antimutagens

These are substances which will interfere with tumor promotion. **Vitamin A** and carotenoids are shown to

reverse precancerous conditions. **Vitamin E** acts as an antioxidant, preventing the damage made by free radicals and superoxides. **Vitamin C** regularly given to such persons working with aniline prevented the production of new cancer cases. Tubers, beans and leafy **vegetables** are shown to intercept tumor promotion.

ONCOGENIC VIRUSES

Another etiological factor of carcinogenesis is the integration of viral genes into the host DNA. Thus, the virus genes become part and parcel of the cellular DNA. The drive for multiplication by the virus genome overrules the regulatory checks and balances of the cellular mechanism. So, there is uncontrolled multiplication of the cells. This is called **transformation** by oncogenic virus.

Rous in 1911 demonstrated that sarcoma in avians can be transferred from one animal to another by injecting the soluble fractions. In 1944, Gross finally proved that viruses could be oncogenic. Rous was awarded Nobel Prize in 1966.

A list of important oncogenic viruses in animals is shown in Table 29.1. The list is only representative and is far from exhaustive. A list of possible oncogenic viruses in man is given in Table 29.2.

Burkitt in 1964 reported a type of lymphoma seen mainly in African children. Epidemiology suggested a strong possibility for a transmitting agent for the **Burkitt's lymphoma** (BL). Later, it was proved that the disease is caused by a virus named **Epstein-Barr (EB) virus**. After the viral infection, the activation of **c-myc oncogene**, leads to malignancy.

Table 29.1: Viruses producing tumors in animals

Virus	*Structure*	*Host*	*Type of tumor produced*
Papovavirus group			
SV-40	DNA	Mouse	Sarcoma
Papilloma	DNA	Rabbit	Papilloma
Marek	DNA	Chicken	Lymphoma
Retrovirus type C			
Gross	RNA	Mouse	Leukemia
Rous	RNA	Avian	Sarcoma

Table 29.2: Human oncogenic viruses

Virus	*Abbreviation*	*Associated human cancer*
Epstein-Barr virus	EBV	Burkitt's lymphoma (BL); Nasopharyngeal carcinoma (NPC)
Human papilloma virus	HPV	Uterine cervical carcinoma
Hepatitis B virus	HBV	Hepatoma

ONCOGENES

Oncogenes are Normal Constituents of Cells

These are genes capable of causing cancer (Box 29.1). A definite proof for an oncogene was first demonstrated in Rous sarcoma virus. The full virus produces sarcoma in avians but a strain of virus deficient in a particular gene, could not cause the disease. Hence this gene was named as sarcoma gene, abbreviated as src. However, the same DNA sequences are available in normal avian cells also. This reveals that normal cells do contain DNA sequences similar to viral oncogenes. The oncogenes present in normal cells are also called as **proto-oncogenes**. Proto-oncogenes are important regulatory genes of the cells. In fact, viruses carry these genes accidentally picking them from the host cells.

Many Factors Activate Oncogenes

The oncogenes also provide an explanation for the multifactorial origin of cancer. Thus viruses, chemical carcinogens, chromosome translocations, gamma-rays, spontaneous mutations, and all such other factors may converge into one biochemical abnormality, the activation of oncogenes. This then leads to malignancy. This unified theory is depicted in Figure 29.3.

Antioncogenes or Oncosuppressor Genes

These are the genes, which normally protect the individual from getting the cancer. When the gene is deleted or mutated, cancer results. Antioncogenes are written with capital letters, whereas oncogenes with small letters.

RB gene (retinoblastoma gene)**:** Only when both alleles of the RB gene are deleted (homozygous), retinoblastoma results. The protein produced from this gene is called p105;

BOX 29.1: Oncogens and oncogenes are different

Oncogen is the chemical which produces cancer
Oncogens are the chemicals that produce cancer
Oncogene is the gene causing cancer
Oncogenes are the genes causing cancer

it suppresses cell proliferation, and prevents the activity of various oncogenes.

The p53: A part of short arm of chromosome 17 is deleted in various human cancers. This region is now known to contain an oncosuppressor gene, called **p53**. It is so called because the gene encodes a protein with molecular weight 53,000 Daltons. It blocks cell division until the damage is repaired. Most tumors have a complete absence of p53, whereas others show mutant nonfunctional p53.

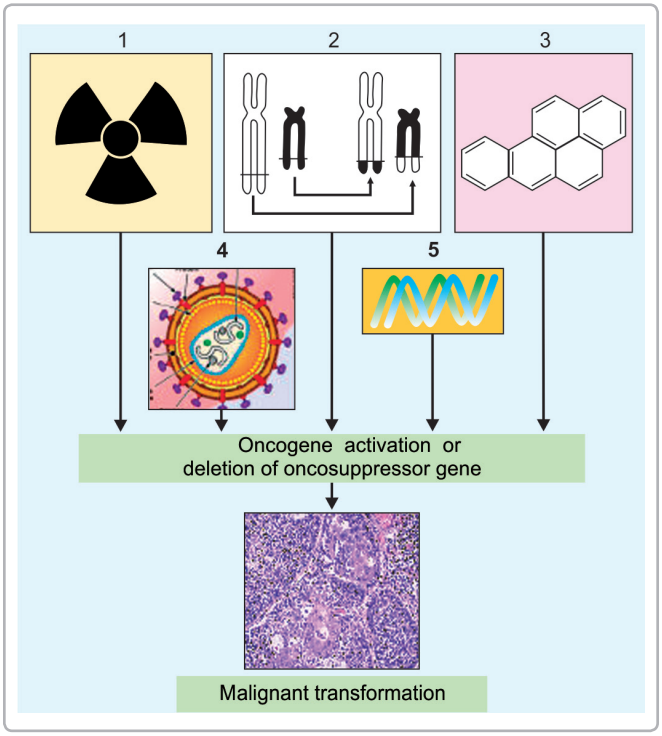


Figure 29.3: Unified concept of carcinogenesis

Oncofetal Antigens

Most of the human cancers show the emergence of *oncofetal antigens* (Fig. 29.4). During the fetal life, a particular gene is active, and the product, a protein is therefore seen in the cell. During the differentiation process, this gene is suppressed and therefore the protein is not seen in adult cells. However, along with the malignant transformation, de-differentiation occurs, the gene is derepressed and the protein is again available in the cell. Such products are classified as oncofetal proteins. The best examples are the appearance of alpha-fetoprotein (AFP) in hepatomas and carcinoembryonic antigen (CEA) in colon cancers. They generally serve as tumor markers.

TUMOR MARKERS

They are also called as **tumor index substances**. They are factors released from the tumor cells, which could be detected in blood and therefore indicate the presence of the tumor in the body. They are useful for the following purposes:

- For follow-up of cancer and to **monitor** the effectiveness of the therapy and also to detect the recurrence of the tumor.
- To facilitate detection of cancer. The presence of tumor marker suggests the **diagnosis**, but caution is to be taken to rule out other nonmalignant conditions
- For **prognosis,** serum level of the marker may indicate roughly the tumor load, which in turn indicates whether the disease is curable or not.

Clinically Important Tumor Markers

- **AFP (alpha-fetoprotein)**. Its molecular weight is 70,000 Daltons (Table 29.3). It is fetal albumin and has

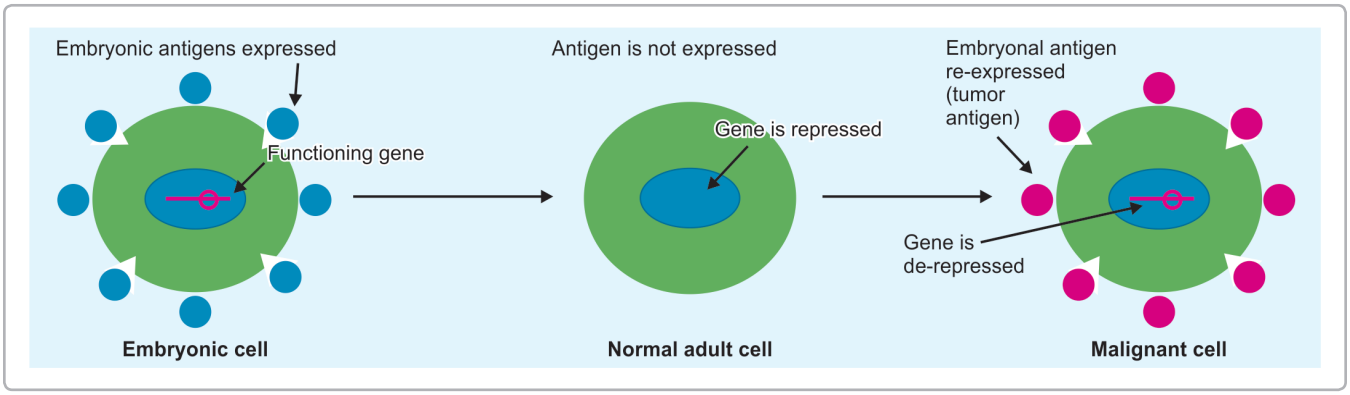


Figure 29.4: Oncofetal antigen

Table 29.3: Tumor markers	
Name	*Serum level increased in*
Oncofetal products	
Alpha fetoprotein (AFP)	Hepatoma, germ cells cancer
Carcinoembryonic antigen (CEA)	Colorectal, gastrointestinal and lung cancer
Enzymes	
Alkaline phosphatase (ALP)	Bone secondaries
Placental ALP (Regan)	Lung, seminoma
Prostate specific antigen (PSA)	Prostate cancer
Hormones and their metabolites	
Beta-HCG	Choriocarcinoma
Vanillylmandelic acid (VMA)	Pheochromocytoma and neuroblastoma
Hydroxyindoleacetic acid	Carcinoid syndrome
Serum proteins	
Immunoglobulins (Ig)	Multiple myeloma, macroglobulinemia
Bence-Jones proteins (in urine)	Multiple myeloma

similarities with adult albumin. It is increased in the circulation of patients with hepatocellular carcinoma, and in pregnancy with fetal malformations of neural tube

- **Carcinoembryonic antigen (CEA)** level is markedly increased in colorectal cancers
- Beta chain of chorionic gonadotropin (**beta-hCG**) is synthesized by normal syncytiotrophoblasts (cells of placental villi). The hCG has alpha and beta subunits. The alpha subunit is identical with those of FSH, TSH and LH. The beta subunit is specific for hCG. It is increased in choriocarcinoma and germ cell tumours. Paraproteinemias and multiple myeloma are described in Chapter 12. Oncofetal proteins and tumour markers are listed in Table 29.3.

A QUICK LOOK

1. Upon entry of the human immunodeficiency virus (HIV), it multiplies within the cells and cannot be detected easily in this period. This is called "window period".
2. Seropositivity indicates presence of antibodies against the viral antigen.
3. This may also be regarded as the carrier state of the disease.
4. Laboratory investigation for the presence of HIV are ELISA to detect presence of *gp120,* Western blot to detect the presence of six components of the virus. RTPCR is used to estimate viral load.
5. The CD4 present on the T helper cells acts as a receptor for the virus.
6. Hypervariability of *gp120* makes it difficult to develop a vaccine against HIV.
7. All cancers are multifactorial in origin. They include genetic, hormonal, metabolic, physical, chemical and environmental factors. Examples of physical carcinogens are X-ray, gamma-ray, ultraviolet ray. Examples of chemical mutagens are aflatoxins, methylcholanthrene, nicotine.
8. Examples of antimutagens include vitamin A, vitamin E, vitamin C and curcumin.
9. Example of viruses producing tumors in animals are polyoma, SV 40, gross, rous, etc.
10. Viruses possibly oncogenic in man are EBV, HPV and hepatitis B.
11. Genes capable of causing cancer are termed oncogenes, e.g. myc, src, ras, abl, erb-B, etc.
12. Genes which normally protect the individual from getting a cancer are called antioncogenes or oncosuppressors, e.g. ***p53***, ***pRB***.
13. Examples of oncofetal antigens are alpha-fetoprotein (AFP) in hepatomas and carcinoembryonic antigen (CEA) in colon cancers.
14. Tumor markers are useful for diagnosis and follow-up of cancer chemotherapy, e.g. CA-125, VMA, placental ALP, prostate specific antigen.

CHAPTER 30

Liver Function Test and Renal Function Test

Chapter at a Glance

The reader will be able to answer questions on the following topics:

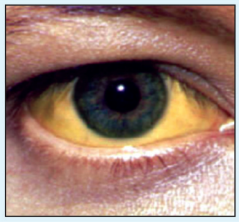
- Serum and urine bilirubin
- Hepatic marker enzymes
- Kidney glomerular functions
- Tubular functions
- Abnormal constituents of urine
- Clearance tests: Inulin, creatinine and urea
- Proteinuria
- Gastric function test

Important liver functions are listed in Table 30.1. Often abnormal liver function will lead to jaundice (Fig. 30.1). Clinically useful liver function tests are broadly classified as:

- Tests to detect hepatic injury:
  - To identify the disease is mild or severe; whether it is acute or chronic.
  - To assess the nature of liver disease, liver cell damage (hepatocellular) or cholestasis (obstructive)
- Tests to assess synthetic functions of liver.

Table 30.1: Major functions of liver

1. Blood glucose regulation
2. Synthesis of carbohydrates
3. Synthesis of fats
4. Synthesis of proteins
5. Detoxification
6. Bile production; help in digestion
7. Heme catabolism and excretion of bilirubin



Yellow color of sclera is seen in jaundice. Normal serum bilirubin value is 1 mg/dL. When it exceeds 2 mg/dL, bilirubin deposits in tissues

Fig. 30.1: Jaundice

Markers of Hepatic Dysfunction

1. Measurement of Bilirubin (Test of Excretory Function of Liver)

Bilirubin is the excretory product formed by the catabolism of heme. It is conjugated by the liver to form bilirubin diglucuronide and excreted through bile (For details refer Chapter 14). Measurement of bilirubin, (in serum and urine) and urobilinogen (in urine) are important tests of liver function.

2. Serum Bilirubin

Normal serum bilirubin level varies from **0.2 to 0.8 mg/dL.** The unconjugated bilirubin (bilirubin-albumin complex) varies from 0.2–0.6 mg/dL and conjugated bilirubin 0–0.2 mg/dL. A rise in serum bilirubin above 1 mg/dL is abnormal (latent jaundice); but jaundice appears only if the level goes above 2 mg/dL. The bilirubin is estimated by **van den Bergh reaction**. Normal serum does not give a positive van den Bergh reaction. When bilirubin is **conjugated**, the purple color is produced immediately on mixing with the

Table 30.2: Urinary findings in jaundice

Type of jaundice	*Bile pigment*	*Bile salt*	*Urobilinogen*
Prehepatic (hemolytic)	Nil	Nil	++
Hepatocellular	++	+	Normal or ˉ
Posthepatic (obstructive)	+++	++	Nil or ˉ

Table 30.3: Classification of jaundice

Type of bilirubin	*Class of jaundice*	*Causes*
Unconjugated	Prehepatic or hemolytic	Abnormal red cells; antibodies; drugs and toxins; thalassemia; hemoglobinopathies
Unconjugated and conjugated	Hepatic or hepatocellular	Viral hepatitis; toxic hepatitis; intrahepatic cholestasis.
Conjugated	Post-hepatic or obstructive	Gallstones; tumors of bile duct or pancreas

reagent, the response is said to be van den Bergh **direct positive.** When the bilirubin is **unconjugated**, the color is obtained only when alcohol is added, and this response is known as **indirect positive**.

If both conjugated and unconjugated bilirubin are present in increased amounts, a purple color is produced immediately and the color is intensified on adding alcohol. Then the reaction is called **biphasic**. 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 **hepatocellular** jaundice, a biphasic reaction is observed, because both conjugated and unconjugated bilirubins are increased (Table 30.2).

