



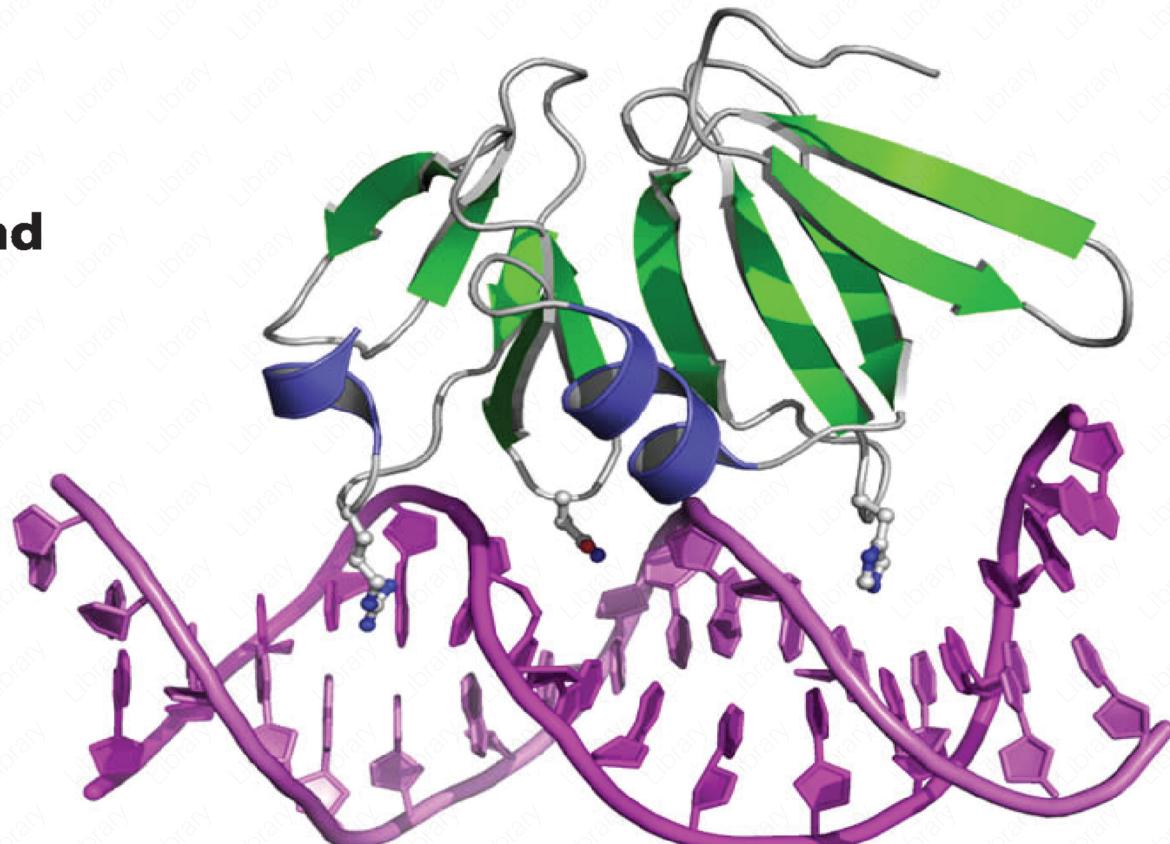
Manjula Shantaram



Biochemistry & Nutrition for BSc (Nursing)

Forewords
BM Hegde
Susan Anand

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Biochemistry and Nutrition for BSc (Nursing)

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Biochemistry and Nutrition for BSc (Nursing)

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*With modesty and respect
to
The Honorable Chancellor of Yenepoya University
and
The Chairman of Islamic Academy of Education
Al-Haj Yenepoya Abdulla Kunhi*

Foreword

In the electrobiochemical model of human physiology, biochemistry forms a very important foundation. Medical students, who do not have a deeper understanding of chemistry, find it hard to grasp the principles of biochemistry easily in their first year in the medical school. A good textbook that explains the various nuances of that subject in an easy-to-understand style is the need of the hour. Dr Manjula Shantaram's book fills that void very well.

As a good student and a professor of biochemistry in a medical school, Manjula is well suited to write the book. I was pleased to go through the chapters written in simple English and lucid style. The chapters are well thought out and well presented. She has followed the syllabus of the University in letter and spirit.

The style is praiseworthy. The subject is divided into small side headings that are very important to understand the subject as also to answer questions in the examination. The chapter on the energy requirement of the body is very well presented to make the subject so easy even for a novice to understand and remember easily. The other chapters follow suit. Balanced diet chapter gives clear-cut diagrams that can easily be replicated in the examination papers.

Nutritional importance of biomolecules chapter has some beautiful pictures that make learning a pleasure. Similar pictures adorn other chapters in relevant pages. The font, layout, as also the printing are excellent. I would have loved to have a textbook like this if I were a student now in first MBBS class. I dare say that this book could be a valuable addition to any good practicing doctor's library as a ready reference to give a patient a diet chart or to understand the nuts and bolts of metabolic abnormalities, like diabetic ketoacidosis. All in all, this is a praiseworthy addition to the hoards of textbooks in the medical schools.

While I compliment Manjula for bringing out this book, I commend the publishers for an excellent job well done. I can easily rate this book very high and wish that the book benefits a large section of students for years to come although, I hope, Manjula will have newer editions fast enough to keep pace with the rapid pace of information pouring into this field of knowledge.

BM Hegde

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Former Vice Chancellor, Manipal University, Manipal, India

Foreword

Comprehensive Nursing Practice is based on application of knowledge derived from physical, biological and behavioral sciences, medicine and nursing. Hence, the importance of sound knowledge of biochemical processes in diseases cannot be undermined. The subject of biochemistry becomes meaningful and interesting to students only when they are able to discern normal biochemical composition and functioning of human body and specific alterations in the biochemistry in disease conditions.

I congratulate the author of this book Dr Manjula Shantaram, Associate Professor, Department of Biochemistry, Yenepoya Medical College, Deralakatte for her venture of writing a textbook for nursing students, which is written in a simple and lucid language keeping in mind the needs of the students for a user-friendly textbook.

Susan Anand
Former Principal
Yenepoya Nursing College
Mangalore, Karnataka, India

Preface

This textbook *Biochemistry and Nutrition for BSc (Nursing)* is compiled with the objective of providing a simple, concise, comprehensive and yet easily understandable course material of biochemistry for BSc Nursing students.

The chapters are selected as per the latest syllabus prescribed by Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, Karnataka, India for graduate nurses in the first year of their course.

Since there are very few textbooks available on biochemistry for graduate nurses, I have made an attempt to venture into this area. While evaluating the RGUHS answer scripts of Biochemistry of BSc Nursing students, I had determined to complete the compilation process which was begun a couple of months ago.

Instead of blaming the students for their bad answers, let us provide them a textbook with easy-to-understand text, self-explanatory flow charts, attractive diagrams, self-assessment, multiple choice questions and repeatedly asked questions in the RGUHS examinations during the last ten years. This would definitely help the students to equip themselves better to face the University examinations.

I welcome criticism along with useful suggestions and comments from the students and faculty for the improvement of this textbook.

Manjula Shantaram

Acknowledgments

I am grateful to the almighty God for giving me strength, perseverance, patience, health and time to compile the matter for this textbook.

I gratefully acknowledge the blessings of my mother, late Smt Savithri R Kamath, father, Dr Ranganath R Kamath and my aunt, Dr Meera R Kamath for instilling in me the joy of teaching, love of scholarship and the value of being organized.

I am thankful to my husband, Mr Shantaram Gadiyar and son, Mr Vivek Gadiyar who graciously overlooked my preoccupied nature while I spent long hours with chapters of this book.

I am grateful to Dr Ronald Roche of Microbiology Department of Yenepoya Medical College for having contributed a small chapter on immunological aspects. I am thankful to Dr Ganesh Prasad V and Dr Nivedita L Rao of Biochemistry Department for their contribution in some chapters.

This book would never have been accomplished without the encouragement and support of my colleagues and associates, Mrs Vidya Bernhardt, Mr Eric Christopher Lobo and Dr U Raghavendra.

My heartfelt thanks are due to Ms Pramila for her excellent clerical work.

My gratitude is extended to M/s Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India for bringing out this book to my utmost satisfaction.



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Abbreviations

ACP	: Acyl carrier protein
ADP	: Adenosine diphosphate
AMI	: Antibody-mediated immunity
AMP	: Adenosine monophosphate
APC	: Antigen presenting cells
AST	: Aspartate transaminase
ATP	: Adenosine triphosphate
BPG	: 2,3 Bisphosphoglycerate
CMI	: Cell-mediated immunity
CNS	: Central nervous system
CoA	: Coenzyme A
CP	: Ceruloplasmin
CRP	: C-reactive protein
CSF	: Cerebrospinal fluid
CTP	: Cytidine triphosphate
Cyt	: Cytochrome
DAG	: Diacyl glycerol
DCT	: Distal convoluted tubules
DNA	: Deoxyribonucleic acid
ECF	: Extracellular fluid
EF	: Elongation factor
ETC	: Electron transport chain
FA	: Fatty acid
FAB	: Fragment for antigen binding
FAD	: Flavin adenine dinucleotide
FFA	: Free fatty acid
FMN	: Flavin mononucleotide
GABA	: Gamma-aminobutyric acid
GAG	: Glucosaminoglycans
GDP	: Gaunosine diphosphate
GFR	: Glomerular filtration rate
GTT	: Glucose tolerance test
GTP	: Gaunosine triphosphate
Hb	: Hemoglobin
HDL	: High density lipoprotein
HLA	: Human leukocyte antigens
HMGCoA	: β Hydroxy β methyl glutaryl CoA

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HMP shunt	: Hexose monophosphate shunt
ICF	: Intracellular fluid
IDL	: Intermediate density lipoproteins
IFNs	: Interferons
ILs	: Interleukins
LCAT	: Lecithin cholesterol acyltransferase
LDH	: Lactate dehydrogenase
LDL	: Low density lipoprotein
MAG	: Monoacyl glycerol
MHC	: Major histocompatibility complex
MPO	: Myeloperoxidase
mRNA	: Messenger RNA
NAD	: Nicotinamide adenine dinucleotide
NADH	: Nicotinamide adenine dinucleotide reduced
NADP	: Nicotinamide adenine dinucleotide phosphate
NMN	: Nicotinate mononucleotide
NMR	: Nuclear magnetic resonance
PABA	: Para-amino benzoic acid
PBI	: Protein bound iodine
PCT	: Proximal convoluted tubules
PEPCK	: Phospho enol pyruvate carboxy kinase
PFK	: Phosphofructokinase
PLP	: Pyridoxal phosphate
PTH	: Parathyroid hormone
PUFA	: Polyunsaturated fatty acid
QPRT	: Quinolinate phosphoribosyl transferase
RBC	: Red blood cell
RER	: Rough endoplasmic reticulum
RF	: Release factor
RNA	: Ribonucleic acid
ROS	: Reactive oxygen species
SER	: Smooth endoplasmic reticulum
SGOT	: Serum glutamate oxaloacetate transaminase
SGPT	: Serum glutamate pyruvate transaminase
SOD	: Superoxide dismutase
TA	: Titrable acidity
TAG	: Triacyl glycerol
TCA	: Tricarboxylic acid cycle
TG	: Triglyceride
TNFs	: Tumor necrosis factors
TPP	: Thiamine pyrophosphate
t-RNA	: Transfer ribonucleic acid
UDP	: Uridine diphosphate
VLDL	: Very low density lipoprotein
α -KG	: α -Ketoglutarate



1.1: DEFINITION AND SCOPE

Biochemistry is the chemistry of life and it explains all cellular or biological events in chemical terms. The chemical reactions that occur in biological systems are called biochemical reactions. The scope of biochemistry is as vast as life itself. Every aspect of life such as birth, growth, reproduction, aging and death involves biochemistry.

Biochemists study the chemical processes that occur in microorganisms, plants, insects, fish, birds, lower and higher mammals, human beings. Biochemistry of less complex forms of life is of direct relevance to human biochemistry.

Knowledge of biochemistry is essential to all life sciences such as genetics, physiology, immunology, pharmacology, pharmacy, toxicology, pathology, microbiology, zoology and botany. These branches employ biochemical approaches almost exclusively.

From a biochemical view point, health may be considered as that situation in which all of those thousands of intra- and extracellular reactions that occur in the body are proceeding at the rates proportionate with its maximal survival in the physiologic state.

Biochemical research influences on nutrition since dietary intake of a number of chemicals is mandatory for the maintenance of health. Chief chemicals are vitamins, certain amino acids, certain fatty acids, various minerals and water.

Biochemical studies contribute to diagnosis, prognosis and treatment of diseases. If a particular vitamin is deficient in the diet, a deficiency disease may result, such as scurvy or rickets (due to lack of intake of vitamins C and D respectively). These diseases are treated by administration of the appropriate vitamins. The condition called phenyl ketonuria (PKU) if untreated may lead to severe mental retardation in infancy. It results due to the deficiency or absence of enzyme that converts amino acid phenyl alanine to the amino acid tyrosine. A diet low in phenyl alanine is being given as a treatment to the affected infants.

Biochemical investigations are used to reveal the causes of diseases, suggest rational and effective treatments, make available screening tests

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for early diagnosis, assist in monitoring progress and help in assessing response to therapy.

1.2: THE CELL

A cell is the basic, functional and structural unit of all forms of life. On the basis of differences in cell structure, all life forms are divided into two major classes. These are prokaryotes and eukaryotes. Prokaryotes are simple cells and mostly, the individual cell itself is the organism. They contain a cell wall and the cytoplasm is not divided into compartments. Bacteria and primitive green algae are the examples of prokaryotes. All other organisms like plants, animals, fungi, protozoa, yeast and true algae are multicellular and are called eukaryotes.

Biological Importance

- All higher living organisms including humans are made up of cells.
- Human body contains a wide variety of cells that differ in structure and function.
- Human cell contains subcellular structures like nucleus, mitochondria, lysosomes, peroxisomes, Golgi bodies, endoplasmic reticulum, etc.
- Some diseases are due to lack of subcellular structures. For example, Zellweger's syndrome is due to lack of functional peroxisomes.
- Lysosomal enzymes are involved in spreading of cancer.
- Growth of cells requires cell division. Cell cycle encompasses all the events of cell division.
- Cells are not immortal. They have finite life span and hence humans are not immortal.
- Cell death is crucial for shaping of organs during development and for recovery from injuries.

MOLECULAR COMPOSITION OF CELL

Water

Water accounts for about 70 to 75 percent of the weight of the cell. Other cellular constituents are either dissolved or suspended in water.

Organic Compounds

- Organic compounds account for 25 to 30 percent of the cell weight.
- They are nucleic acids, proteins, polysaccharides (carbohydrates) and lipids. Proteins account for 10 to 20 percent of the weight of the cell. Nucleic acids account for 7 to 10 percent of the cell weight. Polysaccharides usually account for 2 to 5 percent of the cell weight. About 3 percent of cell weight is due to lipids. Lipid content may be

higher in adipocytes or fat cells. Proteins may account for more of cell weight in cells like erythrocytes.

- Other low molecular weight organic compounds may account for 4 percent of cell weight. They are monosaccharides, amino acids, fatty acids, purine and pyrimidine nucleotides, peptides, hormones, vitamins and coenzymes.

Inorganic Compounds

- Inorganic compounds account for the rest of the cell weight.
- They are cations like sodium, potassium, calcium, magnesium, copper, iron and anions like chloride, phosphate, bicarbonate, sulphate, iodide and fluoride.

Eukaryotic Cell Structure and Function

In eukaryotes, cells aggregate to form tissues or organs and these are further organized to form whole organism. In humans, eukaryotic cells exist in large number of sizes and shapes to perform several functions. For example, nerve cells differ from liver cells which differ from muscle cells and they differ in function also. Though the eukaryotic cells differ in size and shapes, they have certain common structural features. Further, eukaryotes contain subcellular structures and well defined nucleus. Cells are surrounded by membranes. It separates the cells from their surroundings and it is called plasma or cell membrane. The other subcellular organelles are also composed in part by membranes.

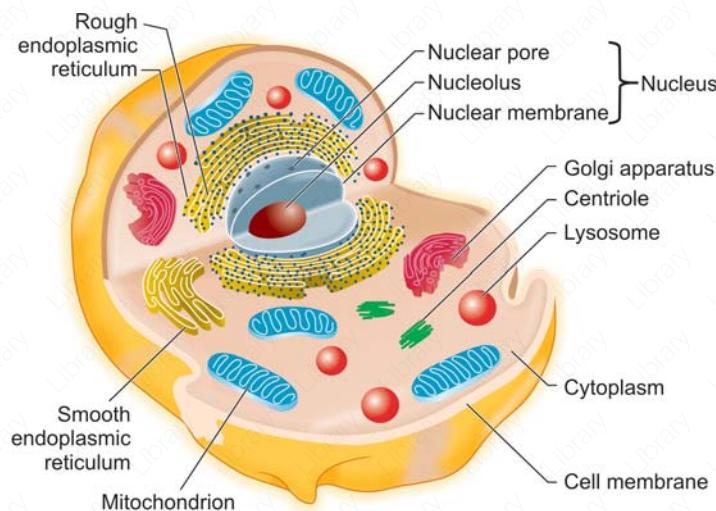


Fig. 1.2.1: A diagrammatic representation of a typical eukaryotic cell

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Subcellular Organelles and their Functions

Table 1.2.1 depicts biochemical functions of subcellular organelles of the eukaryotic cell.

Table 1.2.1: Biochemical functions of subcellular organelles of the eukaryotic cell

<i>Subcellular organelles</i>	<i>Function</i>
Plasma membrane	Transport of molecules in and out of cell, receptors for hormones and neurotransmitters
Lysosome	Intracellular digestion of macromolecules and hydrolysis of nucleic acid, protein, glycosaminoglycans, glycolipids, sphingolipids
Golgi bodies	Post-transcriptional modification and sorting of proteins and export of proteins
Rough endoplasmic reticulum	Biosynthesis of protein and secretion of protein
Nucleus	Storage of DNA, replication and repair of DNA, transcription and post-transcriptional processing
Peroxisomes	Metabolism of hydrogen peroxide and oxidation of long chain fatty acids
Nucleolus	Synthesis of r-RNA and formation of ribosomes
Mitochondrion	ATP synthesis, site for tricarboxylic acid cycle, fatty acid oxidation, oxidative phosphorylation, part of urea cycle and part of haeme synthesis
Smooth endoplasmic reticulum	Biosynthesis of steroid hormones and phospholipids, metabolism of foreign compounds (cytochrome P ₄₅₀ detoxification)
Cytosol	Site for glycolysis, pentose phosphate pathway, part of gluconeogenesis, urea cycle and haeme synthesis, purine and pyrimidine nucleotide synthesis

CELL MEMBRANE

Structure

- Cell membrane is the outer most structure of the cell and decides its shape.
- It is a lipid bilayer and consists of proteins and small amount of carbohydrates also.

Functions

- Cell membrane is fluid and dynamic.
- It is semi-permeable, only selected compounds are allowed to pass through from outside. The selective permeability is responsible for the maintenance of internal environment of the cell and, for creating potential difference across the membrane.

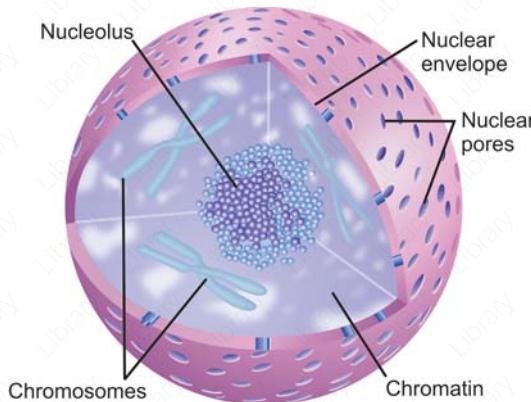


Fig. 1.2.2: Cell nucleus

- The modification of cell membrane results in formation of specialized structures like axon of nerves, microvilli of intestinal epithelium and tail of spermatids.

NUCLEUS

Structure

- Center of the cell is nucleus.
- It is surrounded by a double layered membrane of about $250\text{-}400\text{\AA}$ thick.
- The two layers of nuclear membrane are an outer and inner membrane (layer). The two membranes fuse periodically to produce nuclear pores. Exchange of material between nucleus and rest of the cell occurs through nuclear pores.
- The outer nuclear membrane is continuous with other cytomembranes. In some eukaryotic cells like erythrocyte the nucleus is absent. In spermatozoa, nucleus accounts for 90 percent of the cell whereas in other cells nucleus accounts for less than 10 percent of the cell. In prokaryotes the nucleus is not well defined and is called incipient nucleus.

Functions

- Nucleus is the information center of eukaryotic cell. More than 90 percent of the cellular DNA is present in nucleus. It is mainly concentrated in the form of chromosomes.
- Human cell contains 46 chromosomes. These chromosomes are composed of nucleoprotein chromatin which consists of DNA and histone proteins. Some RNA may also be present in nucleus.
- In prokaryotes the DNA is present as thread in the cytosol.

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NUCLEOLUS

Structure and Function

These are small dense bodies present in the nucleus. Their number varies from cell to cell. There is no membrane surrounding them. They are continuous with nucleoplasm. Protein accounts for 80 percent of nucleolus, remainder is DNA and RNA.

Nucleoplasm: It is also known as nuclear matrix. It contains enzymes involved in the synthesis of DNA and RNA.

CYTOSOL/CYTOPLASM OR CELL SAP

Structure

- The extra cell content that possesses both organelles and other materials constitutes cytoplasm. Material other than subcellular components in the cytoplasm makes up the cytosol or cell sap.
- Sometimes, the soluble portion of the cell is referred to as cytosol. Cytoplasm accounts for 70 to 75 percent weight of the cytosol.

Functions

- Numerous enzymes, proteins and many other solutes are found in cytosol.
- Cytosol is the main site for glycolysis, HMP shunt, activation of amino acids and fatty acid synthesis.

MITOCHONDRIA

Structure

- Mitochondria are the second largest structures in the cell.
- Generally mitochondria are ellipsoidal in shape and can assume a variety of shapes.
- The length of typical mitochondria is about 7 microns and has a diameter of 1 micron.
- Mitochondria consist of outer and inner membranes. The outer membrane is composed of equal amount of proteins and lipids.
- The lipids are mainly phospholipids and cholesterol. The outer membrane functions as limiting membrane and is permeable to many compounds.
- The inner membrane consists of 75 percent protein and remainder is lipid.
- Cardiolipin is the important phospholipid of inner mitochondrial membrane.

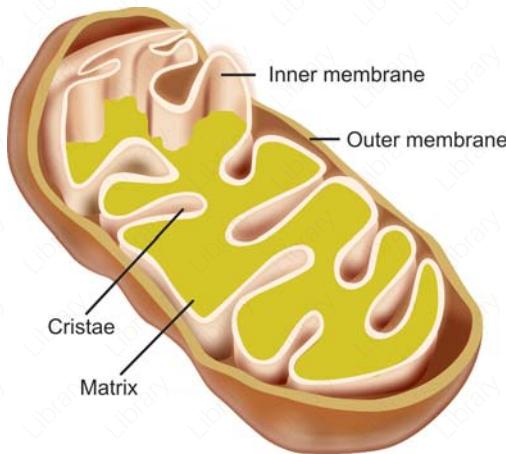


Fig. 1.2.3: Mitochondria inner structure

- The inner membrane is convoluted to form a number of invaginations known as cristae extending to matrix.
- These cristae are covered with knob like structures, which are composed of a head piece, stalk and a base piece.

Functions

- Mitochondria are the power houses of the cell. They are responsible for the production of energy in the form of ATP. The knob like structures function in electron transport and oxidative phosphorylation.
- The number of mitochondria ranges from 1 to 100 per cell, depending on the type of cell and its function. Several factors influence the size and number of mitochondria in cells.
- In highly metabolically active cells mitochondria are more and large.
- Location of mitochondria in the cell also depends on types and functions of the cell. In the liver cell mitochondria are scattered and may range up to 2000, whereas in the kidney they may range up to 300.
- Mitochondria also contain other energy producing pathways like citric acid cycle, fatty acid oxidation and ketone body oxidation.
- Some reactions of gluconeogenesis and urea cycle also occur in mitochondria.
- Mitochondria are capable of synthesizing some of their proteins.
- Mitochondria contain some DNA known as mitochondrial DNA and ribosomes.

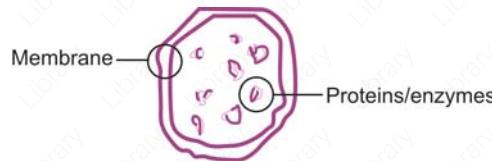


Fig. 1.2.4: Lysosome structure

LYSOSOMES

Structure

- They are small vesicles present in cytoplasm.
- They are surrounded by a membrane. Lysosomes are called “suicide bags” of the cell (Fig. 1.2.4).

Functions

- Lysosomes are rich in hydrolytic enzymes which are active at acidic pH. The lysosomal enzymes digest the molecules brought into the cell by phagocytosis.
- Macrophages are rich in lysosomes.

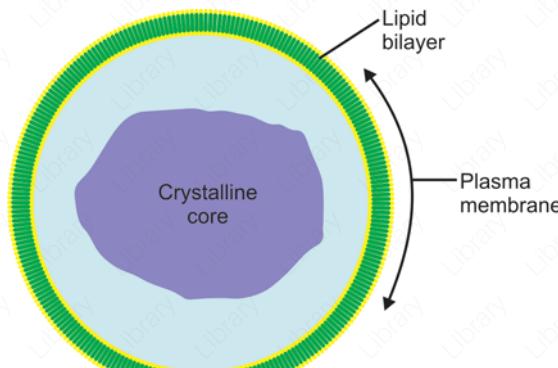
Biological Importance

- Lysosomal enzymes are involved in bone remodeling and intracellular digestion.
- Disease, shock or cell death causes rupture of lysosomes and release of enzymes. In some organisms lysosomal enzymes are responsible for the cell death of larval tissues.
- Lack of one or more of lysosomal enzymes can cause accumulation of materials in the cell, resulting in lysosomal diseases.
- In some diseases like arthritis and muscular dystrophy, lysosomal enzymes are released to cause uncontrolled destruction of surrounding tissues.
- Lysosomal proteases called cathepsins are involved in spreading of cancer (metastasis).

PEROXISOMES

Structure

Peroxisomes are also small vesicles surrounded by a membrane. They are also known as microbodies (Fig. 1.2.5).

**Fig. 1.2.5:** Peroxisome

Functions

- They contain enzymes of H_2O_2 metabolism. The concentration of protein in peroxisomes is very high and, they may occur in crystalline form. The enzymes of H_2O_2 catabolism present in peroxisomes are peroxidase and catalase.
- Peroxisomes also contain other enzymes like D, L-amino acid oxidase, uric acid oxidase and L-hydroxy fatty acid oxidase that generates H_2O_2 . Glycerophospholipids are also synthesized in peroxisomes.

Biological Importance

- Lack of peroxisomes result in Zellweger's syndrome.

CYTOMEMBRANES

There is an extensive network of membranes in the cytoplasm. They are called cytomembranes. They are divided into endoplasmic reticulum and Golgi complex. The endoplasmic reticulum is further subdivided into rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER).

Rough Endoplasmic Reticulum

Structure

- It is continuous with the outer nuclear membrane.
- The cytoplasmic surface of rough endoplasmic reticulum is coated with ribosomes. Membrane enclosed channels of endoplasmic reticulum are called cisternae. The ribosomes are complexes of RNA and protein. Figure 1.2.6 shows endoplasmic reticulum.

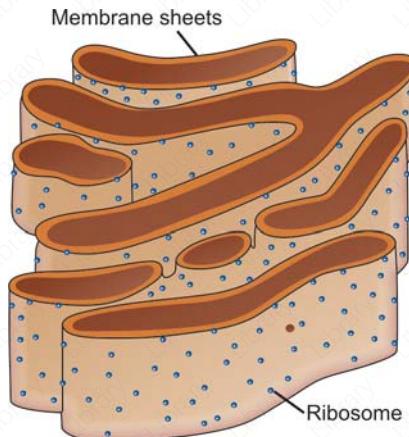


Fig. 1.2.6: Endoplasmic reticulum

Functions

- Ribosomes and rough endoplasmic reticulum are involved in protein synthesis.
- Synthesized protein enters cisternae and is later extruded.

SMOOTH ENDOPLASMIC RETICULUM

Structure

- It is continuous with rough endoplasmic reticulum. It differs from RER by the absence of ribosomes. When isolated, SER is known as microsomes.

Functions

- SER of intestinal cells is involved in formation of triglycerides.
- In the adrenal cortex SER is the site of steroid formation.
- Cytochrome P₄₅₀ dependent mono-oxygenases are present in the liver cell SER.

GOLGI BODIES (FIG. 1.2.7)

Structure

- It consists of a cluster of paired cytomembranes. The margins of these cytomembranes are flattened.
- It also contains several small vesicles, which are pinched off from the flattened margins of membranes.

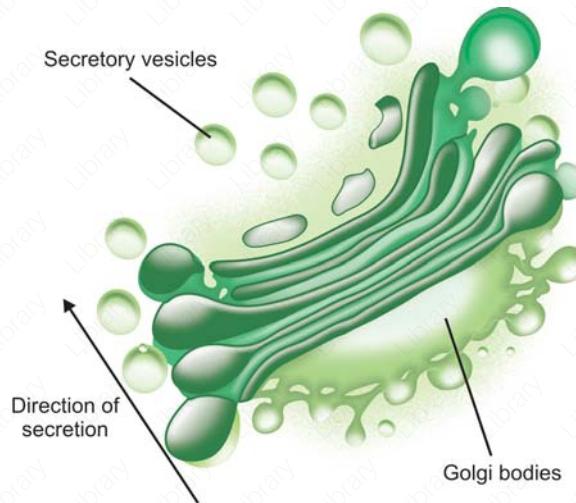


Fig. 1.2.7: Golgi bodies

Functions

- The Golgi bodies are well developed in cells which are involved in secretion. Material produced in the cell for export is processed by the Golgi body and is packaged as vesicle and is pinched off. The vesicles fuse with plasma membrane and their content is released to the exterior by the process known as exocytosis. The digestive enzymes of pancreas and insulin hormones are produced and released in this way.
- Golgi bodies help in the formation of other subcellular organelles like lysosomes and peroxisomes.
- Golgi bodies are involved in protein targeting. It directs proteins to be incorporated into membranes of other subcellular structures. It is also involved in glycosylation and sulfation of proteins.

Biological Importance

Some cases of diabetes are due to defective processing of insulin in Golgi complex.

Vacuoles

Some animal cells contain vacuoles. They are membrane enclosed vesicles containing fluid. Mostly they contain nutrients.

Cell Coat

Some mammalian cells contain a thin coat known as the cell coat on the outer surface of the cell membrane. The cell coat is flexible and sticky. It is composed of mucopolysaccharides, glycolipids and glycoproteins. The adhesive properties of cell and organization of tissue is controlled by the cell coat.

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CYTOSKELETONS (FIG. 1.2.8)

These are filament like structures made up of proteins present in the cytoplasm. Non-muscle cells perform mechanical work with this intracellular network of proteins. Cytoskeletons are microfilaments, myosin fibers, microtubules and intermediate filaments.

Comparison between prokaryotic and eukaryotic cells are depicted in Table 1.2.2.

Table 1.2.2: Comparison between prokaryotic and eukaryotic cells

Characteristics	Prokaryotic cell	Eukaryotic cell
1. Size	Small (generally 1-10 μm)	Large (generally 10-100 μm)
2. Cell membrane	Cell is enveloped by a rigid cell wall	Cell is enveloped by a flexible plasma membrane
3. Cell wall	Non-cellulosic	Cellulosic only in plants
4. Subcellular organelles	Absent	Distinct organelles are found (e.g. mitochondria, nucleus, lysosomes)
5. Nucleus	Not well defined; DNA is found as nucleoid, histones are absent. Nuclear envelope absent	Nucleus is well defined, surrounded by a membrane; DNA is associated with histones
6. Cell division	Usually fission and no mitosis	Mitosis or meiosis
7. Cytoplasm	Organelles and cytoskeleton absent	Contains organelles and cytoskeleton (a network of tubules and filaments)
8. Chromosomes	Single	Multiple
9. Nucleolus	Absent	Present
10. Ribosomes	70S (50S + 30S)*	80S (60S + 40S)*
11. Endomembranes	Absent	Present
12. Mitochondria	Respiratory and photosynthetic enzymes in the plasma membrane	Present
13. Chloroplast	Absent	Present in plant cells
14. Exocytosis and endocytosis	Absent	Present
15. Locomotion	Single fibril, flagellum	Cilia and flagella

* S refers to the Svedberg sedimentation unit which is a function of the size and shape of molecules.

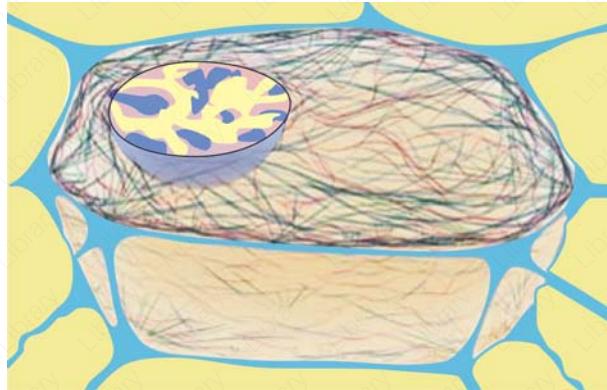


Fig. 1.2.8: Cytoskeleton

MULTIPLE CHOICE QUESTIONS

1. The subcellular organelle which is the primary consumer of respiratory oxygen is:
A. Mitochondrion B. Lysosome
C. Peroxisome D. Nucleus
 2. Smooth endoplasmic reticulum is associated with:
A. Protein synthesis B. Lipid synthesis
C. Synthesis of ketone bodies D. Pentose phosphate pathway of glucose oxidation
 3. Intracellular digestive enzymes are present in:
A. Cytosol B. Microsomes
C. Lysosomes D. Golgi apparatus
 4. The cellular organelle is designated as the power house of the cell is:
A. Lysosome B. Mitochondrion
C. Golgi body D. Nucleus
 5. Plasma membrane refers to:
A. The inner membrane of the cell B. The outer membrane of the cell
C. The mitochondrial membrane D. The outer nuclear membrane

ANSWERS

1. A 2. B 3. C 4. B 5. B

MOST LIKELY QUESTIONS

Long Essay

1. Draw an animal cell diagram and label various cell organelles. Write functions of mitochondria, Golgi bodies and lysosomes.

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Short Essays

1. Give the structure and functions of endoplasmic reticulum.
2. Name organic substances present in cell.
3. What are the functions of nucleolus?
4. Write a note on peroxisomes.
5. Draw a neatly labeled diagram of mitochondrion and write its importance.
6. On what basis the mitochondrion is described as "The Power House of the Cell"?

Short Answers

1. What are cytoskeletons?
2. What are lysosomes?

1.3: MICROSCOPY

A microscope (Greek: micron = Small + skopein = to look at) is an instrument for viewing objects that are too small to be seen by the naked or unaided eye. The science of investigating small objects using such an instrument is called microscopy. The term microscopic means minute or very small, not visible with the eye unless aided by a microscope.

The most common type of microscope and the first to be invented is the optical microscope. This is an optical instrument containing one or more lenses that produce an enlarged image of an object placed in the focal plane of the lens.

Microscopes can largely be separated into two classes, optical theory microscopes and scanning probe microscopes.

Optical theory microscopes are microscopes which function through the optical theory of lenses in order to magnify the image generated by the passage of wave through the sample. The waves used are either electromagnetic in optical microscopes or electron beams in electron microscopes. The types are the Compound Light, Stereo, and the Electron microscope.

Optical Microscopes

Optical microscopes, through their use of visible wavelengths of light, are the simplest and hence most widely used type of microscopes. Today compound microscopes, i.e. especially those with a series of lenses, have several uses in many fields of science, particularly biology and geology.

Optical microscopes use refractive lenses, typically of glass to focus light into the eye or another light detector. Typical magnification of a light microscope is up to $1500\times$ with a theoretical resolution of around 0.2 micrometers.



The Components of the Microscope

Basic optical microscope elements:

1. Ocular lens, or eyepiece
2. Objective turret
3. Objective lenses
4. Coarse adjustment knob
5. Fine adjustment knob
6. Object holder or stage
7. Mirror
8. Diaphragm and condenser
9. Mechanical stage

All optical microscopes share the same basic components:

Eyepiece: A cylinder containing two or more lenses to bring the image to focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification.

Typical magnification values for eyepieces include 5 \times , 10 \times and 2 \times . In some high performance microscopes, the optical configuration of the objective lens and eyepiece are matched to give the best possible optical performance.

Objective lens: A cylinder containing one or more lenses to collect light from the sample. At the lower end of the microscope tube one or more objective lenses are screwed into a circular nose piece which may be rotated to select the required objective lens. Typical magnification values of objective lenses are 4 \times , 5 \times , 10 \times , 20 \times , 40 \times , 80 \times and 100 \times . Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

Stage: A platform below the objective which supports the specimen being viewed. In the center of the stage is a circular hole through which light passes to illuminate the specimen. The stage usually has arms to hold slides (rectangular glass plates with typical dimensions of 25 mm by 75 mm, on which the specimen is mounted).

Illumination source: Below the stage, light is provided and controlled in a variety of ways. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own controllable light source that is focused through an optical device called a condenser, with diaphragms and filters available to manage the quality and intensity of the light.

The whole of the optical assembly is attached to a rigid arm which in turn is attached to a robust U shaped foot to provide the necessary rigidity. The arm is usually able to pivot on its joint with the foot to allow the viewing angle to be adjusted. Mounted on the arm are controls for focusing,



Fig. 1.3.1: Microscopy

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typically a large knurled wheel to adjust coarse focus, together with a smaller knurled wheel to control fine focus.

Updated microscopes may have many more features, including transmission, illumination, phase contrast microscopy and differential interference contrast microscopy and digital cameras.

On a standard compound optical microscope, there are three objective lenses: A scanning lens ($4\times$), low power lens ($10\times$) and high power lens ($40\times$). Advanced microscopes often have a fourth objective lens, called an oil immersion lens. To use this lens, a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective element is immersed in the oil film. Such immersion lenses are designed so that the refractive index of the oil and of the cover slip is closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a power of $100\times$.

The actual power or magnification of an optical microscope is the product of the powers of the ocular (eyepiece), usually about $10\times$, and the objective lens being used.

Compound optical microscopes can produce a magnified image of a specimen up to $1000\times$ and, at high magnifications, are used to study thin specimens as they have a very limited depth of field.

How a Microscope Works?

Optical Path in a Typical Microscope

The optical components of a modern microscope are very complex and for a microscope to work well, the whole optical path has to be very accurately set up and controlled. Despite this, the basic optical principles of a microscope are quite simple.

The objective lens is, at its simplest, a very high powered magnifying glass, i.e. a lens with a very short focal length. This is brought very close to the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By careful focusing a rather dim image of the specimen, much enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement.

In most microscopes, the eyepiece is a compound lens, with one component lens near the front and one near the back of the eyepiece tube. This forms an air-separated couplet.

MOST LIKELY QUESTIONS

Short Essays

1. Draw a neatly labeled diagram of an optical microscope.
2. How does a microscope work?

Chapter

2

Structure and Functions of Cell Membrane

2.1: CELL MEMBRANE

The cell is enveloped by a thin membrane called cell membrane or plasma membrane. Cell membrane separates cells from their external environment and divides the interior of the cell into compartments. Cell membrane is 75 to 90 Å thick. The fluid outside the cell membrane is called extracellular fluid (ECF), while that inside the cell, covered by the cell membrane is called intracellular fluid (ICF).

Structure and Chemical Composition of Cell Membrane

- Cell membranes mainly consist of lipids, proteins and smaller proportion of carbohydrates that are linked to lipids and proteins.
- The chemical composition of cell membranes varies widely as shown in Table 2.1.1.
- The basic organization of biologic membranes is illustrated in Figure 2.1.1.
- Membranes are asymmetric. The two faces of a membrane are different. The outer and inner surfaces of all known biological membranes have different components and different enzymatic activities.
- Membranes are fluid structures. The unsaturated fatty acids bound to phospholipids contribute to the fluid state of the membrane. At body temperature lipids are in a fluid state and this fluidity of the membrane is essential for the normal functioning to occur, e.g. exocytosis and endocytosis and lysosomal activity.

Table 2.1.1: Chemical composition of cell membranes

Membrane	Chemical composition by weight		
	Protein (percent)	Lipid (percent)	Carbohydrate (percent)
Erythrocyte	49	43	8
Outer mitochondrial membrane	50	46	4
Inner mitochondrial membrane	75	23	2
Myelin	20	75	5

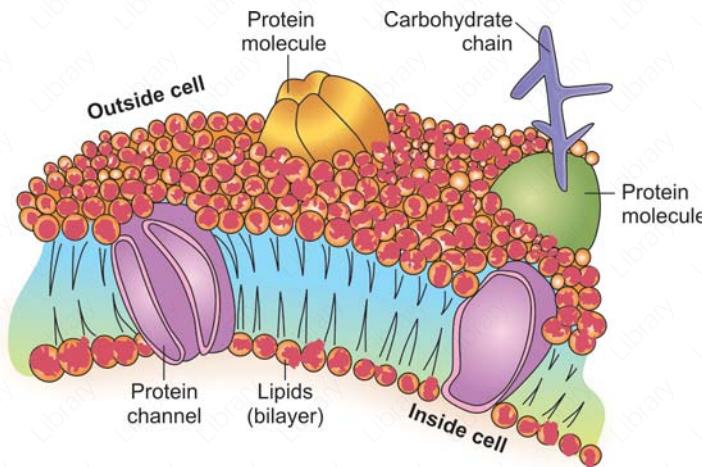


Fig. 2.1.1: The basic organization of biological membrane

- The cell membrane is anchored to the cytoskeleton which is a network of microfilaments and microtubules that interact with each other and with the components of the plasma membrane.

Membrane Lipids

- The major classes of membrane lipids are:
 - Phospholipids
 - Glycolipids and
 - Cholesterol
 They are all amphipathic molecules having both hydrophobic and hydrophilic ends.
- Membrane lipids spontaneously form bilayer in aqueous medium, burying their hydrophobic tails and leaving their hydrophilic ends exposed to the water.

Functions of Cell Membrane

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

Fluid Mosaic Model of Cell Membrane

- In 1972, Singer and Nicolson postulated a theory of membrane structure called the fluid mosaic model.

- The plasma membrane is composed of different kinds of macromolecules like phospholipids, integral proteins, peripheral proteins, glycoproteins, glycolipids and cholesterol.
- The bilayer is fluid because the hydrophobic tails of its polar lipids consist of an appropriate mixture of saturated and unsaturated fatty acids that is fluid at normal temperature of the cell.
- This lipid bilayer has a dual role having both as a solvent for integral membrane proteins and a permeability barrier.
- Proteins are interspersed in the lipid bilayer, of the plasma membrane, producing a mosaic effect.
- The peripheral proteins laterally float on the surface of the predominantly phospholipid molecules, whereas the integral proteins are completely submerged in the hydrocarbon core.
- Fluid mosaic model allows the membrane proteins to move around laterally in two dimensions unless restricted by special interactions and that they are free to diffuse from place to place within the plane of the bilayer.
- The fluid-mosaic model can explain many of the physical, chemical and biological properties of membranes and has been widely accepted as the most probable molecular arrangement of lipids and proteins of membranes.

CYTOSKELETON

These are filament-like structures made up of proteins present in cytoplasm. Non-muscle cells perform mechanical work with these intracellular networks of proteins.

- a. *Microfilaments*: They are made up of filamentous proteins called actins. They form a meshwork just underlying the cell membrane of many cells and are referred to as stress fibers. Microfilaments help muscle contraction, cellular movement and maintain shape of the cell. Microvilli are maintained due to the presence of bundles of actin filaments.
- b. *Microtubules*: Tubulin is the building block of microtubules. Dendrites, axons of nerve cells and sperm cells contain microtubules. The sperm cell moves with the help of flagellum, a microtubule. These cytoskeletons are involved in the maintenance of cell shape, cell division, cell motility, phagocytosis, endocytosis and exocytosis.
- c. *Intermediate filaments*: They are not involved in movement of cell. They are stable components of cytoskeleton. Neurofilament of neurons, glial filaments of glial cells and keratin of epithelial cells are some examples of intermediate filaments.

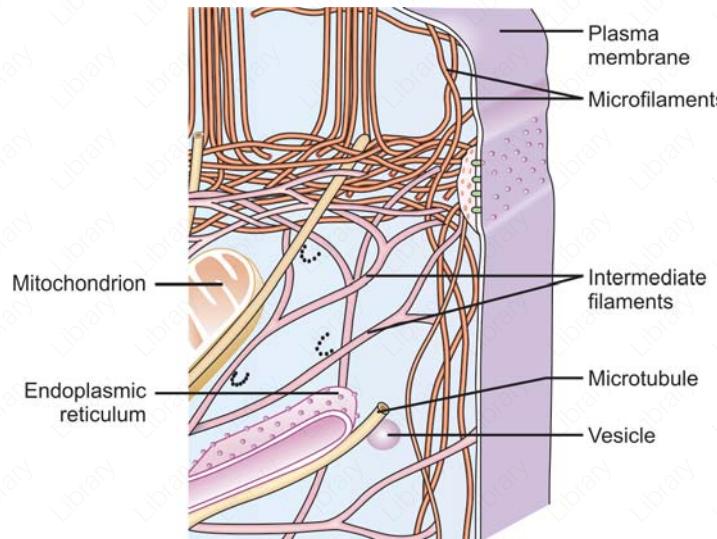


Fig. 2.1.2: Cytoskeleton

2.2: TRANSPORT MECHANISMS

Several transport systems (transporters) present in the membrane regulate the flow of solute molecules between the cell and its surroundings. Further, they are involved in the:

1. Regulation of cell volume
2. Maintenance of intracellular pH required for optimum activity of cellular enzymes
3. Uptake and concentration of nutrients from the environment
4. Removal of toxic substances
5. Generation of ionic gradients across the membrane which are essential for nerve impulse transmission and muscle contraction.

These transport systems may move one solute molecule in any one direction. Then the process is known as uniport. If the transport system moves two solute molecules in same direction, then the process is symport (co-transport). Sometimes the transport system moves two solute molecules in opposite directions, and then the process of transport is known as antiport.

The permeability of the biological membrane is highly selective. The lipid or non-polar molecule can easily pass through the membrane because of their solubility in the membrane lipid bilayer. Even water and uncharged molecules of a smaller size are freely permeable to membrane. Charged solute molecules and molecules like proteins and carbohydrates are not permeable.

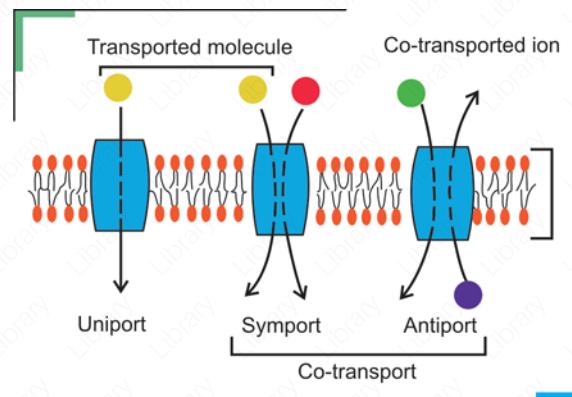


Fig. 2.2.1: Transport mechanisms

Transport mechanisms (Fig. 2.2.1) are classified into passive and active; passive transport is again subclassified into simple diffusion and facilitated diffusion. Ion channels are specialized carrier systems. Ion channels allow passage of molecules in accordance with the concentration gradient, but pumps can drive molecules against the gradient using energy.

Simple Diffusion

Solutes and gases enter into the cells passively. They are proportional to 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. However, it is a very slow process.

Facilitated Diffusion (Fig. 2.2.2)

This is a carrier-mediated process. Important features of facilitated diffusion are:

1. Structurally similar solutes can competitively inhibit the entry of the solutes.
2. Facilitated diffusion can operate bidirectionally.
3. This mechanism does not require energy but the rate of transport is more rapid than diffusion process.
4. It is dependent on concentration gradient.

Active Transport

The salient features of active transport are:

1. This form of transport requires energy. About 40 percent of the total energy expenditure in a cell is used for the active transport system.

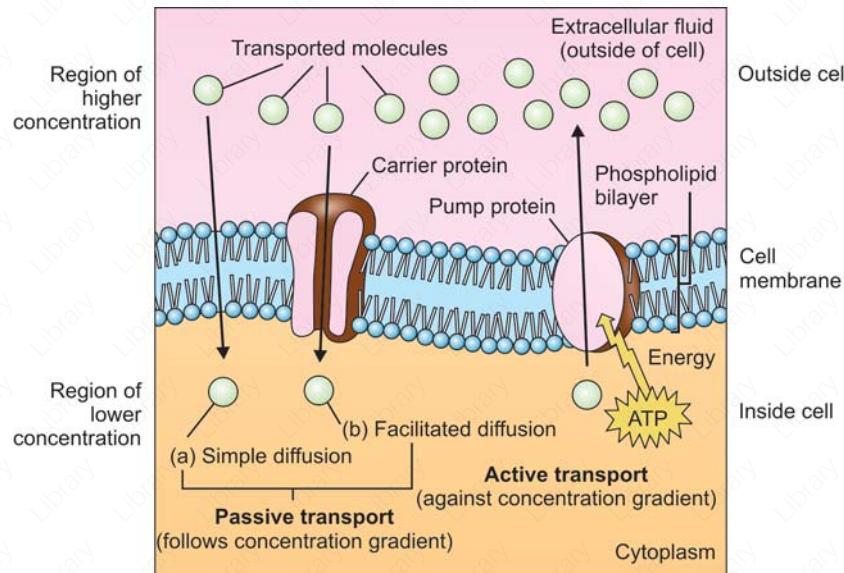


Fig. 2.2.2: Facilitated diffusion

2. The active transport is unidirectional.
3. It requires specialized integral proteins called transporters.
4. The transport system is saturated at higher concentration of solutes.
5. The transporters are susceptible to inhibition by specific organic or inorganic compounds.

Sodium Pump

It is the best example for active transport. Cell has high concentration of potassium inside the cell but has low intracellular sodium; this is maintained by the sodium-potassium activated ATPase, generally called as sodium pump. The ATPase is an integral protein of the membrane. It has binding sites for ATP and sodium on the inner side and the potassium binding site is located outside the membrane.

The hydrolysis of one molecule of ATP can result in expulsion of 3 Na^+ ions and influx of 2 K^+ ions. The first event is binding of sodium to the cytosolic side of the pump. Some subunits of ATPase are phosphorylated by ATP, which results in aversion of the binding site and release of Na^+ on the extracellular side. The binding of potassium removes the phosphate. This would again revert the binding site to its original orientation. K^+ is released inside the cell. Ion transport and ATP hydrolysis are tightly coupled.

Endocytosis

This is another form of transport whereby extracellular material is incorporated into the cytoplasm. Pinocytosis is one of the methods of endocytosis. Pinocytosis can be a selective process mediated by receptors on the cell membrane or it can be non-selective.

Small invaginations are formed on the cell membrane, the edges of which finally fuse to form a vesicle enclosing the extracellular material which now becomes intracellular. The vesicle migrates into the cytoplasm and gets adherent to a lysosome. The lysosomal enzymes act on the membranous wall of the vesicle to release the contents of the vesicle into the cytoplasm. Receptors, if any, are recycled back to cell surfaces.

Osmosis

Osmosis is defined as the spontaneous flow of water into a solution or from a more dilute to a more concentrated solution when the two solutions are separated from each other by a semi-permeable membrane.

Osmosis occurs in the direction opposite to that in which diffusion occurs. The animal membranes are not completely "semi-permeable". The artificial membrane, $\text{Cu}_2\text{Fe}(\text{CN})_6$, is the most selective. This is prepared by keeping copper crystals in the pores and dissolved in $\text{K}_4\text{Fe}(\text{CN})_6$ solution.

Experiment

In Figure 2.2.3, the semi-permeable membrane is fitted at the mouth of the thistle funnel. The thistle funnel is provided with a sucrose solution up to a mark and inverted over water in a trough. After sometime it has been found that the level of sucrose solution is increased in the thistle funnel

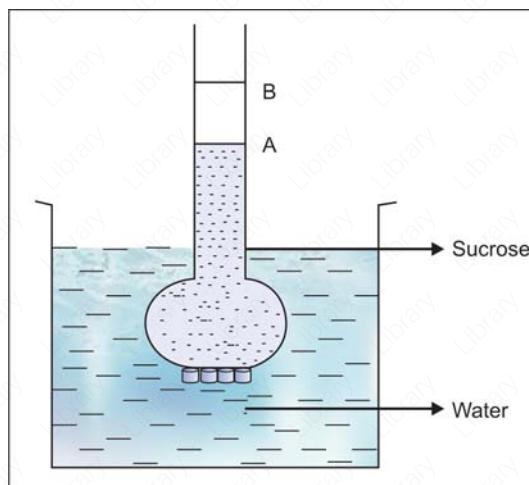


Fig. 2.2.3: Osmosis and osmotic pressure

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owing to the entry of water through the semi-permeable membrane. The flow of water will be prevented when both the pressure (inside and outside) are equal. The excess pressure can be measured by a mercury manometer.

Definition

Osmotic pressure can be defined as the excess pressure which must be applied to a solution to prevent the flow of solvent of low osmotic pressure when they are separated by a perfectly semi-permeable membrane.

Physiological Importance

1. Absorption from gastrointestinal tract, fluid interchanges in various compartments of the body follow the principle of osmosis.
2. The osmotic pressure of plasma proteins regulates water to flow from the protein-free intestinal fluid into the blood vessels.
3. Living red cells if suspended in 0.92 percent NaCl solution, they neither gain nor lose water. Briefly speaking, intracellular fluid of red cells is isotonic with the red cell membrane in 0.92 percent NaCl solution.

Application of Osmosis

1. The purgative action of Epsom ($MgSO_4 \cdot 7H_2O$) or Glauber's ($Na_2SO_4 \cdot 10H_2O$) salts is an osmotic phenomenon. A strong solution of a salt in the intestine prevents absorption of water or withdrawal of water from the body causing dilution of the intestinal contents.
2. The pain caused by contact of sugar with exposed nerves of teeth is due to the osmotic withdrawal of water from the exposed area by strong sugar solution.
3. The pain experienced by the application of salt on the cut of the skin follows the same osmotic phenomenon as in No. 2.
4. Water or salts (chiefly NaCl) are excreted by the kidney to keep the blood isotonic with the cells. Hence, osmosis is of great importance in the process of urine secretion.
5. The clinical application of osmotic force is the injection of hypertonic solution of magnesium sulphate to reduce the volume of the brain or lower the pressure of cerebrospinal fluid.
6. Hemolysis is caused by the dilution of RBC by the osmotic phenomenon.

Filtration

Filtration is a mechanical/physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium to fluid flow through which the fluid can pass, but the solids (or at least part of the solids) in the fluid are retained. It has to be emphasized that the

separation is not complete, and it will depend on the pore size and the thickness of the medium as well as the mechanism that occur during filtration.

Filtration is used for the purification of fluids, for instance, separating dust from the atmosphere to clean ambient air.

Filtration as a physical operation is very important in chemistry for the separation of materials of different chemical composition in solution (or solids which can be dissolved) by first using a reagent to precipitate one of the materials and then use a filter to separate the solid from the other material(s).

The filtration process separates particulates and fluid from a suspension, and the fluid can be either a liquid or a gas (or a supercritical fluid). To separate a mixture of chemical compounds, a solvent is chosen which dissolves one component, while not dissolving the other. By dissolving the mixture in the chosen solvent, one component will go into the solution and pass through the filter, while the other will be retained. This is one of the most important techniques used by chemists to purify compounds.

There are many different methods of filtration; all aim to attain the separation of two or more substances. This is achieved by some form of interaction between the substance or objects to be removed and the filter. In addition, the substance that is to pass through the filter must be a fluid, i.e. a liquid or gas.

The simplest method of filtration is to pass a solution of a solid and fluid through a porous interface so that the solid is trapped, while the fluid passes through. This principle relies upon the size difference between the particles making up the fluid, and the particles making up the solid. In the laboratory, a Buchner funnel is often used, with a filter paper serving as the porous barrier.

The kidney's ability to perform many of its functions depends on the three fundamental functions, out of which filtration is one of them apart from re-absorption and secretion.

Filtration in Kidney

The blood is filtered by nephrons, the functional units of the kidney. Each nephron begins in a renal corpuscle, which is composed of a glomerulus enclosed in a Bowman's capsule. Cells, proteins, and other large molecules are filtered out of the glomerulus by a process of ultrafiltration, leaving an ultrafiltrate that resembles plasma (except that the ultrafiltrate has negligible plasma proteins) to enter Bowman's space.

The ultrafiltrate is passed through, in turn, the proximal tubule, the loop of Henle, the distal convoluted tubule, and a series of collecting ducts to form urine.

2.3: ACID-BASE BALANCE

The normal pH of the blood is slightly alkaline and is maintained in the narrow range of 7.35 to 7.45. That is because, body enzymes can function optimally only at a particular pH to drive metabolic reactions. Thus, life can be sustained only if pH is maintained within specified limits. Maintenance of pH is important because during metabolic reactions, acids are produced which include volatile acids like carbonic acid (20,000 mEq/day) and non-volatile acids like lactic acid, keto acids, sulfuric acid, phosphoric acid (60-80 mEq/day). Some amount of bicarbonate is generated from organic acids. These can profoundly alter pH of body fluids. Thus for proper understanding of acid-base balance, knowledge of acids, bases and pH is essential.

According to proton transfer theory of Bronsted and Lowry (1923), acids are proton donors and bases are proton acceptors.

Example:

- $\text{HCl} \leftrightarrow \text{H}^+ + \text{Cl}^-$
 - $\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$
(Acid) (Conjugate base)
 - $\text{NH}_3 + \text{H}^+ \leftrightarrow \text{NH}_4^+$
 - $\text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{H}_2\text{CO}_3$
(Base) (Conjugate acid)

Strong acids dissociate completely in a solution to give very high H⁺ concentration.

Example: HCl, HNO₃, H₂SO₄, etc.

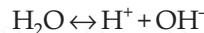
Weak acids do not ionize completely. Hence, H^+ ions are less

Example: H_2CO_3 , CH_3COOH , etc.

Conjugate bases of strong acids (HCl) are weak bases (Cl^-) and conjugate bases of weak acids (H_2CO_3) are strong bases (HCO_3^-).

Dissociation of Water

Water can dissociate to give H^+ and OH^- to a very negligibly small extent.



The degree of dissociation increases with temperature. The concentration of H^+ ions in pure water will always be 1/100,00,000 gram moles/liter at $25^\circ C$.

Naturally, OH^- ion concentration will also be the same.

$$H^+ = 10^{-7} \text{ gram moles/liter}$$

$$\text{OH}^- = 10^{-7} \text{ gram moles/liter}$$

$$K_{w} = H^+ \times OH^-$$

$$w = 10^{-7} \times 10^{-7}$$

$$K_w = 10^{-14}$$

^w
(Ionic product of water)

This is not only for water, but for all solutions.

Any increase in H^+ is accompanied by decrease in OH^- so that product (K_w) is constant.

pH

pH is the negative logarithm of hydrogen ion concentration and it is inversely related to H^+ concentration.

$$pH = -\log H^+ \text{ or } \log \frac{1}{[H^+]}$$

pH scale is a logarithmic scale from 0 to 14. pH of pure water is 7 and it is neither acidic nor alkaline but neutral.

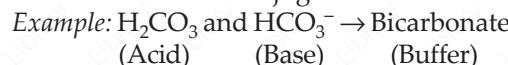
Buffer

Buffer is a system that resists any change in its pH when a small amount of acid or alkali is added to it. It comprises two major components: A weak acid (HA) and its conjugate base (A^-) and it is prepared by mixing:

1. Weak acid and its salt formed with a strong base:



2. Weak acid and its conjugate base.



3. Weak base and its salt formed with strong acid.



4. Mixture of 2 salts of polybasic acids.



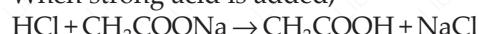
Factors Affecting pH of a Buffer

1. *The value of pK*: Lower the value of pK, lower is the pH of the solution.
2. *The ratio of salt to acid concentrations*: Actual concentration of salt and acid in a buffer solution may be varied widely, with no change in pH, as long as the ratio of the concentrations remains the same.

Mechanism of Action

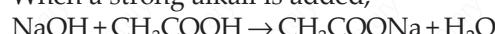
For example, take acetate buffer:

- When strong acid is added,



Since the CH_3COO^- ions neutralize the H^+ ions and the equilibrium shifts to left, there is no much change in the pH.

- When a strong alkali is added,

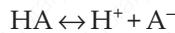


Since the H^+ ions neutralize the OH^- ions and the equilibrium shifts to right, there is no much change in the pH of the buffer.

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Determination of pH of buffers: Henderson Hasselbalch Equation:

Let us consider a weak acid (HA) dissociation:



(Acid) (Base)

For the above reaction, the dissociation constant K_a is,

$$K_a = \frac{[\text{H}^+] [\text{A}^-]}{[\text{HA}]} \quad (\text{From the Law of Mass Action})$$

$$[\text{H}^+] [\text{A}^-] = K_a [\text{HA}]$$

$$[\text{H}^+] = K_a \frac{[\text{HA}]}{[\text{A}^-]}$$

Taking -ve logarithms on both sides,

$$-\log \text{H}^+ = -\log K_a - \log \frac{[\text{HA}]}{[\text{A}^-]}$$

$$\text{pH} = \text{pKa} - \log \frac{[\text{Acid}]}{[\text{Base}]}$$

$$= \text{pKa} + \log \frac{[\text{Base}]}{[\text{Acid}]}$$

For a buffer, base components is usually salt.

Henderson-Hasselbalch Equation

$$\text{pH} = \text{pKa} + \log \frac{[\text{Salt}]}{[\text{Acid}]}$$

pH at which the acid is half ionized is called pKa of an acid which is constant at a particular temperature and pressure.

Applications of Henderson-Hasselbalch Equation

1. To find out pH of a buffer.
2. To find out the concentrations of salt and acid to be added to prepare a buffer of required pH.
3. To predict the limits of compensation of body buffers.
4. To assess the acid-base status.

Buffering Capacity

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. Buffering capacity is determined by the absolute concentration of the salt and acid. When the

ratio between salt and acid is 10:1, the pH will be 1 unit higher than the pKa. When the ratio between salt and acid is 1:10, the pH will be 1 unit lower than the pKa.

Buffer is most effective when:

Salt = Acid

$$\begin{aligned} \text{pH} &= \text{pKa} + \log 1 \\ &= \text{pKa} + 0 \end{aligned}$$

$$\text{pH} = \text{pKa}$$

Therefore, buffering capacity is maximum when its pH = pKa.

A buffer is effective +1 or -1 unit on either side of pKa, e.g. Phosphate buffer pKa = 6.8.

It is most effective at pH of 6.8 but can operate in the pH range of 5.8 to 7.8 ± 1 pKa.

Determination of pH in laboratory is by using ion sensitive electrodes.

Maintenance of Acid-Base Balance

Three mechanisms are involved to maintain pH in the body.

- i. Buffers of the body
- ii. Respiratory mechanism
- iii. Renal mechanism

These mechanisms are inter-related.

- i. Buffers of the body:
 - a. Extracellular (plasma) buffers
 - b. Intracellular (inside the cells) buffers
- a. *Extracellular buffers:* 
- Bicarbonate buffer $[\text{NaHCO}_3]/\text{H}_2\text{CO}_3$ or $\text{HCO}_3^-/\text{H}_2\text{CO}_3$
 Phosphate buffer $[\text{Na}_2\text{HPO}_4]/\text{NaH}_2\text{PO}_4$ or $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$
 Protein buffer $[\text{Pr}^-/\text{HPr}]$
- b. *Intracellular buffers:* 
- Phosphate buffer $[\text{Na}_2\text{HPO}_4]/\text{NaH}_2\text{PO}_4$ or $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$
 Protein buffer $[\text{Pr}^-/\text{HPr}]$
 Hemoglobin buffer $[\text{Hb}^-/\text{HHb}]$

Bicarbonate Buffer

It is a principal buffer in extracellular fluid (ECF) like plasma. It has HCO_3^- (or NaHCO_3) as base component and H_2CO_3 as acid component. Its pka value is 6.1. To have a pH of 7.4, the ratio of $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ should be 20. Only then, this buffer shows maximum efficiency.

$$[\text{HCO}_3^-] = 24 \text{ m moles}$$

$$[\text{H}_2\text{CO}_3] = 1.2 \text{ m moles}$$

$$\begin{aligned} \text{pH} &= 6.1 + \log \frac{24}{1.2} \\ &= 6.1 + \log 20 \\ &= 7.4 \end{aligned}$$

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Alkali Reserve

Plasma HCO_3^- concentration is 20 times higher than H_2CO_3 level. HCO_3^- ensures buffering of all acids produced in the body. Thus, HCO_3^- gives protection against pH changes produced by acids. Hence, the plasma HCO_3^- content is called the "alkali reserve".

Advantages of Bicarbonate Buffer

- The concentration of its components can be easily controlled by the body. HCO_3^- is regulated by kidneys, hence it is called the metabolic component and H_2CO_3 is regulated by lungs, hence it is called the respiratory component.
- HCO_3^- level is sufficiently high to buffer acid load.

Disadvantages of Bicarbonate Buffer

Efficiency of bicarbonate buffer is less at plasma pH of 7.4, since its pka is 6.1 (pH is far away from pka).

Phosphate Buffer ($\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$)

Phosphate buffer comprises of dibasic phosphate (HPO_4^{2-}) as base and monobasic phosphate (H_2PO_4^-) as acid component. It has a pK_a of about 6.8 and it is a principal intracellular buffer.

Advantages

It can operate effectively, since its pK_a is closer to blood pH of 7.4.

Disadvantages

- Concentration of phosphate buffer in plasma is too low.
- Components of buffer cannot be easily controlled.

Protein Buffers (Pr^-/HPr)

Protein buffers are of considerable importance in plasma and intracellular fluids. It is too low in concentration in lymph, interstitial fluids and cerebrospinal fluid (CSF). At pH 7.4, many plasma proteins exist as anions as Pr^- which can accept H^+ forming conjugate acid HPr. Thus, Pr^-/HPr pair serves as a buffer. Buffering capacity of plasma proteins is much less than Hb.

Hemoglobin buffer system: Most important buffer in RBC is hemoglobin (Hb^-/HHb) buffer system. It is the major buffer system of blood as well as erythrocytes. Hb operates only in erythrocytes.

The pK value of imidazole group (7.3) of histidine part of hemoglobin is close to body pH and hence Hb buffer system is effective at body pH. Further, high Hb concentration (14g/dl) makes it the major buffer of blood.

Buffers can act quickly but not permanently. 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 mechanisms (Role of lungs in acid-base balance): Lungs work in close association with bicarbonate buffer system. They maintain acid-base balance mainly by 2 mechanisms.

- a. Regulation of H_2CO_3 level of plasma.
- b. Excretion of CO_2 produced in the body by various metabolisms.

Regulation of pH by Regulating H_2CO_3 Levels

It is mediated by respiratory center of medulla. When plasma pH falls (acidosis), respiratory center is stimulated and thus respiratory rate is increased (hyperventilation). More CO_2 is exhaled and H_2CO_3 level falls. With this, $\text{HCO}_3^- / \text{H}_2\text{CO}_3$ ratio increases (as per Henderson-Hasselbalch equation) and pH is restored to normal.

When plasma pH increases (alkalosis), respiratory center is depressed and thus respiratory rate is lowered. Hence, CO_2 is accumulated and H_2CO_3 level is raised. $\text{HCO}_3^- / \text{H}_2\text{CO}_3$ ratio is decreased and pH is restored to normal.

Advantages

It is a very rapid mechanism (2-3 minutes), because respiratory center is highly sensitive to pH changes.

Disadvantages

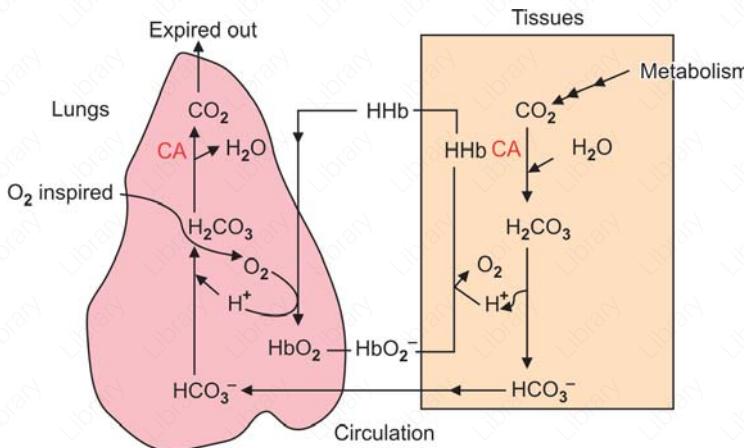
1. It does not ensure complete restoration of pH.
2. It cannot function in respiratory diseases.

Excretion of CO_2 by Lungs

Lungs excrete CO_2 produced by metabolism. CO_2 in H_2O forms H_2CO_3 which can alter plasma pH. Lungs excrete CO_2 and thus pH alteration of plasma is prevented.

Mechanism

- a. *In the lungs:* The formation of oxyHb (HbO_2) from deoxygenated Hb (HHb) must release H^+ ions, which will react with HCO_3^- to form H_2CO_3 . Because of low CO_2 tension in the lungs, the equilibrium then shifts towards the production of CO_2 , which is continually eliminated in the expired air.
- b. *In the tissues:* Due to reduced O_2 tension and local acidity, oxyHb (HbO_2) dissociates delivering O_2 to the cells and deoxygenated Hb (HHb) is formed. At the same time, CO_2 produced as a result of metabolism in



CA = Carbonic anhydrase

Fig. 2.3.1: Sequence of events that occur in lungs and tissues is shown schematically

the cells, is hydrated to form H_2CO_3 , which ionizes to form H^+ and HCO_3^- .

Deoxygenated Hb acting as an anion, accepts the H^+ ions, forming the so called acid-reduced Hb. Very little change in pH occurs because the newly arrived H^+ ions are buffered by formation of a very weak acid. CO_2 is thus transported as HCO_3^- and it is transported without much changes in plasma pH. It is called **isohydric transport**. The H^+ ions are buffered by the deoxy hemoglobin and this is called **Haldane effect**.

Renal Mechanisms (Role of Kidneys in Acid-base Balance)

Kidneys achieve second line of defense in acid-base balance. Though action is slow, it is complete. Important function of kidneys is to regulate the pH of the extracellular fluid. They excrete urine (pH around 6) with a pH lower than that of ECF (pH = 7.4). This is called acidification of urine. The pH of urine may vary from as low as 4.5 to as high as 9.8 depending on the amount of acid excreted. Urine is normally bicarbonate free.

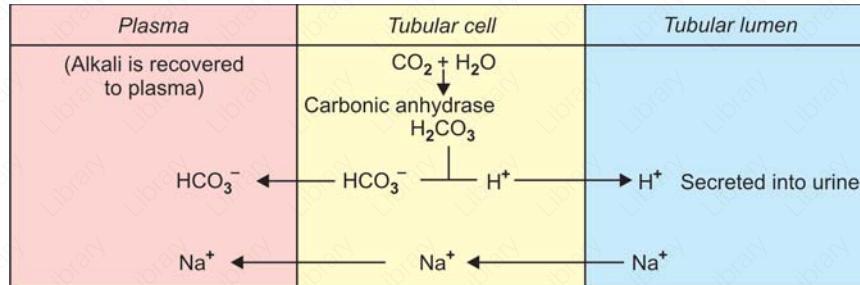
Acidification of Urine

Primary action of kidneys in maintaining acid-base balance is by secretion of H^+ into glomerular filtrate.

This process is aided by three mechanisms:

1. Bicarbonate mechanism
2. Phosphate mechanism
3. Ammonia mechanism

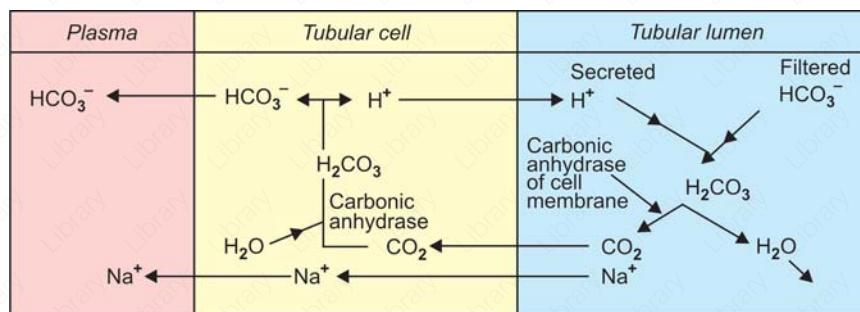
Acidification of Urine: Excretion of H^+



The process starts in proximal convoluted tubules (PCT) and continues up to distal convoluted tubules (DCT). As urine passes down the tubule, it becomes more and more acidic. H^+ concentration in glomerular filtrate goes on increasing. Further secretion of H^+ is not possible due to high concentration gradient of H^+ across renal tubular cell. To overcome this problem H^+ ions should be continuously buffered as and when they are secreted, which is by three mechanisms.

1. Bicarbonate mechanism
2. Phosphate mechanism
3. Ammonia mechanism

Bicarbonate Mechanism

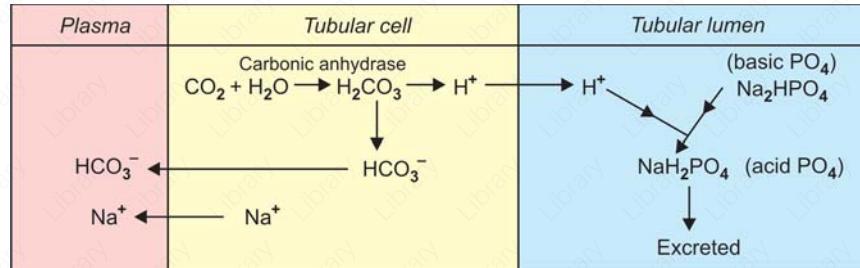


Site: Proximal tubular epithelial cells.

The secreted H^+ combines with filtered HCO_3^- forms H_2CO_3 which is broken down to CO_2 and H_2O . Later CO_2 diffuses into tubular cell, where reaction is reversed, HCO_3^- is returned to plasma. The net effect H^+ is buffered and HCO_3^- is effectively reabsorbed and returned to blood. In the renal tubular cells H^+ ions are exchanged with the reabsorbed Na^+ instead of K^+ .

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Phosphate Mechanism



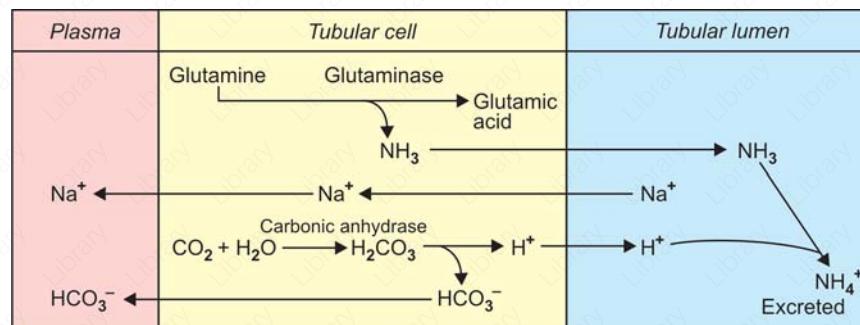
Site: Distal tubular epithelial cells.

Glomerular filtrate has abundant phosphate buffer having Na_2HPO_4 and NaH_2PO_4 as basic and acid components respectively. As the urine is acidified when it passes down the tubule, H^+ ions are taken up by basic phosphate (Na_2HPO_4). As a result, more and more NaH_2PO_4 is formed, which is excreted. Acid and basic phosphate pair is called urinary buffer. NaH_2PO_4 is called titrable acid of urine and its level in urine is titrable acidity (TA).

In Urine

When plasma pH decreases, NaH_2PO_4 is elevated and hence titrable acid is increased. When pH increases, NaH_2PO_4 is lowered and hence titrable acid is decreased. Therefore, titrable acidity is an indicator of acid-base status.

Ammonia Mechanism



Site: DCT (Distal convoluted tubules)

The process is mediated by glutaminase enzyme present in tubular cell. This mechanism traps H^+ ions and helps to excrete H^+ without much change in pH of urine.

SUMMARY

Mechanism	H^+ in urine is buffered by	Product formed and its fate
Bicarbonate mechanism	HCO_3^-	$CO_2 \longrightarrow$ Returned to tubular cell
Phosphate mechanism	Na_2HPO_4 (basic phosphate)	$NaH_2PO_4 \longrightarrow$ Excreted as titrable acid in urine
Ammonia mechanism	NH_3	$NH_4^+ \longrightarrow$ Excreted in urine

Disturbances in Acid-Base Balance

Acidosis: Clinical condition that arises because of decreased pH of blood.

Alkalosis: Clinical condition that arises because of increased pH of blood.

Acidosis

- Metabolic acidosis:** Decreased plasma pH, because of primary HCO_3^- deficit (Decrease in metabolic component).
- Respiratory acidosis:** Decreased plasma pH, because of primary H_2CO_3 excess (Increase in respiratory component).