3. Urinary Bilirubin

In all cases of jaundice, urine should be examined for the presence of bile pigments (bilirubin), bile salts and urobilinogen. Only conjugated bilirubin is soluble in water and is excreted in urine. Hence in prehepatic jaundice, when the unconjugated bilirubin is increased in blood, it does not appear in urine; hence called **acholuric jaundice**. But in obstructive jaundice, conjugation of bilirubin is taking place, which could not be excreted through the normal passage, and so it is regurgitated back into blood stream; this is then excreted through urine. So in obstructive jaundice, urine contains bilirubin; hence in old literature, it is called **choluric** jaundice.

4. Urinary Urobilinogen

In cases of obstruction, bile is not reaching the intestine and so urobilinogen may be decreased or absent in urine. Urobilinogen is absent in urine, when there is obstruction to bile flow. The **first indication of the recovery is the reappearance of urobilinogen** in urine. In urine, bilirubin is detected by Fouchet's test and urobilinogen by Ehrlich's test. The findings in urine in different types of jaundice are shown in Table 30.2. Table 30.3 shows the classification and causes for jaundice. Bilirubin synthesis, excretion and jaundice are described in detail in Chapter 14.

Causes of Jaundice

The most common cause for hepatocellular jaundice is the infection with hepatitis viruses (viral hepatitis). It may be due to **hepatitis A virus** (HAV), which is transmitted by the intake of contaminated food and water. Type A disease is usually self-limited. Infection by **hepatitis B virus** (HBV) is transmitted mainly through serum contamination. The virus is highly contagious and can be destroyed only by boiling for 20 minutes. It is a DNA virus, which destroys the hepatic cells. The **surface antigen** (HBs) (also called Australia antigen, because it was first demonstrated in an Australian) is seen in the circulation of patients.

About 5% of world population is the carriers of HBV. In most cases of hepatitis B infection, complete recovery is possible. But in about 1% cases, patients go into hepatic coma and eventual death. In another 2–5% cases, the disease goes to a chronic carrier state. About 1% cases go for chronic **cirrhosis** and eventual hepatic failure. In fact, the most common cause for cirrhosis in developing countries is the hepatitis virus.

In a small fraction of such cases, development of hepatocellular carcinoma is also noticed. Thus, HBV is an **oncogenic virus**.

Medical personnel, including dental practitioners should take the hepatitis B vaccination. Transmission of the virus through faulty sterilization of medical and dental equipment has been reported.

Tests Based on Synthetic Function of Liver

1. Serum Albumin level

Almost all the plasma proteins except immunoglobulins are synthesized by the liver. Serum **albumin** (For details refer Chapter 12) is quantitatively the most important protein synthesized by the liver, and reflects the extent

of functioning liver cell mass. A reversal in A/G ratio is often the rule in cirrhosis, due to hypoalbuminemia and associated hyper gammaglobulinemia (For details refer Chapter 12).

Normal albumin level in blood is 3.5 to 5 g/dL; and globulin level is 2.5 to 3.5 g /dL.

2. Prothrombin Time (PT)

Since prothrombin is synthesized by the liver, it is a useful indicator of liver function. The half-life of prothrombin is 6 hours only; therefore PT indicates the present function of the liver. PT is prolonged only when liver loses more than 80% of its reserve capacity.

3. Alpha-Fetoprotein (AFP)

It is a tumour marker (For details refer Chapter 29). Its level in blood is markedly increased in hepatocellular carcinoma. In chronic hepatitis and in cirrhosis, there may be mild elevation.

Tests Based on Serum Enzymes (Liver Enzyme Panel)

1. Enzyme Indicating Hepatocellular Damage

Normal serum ALT (alanine aminotransferase) is 10–35 IU/L. The level of **aminotransferases** (ALT and AST) in serum are elevated in all liver diseases. **Very high levels** (more than 1000 units) are seen in acute hepatitis (viral and toxic drugs) and in ischemia due to cardiac failure. **Moderate elevation** of aminotransferases often between 100–300 U/L are seen in **alcoholic** and nonalcoholic chronic hepatitis. M**inor elevation** less than 100 U/L is seen in chronic viral hepatitis.

2. Markers of Obstructive Liver Disease

Alkaline Phosphatase (ALP)

Very high levels of alkaline phosphatase (ALP) are noticed in patients with cholestasis or hepatic carcinoma. (Table 30.4). In parenchymal diseases of the liver, mild elevation of ALP is noticed (Table 30.5). ALP is also increased in bone diseases.

Gamma-Glutamyl Transferase (GGT)

Elevated levels of GGT are observed in chronic alcoholism. In liver diseases, GGT elevation parallels that of ALP and is very sensitive of biliary tract disease.

Table 30.5: Functions of kideny at a glance

1. Excretion of urea and other wastes
2. Keeping water balance
3. Excretion of sodium (effect on BP)
4. Excretion of potassium (effect on heart)
5. Excretion of hydrogen ions (maintenance of pH)
6. Activation of vitamin D (effect on bone)
7. Production of erythropoietin (effect on RBCs)
8. Filtration: 180 liters/day of water with all sodium, chloride, sugar and amino acids
9. Reabsorption: 178.5 liters reabsorbed; all glucose and amino acids reabsorbed; most of sodium and chloride reabsorbed
10. Secretion: Acids, bases, toxins, drug metabolites, urea, creatinine

Table 30.4: Tests useful to distinguish different types of jaundice

Specimen	*Test*	*Prehepatic or hemolytic or retention jaundice*	*Hepatocellular jaundice*	*Posthepatic or obstructive or regurgitation jaundice*
Blood	Unconjugated bilirubin (van den Bergh indirect test)	++	++	Normal
Blood	Conjugated bilirubin (van den Bergh direct test)	Normal	Excretion is rate-limiting. It is the first impaired activity. In early phase, it is increased	++
Blood	Alkaline phosphatase (40–125 U/L)	Normal	2–3 times increased	10–12 times
Urine	Bile salt (Hay's test)	Absent	Absent	Present
Urine	Conjugated bilirubin (Fouchet's)	Absent	Present	Present
Urine	Urobilinogens (Ehrlich test)	+++	Earliest manifestation of recovery is presence of bilinogen in urine	Absent
Feces	Urobilins	++	Normal or decreased	Clay colored

RENAL FUNCTION TESTS

The functions of kidney are shown in Table 30.5. Changes seen in kidney functions during diseases are shown in Table 30.6. The classification of renal functional tests are shown in Table 30.7.

Table 30.6: Summary of renal function tests

Glomerular dysfunction	*Tubular dysfunction*
Blood urea ↑	Urine concentration ↓
Blood creatinine ↑	Dilution test abnormal
Inulin clearance ↓	Uric acid excretion ↓
Creatinine clearance ↓	Blood uric acid ↑
Urea clearance ↓	
PAH clearance ↓	Acidification of urine ↓
Proteinuria present	Amino aciduria present
Urine volume ↓	Urine volume ↑
Specific gravity ↑	Specific gravity ↓
Blood phosphate ↑	Blood phosphate ↓

Table 30.7: Classification of renal function tests

I. On the basis of functions:
   a. Those measuring glomerular filtration rate
   b. Those study tubular function
II. On the basis of the clinical applications:
   1. Routine clinical tests:
      a. Complete urinalysis
      b. Measurement of NPN in blood
      c. Measurement of serum electrolytes
   2. Markers of glomerular filtration rate:
      Clearance tests
   3. Markers of glomerular permeability:
      Proteinuria
   4. Markers of tubular function:
      a. Urinary and plasma osmolality
      b. Concentration and dilution tests
      c. Tests to assess renal acidification

Glomerular Function

When the blood is perfused through the Bowman's capsule, an ultrafiltrate of the blood is produced in glomerulus, while the cells and proteins are retained in the blood. The sieves of the glomeruli are such that hemoglobin (mol. wt. 67,000) is passed through to the urine, while albumin (mol. wt. 69,000) is retained in the blood. Therefore the earliest manifestation in the abnormality of the glomeruli is the **appearance of albumin** in urine.

Glomerular Filtration Rate (GFR)

GFR is decreased when BP is below 80 mm of mercury. The GFR is reduced when there is obstruction in the renal flow (calculi, enlarged prostate, etc.). It also decreases with age. The glomerular filtration rate (GFR) is 120–125 mL per minute in a person with 70 kg body weight. Glomerular filtrate formed is about 170 to 175 liters per day, out of which only 1.5 liters are excreted through urine. This means that most of the water content of glomerular filtrate is reabsorbed.

Functions of the Tubules

When the glomerular filtrate is formed, it contains almost all the crystalloids of plasma. In the proximal convoluted tubules, about 70% water, Na^+ and Cl^- as well as 100% glucose, amino acids and K^+ are reabsorbed. Some amount of urea, phosphate and calcium are partially absorbed. (Table 30.8). The major processes occurring in renal tubules are the reabsorption or secretion of solutes and reabsorption of water.

Renal Threshold

Compounds whose excretion in urine are dependent on blood level are known as **threshold substances**. At normal or low plasma levels, they are completely reabsorbed and

Table 30.8. Handling of solutes by the renal tubules (GF = Glomerular filtrate; PCT = proximal convoluted tubules; DCT = distal convoluted tubules)

Compound	*Mode of handling by tubules*	*Relative concentration*
Creatinine	Neither reabsorbed nor secreted	GF = Urine
Uric acid	90% is first absorbed in PCT; but later secreted in DCT	GF approx. = Urine
Urea	About 40% reabsorbed in PCT	GF > Urine
Sodium	Partially reabsorbed	GF > Urine
Glucose	Completely reabsorbed	GF >> Urine
Amino acid	Completely reabsorbed	GF >> Urine

are not excreted in urine. But when the blood level is elevated, the tubular reabsorptive capacity is saturated, so that the excess will be excreted in urine. The renal threshold of a substance is the plasma level above which the compound is excreted in urine. For glucose, the renal threshold is 180 mg/dL In other words, glucose starts to appear in urine when blood level is more than 180 mg/dL. In abnormal conditions, the renal threshold may be lowered so that even at lower blood levels, compounds are excreted in urine, e.g. renal glycosuria (glucose). Renal threshold for calcium is 10 mg/dL.

Reabsorption of Water

The glomerular filtration rate (GFR) is about 125 mL/minute. In the proximal convoluted tubules, most of this is reabsorbed. Since Na^+, Cl^- and HCO_3^- ions are absorbed, water has to move along with the solutes to maintain the osmolality. Hence, this is called **obligatory** reabsorption of water. Here sodium is again reabsorbed, but water absorption is less so that, urine is hypotonic at this level. Here again water is reabsorbed, but it is under the influence of ADH. Therefore, this is called **facultative** reabsorption of water.

ADH secretion, in turn, is controlled by hypothalamic osmoreceptors. The osmolality of plasma is the stimulus for modulating ADH secretion. Thus, when urine reaches the collecting ducts, the flow rate is only about 1 mL/min, and the urine is always hypertonic. **Osmotic diuretics** act by interfering with reabsorption of solute so that more water is obligatorily excreted along with the solute. Osmotic diuretics mainly act at the proximal convoluted tubules, e.g. **mannitol**. **Frusemide** acts on the ascending limb of loop of Henle, inhibiting chloride reabsorption along with Na^+ and water. So, chances of K^+ depletion are present.

NONPROTEIN NITROGEN (NPN)

These include urea, creatinine and uric acid. The major route of excretion of these compounds is urine. In kidney dysfunction, the levels of these compounds are elevated in plasma. Of the three, **creatinine estimation is the most specific** and sensitive index of renal function. Normal blood and urine levels of these parameters are shown in Table 30.9.

MARKERS OF GFR

Clearance Tests

Measurement of the clearance is predominantly a test of glomerular filtration rate (GFR) (Fig. 30.2). Measurement of glomerular filtration rate (GFR) provides the most useful general index for the assessment of the severity of renal damage. A decrease in the renal function is due to the loss of functional nephrons, rather than a decrease in the function of individual nephron. The relation between clearance value and GFR is shown in Table 30.10 and Fig. 30.2. GFR cannot be measured directly, it is estimated from the clearance of a filtration marker.

A substantial kidney damage occurs before GFR is decreased. Normal GFR for young adults is 120–130 mL/mt/1.73M^2. GFR is constant in a normal individual, but may vary among people with normal kidney function. A decline with age is significant and more than 25% of people older than 70 years may have a GFR less than 60 mL/mt.

Definition

- *Clearance is defined as the quantity of blood or plasma completely cleared of a substance per unit time and is expressed as milliliter per minute*

Table 30.9: Very commonly employed tests to assess kidney functions

Constituent	*Normal blood level*	*Urinary excretion*	*Factors affecting urinary excretion*
Urea	15–40 mg/dL	15–30 g/day	Dietary proteins, rate of protein catabolism, renal blood flow, ECF volume
Creatinine	0.7–1.4 mg/dL (Males) 0.4–1.3 mg/dL (Females)	1–2 g/day	GFR, tubular secretion, age, sex, muscle mass
Uric acid	3–7 mg/dL (Males) 2–5 mg/dL (Females)	0.5–0.8 g/day	Rate of purine catabolism, rate of tubular excretion
Sodium	135–142 mmol/L		State of hydration, dietary sodium, renal function
Potassium	3.5–5.2 mmol/L		Dietary potassium, acid-base balance, renal function
Calcium	9–11 mg /dL		Dietary calcium, PTH, calcitonin, renal function

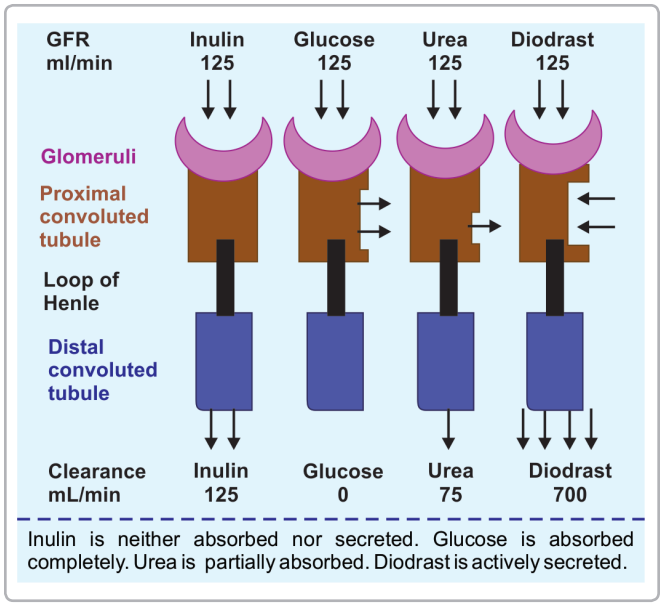


Figure 30.2: Tubules handle substances differently

- It is the **mL of plasma** which contains the amount of that substance excreted by the kidney within a minute
- It estimates the amount of plasma that must have passed through the glomeruli per minute with complete removal of that substance to account for the substance actually appearing in the urine.

$$\text{Clearance} = \frac{\text{mg of substance excreted per minute}}{\text{mg of substance per mL of plasma}}$$

It is calculated by using the formula:

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

where U = concentration of the substance in urine; P = concentration of the substance in plasma or serum and V = the mL of urine excreted per minute. The value is expressed as mL/minute.

If the substance is freely filtered across the capillary wall, and neither secreted nor reabsorbed, then its clearance is equal to glomerular filtration rate. A substance which meets these requirements is an ideal filtration marker. If the substance is also secreted by the tubules, the clearance exceeds GFR. For those which are reabsorbed by tubules, clearance is less than GFR (Fig. 30.2).

Endogenous markers are urea and creatinine. None of these markers are ideal, but creatinine is the best out of all of them.

Creatinine Clearance Test

Importance of Creatinine Clearance

- Creatinine is a waste product, formed from creatine phosphate (For details refer Chapter 11). This conversion is a **spontaneous, non-enzymatic,** and is dependent on total muscle mass of the body. It is not affected by diet, age or exercise
- Since the production is continuous, the blood level **will not fluctuate** much, making creatinine an ideal substance for clearance test.