Alkalosis

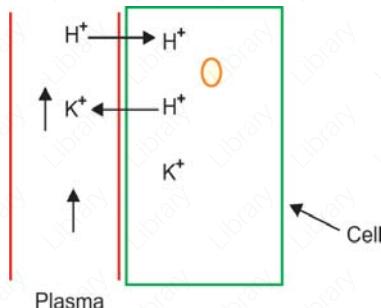
- Metabolic alkalosis:** Increased plasma pH, because of primary HCO_3^- excess (Increase in metabolic component).
- Respiratory alkalosis:** Increased plasma pH, because of primary H_2CO_3 deficit (Decrease in respiratory component).

To encounter the acid-base disturbances, the body gears up its homeostatic mechanism and makes every attempt to restore the pH to normal level (7.4). This is referred to as compensation which may be partial or full. Sometimes, the acid-base disorders may remain uncompensated.

Potassium ion (K^+) is mainly an intracellular ion. Normal serum K^+ level is 3.5 to 5 mEq/liter. Its level is altered in acid-base disturbance.

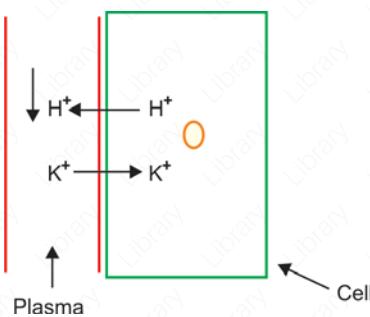
Serum Potassium Levels in Acidosis and Alkalosis

In Acidosis



When plasma pH is lowered, H^+ concentration is increased. Then H^+ ions enter the cells. To maintain electrical neutrality, K^+ comes out and thus serum K^+ levels increase resulting in hyperkalemia.

In Alkalosis



When plasma pH is increased, H^+ concentration is decreased. H^+ ions come out of the cells. To maintain electrical neutrality, K^+ enters the cells and thus serum K^+ levels decrease resulting in hypokalemia.

Hence, when treating a patient with acid-base disturbance, care should be taken to maintain serum potassium levels also. So, when acidosis is corrected, K^+ should be supplemented to prevent hypokalemia.

Metabolic Acidosis

It is a clinical condition arising from decreased plasma pH, because of "primary bicarbonate deficit". It is the most common acid-base disorder.

Causes

1. Increased acids in body:
 - a. Exogenous acids:
 - i. Ingestion of acidic salts like NH_4Cl
 - ii. Accidental ingestion of acids
 - iii. Drugs which are acidic in nature, e.g. aspirin, salicylates.
 - b. Endogenous acids:
 - i. Lactic acid as seen in lactic acidosis
 - ii. Ketoacids as seen in diabetes and starvation, e.g. diabetic ketoacidosis
 - iii. High fever
 - iv. Violent exercise
 - v. Shock, hemorrhage and anoxia.
2. Failure to excrete acid load of body, which occurs in renal failure
3. Loss of HCO_3^- due to severe diarrhea and renal failure.

Laboratory Findings

Blood: pH is lowered; HCO_3^- is decreased; H_2CO_3 is normal.

Urine: NH_4^+ excretion is increased; titrable acidity (NaH_2PO_4) is increased.

Compensatory Mechanisms

1. Primary compensation by lungs:
 - a. Respiratory rate and depth increases (Deep acidotic breathing)
 - b. More CO_2 is washed off
 - c. H_2CO_3 level decreases
 - d. pH is restored to normal.
2. Secondary compensation is by kidney:
 - a. Urinary H^+ excretion increases
 - b. HCO_3^- re-absorption increases
 - c. NH_4^+ and titrable acidity excretion increases.

Respiratory Acidosis

It is a clinical condition where pH of blood is decreased due to primary H_2CO_3 excess. Here basic defect is the decreased CO_2 excretion by lungs, due to CO_2 retention, increased formation of H_2CO_3 which results in fall of plasma pH.

Causes

Any disease which causes decrease in CO_2 excretion from lungs, due to:

- a. Pneumonia (where gaseous exchange in alveoli is inhibited)
- b. Obstruction to respiratory tract as in asthma (bronchospasm) and because of presence of foreign body
- c. Diseases of alveoli
- d. CNS damage causing decreased respiration and due to intake of drugs like morphine (CNS depressants).

Laboratory Findings

Blood: pH is lowered; HCO_3^- is normal; H_2CO_3 is increased.

Urine: NH_4^+ excretion is increased; titrable acidity (NaH_2PO_4) is increased.

Compensatory Mechanisms

Since lungs are affected, compensation by lungs is not possible.

Compensation is mainly by kidneys:

- i. H^+ excretion increases
- ii. Glutaminase levels increase
- iii. HCO_3^- reabsorption increases
- iv. Titrable acidity and NH_4^+ excretion increase

Steps for Treatment of Acidosis

Treat the underlying cause:

1. Sodium bicarbonate infusion must be given.
2. Take care of serum electrolytes especially K^+ .

Metabolic Alkalosis

It is a clinical condition where pH of blood is increased due to "primary HCO_3^- excess". There is increased alkali reserve. When HCO_3^- increases,

ratio increases. Hence pH is increased.

Causes

1. Excess loss of acid from the body:
 - a. Violent vomiting leading to loss of HCl
 - b. Pyloric obstruction.
2. Excessive intake of alkali:
 - a. Excess bicarbonate therapy
 - b. Alkaline drugs given as antacids for gastric ulcers.
3. K^+ deficiency is observed resulting in hypokalemia.

Biochemical Findings

Blood: pH is increased; HCO_3^- is increased; H_2CO_3 is normal.

Urine: pH = alkaline (hyperaldosteronism); pH = acidic (paradoxic aciduria); Titrable acidity and NH_4^+ excretion is decreased; HCO_3^- excretion is increased.

Compensatory Mechanisms

Primary compensation by lungs: Increase in pH leads to depression of respiratory center. Respiratory rate is lowered (hypoventilation) and so CO_2 is retained. With the increased H_2CO_3 levels, pH is restored to normal.

Secondary Compensation by Kidneys

1. HCO_3^- reabsorption is decreased and hence HCO_3^- excretion increases in urine.
2. $H^+ - Na^+$ exchange is inhibited. Thus, there is H^+ retention.
3. Secretion of H^+ into urine is lowered.
4. NaH_2PO_4 and NH_4^+ excretion is lowered. By these mechanisms, ratio is brought back to 20. Then, pH is restored to normal.

Other conditions that may co-exist with alkalosis:

- Hypokalemia
- Tetany due to decreased ionic Ca level.

Respiratory Alkalosis

It is a clinical condition wherein pH of blood is increased due to primary H_2CO_3 deficit. It is mainly due to excess CO_2 excretion. Then plasma H_2CO_3

level decreases and $\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$ ratio increases and thus pH increases.

Causes

It is mainly due to excess CO_2 excretion by lungs.

- In hysterical hyperventilation (due to psychological cause)
- Stimulation of respiratory center due to:
 - Drugs like salicylates
 - Diseases of brainstem.
- Injudicious use of ventilators in hospital.

Biochemical Findings

Blood: pH is increased; HCO_3^- is normal; H_2CO_3 is decreased.

Urine: Titrable acidity and NH_3^- excretion is decreased; HCO_3^- excretion is increased.

In respiratory alkalosis, main compensation is by kidneys which is exactly opposite of respiratory acidosis.

Mechanism

- HCO_3^- reabsorption is decreased
- H^+ Excretion is decreased
- Glutaminase enzyme is not stimulated. Hence, NH_4^+ excretion is lowered.
- Titratable acid (NaH_2PO_4) excretion is lowered.

By all these mechanisms, more of HCO_3^- is excreted out and H^+ is conserved.

Hence, $\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$ ratio brought back to normal.

Thus, normal pH is maintained.

Anion Gap

In plasma, usually measured cations are Na^+ and K^+ which account for majority of cations. Usually measured anions are HCO_3^- and Cl^- , which

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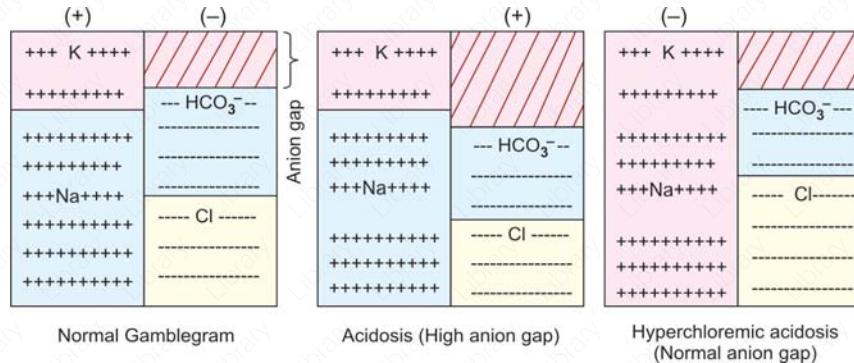
account for only 85 percent of anions. Hence, measurable cations are greater than measurable anions. In plasma, the concentration of cations and concentration of anions are always equal, to maintain electrical neutrality. Anion gap is defined as difference between measurable cations and measurable anions.

Anion gap is due to unmeasured anions in plasma which are mainly protein anions (at physiological pH, proteins mainly exist as anions). Numerical value is 12 ± 5 mEq/L

$$\text{Anion gap} = (\text{Measurable cations}) - (\text{Measurable anions})$$

$$\begin{aligned} & (\text{Na}^+ + \text{K}^+) - (\text{HCO}_3^- + \text{Cl}^-) \\ &= \text{Na}^+ + \text{K}^+ - \text{HCO}_3^- - \text{Cl}^- \\ &= 12 \text{ mEq/L.} \end{aligned}$$

Anion gap is mainly because of unmeasured anions which are due to PO_4^{2-} , SO_4^{2-} , lactic acid, keto acids and protein anions.



Significance of Anion Gap

- Increased anion gap is seen in conditions where there is increase in unmeasured anions.

Example:

- Diabetic ketosis is due to increased keto acids
- Lactic acidosis is due to increased lactic acid
- Renal failure is due to increased fixed acids.

Thus, anion gap gives an idea regarding acid-base status of the body.

- Anion gap can be used for quality control assessments in laboratory (To check the reliability of electrolyte estimation). If anion gap value in a normal person is altered, then it signifies that one or more estimation of electrolyte is faulty.

Normal Range of Human Serum Electrolytes

Normal levels for reference:

pH = 7.4

$\text{HCO}_3^- = 24$ mmoles/L

pCO_2 = 40 mm of Hg.
 K^+ = 3.5-5 mEq/L
 Na^+ = 135-145 mEq/L
 Cl^- = 95-105 mEq/L
 HCO_3^- = 22-26 mEq/L

SUMMARY

Condition	Basic defect	Causes	Blood				Urine	
			pH	HCO_3^-	H_2CO_3	TA	NH_4^+	pH
Metabolic acidosis	Primary HCO_3^- deficit	1. Acid intake 2. Diabetic ketosis 3. Lactic acidosis 4. Diarrhea	↓↓	↓↓	N	↑↑	↑↑	Highly acidic
Respiratory acidosis	Primary H_2CO_3 excess	1. Obstructive lung diseases 2. Asthma 3. Pneumonia 4. CNS causes	↓↓	N	↑↑	↑↑	↑↑	Highly acidic
Metabolic alkalosis	Primary HCO_3^- excess	1. Vomiting 2. Alkaline drugs 3. HCO_3^- therapy	↑↑	↑↑	N	↓↓	↓↓	Alkaline
Respiratory alkalosis	Primary H_2CO_3 deficit	1. Hysteria 2. Excess ventilation 3. CNS causes	↑↑	N	↓↓	↓↓	↓↓	Alkaline

For assessment of acid-base status in laboratory:

1. Heparinised plasma should not be exposed to atmospheric oxygen
2. Estimation should be done within half an hour after the collection of sample
3. pH is measured using pH meter
4. HCO_3^- , H_2CO_3 , pCO_2 , Na^+ , K^+ , etc. are measured by ion sensitive electrodes.

Cases of Acid-Base Imbalance (Uncompensated)

pH	HCO_3^-	pCO_2	Diagnosis
6.9	14	42	Metabolic acidosis
7.57	26	22	Respiratory alkalosis
7.21	26	60	Respiratory acidosis
7.6	36	42	Metabolic alkalosis

MOST LIKELY QUESTIONS

Long Essays

1. Describe the various mechanisms by which acid-base status in the plasma is maintained within normal limits.
2. How normal pH of blood is maintained? Explain.

Short Essays

3. Metabolic acidosis.
4. Respiratory acidosis.
5. Role of bicarbonate buffer system.
6. Metabolic alkalosis.
7. Describe various mechanisms of acid-base balance.
8. Respiratory alkalosis.
9. Role of lungs in acid-base balance.
10. Role of kidneys in acid-base balance.

Short Answers

11. What is the normal pH of blood? Name two plasma buffers.
12. Anion gap.
13. Alkali reserve.
14. Plasma buffers.
15. Intracellular buffers.
16. Define a buffer. Give examples and explain the mechanism of action of a buffer.
17. Define pH. Write Henderson-Hasselbalch equation.
18. Give normal values of pCO_2 , HCO_3^- , anion gap.
19. Glutaminase.
20. Carbonic anhydrase.
21. Buffers of urine.

2.4: ELECTROLYTES

Electrolytes are positively and negatively charged ions which are in solution in all body fluids. Normal cellular functions and survival requires electrolytes which are maintained within narrow limits. The concentrations of electrolytes are expressed as milliequivalent/liter (mEq/L).

Distribution of Electrolytes

- Total concentration of cations and anions in each compartment (ECF and ICF) is equal to maintain electrical neutrality. The concentration of

Table 2.4.1: Electrolyte content of ECF and ICF

Ions	Extracellular fluid (mEq/L)	Intracellular fluid (mEq/L)
Cations		
Na ⁺	142	10
K ⁺	5	150
Ca ⁺⁺	5	2
Mg ⁺⁺	3	40
Total	155	202
Anions		
Cl ⁻	103	2
HCO ₃ ⁻	27	10
HPO ₄ ²⁻	2	140
SO ₄ ²⁻	1	5
Organic acids	6	5
Protein	16	40
Total	155	202

electrolytes in extracellular and intracellular fluids is shown in Table 2.4.1. There are striking differences in composition between the two fluids:

- Sodium is the principal cation of the extracellular fluid and compromises over 90 percent of the total cations, but has a low concentration in intracellular fluid and constitutes only 8 percent of the total cations.
- Potassium by contrast is the principal cation of intracellular fluid and has a low concentration in extracellular fluid.
- Similar differences exist with the anions. Chloride (Cl⁻) and bicarbonate (HCO₃⁻) predominate in the extracellular fluid, while phosphate is the principal anion within the cells.

The term electrolytes applied in medicine to the four ions in plasma, (Na⁺, K⁺ Cl⁻ and HCO₃⁻) exert the greatest influence on water balance and acid-base balance. Other ions that are important in clinical chemistry (Ca²⁺, phosphate, Mg²⁺ and trace elements) are discussed in the Chapter on Mineral Metabolism.

The body water balance is closely linked to the balance of dissolved electrolytes, the most important of which are Na⁺ and K⁺. The osmotic pressure of extracellular fluid is determined by the concentration of Na⁺ since, with its associated anions, it accounts for over 90 percent of the osmolality and thus Na⁺ concentration determines the extracellular fluid volume because water flows from or into other compartment to restore osmotic homeostasis if disturbed. K⁺ similarly determines intracellular osmolality to a large extent.

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MOST LIKELY QUESTIONS

1. What are the normal serum sodium and potassium levels?
2. Why a low sodium diet is prescribed for a hypertensive patient?
3. Write the normal serum electrolyte levels.
4. Write the normal ranges of the following:
 - a. Serum sodium
 - b. Serum potassium
5. Write the normal blood level of potassium.

Chapter

3

Composition and Metabolism of Carbohydrates

3.1: CHEMISTRY OF CARBOHYDRATES

Carbohydrates are the most abundant organic molecules in nature, literally means hydrates of carbon (Carbon, hydrogen and oxygen).

Definition

Carbohydrates are defined as polyhydroxy aldehydes or ketones or compounds which produce them on hydrolysis.

Example: Glucose, fructose, sucrose, starch, lactose, glycogen, etc.

The term sugar is applied to carbohydrates which are soluble in water, crystalline in nature and are sweet in taste.

Functions

1. Carbohydrates are the main sources of energy in the body.
2. They are precursors for many organic molecules (fats, amino acids).
3. They also serve as the storage form of energy (glycogen).
4. The sugars, ribose and deoxyribose are essential constituents of nucleic acids.
5. Carbohydrates (as glycoproteins and glycolipids) participate in the structure of cell membrane.
6. Lactose of milk serves as the important nutrient for all young mammals.
7. Carbohydrates are the structural components of many organisms.

Example: Cellulose of plants, cell wall of microorganisms, etc.

Classification of Carbohydrates

1. They are often, referred to as “saccharides”.
2. Broadly classified into three groups.
 - a. Monosaccharides
 - b. Oligosaccharides
 - c. Polysaccharides.

Monosaccharides: These are the simplest group of carbohydrates and are often referred to as simple sugars.

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Definition: They are the simplest carbohydrates and cannot be further hydrolyzed into simple carbohydrate molecules.

Most monosaccharides have general formula $C_nH_{2n}O_n$ or $C_n(H_2O)_n$

Example: Glucose, fructose, ribose.

Oligosaccharides: Oligosaccharides contain 2-10 monosaccharide molecules joined by glycosidic bonds which are liberated on hydrolysis.

They are further subdivided into disaccharides, trisaccharides, tetrasaccharides, pentasaccharides etc.

Disaccharides: On hydrolysis produce two molecules of the same or different monosaccharides.

<i>Example:</i>	Maltose	\rightarrow	Glucose + Glucose
	Sucrose	\rightarrow	Glucose + Fructose
	Lactose	\rightarrow	Glucose + Galactose

General formula $C_n(H_2O)_{n-1}$

<i>Example:</i>	a. Trisaccharides	\rightarrow	Raffinose
	b. Tetrasaccharides	\rightarrow	Stachyose
	c. Pentasaccharides	\rightarrow	Verbascose

Polysaccharides

Macromolecular carbohydrates, each composed of many (> 10) monosaccharide molecules linked by glycosidic bond are further subdivided into:

- Homopolysaccharides (Homoglycans)
- Heteropolysaccharides (Heteroglycans)

Homopolysaccharides

They contain monosaccharide units of a single type and may be represented by the general formula $(C_6H_{10}O_5)_n$.

Example: Starch present in plants, rice, etc. Glycogen present in liver and muscle.

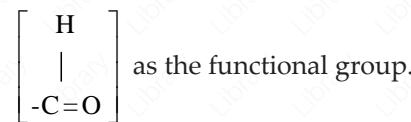
Heteropolysaccharides

They possess two or more different types of monosaccharide units or their derivatives.

Example: Hyaluronic acid, heparin, keratin sulphate, chondroitin sulphate, etc.

MONOSACCHARIDES

Aldoses: Monosaccharides having an aldehyde (CHO) group



Example: Glyceraldehyde, glucose, galactose, mannose, etc.

Ketoses: Monosaccharides having keto ($C=O$) group as the functional group.

Example: Erythrulose, ribulose, fructose, etc.

Common Monosaccharides

No. of carbon-atoms	Generic name	Aldose	Ketose
3	Trioses	Glyceraldehyde	Dihydroxy acetone
4	Tetroses	Erythrose	Erythrulose
5	Pentoses	Ribose	Ribulose
6	Hexoses	Glucose	Fructose
7	Heptoses	Glucoheptose	Sedoheptulose

Monosaccharides and their biochemical importance:

- 1. Glyceraldehyde (3 Carbon) - Glyceraldehyde is an intermediate in glycolysis
- 2. D - Ribose (5 Carbon) - For the structure of RNA and nucleotides, co-enzyme (ATP, NAD⁺)
- 3. D - Glucose (6 Carbon) - Most predominant sugar in human body excreted in urine in diabetes.
- 4. D - Galactose (6 Carbon) - Constituents of lactose = Galactose + Glucose
- 5. D - Fructose (6 Carbon) - Fruit sugar

ISOMERISM

Isomers are compounds with the same molecular formula but different structural formulae.

- i. *Aldose-ketose isomerism:* Aldose-ketose isomerism is the existence of an aldose and a ketose with the same molecular formula.

Example: Glucose-aldohexose, ribose-aldohexoses, fructose-ketohexose, ribulose- ketopentose.

- ii. *Stereoisomerism:* Compounds having same structural formula but differ in spatial configuration is known as stereoisomers. Stereoisomerism is an important character of monosaccharides.

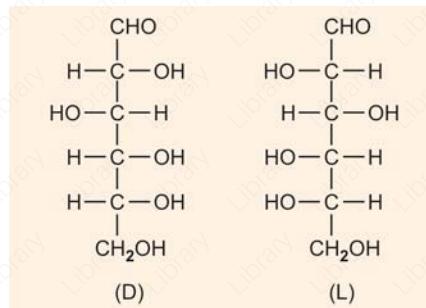
The presence of one or more asymmetric carbon atoms in the molecule may result in the existence of compounds in more than one structural configuration due to different 3-dimensional spatial arrangement of the groups or atoms held by its asymmetric carbons. This is known as stereoisomerism.

D and L Isomers

They are the mirror images of each other.

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Example: D and L isomers of glucose



Optical Activity

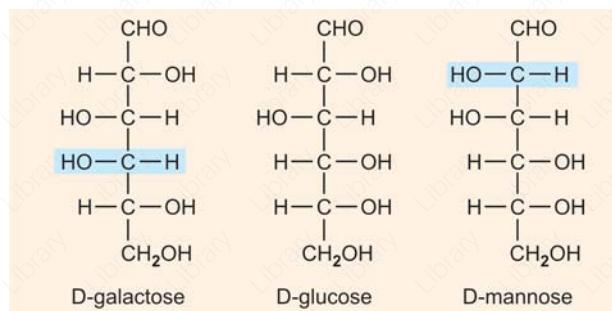
When a beam of polarized light is passed through a solution of an optical isomer, it will be rotated either to the right, dextrorotatory (+) or left, levorotatory (-).

Epimers

If a monosaccharide differ from each other in their configuration around a single specific carbon atom (other than anomeric) then they are referred to as epimers to each other.

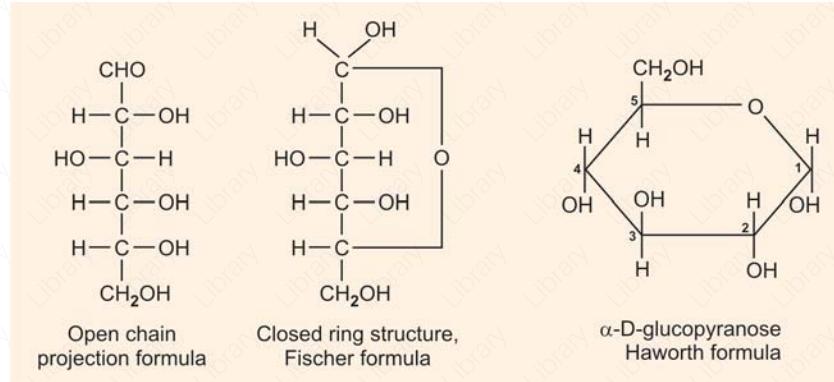
Example: Glucose and galactose are C₄-epimers; glucose and mannose are C₂-epimers.

Example:

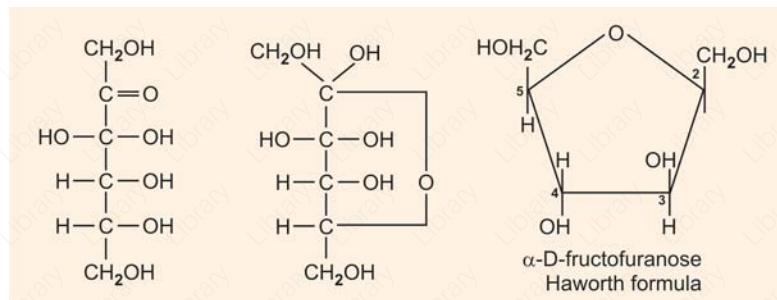


Example: Glucose to galactose and vice versa is known as epimerization. Epimers are also called diastereoisomers.

Different Representations of D-glucose



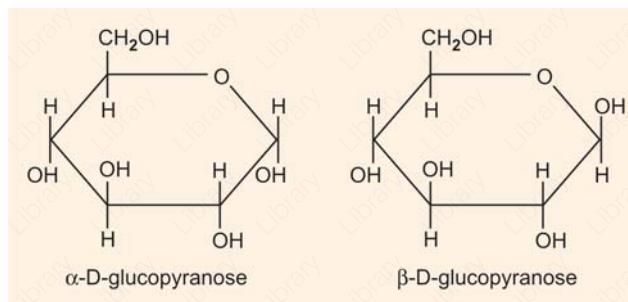
Different Representations of D-fructose



Anomers

Anomers are those which differ from each other in configuration only around anomeric carbon atom.

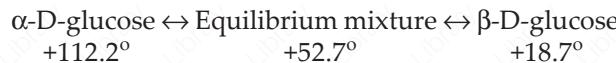
Example: α and β D- glucose.



Note: Pyranose ring is formed as a result of hemiacetal formation between OH of (5th carbon) and CHO (first carbon). As a result carbon atom 1 becomes asymmetric which is called anomeric carbon atom.

Mutarotation

It is defined as change in the specific optical rotation representing the interconversion of α and β forms of D-glucose to an equilibrium mixture.



At equilibrium, each solution contains $1/3\alpha$ -form and $2/3\beta$ -form of glucose.

Note: Fructose also exhibits mutarotation. In this case pyranose ring is converted to furanose ring till equilibrium is attained (-92°).

REACTIONS OF MONOSACCHARIDES

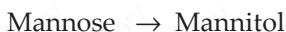
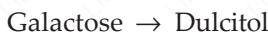
1. Oxidation

Oxidation of aldehyde group results in the formation of gluconic acid.
Oxidation of terminal OH group leads to the production of glucuronic acid.

Oxidation of both aldehyde and terminal groups leads to saccharic acid.

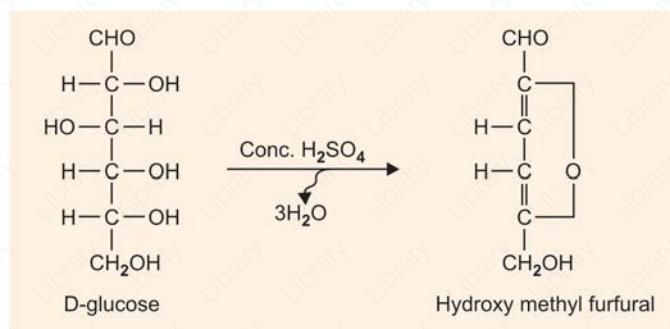
2. Reduction

- a. When treated with reducing agents monosaccharides give corresponding hydroxy alcohols.



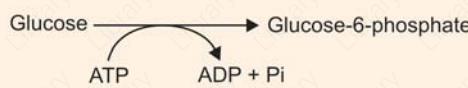
3. Dehydration

When treated with concentrated H_2SO_4 they undergo dehydration to form furfural.



4. Esterification

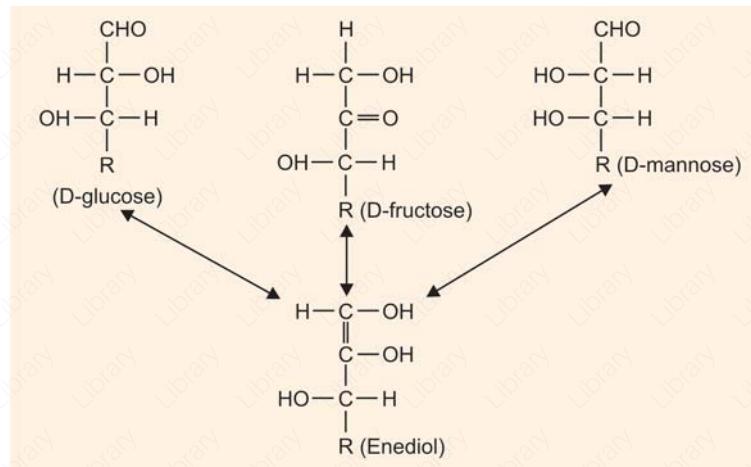
Esterification of carbohydrate with phosphoric acid is a common reaction in metabolism.



5. Enediol Formation or Tautomerization

The process of shifting a hydrogen atom from carbon atom to another to produce enediols is known as tautomerization.

In the mild alkaline solution, carbohydrates containing a free sugar group will tautomerize to form enediols where 2 hydroxyl groups are attached to the double bonded carbon.



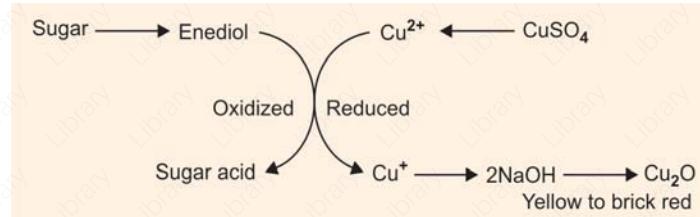
When glucose is kept in alkaline solution for several hours, it undergoes isomerization to form D-fructose and D-mannose. This oxidation is known as the lobry de Bruyn-Von reaction.

6. Reducing Properties

Sugars are classified as reducing (all monosaccharides) or non-reducing (sucrose), based on the reactions in the tests given below:

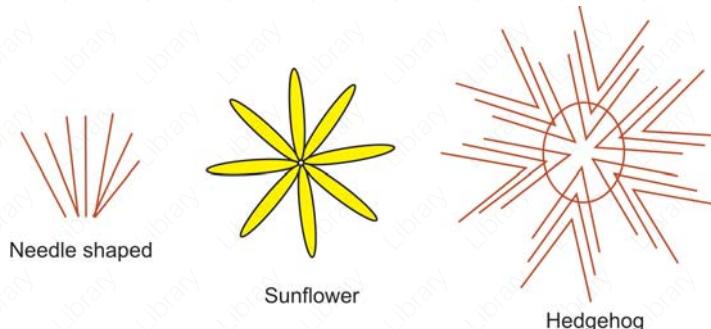
1. Benedict's test
2. Fehling's test
3. Barfoed's test

Benedict's Test



7. Osazone Formation

- Phenylhydrazine in acetic acid, when boiled with reducing sugars forms corresponding osazones.
- Glucose and fructose give needle shaped osazones.
- Maltose gives sunflower shaped osazones.
- Lactose gives hedgehog shaped osazones.

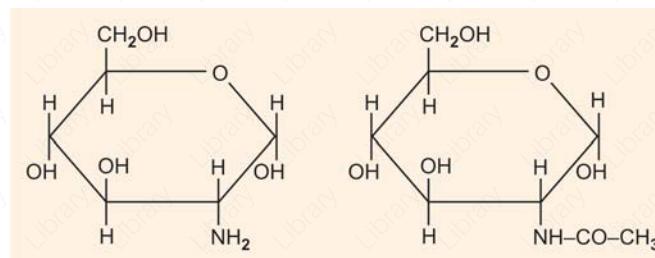


8. Glycosides

Glycosides are formed when the hemiacetal or hemiketal hydroxide group of carbohydrates reacts with a hydroxyl group of another carbohydrate or non-carbohydrate. The bond so formed is known as glycosidic bond.

9. Amino Sugars

When one or more hydroxyl groups of the monosaccharides are replaced by amino groups, the products formed are amino sugars.



Example:

1. Glucosamine
2. N-acetyl glucosamine

10. Deoxy Sugars

Oxygen of the hydroxyl group may be removed to form deoxy sugars.

Example: Deoxy ribose.

DISACCHARIDES

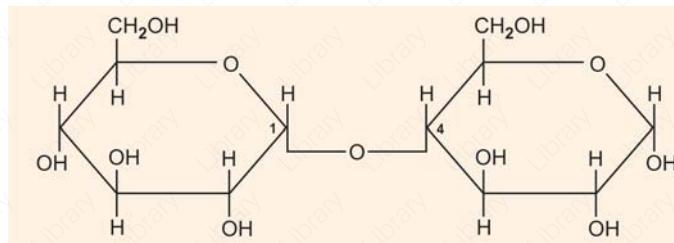
When two monosaccharides are combined together by glycosidic linkage a disaccharide is formed

Disaccharides

- a. Reducing (Maltose, lactose, isomaltose)
- b. Non reducing (Sucrose).

1. Maltose ($C_{12}H_{22}O_{11}$)

It is produced during the course of digesting starch by the enzyme amylase (pancreatic). It is a reducing disaccharide. Maltose gives sunflower shaped osazone crystals. It is a water soluble dextro rotatory disaccharide. Maltose is composed of 2 α -D - glucose units held together by α (1 → 4) glycosidic bond.



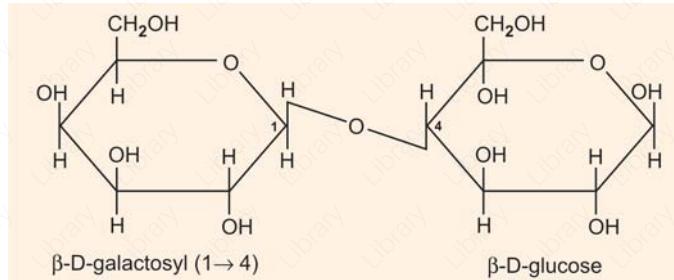
The free aldehyde group present in C₁ of second glucose answers the reducing reactions. In isomaltose, the glucose units are held together by α (1 → 6) glycosidic linkage.

Maltose is hydrolyzed by intestinal enzyme maltase or by boiling with dilute HCl to give 2 molecules of glucose.

2. Lactose

Lactose or milk sugar is a water soluble dextrorotatory disaccharide. It is composed of β -D-galactose and β -D-glucose held together by β (1 → 4) glycosidic bond.

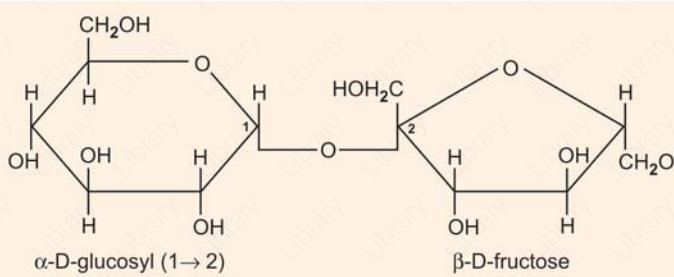
The anomeric carbon of C₁ glucose is free, hence lactose exhibits reducing properties and forms hedgehog shaped osazones. Lactose is hydrolyzed by intestinal enzyme lactase to give glucose and galactose.



Sucrose

It is a water soluble disaccharide present in sugar cane, sugar beet and ripened sweet fruits like pineapple.

Sucrose is made up of α -D-fructose held together by ($\alpha_1\text{-}\beta_2$) glycosidic bond.



The reducing groups of glucose and fructose are involved in the glycosidic bond, hence sucrose is a non-reducing sugar and it cannot form osazones. Sucrose is an important source of dietary carbohydrates. Intestinal enzyme sucrase hydrolyses sucrose to glucose and fructose which are absorbed.

Inversion of Sugar

Sucrose is dextrorotatory (+65.5°). But when hydrolyzed, sucrose becomes levorotatory (-28.2°). The process of change in optical rotation from dextrorotatory (+) to levorotatory (-) is referred to as inversion.

Invert Sugar

The hydrolyzed mixture of sucrose, containing glucose and fructose is known as invert sugar.

Polysaccharides

They consist of repeated units of monosaccharides or their derivatives held together by glycosidic bond.

Polysaccharides

- a. Homopolysaccharides
- b. Heteropolysaccharides

Homopolysaccharides

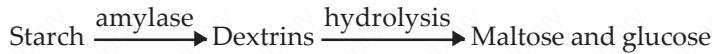
1. Starch

It is the reserve carbohydrate of plant kingdom. They are present abundantly in potatoes, tapioca, rice, wheat and other food grains.

Starch consists of 2 polysaccharide components — water soluble amylose and a water insoluble amylopectin.

Amylose is a long un-branched chain with 200-1000 D-glucose units held by α (1 \rightarrow 4) glycosidic linkages.

Amylopectin is a branched chain with α (1 \rightarrow 6) glycosidic bonds at the branching points and α (1 \rightarrow 4) linkages everywhere else.



2. Dextrans

They are breakdown products of starch by the enzyme amylase or dilute acids.

3. Inulin

It is a long chain homoglycan composed of D-fructose. It occurs in dahlia, onion, garlic, etc.

It is clinically used to find renal clearance value and glomerular filtration rate (GFR).

4. Glycogen

It is the reserve carbohydrate in animals and often referred to as "animal starch". It is stored in liver and muscle. Glycogen is composed of glucose units joined by α - 1, 4 and α - 1, 6 glycosidic linkages. Its structure is similar to amylopectin with more number of branches.

5. Cellulose

It is the chief carbohydrate in plants. It is the predominant constituent of plant cell wall. It is made up of β -D-glucose units linked by β (1 \rightarrow 4) glycosidic bonds.

Cellulose is not digested by human enzyme. It is a major constituent of dietary fiber.

Heteropolysaccharides

Mucopolysaccharides are negatively charged heteropolysaccharides. Mucopolysaccharides or glycosaminoglycans (GAG) are mainly made up of amino sugars and uronic acids.

Functions of Heteropolysaccharides

- i. Essential components of tissue structure.
- ii. Collagen and elastin fibers embedded in a matrix or ground substance which is predominantly composed of GAG.
Example: Hyaluronic acid, heparin, chondroitin sulphate, dermatan sulphate, keratin sulphate, etc.
1. *Hyaluronic acid:* It is present in vitreous humor, skin, synovial fluid, umbilical cord and ovum.
It is composed of N-acetyl glucosamine and glucuronic acid held together by β (1 \rightarrow 3) glycosidic bond.
It acts as a lubricant and a shock absorbant in joints and it further acts as a cementing substance and contributes semi-permeability to membrane.
2. *Heparin:* It occurs in liver, lungs, thymus, blood, etc. and consists of sulfated glucosamine and glucuronic acid (or iduronic acid).
It acts as an anticoagulant and also as an enzyme.
3. *Chondroitin sulphate:* It occurs in skin, heart, valves, bones, tendons, cartilages, etc. It is composed of glucuronic acid and N-acetyl galactosamine sulphate. It is required for synthesis of osteocytes and cartilages. It is a constituent of basement membrane and cell surface.
4. *Dermatan sulphate:* Mostly it occurs in the skin, blood vessels, etc. and composed of iduronic acid and N- acetyl galactosamine sulphate.
5. *Keratan sulphate:* It is the only GAG which does not contain any uronic acid. It is present in cornea and tendon. It is composed of galactose and N-acetyl glucosamine sulphate.

Glycoproteins

They are proteins to which carbohydrates are covalently attached.

Glycoproteins	Carbohydrate content
Immunoglobulin IgG	Less than 4 %
Glycophorin of red blood cell membrane	More than 20 %
Mucin of stomach	More than 60 %

Proteoglycans

They are also proteins to which carbohydrates are covalently attached but the carbohydrate differs chemically from those attached to

glycoproteins. The carbohydrate may be glucosamine or galactosamine and or their acetyl derivatives, uronic acid and sulphate groups. Proteoglycans are also called mucoproteins or mucoids.

MULTIPLE CHOICE QUESTIONS

1. Glucose on reduction forms:
A. Dulcitol B. Mannitol
C. Sorbitol D. Mannitol and Sorbitol
2. Table sugar is:
A. Glucose B. Lactose
C. Sucrose D. Maltose
3. Inulin is the polymer of:
A. Fructose B. Ribose
C. Ribulose D. Fucose
4. A disaccharide linked by alpha 1-4 glycosidic bond is:
A. Lactose B. Maltose
C. Sucrose D. Cellubiose
5. The glycosaminoglycan which functions as a lubricant and shock absorber in synovial fluid is:
A. Heparin B. Hyaluronic acid
C. Keratan sulphate D. Chondroitin 4-sulphate

ANSWERS

1. C 2. C 3. A 4. B 5. B

MOST LIKELY QUESTIONS

Long Essays

1. What are carbohydrates? Classify them with examples. Write a note on their functions.
2. Define polysaccharides. Give examples and explain the structure of biologically important polysaccharides.
3. Discuss the structure and function of lactose, maltose, sucrose, glucose and fructose.
4. Give an account of different isomerism exhibited by monosaccharides.

Short Essays

1. Inversion in sucrose.
2. Reducing sugars.
3. Isomerism in monosaccharides.
4. Biologically important derivatives of monosaccharides.
5. Classification of carbohydrates.
6. Name important disaccharides. Write a note on their structure and biomedical importance.

Short Answers

7. Compare and contrast structure of starch and glycogen.
8. Difference between muscle glycogen and liver glycogen.
9. Cellulose.
10. Epimers.
11. Structure of lactose.
12. Structure of maltose.
13. Structure of sucrose. Why is it non-reducing?
14. Why is starch non-reducing? What is the test done to identify starch?
15. Write the structure of glucose.
16. Epimers.
17. Epimerism.
18. DL isomerism.
19. Anomerism.
20. Anomers.
21. Structure and importance of fructose.
22. Amino sugars.
23. Difference between amylose and amylopectin.
24. Hyaluronic acid.
25. Heteropolysaccharides.
26. Cellulose.
27. Difference between ribose and deoxyribose.
28. Milk sugar.

3.2: DIGESTION AND ABSORPTION OF CARBOHYDRATES

Digestion and Absorption from Gastrointestinal Tract

<i>Organ</i>	<i>Major functions</i>
Mouth	- Saliva containing α -amylase is produced and polysaccharides are partially digested.
Stomach	- Gastric juice with HCl and proteases is produced and proteins are partially digested.
Pancreas	- Many enzymes and NaHCO_3 required for intestinal digestion are released.
Liver	- Bile acids are synthesized.
Gallbladder	- Bile is stored.
Small intestine	- Foodstuffs are finally digested and digested products are absorbed.
Large intestine	- Electrolytes are absorbed and bacterial utilization of certain undigested and/or unabsorbed foods.

Figure 3.2.1 depicts the diagrammatic representation of gastrointestinal tract.

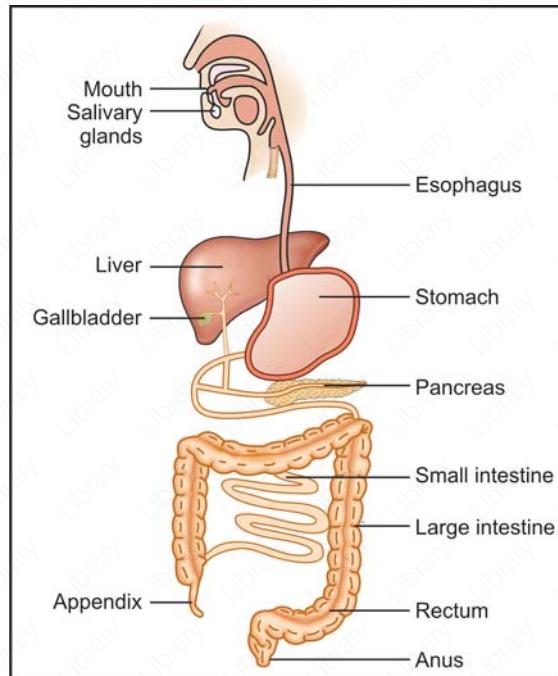


Fig. 3.2.1: Diagrammatic representation of gastrointestinal tract

Definition

Digestion involves the hydrolysis of complex organic molecules of food stuffs into smaller and preferably water soluble molecules with the help of enzymes of the digestive juice, which can be easily absorbed by the GI tract for utilization by the organism.

Movement of completely digested food particles into the blood or lymph through intestine is termed as absorption.

There are two pathways for the transport of materials absorbed by the intestine:

Hepatic portal system—leads directly to the liver transporting water soluble nutrients.

Lymphatic vessels—lead to the blood by way of the thoracic duct and transport lipid soluble nutrients.

Based on sites, digestion is of 3 types: Extracellular, surface and intracellular digestion.

Digestive juices are: Saliva, gastric juice, bile, pancreatic juice, intestinal juice.

Absorption of a substance into any cell involves its passage across the plasma membrane by transport mechanisms (Fig. 3.2.2).

1. Passive transport
2. Active transport

1. Passive Transport

- a. Simple diffusion
 - b. Facilitated diffusion
- a. *Simple diffusion:* It is a physical process and carrier protein is not required. Cellular work and energy expenditure are not involved. Diffusion occurs down the concentration gradient
- b. *Facilitated diffusion:* It is a carrier-mediated process (carriers are required). It does not require energy and is dependent on concentration gradient.

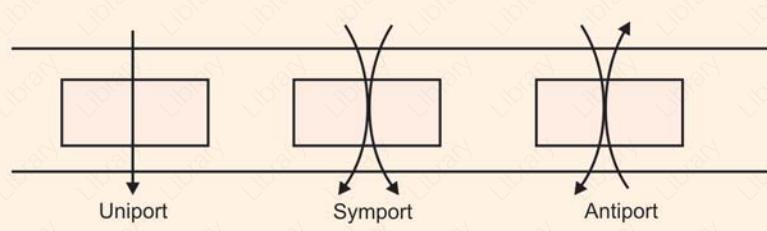


Fig. 3.2.2: Transport systems

2. Active Transport

It requires energy and transporters which are specialized integral proteins. Transporters are susceptible to inhibition by specific organic or inorganic compounds. Best example for active transport is sodium pump.

Carrier-mediated transports are of 3 types:

1. Uniport - Transmembrane transport of a single substrate (Example: Fructose or mannose transport).
2. Symport - Simultaneous transport of two substrates in the same direction across the membrane (Na^+ -glucose transport).
3. Antiport - Transport of 2 substrates simultaneously in opposite directions across the membrane (Example: Na^+ - K^+ antiport).

Digestion of Carbohydrates

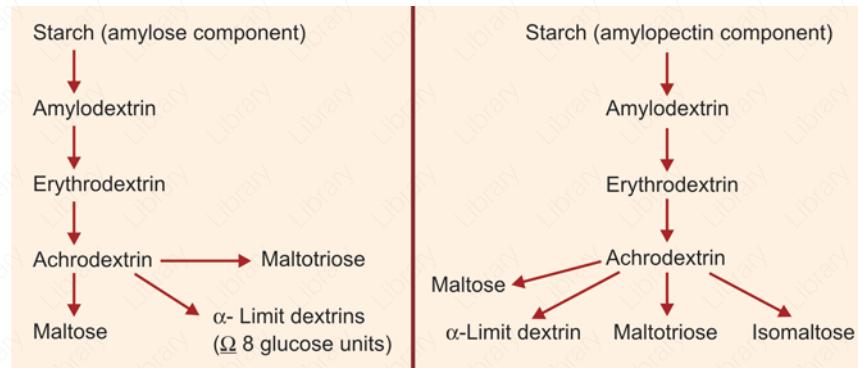
Dietary carbohydrates: Starch is the main dietary carbohydrate and small amounts of monosaccharides, disaccharides, glycogen (for non-vegetarians) and dietary fibers are also included in carbohydrate diet.

Stages: Carbohydrate digestion takes place in 4 stages with the following juice of GI tract.

1. Saliva
2. Gastric juice

3. Pancreatic juice
4. Intestinal juice.

Digestion by saliva: Salivary α -amylase (Ptyalin)

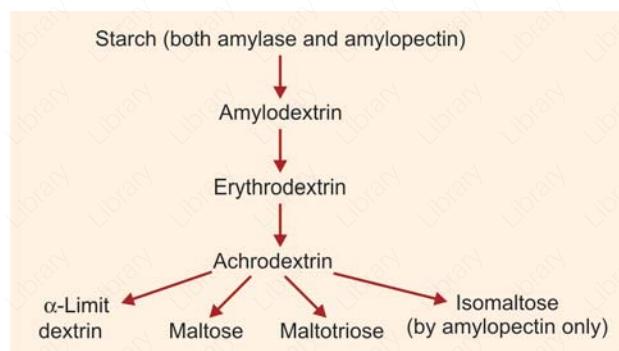


Digestion by Gastric Juice

Gastric HCl hydrolyzes some sucrose into glucose and fructose. HCl stops the action of salivary amylase.

Digestion by Pancreatic Juice

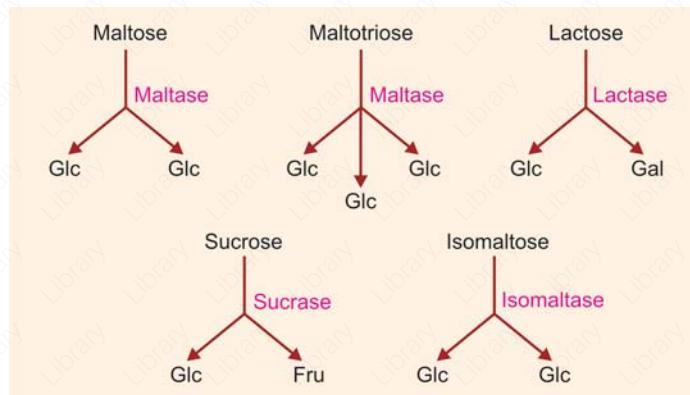
Pancreatic amylase (pH 6.9-7.2) hydrolyses starch into maltose, maltotriose, isomaltose and α -limit dextrin.



Digestion by Intestinal Juice

Most enzymes are glycoproteins and attached to the membranes of small intestinal cells.

Enzymes are intestinal amylase, isomaltase, maltase, lactase, sucrase, β -glycosidase and trehalase.

Digestion of Disaccharides by Disaccharidases

Glycogen in the diet is broken down in a similar way like that of amylopectin part of starch. Ultimately, by the action of all the digestive enzymes, carbohydrates are broken down to glucose, fructose, galactose and also pentoses.

Absorption of Monosaccharides

Only monosaccharides are absorbed by the intestine (upper jejunum and duodenum).

Minute quantities of disaccharides that may be absorbed are immediately eliminated through kidneys.

Pentoses are absorbed into the blood by simple diffusion.

Fructose and mannose are absorbed into the blood by facilitated diffusion (protein carrier on the membrane).

Glucose and galactose are absorbed by "secondary active transport process" and it is powered by the sodium pump (Fig. 3.2.3).

Absorption rate of galactose is more than glucose, while fructose is absorbed at a lesser rate than glucose.

Abnormalities of Digestion and Absorption of Carbohydrates*Lactose Intolerance*

1. An inborn deficiency of lactase in the intestinal mucosa produces a failure of lactose digestion and absorption and a consequent intolerance of dietary lactose. This is due to inherited lactase deficiency.

Primary low lactase activity: A gradual decline in lactase activity in the intestine with increasing age may produce similar lactose intolerance.

Secondary low lactase activity: Lactose intolerance also results from intestinal lesions in sprue, gastroenteritis, Kwashiorkor and colitis.

In all cases of lactase deficiency, intestinal fermentation of undigested lactose produces large volumes of gases. This leads to bloating,

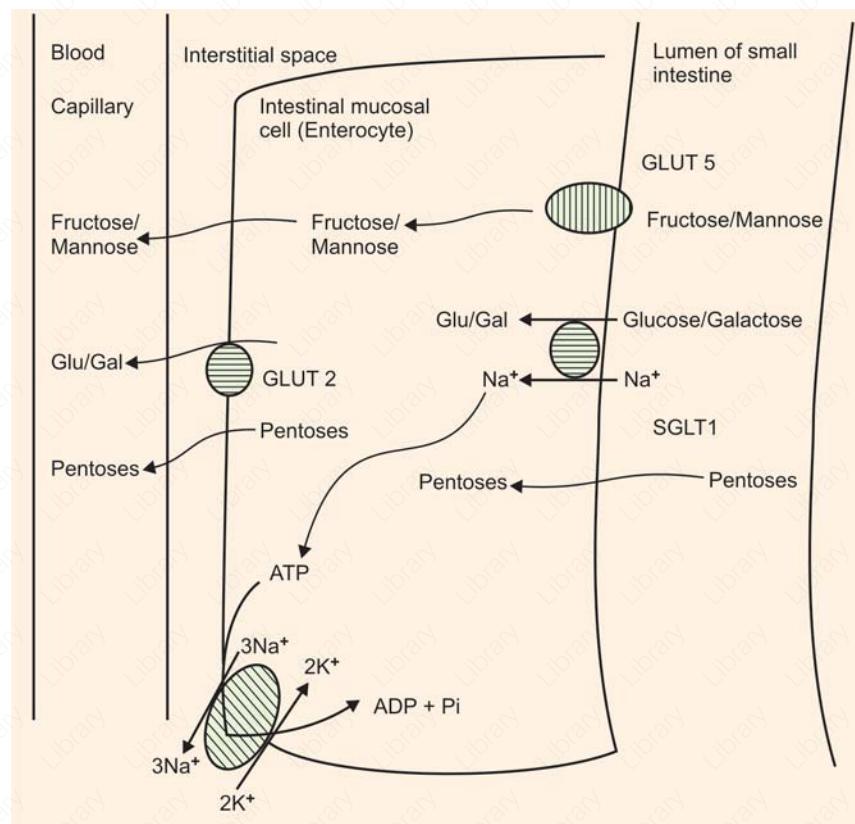


Fig. 3.2.3: Transport of monosaccharides across intestinal mucosal cell

flatulence, intestinal cramps and abdominal pain. The osmotic effect of unabsorbed lactose draws and retains large volumes of water in the intestinal contents, producing diarrhea.

Lactosuria: Some of the undigested lactose may get absorbed causing lactose to appear in the urine.

2. **Sucrose-isomaltose intolerance:** A rare inborn deficiency of both sucrase and isomaltase in the intestinal mucosa causes a failure of sucrose and isomaltose digestion, leading to the intestinal fermentation of undigested sucrose and isomaltose, leading to flatulence, diarrhea and intestinal cramps.

Sucrosuria: Undigested sucrose is absorbed and eliminated in urine.

3. **Monosaccharide malabsorption:** Due to defect in the Na^+ -glucose cotransporter carrier mechanism (SGLT-1), glucose and galactose are absorbed very slowly. Fermentation of these sugars leads to diarrhea, flatulence and abdominal pain. Water retention in the intestine due to osmotic effect of sugars leads to diarrhea.

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MULTIPLE CHOICE QUESTIONS

- Lactose intolerance is caused by the deficiency of:
A. Amylase B. Lactase
C. β -glycosidase D. Isomaltase
 - Symport is:
A. Transport of 2 substrates simultaneously in opposite directions across the membrane
B. Transmembrane transport of a single substrate
C. Simultaneous transport of two substrates in the same direction across the membrane
D. It is a physical process and carrier protein is not required
 - The disease characterized by impairment in the absorption of neutral amino acids is:
A. Hartnup's disease B. Pellagra
C. Albinism D. Cataract
 - The cellulose is not digested in humans due to lack of the enzyme that hydrolyses _____.
A. α -1,4 glycosidic linkage
B. α -1,6 glycosidic linkage
C. β -1,6 glycosidic linkage
D. β -1,4 glycosidic linkage
 - Bile salts form _____ with lipids
A. Chylomicrons B. Alcohols
C. Esters D. Mixed micelles

ANSWERS

1. B 2. C 3. A 4. D 5. D

MOST LIKELY QUESTIONS

1. Name the digestive enzymes of pancreas. Give their functions
 2. Digestion and absorption of carbohydrates.
 3. How are carbohydrates digested and absorbed?
 4. Absorption of glucose in the intestine.
 5. Glucose transporters.

3.3: CARBOHYDRATE METABOLISM

INTRODUCTION

Carbohydrates are obtained mainly from food but are also produced in the body from amino acids, glycerol, propionate and lactate. Carbohydrates are mainly utilized for energy production. They are also

used in synthesizing glycolipids, glycoproteins, mucoproteins, nucleotides, nucleic acids, glycerol, porphyrins and many amino acids.

The chemical reactions that occur in the body can be divided into three stages (Fig. 3.3.1):

- Digestion and absorption (primary metabolism) Example: Starch is digested and absorbed in the form of glucose.
- Intermediary metabolism (secondary metabolism) involves 3 types of “pathways” with series of reactions (Fig. 3.3.2).
 - Catabolic pathways:** Products of primary metabolism are further broken down to produce energy.
 - Anabolic pathways:** Products of primary metabolism are converted to larger molecules. In other words, process of synthesis which consumes energy.
 - Amphibolic pathway:** It has a dual role, both catabolic and anabolic.

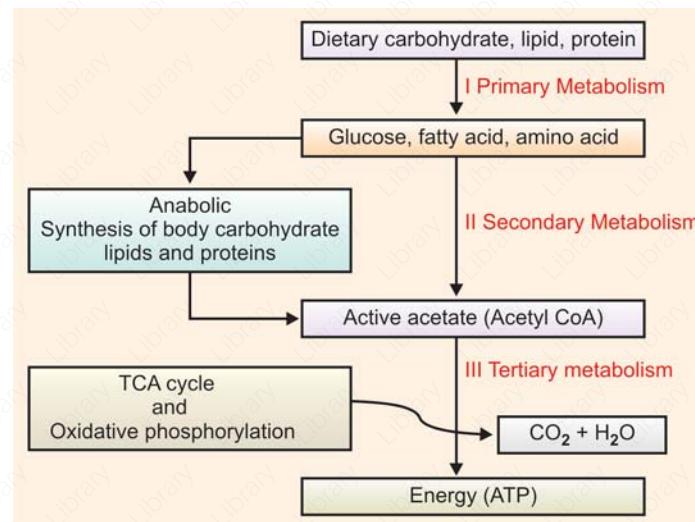


Fig. 3.3.1: Schematic diagram indicating chemical reactions taking place in the body

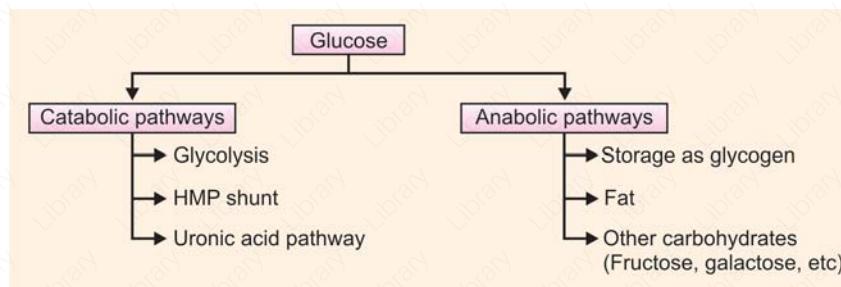
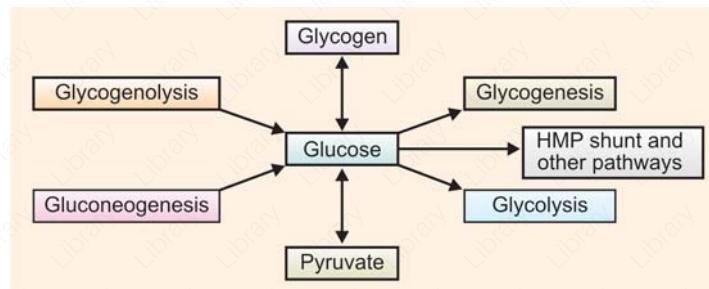


Fig. 3.3.2: Glucose metabolism

**Fig. 3.3.3:** Overview of carbohydrate metabolism

III. Tertiary metabolism: Metabolism of acetyl COA (Active acetate) to produce energy involves TCA cycle and oxidative phosphorylation. Figure 3.3.3 gives an overview of carbohydrate metabolism.

Glycolysis (Embden-Meyerhof pathway)

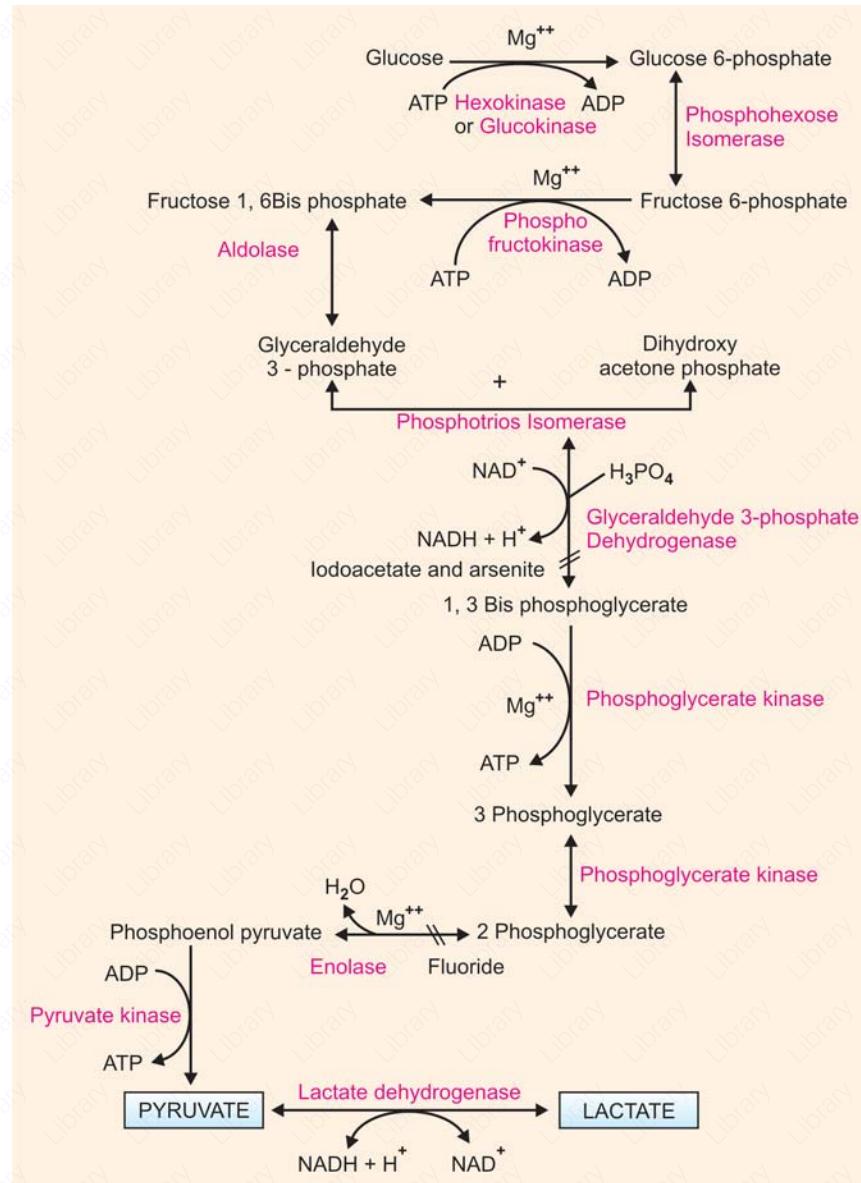
It is a major pathway of glucose breakdown and occurs in almost all cells of the body. It takes place in the cytosol of the cell.

Importance

- Glycolysis provides energy for cells.
- It is a unique pathway since it can operate both in aerobic and anaerobic conditions. The end product in aerobic condition is pyruvate and in anaerobic condition it is lactate.
- It is the best pathway for quick supply of energy as in vigorously contracting muscle.
- Any defect in glycolysis will lead to hemolysis and early fatigue.
- Intermediates of glycolysis are required for synthesis of non-essential amino acids, glycerol and fatty acids.

Difference between glucokinase and hexokinase

Glucokinase	Hexokinase
<ul style="list-style-type: none"> It is present in liver K_m is high, and hence low affinity for glucose Phosphorylation of only glucose Stimulated by glucose and insulin Not inhibited by glucose-6-phosphate 	<ul style="list-style-type: none"> It is present in all tissues K_m is low, and hence high affinity for glucose Phosphorylation of hexoses Not affected by insulin Allosterically inhibited by glucose

Glycolytic Pathway

Reactions of Aerobic Glycolysis

- In the first step the glucose is irreversibly activated to glucose-6-phosphate in the cell. This step is catalyzed by hexokinase enzyme. This requires Mg²⁺ and ATP. In liver, glucokinase is the specific enzyme which also catalyzes this reaction at higher concentration of glucose. Glucose-6 phosphate is impermeable to the cell membrane. It is a central molecule with a variety of metabolic fates such as glycolysis, glycogenesis, gluconeogenesis and HMP shunt.

2. Next the glucose-6-phosphate is isomerized to fructose-6-phosphate by phosphoglucomutase enzyme.
3. Fructose-6-phosphate is then irreversibly phosphorylated by phosphofructokinase enzyme to fructose 1,6-bisphosphate. Fructose 1,6-bisphosphate contains two phosphoric acid groups at C1 and C6 of fructose via phosphate ester bond.
4. Later fructose 1,6-bisphosphate molecule (six carbon sugar) is cleaved by aldolase enzyme to yield glyceraldehyde-3-phosphate and dihydroxy acetone phosphate (2, 3-carbon sugars-triose).
5. Dihydroxy acetone phosphate formed in the above step is converted back to glyceraldehyde 3-phosphate by phosphotriose isomerase enzyme.
6. Now we have two molecules of glyceraldehyde-3-phosphate molecules, which gets oxidized to 1,3-bisphosphoglycerate by the action of glyceraldehyde 3-phosphate dehydrogenase enzyme. This step utilizes inorganic phosphate (pi) to convert glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate. In 1,3-bisphosphoglycerate the phosphate group at carbon atom number 1 is high-energy group. Iodoacetate and arsenite inhibit the enzyme glyceraldehyde 3-phosphate dehydrogenase.

During oxidation of glyceraldehyde-3-phosphate the reducing equivalents are transferred to the acceptor NAD⁺ (Nicotinamide adenine dinucleotide). The (reduced) NADH under aerobic conditions enters into mitochondria and produces 3 molecules of ATP through its passage into electron transport chain or respiratory chain. This type of formation of energy currency ATP through respiratory chain is called as oxidative phosphorylation.

7. In the next step, the high-energy compound 1,3-bisphosphoglycerate transfers its high-energy to ADP to form ATP resulting in the formation of 3-phosphoglycerate. This reaction is catalyzed by phosphoglycerate kinase enzyme. This type of formation of energy currency ATP by high-energy substrate is called as **substrate level phosphorylation**.
 8. 3-phosphoglycerate is then isomerized to 2-phosphoglycerate by phosphoglycerate mutase enzyme.
 9. 2-phosphoglycerate is then converted to one more high-energy compound called phosphoenolpyruvate. This reaction is catalyzed by enolase enzyme. The activity of this enzyme is completely inhibited by fluoride. Hence, fluoride is used during blood collection for glucose estimation. This prevents the utilization of glucose by RBC.
 10. Later phosphoenolpyruvate is converted to pyruvate by pyruvate kinase enzyme. In this step one molecule of ATP is formed by substrate level phosphorylation.
- Under aerobic conditions pyruvate is the end product of glycolysis. Hence, pyruvate is then converted into acetyl CoA or oxaloacetate in the mitochondria.

Anaerobic glycolysis: Under anaerobic condition pyruvate is reduced to lactate by lactate dehydrogenase enzyme. This step utilizes the reducing equivalent from NADH formed in the earlier step and regenerates NAD⁺. Thus, the number of ATP produced will be less in anaerobic condition. Lactate accumulation in muscle leads to muscle cramps and fatigue. In thiamine deficient alcoholics, pyruvate is rapidly converted to lactate resulting in lactic acidosis.

The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited. Glycolysis in the erythrocytes leads to lactate production, since mitochondria are absent in RBCs. Brain, retina, skin, renal medulla and gastrointestinal tract derive most of their energy from glycolysis.

Regulation of Glycolysis

Insulin favors glycolysis by activating 3 enzymes which catalyze irreversible steps, they are:

- i. Hexokinase (or glucokinase)
- ii. Phosphofructokinase (PFK)
- iii. Pyruvate kinase

Glucocorticoids inhibit glycolysis and favor gluconeogenesis. PFK is the most important regulatory enzyme. It is allosterically inhibited by ATP, citrate and low pH and allosterically activated by AMP, fructose 2,6-bisphosphate.

<i>Enzyme</i>	<i>Inducers and activators</i>	<i>Inhibitors</i>
PFK	Insulin, AMP Fructose 2, 6-bisphosphate	Glucagon, citrate, ATP low pH
Pyruvate kinase	Insulin Fructose- 1,6 bis phosphate	ATP, glucagon
Glucokinase	Insulin	Glucagon

Pasteur effect: In the aerobic condition, the levels of glycolytic intermediates from fructose 1,6-bisphosphate onwards decrease while the earlier intermediate accumulate. This clearly indicates that Pasteur effect is due to the inhibition of the enzyme phosphofructokinase of glycolysis. The inhibitory effect of citrate and ATP produced in the presence of oxygen on phosphofructokinase explains the Pasteur effect (Plenty of oxygen leads to increase in respiration which in turn increases ATP production. This inhibits phosphofructokinase).

Crabtree effect: Basically this is opposite to that of Pasteur effect. Plenty of glucose will inhibit oxygen utilization.

Energetics of Glycolysis

1. *Anaerobic glycolysis*

Energy spent/molecule of glucose =

i. Hexokinase	1 ATP
ii. PFK	1 ATP
	Total = 2 ATP

Energy generated/molecule of glucose =

i. Phosphoglycerate kinase	$1 \text{ ATP} \times 2$	= 2 ATP
ii. Pyruvate kinase	$1 \text{ ATP} \times 2$	= 2 ATP
	Total	= 4 ATP

Therefore NET GAIN OF ATP = $4 - 2 = 2 \text{ ATP}$

2. *Aerobic glycolysis*

Energy spent/molecule of glucose =

i. Hexokinase	1 ATP
ii. PFK	1 ATP
	Total = 2 ATP

Energy generated/molecule of glucose =

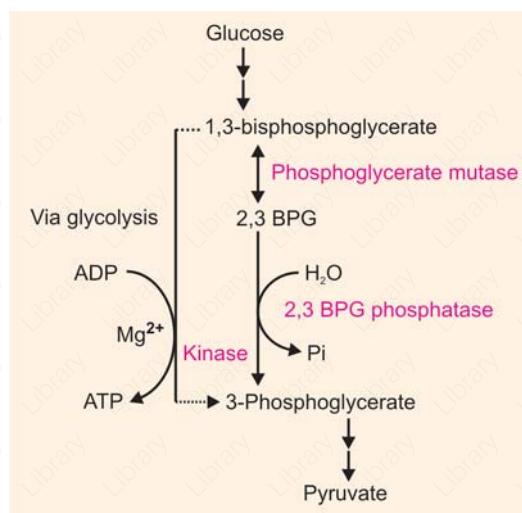
i. Glyceraldehyde 3-Ph DH	$1 \times \text{NADH} \times 2 = 3 \times 2$	= 6 ATP
ii. Phosphoglycerate kinase	$1 \text{ ATP} \times 2$	= 2 ATP
iii. Pyruvate kinase	$1 \text{ ATP} \times 2$	= 2 ATP
	Total	= 10 ATP

Therefore NET GAIN = $10 - 2 = 8 \text{ ATP}$

Rapoport-Luebering Cycle (BPG Shunt)

This is a supplementary pathway to glycolysis and occurs in RBCs of man. It is mainly concerned with production of 2,3-bisphosphoglycerate (2,3 BPG).

Pathway



Significance

- i. 2, 3 BPG binds to hemoglobin. It displaces oxygen from hemoglobin (reduces affinity of Hb for oxygen). Thus, oxygen delivery to tissues is favored.
$$2,3\text{-BPG} + \text{Hb O}_2 \rightarrow \text{Hb} - 2,3\text{-BPG} + \text{O}_2$$
- ii. This pathway does not produce ATP (as ATP generating step is bypassed)
- iii. Under hypoxic conditions, 2, 3 BPG is increasing.

TCA CYCLE (KREBS CYCLE, TRICARBOXYLIC ACID CYCLE, CITRIC ACID CYCLE)

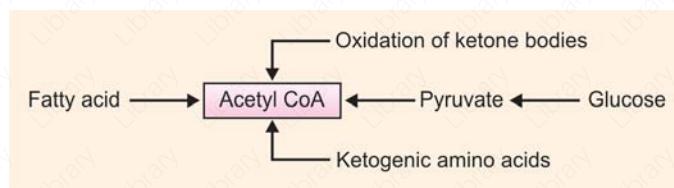
Significance

It is a final common pathway for oxidation of carbohydrates, fats and proteins. It provides abundant ATP for the body and also provides substrates for respiratory chain. It is amphibolic in nature showing both catabolic and anabolic features. Since final common oxidation takes place in this cycle it is called catabolic. TCA cycle provides substrates for synthesis of heme, nonessential amino acids, fats, glucose, etc. and hence called anabolic.

Features

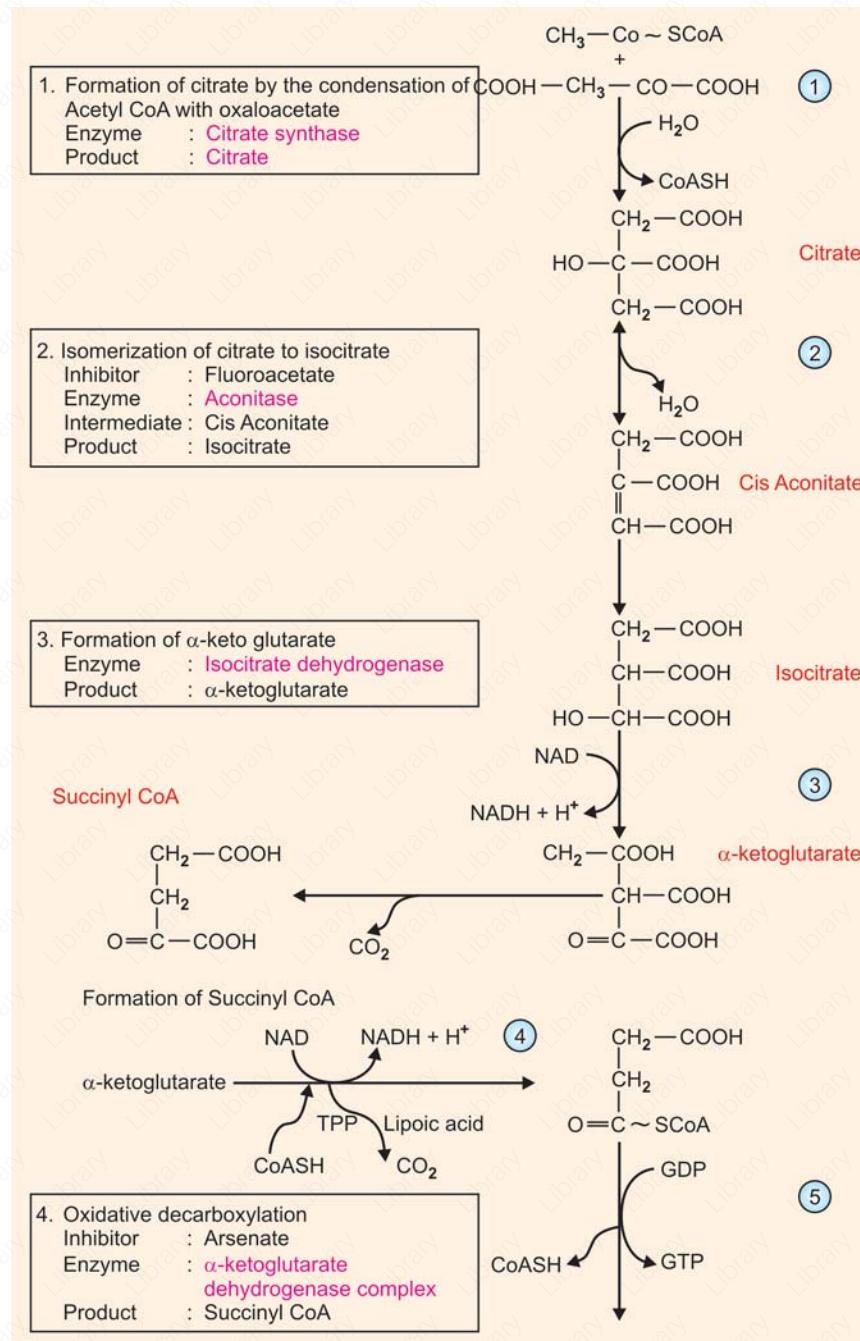
TCA cycle oxidizes Acetyl CoA to CO_2 and H_2O with liberation of energy. It is strictly aerobic and purely mitochondrial.