Normal Serum Creatinine Level

Adult males, 0.7–1.4 mg/dL
Adult females, 0.6–1.3 mg/dL
Children, 0.4–1.2 mg/dL.

The kidney reserve is such that about 50% kidney function must be lost before creatinine level in blood is raised. *Creatinine level more than 1.5 mg/dL indicates impairment of renal function.*

Creatinine Clearance Test

Creatinine clearance = (U/P) × V
where U is the urine creatinine concentration, P is the plasma creatinine concentration and V is the urine flow in mL/min (The 24 hour urine collection is not necessary for the creatinine clearance test).

Reference Values for Creatinine Clearance

- Males 85–125 mL/min.
- Females 80–115 mL/min.

Table 30.10: Relationship of GFR with clearance

Mechanism	*Result*	*Example*
Substances filtered; neither reabsorbed nor excreted	GFR = clearance	Inulin
Substance filtered; reabsorbed and excreted	GFR ≅ clearance	Uric acid
Substances filtered; partially reabsorbed	Clearance < GFR	Urea, creatinine
Substances filtered; secreted but not reabsorbed	Clearance > GFR	Diodrast, PAH

- When corrected for surface area, the creatinine clearance value will become the same for males, females and children, which is about 100 mL/min./1.73 sqm.

Interpretation of Creatinine Clearance

A decreased creatinine clearance is a very sensitive indicator of a reduced **glomerular filtration rate**. The importance of creatinine clearance is in the **early detection** of functional impairment of kidney without overt signs and symptoms.

Urea Clearance Test

Since the factors influencing the production and excretion of urea by the kidney are influenced by widely varying factors, neither urea clearance nor blood urea are used today as an index of kidney function. Urea forms 80% of total urinary solutes. Urine is roughly a 2% solution of urea.

Urea clearance is the number of mL of blood which contains the urea excreted in a minute by the kidneys. Urea is freely filtered by the glomerulus, but about 40% is reabsorbed actively by the tubules. If the urea clearance value is below 75% of the normal, it is considered to be **abnormal**. The values fall progressively with increasing renal failure.

Blood Urea Level

Urea is the end-product of protein metabolism (For details refer Chapter 11). Normal value is 20 to 40 mg/dL. The serum concentration of urea generally increases as the **age** advances.

A. Prerenal conditions

Dehydration, which may occur in severe vomiting, intestinal obstruction, severe diarrhea, etc. may lead to very high values for serum urea.

B. Renal diseases

Serum urea is increased in all forms of kidney diseases. In **acute glomerulonephritis** values may be as high as 300 mg/dL. In early stages of **nephrosis**, serum urea may be normal, but in late stages serum urea increases along with the increasing renal failure. In malignant **hypertension** and in chronic **pyelonephritis**, the values may reach very high levels.

C. Postrenal causes

When urine flow is obstructed, the glomerular filtration rate is decreased, with corresponding increase in serum urea.

- **Stones** in the urinary tract
- Stricture of urethra
- Enlarged **prostate**
- Tumors of bladder are examples.

MARKERS OF GLOMERULAR PERMEABILITY

Glomerular proteinuria: The glomeruli of kidney are not permeable to substances with molecular weight more than 69,000 and so plasma proteins are absent in normal urine. When glomeruli are damaged or diseased, they become more permeable and plasma proteins may appear in urine. The smaller molecules of albumin pass through damaged glomeruli more readily than the heavier globulins. Albuminuria is always pathological. Large quantities (a few grams per day) of albumin are lost in urine in nephrosis. Small quantities are seen in urine in acute nephritis.

Overflow proteinuria: When small molecular weight proteins are increased in blood, they overflow into urine. For example, hemoglobin having a molecular weight of 67,000 can pass through normal glomeruli, and therefore, if it exists in free form (as in hemolytic conditions), hemoglobin can appear in urine (**hemoglobinuria**).

Yet another example is the **Bence-Jones Proteins** (monoclonal light chains produced by plasmacytomas) (For detail refer Chapter 12).

Microalbuminuria or minimal albuminuria or pauci-albuminuria is identified, when small quantities of albumin (50–300 mg/day) is seen in urine. It is an early indication of nephropathy in patients with diabetes mellitus and hypertension. It is an indicator of future renal damage.

TESTS FOR TUBULAR FUNCTION

Specific Gravity of Urine

The simplest test of tubular function is the measurement of the specific gravity (SG) of urine.

Specific gravity depends on the concentration of solutes, whereas osmolality depends on the number of osmotically active particles.

Concentration Test

In moderate forms of kidney damage, the inability to excrete the waste products may be counterbalanced by large urine output. Thus, the **earliest manifestation** of renal disease may be the difficulty in concentrating the

urine. The patient is allowed no food or water for 12 hours. Then, the specific gravity may be as high as 1.032.

As the disease progresses and tubular concentrating capacity is lost, the specific gravity becomes fixed, at 1.010 (300 mosmols/Kg). It is then called **isosthenuria**.

The measurement of the volume of urine excreted during the day and the night is another simple index of tubular function. Normally night volume is only half of the day volume. But an increased excretion of urine at night or **nocturia** is an early indication of tubular dysfunction.

Dilution Tests

Bladder is emptied at 7 AM and a water load is given (1200 mL over the next 30 minutes). Hourly urine samples are collected for the next 4 hours separately. Volume, specific gravity and osmolality of each sample are measured. A normal person will excrete almost all the water load within 4 hours and the specific gravity of at least one sample should fall to 1.003. The test is more sensitive and less harmful than concentration test.

Urinary Acidification

The **most useful test** is acid loading test. Give ammonium chloride (NH_4Cl), which is dissociated into $NH4^+$ and Cl^-. In the liver the NH_4^+ is immediately converted into urea. Therefore Cl^- ions are counter balanced by H^+ to produce HCl, a powerful acid. It is then excreted through urine so as to produce acidification. Urine is collected hourly, from 2 to 8 hours after ingestion. The pH and acid excretion of each sample is noted. At least one sample should have a pH of 5.3 or less and ammonia excretion should be 30–90 mmol/hour.

In chronic renal failure, the pH may be low, due to coexisting metabolic acidosis, but the ammonia excretion is less. In renal tubular acidosis, the pH 5.3 is not achieved.

A QUICK LOOK

1. Bilirubin is estimated by van den Bergh reaction. Normal serum does not give a positive van den Bergh reaction.
2. When bilirubin is conjugated, the purple color is produced immediately on mixing with the reagent, the response is said to be van den Bergh direct positive. When the bilirubin is unconjugated, the color is obtained only when alcohol is added, and this response is known as indirect positive.
3. The most common cause for hepatocellular jaundice is the infection with hepatitis viruses (viral hepatitis).
4. Elevated levels of gamma-glutamyl transferase (GGT) are observed in chronic alcoholism, pancreatic disease, myocardial infarction, renal failure, chronic obstructive pulmonary disease and in diabetes mellitus.
5. The GFR of a person with 70 kg body weight is 120–125 mL per minute.
6. Acetazolamide, a carbonic anhydrase inhibitor, will cause diuresis by decreasing reabsorption of bicarbonate, sodium and water.
7. The glomeruli of kidney are not permeable to substances with molecular weight more than 69,000.
8. Large quantities (up to a few gram per day) of albumin are lost in urine in nephrosis.
9. Microalbuminuria is seen as a complication of diabetes mellitus and hypertension.
10. Ketonuria may be detected by Rothera's test.
11. Non-protein Nitrogen includes urea, creatinine and uric acid. Minor components of NPN are urobilinogen, indican, ammonia and amino acids.
12. Clearance is defined as the quantity of blood or plasma completely cleared of a substance per unit time and is expressed as mL per minute.
13. Inulin is neither absorbed nor secreted by the tubules. Therefore, inulin clearance is a measure of GFR.
14. Creatinine coefficient is the urinary creatinine expressed in mg/kg body weight. The value is elevated in muscular dystrophy. Normal range is 20–28 mg/kg for males and 15–21 mg/kg for females.
15. Maximum urea clearance is found to be 75 mL/min in normal.

CHAPTER 31

Hormones

Chapter at a Glance

The reader will be able to answer questions on the following topics:

- Signal transduction
- Cyclic AMP and G proteins
- Hormones acting through calcium
- Hormone response element
- Synthesis of steroid hormones
- Biological effects of glucocorticoids
- Adrenal hyper- and hypofunction
- Ovarian hormones
- Testicular hormones
- Synthesis and effects of thyroid hormones
- Assessment of thyroid function
- Hyperthyroidism and hypothyroidism

INTRODUCTION

The classical **definition** of a hormone is "that released from ductless or endocrine glands directly to the blood". A more modern definition of a hormone is that which is *synthesized by one type of cells and transported through blood to act on another type of cells.* Based on mechanism of action, the hormones may be classified into two (Table 31.1).

I. Hormones with cell surface receptors

II. Hormones with intracellular receptors.

1. Hormones Acting through Cyclic AMP (cAMP)

Adenyl cyclase or adenylate cyclase converts ATP to cAMP (3',5'-cyclic AMP), and phosphodiesterase hydrolyses cAMP to 5' AMP (Fig. 31.1). Table 31.1 contains the list of hormones mediated through cyclic AMP.

Signal Transduction through G Protein

The extracellular messenger, the hormone (H) combines with the specific receptor (R) on the plasma membrane (Fig. 31.2). The H-R complex activates the regulatory component of the protein designated as G protein or nucleotide regulatory protein. G proteins are so named, because they are bound to GTP. The G protein is a membrane protein consisting of alpha, beta and gamma subunits (Fig. 31.2).

G protein Activates Adenyl Cyclase

When the hormone receptor complex is formed, the activated receptor stimulates the G protein, which carries the excitation signal to adenylate cyclase. (Fig. 31.3-2).

The hormone is not passed through the membrane; but only the signal is passed; hence this mechanism is called **signal transduction.** The adenyl cyclase is embedded in the plasma membrane (Fig. 31.3).

Subunit Activation of G Protein

The inactive G protein is a trimer with alpha, beta and gamma subunits. The active alpha G–GTP is immediately inactivated by GTPase. The G-alpha–GDP form is inactive (Fig. 31.3-1). The binding of hormone to the receptor triggers a configurational change in the G protein which induces the release of bound GDP and allows GTP to bind. The alpha subunit, which binds the GTP, dissociates from the beta gamma subunits. Adenylate cyclase is activated by G-alpha-GTP (Fig. 31.3-2).

Table 31.1: Mechanism of action of hormones (for expansions of abbreviations, see Appendix I)

Group	*Mechanism of action*	*Examples of hormone*
I	Hormones that bind to intracellular receptors	Glucocorticoids, estrogens, progesterone, androgens and thyroid hormones
II A	Hormones bind with cell surface receptors with cAMP as the second messenger	ACTH, ADH, FSH, LH, TSH, PTH, glucagon, calcitonin
II B	Hormones having cell surface receptors; cGMP as second messenger	ANF (atrial natriuretic factor), nitric oxide
II C	Hormones having cell surface receptors; second messenger is calcium or phosphatidyl inositol (PIP2)	TRH, GnRH, catecholamines, acetylcholine
II D	Hormones having cell surface receptors and mediated through tyrosine kinase	Insulin
II E	Hormones having cell surface receptors, but intracellular messenger is a kinase or utilize phosphatase cascade	IL, GH, Leptin

Abbreviations: ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; LH, Luteinizing hormone; TSH, thyroid-stimulating hormone; PTH, parathyroid hormone; TRH, Thyrotropin releasing hormone

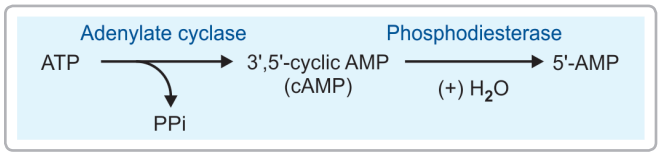


Figure 31.1: Synthesis and degradation of cyclic AMP

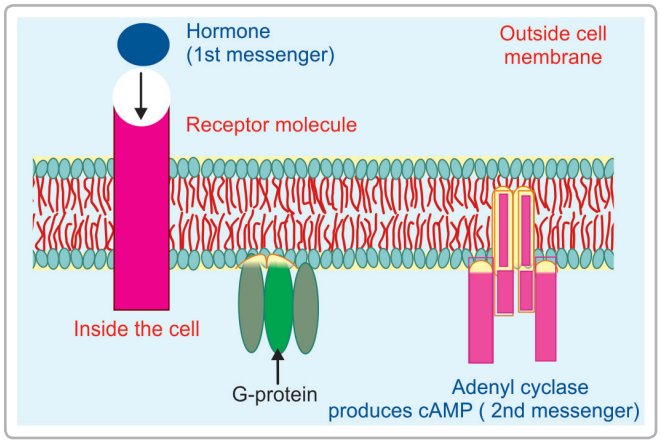


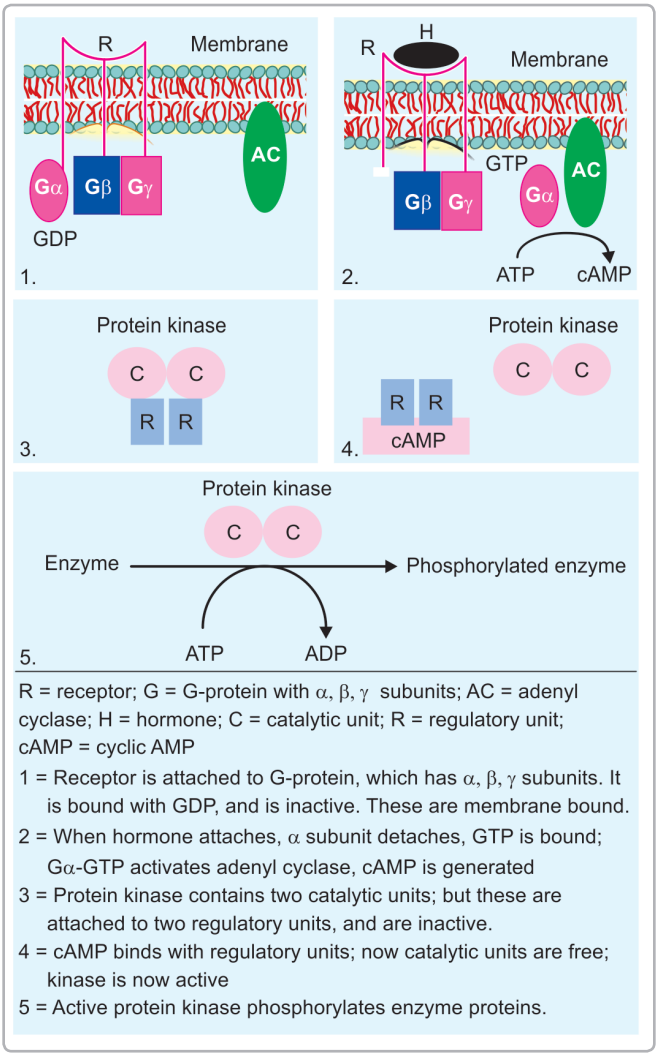
Figure 31.2: Hormone binding activates G-protein

Inactivation

The activation is switched off when the GTP is hydrolysed to GDP by the GTPase activity of the alpha subunit. This is a built-in mechanism for deactivation. The alpha subunit, which is bound to GDP, can re-associate with beta and gamma subunits. The GTP-GDP exchange rate decides the activity of adenyl cyclase.

Second Messenger Activates Protein Kinases

The cAMP (second messenger), in turn, activates the enzyme, protein kinase. This kinase is a tetrameric



R = receptor; G = G-protein with α, β, γ subunits; AC = adenyl cyclase; H = hormone; C = catalytic unit; R = regulatory unit; cAMP = cyclic AMP

1 = Receptor is attached to G-protein, which has α, β, γ subunits. It is bound with GDP, and is inactive. These are membrane bound.

2 = When hormone attaches, α subunit detaches, GTP is bound; Gα-GTP activates adenyl cyclase, cAMP is generated

3 = Protein kinase contains two catalytic units; but these are attached to two regulatory units, and are inactive.