Sources of Acetyl CoA



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Reactions

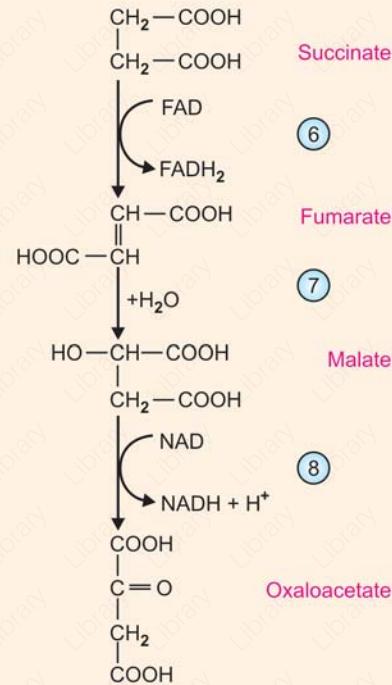


5. Formation of succinate
Substrate level phosphorylation
Enzyme : **Succinate thiokinase**
Product : Succinate

6. Formation of fumarate
Inhibitor : Malonate
Enzyme : **Succinate dehydrogenase**
Product : Fumarate

7. Formation of malate
Enzyme : **Fumarase**
Product : Malate

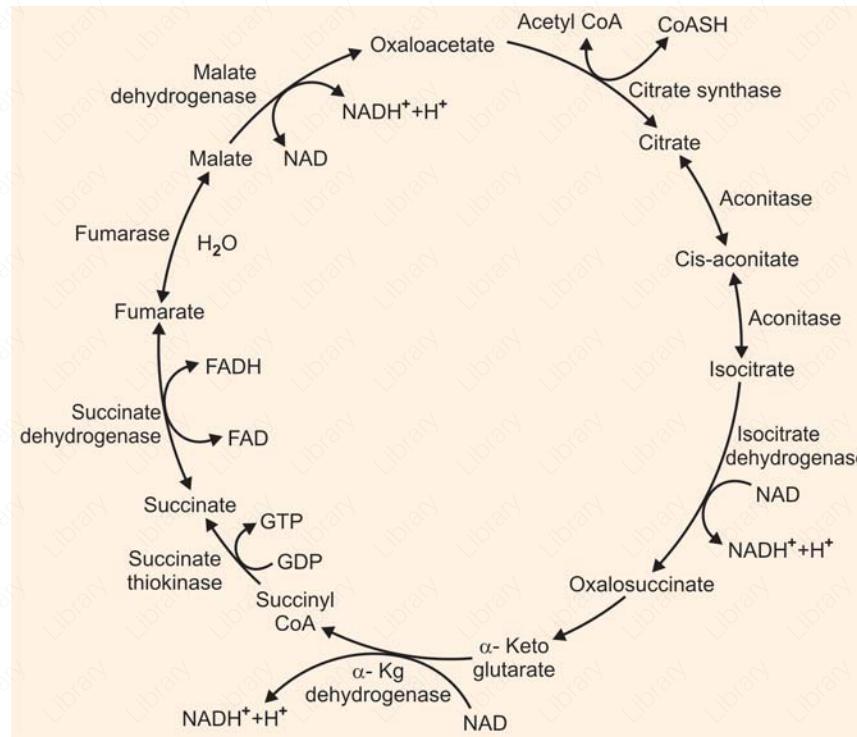
8. Formation of oxaloacetate
Enzyme : **Malate dehydrogenase**
Product : Oxaloacetate



Energy Liberating Steps

<i>Reaction catalyzed by</i>	<i>Method of production</i>	<i>No. of ATP produce</i>
1. Isocitrate dehydrogenase	Respiratory chain - oxidation of NADH	3
2. α-KG Dehydrogenase complex	Oxidation of NADH by respiratory chain	3
3. Succinate thiokinase	Substrate level phosphorylation	1
4. Succinate dehydrogenase	Oxidation of FADH ₂ by respiratory chain	2
5. Malate dehydrogenase	Oxidation of NADH by respiratory chain	3
Total 12 ATP / molecule of Acetyl CoA		

Reactions of TCA Cycle



Amphibolic Role of TCA Cycle

- Catabolic role of TCA cycle:* It serves as final common pathway for oxidation.
- Anabolic role of TCA cycle:* Many intermediates of TCA cycle are used for synthetic reactions. They are:
 - Citrate can be used for fatty acid synthesis
 - α-ketoglutarate to glutamate and oxaloacetate can give rise to aspartate. Thus nonessential amino acids can be synthesized.
 - Succinyl CoA acid is used in heme synthesis.
 - Malate → Pyruvate → Glucose

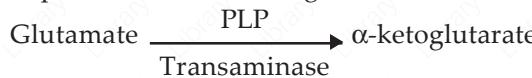
↑

Oxaloacetate

Anaplerosis

The reactions concerned to replenish the intermediates of TCA cycle are called anaplerotic reactions or anaplerosis (Fig. 3.3.4).

- Replenishment of α-ketoglutarate



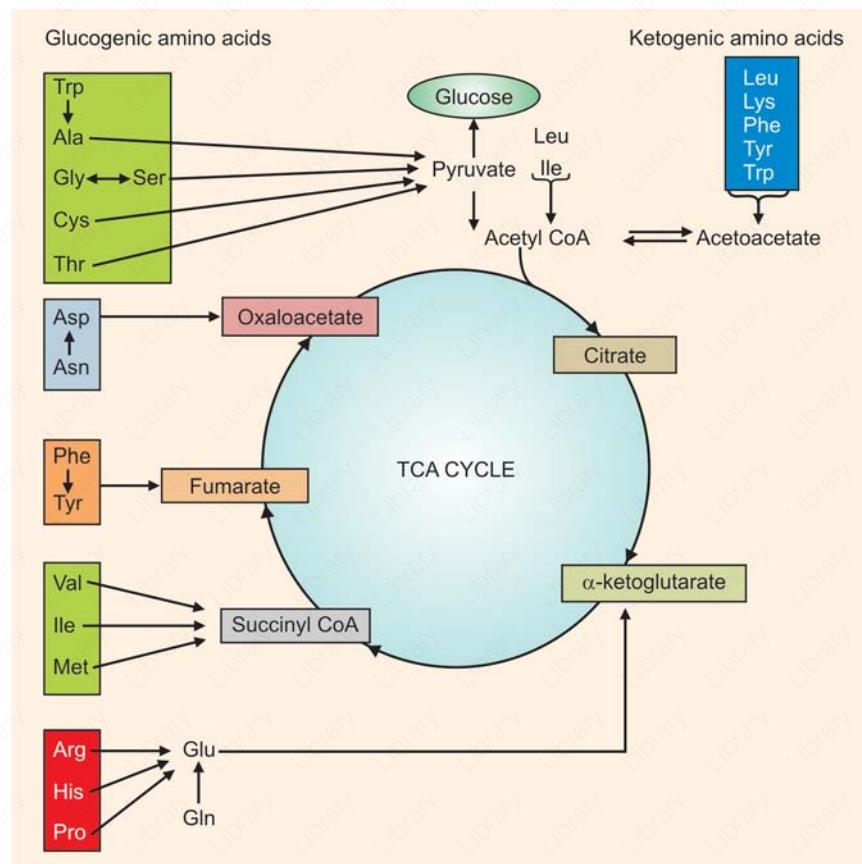


Fig. 3.3.4: TCA cycle—a common pathway

b. Replenishment of Succinyl CoA

Oxidation of Odd chain fatty acid

↓

Propionyl CoA

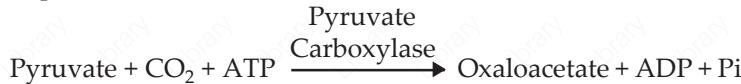
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Methyl malonyl CoA

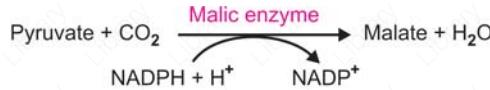
↓ Racemase

Succinyl CoA

c. Replenishment of oxaloacetate



d. Replenishment of Malate



GLUCONEOGENESIS

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors. Substrates for gluconeogenesis are lactate, pyruvate, glucogenic amino acids as major substrates and to a lesser extent propionate and glycerol.

Significance of Gluconeogenesis

Maintenance of blood glucose level within normal limits, because:

- i. Some tissues are exclusively dependant on glucose for energy
Example: Nervous tissue, RBC.
- ii. Skeletal muscle during anaerobic glycolysis.
- iii. For synthesis of other carbohydrates.
- iv. For providing oxaloacetate for TCA cycle.

Gluconeogenesis occurs in starvation (>18 hours) and diabetes mellitus in the tissues mainly in liver and kidney. Subcellular site is mainly cytosol, but some reactions are mitochondrial.

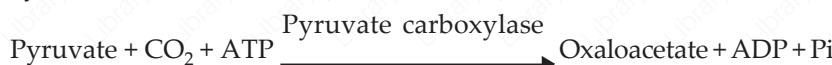
Reactions

Essentially, reverse of glycolysis except 3 irreversible steps of glycolysis which are bypassed by specific enzymes of gluconeogenesis. They are:

<i>Irreversible glycolytic enzyme</i>	<i>Corresponding enzymes in gluconeogenesis</i>
Hexokinase	iv. Glucose 6-phosphatase (Glucose 6-PO ₄ to Glucose)
Phosphofructokinase	iii. Fructose 1,6 bisphosphatase (Fructose 1, 6-bisphosphate to Fructose 6-PO ₄)
Pyruvate kinase	i. Pyruvate carboxylase (Pyruvate to Oxaloacetate) ii. Phosphoenol pyruvate carboxy kinase (PEPCK) (Oxaloacetate to Phosphoenol pyruvate)

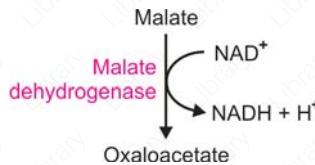
Reactions

- Synthesis of Oxaloacetate (Mitochondrial)



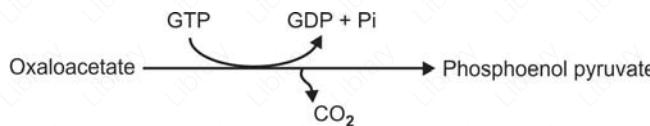
- Transport of Oxaloacetate to cytosol (by Malate Shuttle)





3. Synthesis of phosphoenol pyruvate

Phosphoenol pyruvate carboxy kinase (PEPCK)

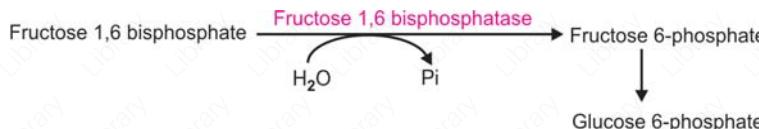


4. Synthesis of Fructose 1,6 bisphosphate

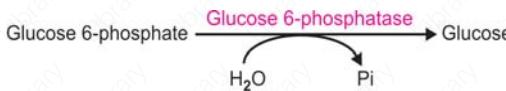
By reversal of glycolysis



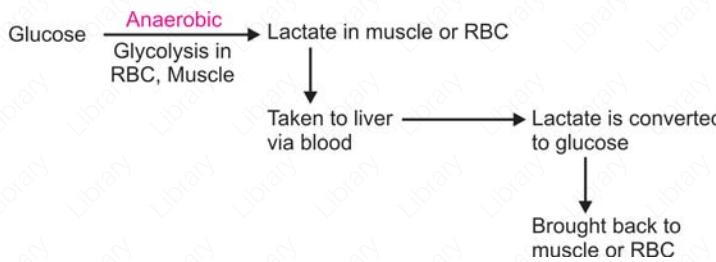
5. Synthesis of Fructose 6-phosphate



6. Synthesis of glucose by glucose 6-phosphatase

**Cori's Cycle (Lactic Acid Cycle)**

It is a cycle that operates for efficient utilization of lactate (Fig. 3.3.5).



Lactic acid is the major end product in muscle in anaerobic glycolysis. Muscle tissue is incapable of re-synthesizing glucose from lactate. The conversion takes place entirely in the liver. Muscle lactate is transported to the liver by the blood. In the liver, it is converted to glucose and glycogen by the enzymes concerned in gluconeogenesis. Liver glycogen is converted to glucose which is carried back to muscle by blood. This conversion of

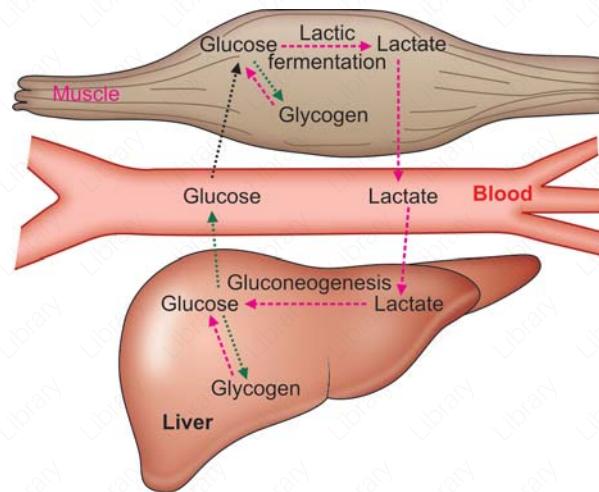


Fig. 3.3.5: Cori's cycle

muscle lactate to glucose in liver and its reentry into muscle is called "Cori's Cycle".

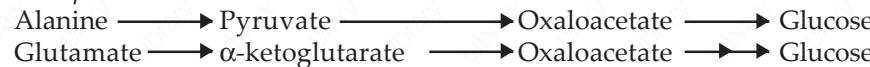
Glucose – Alanine Cycle (Gluconeogenesis from Alanine)

Glucose alanine cycle occurs in starvation. There is a continuous transport of amino acids from muscle to liver. Alanine dominates among the amino acids. Pyruvate in skeletal muscle undergoes transamination to produce alanine which is transported to liver and used for gluconeogenesis. Glucose produced by this process is made available for muscle.

Gluconeogenesis from Amino Acids

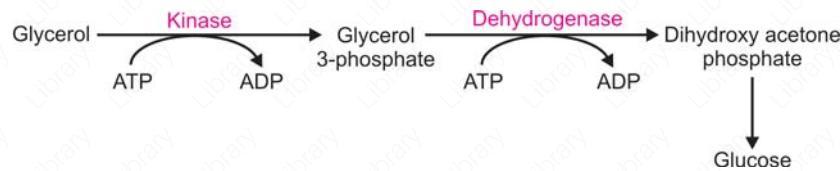
The carbon skeleton of glucogenic amino acids results in the formation of pyruvate or the intermediates of citric acid cycle which ultimately results in the synthesis of glucose.

Example:



Gluconeogenesis from Glycerol

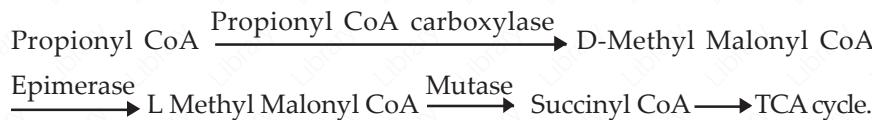
By hydrolysis of fats, glycerol is liberated in the adipose tissue. The enzyme glycerokinase found in liver and kidney activates glycerol to glycerol 3-phosphate which is converted to dihydroxy acetone phosphate by glycerol 3-phosphate dehydrogenase. Dihydroxy acetone phosphate is an intermediate in glycolysis and can be conveniently used for the production of glucose.



Gluconeogenesis from Propionate

Three carbon propionyl CoA is produced by oxidation of odd chain fatty acids and breakdown of some amino acids like methionine and isoleucine. Propionyl CoA carboxylase acts on this in presence of ATP and biotin and converts to methyl malonyl CoA which is then converted to succinyl CoA in presence of vit. B₁₂ coenzyme. Succinyl CoA formed from propionyl CoA enters gluconeogenesis via citric acid cycle.

Propionate is an important precursor for gluconeogenesis in ruminants like cattle.



Gluconeogenesis from Fat

Even chain fatty acids on oxidation produce acetyl CoA which cannot be converted to pyruvate. Hence even chain fatty acid cannot serve as precursors for glucose formation.

Regulation of Gluconeogenesis

- Allosteric modulation of pyruvate carboxylase:* During starvation, acetyl CoA accumulates in the liver due to excessive lipolysis in adipose tissue. Acetyl CoA allosterically activates pyruvate carboxylase resulting in enhanced glucose production.

- Regulation of Fructose 1,6-bisphosphatase:* ATP and citrate are the activators of fructose 1,6-bisphosphatase and hence gluconeogenesis is increased.

But the high level of AMP in liver cells inhibits the enzyme and thus reduces gluconeogenesis. In glucose shortage, the more secreted glucagon stimulates gluconeogenesis by decreasing the concentration of fructose 2,6-bisphosphate which in turn inhibits phosphofructokinase and activates the enzyme fructose 1,6-bisphosphatase.

- Hormonal regulation:* All key enzymes of gluconeogenesis are activated by glucagon and glucocorticoids and depressed by insulin.

All these mechanisms ensure that glycolysis and gluconeogenesis are "Reciprocally Regulated" so that when one is active, the other one is inactive.

GLYCOGEN METABOLISM

Glycogen is stored in liver and muscle in cytoplasm as granules. Liver glycogen is concerned with export of glucose for circulation to maintain blood sugar and muscle glycogen provides glucose for glycolysis within the muscle itself providing energy during exercise.

Metabolism of glycogen:

- Synthesis (Glycogenesis)
- Breakdown (Glycogenolysis)

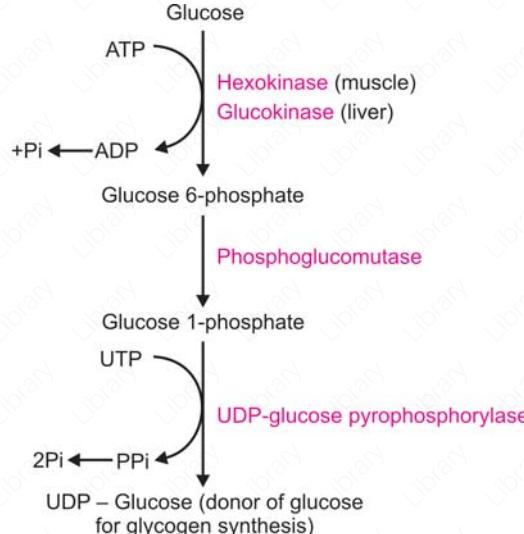
Glycogenesis (Synthesis of Glycogen)

Glycogenesis occurs in liver and muscle when plenty of glucose is available. It is a cytosolic pathway requiring ATP and UDP glucose.

Steps Involved in Glycogenesis

- Activation of glucose
- Synthesis of linear chain of glucose
- Formation of branches and elongation of chains to form glycogen.

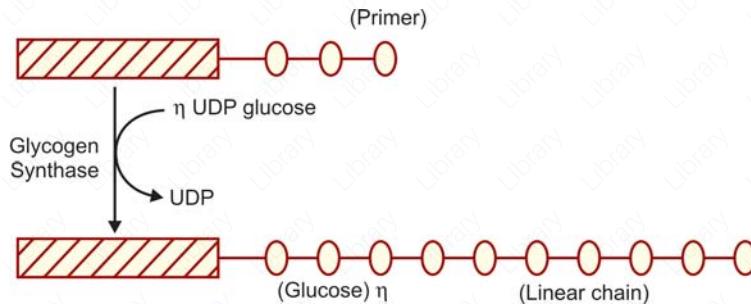
i. Activation of glucose to form UDP glucose



- Synthesis of linear chain of glucose: It requires glycogen primer which is a protein called glycogenin with 3-4 glucose attached to it.



Glycogen synthase sequentially attaches glucose residues from UDP glucose by creating α , 1-4 link. It is done from non-reducing end of molecule.



iii. Formation of branches (Fig. 3.3.6)

- It involves "branching enzyme".
- It transfers a linear chain of 6-8 glucose residues to another chain of glucose, forming α -1,6 linkage, thus branching takes place.
- To the newly formed branch, glycogen synthase again adds glucose units by α , 1-4 linkages with further branching and elongation to form glycogen.

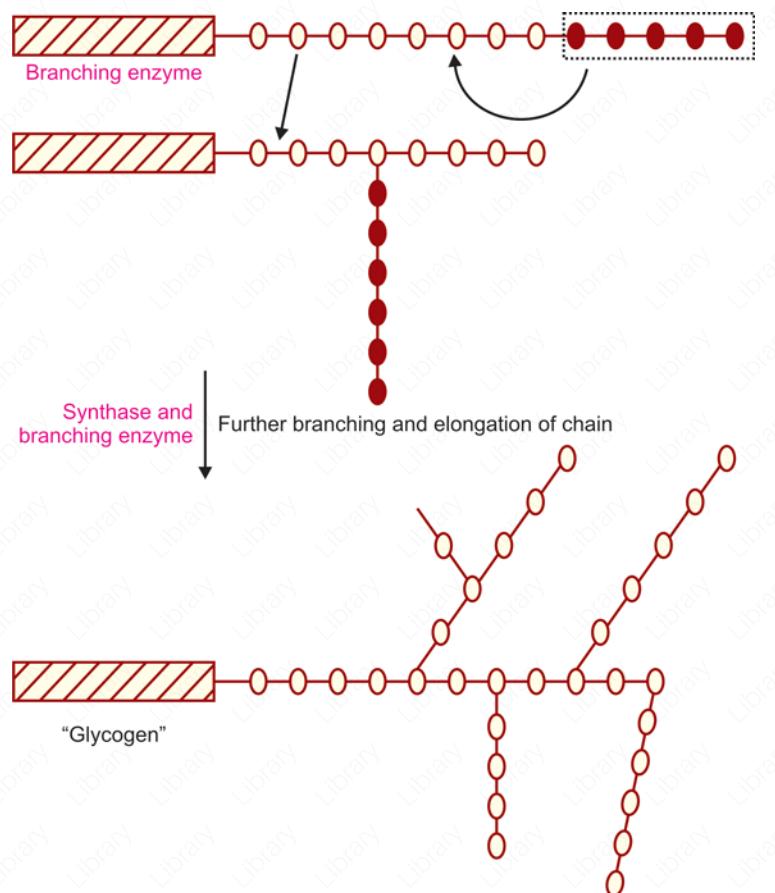


Fig. 3.3.6: Schematic diagram of glycogenesis (mechanism of branching)

Glycogenolysis

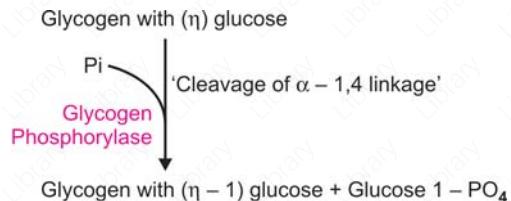
It is the breakdown of glycogen and occurs in liver when blood glucose level decreases and in muscle during its contraction.

Sub-cellular site: Cytosol

It is NOT reversal of glycogenesis

It involves:

- i. Phosphorolysis of glucose residues by glycogen phosphorylase
 - ii. Debranching (Fig. 3.3.7).
 - i. *Glycogen phosphorylase (key enzyme)*



Action of glycogen phosphorylase continues till it reaches a glucose residue, 3-4 glucose units away from a branch point.

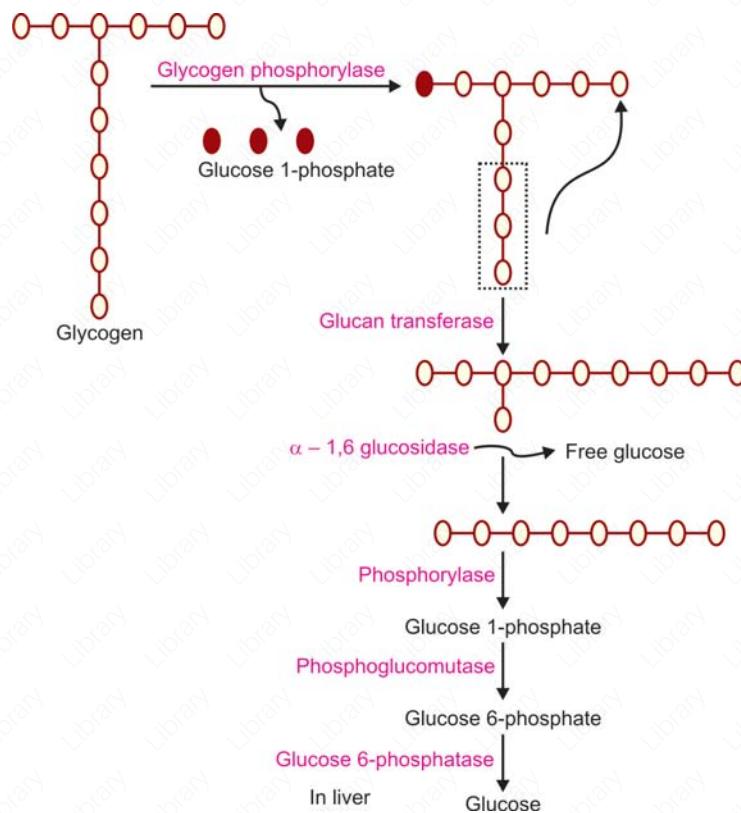


Fig. 3.3.7: Schematic representation of glycogenolysis (mechanism of debranching)

- ii. Debranching enzyme has 2 activities:
- Glucan transferase activity: It transfers a block of 3 glucose residues to another chain. Now branch point is exposed.
 - α , 1-6 glucosidase activity: It hydrolyses α , 1-6 linkage at branch point, releasing free glucose.

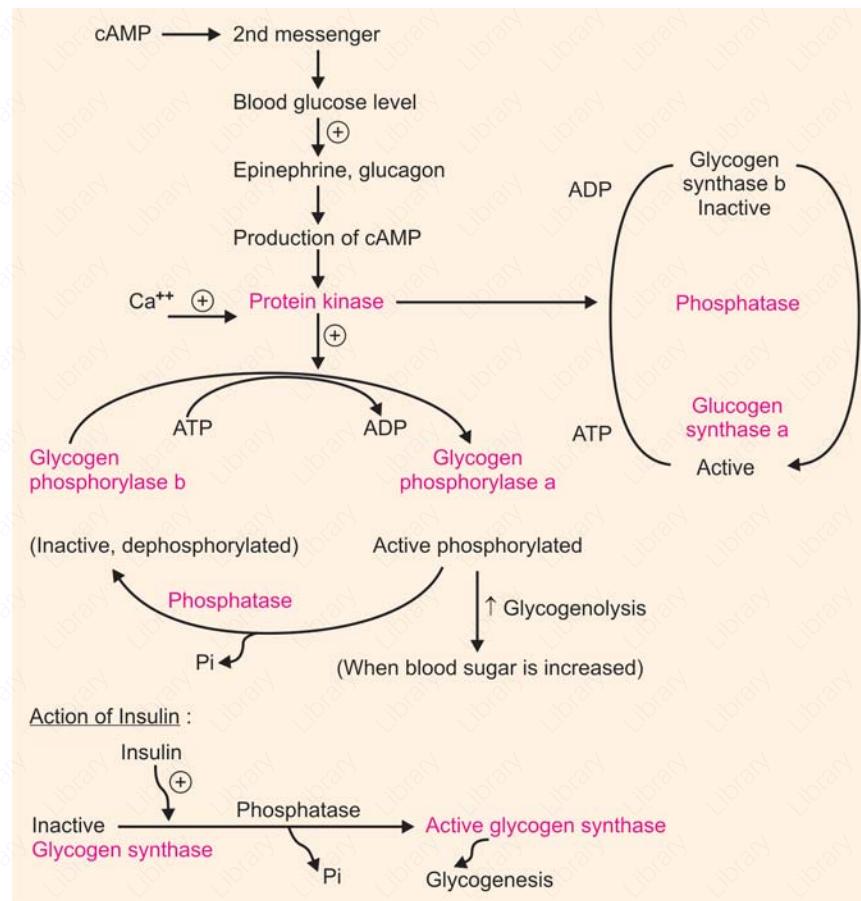
Regulation of Glycogen Metabolism

Key enzymes of glycogen metabolism are glycogen synthase and glycogen phosphorylase. These are "reciprocally regulated"

- Covalent modification - mediated by hormones
- Allosteric regulation - mediated by substrates.

Covalent Modification

- Addition or removal of phosphate group modifies the activities of key enzymes (Phosphorylation and dephosphorylation).



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- Enzyme is active in only one form.
 - Glycogen phosphorylase is active in phosphorylated form
 - Glycogen synthase is active in de-phosphorylated form
 - Thus these enzymes exist in 2 forms:
 - Active form
 - Inactive form and get activated by suitable signal.
- Covalent modification of enzymes by hormones is cAMP mediated.

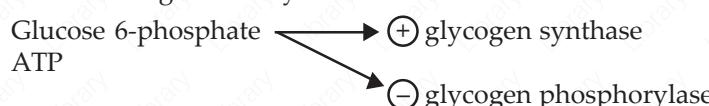
Glycogen Storage Diseases

In born errors where there is accumulation of abnormal quantity or type of glycogen.

Type	Name	Enzyme defect	Features
I	von Gierke's disease	Glucose 6-phosphatase	Fasting hypoglycemia Accumulation of glycogen in liver and muscle Death by liver failure
II	Pompe's	Lysosomal α -1, 4 glucosidase	Fatal, glycogen accumulation in heart, liver, kidney
III	Cori's	Debranching enzyme	Accumulation of abnormal glycogen in tissues, hypoglycemia
IV	Anderson's (Amylo Pectinosis)	Branching enzyme	Accumulation of abnormal glycogen in tissue
V	McArdle's	Muscle glycogen phosphorylase	Decreased exercise tolerance; fatigue, cramps
VI	Her's	Liver glycogen phosphorylase	Hypoglycemia
VII	Tarui's	PFK	Accumulation of glycogen in liver Hemolysis

Stimulus	Hormone	Mediator	End result
↓ Blood glucose and exercise	Glucagon Epinephrine Ca^{++}	cAMP and protein kinase	Activation of glycogen phosphorylase Inhibition of glycogen synthase ↑↑ Glycogenolysis
↑↑ Blood glucose	Insulin	Phosphatase	Activation of glycogen synthase ↑↑ Glycogenesis

Allosteric regulation by substrates



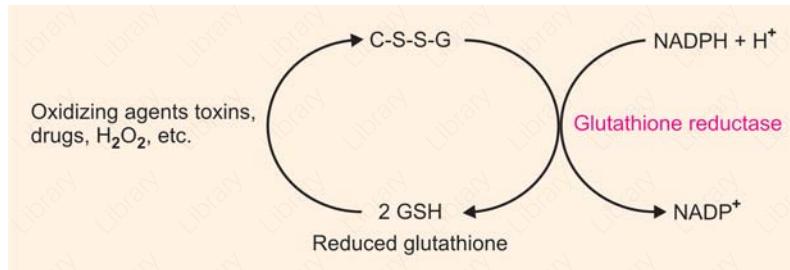
Thus, these 2 enzymes are reciprocally regulated.

HMP SHUNT (HEXOSE MONOPHOSPHATE) PATHWAY

It is also called pentose phosphate pathway or direct oxidative pathway or Dicken-Horecker pathway. It is an alternate pathway for metabolism of glucose. About 10 percent glucose/day enters this pathway. 30 percent Glucose is metabolized by HMP shunt pathway in RBC and liver.

Significance

- Production of NADPH (not concerned with ATP production).
 - Production of ribose for nucleotide synthesis.
- NADPH is used as hydrogen donor in "reductive biosynthesis" mainly, the synthesis of fatty acids, cholesterol, steroids.
 - NADPH is required for regeneration of "reduced glutathione" which is required for:
 - Preserving RBC membrane integrity.
 - Keeping iron of Hb in ferrous (Fe^{++}).
 - Prevents oxidative damage to cell.
 - Keeps many enzymes (- SH containing) in the form.



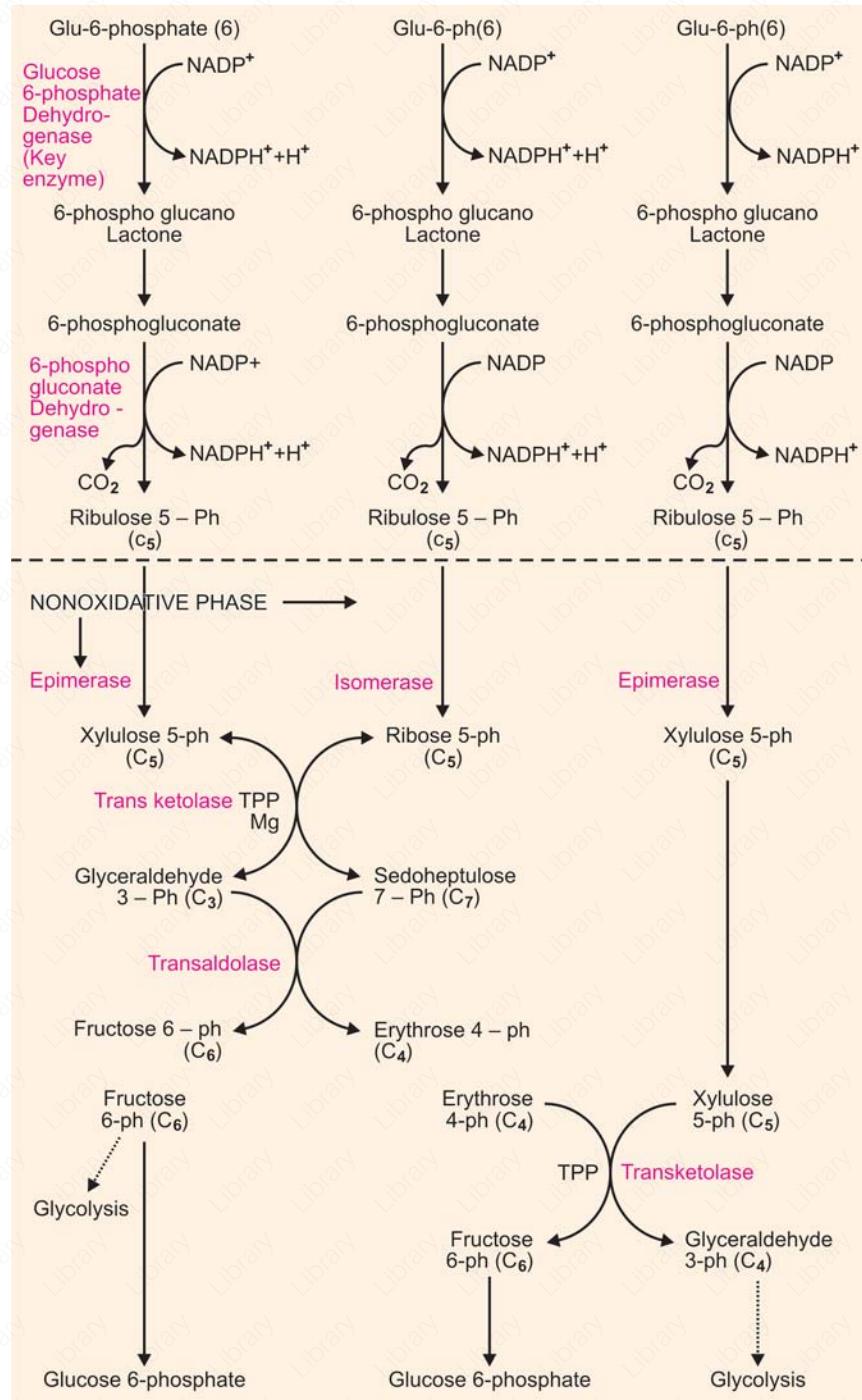
- NADPH is required as coenzyme for many detoxification reactions
- NADPH is required by WBC for destruction of foreign organisms
- NADPH is needed for maintaining the transparency of lens of eyes.

Pathway

HMP shunt pathway occurs in all cells and takes place in cytosol. It has two phases.

- Oxidative phase is irreversible and generates NADPH while CO_2 is removed.
- Non-oxidative phase is reversible and generates ribose phosphate and glucose 6-phosphate.

Oxidative Phase



Regulation

Key enzyme is glucose 6-phosphate dehydrogenase (G-6-PD). NADP activates while NADPH inhibits.

G-6-PD deficiency is an inherited sex linked trait. Although the deficiency occurs in all the cells of the affected individuals, it is more severe in RBC.

In RBCs, HMP shunt is the only means of providing NADPH. Decreased activity of G-6-PD decreases the synthesis of NADPH in RBC. This results in the storage of Methemoglobin and peroxides in RBC leading to hemolysis.



↓
Oxidation of RBC membrane unchecked

↓
Hemolysis

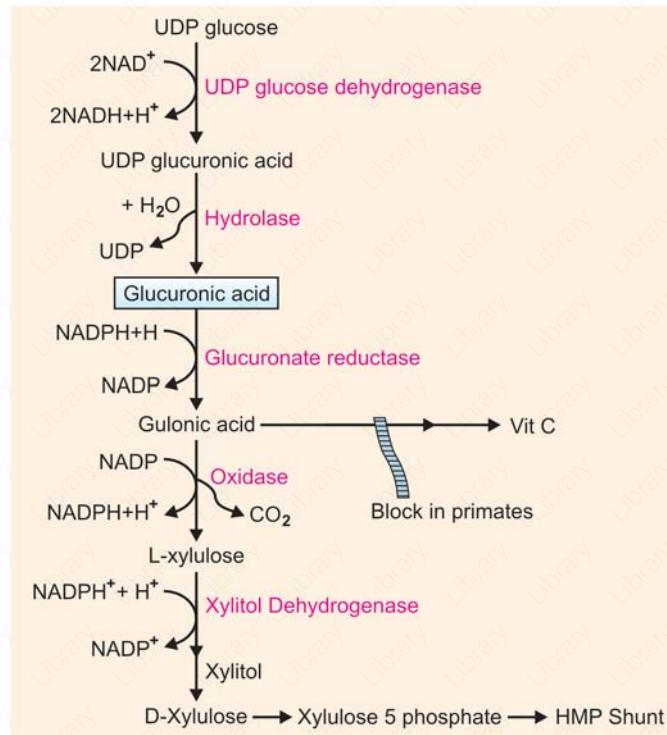
↓
Hemolytic anemia and jaundice

URONIC ACID PATHWAY

It is the alternative oxidative pathway for glucose. Glucose is converted to glucuronic acid, ascorbic acid and pentoses.

Significance

- It provides glucuronic acid which is required for
 - i. Conjugation reactions involved in detoxification of drugs, bilirubin etc.
 - ii. Synthesis of heteropolysaccharides (GAG)
- It provides vitamin C in lower animals (not in primates).



Essential Pentosuria

This is a rare genetic harmless disorder related to the deficiency of xylitol dehydrogenase which is an NADP dependent enzyme.



As a result, accumulation of L-xylulose and its excretion in urine. It is detected by Bial's test with [Test for pentoses] urine of patient.

Metabolism of Fructose

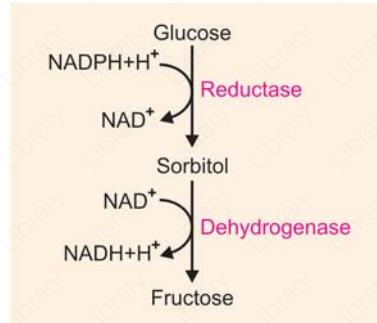
Fructose is not a dietary essential sugar. It is seen in large quantities in semen (provides energy for spermatozoa). It is not seen in significant amounts in blood.

Metabolism of Fructose:

1. Synthesis
2. Break down

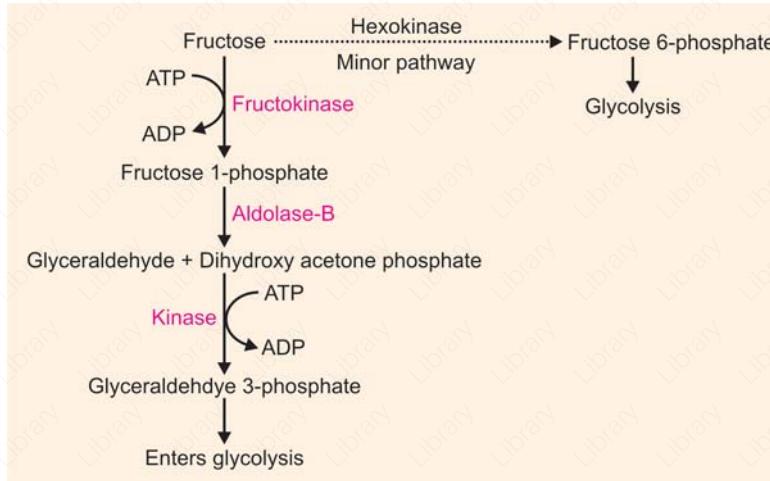
Synthesis

From glucose by sorbitol pathway:



In diabetes mellitus, there is increase in blood glucose which in turn produces more sorbitol leading to the complications of diabetes (neuropathy, retinopathy).

Catabolism



Errors of Metabolism of Fructose

1. *Hereditary fructose intolerance:* This is due to the absence of the enzyme Aldolase B. Hereditary fructose intolerance causes intracellular accumulation of fructose 1 phosphate, severe hypoglycemia, hepatic failure and jaundice. Fructose-1 phosphate inhibits liver phosphorylase and blocks glycogenolysis leading to hypoglycemia. Manifestations are-vomiting, loss of appetite, failure to thrive, fainting are observed. Early detection and intake of diet free from fructose and sucrose are advised.

2. *Essential fructosuria:* Due to the deficiency of the enzyme hepatic fructokinase, fructose is not converted to fructose-1 phosphate. This is an asymptomatic condition with excretion of fructose in urine which results in +ve Benedict's test and +ve Seliwanoff's test. Treatment involves the restriction of dietary fructose.

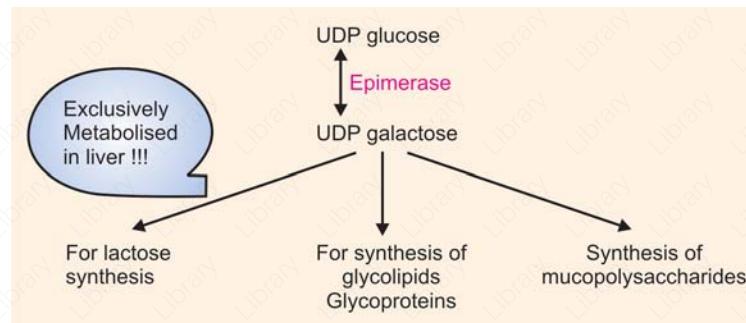
Metabolism of Galactose

Galactose is a constituent of milk sugar (Lactose). It can be synthesized from glucose.

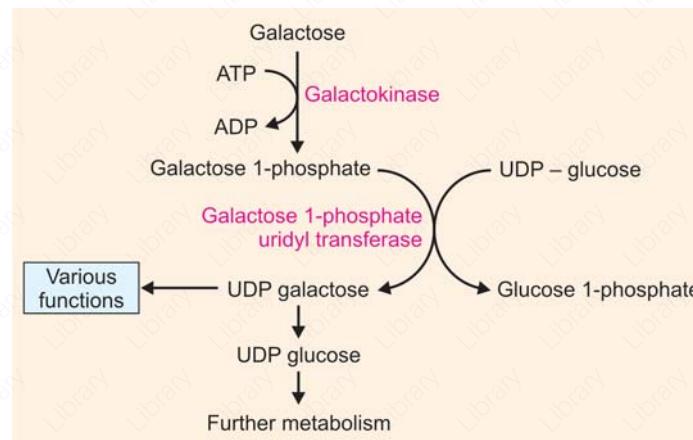
Functions

Galactose is required for lactose synthesis in lactating mammary glands. It is also needed for the synthesis of glycolipids, glycoproteins and mucopolysaccharides. UDP galactose is an active donor of galactose for many synthetic reactions. Galactose is not an essential nutrient since UDP glucose can be converted to UDP galactose by the enzyme UDP hexose-4 epimerase.

Synthesis of Galactose from Glucose



Catabolism: In Liver



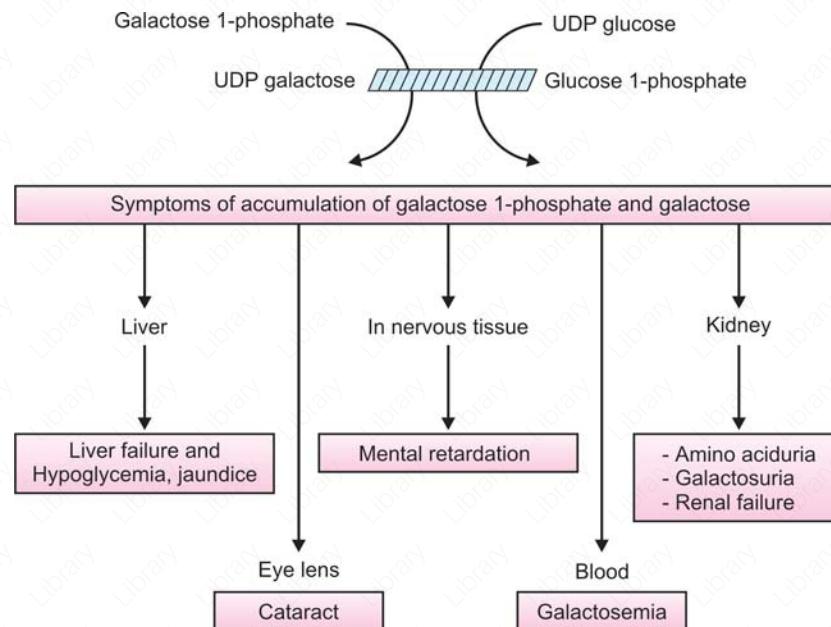


Fig. 3.3.8: Galactosemia

Inborn Error

Galactosemia: It is the serious error of galactose metabolism due to the deficiency of galactose 1 phosphate uridyl transferase (Fig. 3.3.8).

Symptoms

Intolerance to milk, fainting, mental retardation, cataract, renal failure and liver failure.

Deficiency of galactokinase leads to galactosemia but symptoms are mild.

Determination of Glucose in Body Fluids (Blood, urine and cerebrospinal fluid)

There are many methods available to measure the blood glucose level. They are:

1. Enzymatic method
 - a. Hexokinase method
 - b. Glucose oxidase-peroxidase method (GOD-POD)
 - c. Glucose dehydrogenase method.
2. Polarographic method
 - a. PO_2 electrode method

3. Chemical methods
 - a. O-Toluidine method
 - b. Folin Wu method
 - c. Nelson Somogyi's method

Sample Collection and Processing

- The blood sample is collected and processed. The selection of serum or plasma depends on the methods used.
- The serum is free of fibrinogen.
- Plasma contains fibrinogen.
- The fasting whole blood glucose concentration is 12 percent lower than plasma glucose.
- Usually, venous blood samples are used for the glucose estimation.
- If the serum is used then, the assay should be done within 30 minutes of sample collection, since it does not contain any preservatives to stop the process of glycolysis.
- Glycolysis decreases serum glucose by 5-7 percent/hour in normal uncentrifuged blood kept at room temperature.
- Glycolysis can be inhibited by adding the sodium iodoacetate or sodium fluoride which stabilizes the glucose level.
- Fluoride ions prevent glycolysis by inhibiting the enzyme enolase. Fluoride is also a weak anticoagulant because it binds Ca^{2+} ions.
- Potassium oxalate 2 mg and sodium fluoride 2 mg/ml of blood are added to prevent clotting.
- The vacutainer available contains the fluoride.

The blood glucose done at three intervals are:

Fasting (FBS)

Fasting blood sugar is estimated 8-10 hours after the last meal. This is usually done in the early morning. The person should not consume anything other than water.

Postprandial (PPBS)

Postprandial blood glucose is estimated usually 2 hours after a normal meal or breakfast.

Random (RBS)

The random blood glucose can be measured at any time of the day irrespective of the food taken.

Glucose Tolerance Tests (GTT)

- A normal person should be able to reduce a glucose load from his blood within a specified time. This is known as normal tolerance.
- If the person has an elevated blood glucose concentration for longer than the normal time, the condition is called as reduced tolerance.
- If the glucose concentration becomes very low or normal very early than the normal time then the condition is called as increased tolerance.
- The tests that are used to measure these changes in blood glucose after a glucose load are called glucose tolerance tests.

There are 2 types:

- i. Oral
- ii. Intravenous GTT.

These are mainly used in the detection of diabetes. Oral GTT is more commonly used in all the laboratories. It is convenient to give glucose through oral route.

Indications for GTT

1. From the family history of diabetes mellitus
2. Signs and symptoms comparable with diabetics without any complications
3. Glucosuric patients with normal fasting blood sugar
4. Border line glucose in PPBS
5. Reactive hypoglycemia for 3 hours or longer period after food intake.
6. Pregnancy with history of abortions, stillbirths or a large baby.

Preparation of the Patients

1. Patient should not be under fear or anxiety about the possibility of being a diabetic. If so, it can lead to false positive results. So it is the duty of the technician to prepare the patient emotionally or mentally to be calm.
2. Adequate carbohydrate intake. Before the test the patient should have been on a diet containing at least 150 g of carbohydrate per day with low fat for at least 3 days. An adequate deposit of glycogen in the liver and other tissues is essential for the production of a normal response. If the subject is in a state relatively low carbohydrate diet for sometime before the test, the rise in blood sugar levels following the ingestion of glucose will be more pronounced and its fall to the normal level is delayed.
3. It is desirable for the subject to fast for 10 to 12 hours before the test.
4. The test patient must not have ingested tea or coffee on the day of test.
5. The patient should not have excessively exercised or be physically strained.
6. If the patient is not well the test should be postponed.
7. The patient should not receive any drugs for at least 3 days before the test.

Factors Affecting GTT

1. Factors associated with hyperglycemia: aldosterone, catecholamines, diphenylhydantoin (DPH), nicotine, oral contraceptives, thiazides, glucagon and growth hormone.
2. Factors associated with hypoglycemia: Ethanol, INH and sulfonamide drugs.
3. The age factor is also important. The glucose tolerance tends to become lower in old age.

Method

The test is usually carried out in the early morning after fasting overnight.

Fasting blood sample and urine are then collected. 75 (or 100 g) of glucose dissolved in about 150-200 ml of water is given to drink.

Venous blood for the estimation of blood glucose is collected at $\frac{1}{2}$ hour intervals for 2- $\frac{1}{2}$ hours or hourly intervals for 3 hours after the ingestion of glucose. Urine specimens are also collected at the same time.

Blood glucose is estimated in each sample and the urine is tested for the presence of the sugar (qualitative Benedict's test).

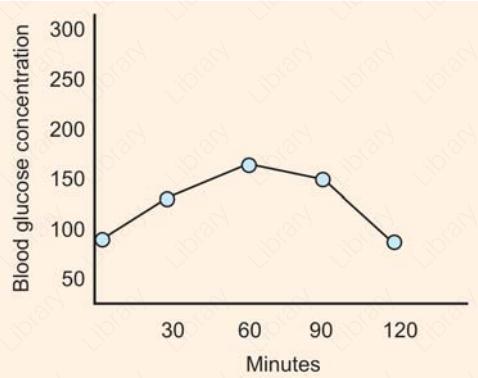
INTERPRETATION

Normal Glucose Tolerance Curve

The normal curve has the following features.

1. The fasting blood glucose in this category is usually within the range of 60-100 mg/dl.
2. The blood glucose does not rise above 160 mg/dl.
3. The blood glucose at 2 hour after the load is 110 mg/dl.
4. The urine remains free of glucose throughout the test.
5. The timing of the peak value is not defined as a part of the normal pattern of response but it is usually seen either in the 30 minutes or 60 minutes blood sample.

Sample no.	Mg glucose /100 ml blood	Urine glucose
Fasting	90	Negative
30 minutes	120	Negative
60 minutes	150	Negative
90 minutes	140	Negative
120 minutes	90	Negative



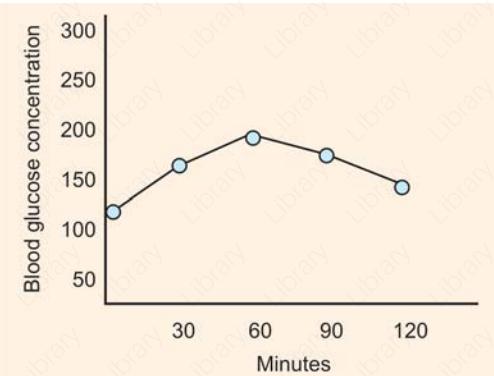
Abnormal Glucose Tolerance Response

Reduced glucose tolerance:

The features are:

1. The fasting level is above 120 mg.
2. The glucose level crosses 200 mg/100 ml in 30 to 60 minutes.
3. The blood glucose level is more than 110 mg/dl even after 2 hours.
4. There may be glucose in at least two of the urine specimens.

Sample no.	Mg glucose /100 ml blood	Urine glucose
Fasting	120	Negative
30 minutes	160	Positive
60 minutes	200	Positive
90 minutes	180	Positive
120 minutes	150	Negative



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MULTIPLE CHOICE QUESTIONS

1. Blood glucose level after an overnight fasting in a normal adult is:
A. 50-60 mg/dl B. 60-70 mg/dl
C. 70-80 mg/dl D. 60-90 mg/dl
2. Glycogen synthesis takes place in liver and muscle in a state of:
A. Hypoglycemia B. Normoglycemia
C. Hyperglycemia D. Starvation
3. The enzymes of the Embden-Meyerhof pathway are located in:
A. Mitochondria
B. Smooth endoplasmic reticulum
C. Extra-mitochondrial soluble fraction
D. Lysosomes
4. Muscle glycogen does not contribute glucose to blood but gives rise to a gluconeogenic substrate:
A. Pyruvic acid B. Lactic acid
C. Glycerol D. Glucose-6-PO₄
5. All of the following are key enzymes of gluconeogenesis *except*:
A. Aldolase B. Pyruvate carboxylase
C. Glucose-6-phosphatase D. Fructose 1, 6-diphosphatase

ANSWERS

1. C 2. C 3. C 4. A 5. A

MOST LIKELY QUESTIONS

Long Essays

1. Describe HMP shunt pathway with its significance.
2. Give an account of glycogen metabolism.
3. Trace pathway for TCA cycle. Justify it is a final common metabolic pathway for oxidation of foodstuffs.
4. Define gluconeogenesis. Trace pathway for gluconeogenesis from propionate.
5. Discuss synthesis of glucose from non-carbohydrate sources.
6. Reactions of TCA cycle and its regulation.
7. Write an account on steps involved in glycolysis. Write a note on its energetics.
8. Enumerate steps of glycolysis. Write a note on significance of 2, 3 BPG and substrate level phosphorylation in glycolysis.
9. What is normal fasting blood sugar level? What is diabetes mellitus? Write in detail the metabolic changes that take place in diabetes mellitus.
10. What is diabetes mellitus? How blood sugar level is maintained and regulated? Write a note on action of insulin.

Short Essays

1. Explain glycogenesis and its regulation.
2. Explain glycogenolysis and its regulation.
3. Sorbitol pathway and diabetes mellitus.
4. Action of insulin.
5. Note on uronic acid pathway and its importance.
6. Significance of HMP shunt pathway.
7. Metabolism of galactose.
8. Metabolism of fructose.
9. Glycogen storage diseases.
10. Name the key gluconeogenesis enzymes. Explain how lactate is converted to glucose.
11. Energetics of glucose mediation.
12. Inhibitors of Kreb's cycle.
13. UDP-glucose.
14. 2,3 - BPG.
15. Rapaport Leubering cycle.
16. Glucose 6-phosphate dehydrogenase.
17. Conversion of pyruvate to acetyl CoA.
18. What is glucose tolerance test? How is it performed? How is the test interpreted?
19. Essential fructosuria.
20. Cori's cycle.
21. Energetics of TCA cycle.
22. TPP in carbohydrate metabolism.

Short Answers

1. Importance of pyruvate dehydrogenase enzyme.
2. Role of fructose 1,6 bisphosphate.
3. Irreversible steps of glycolysis.
4. Importance of glucose 6 phosphate dehydrogenase.
5. Energetics of glycolysis.
6. Substrate level phosphorylation.
7. Write the reactions involving substrate level phosphorylation in TCA. cycle and glycolysis.
8. Renal glycosuria.
9. Serum amylase.
10. Lactate dehydrogenase and its clinical importance.
11. Energy yielding steps of glycogen.
12. Cori's cycle.
13. Key gluconeogenic enzymes.
14. What is gluconeogenesis?
15. What are glycosides? Name glycosides of biological importance.
16. Dietary fiber.

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17. Glucose-alanine cycle and its significance.
18. Entry of glucose into the cells.
19. Oxidative decarboxylation of pyruvate.
20. Galactosemia.
21. Sources of acetyl CoA
22. Role of liver in blood sugar regulation.
23. Anaplerosis.
24. Mention biochemical defect in hereditary fructose intolerance and galactosemia.
25. Galactitol and its significance.
26. Primer for glycogen synthesis.
27. Five vitamins required by pyruvate dehydrogenase.



4.1: CHEMISTRY OF LIPIDS

INTRODUCTION

Lipids are organic substances insoluble in water and soluble in organic solvents such as ether, alcohol, acetone, etc.

They are small molecules, not polymers, related to fatty acids and utilized by living cells.

Functions of Lipids

1. Lipids are storage form of energy = 9.1 K cal of energy/gram of lipid.
2. They form components of the cell membrane (phospholipids and cholesterol).
3. They provide insulation against changes in external temperature (subcutaneous fat).
4. They protect internal organs by providing a cushioning effect.
5. They are metabolic regulators: prostaglandins, leukotrienes, thromboxanes, steroid hormones.
6. They act as surfactants in the lungs – Dipalmitoyl lecithin acts as a surfactant and maintains the surface tension in the alveoli without which they will collapse.
7. They act as detergents and emulsifying agents – Bile salts act as emulsifying agents of the dietary lipids during their digestion and absorption.
8. They act as electrical insulators – Sphingomyelin on the myelin sheath around myelinated nerves helps in quick conduction of impulses across the nerves.
9. The absorption of the fat soluble vitamins from the diet is facilitated by the dietary lipids.
10. They give shape and contour to the body.
11. Lipids give taste and palatability to food.

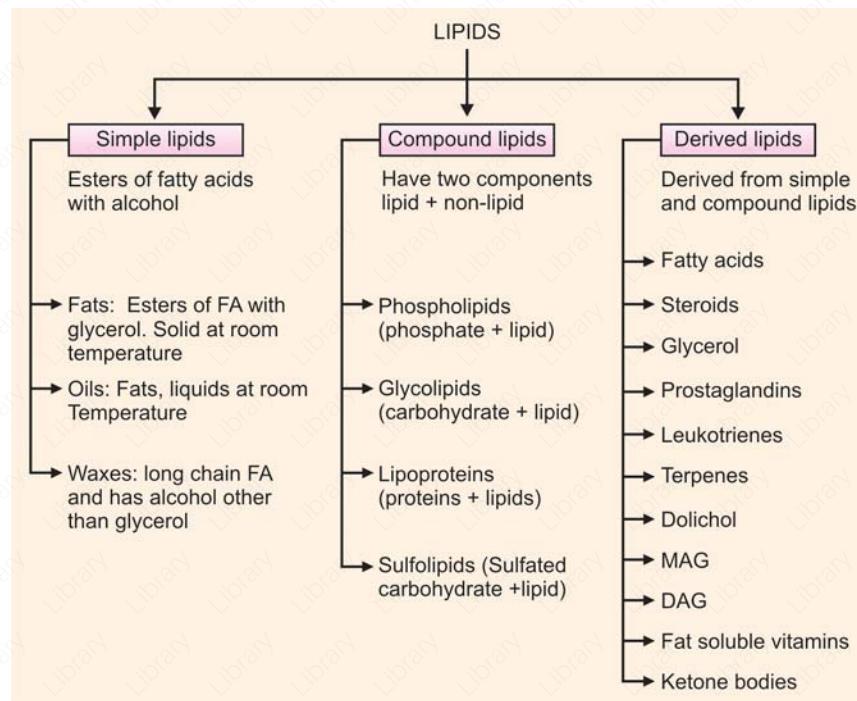
Clinical Applications

1. Obesity – excessive fat deposits cause obesity, which may lead to heart attacks.
2. Atherosclerosis – depositions of cholesterol and cholesterol containing lipids in the blood vessels leading to hypertension.
3. Diabetic ketosis – decreased carbohydrate metabolism and increased lipid metabolism leads to formation of ketone bodies.

CLASSIFICATION OF LIPIDS

Bloor (1925) classified lipids based on chemical nature.

1. Simple lipids
 2. Compound lipids
 3. Derived lipids
1. *Simple lipids*: Esters of fatty acids (FA) with alcohol. Simple lipids are further classified into:
 - a. *Fats*: Esters of fatty acids with glycerol. These are solid at room temperature.
 - b. *Oils*: Fats, liquids at room temperature.
 - c. *Waxes*: Have long chain fatty acids and an alcohol other than glycerol.



2. **Compound lipids:** Esters of fatty acids with alcohol and in addition have other groups.
 - a. **Phospholipids:** Contain phosphate groups, in addition to lipid part.
Example: Lecithin, Sphingomyelins.
 - b. **Glycolipids:** Contain carbohydrate groups along with lipid part.
Example: Cerebrosides, Gangliosides.
 - c. **Lipoproteins:** Complexes of lipids and proteins.
Example: Chylomicrons, High Density Lipoproteins (HDL)
 - d. **Sulfolipids:** Contain sulfated carbohydrates with lipid part.
Example: Sulfatides.
3. **Derived lipids:** Derived from simple and compound lipids after hydrolysis.
Example: Fatty acids, steroids and sterols, glycerol, prostaglandins, leukotrienes, terpenes, dolichol, monoacyl glycerol (MAG), diacylglycerol (DAG), fat soluble vitamins, ketone bodies.

Miscellaneous lipids: Compounds having characters of lipids.

Example: Carotenoids, squalene, hydrocarbons in beeswax, terpenes.

Neutral lipids: Uncharged lipids.

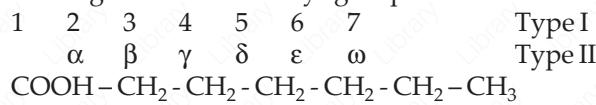
Example: Monoacyl glycerol (MAG), diacyl glycerol (DAG), triacyl glycerol (TAG).

FATTY ACIDS

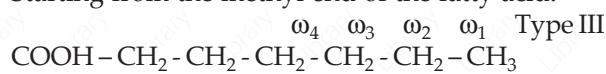
These are the simplest forms of lipids. Their functional group is $-COOH$. Hence, fatty acids are carboxylic derivatives of hydrocarbons. Fatty acids exist in free form, ester form and amide form.

Nomenclature

Starting from the carboxyl group:



Starting from the methyl end of the fatty acid:



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Classification of Fatty Acids

1. Based on number of carbon atoms:

- Even chain fatty acids: Fatty acids having even number of carbon atoms.

S.No.	Name of fatty acid	No. of C atoms	Molecular formula	Sources
1.	Acetic acid	2	CH ₃ COOH	Vinegar
2.	Butyric acid	4	C ₃ H ₇ COOH	Butter
3.	Caproic acid	6	C ₅ H ₁₁ COOH	Butter
4.	Caprylic acid	8	C ₇ H ₁₅ COOH	Butter
5.	Capric acid	10	C ₉ H ₁₉ COOH	Coconut oil
6.	Lauric acid	12	C ₁₁ H ₂₃ COOH	Coconut oil
7.	Myristic acid	14	C ₁₃ H ₂₇ COOH	Coconut oil
8.	Palmitic acid	16	C ₁₅ H ₃₁ COOH	Human fat
9.	Stearic acid	18	C ₁₇ H ₃₅ COOH	Human fat
10.	Arachidic acid	20	C ₁₉ H ₃₉ COOH	Arachis oil

- Odd chain fatty acids – having odd number of carbon atoms.

Propionic acid	3 carbon	C ₂ H ₅ COOH
Valeric acid	5 carbon	C ₄ H ₉ COOH

Most of the fatty acids synthesized in the body are even chain fatty acids.

But odd chain fatty acids are produced during metabolism.

2. Based on the length of the carbon chain

- Short chain fatty acids : 2 to 6 carbon atoms
- Medium chain fatty acids : 8 to 14 carbon atoms
- Long chain fatty acids : 16 to 24 carbon atoms

3. Based on presence or absence of double bonds

Saturated and unsaturated fatty acids

- Saturated fatty acids are those which have no double bonds.

Example: All the odd and even chain fatty acids

- Unsaturated fatty acids are those which have double bonds.

Depending upon the number of double bonds present, the fatty acids are further classified into:

i. Monoenoic acids have one double bond.

Example: Palmitoleic acid C16 : 1 : 9 – body fat

Oleic acid C18: 1: 9 – body fat

Nervonic acid C24 : 1: 15 – brain

ii. Polyunsaturated fatty acids (PUFA) have two or more number of double bonds.

Dienoic: 2 double bonds, linoleic acid

C₁₈ : 2 : Δ^{9, 12}, occur in vegetable oils.

Trienoic: 3 double bonds, linolenic acid

C₁₈ : 3 : Δ^{9, 12, 15}, vegetable oils.

Tetraenoic: 4 double bonds, arachidonic acid

$C_{20}:4:\Delta^{5,18,11,14}$, vegetable oils, phospholipids

Pentaenoic: 5 double bonds, timnodonic acid

C₂₀:5:Δ^{5, 8, 11, 14, 17}, fish oils and brain

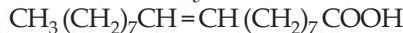
Hexaenoic: 6 double bonds, Cervonic acid

$C_{22}:6:\Delta^{4,7,10,13,16,19}$, fish oils and brain

4. Based on the groups present in the side chain:

- a. *Saturated Fatty Acids*: Only C, H and no double bonds.

- b. *Unsaturated Fatty Acids*: are made up of only C, H and double bonds.



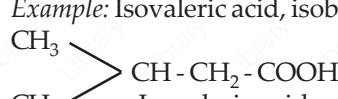
Unsaturated F.A are also designated as:

ω 3 family : Timnodonic and linolenic acids

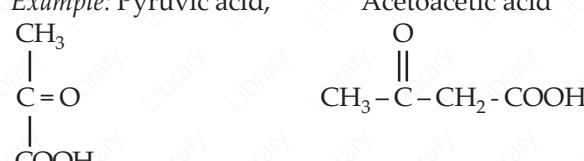
ω 6 family : Linoleic an

- c. *Substituted family acids:* Groups that can be substituted are methyl,

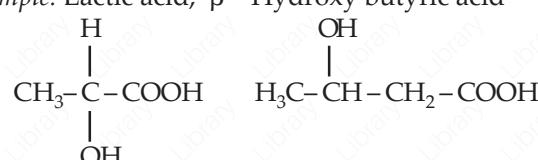
- hydroxy, keto, and cyclic.



- iii) *Keto:* CH_3 Isovaleric acid
 Farnesyl Pyruvate acid Acetoacetic acid

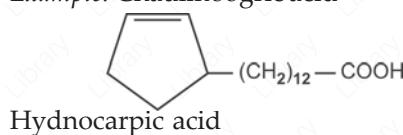


- iii. *Hydroxy:* Example: Lactic acid, β -Hydroxy butyric acid



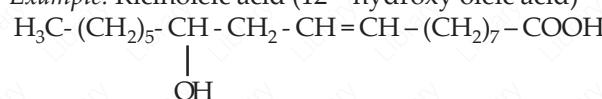
- iv. Cyclic: Cyclopentenyl ring system is present in the fatty acids.

Example: Chaulmoogric acid



A combination of two or more of the above can occur.

Example: Ricinoleic acid (12-hydroxy oleic acid)



5. Based on whether these can be synthesized in the body:
 - a. *Essential fatty acid*: Cannot be synthesized in the body.
 - b. *Non-essential fatty acid*: Can be synthesized in the body.

Essential Fatty Acids (EFAs)

Definition: EFAs are those which cannot be synthesized in the body and have to be taken in through the diet.

Essential fatty acids are linoleic, linolenic and arachidonic acid. Human body does not have the enzyme systems to insert double bonds beyond the position of C₉ to C₁₀.

Functions of Essential Fatty Acids

1. These are the constituents of the cell membrane and membranes of the mitochondria.
2. These are involved in the esterification of cholesterol and thus help in the transport and metabolism.
3. Arachidonic acid is the precursor of hormone like substances – prostaglandins, leukotrienes and thromboxanes.
4. These are essential for normal growth and health.
5. These prevent fatty liver.
6. EFAs are hypocholesterolemic and therefore they are anti-atherogenic in effect.

Deficiency of Essential Fatty Acids

Deficiency results in *phrynodermia* or toad skin characterized by the presence of horny eruptions on the posterior and lateral parts of limbs, on the back and buttocks, loss of hair and poor wound healing.

It also results in eczematous lesions, arterial degeneration and fatty liver in man. Hypercholesterolemia occurs in deficiency of EFA. Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as free fatty acids, a transport form found in the plasma.

Distribution of Fatty Acids in Human Fat

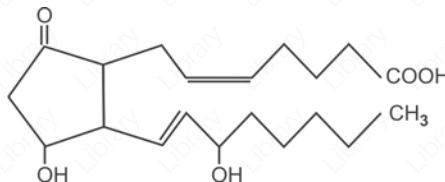
Oleic acid	- 50 percent
Palmitic acid	- 25 percent
Linoleic acid	- 10 percent
Stearic acid	- 5 percent
Others	- 10 percent

Composition of fatty acids in olive oil: 79 percent Oliec acid + 11 percent Saturated fatty acid + 7 percent Linoleic acid.

Composition of fatty acids in almond oil: 71 percent Oliec acid + 5 percent Saturated F.As + 21 percent Linoleic acid.

Prostaglandins (PGs): These are derived from arachidonic acid. They are unsaturated, C₂₀ compounds. They have cyclo-pentane ring in the middle and double bonds in the linear part.

If there is one double bond, it is called PG₁, 2 double bonds, PG₂ and 3 double bonds, PG₃.



Structure of PGE₂

Based on the substituent groups, such as CH₃, OH, keto groups in the linear part, they are denoted as PGD, PGE, PGF, PGG and PGH.

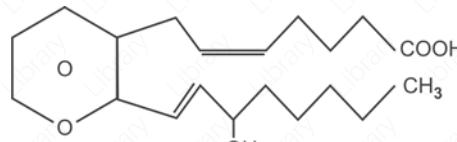
Nomenclature is done combining number of double bonds and types of substituent groups. Example: PGE₂

PGs occur in animal tissues, body fluids, prostate, seminal vesicles, renal medulla, thymus, blood plasma and semen.

Functions: PGs are called local hormones.

1. They have lower blood pressure.
2. They inhibit gastric secretion.
3. PGs decrease immune response.
4. They help in induction of labor

Thromboxanes: They are derived from arachidonic acid. They are C₂₀ compounds and contain oxane ring in the middle.



Structure of TXA₂

They are unsaturated compounds with double bonds in the linear part of the structure. If there is one double bond it is denoted as TX₁, with 2 double bonds TX₂ and with 3 double bonds TX₃.

Substituent groups in the linear part can be CH₃, OH, C=O and based on the position of these groups, thromboxanes are designated as TXA, TXB. Nomenclature depends by combining number of double bonds and types of substituent groups.

Thromboxanes occur in animal tissues, body fluids, blood platelets, polymorphs, lung, brain and smooth muscle cells.

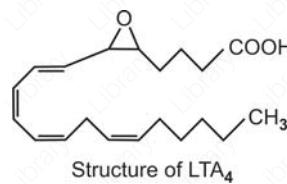
Functions

Thromboxanes increase platelet aggregation and promote thrombosis.

Leukotrienes

They are derived from arachidonic acid. These are C₂₀ compounds. These occur in animal tissues, body fluids, mast cells, leukocytes, lungs, liver, brain and other tissues.

1. They cause contraction of smooth muscle
2. Leukotrienes constrict the bronchioles
3. They increase vasoconstriction
4. They attract the leukocytes (WBC) to the inflammatory site.



Isomerism of Unsaturated Fatty Acids

Unsaturated fatty acids contain one or more double bonds. They show reactions of carboxylic group and show properties due to presence of double bond.

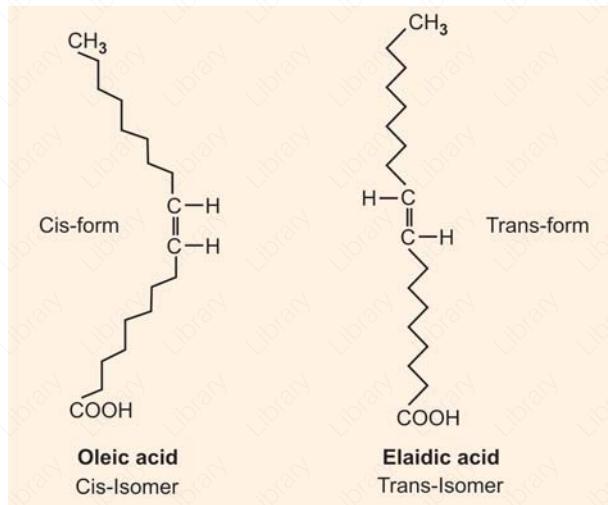
Cis Trans Isomerism (Geometric isomerism)

Unsaturated fatty acids exhibit geometric isomerism at the double bonds. All naturally occurring fatty acids have cis configuration. In the body, during metabolism trans fatty acids are formed.

$\begin{array}{c} \text{CH}_3 - (\text{CH}_2)_7 - \text{CH} \\ \\ \text{HOOC} - (\text{CH}_2)_7 - \text{CH} \end{array}$ Cis-form (Oleic acid) More reactive, less stable Melting point 13°C	$\begin{array}{c} \text{CH} - (\text{CH}_2)_7 - \text{CH}_3 \\ \\ \text{HOOC} - (\text{CH}_2)_7 - \text{CH} \end{array}$ Trans-form (Elaidic acid) Less reactive, more stable Melting point 45°C
--	--

Depending on the orientation of the groups around the double bond axis, unsaturated fatty acid exhibits geometric isomerism. If the atoms or acyl groups are present on the same side of the double bond, it is a Cis configuration. In the Cis isomeric form, there is a molecular bending at the double bond.

Trans isomers of fatty acids with their characteristic bonds will compactly pack the membrane structure.

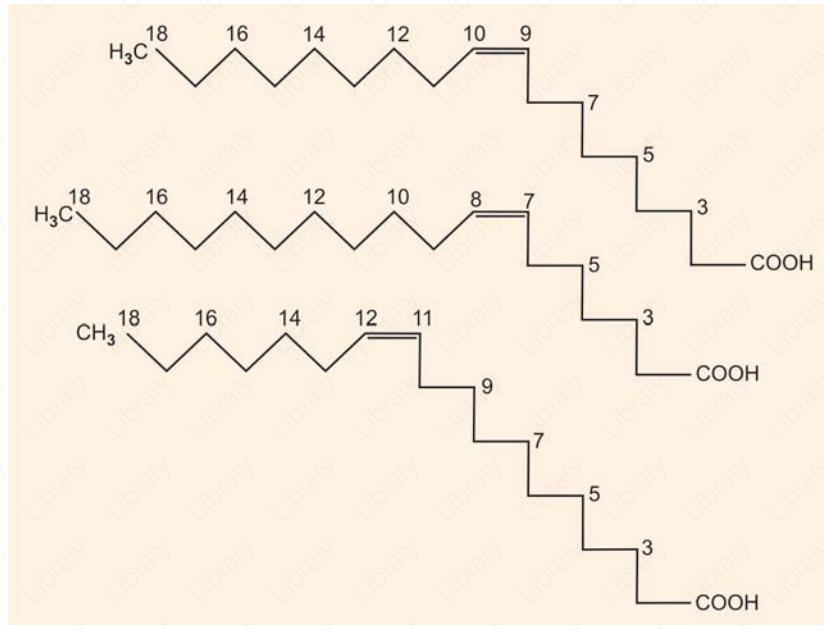


Significance of Cis-trans Isomerism

1. Trans-isomers of fatty acids bring close packing since they are straight chain fatty acid.
2. Cis-isomers prevent close packing of fatty acids, hence there is fluidity of the membranes.

Positional isomerism: This results from variations in the locations of double bonds in the molecule.

Different isomers of an acid possess the same number of double bonds, but at different positions in the molecular chain.



Positional isomers of an octadecenoic acid

CHEMICAL PROPERTIES

1. Salt Formation

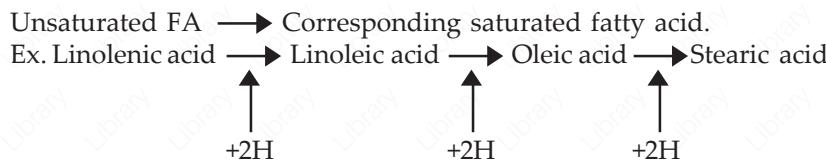
Fatty acid + alkali \longrightarrow alkali salt of fatty acid

Alkali used: NaOH, KOH, Mg(OH)₂, Ca(OH)₂, Al(OH)₃

Significance

1. Na⁺ and K⁺ salts form commercial soaps
2. Ca⁺⁺ and Mg⁺⁺ salts form soaps for hard water
3. Ca⁺⁺ salts are used in grease
4. Al⁺⁺⁺ salts are used in gels.

2. Hydrogenation



Requirement: Elevated temperature and high pressure and catalysts such as Ni, Pt, Pd, Cu.

Significance: Formation of hydrogenated oils. Example: Vanaspati, Dalda.

3. Esterification

Anhydrous HCl

- a. Fatty acid + alcohol \longrightarrow Esters of Fatty acid
- b. With Glycerol
 - Glycerol + 3FFA \longrightarrow Triglyceride or Triacylglycerol
 - Glycerol + FFA \longrightarrow Monoacyl glycerol (MAG)
 - MAG + FFA \longrightarrow Diacyl glycerol (DAG)
 - DAG + FFA \longrightarrow Triacylglycerol (TAG)
- c. Amide formation with sphingosine:
Sphingosine + free fatty acid \longrightarrow Ceramide

Significance of Esterification

- a. TAG is called a neutral fat.
- b. Ceramide is a component of Sphingomyelins and Glycolipids.

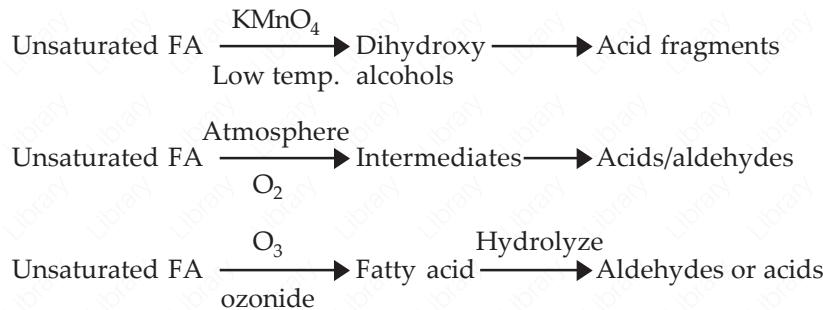
4. Halogenation

Unsaturated Fatty acids + halogens \longrightarrow Halogenated Fatty acids
(F, Cl, Br, I)

Significance

Controlled halogenations are a quantitative evaluation of the degree of unsaturation.

5. Oxidation



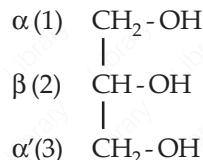
Significance

- Identification of the acid fragments to study the position of the double bonds.
- Unsaturated fatty acids, when exposed to O_2 undergo peroxidation, leading to rancidity of fats.
- All fatty acids undergo oxidation in the body to give energy. β -Oxidation is the major process by which acids are oxidized.