4 = cAMP binds with regulatory units; now catalytic units are free; kinase is now active

5 = Active protein kinase phosphorylates enzyme proteins.

Figure 31.3: Action of hormone through G-protein

molecule having two regulatory (R) and two catalytic (C) subunits (R2 C2) (Fig. 31.3-3). This complex has no activity. But cAMP binds to the regulatory subunit and dissociates the tetramer into regulatory and catalytic subunits (Fig. 30.3-4). The catalytic subunit is now free to act.

Protein Kinase Phosphorylates the Enzymes

The catalytic subunit then transfers a phosphate group from ATP to different enzyme proteins (Fig. 31.3-5). Phosphorylation usually takes place on the OH groups of **serine, threonine or tyrosine** residues of the substrates. The enzymes may be activated or inactivated by this phosphorylation. This is an example of **covalent modification**. Examples of the cascade activation of enzymes by the hormones through cyclic AMP are glycogen phosphorylase (Fig. 5.19) and hormone sensitive lipase (Fig. 9.10). The hormones that are acting through cyclic AMP are enumerated in Table 31.1.

There are many G proteins

About 30 different G proteins are identified, each being used for different signal transduction pathways. The G protein, which stimulates adenyl cyclase, is called Gs (**G stimulatory**) and the opposite group is called Gi (**G inhibitory**).

There are many protein kinases

More than thousand protein kinases are now known. Some important hormone responsive protein kinases are, cAMP-dependent kinases and insulin-dependent tyrosine kinase. All the known effects of cAMP in eukaryotic cells result from activation of protein kinases.

Phosphatases

Phosphorylation of the enzyme causes the effect of the hormonal action; this is terminated by **dephosphorylation** (removal of phosphoric acid) by **phosphatases**. For example, glycogen phosphorylase becomes inactive in the dephosphorylated state. But, glycogen synthase is active in dephosphorylated state (Fig. 5.18). Certain enzymes are activated by dephosphorylation. Protein kinases as well as protein phosphatases are involved in the action of different hormones.

2. Action of Hormones Acting through Calcium

The intracellular concentration of calcium is much lower than the extracellular concentration. Hormones can increase the cytosolic calcium level by the following mechanisms:

A. By altering the permeability of the membrane.

B. The action of Ca-H^+-ATPase pump which extrudes calcium in exchange for H^+.

C. By releasing the intracellular calcium stores.

D. Calmodulin, the calcium dependent regulatory protein within the cell has four calcium binding sites. When calcium binds, there is a conformational change to the calmodulin, which has a role in regulating various kinases. Intracellular calcium acts as a mediator of hormone action either independently or in conjunction with cAMP.

3. Hormones Acting through PIP2 Cascade

The intracellular messengers generated from phosphatidyl inositol bisphosphate (PIP2), a membrane phospholipid, are inositol-triphosphate (IP3) and diacylglycerol (DAG). The binding of hormones like serotonin to cell surface receptor triggers the activation of the enzyme **phospholipase-C** which hydrolyses the phosphatidyl inositol to diacylglycerol. The effect of GTP on this process suggests the involvement of a G protein. **I P3** can release Ca^{++} from intracellular stores, such as from endoplasmic reticulum and from sarcoplasmic reticulum. The elevated intracellular calcium then triggers processes like smooth muscle contraction, glycogen breakdown and exocytosis.

4. Role of Cyclic GMP

It is formed from GTP by the action of **guanyl cyclase.** Several compounds have been found to increase the concentration of cGMP by activating guanyl cyclase. These include drugs like nitroprusside, nitroglycerine, sodium nitrite and atriopeptides (a group of peptides produced by atrial cardiac tissue). All these compounds act as potent vasodilators, by inhibiting the phosphodiesterase. Cyclic GMP activates cGMP-dependent protein kinase, which phosphorylates smooth muscle myosin, leading to relaxation and vasodilatation. Cyclic GMP is also involved in the rhodopsin cycle (For details refer under vitamin A, Chapter 15).

6. Hormones with Intracellular Receptors

The hormones in this group include the steroid hormones and thyroid hormones. They diffuse through the plasma membrane and bind to the receptors in the cytoplasm (Fig. 31.4). The hormone receptor (HR) complex is formed in the cytoplasm. The complex is then translocated to the nucleus. Steroid hormone receptor proteins have a molecular weight of about 80–100 kD. In the nucleus, the HR binds to the **hormone response elements** (HRE) or steroid response elements (SRE). The SRE acts as an enhancer element and when stimulated by the hormone,

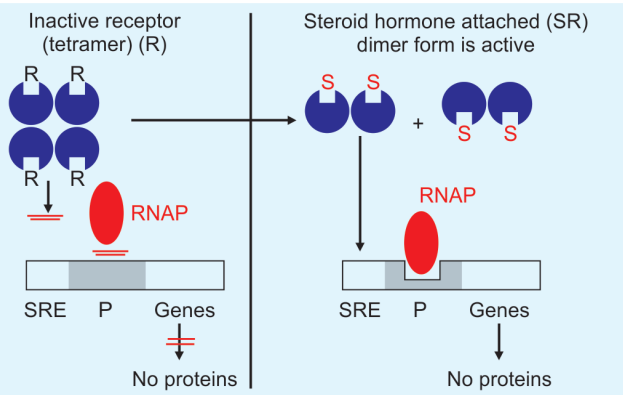


Figure 31.4: Steroid hormone enters nucleus and then acts on the gene

would increase the transcriptional activity (Fig. 31.4). The newly formed mRNA is translated to specific protein, which brings about the metabolic effects. Best example of the effect of hormones on a gene is the synthesis of calcium binding protein by calcitriol (For details refer Chapter 15, under vitamin D).

ADRENAL CORTICAL HORMONES

The adrenal cortex has three different zones each responsible for production of different classes of steroid hormones (C21, C19 and C18). The smallest and outermost zona glomerulosa produces the C21 steroids, mineralocorticoids. They have effects on water and electrolyte balance. The middle zone of the adrenal cortex, the zona fascicularis produces the glucocorticoids mainly; and adrenal androgens and estrogens to a lesser extent. The innermost zona reticularis produces the androgens (C19) and estrogens (C18).

Synthesis of Steroid Hormones

Cholesterol (Fig. 31.5-A) is first acted upon by **desmolase** and a 6-carbon unit is cleaved off, forming the 21 carbon steroid, **pregnenolone** (Fig. 31.5-B). It is a common precursor for all the steroid hormones. This is the **rate limiting** step for synthesis of all steroid hormones. **Progesterone** is the first steroid hormone formed from pregnenolone in two steps. The hydroxyl group in the 3rd position is converted to a keto group by a dehydrogenase and the Δ5 double bond shifted to Δ4 (Fig. 31.5-C). Progesterone is further converted into 21 C Cortisol

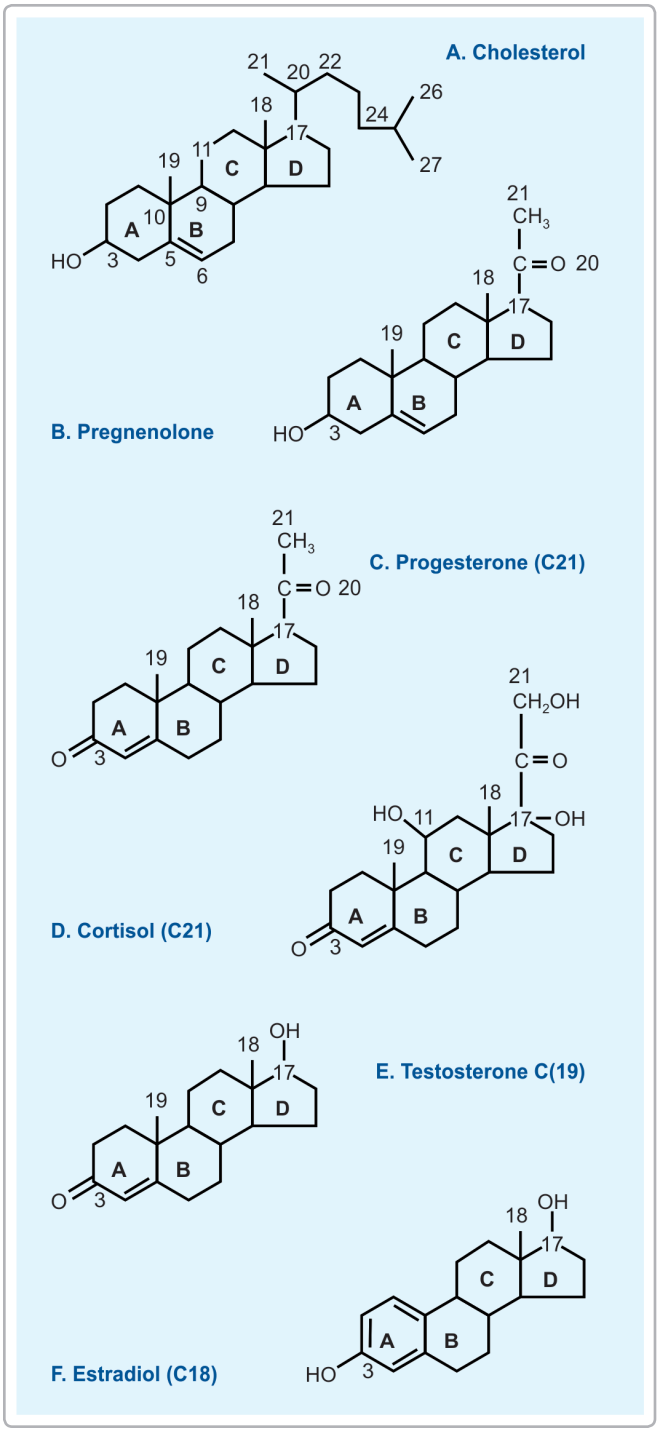


Figure 31.5: Steroid hormones

(glucocorticoid) (Fig. 31.5-D), 19 C Testosterone (male sex hormone) (Fig. 31.5-E) and 18 C Estradiol (female sex hormone) (Fig. 31.5-F). The major adrenal glucocorticoid is cortisol. The major mineralocorticoid is aldosterone. Adrenocorticotropic hormone (ACTH) stimulates the

synthesis of all steroid hormones by activating desmolase so that the availability of pregnenolone is increased.

Secretion of Adrenal Hormones

Secretion of all adrenocortical hormones is under the control of ACTH. The diurnal variation of secretion of cortisol (highest values early in the morning and minimum at night) parallels the pulsatile release of ACTH from anterior pituitary under the influence of CRF. Cortisol exerts the negative feedback effect on ACTH secretion. All steroid hormones act through intracellular messengers and increase the rate of transcription. Cortisol in blood is bound to **cortisol binding globulin** (CBG) or transcortin.

Biological Effects of Glucocorticoids

The glucocorticoids, as the name suggests, mainly affect metabolism of glucose. The major biological effects of glucocorticoids are given in Table 31.2.

Assessment of Glucocorticoid Secretion

Basal Level of Cortisol

The plasma cortisol level is determined by radio-immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or chemiluminescent immunoassay (CLLA). The normal range is 5–25 microgram/dL at 9 AM and 2–5 microgram/dL at 10 pm. A loss of diurnal rhythm may be an early indication of disease.

Estimation of Urinary Free Cortisol

The free cortisol in plasma is the biologically active fraction. A definite fraction of the unbound cortisol is excreted in urine unchanged. Estimation of this fraction is a sensitive index of adrenal activity. High levels are seen in hyperfunction and low levels in hypoactivity.

Plasma ACTH

Suppressed ACTH levels are seen in hyperadrenalism and high ACTH levels in hypoadrenalism as well as in *Cushing's disease*. In hyperadrenalism due to ectopic ACTH secretion, ACTH levels are elevated.

Assessment of Adrenal Androgen Secretion

These tests are done in cases of suspected adrenogenital (AG) syndrome. There is excessive production of adrenal androgens, leading to virilism and hirsutism.

Adrenal Hyperfunction

Hyperactivity of adrenal cortex may be due to primary defect in adrenal gland itself (**Cushing's syndrome**) or secondarily by excessive production of ACTH from pituitary (**Cushing's disease**) or ectopic ACTH production by other malignant tumors.

Adrenal Hypofunction

The most common cause of adrenal hypofunction is primary adrenal insufficiency or **Addison's disease**. It is characterized by tiredness, dehydration, hyponatremia and hyperpigmentation (due to high ACTH levels and its MSH activity).

SEX HORMONES

These are secreted by the gonads in response to pituitary gonadotropins (LH and FSH).

Table 31.2: Effects of glucocorticoids

System	*Effect*
Carbohydrates	Activity of transaminases and gluconeogenic enzymes (PC, PEPCK, FDP and GP) are stimulated, increasing gluconeogenesis. Glycolytic enzymes (GK, PFK and PK) are suppressed. All of them lead to hyperglycemia (Diabetogenic)
Lipids	Facilitate lipolytic hormones leading to hyperlipidemia
Proteins and nucleic acids	Catabolism of proteins and nucleic acids increased
Fluid and electrolytes	Retention of sodium and water
Bone and calcium	Decreased serum calcium by inhibiting osteoblast function, leading to osteoporosis
Secretory action	Stimulates secretion of gastric acid and enzyme. Induces acid peptic disease
Connective tissue	Impaired collagen formation. Poor wound healing
Immune system	Immunosuppressant. Lysis of lymphocytes. Anti-inflammatory and antiallergic

Abbreviations: PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; FDP, fructose diphosphatase; GP, glucose-6-phoshatase; PFK, phosphofructokinase

Ovarian Hormones

They are C18 estrogens, C19 androgens and C21 progesterone. These are produced by the ovarian follicles. The follicular thecal cells produce C19 androgens. These are converted to C18 estrogens by granulosa cells, by aromatization of ring A and loss of C19 methyl group (Fig. 31.5-F). **Estradiol** is the most important estrogen. Estradiol is bound to plasma SHBG (**sex hormone binding globulin**).

Regulation of Ovarian Hormones

FSH influences follicles to ripen, which produces estrogen. Estrogen level gradually increases in the second week of the menstrual cycle. Estrogen level is maximum 24 hours before the **LH peak**. High doses of estrogen can suppress the LH release and, therefore effective as contraceptive. LH level peaks 16 hours before the ovulation. The surge of LH induces the ovulation. The corpus luteum then starts secreting progesterone. LH is required for maintenance of corpus luteum. If implantation of embryo occurs (day 22–24), the LH function is taken over by the hCG (human chorionic gonadotropin), produced by the embryo. The hCG can be detected 5–7 days after missing a period. Certain breast cancers, especially in perimenopausal women are estrogen-dependent. In such patients, estrogen receptor antagonists (**Tamoxifen**) will block the estrogen receptors, and cancer cells tend to die.

Testicular Hormones

In humans, **testosterone** (Fig. 31.5-E) is the major male hormone, while in animals, it is androstenedione. The Leydig cells (interstitial cells), secrete the androgens, under the influence of LH. LH is also called **ICSH** (interstitial cell stimulating hormone). FSH binds to **Sertoli cells** (basement membrane cells of seminiferous tubules) and promotes the synthesis of androgen binding protein (ABP). Thus high concentration of androgen is made available locally at the seminiferous tubules, at the site of spermatogenesis. Androgens stimulate spermatogenesis, produce hypertrophy of prostate, seminal vesicles, muscle, bone and kidney cells. It is anabolic. **Dihydro testosterone** (DHT) is the cause for the benign prostate hypertrophy, that affects more than 75% of men over the age of 60 years.

THYROID HORMONES

Synthesis of Thyroxine

Uptake of Iodine

Iodine metabolism is described in Chapter 17. Thyroid gland takes up and concentrates iodine (Step 1 in Fig. 31.6). This step is inhibited by **thiocyanate** and **perchlorate**. This step is stimulated by TSH.