Alcohols Present in Lipids

1. Glycerol: Trihydroxy alcohol

Nomenclature:

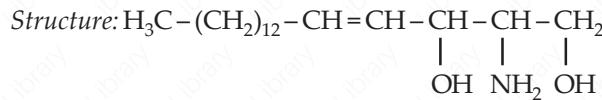


Glycerol is capable of forming 3 ester bonds with 3 molecules of fatty acids (same or different types) to form triglycerides. Diglycerides and monoglycerides are intermediates.

Significance: Glycerol is the component of fats, oils and phosphoglycerides.

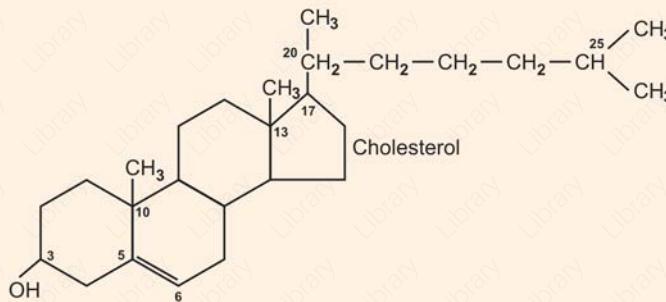
2. Sphingosine: It contains 18 carbon atoms, 2 hydroxyl groups at C_1 and C_3 , one amino group at C_2 which reacts with COOH group of fatty acid to form ceramide.

It has a double bond between C_4 and C_5 .



Significance: Sphingosine is a component of sphingolipids and glycolipids.

3. *Cholesterol:* It is a 27 carbon compound and consists of Cyclopentanoperhydrophenanthrene ring structure/steroid nucleus. It has one hydroxyl group at C₃ which forms one ester bond with a fatty acid to form cholesterol ester. It has one double bond between C₅ and C₆, 5 methyl groups at C₁₀, C₁₃, C₂₀, C₂₅ (2) and a side chain of 8 carbon atoms at C₁₇.



Functions of Cholesterol

1. It is a structural component of cell membrane
2. It is an essential ingredient in the structure of lipoproteins. Lipids in the body are transported in that form.
3. Fatty acids are transported to liver as cholesteryl esters for oxidation.

Characteristic Features

- Cholesterol is present in tissues and in plasma lipoproteins either as free cholesterol or combined with a long chain fatty acid, as cholesterol ester.
- It is synthesized in many tissues from acetyl CoA.
- It is ultimately eliminated from the body in the bile as cholesterol or bile salts.
- Cholesterol is the precursor of all other steroids in the body such as
 1. Corticosteroids – Mineralocorticoids and glucocorticoids
 2. Sex hormones – Estrogen, testosterone, progesterone
 3. Bile acids – Glycocholic acid, taurocholic acid
 4. Vitamin D
- Cholesterol is typically a product of animal metabolism and so, occurs in foods of animal origin such as egg yolk, meat, liver and brain.
- Normal total serum cholesterol level in young adults is 150 – 240 mg percent.

Bile Acids

Primary Bile Acids

1. Cholic acid
2. Chenodeoxy cholic acid

Secondary Bile Acids

1. Deoxycholic acid
2. Lithocholic acid

Bile Salts

Sodium (Na) and Potassium (K) salts of primary bile acids.

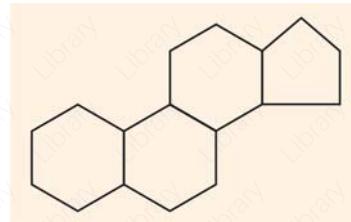
- Glycocholic acid
- Taurocholic acid
- Glyco chenodeoxycholic acid
- Tauro chenodeoxycholic acid
- Neutral sterols: Coprostanol, Cholestanol – formed by the intestinal bacteria in the intestine and excreted in the feces.

Higher Monohydroxy Alcohols

Myricyl alcohol, Cetyl alcohol.

Significance: Found as waxes from beeswax, plant wax used for making polishes, etc.

Steroids: Such as bile acids and steroid hormones are hydrocarbons – have fused, reduced, tetracyclic C_{17} ring system, “Cyclopentanoperhydro-phenanthrene” Nucleus.



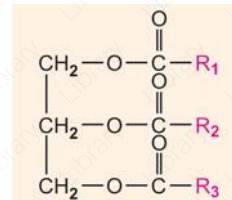
Cyclopentano-perhydro-phenanthrene nucleus

Sterols are steroids with a $C_3 - OH$ and a $C_8 - C_{10}$ carbon chain on C_{17} , but without carboxyl and carbonyl substituents.

Triacylglycerols (TAG)/Neutral Fats/Triglycerides (TG)

Esters of fatty acids with glycerol (MG, DG, TG)

Simple TGs – Carry 3 identical F.A. residues, *Example:* Tripalmitin



Mixed TGs – Carry more than one type of FA Example: α' Oleo – $\alpha\beta$ dipalmitin.

Fats remain stored as fat droplets in fat-cells of animals. They are oxidized in mitochondria to give energy. The layer of fat as adipose tissue under the skin and around internal organs provides thermo insulation and also acts as shock absorbing cushions.

Physical properties of TGs depend on the nature of fatty acids. TGs with higher proportion of unsaturated fatty acids or short and medium chain TGs are liquids at 20°C and are usually called oils. Animal fat is made up of generally saturated fatty acids and it is called hard fat. High proportion of unsaturated fatty acids in animal fat is called soft fat.

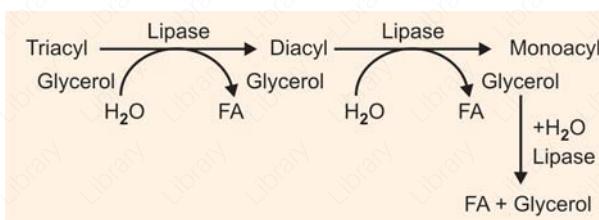
Chemical Properties

Saponification



Hydrolysis

In the body,



Hydrogenation of Fats

Unsaturated fats $\xrightarrow{\hspace{1cm}}$ saturated fats

(Vegetable oils) Ni/Pt Catalyst

Significance: Hydrogenation prevents rancidity. Partial hydrogenation of vegetable oils (cotton seed/sunflower) is called vanaspati.

Rancidity

Represents deterioration of fats and oils resulting in unpleasant taste. It is due to auto-oxidation, splitting and aldehyde formation. Fats with unsaturated fatty acids are more susceptible to rancidity. Agents for rancidity are air, sunlight, ultraviolet light, moisture, O_2 , O_3 , micro-organisms, ionizing radiations, environmental pollution, and cigarette smoke. There are two types of rancidity – hydrolytic and oxidative (enzymatic, O_2 and O_3).

Hydrolytic rancidity: It is due to partial hydrolysis of the triacyl glycerol molecules due to traces of hydrolytic enzymes present in naturally occurring fats and oils.

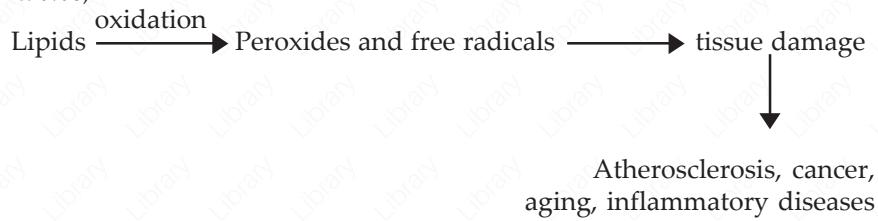
Oxidative rancidity: is the result of partial oxidation of unsaturated fatty acid due to free radicals, with formation of epoxides and peroxides of small molecular weight fatty acids. Partial oxidation, if occurs *in vivo* will affect the integrity of biomembranes, leading to cell death.

Antioxidants are the substances which prevent oxidative rancidity. Examples for antioxidants are tocopherols (vitamin E), hydroquinone, gallic acid, α -naphthol. These are used for commercial preparation of fats.

Butylatd hydroxy anisole (BHA), Butylatd hydroxy toluene (BHT) are used in food preservation.

Peroxidation

In vivo,



Antioxidants are the Compounds which destroy free radicals. *Example:* vitamin E, vitamin C, β carotene, urate, SOD (Superoxide dismutase).

Free radical is any molecule which has one or more unpaired electrons. *Example:* superoxide anion radical O_2^\bullet , hydroxyl radical OH^\bullet . They are highly reactive. Targets are PUFA of cell membrane, proteins, enzymes and membrane ion transporters, DNA. As a result cell injury and death occurs. Formation of free radicals is by biochemical redox reactions with O_2 , controlled inflammatory reactions (phagocytosis) and in response to UV light and environmental pollutants. Free radicals initiate a series of chain reactions which continue till all free radicals are removed from the body by antioxidant system.

Characterization of Fats

1. Saponification Number

Number of mgs of KOH required to saponify one gram of fat/oil.

Human fat	:	194 – 198
Butter	:	210 – 230
Coconut oil	:	253 – 262
Caster oil	:	175 – 180

Significance: Saponification number is inversely proportional to length of the fatty acid.

2. Iodine Number

Number of grams of iodine absorbed by 100 grams of fat/oil.

Coconut oil	:	7 – 10
Butter	:	25 – 28
Palm oil	:	45 – 55
Olive oil	:	80 – 85
Sunflower oil	:	125 – 135
Linseed oil	:	175 - 200

Significance: It is an index of degree of unsaturation.

Waxes: They are wide spread in nature and form secretions of insects, protective coatings of skin and fur of animals, leaves and fruits of plants.

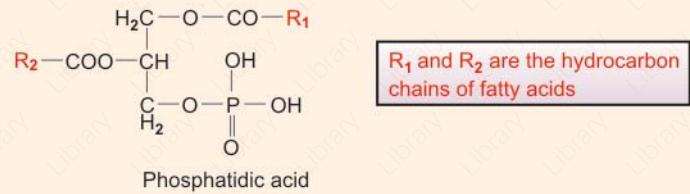
Example: Lanolin or wool fat, beeswax, whale sperm oil, etc.

Esters of higher fatty acids with higher mono-hydroxy aliphatic alcohols and so have very long straight chains of 60 – 100 carbon atoms.

Phospholipids: They are compound lipids and fatty acid esters containing in addition, a phosphate group and a nitrogenous or non-nitrogenous group (e.g. Choline, ethanolamine, serine and inositol). Phospholipids are broadly classified into phosphoglycerides, containing glycerol as the alcohol (Glycerophospholipids) and sphingomyelins containing the nitrogenous alcohol sphingosine instead of glycerol (Sphingophospholipids).

Most phospholipid molecules have a charged polar head-group consisting of the phosphate and the nitrogenous base, and nonpolar fatty acid tails. Such molecules, carrying both polar and nonpolar groups are called amphipathic molecules.

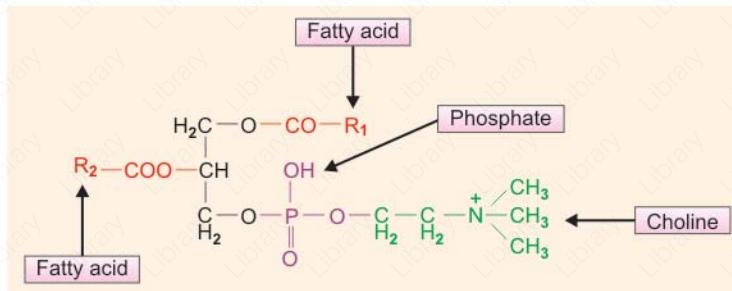
Phosphoglycerides (Glycerophospholipids) are derivatives of phosphatidic acid, which is the simplest phospholipid, composed of glycerol, phosphoric acid and two fatty acid molecules.



Phosphoglycerides are classified into:

1. Lecithins (Phosphatidylcholines)
2. Cephalins (Phosphatidylethanolamines)
3. Lipoamino acids (Phosphatidyl serines, phosphatidylthreonines and phosphatidylhydroxyprolines)
4. Plasmalogens (*Ex*: Phosphatidyl choline)
5. Nitrogen free phosphoglycerides (*Ex*: Lipositol)

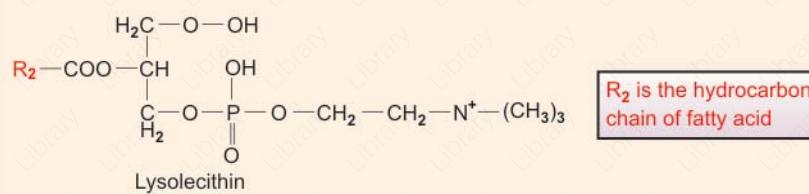
Lecithins: Composed of glycerol, two fatty acid molecules, a phosphate group and a nitrogenous group, choline.



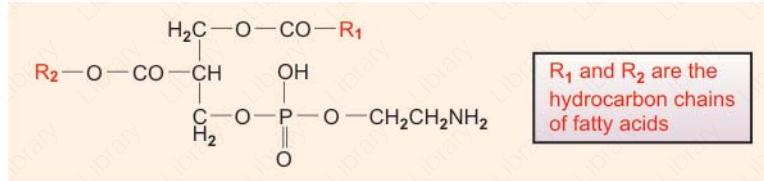
Lecithins are found in animal tissues such as brain, liver, cardiac muscle and blood. They form stable emulsions in water due to micelle formation because of their zwitterion existence.

1. *Dipalmitoyl lecithin*: is an important phosphatidylcholine found in lungs. Surface active agent prevents the adherence of inner surface of lungs due to surface tension. Absence of dipalmitoyl lecithin causes "Respiratory distress syndrome" in infants.
2. *Lysolecithin*: is formed by removal of the fatty acid either at C₁ or C₂ of lecithin.

Cephalins: Here nitrogenous base is ethanolamine.



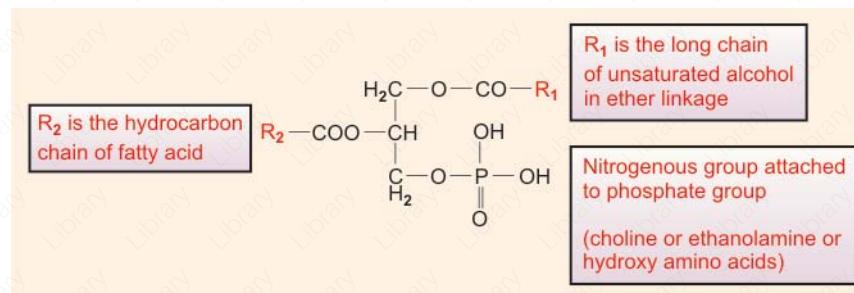
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Cephalins are present in brain, liver, cardiac muscle and erythrocytes. They exist as zwitterions at specific isoelectric pH.

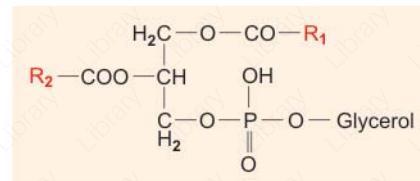
Lipoamino acids: Choline or ethanolamine is replaced by a hydroxy amino acid such as serine, threonine or hydroxy proline. Carboxyl group of amino acids makes it more acidic. It occurs in brain and erythrocytes.

Plasmalogens: α -Carbon of glycerol is linked to an unsaturated alcohol by an ether linkage, instead of being linked to a fatty acid. It occurs in brain, cardiac muscle and erythrocytes and found in bio-membranes.



Nitrogen-free phosphoglycerides: Here nitrogenous groups are replaced by non-nitrogenous substances.

i. *Phosphatidylglycerols:* Nitrogenous group is replaced by another glycerol.



ii. *Diphosphatidylglycerols:* They bear two molecules of phosphatidic acids joined by a molecule of glycerol. (ex: cardiolipin in cardiac mitochondria).

Phosphatidic acid — Glycerol — Phosphatidic acid.

iii. *Lipositols* (Phosphatidylinositol): They resemble lecithins in composition, but contain inositol in place of choline. They are found in soyabean, cardiac muscle, brain and liver.

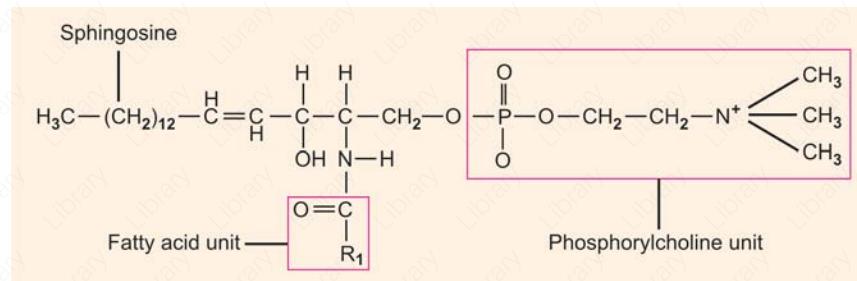
Fatty acid —— Glycerol —— Fatty acid

|
Phosphate — inositol

Phosphatidyl inositol bisphosphate plays a vital role in the mediation of hormone action on biomembranes.

Sphingomyelins: Here sphingosine is the alcohol.

Ceramide is formed when single fatty acid residue is bound to the NH₂ group of sphingosine.



It is found in blood, brain, cardiac muscle and liver. At specific isoelectric pH, it exists as zwitterions. It functions as detergent and component of bio-membranes due to amphipathic nature.

Liposomes

When a lipid bilayer closes on itself a spherical vesicle called as liposome is formed.

Functions

1. Liposomes are used as carriers of certain drugs to specific sites of body where they act. They can deliver drugs directly into cell because they easily fuse with cell membranes.
2. They are used in cancer therapy to deliver drugs only to cancer cells.
3. In gene therapy also they are used as vehicles for genes.

Sphingolipids

1. *Sphingomyelins:* They are phospholipids and CH₂OH group of ceramide remains esterified with phosphocholine (already discussed above).
2. *Glycolipids/Glycosphingolipids:* In their molecules, the CH₂OH group of ceramide remains bound by glycosidic linkage to either a monosaccharide or an oligosaccharide.

Glycolipids have Three Major Classes

Cerebrosides/Galactolipids: They are ceramide-carbohydrate complexes, amphipathic in nature. Carbohydrate may be charged or uncharged.

- CH₂-OH group of ceramide is connected by a β glycosidic linkage to the C of a D-galactose residue. It possesses uncharged head group and is natural in reaction. In glucocerebrosides, D-glucose residue is present instead of D-galactose. Depending on nature of fatty acid residue, cerebrosides are classified into four main types. They are important components of nerve tissue.

Example:

Kerasin – Lingnocerate (Saturated, 24 carbon fatty acid)

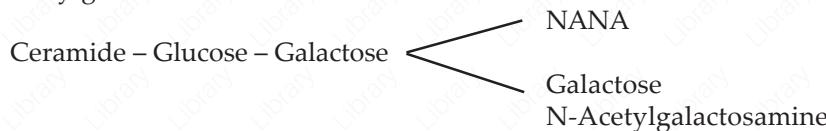
Nervon – Nervonate (Unsaturated, 24 carbon fatty acid)

Cerebron – Cerebronate (α -Hydroxylignocerate)

Oxyneron- Oxyneronate (α -Hydroxyneronate)

2. **Sulfatides/Sulfolipids:** They are cerebroside sulfates. Sulfated galactose has polar head which is negatively charged and hence acidic. They occur in liver, kidney, salivary glands, white matter of brain and are important components of membranes of nervous tissue.

3. **Gangliosides:** They are ceramide – oligosaccharide complexes. A simple ganglioside/monosialoganglioside is composed of a ceramide bound to an oligosaccharide containing a single sialic acid (NANA), three molecules of hexoses (glucose and galactose) and one N-acetylgalactosamine.



Higher gangliosides are namely di, tri, tetra, penta-sialogangliosides carry 2, 3, 4, 5, sialic acid residues, respectively. Gangliosides are found in spleen, RBC, nervous tissue. These are found on surface of membranes. Polar head groups are negatively charged, sialic acid is acidic in reaction. Major brain gangliosides are G_{m1} , G_{m2} , G_{m3} .

Globosides are the ceramides with oligosaccharides containing two or more hexoses or hexosamines attached to a ceramide molecule.

Ceramide + Galactose + Glucose = Lactosyl ceramide which is a component of RBC membrane.

Gm_2 ganglioside accumulates in Tay-Sachs disease, defective enzyme being hexosaminidase A.

Niemann-Pick disease is an inherited disorder. Here, defective enzyme is sphingomyelinase. Accumulation of sphingomyelins in liver and spleen result in the enlargement of these organs. Victims of this disease suffer from severe mental retardation and death may occur in early childhood.

Lipotropic Factors

Lipotropic factors are the substances which facilitate mobilization of fats from liver. Various lipotropic agents are choline, betaine, methionine and

inositol. They convert TGs to phospholipids and thus help in normal transport and utilization of lipids, especially in liver. Deficiency of lipotropic factors results in increased fat content of liver called "Fatty liver".

Functions of Phospholipids

1. With proteins, they form the structural components of membranes and regulate membrane permeability.
2. In mitochondria, they are responsible for maintaining the conformation of electron transport chain components and thus help in cellular respiration.
3. They combine with polar and nonpolar compounds in the cell, due to their amphipathic nature.
4. They help in the absorption of fat from the intestine.
5. They are essential for the synthesis of different lipoproteins and thus participate in transport of lipids.
6. They are regarded as lipotropic factors since they prevent accumulation of fat in liver (fatty liver).
7. Arachidonic acid which is liberated from phospholipids, serves as a precursor for the synthesis of Eicosanoids.
8. They help in reverse cholesterol transport and thus help in removal of cholesterol from the body.
9. They act as surfactants (agents lowering surface tension).
10. Cephalins participate in blood clotting.
11. Phosphatidylinositol are involved in signal transmission across membrane.
12. They are essential components of bile, acting as detergents and help in solubilization of cholesterol.

MULTIPLE CHOICE QUESTIONS

1. The phospholipid that produces second messengers in hormonal action is:
A. Plasmalogen B. Phosphatidylinositol
C. Lecithin D. Cardiolipin
2. Niemann-Pick disease is due to the accumulation of Sphingomyelins in:
A. Liver B. Spleen
C. Liver and spleen D. Kidney
3. The glycolipid that contains N-acetylneurameric acid is:
A. Cerebroside B. Globoside
C. Sulphatide D. Ganglioside

ANSWERS

1. B 2. C 3. D

MOST LIKELY QUESTIONS

Long Essays

1. Classify lipids giving suitable examples. Add a note on the physiological importance of lipids.
2. Classify phospholipids with suitable examples.

Short Essays

1. Discuss the formation and functions of any two Phospholipids.
2. Write the functions of cholesterol.

Short Notes

1. Name one each of phospholipid and a steroid.
2. Name any *four* compounds synthesized from Cholesterol.
3. Triglycerides
4. Cholesterol
5. Name the major plasma lipoproteins
6. What is a ceramide?
7. Dipalmitoyl lecithin
8. Cerebrosides

4.2: DIGESTION OF LIPIDS

Dietary lipids constitute 90-95 percent fat and oil, remaining dietary lipids are from phospholipids, cholesterol, cholesteryl esters and free fatty acids. Intake of lipids in poorer sections of society is less than 60 grams/day. In developed countries, an adult ingests approximately 60-150 g/day.

Partition theory: The entire amount of food fat need not be digested completely before absorption. Of the dietary triglycerides (TGs), 25-30 percent is totally hydrolyzed by lipases into free fatty acids and glycerol. 60-70 percent of the food fat is partially hydrolyzed into fatty acids and monoacylglycerols (MAGs). Five percent of dietary fat is absorbed without any enzymatic digestion.

Stages of Lipid Digestion

Digestion in the oral cavity: There is no significant digestion in the oral cavity. A lingual lipase from Ebner's gland is demonstrated.

Digestion in the Stomach

Lingual lipase from the mouth that enters stomach along with the food, acts on short chain TGs which are present in milk, butter, ghee and coconut oil. Lingual lipase is more significant in infants. Gastric lipase secreted by chief cells act on short chain TGs. Thirty percent digestion of TGs occurs in stomach.

Gastric lipase "tributyrase" acts on TGs of short chain and medium chain fatty acid (Butter fat "tributyrin"). It also acts on TGs with unsaturated fatty acid.

Digestion in the Intestine

With co-ordinated actions of bile salts, fats are acted upon by pancreatic colipase, lipase and intestinal lipase. Pancreatic and intestinal phospholipases and intestinal lysophospholipase act on phospholipids. Pancreatic cholesterol esterase act on cholesteryl esters.

Fats are digested by the coordinated actions of bile salts, pancreatic co-lipase, lipase and intestinal lipase.

Action of Bile Salts, Pancreatic Lipase and Co-lipase

Functions of bile: Bile salts in bile lower surface tension

Emulsification of fat: Bile salts lower surface tension. These convert large lipid droplets into smaller ones (emulsification). They emulsify the fat droplets in the intestine.

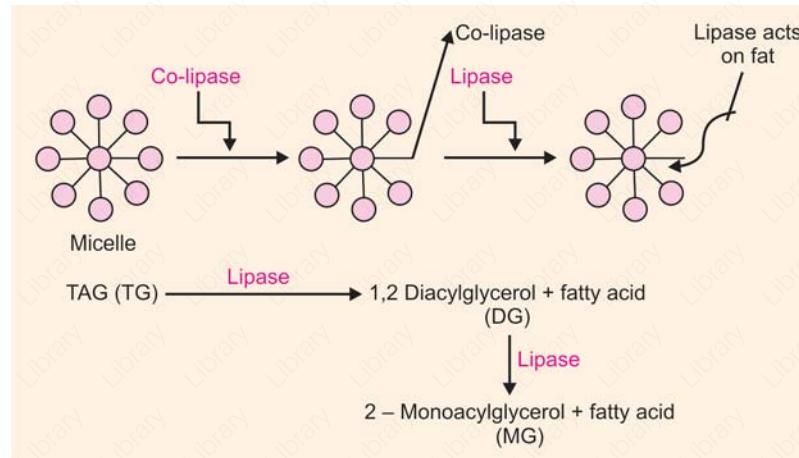
1. Emulsification increases the surface area of the particles for enhanced activity of enzymes.
2. *Neutralization of acid:* Bile, having a pH slightly above 7, neutralizes the acid chyme from the stomach and prepares it for digestion in the intestine.
3. *Excretion:* Bile is an important vehicle for bile acid and cholesterol excretion, but it also removes many drugs, toxins, bile pigments and various inorganic substances such as Cu, Zn and Hg.

Pancreatic Co-lipase

Inactive pro-colipase is activated by trypsin into co-lipase and deca peptide.

Pancreatic lipase: It is active only in the presence of co-lipase. It acts as esterase acting on primary ester linkage. Its action on secondary ester linkage is very low. Activation of prolipase involves removal of a pentapeptide. Pentapeptide is named "enterostatin" which acts as satiety signal.

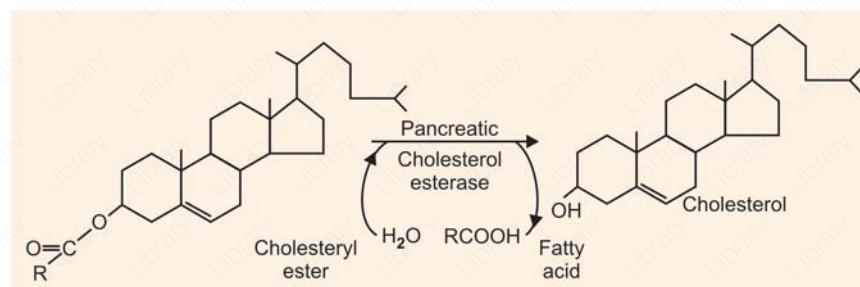
Breakdown of Emulsified Fat



Ca^{+} forms insoluble calcium soap, as and when there is the release of free fatty acid by the action of pancreatic lipase. Sometimes, isomerase enzyme of enterocytes converts 2-MG to 1-MG on which pancreatic lipase acts to form glycerol and free fatty acid. Intestinal lipase in the enterocytes acts on unhydrolysed fat, which enters it.

Action of phospholipases: Trypsin activates its proenzyme into phospholipase A₂ (duodenum). Phospholipase A₂ acts on secondary ester linkages in the phospholipids and form lysophospholipids. Intestinal phospholipase also form lysophospholipids, which are further acted upon by intestinal lysophospholipases to form free fatty acid and glycerylphosphocholine (ethanolamine).

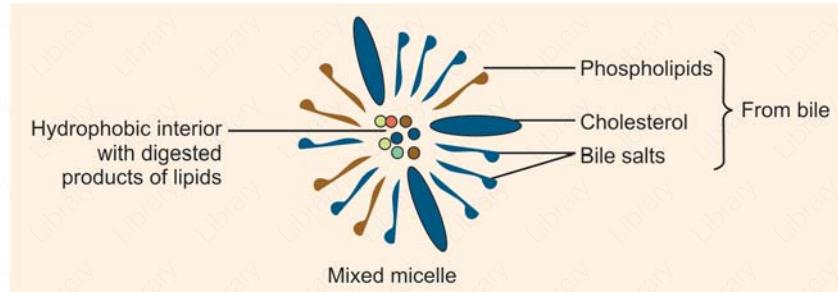
Action of cholesterol esterase (cholesterol ester hydrolase): is activated by bile salts.



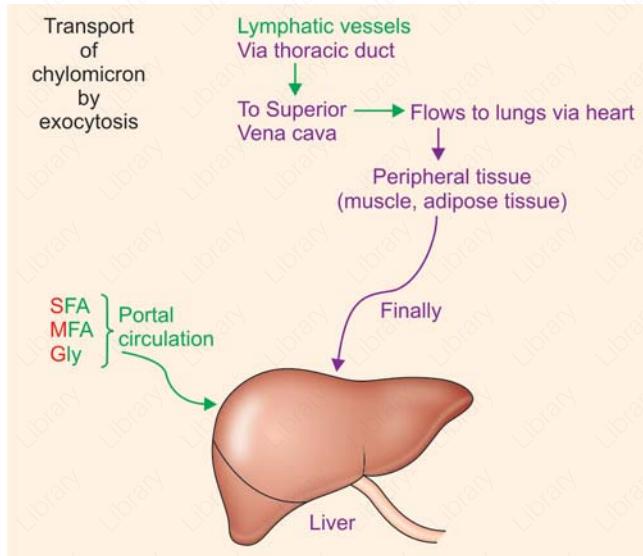
Alkaline Phosphatase (phosphoesterase)

Alkaline phosphatase in the luminal membrane at pH between 8-9, acts on glycerophosphates and form glycerol and inorganic phosphate. Ultimately partial digestion of lipids leads - 2- MG, 1-MG, glycerol, free

fatty acid (Calcium soap), lysophospholipids, glycerophosphocholine with triglycerides, phospholipids, cholesterol esters and cholesterol. All these, along with bile salts form mixed micelle.



Circulation



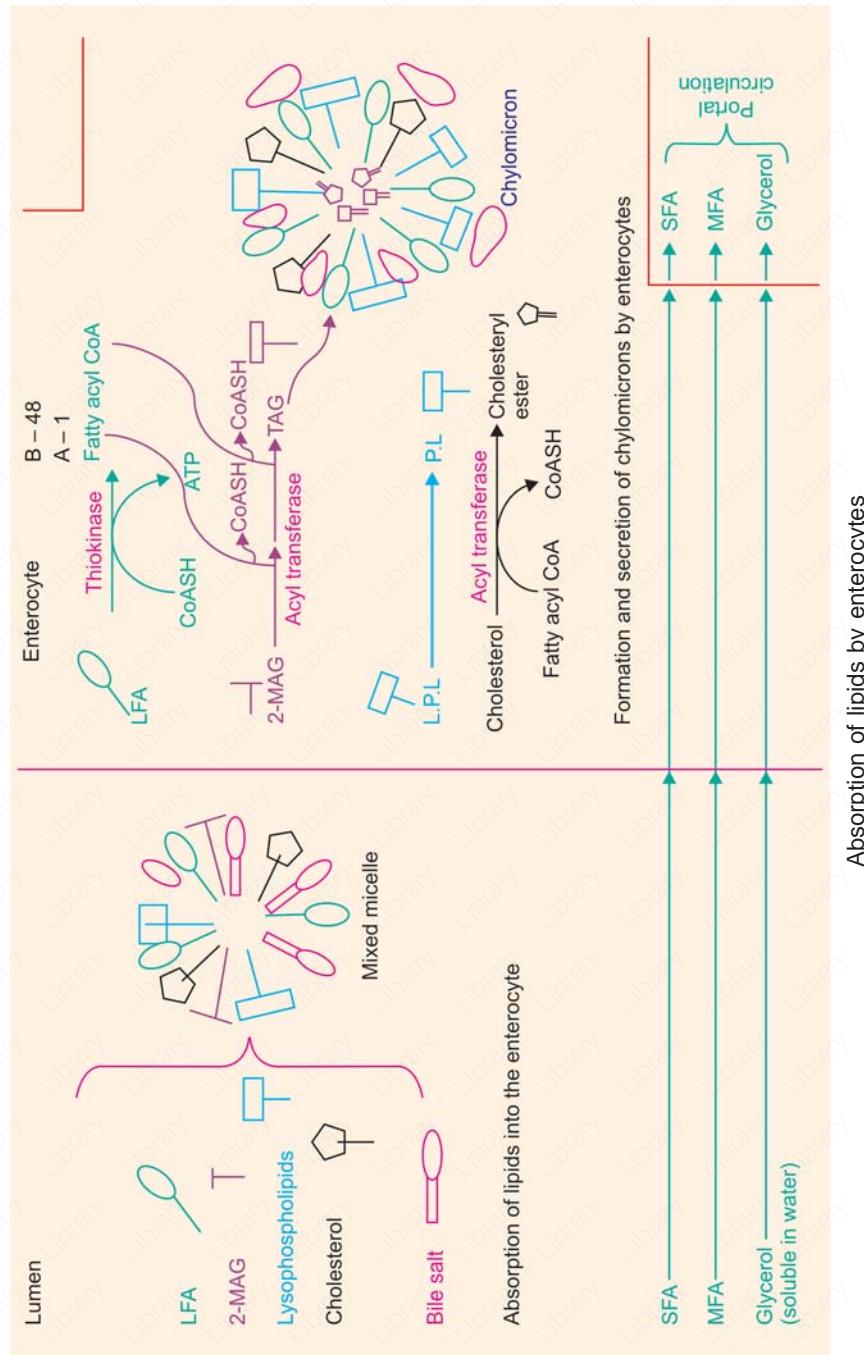
Steatorrhea: is a condition characterized by the loss of fats in the feces.

It may be due to

1. A defect in the secretion of bile or pancreatic juice into the intestine.
2. Impairment in the lipid absorption by the intestinal cells.

Large amount of unabsorbed fat makes the feces bulky, greasy, frothy, clay colored and formless. Steatorrhea is commonly seen in disorders associated with pancreas, biliary obstruction, severe liver dysfunction.

Putrefaction: The process of microbial degradation of food remnants and the presence of excreted materials in the large intestine. Putrefaction of proteins and amino acids produces toxic amines, called "ptomaines" such as,



agmatine and putrescine from arginine, histamine from histidine, cadaverine from lysine, indoethanolamine from tryptophan, tyramine from tyrosine.

Other putrefaction products are phenols, skatoles, indoles, p-cresols, benzoic acid, methyl and ethyl mercaptans, some free amino acids and considerable volumes of gaseous CH_4 , H_2S , H_2 , NH_3 and CO_2 from protein putrefaction.

Cholestanol and coprostanol are the putrefied products of cholesterol. Deoxycholic acid and lithocholic acid are the secondary bile acids derived from primary bile acids such as cholic acid chenodeoxycholic acid. Choline is putrefied into neurine and bilirubin is putrefied into Urobilinogen. Most of the products are eliminated in the feces.

Absorption of Lipids by Enterocytes

Absorption takes place in duodenum, jejunum and proximal ileum of small intestine. Glycerol and small chain and medium chain fatty acids (chain less than 14 carbons) are directly absorbed from the intestinal lumen into the portal vein and taken to the liver, and are immediately utilized for energy.

2 MAG, long chain fatty acid and a small amount of 1 MAG leave the oil phase of lipid emulsion and diffuse into the mixed micelles consisting of bile salts, phospholipids (lecithin) and cholesterol, furnished by the bile.

The mixed micelles are spherical particles with a hydrophilic exterior and hydrophobic interior core. Because the micelles are soluble, they allow the products of digestion to be transported through the aqueous environment of the intestinal lumen to the brush border of the mucosal cells, where they are absorbed into the intestinal epithelium.

The bile salts pass on to the ileum and return to the liver to be re-excreted (enterohepatic circulation). About 98 percent of dietary lipids are normally absorbed.

Once inside the enterocyte, the long chain fatty acids are re-esterified to form TGs.

Fatty acids are first activated to fatty acyl CoA by the enzyme acyl CoA synthetase. This needs lysis of 2 high energy phosphate bonds. Two such activated fatty acids react with MAG to form TAG. Majority of molecules take up this MAG pathway.

Free-glycerol is not available in the cell since it directly enters the blood stream. But cells can convert glucose to glycerol phosphate and then add 3 molecules of acyl groups to synthesize TAG. The TAG, cholesterol ester and phospholipid molecules along with apoproteins B 48 and A₁ are incorporated into chylomicrons. 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 systemic circulation. The serum may appear milky after a high fat meal.

MULTIPLE CHOICE QUESTIONS

1. The vehicles for the transport of lipids from the intestinal lumen to the membrane of mucosal cells are:
A. Chylomicrons B. Mixed micelles
C. Bile salts D. Apolipoproteins
2. Long chain fatty acids are activated in the intestinal cells into acyl CoA derivatives by:
A. Thiophorase B. Thioesterase
C. Thiokinase D. Thiolase
3. Cholesterol esterase cleaves _____ to produce cholesterol and free fatty acids:
A. Cholesteryl esters B. Triacylglycerol
C. Lecithin D. Plasmalogen

ANSWERS

1. B 2. C 3. A

MOST LIKELY QUESTIONS

1. Discuss digestion and absorption of lipids.
2. Give the functions of bile in digestion.
3. Name the digestive enzymes of pancreas. Give their functions.
4. Digestion of lipids.
5. Bile salts.
7. Name bile acids and mention about the functions of bile salts.
8. Lipolytic enzymes

4.3: METABOLISM OF FATTY ACIDS**FATTY ACID OXIDATION**

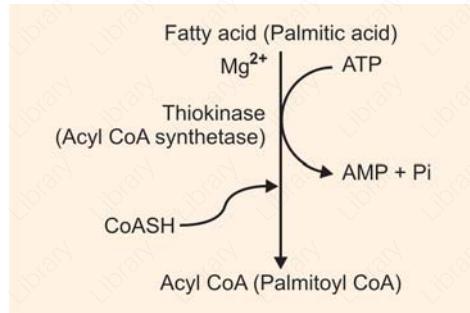
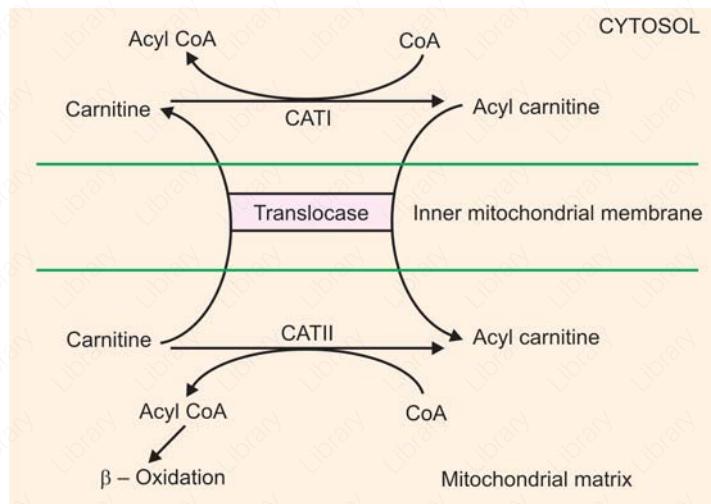
Fatty acids in the body are mostly oxidized by β -oxidation. It may be defined as the oxidation of fatty acids on the β -carbon atom. It is a cyclic process. In each cycle, active fatty acid (acyl CoA) is degraded to give acetyl CoA (2 carbon compound) and acyl CoA two carbon less than original acyl CoA.