Oxidation of Iodine

The iodide taken up by the thyroid cell is oxidized to active iodine (Step 2 in Fig. 31.6). The thyroid is the only organ which can perform this oxidation step. This is catalyzed by the enzyme **thyroperoxidase**. with the help of **NADPH** which is generated by the hexose monophosphate shunt pathway. This second step is stimulated by TSH and inhibited by **antithyroid drugs** such as thiourea, thiouracil and methimazole.

Iodination

Then thyroglobulin (Tgb) is iodinated. **Thyroglobulin** is synthesized by the thyroid follicular cells. It is a large protein with about 5000 amino acids (660 kD). Iodination of the tyrosine is taking place on the intact Tgb molecule in the follicular space. Thus mono-iodo tyrosine (MIT) and di-iodo tyrosine (DIT) are produced. Structures of thyroid hormones are shown in Fig. 31.7.

Coupling

Some of the tyrosine residues in the thyroglobulin are aligned opposite each other, and are coupled (step 4, Fig. 31.6). When two DIT molecules couple, one molecule of tetra-iodo thyronine (T4) is formed (Fig. 31.7). The Tri-iodo thyronine (T3) may be formed by de-iodination of T4. Under normal conditions, 99% of the hormone produced by the thyroid gland is T4. The iodination and coupling are taking place in the borders of the follicular cells.

Storage

The thyroid gland is unique, in that it is the only endocrine gland to store appreciable amounts of the hormone (Step 5 in Fig. 31.6). It is stored as colloid in the thyroid acini.

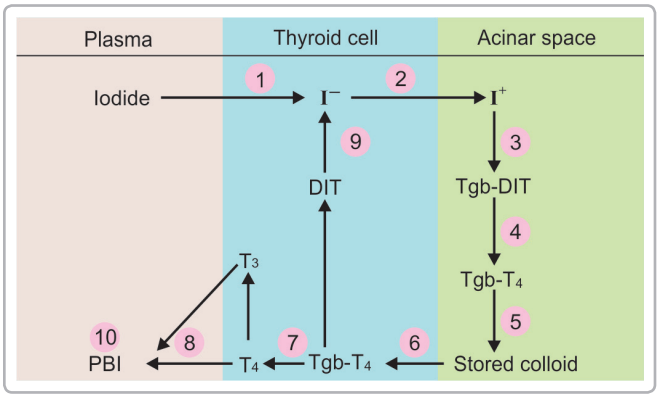


Figure 31.6: Metabolism of thyroid hormones

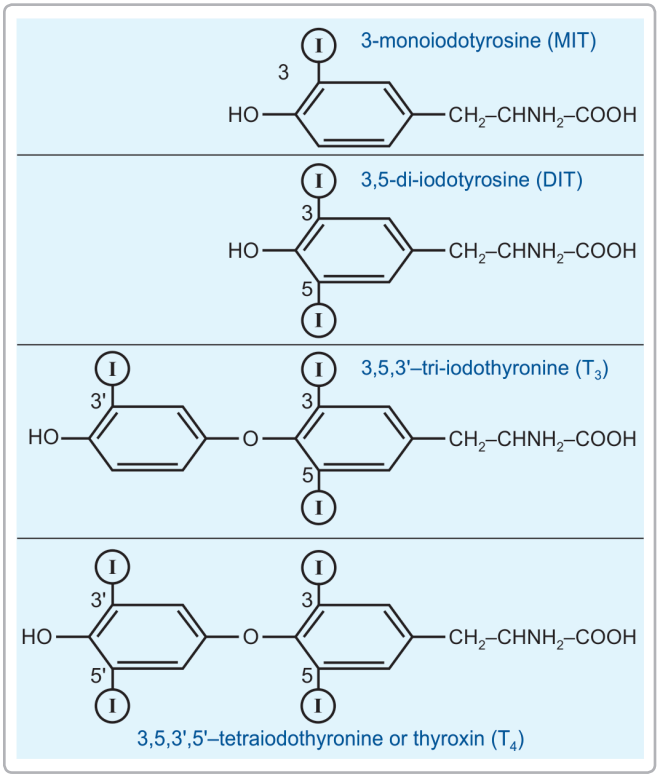


Figure 31.7: Thyroid hormones and precursors

Release

When necessity arises, T4 is liberated by hydrolysis by specific proteases. This activity is markedly enhanced by TSH. (Steps 6 and 7, Fig. 31.6). The T4 thus generated is released into the bloodstream.

Salvage of Iodine

The MIT and DIT that are not utilized are deiodinized and salvaged for reutilization inside the cell itself (Step 9 in Fig. 31.6).

Transport of Thyroid Hormones

Thyroid hormones are transported in plasma by proteins (Step 10 in Fig. 31.6). Total protein bound iodine (PBI) is about 10 microgram/dL; out of which T4 constitutes 8 microgram/dL. The thyroxine binding globulin (TBG) carries T4 and T3. T3 is biologically more active. T4 is a prohormone which is deiodinated to T3.

Mechanism of Action of Thyroid Hormone

The hormone attaches to specific nuclear receptors. Then the receptor-hormone complex binds to the DNA. The T3 receptor complex binding sequence in the DNA is called thyroid responsive element (TRE). This increases transcription activity of genes.

Metabolic Effects of Thyroid Hormones

The hormone shows action on every cell of the body. Calorigenic effect or **thermogenesis** is the major effect of thyroid hormone. One mg of T4 will produce an excess of 1000 kcal. Basal metabolic rate (BMR) is increased. Thyroxine increases cellular metabolism. Higher concentration of T3 causes **protein catabolism** and negative nitrogen balance. Loss of body weight is a prominent feature of hyperthyroidism. **Cholesterol** degradation is increased and hence cholesterol level in blood is decreased, which is another hallmark of hyperthyroidism.

Assessment of Thyroid Function

Assay of Hormones

Measurements of T4 , T3 and TSH levels in blood by ELISA form the basis of laboratory diagnosis of thyroid diseases. ELISA technique is described in Chapter 28. In hyperthyroidism, thyroid hormone levels are increased. Both T3 and T4 levels are increased, while TSH is reduced due to feedback inhibition. In hypothyroidism, T3 and T4 are reduced; but TSH level is increased. But when hypothyroidism is due to hypothalamic or pituitary defect, then TSH, T3 and T4, all are decreased.

Free T3 and T4

The free hormones are the really active molecules. Nowadays, very sensitive ELISA techniques are available to quantitate this free fraction. The values of free hormones are not affected by the amount of carrier proteins in the blood.

Plasma TSH

In primary hypothyroidism, TSH level is elevated due to lack of feedback. But in secondary hypothyroidism, TSH level as well as T3 and T4 levels are low; this could point to a pituitary or hypothalamic cause. Hyperthyroidism due to primary thyroid disease has high T3 and T4 levels, but suppressed TSH levels. Hyperthyroidism due to pituitary cause is indicated by high TSH, T3 and T4 levels.

Detection of Thyroid Antibodies

In Grave's disease, the presence of thyroid stimulating immunoglobulin (TSIg), also known as long acting thyroid stimulator (LATS) is seen in circulation. The LATS can bind to TSH receptors on thyroid gland and produce stimulation which is not under feedback control. The TSIg is an antibody generated against the TSH receptor.

In Hashimoto's thyroiditis anti TPO antibodies (antimicrosomal antibodies), antithyroglobulin

antibodies, and antinuclear antibodies are detected in the circulation. They produce cell destruction and eventual hypothyroidism.

Abnormalities of Thyroid Function

Diseases of the thyroid are the most common afflictions involving the endocrine systems. The commonest types of thyroid diseases are hyperthyroidism (excess secretion), hypothyroidism (decreased secretion) and goiter (enlargement of thyroid gland). Goiter may or may not be associated with abnormal function, e.g. euthyroid goiter (diffuse enlargement); nodular goiter which may lead to hyperfunction, or iodine deficiency goiter which may result in hypothyroidism.

Hyperthyroidism

i. This is often referred to as thyrotoxicosis. It is a syndrome resulting from sustained high levels of thyroid hormones. Patients have an increased rate of metabolism, weight loss, tachycardia, fine tremors, sweating, diarrhea, emotional disturbances, anxiety and sensitivity to heat.

ii. Common causes for hyperthyroidism are

- **a.** Grave's disease (autoantibodies)
- **b.** Toxic goiter
- **c.** Excess intake of thyroid hormones
- **d.** Rarely TSH secreting tumors of pituitary can lead to hyperthyroidism. Table 31.3 summarises the laboratory findings in common types of hyperthyroidism.

Hypothyroidism

This disorder results from low levels of circulating thyroid hormones. Most common cause is primary thyroid disease, often auto-immune in nature, leading to myxedema in adults. Women are more affected than males. Symptoms are lethargy, tolerance to heat, cold intolerance, slow heart rate, weight gain, dry coarse skin, slow responses and sluggishness. In children, hypothyroidism produces mental and physical retardation, known as **cretinism**. The TBG may be elevated due to maternal hyperestrogenism and therefore total T4 and T3 may be normal. The lack of feedback will give elevated TSH level also. Prompt diagnosis and treatment are important in cretinism since any delay in starting replacement may lead to irreversible damage. Maternal hypothyroidism may also cause congenital hypothyroidism. This condition is included in the new born screening programmes. Secondary hypothyroidism may result from pituitary or hypothalamic causes. The measurement of TSH level and TRH test will help to differentiate the different types (Table 31.4).

Table 31.3. Lab findings in hyperthyroidism

	Plasma total T3 and T4	*Plasma TSH*	*Response to TRH*
Grave's disease	increase	Decrease	Nil
Toxic goiter	increase	Decrease	Nil
T3 toxicosis	T3 increase, T4 normal	Decrease	Sluggish
Excess intake of thyroxin	increase	Decrease	Sluggish

Table 31.4. Laboratory findings in hypothyroidism

	Plasma total T3 and T4	*Plasma TSH*	*Response to TRH*
Primary hypothyroidism	Decrease	Increase	Exaggerated response
Secondary hypothyroidism	Decrease	Decrease	No response

Euthyroid Goiter

Iodine deficiency may lead to euthyroid goiter. There is raised TSH level which would produce continued stimulation of gland leading to hyperplasia and goiter. Hormone levels are seen in the lower limits of the normal values.

INSULIN

The word "insulin" is derived from Latin, insula, meaning island (islet). In 1869, Langerhans identified the alpha and beta cells in islets of pancreas. In 1889, von Mering and Minkowski produced experimental diabetes by pancreatectomy. In 1922, Banting and Best extracted insulin from pancreas. Insulin was the first hormone to be isolated in a pure form. They injected the extract to a diabetic dog, Marjorie, who was kept alive by regular insulin injections. For this work, Banting was awarded Nobel prize in 1923. In 1954, Sanger studied the amino acid sequence of insulin (Nobel prize in 1958).

Structure of Insulin

Insulin is a protein hormone with 2 polypeptide chains. The A chain has 21 amino acids and B chain has 30 amino acids. These two chains are joined together by two interchain disulfide bonds. There is also an intrachain

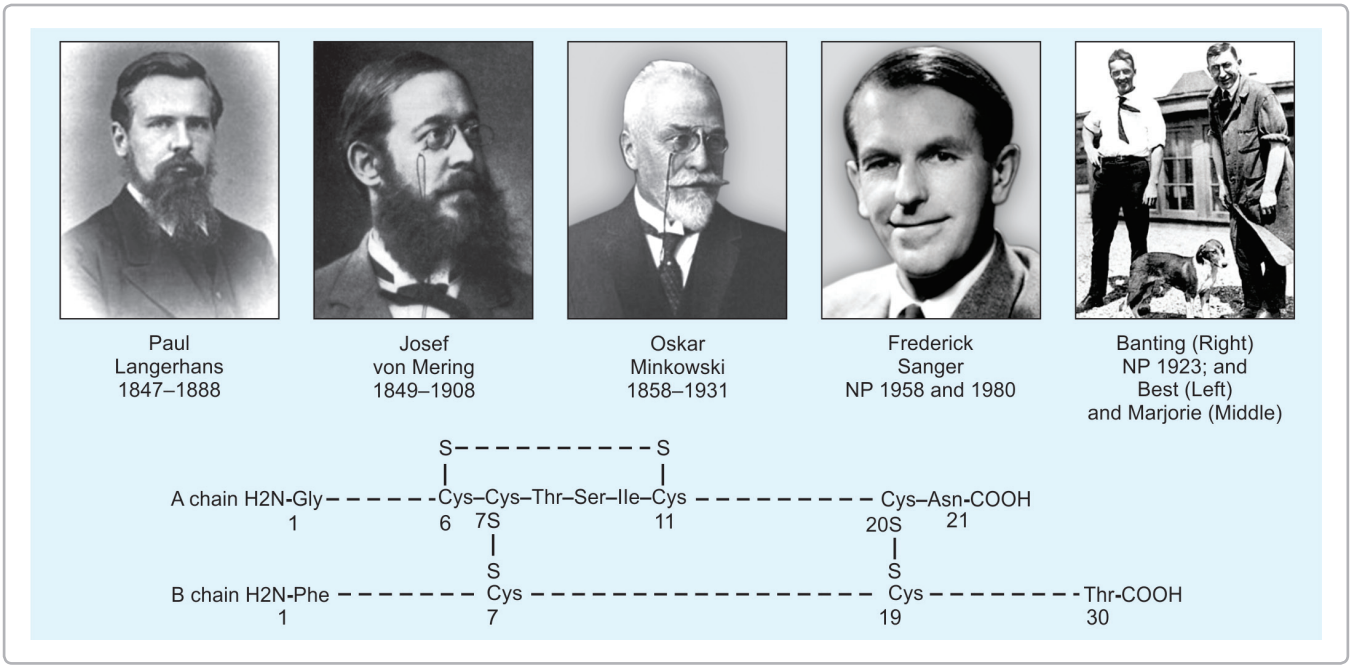


Figure 31.8: Primary structure of human insulin

disulfide link in A chain between 6th and 11th amino acids (Fig. 31.8).

Biosynthesis of Insulin

Insulin is synthesized and secreted by the beta-cells of the islets of Langerhans of pancreas. Insulin is synthesized as a larger precursor polypeptide chain, **proinsulin** with 86 amino acids. This is then cleaved by a protease (Fig. 31.9). Thus, **C-peptide** or connecting peptide with 33 amino acids is removed. Insulin with 51 amino acids is thus formed (Fig. 31.8). Insulin and C-peptide are synthesised and secreted in equimolar quantities.

Factors Increasing Insulin Secretion

Glucose: Glucose is the major stimulant of insulin secretion. As blood glucose level increases, the insulin secretion also correspondingly increases. The beta cells have **GluT 2 receptors**, which act as the sensors of blood sugar level.
Gastrointestinal hormones: Insulin secretion is enhanced by secretin, pancreozymin and gastrin. After taking food, these hormones are increased.

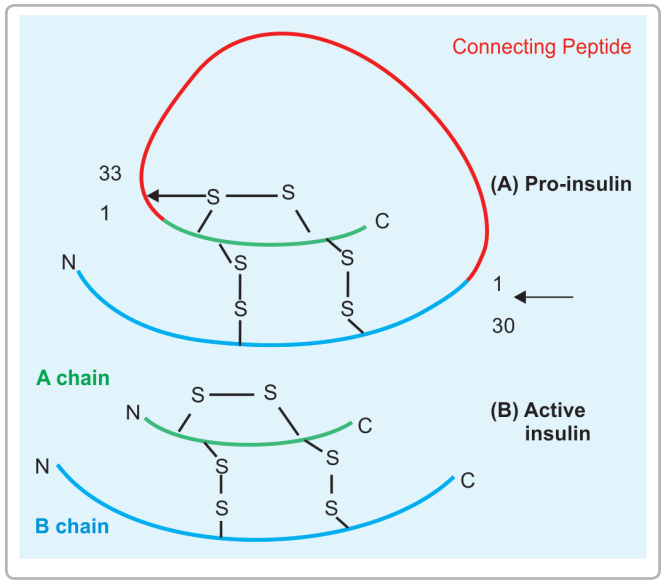


Figure 31.9: Conversion of proinsulin to active insulin. Arrows show site of action of proteolytic enzymes

Physiological Actions of Insulin (Mechanisms of Action of Insulin, Metabolic Effects of Insulin)

Insulin plays a central role in regulation of the metabolism of carbohydrates, lipids and proteins (Table 31.5).

Insulin Receptors

Insulin acts by binding to a plasma **membrane receptor** on the target cells. In diabetes mellitus type 2, target tissue becomes less sensitive to insulin.

Table 31.5: Biological effects of insulin				
Metabolism	*Key enzyme*	*Action of insulin on the enzyme*	*Direct effect*	*Overall effect*
Carbohydrate	Glucokinase	Stimulation	Glycolysis favored	Hypoglycemia
	Phosphofructokinase	Stimulation	Glycolysis favored	Hypoglycemia
	Pyruvate kinase	Stimulation	Glycolysis favored	Hypoglycemia
	Pyruvate carboxylase	Inhibition	Gluconeogenesis	Hypoglycemia
	PEPCK	Inhibition	Gluconeogenesis	Hypoglycemia
	Fructose-1,6-bisphosphatase	Inhibition	Gluconeogenesis	Hypoglycemia
	Glycogen synthase	Activation	Glycogen deposition	Hypoglycemia
	Glycogen phosphorylase	Inactivation	Glycogen deposition	Hypoglycemia
Lipid	Acetyl-CoA carboxylase	Stimulation	Lipogenesis favored	Glucose is used for lipogenesis; hence hypoglycemia
	Hormone sensitive lipase	Inhibition	Lipolysis inhibited	Decreased ketogenesis
Protein	Transaminases	Inhibition	Catabolism inhibited	General anabolism
	RNA polymerase	Favored	Protein synthesis	General anabolism

Uptake of Glucose by Tissues

Insulin facilitates the membrane **transport of glucose**. In diabetes mellitus, the transporter, GIuT4 is reduced. However, glucose uptake in liver (by GluT2) is independent of insulin.

Utilization of Glucose

Glycolysis is stimulated by insulin. The activity and amount of key glycolytic enzymes (glucokinase, phosphofructokinase and pyruvate kinase) are increased. Glycogen synthase enzyme is activated, and so insulin favors glucose storage as **glycogen**. Insulin favors synthesis of fatty acid from glucose and so glucose utilization is increased.

Hypoglycemic Effect

Insulin lowers the blood glucose level by promoting utilization and storage. **Gluconeogenesis is inhibited** (Chapter 5). Insulin **inhibits glycogenolysis** by favoring the inactivation of glycogen phosphorylase and inhibiting the glucose-6-phosphatase. The net effect of all these three mechanisms, blood glucose level is lowered.

Lipogenesis

Lipogenesis is favored by providing more acetyl-CoA. Insulin increases the activity of **acetyl-CoA carboxylase** and provides glycerol for esterification of fatty acids to TAG (For details refer Chapter 9).

Antilipolytic Effect

Insulin inhibits lipolysis in adipose tissue due to inhibition of **hormone sensitive lipase** (Fig. 9.10).

Antiketogenic Effect

Insulin depresses **HMG-CoA synthase** and so ketogenesis is decreased. Insulin deficiency leads to diabetes mellitus, which is given in detail in Chapter 6.

HYPERGLYCEMIC HORMONES

1. Glucagon
2. Epinephrine or adrenaline
3. Glucocorticoids
4. ACTH
5. Growth hormone
6. Thyroxine

All these are anti-insulin hormones.

GLUCAGON

It is a polypeptide hormone with 29 amino acids. It is secreted by the alpha cells of pancreas. The major regulator of secretion of glucagon is glucose. An increase in blood glucose level inhibits secretion of glucagon.

Physiological Actions of Glucagon

Glucagon is the most potent **hyperglycemic** hormone. It is **anti-insulin** in nature. Therefore, the net effect is

decided by the insulin-glucagon ratio. Glucagon is mainly **glycogenolytic**. The active form of glycogen phosphorylase is formed under the influence of glucagon. Liver is the primary target for the glycogenolytic effect of glucagon. It depresses glycogen synthesis. **Gluconeogenesis** is favored by glucagon by inducing enzymes like PEPCK, glucose-6-phosphatase and fructose-1,6-bisphosphatase.

Other Important Hormones

Regulation of carbohydrate metabolism in general depends on balance between insulin and anti-insulin hormones. Regulation of blood sugar and diabetes mellitus are described in Chapter 6. Details of Epinephrine (Adrenaline) are given in Chapter 11, under Tyrosine metabolism.

A QUICK LOOK

1. Definition of a hormone is that is synthesized by one type of cells and transported through blood to act on another type of cells.
2. G protein is a peripheral membrane protein consisting of alpha, beta and gamma subunits. When GTP is attached, it becomes active. It then activates the enzyme adenylate cyclase.
3. G proteins are used for different signal transduction pathways. There are 2 major types of G proteins; Gs (G stimulatory) and Gi (G inhibitory).
4. G protein activity can be inhibited by GTPase.
5. Examples of hormones that use cyclic AMP as second messenger are ACTH, FSH, LH, PTH etc.
6. Example of a hormone that use cGMP as second messenger is ANF.
7. Examples of hormones that use calcium or PIP2 as second messenger are TRH, GnRH, CCK, etc.
8. Examples of hormones whose actions are mediated via a tyrosine kinase mechanism are Insulin, EGF, PDGF, NGF, etc.
9. Cholera toxin binds to a ganglioside on intestinal mucosal cell, which leads to ribosylation of the alpha subunit of Gs protein. Pertussis toxin ribosylates the alpha subunit of Gi protein and prevents the Gi-GDP complex from interacting with the activated receptor.
10. Diacylglycerol (DAG), the second messenger formed by the hydrolysis of PIP2 activates protein kinase C which in turn phosphorylates other target proteins.
11. Hormones bind to the specific area of the gene, referred to as the hormone response element (HRE). For example, thyroid hormones and steroid hormones.
12. The adrenal cortex has three different zones each responsible for production of different classes of steroid hormones (C21, C19 and C18).
13. Zona glomerulosa produces the C21 steroids, mineralocorticoids.
14. Zona fascicularis produces the glucocorticoids mainly; and adrenal androgens and estrogens to a lesser extent.
15. Zona reticularis produces the androgens (C19) and estrogens (C18).
16. Approximately 70% of cortisol in blood is bound to an alpha-1-globulin called cortisol binding globulin (CBG) or transcortin.
17. Hyperactivity of adrenal cortex may be due to primary defect in adrenal gland itself (Cushing's syndrome) or secondarily by excessive production of ACTH from pituitary (Cushing's disease) or ectopic ACTH production by other malignant tumors.
18. Congenital deficiency of steroid hydroxylases leading to deficient secretion of cortisol is the cause for Adrenogenital syndrome.
19. Goitrogens that decrease utilization of iodine are found in food stuffs, e.g. Thiocyanates in cabbages and tapioca, thiourea in mustard seeds. Goitrogens are seen in cassava, maize, millet, bamboo shoots, sweet potatoes and beans.
20. The main hormones secreted by the thyroid gland are triiodothyronine (T3) and tetraiodothyronine or thyroxine (T4).
21. Measurement of T4 and T3 levels in blood are done by RIA or ELISA.
22. Cholesterol is increased in blood in hypothyroidism.
23. Grave's disease and Hashimoto's thyroiditis are produced by autoimmune mechanisms.
24. Most common cause of hypothyroidism is primary thyroid disease, often autoimmune in nature, leading to myxedema in adults. Women are more affected than males.
25. In children, hypothyroidism produces mental and physical retardation, known as cretinism.
26. Euthyroid goiter can result from iodine deficiency.

Appendices

APPENDIX I

ABBREVIATIONS USED IN THIS BOOK

A = adenine
A = alanine
Å = Angstrom unit (10^{-10} m)
ACAT = acyl cholesterol acyltransferase
ACP = acid phosphatase
ACP = acyl carrier protein
ADH = antidiuretic hormone (vasopressin)
ADH = alcohol dehydrogenase
ADP = adenosine disphosphate
AFP = alpha fetoprotein
AIDS = acquired immunodeficiency syndrome
Ala = alanine
ALA = (delta) aminolevulinic acid
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AMP = adenosine monophosphate
Arg = arginine
Asn = asparagine
Asp = aspartic acid
AST = aspartate aminotransferase
ATP = adenosine triphosphate

BMR = basal metabolic rate
bp = base pair

C = carbon
C = cytosine
C = cysteine
Ca = calcium
cal = calorie
cAMP = cyclic AMP (3',5'–cyclic AMP)
CEA = carcinoembryonic antigen
CK = Creatine kinase
CMP = cytidine monophosphate
CO = carbon monoxide
CO2 = carbon dioxide
CoA = (CoA-SH); co-enzyme A
CoQ = coenzyme Q
CPS = carbamoyl phosphate synthetase
CRP = C-reactive protein
CTP = cytidine triphosphate
Cu = copper
Cys = cysteine

D = aspartic acid
D = Dalton (molecular weight or molecular mass)
dATP = deoxyadenosine triphosphate
DHU = dihydrouracil
DIT = di-iodotyrosine
DNA = deoxy ribonucleic acid
dNTP = deoxynucleoside triphosphate
DOPA = dihydroxyphenylalanine

E = glutamic acid
EF = elongation factor (protein synthesis)
ELISA = enzyme-linked immuno sorbent assay
ESR = erythrocyte sedimentation rate
ETC = electron transport chain
F = phenyl alanine

FAD = flavin adenine dinucleotide
FADH2 = reduced FAD
Fe = iron
FFA = free fatty acid
FMN = flavin mononucleotide

g = gram
G = glycine
G = guanine
GABA = gamma amino butyric acid
GAG = glycosaminoglycans
Gal = galactose
GalNAc = N-acetylgalactosamine
GDP = guanosine diphosphate
GFR = glomerular filtration rate
GGT = gamma-glutamyl transaminase
Gln = glutamine
Glu = glutamic acid
Glu = glucose
Gly = glycine
GOT = glutamate oxaloacetate transaminase
GPD = glucose-6-phosphate dehydrogenase
GPT = glutamate pyruvate transaminase
GSH = glutathione
GTT = glucose tolerance test
GTP = guanosine triphosphate

H = hydrogen
H = histidine
Hb = hemoglobin
HbA1c = glycated hemoglobin
HbS = hemoglobin sickle cell
HDL = high density lipoprotein
His = histidine
HIV = human immunodeficiency virus
HMG CoA = beta-hydroxy beta-methylglutaryl-CoA

I = isoleucine
IDL = intermediate density lipoprotein
IF = initiation factor
Ig = immunoglobulin
Ile = isoleucine
IMP = inosine monophosphate

K = lysine
K = potassium
kbp = kilo base pair
kcal = kilocalorie
kD = kilo daltons (see D)
Km = Michaelis constant

L = Leucine
LCAT = lecithin cholesterol acyltransferase
LDH = lactate dehydrogenase
LDL = low density lipoproteins
Leu = leucine
Lp = lipoproteins
LpL = lipoprotein lipase
Lys = lysine

M = molar
M = methionine
MAG = mono acyl glycerol
MAO = mono amine oxidase
MCFA = medium chain fatty acid
Met = methionine
mEq = milli equivalents
mg = milligram
mM = milli molar
mmol = milli mole
mol wt = molecular weight
mRNA = messenger RNA

N = asparagine
N = nitrogen
Na = sodium
NAD^+ = nicotinamide adenine dinucleotide
NADH = reduced nicotinamide adenine dinucleotide
$NADP^+$ = nicotinamide adenine dinucleotide phosphate
NADPH = reduced nicotinamide adenine dinucleotide phosphate
NANA = N-acetylneuraminic acid
NEFA = non-esterified fatty acid
ng = nanogram (10^{-9} gram)
NTP = nucleoside triphosphate

O = oxygen
P = proline
P = phosphorus
PAPS = phospho adenosine phospho sulfate
PDH = pyruvate dehydrogenase
PEM = protein energy malnutrition
PEPCK = phosphoenolpyruvate carboxykinase
PFK = phospho fructo kinase
pH = hydrogen ion concentration

Phe = phenyl alanine
Pi = inorganic phosphate
pI = Iso-electric point
pKa = dissociation constant of acid
PLP = pyridoxal phosphate
PPi = inorganic pyrophosphate
Pro = proline
PRPP = phosphoribosyl pyrophosphate
PTH = parathyroid hormone
PUFA = poly unsaturated fatty acid

Q = glutamine
R = arginine
RBP = retinol binding protein
RDA = recommended daily allowance
RIA = radio immuno assay
RNA = ribonucleic acid
RNase = ribonuclease
rRNA = ribosomal RNA

S = serine
SAH = S-adenosyl-l-homocysteine
SAM = S-adenosylmethionine
SCFA = short chain fatty acid
SDA = specific dynamic action
Ser = serine
SH = sulfhydryl (group)
sRNA = soluble RNA

T = threonine
T = thymine
TAG = triacylglycerol
TBG = thyroxine binding globulin
TG = triglyceride
THFA = tetrahydrofolic acid
Thr = threonine
TPP = thiamine pyro phosphate
tRNA = transfer RNA
Trp = tryptophan
Tyr = tyrosine

m = micro meter (10^{-6} meter)
mL = micro liter
mM = micro molar
UMP = uridine mono phosphate (uridylic acid)
UTP = uridine tri phosphate

V = valine
Val = valine
VLDL = very low density lipoprotein
VMA = vanillylmandelic acid
Vmax = maximal velocity
W = tryptophan
XMP = xanthosine monophosphate
Y = tyrosine

APPENDIX II

Normal Values (Reference Values)

P = plasma; B = blood; S = serum; E = erythrocyte; U = urine; CSF = cerebrospinal fluid; pg = picogram; ng = nanogram; µg = microgram; mg = milligram; d = day

Analyte	*Sample*	*Units*
Alanine amino transferase (ALT/SGPT) Male: Female:	S	13–35 IU/L 10–30 IU/L
Albumin	S	3.5–5 g/dL
Alkaline phosphatase (ALP)	S	40–125 IU/L
Alpha fetoprotein (AFP)	S	5–15 µg/L
Aspartate aminotransferase (AST/SGOT)	S	8–20 IU/L
Bicarbonate (HCO_3^-)	S	22–26 mEq/L
Bilirubin, total	S	0.2–1 mg/dL
Calcium	S	9–11 mg/dL
Ceruloplasmin	S	25–50 mg/dL
Chloride	S/P	96–106 mEq/L
Chloride	CSF	120–130mEq/L
Cholesterol, Total	S/P	150–200 mg/dL
(HDL fraction) Male Female	S	30–60 mg/dL 35–75 mg/dL
(LDL fraction) 20–29 year 30–39 year		60–150 mg/dL 80–175 mg/dL
Creatine	S	0.2–0.4 mg/dL
Creatine kinase (CK)		
Female	S	10–80 U/L
Male	S	15–100 U/L
Creatinine	S U	0.7–1.4 mg/dL 15–25 mg/kg/d
Ferritin Male Female	S	3–30 mg/dL 2–12 mg/dL
Fibrinogen	P	200–400 mg/dL
Globulins	S	2.5–3.5 g/dL
Glucose (Fasting)	P CSF	70–110 mg/dL 50–70 mg/dL
Hemoglobin Male Female	B B	14–16 g/dL 13–15 g/dL
Hemoglobin A_2	E	2–3% of total
HbA1C (glycohemoglobin)		4–8 % of total
Immunoglobulins	S	

Analyte	*Sample*	*Units*
IgG		800–1200 mg/dL
IgM		50–200 mg/dL
IgA		150–300 mg/dL
Iron	S	100–150 mg/dL
Iron binding capacity	S	250–400 mg/dL
Lactate dehydrogenase	S	100–200 IU/L
Lipids–Total	S	400–600 mg/dL
Lipoproteins Alpha	S	40 mg/dL
Beta		180 mg/dL
Nonesterified fatty acids	P	10–20 mg/dL
Parathyroid hormone	S	10–25 ng/L
pCO_2, arterial	B	35–45 mm Hg
pH	B	7.4
Phosphate	S	3–4 mg/dL
	U	1 g/day
Phospholipids		150–200 mg/dL
pO_2 arterial	B	90–100 mm Hg
Potassium	S	3.5–5 mEq/L
Proteins—total	S	6–8 g/dL
Prothrombin	P	10–15 mg/dL
Sodium	S	136–145 mEq/L
T_3 (Tri iodothyronine)	S	120–190 ng/dL
T_4 (Thyroxine)	S	5–12 mg/dL
TRH	S	5–60 ng/L
TSH	S	0.5–5 mU/mL
Triglycerides, fasting Male Female	S	 50–200 mg/dL 40–150 mg/dL
Urea	S	20–40 mg/dL
Uric acid Male Female	S/P S/P	3.5–7 mg/dL 3.0–6 mg/dL
Vitamin A	S	15–50 mg/dL
Vitamin C (Ascorbic acid)	P	0.4-1.5 mg/dL
Vitamin D3 (Calcitriol)	S	1.5-6 mg/dL
Vitamin E	S	0.5--1.8 mg/dL

APPENDIX III

Recommended Daily Allowance (RDA) of Essential Nutrients

Sl. No.	Nutrient	Requirement per day
1.	**Proteins**	
	Adult	
	Males	1 g/kg
	Females	1 g/kg
	Children	
	Infants	2.4 g/kg
	Upto 10 years	1.75 g/kg
	Boys	1.6 g/kg
	Girls	1.4 g/kg
	Pregnancy and lactation	
	Pregnancy	2 g/kg
	Lactation	2.5 g/kg
2.	**Essential amino acids**	
	Phenylalanine	14 mg/kg
	Leucine	11 mg/kg
	Lysine	9 mg/kg
	Valine	14 mg/kg
	Isoleucine	10 mg/kg
	Threonine	6 mg/kg
	Methionine	14 mg/kg
	Tryptophan	3 mg/kg
3.	**Fat soluble vitamins**	
	Vitamin A	
	Adult	750 µg
	Children	400 to 600 µg
	Pregnancy	1000 µg
	Lactation	1200 µg
	Vitamin D	
	Adult	5 µg
	Children (preschool)	10 µg
	Pregnancy and lactation	1200 µg
	Vitamin E	
	Adult males	10 mg
	Females	8 mg
	Old age	10 mg
	Pregnancy	11 mg
	Vitamin K	
	Adult	50 to 100 µg
	Children	1 µg/kg
4.	**Water soluble vitamins**	
	Thiamine (B_1)	
	Adult	1–1.5 mg
	Riboflavin (B_2)	
	Adult	1.5 mg
	Pregnancy and lactation	1.7–2 mg
	Niacin	
	Adult	20 mg
	Pregnancy	22 mg
	lactation	25 mg
	Pyridoxine (B_6)	
	Adult	2 mg
	Pregnancy	2.5 mg
	Pantothenic acid	10 mg
	Biotin	200–300 µg
	Folic acid	
	Adult	100 µg
	Pregnancy	300 µg
	Lactation	150 µg
	B_{12}	
	Adult	1 mg
	Pregnancy and lactation	1.5 mg
	Ascorbic acid	
	Adult	70 mg
	Pregnancy and lactation	100 mg
5.	**Minerals**	
	Calcium	
	Adult	0.5 g
	Children	1 g
	Pregnancy and lactation	1.5 g
	Phosphorus	500 mg
	Magnesium	400 mg
	Manganese	5–6 mg
	Sodium	5–10 g

Sl. No.	*Nutrient*	*Requirement per day*
	Potassium	3–4 g
	Iron	
	Males	15–20 mg
	Females	20–25 mg
	Pregnancy	40–50 mg

Sl. No.	*Nutrient*		*Requirement per day*
	Copper	Adult	1.5–3 mg
	Iodine	Adult	150–200 µg
	Zinc	Adult	8–10 mg
	Selenium	Adult	50–100 µg

APPENDIX IV

Composition of Nutrients in Selected Common Food Materials

Food materials		*Protein g/100 g*	*Fat g/100 g*	*Carbohydrate g/100 g*	*Energy kcal/100 g*	*Calcium mg/100 g*	*Iron mg/100 g*	*Vit. A IU/100 g*	*Vit. B1 µg/100 g*
I.	**Cereals:**								
1.	Wheat flour, whole	12.1	1.7	72.2	358	35	7.3	-	70
2.	Rice, raw, milled	6.9	0.4	79.2	348	10	1.0	-	50
3.	Sorghum, Juar, Cholam	10.4	1.9	74.0	335	30	6.2	136	345
II.	**Legumes and pulses:**								
1.	Bengal gram, (Channa)	17.1	5.3	61.2	361	190	9.8	316	300
2.	Peas (Mattar) dried	19.7	1.1	56.6	315	70	4.4	-	450
3.	Soyabean	43.2	19.5	20.9	432	240	11.5	710	730
III.	**Vegetables, A group, (low calorie)**								
1.	Amaranth (lal cholai)	4.9	0.5	5.7	47	500	21.4	8,000	50
2.	Cabbage	1.8	0.1	6.3	33	30	0.8	2,000	60
3.	Tomato, ripe	1.0	0.1	3.9	21	10	-	320	120
IV.	**Vegetables, B group, (Medium calorie)**								
1.	Carrot	0.9	0.2	10.7	47	80	1.5	4,000	40
2.	Onion, big (sabola)	1.2	-	11.6	51	180	0.7	25	80
3.	Ladies finger (Bhindi)	2.2	0.2	7.7	41	90	1.5	60	60
V.	**Vegetables, C group (Roots and Tubers)**								
1.	Potato (Aloo)	1.6	0.1	22.9	99	-	0.7	40	100
2.	Tapioca (cassava)	0.7	0.2	38.7	159	50	0.9	-	45
3.	Yam (Ratalu)	1.4	0.1	27.0	115	60	1.3	-	72
VI.	**Fruits**								
1.	Apple	0.3	0.1	13.4	56	-	1.7	-	120
2.	Banana, ripe	1.3	0.2	36.4	153	10	-	-	150
3.	Mango, ripe	0.6	0.1	11.8	50	10	-	4,800	40
4.	Papaya, ripe	0.5	0.1	9.5	40	10	-	3,000	40
VII.	**Milk and milk products**								
1.	Cow's milk	3.3	3.8	4.4	69	100	-	160	50
2.	Buffalo's milk	4.3	8.8	5.3	117	210	-	160	40
3.	Curd (Yogurt) (dahi)	2.9	2.9	3.3	51	120	-	130	-
4.	Cheese (Paneer)	24.1	25.1	6.3	548	790	2.1	275	-
VIII.	**Meat and other products**								
1.	Mutton, muscle	18.5	11.3	0.5	194	150	2.5	30	180
2.	Beef muscle	22.6	2.6	0.5	114	10	0.8	60	150
3.	Fish	22.6	0.6	0.2	91	20	0.9	20	100
4.	Egg, Hen	13.3	13.3	0.2	173	60	2.1	1,200	130

ESSAY QUESTIONS AND SHORT NOTES

Chapter 1: Cell

1-1. Enumerate the cellular organelle and list the functions of different organelles
1-2. Give a labeled diagram of mitochondria and enumerate the funtions.
1-3. Briefly outline the structure of biomembrane with the help of a diagram.
1-4. Define active transport. Explain the different types of active transport with examples.

Write Short Notes on

1-1. Structure of cell membrane.
1-2. Fluid mosaic model.
1-3. Active transport.
1-4. Symport system.

Chapter 2: Amino Acids and Proteins

2-1. Classify amino acids, giving suitable examples.
2-2. How will you classify amino acids based on their nutritional importance?
2-3. Define isoelectric pH and give the importance.
2-4. Give an account of the transamination reactions. Give two suitable examples. What is the metabolic importance of transamination? What is the clinical application of transaminase estimation?
2-5. Describe the primary, secondary and tertiary structures of proteins. What are the forces, which stabilize them?
2-6. What is the primary structure of a protein? Explain with the help of the structure of insulin.
2-7. Explain the structural organization of hemoglobin molecule. How does the alteration in amino acid sequence affect the properties of hemoglobin?
2-8. What are the different techniques used for precipitation of proteins?
2-9. Classify proteins with suitable examples.

Write Short Notes on

2-1. Decarboxylation of amino acids.
2-2. What are essential amino acids?
2-3. Primary structure of proteins.
2-4. Alpha helix of proteins.
2-5. Tertiary structure of proteins.
2-6. Quaternary structure of proteins.
2-7. Denaturation of proteins.
2-8. Conjugated proteins.
2-9. Isoelectric pH.
2-10. Precipitation reactions of proteins.
2-11. Heat coagulation.
2-12. Decarboxylation of amino acids.

Chapter 3: Enzymology

3-1. What are the salient features of the active site of the enzyme?
3-2. Explain the factors affecting the velocity of an enzyme reaction.
3-3. What are the different types of enzyme inhibition? Explain with suitable examples.
3-4. What is competitive inhibition? Explain with two examples of its therapeutic significance.
3-5. What are the differences between competitive and noncompetitive inhibition? Give two examples for competitive inhibition.
3-6. What is meant by Km value, and what is its significance?
3-7. What are isoenzymes? Give examples. What are their clinical significance?
3-8. Write briefly about the enzymes that show variations in serum level in myocardial infarction, and their clinical significance.

Write Short Notes on

3-1. Oxidoreductases.
3-2. Coenzymes.
3-3. Metalloenzymes.
3-4. Active site of enzyme.
3-5. Koshland's induced fit theory
3-6. Zymogens.
3-7. Effect of pH on enzyme activity.
3-8. Optimum temperature.
3-9. Michaelis Constant (Km).
3-10. Competitive inhibition.
3-11. Allosteric inhibition.
3-12. Isoenzymes.
3-13. Clinical significance of LDH.
3-14. Clinical significance of CK.
3-15. Enzyme profile in myocardial infarction.
3-16. Enzyme profile in liver diseases.
3-17. Clinical significance of transaminases.
3-18. Clinical significance of ALP.

Chapter 4: Carbohydrates, Chemistry

Write Short Notes On

4-1. Classification of monosaccharides.
4-2. Reducing disaccharides.
4-3. Nonreducing disaccharide.
4-4. Why sucrose is a non-reducing sugar?
4-5. Transport mechanisms of glucose.
4-6. Anomers.
4-7. Epimerism.

Chapter 5: Metabolic Pathways of Glucose

5-1. Describe digestion and absorption of carbohydrates.
5-2. What is the major catabolic pathway of glucose under anaerobic conditions? Mention the steps in the pathway and indicate the key enzymes.
5-3. Describe the process of glycolysis. Explain how many molecules of ATP are formed in anaerobic and aerobic conditions.
5-4. In anaerobic glycolysis, lactic acid is generated. What is the reason for reduction of pyruvate to lactate?
5-5. What are the irreversible steps in glycolysis? How are these blocks circumvented?
5-6. Trace the pathway of gluconeogenesis. Mention the key enzymes.
5-7. How is glycogen broken down in the body? Explain the hormonal regulation of the pathway.

Write Short Notes on

5-1. Regulation of glycolysis.
5-2. Key enzymes of glycolysis.
5-3. Substrate level phosphorylation.
5-4. 2,3-bisphospho glycerate (2,3-BPG).
5-5. Cori's cycle.
5-6. Substrates for gluconeogenesis.
5-7. Regulation of gluconeogenesis.
5-8. Key enzymes of gluconeogenesis.
5-9. Action of glucagon on glycogenolysis.
5-10. Von-Gierke's disease.
5-11. Glycogen storage diseases.

Chapter 6: Regulation of Blood Glucose

6-1. What is the normal fasting blood glucose level? How is it regulated?
6-2. What are the hormones influencing blood sugar level and how are these hormones acting?
6-3. Discuss the changes in metabolism during diabetic mellitus.
6-4. What are the enzymes influenced by Insulin? What are the derangements seen in diabetes mellitus?
6-5. What are the indications of glucose tolerance test? What precautions are to be taken before doing a GTT? What are the abnormal curves obtained? What is impaired glucose tolerance?
6-6. Name the reducing sugars that may appear in urine and give the differential diagnosis of these clinical conditions.

Write Short Notes on

6-1. Key enzymes influenced by insulin.
6-2. Give the normal blood level of glucose.
6-3. Renal glycosuria.
6-4. Benedict's test.
6-5. Glucose tolerance test (GTT).
6-6. Reducing sugars in urine.
6-7. Glycated hemoglobin.

Chapter 7 : Other Sugars

7-1. Write the reactions of the oxidative phase of the hexose monophosphate shunt pathway. Which tissues have this pathway?
7-2. What is the significance of HMP shunt pathway?
7-3. Describe the process by which galactose is converted into glucose. Indicate the metabolic errors associated with this pathway.

Write Short Notes On

7-1. Significance of HMP shunt pathway.
7-2. Key enzyme of HMP shunt pathway.
7-3. Drug-induced hemolytic anemia.
7-4. Enzyme defect in galactosemia.
7-5. Enzyme defect in congenital cataract.
7-6. Hereditary fructose intolerance.
7-7. Essential pentosuria.
7-8. Metabolism of alcohol.

Chapter 8: Lipids, Chemistry

8-1. Classify lipids, giving examples.
8-2. Name the dietary lipids. Explain digestion and absorption of fats.
8-3. Explain the role of bile salts in the digestion and absorption of dietary lipids. Mention the changes observed in obstructive jaundice.

Write Short Notes on

8-1. Prostaglandins.
8-2. Cyclooxygenase.
8-3. Mechanism of action of aspirin.
8-4. Effect of prostaglandin on smooth muscles.
8-5. Lipid storage diseases.

Chapter 9: Metabolism of Fatty Acids

9-1. Explain the steps of beta-oxidation of palmitic acid, giving energetics.
9-2. Give the sources and fate of Acetyl-CoA.
9-3. Describe the de novo synthesis of fatty acids. What is the coenzyme required, and how is it regulated?
9-4. How are the fatty acids in adipose tissue mobilized and transported to other tissues? Explain the effect of hormones in this process.
9-5. What is fatty liver? Explain the causes of fatty liver. Indicate how lipotropic factors can prevent fatty liver.
9-6. What are ketone bodies? Explain the reactions leading to the formation of them. How are they utilized in the body?
9-7. Name the ketone bodies. Give two conditions characterized by excessive production of ketone bodies. Explain the metabolic derangements and consequences of ketosis.

Write Short Notes on

9-1. Rate limiting enzyme of fatty acid biosynthesis.
9-2. Carnitine.
9-3. Oxidation of odd chain fatty acids.
9-4. Metabolism of propionyI-CoA.
9-5. Effect of insulin on lipolysis.
9-6. Hormone sensitive lipase.
9-7. Fatty liver.
9-8. Lipotropic factors.
9-9. Ketosis.
9-10. Ketogenesis.

Chapter 10: Cholesterol

10-1. Classify lipoproteins. Explain their biological significance.
10-2. What is the normal cholesterol level in plasma? Explain how the cholesterol is transported from liver to peripheral tissues and back?
10-3. What is the normal cholesterol level in plasma? What is its clinical significance? What are the dietary precautions to reduce hypercholesterolemia?
10-4. How are dietary triglycerides absorbed and transported in plasma? Explain briefly the transport of dietary TAG from intestine to liver.
10-5. How are endogenously produced triglycerides transported in plasma? Explain the transport of them from liver.

Write Short Notes on

10-1. Biologically important compounds derived from cholesterol.
10-2. HMG-CoA reductase.
10-3. Regulation of cholesterol synthesis.
10-4. Key enzymes of cholesterol biosynthesis.
10-5. Chylomicrons.
10-6. HDL-cholesterol.
10-7. LDL-cholesterol.
10-8. Prevention of atherosclerosis.
10-9. Polyunsaturated fatty acids.

Chapter 11: Amino Acid Metabolism

11-1. Explain the term transamination. Give one suitable example. What is the metabolic importance of transamination? What is the clinical application of transaminase estimation?
11-2. Describe the reactions of the urea cycle. Discuss the interrelation of urea cycle and citric acid cycle.
11-3. Give details of the steps by which ammonia is detoxified in the brain and in liver.
11-4. Describe the reactions of urea cycle. Discuss the diagnostic significance of blood urea.
11-5. Name six important compounds derived from glycine and indicate their functions.
11-6. Describe the metabolism of methionine. Explain the term transmethylation with suitable examples.
11-7. What is the biochemical basis of homocystinuria? What test you will do to diagnose homocystinuria?
11-8. Describe the steps of catabolism of phenylalanine and tyrosine. Indicate the inborn errors of metabolism associated with this pathway.
11-9. Describe the steps by which catecholamines are synthesized. What is the final excretory product of catecholamines?

Write Short Notes on

11-1. Proteolytic enzymes of gastrointestinal tract.
11-2. Digestion of proteins.
11-3. Transamination.
11-4. Transdeamination.
11-5. Decarboxylation of amino acids.
11-6. Name the important compounds formed from glycine.
11-7. Creatinuria.
11-8. Biological action of glutathione.
11-9. Gamma-aminobutyric acid (GABA).
11-10. Homocystinuria.
11-11. Give 4 examples of transmethylation reactions.
11-12. S-adenosyl methionine (active methionine).
11-13. Phenyl ketonuria.
11-14. Alkaptonuria.
11-15. Albinism.
11-16. Important compounds derived from tyrosine.
11-17. Hydroxyindoleacetic acid (HIAA).
11-18. Synthesis and catabolism of serotonin.
11-19. Carcinoid syndrome.
11-20. Histamine.
11-21. Name the ketogenic amino acids.
11-22. Give the enzyme defect in the following conditions: (a) methyl malonyl aciduria; (b) cystathioninuria; (c) homocystinuria; (d) phenylketonuria; (e) alkaptonuria; (f) albinism.

Chapter 12: Plasma Proteins

12-1. Enumerate the major transport proteins of plasma. Explain the transport of free fatty acids, bilirubin, iron.
12-2. What are the important functions of albumin? Give the major causes and manifestations of hypo-albuminemia.
12-3. Indicate the electrophoresis abnormalities observed in the following conditions: (a) Cirrhosis liver; (b) Nephrotic syndrome and (c) Multiple myeloma.

Write Short Notes on

12-1. Albumin-globulin ratio
12-2. Enumerate transport proteins of blood.
12-3. Ceruloplasmin.
12-4. Transferrin.
12-5. Immunoglobulins.
12-6. Bence Jones proteins.
12-7. Multiple myeloma.
12-8. Immunoglobulin E and its clinical significance
12-9. Give the normal blood level of the following: (a) glucose; (b) albumin; (c) globulins; (d) total protein; (e) cholesterol; (f) creatinine; (g) urea.

Chapter 13: Citric Acid Cycle

13-1. Discuss the formation of acetyl-CoA from pyruvate. How is acetyl-CoA is further metabolized in the citric acid cycle?
13-2. Give an account of the citric acid cycle and explain why it is called the common terminal metabolic pathway.
13-3. Write the steps of citric acid cycle. What is the biological significance of this cycle?
13-4. Describe the reactions of the citric acid cycle. How many ATP molecules are generated per molecule of acetyl-CoA entering the cycle?
13-5. Write the members of the electron transport chain, in the order of redox potentials, and show the steps where ATP is synthesized.
13-6. Define oxidative phosphorylation. Explain the chemiosmotic theory.

Write Short Notes on

13-1. Energy releasing steps of citric acid cycle.
13-2. Sites of production of ATP in ETC.
13-3. Inhibitors of ETC.
13-4. Oxidative phosphorylation.
13-5. Uncouplers of oxidative phosphorylation.
13-6. Chemiosmotic theory.
13-7. Cytochromes.
13-8. Energy rich compounds.

Chapter 14: Heme and Hemoglobin

14-1. Give an account of heme synthesis.
14-2. What is porphyria? Give an account of acute intermittent porphyria.
14-3. Describe the catabolism of heme in the body.
14-4. How bile pigments are formed? Give the significance of their presence in blood and urine.
14-5. Classify jaundice. How do you investigate a case of jaundice?
14-6. Discuss the biochemical alterations seen in blood and urine in different types of jaundice.

14-7. How is bilirubin formed in the body? Describe how it is excreted? Describe the biochemical changes in hepatocellular jaundice and obstructive jaundice.
14-8. What is the difference between hemoglobinopathies and thalassemias? Describe any one of the hemoglobinopathies in detail.
14-9. Give biochemical explanation for the finding that geographical distribution of glucose-6-phosphate dehydrogenase deficiency correlates well with malarial incidence.

Write Short Notes on

14-1. ALA synthase.
14-2. Acute intermittent porphyria.
14-3. Rate limiting step of heme synthesis.
14-4. Regulation of heme synthesis.
14-5. Formation of bilirubin.
14-6. Catabolism of heme
14-7. Hemolytic jaundice.
14-8. Hepatocellular jaundice.
14-9. Obstructive jaundice.
14-10. Urobilinogen.
14-11. Isohydric transport of carbon dioxide.
14-12. Oxygen dissociation curve.
14-13. Abnormal hemoglobins.
14-14. Hemoglobinopathies.
14-15. Sickle cell anemia.
14-16. Hemoglobin S.
14-17. Hemoglobin F.
14-18. Thalassemia.

Chapter 15: Fat Soluble Vitamins

15-1. Describe sources, biochemical functions, requirement and deficiency manifestations of vitamin A.
15-2. Describe the sources, biochemical functions, normal requirement and deficiency manifestations of vitamin D.

Write Short Notes on

15-1. Sources and daily requirement of vitamin A.
15-2. Functions of vitamin A.
15-3. Visual cycle.
15-4. Hypervitaminosis A.
15-5. Provitamins.
15-6. Antivitamins.
15-7. Functions of vitamin D.
15-8. Activation of vitamin D.
15-9. Vitamin D deficiency.
15-10. Tocopherol.
15-11. Biological role of vitamin K.

Chapter 16: Water Soluble Vitamins

16-1. Describe the source, biochemical functions, normal requirement and deficiency manifestations of thiamine.
16-2. Describe the sources, biochemical functions, normal requirement and deficiency manifestations of pyridoxal phosphate.
16-3. Describe sources, biochemical functions, requirement and deficiency manifestations of vitamin C.

Write Short Notes on

16-1. Functions of thiamine pyrophosphate.
16-2. Deficiency of thiamine.
16-3. Beriberi.
16-4. Metabolic role of riboflavin.
16-5. Coenzyme function of niacin.
16-6. Pellagra.
16-7. Functions of pyridoxal phosphate.
16-8. Coenzyme functions of biotin.
16-9. Deficiency of folic acid.
16-10. Folate antagonists.
16-11. Deficiency of vitamin B12.
16-12. Biological role of vitamin B12.
16-13. Absorption of vitamin B12.
16-14. Methylmalonyl aciduria.
16-15. What is the normal daily requirement of: (a) Thiamine; (b) Folic acid; (c) Vitamin B12; (d) Pyridoxine; (e) Riboflavin; (f) Niacin; (g) Vitamin C.

Chapter 17: Mineral Metabolism

17-1. What is the normal blood level of calcium? What are the mechanisms by which calcium homeostasis is maintained?
17-2. Describe the sources, daily requirement, absorption, biochemical functions and deficiency manifestations of iron.
17-3. Name any three trace elements and mention the biological functions of each of them.

Write Short Notes on

17-1. Factors influencing calcium absorption.
17-2. Homeostasis of blood calcium level.
17-3. Hemosiderosis.

17-4. Absorption of iron.
17-5. Transferrin.
17-6. Causes and deficiency manifestations of iron.
17-7. Ceruloplasmin.
17-8. Wilson's hepatolenticular degeneration.
17-9. Sources and requirement of potassium.
17-10. Hyperkalemia.
17-11. Hypokalemia.
17-12. Functions of sodium.
17-13. Metabolic role of zinc.
17-14 Lead poisoning.

Chapter 18: Nutrition

18-1. Define BMR. What are the factors that affect BMR?
18-2. What is a balance diet? How do you prepare a diet for a normal young adult male of sedentary habits?
18-3. What is the nutritional importance of dietary proteins? Explain how the dietary deficiency of proteins will affect growing children.

Write Short Notes on

18-1. Basal metabolic rate.
18-2. Specific dynamic action.
18-3. Biological value of proteins.
18-4. Nitrogen balance.
18-5. Essential amino acids.
18-6. Essential fatty acids.
18-7. Protein calorie malnutrition.
18-8. Kwashiorkor.

Chapter 19: Detoxification

19-1. What are xenobiotics? Describe the role of glutathione in detoxification.
19-2. What is meant by detoxification? Give an account of various detoxification processes.

Write Short Notes on

19-1. Metabolic role of glucuronic acid.
19-2. Detoxification by conjugation.
19-3. Detoxification by oxidation.
19-4. Detoxification by hydrolysis.
19-5. Detoxification of aspirin.
19-6. Detoxification of bilirubin.
19-7. How is each of the following compounds detoxified: (a) aspirin; (b) ethyl alcohol; (c) bilirubin; (d) barbiturates.
19-8. Detoxification of hydrogen peroxide.
19-9. Antioxidants.
19-10. Free radical scavenger mechanisms.
19-11. Reactive oxygen species (Free radicals).

Chapter 20: Acid–Base Balance

20-1. What is the normal pH of blood? Explain the role of plasma buffers and renal mechanisms in the maintenance of acid–base balance of the body.
20-2. Name the important buffer systems in the body. Describe the role of kidney and lungs in the maintenance of acid–base balance.
20-3. What is titratable acidity of urine? What is the role of kidney in maintaining acid–base balance?
20-4. What is metabolic acidosis? Enumerate its causes. What are the compensatory mechanisms?

Write Short Notes on

20-1. Bicarbonate buffer system of blood.
20-2. AIkali reserve.
20-3. Renal acidification of urine.
20-4. Urinary ammonia.
20-5. Role of kidney in the regulation of pH.
20-6. Anion gap.
20-7. Metabolic acidosis.
20-8. Respiratory acidosis.
20-9. Metabolic alkalosis.
20-10. Respiratory alkalosis.
20-11. Give the normal blood level of the following: (a) chloride; (b) bicarbonate; (c) sodium; (d) potassium; (e) pH.

Chapter 22: Saliva and Dental Caries

22-1. What is the composition of saliva?
22-2. Give an account of the composition of teeth.
22-3. Describe the process of caries formation.

Short Notes

22-1. Organic components of saliva.
22-2. Salivary enzymes.
22-3. Calcium apatite.
22-4. Proteins of teeth.
22-5. Relation of sucrose with caries.
22-6. *Streptococcus mutans.*
22-7. Prevention of caries.
22-8. Fluoride and caries.
22-9. Fluorosis.

Chapter 23: Nucleotides

23-1. Give the sources of carbon and nitrogen atoms of purine and pyrimidine rings. How is the de novo synthesis regulated? Indicate the clinical uses of inhibitors of purine nucleotide synthesis.
23-2. Describe the inborn errors of metabolism associated with degradation pathways of purines.

Write Short Notes on

23-1. Sources of carbon and nitrogen atoms in purine ring.
23-2. Regulation of purine synthesis.
23-3. Xanthine oxidase.
23-4. Formation of uric acid.
23-5. Purine catabolism.
23-6. Gout.
23-7. Lesch-Nyhan syndrome.
23-8. Orotic aciduria.

Chapter 24 : DNA Structure, Replication

24-1. Describe the structure of DNA. What are the differences between DNA and RNA?
24-2. What are the salient features of Watson-Crick model of DNA?
24-3. Describe the process of DNA replication. Name two inhibitors of replication.

Write Short Notes on

24-1. Watson and Crick model of DNA.
24-2. Base pairing rule.
24-3. Difference between DNA and RNA.
24-4. DNA polymerase.
24-5. Okazaki pieces.
24-6. Inhibitors of replication.

Chapter 25 : Transcription and Translation

25-1. Describe different types of RNA.
25-2. Give a detailed account of the transcription process. How is it regulated? Name inhibitors of transcription.
25-3. What is a codon? Describe the salient features of genetic code.
25-4. Describe the phases of activation, initiation, elongation and termination of biosynthesis of protein.
25-5. Describe the steps of protein synthesis.

Write Short Notes on

25-1. RNA polymerase.
25-2. Post-transcriptional modifications.
25-3. Introns and exons.
25-4. Inhibitors of transcription.
25-5. Ribosomes.
25-6. Initiation of translation.
25-7. Translocation.
25-8. Structure and function of tRNA.
25-9. Genetic code.
25-10. Degeneracy of codons.
25-11. Post-translational modifications.
25-12. Inhibitors of protein biosynthesis.

Chapter 26 : Control of Gene Expression

26-1. Explain with suitable examples, how mutations result in abnormal proteins.
26-2. What is mutation? What are mutagens? Describe point mutation and frameshift mutation.

Write Short Notes on

26-1. Induction.
26-2. Repression.
26-3. Operon concept.
26-4. Mutations.
26-5. Mutagens.
26-6. Point mutation.
26-7. Frameshift mutation.

Chapter 27 : Recombinant DNA Technology

27-1. Describe recombinant DNA technology. What are the important applications of the technique?

Write Short Notes on

27-1. Restriction endonucleases.
27-2. Gene therapy.

Chapter 28 : Laboratory Techniques

28-1. Give the salient features of electrophoresis. What are the abnormalities that you could detect in serum electrophoresis?
28-2. Describe the principle and applications of RIA.

Write Short Notes on

28-1. Electrophoresis.
28-2. Paper chromatography.
28-3. Southern blotting.

28.4. Thin layer chromatography.
28-5. Radioimmunoassay.
28-6. ELISA (enzyme-linked immunosorbent assay).

Chapter 29 : AIDS and Cancer

Write Short Notes on

29-1. Laboratory findings of HIV infection.
29-2. Oncogenes.
29-3. Oncosuppressor genes.
29-4. Tumor markers.
29-5. Alpha fetoprotein.
29-6. Name the tumor marker most appropriate for the following: (a) prostate carcinoma; (b) choriocarcinoma; (c) colon cancer; (d) hepatoma; (e) pheochromocytoma; (f) carcinoid syndrome; (g) bone metastasis.

Chapter 30: Liver and Kidney Function Tests

30-1. Enumerate liver function tests and describe in detail any two of them with clinical significance.
30-2. Classify jaundice. How do you investigate a case of jaundice?
30-3. How creatinine clearance test is done? What is its diagnostic significance?
30-4. How urea clearance test is done? What is its clinical significance?

Write Short Notes on

30-1. Enzymes used as liver function tests.
30-**2.** Albumin globulin ratio.
30-3. van den Bergh test.
30-4. Clinical significance of serum bilirubin level.
30-5. Proteinuria.
30-6. Creatinine clearance test.
30-7. Urea clearance test.
30-8. Give the normal blood level of the following: (a) glucose; (b) albumin; (c) Globulins; (d) total protein; (e) creatinine; (f) urea; (g) chloride; (h) bicarbonate; (i) sodium; (j) potassium.

Chapter 31 : Hormones

31-1. What is cyclic AMP? What is its metabolic importance?
31-2. Describe the synthesis and secretion of thyroxine.
31-3. Enumerate the thyroid function tests. Describe any one of them in detail.

Write Short Notes on

31-1. G-proteins
31-2. Transduction of message
31-3 Second messenger
31-4. Biological effects of glucocorticoids
31-5. TSH stimulation test.
31-6. T3 suppression test.

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