Site: Most of the tissues except brain, RBC and adrenal medulla.

Subcellular site: Mitochondria since various enzymes for the oxidation are present.

Stages of β -Oxidation: Three stages.

- i. Activation of fatty acids occurring in the cytosol.
- ii. Transport of fatty acids into mitochondria
- iii. β -Oxidation proper in the mitochondrial matrix.

I. Fatty Acid Activation**II. Transport of Fatty Acids into Mitochondria**

Carnitine: It is a transporter consisting of β - hydroxy γ - trimethyl amino butyrate.

CAT - Carnitine Acyl Transferase (I and II)

Role of carnitine: Acyl CoA cannot pass through inner mitochondrial membrane. A specialized carnitine carrier system transports acyl group across the inner mitochondrial membrane.

Steps

- i. Carnitine acyl transferase I (CAT I) present on outer surface of inner mitochondrial membrane transfers acyl group to carnitine.
- ii. Acyl carnitine is transported across the membrane to mitochondrial matrix by carrier protein translocase.

- iii. CAT II found on the inner surface of inner membrane converts acyl-carnitine to acyl CoA.
- iv. Carnitine released returns to cytosol for reuse.

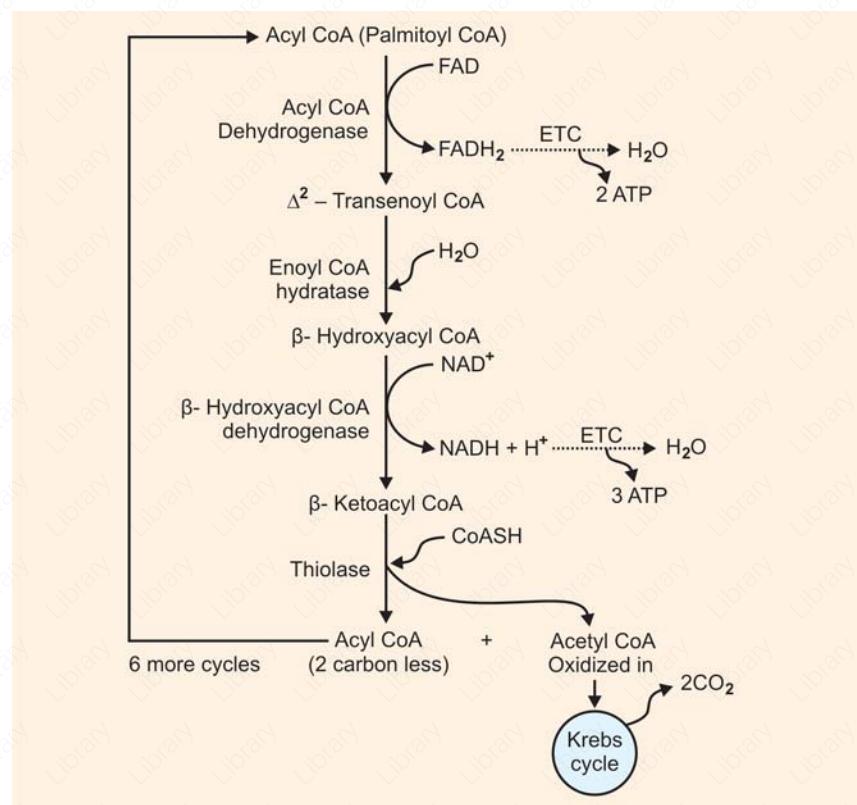
III. β - Oxidation Proper

Subcellular site: Mitochondrial matrix

Involves four steps:

- i. Oxidation
- ii. Hydration
- iii. Oxidation
- iv. Thiolytic cleavage

Summary of β - Oxidation of Palmitoyl CoA



Energetics of Palmitic Acid Oxidation

Mechanism	<i>ATP yield</i>
I. β – Oxidation proper (7 cycles):	
7 FADH ₂ (oxidized by ETC, each FADH ₂ gives 2 ATP)	14
7 NADH (oxidized by ETC, each NADH gives 3 ATP)	21
II. From 8 acetyl CoA oxidized by citric acid	
Cycle, each acetyl CoA provides 12 ATP	<u>96</u>
Total energy from one mole of palmitoyl CoA	131
Energy utilized for activation (formation of palmitoyl CoA)	<u>- 2</u>
Net yield of oxidation of one mole of palmitate	<u>129</u>

Regulation of β -Oxidation

- Supply of fatty acids: Use of fatty acids by tissues is proportional to the plasma free fatty acid level and so fatty acid oxidation is regulated at the level of adipose tissue metabolism (for example: During uncontrolled diabetes and fasting).
- Uptake of fatty acids: Oxidation is also regulated by the enzymes which regulate entry of these fatty acids into mitochondrial matrix. (CAT I is inhibited by malonyl CoA).

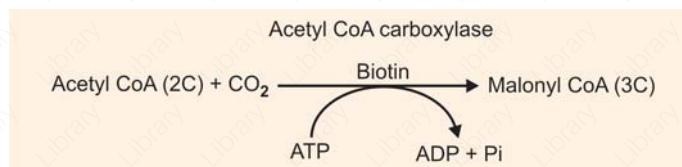
DE NOVO (NEW, TOTAL) SYNTHESIS OF FATTY ACIDS

Extramitochondrial Pathway

Cytosolic pathway: Occurs in liver, adipose tissue, lactating mammary glands, kidney, brain and lungs.

Acetyl CoA is the source of carbon atoms and NADPH from HMP shunt is the source of reducing equivalents.

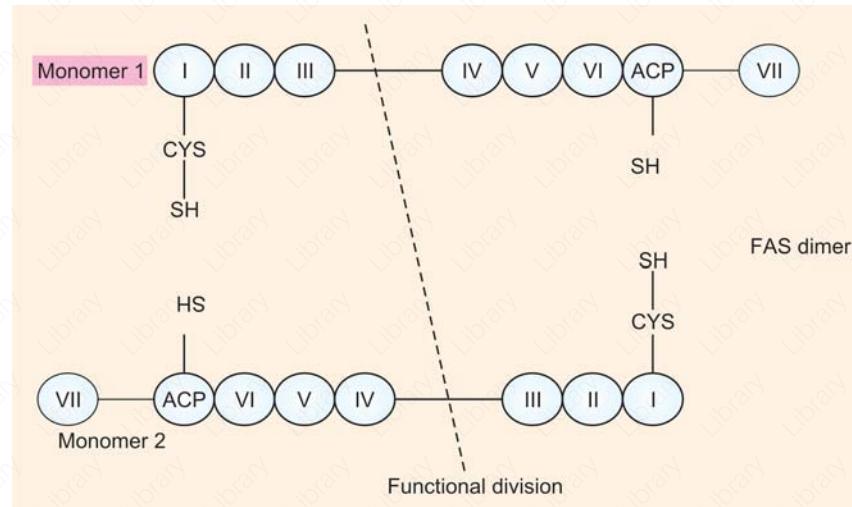
I step: Formation of Malonyl CoA



Acetyl CoA carboxylase is the regulatory enzyme.

II step: Reactions of fatty acid synthase complex (FAS complex)

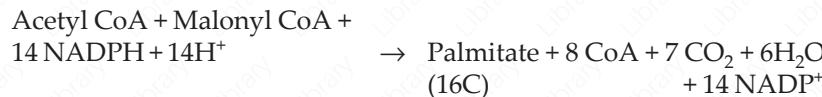
It is a multienzyme complex. It is a dimer with 2 identical monomers. Each monomer has 7 enzymes and acyl carrier protein (ACP). Each functional unit consists of one half of a monomer interacting with complementary half of other monomer.



Enzymes of FAS Complex

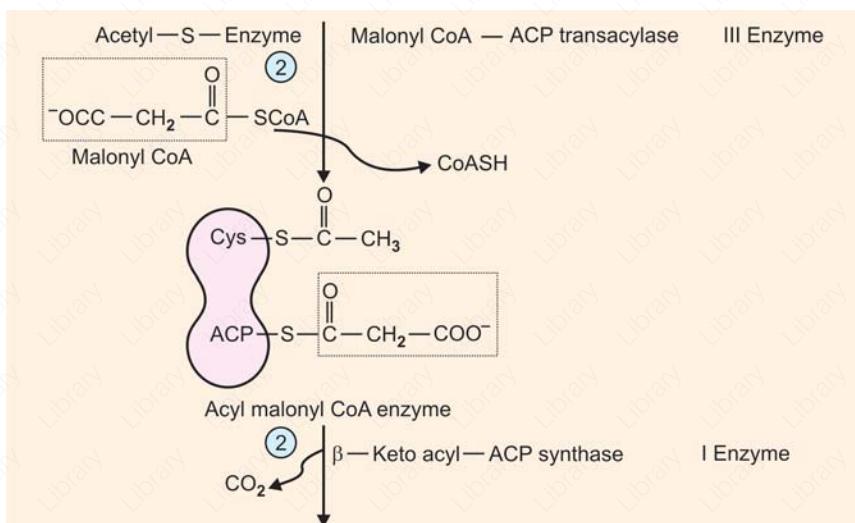
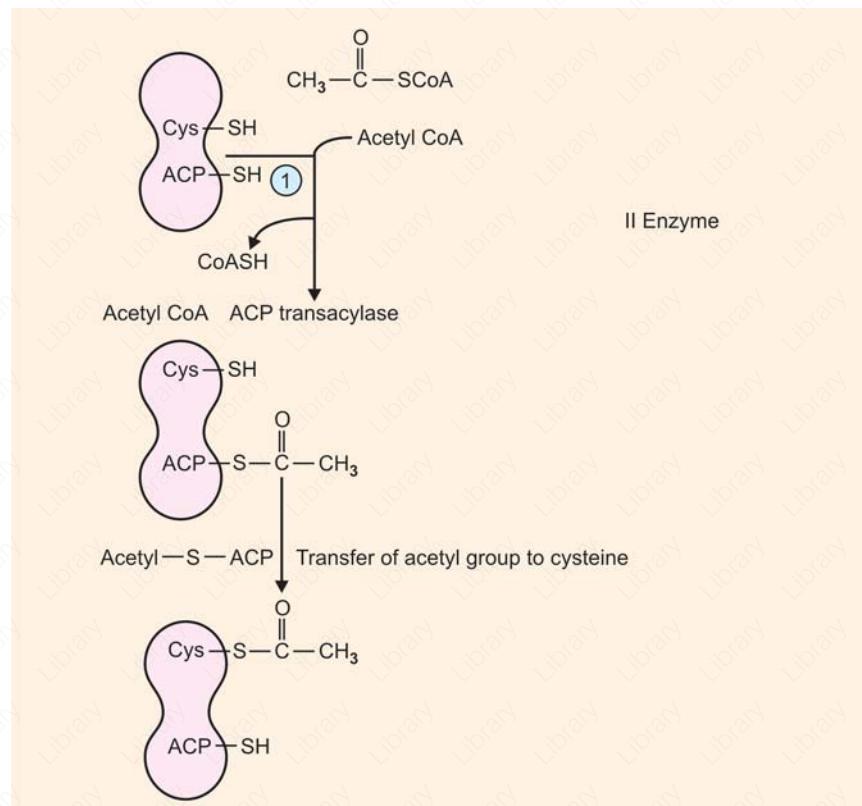
I	=	Ketoacyl synthase
II	=	Acetyl transacylase
III	=	Malonyl transacylase
IV	=	Dehydratase
V	=	Enoyl reductase
VI	=	Ketoacyl reductase
VII	=	Thioesterase
ACP	=	Acyl Carrier Protein

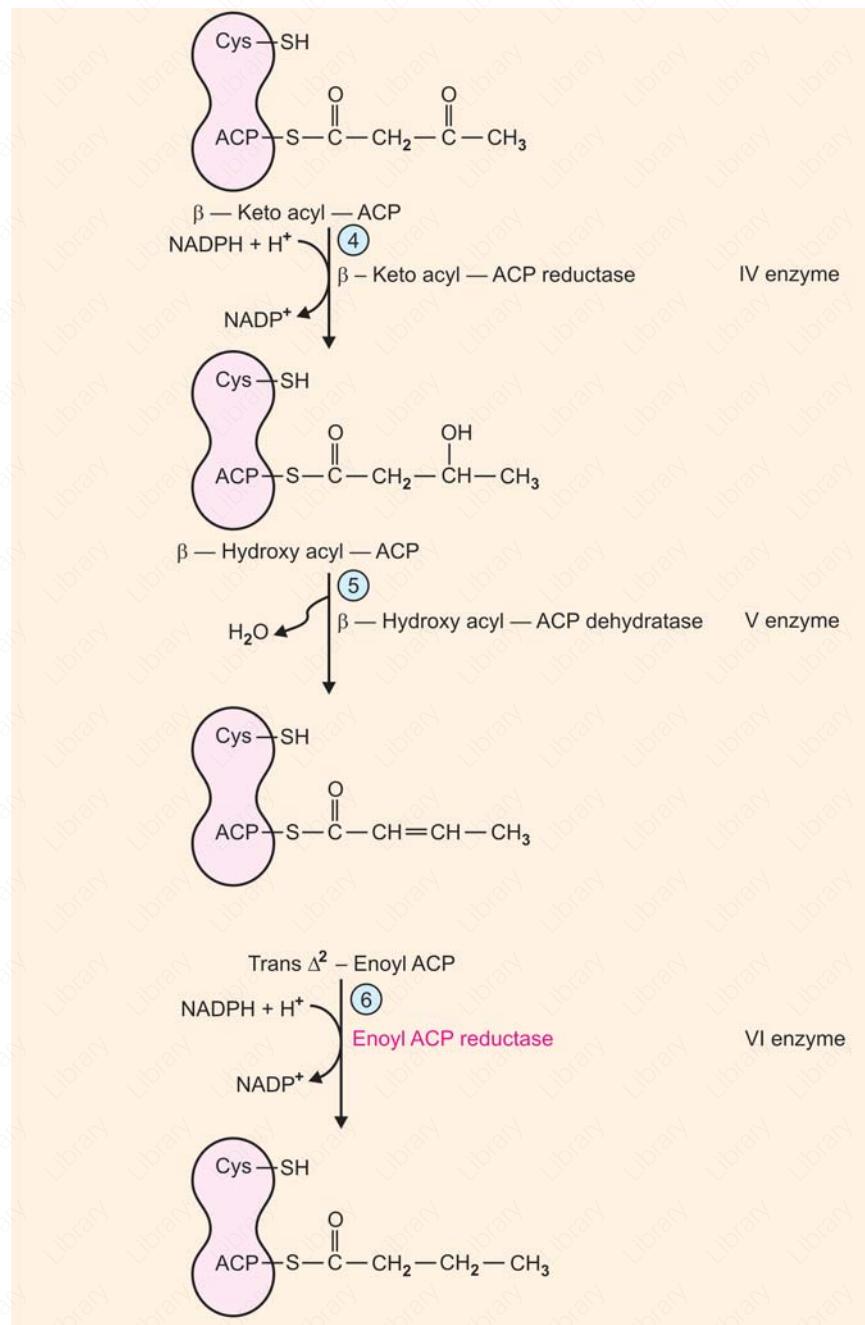
Overall equation for palmitate synthesis is:

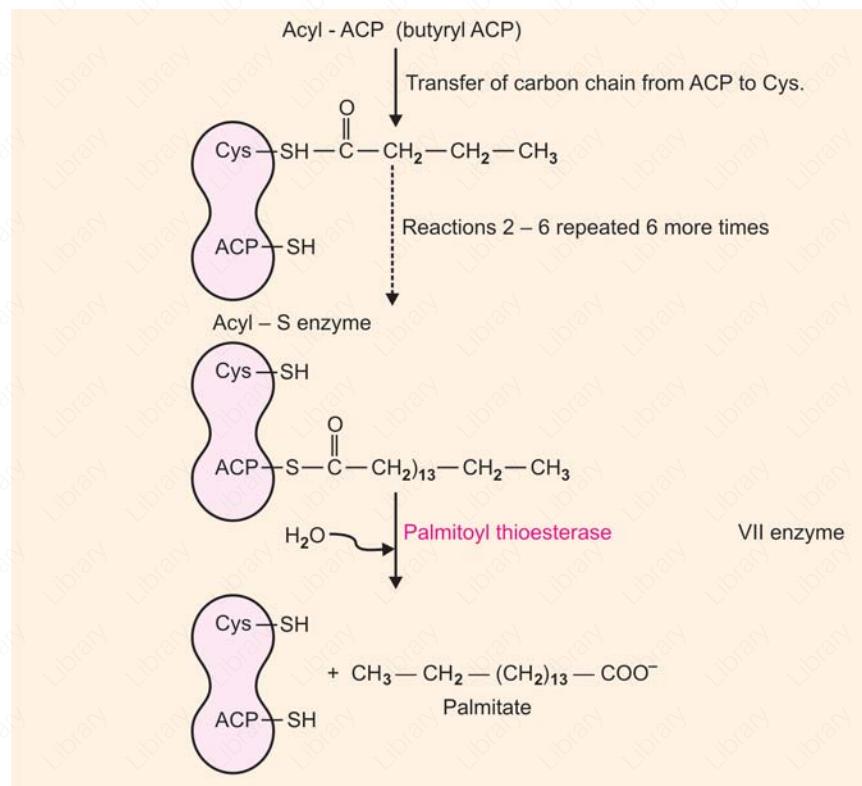


Reactions

1. Acetyl CoA is transferred to Cys-SH of one monomer by enzyme II
2. Malonyl CoA is transferred to SH of ACP of other monomer by enzyme II
3. Condensation of acetyl and malonyl groups by enzyme I, CO_2 released, -Cys – SH becomes free.
4. Reduction of β -Keto acyl group by enzyme VI to β -hydroxy acyl group, NADPH is used.
5. Dehydration of hydroxy acyl group to α , β -unsaturated acyl group by enzyme IV
6. Reduction of unsaturated acyl group to acyl group by enzyme V, NADPH is used.







7. New malonyl CoA combines with - SH of ACP, acyl group transferred to - Cys - SH.
8. Sequence of reactions repeated 6 more times.

Regulation of Fatty Acid Synthesis

Short-term regulation is done by acetyl-CoA carboxylase, which catalyzes the first committed step of fatty acid synthesis. This enzyme is inhibited by palmitoyl CoA.

Long-term regulation is by insulin stimulation and by starvation, thus inhibiting the synthesis of acetyl-CoA carboxylase enzyme and fatty acid synthase complex. Presence of PUFA in the diet also decreases the concentrations of these enzymes. Starvation and regular exercise increase β -oxidation enzymes and decrease fat synthesis enzymes.

Energetics of Fatty Acid Synthesis

- a. One high energy bond of ATP is spent in producing each Acetyl CoA from citrate by ATP-Citrate lyase in the cytoplasm.
Therefore, 7 high energy phosphate bonds are spent in providing 8 acetyl-CoA molecules for synthesizing one palmitate molecule.
- b. Seven of the acetyl CoA molecules have to be carboxylated to seven malonyl-CoA molecules by acetyl CoA carboxylase, each involving the expenditure of one high energy bond of ATP.
Therefore, 7 high energy phosphate bonds are spent in providing seven malonyl-CoA molecules for one palmitate.

Regulation of Fatty Acid Synthesis

Regulation of fatty acid synthesis is controlled by-enzymes, metabolites, end products, hormones and dietary manipulations.

1. *Acetyl CoA carboxylase:* It controls committed step in fatty acid synthesis. Citrate promotes polymer formation and favors fatty acid synthesis. Palmitoyl CoA and malonyl CoA cause depolymerization of the enzyme and so inhibits fatty acid synthesis.
2. *Hormonal influence on acetyl CoA carboxylase:* Glucagon, epinephrine and norepinephrine inactivate the enzyme by cAMP - dependent phosphorylation = (inhibits fatty acid synthesis).
Insulin promotes fatty acid synthesis by dephosphorylation
3. *Dietary regulation:* Consumption of high carbohydrate diet and fat-free diet increases the synthesis of the enzyme Acetyl CoA carboxylase and fatty acid synthase. Fasting and high fat diet decrease the synthesis of these 2 enzymes and so decreases the fatty acid synthesis.

MULTIPLE CHOICE QUESTIONS

1. Carnitine has an important role in:
A. Fatty acid synthesis B. Fatty acid activation
C. Transport of acyl CoA D. β -oxidation proper
2. Fatty acid synthase is a multi-enzyme complex and a dimer with two identical monomers containing:
A. 7 enzymes and acyl carrier protein B. 5 enzymes and acyl carrier protein
C. 7 enzymes D. Acyl carrier protein
3. Net yield of oxidation of one molecule of palmitic acid is:
A. 131 B. 96
C. 130 D. 129

ANSWERS

1. C 2. A 3. D

MOST LIKELY QUESTIONS

Long Essays

1. Give an account of the beta oxidation of fatty acids with a note on energetics of palmitic acid oxidation.
2. What is carnitine? What is its role in oxidation of fatty acids?

Short Essays

1. Beta oxidation of fatty acids.
2. Energetics of beta oxidation of fatty acids.
3. Fatty acid synthase complex.

Short Answer

1. What is carnitine? Mention its biochemical role.

4.4: METABOLISM OF TRIACYLGLYCEROLS

Fat Metabolism in Adipose Tissue

Adipose tissue: It is the storage site for excess calories ingested. Triglycerides stored in the adipose tissue are not inert. They undergo a daily turnover with new TG molecules being synthesized and a definite fraction being broken down.

White adipose tissue: It is mainly concerned with energy storage. Adipocytes have very few mitochondria.

Brown adipose tissue: It is involved in thermogenesis. Cells have numerous mitochondria. It is important in newborn babies and adult hibernating animals.

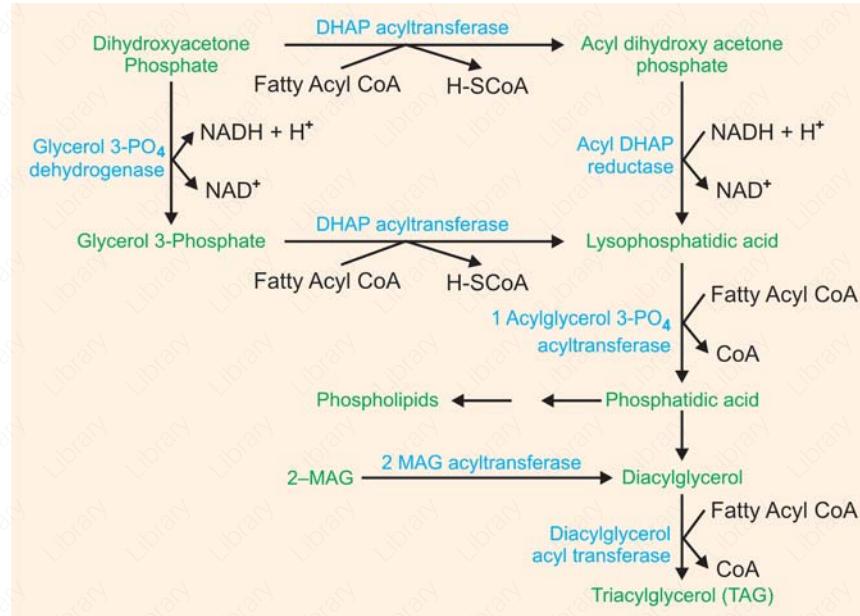
Synthesis of Triglycerides (TAG)

Major sites: Liver and adipose tissue.

In adipose tissue, TAG is stored for energy. In liver, TAG is mainly secreted as VLDL and is transported.

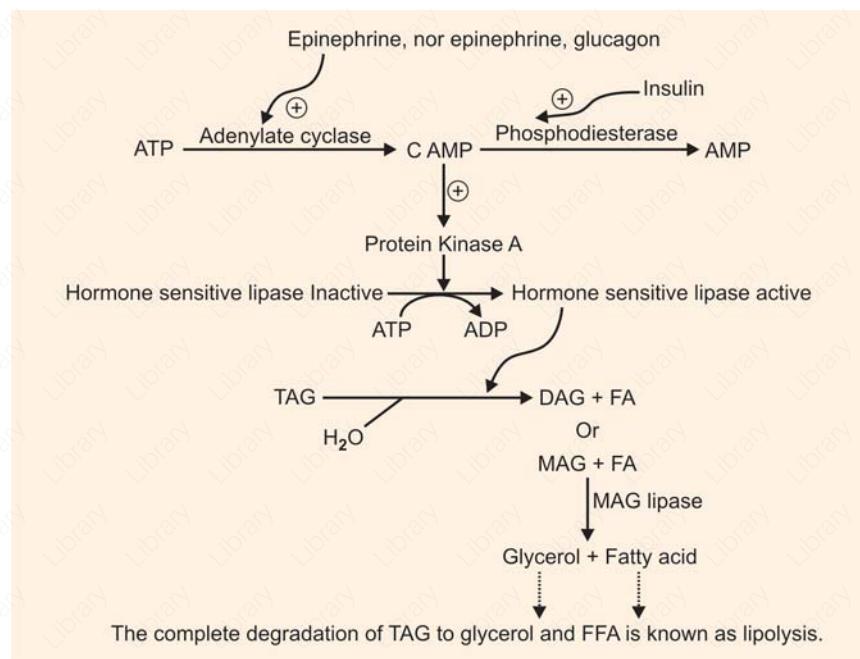
136 Biochemistry and Nutrition for BSc (Nursing)

Synthesis of Triacylglycerol



ACTION OF HORMONES ON LIPOLYSIS

TAG breakdown in adipose tissue is under hormonal control.



Fate of Glycerol

Glycerol kinase is absent in adipose tissue. So, there is no phosphorylation of glycerol in adipose tissue. Glycerol is transported to liver and gets activated to glycerol 3-phosphate. Glycerol-3-phosphate is used for the synthesis of TAGs and phospholipids.

Fate of Free Fatty Acids

Free fatty acids released by lipolysis in the adipocytes enter the circulation and are transported in a bound form to albumin. Free fatty acids enter various tissues and are utilized for the energy. Ninety-five percent of energy obtained from fat comes from oxidation of fatty acids. Role of Hormone sensitive TG lipase: It removes the fatty acid either from carbon 1 or 3 of the TAG to form DAG.

Role of Lipoprotein Lipase

It is present in the capillary walls of adipose tissue, cardiac and skeletal muscle, besides other tissues. It hydrolyses a portion of TAGs present in chylomicrons and VLDL to liberate free fatty acid and glycerol. Lipoprotein lipase is activated by Apo CII.

Deposition of TAG

TAG is deposited in adipose tissue which is widely distributed in the body.

Obesity

An abnormal increase in the body weight due to excessive fat deposition leads to obesity. Over eating coupled with lack of physical exercise contribute to obesity.

Fatty Liver

The normal concentration of lipid, mostly phospholipid in liver is around 5 percent. Liver is not a storage organ for fat. In certain conditions, TAGs accumulate excessively in the liver, resulting in fatty liver. Fatty liver is associated with fibrotic changes and cirrhosis. It may occur due to two main causes.

1. *Increased TAG synthesis:* In diabetes mellitus, starvation, alcoholism and high intake of fat diet increased mobilization of fatty acids is observed leading to fatty liver. Alcohol inhibits fatty acid oxidation and promotes fat synthesis and deposition.
2. *Impairment of lipoprotein synthesis:* The synthesis of VLDL in the liver requires phospholipids and apoprotein B. Fatty liver may be caused if there is a defect in phospholipid synthesis or a block in apoprotein formation or a failure in secretion of lipoproteins.

Lipotropic Factors

They are the substances which prevent fatty liver by promoting TAG mobilization from the liver in the form of VLDL. Choline, methionine and betaine are the lipotropic factors.

MULTIPLE CHOICE QUESTIONS

1. The storage form of lipid in the adipose tissue is:
A. Monoacylglycerol B. Diacylglycerol
C. Triacylglycerol D. Fatty acids
2. Brown adipose tissue is involved in:
A. Glycogenesis B. Ketogenesis
C. Atherosclerosis D. Thermogenesis
3. Glycerol kinase is absent in:
A. Liver B. Adipose tissue
C. Muscle D. Heart

ANSWERS

1. C 2. D 3. B

MOST LIKELY QUESTIONS

Long Essay

1. How are dietary triglycerides absorbed and transported in plasma?

Short Essays

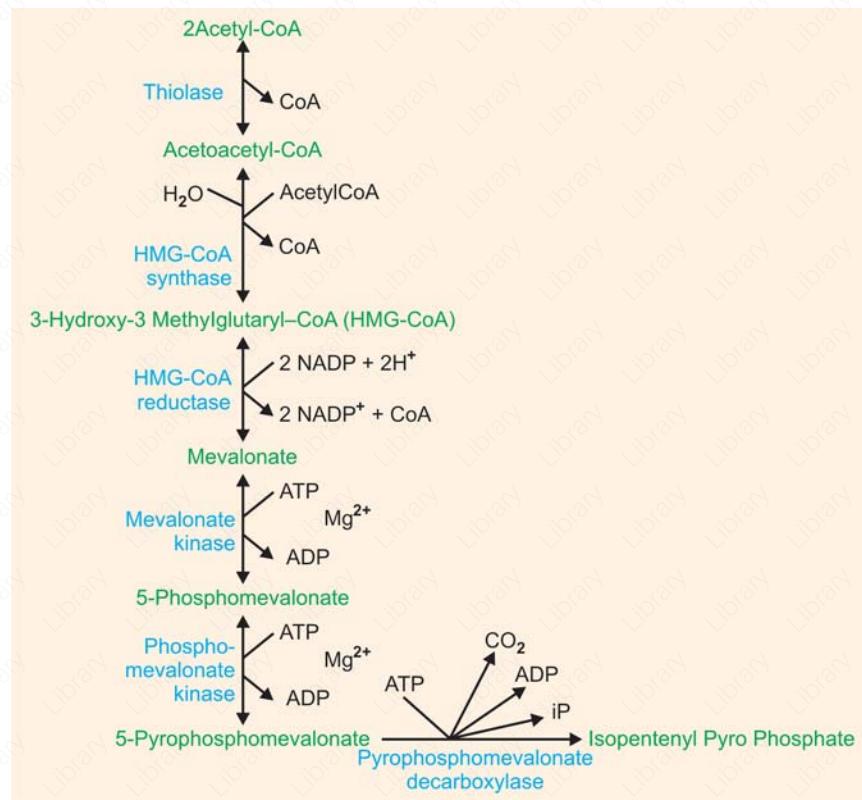
1. Fatty liver with reference to functions of VLDL.
2. Fatty liver and lipotropic factors.

Short Answer

1. Lipotropic factors.

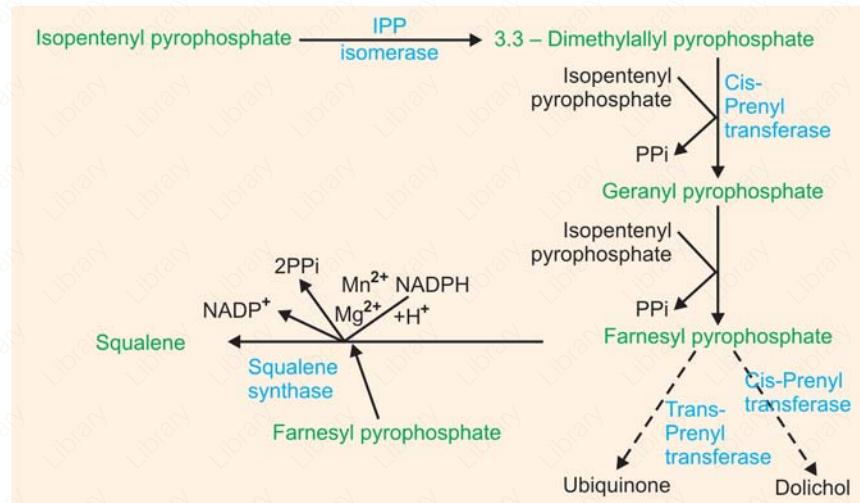
4.5: CHOLESTEROL METABOLISM

Cholesterol Biosynthesis

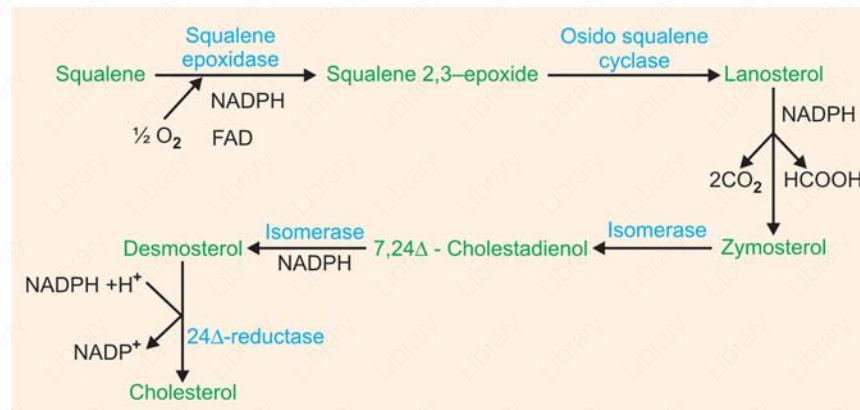


Major sites of cholesterol biosynthesis are liver, adrenal cortex, testes, ovaries and intestine. Liver is responsible for 80 percent of the endogenous cholesterol synthesis. All nucleated cells can synthesize cholesterol, including arterial walls. The enzymes involved in the synthesis are partly located in the endoplasmic reticulum and partly in the cytoplasm.

Synthesis of Squalene



Squalene to Cholesterol



Cholesterol Transport

Cholesterol is transported in the blood and taken up by cells in lipoprotein complexes.

Cholesterol synthesized by the liver is either converted to bile acids for use in the digestive process or esterified by ACAT (Acyl-CoA: Cholesterol acyl transferase) to form cholesteryl esters which are secreted into the bloodstream as part of lipoprotein complexes called Very Low Density Lipoproteins (VLDL).

As the VLDL circulate, their component TAG and most types of apolipoproteins are removed in the capillaries of muscle and adipose tissues, sequentially converting the VLDL to intermediate density Lipoproteins (IDL) and then to Low Density Lipoproteins (LDL).

Dietary cholesterol, cholesteryl esters and TAGs are transported in the blood by intestinally synthesized lipoprotein complexes called chylomicrons. After removal of their TAGs at the peripheral tissues, the resulting chylomicron remnants bind to specific liver cell remnant receptors and taken up by endocytosis.

In the liver, dietary cholesterol is either used in bile acid biosynthesis or packaged into VLDL.

Atherosclerosis

Elevation of lipids in blood leads to the deposition of cholesterol on the arterial walls, leading to atherosclerosis.

The coronary and cerebral vessels are more commonly affected.

Thrombo-embolic episodes in these vessels lead to ischemic heart disease and cerebrovascular accidents.

Normal Levels	
Total Serum Cholesterol	150 – 240 mg/dL
LDL Cholesterol	< 190 mg/dL
HDL Cholesterol	Males: 45 +/- 12 mg/dL Females: 55 +/- 12 mg/dL
Triacylglycerol (TAG)	< 165 mg/dL

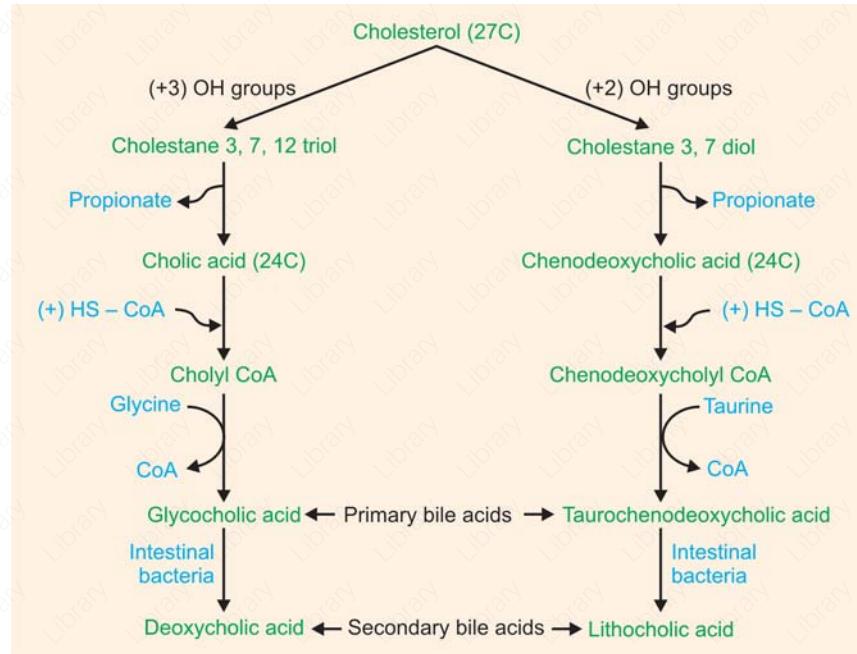
Regulation of Cholesterol Biosynthesis

There are three ways in which the cellular cholesterol supply is maintained:

1. By regulating the HMG-CoA reductase, the rate limiting enzyme in the *de novo* pathway: By short-term regulation of the enzyme's catalytic activity and by long-term regulation by modulating the rate of enzyme synthesis and degradation.
2. By regulating the rate of LDL receptor synthesis. High intracellular concentration of cholesterol, suppresses LDL receptor synthesis whereas low cholesterol concentration stimulates it.
3. By regulating the rate of esterification, the removal of free cholesterol is possible.

ACAT (*Acyl-CoA: Cholesterol acyl transferase*) which catalyzes intracellular cholesterol esterification is regulated by reversible phosphorylation and by long-term control.

FORMATION OF BILE SALTS



VITAMIN D

Structure

Vitamin D refers to a group of 2 steroid compounds that exhibit vitamin D activity. Vitamin D₂ (ergocalciferol) is produced in the laboratory by irradiation of the plant sterol, ergosterol used in pharmaceuticals. Vitamin D₃ (cholecalciferol) is the form obtained from animal sources in the diet or made in the skin by the action of ultraviolet light from sun light on 7-dehydrocholesterol. These two forms differ slightly in the structure of their side chains.



Structure of active form of vitamin D₃

Source

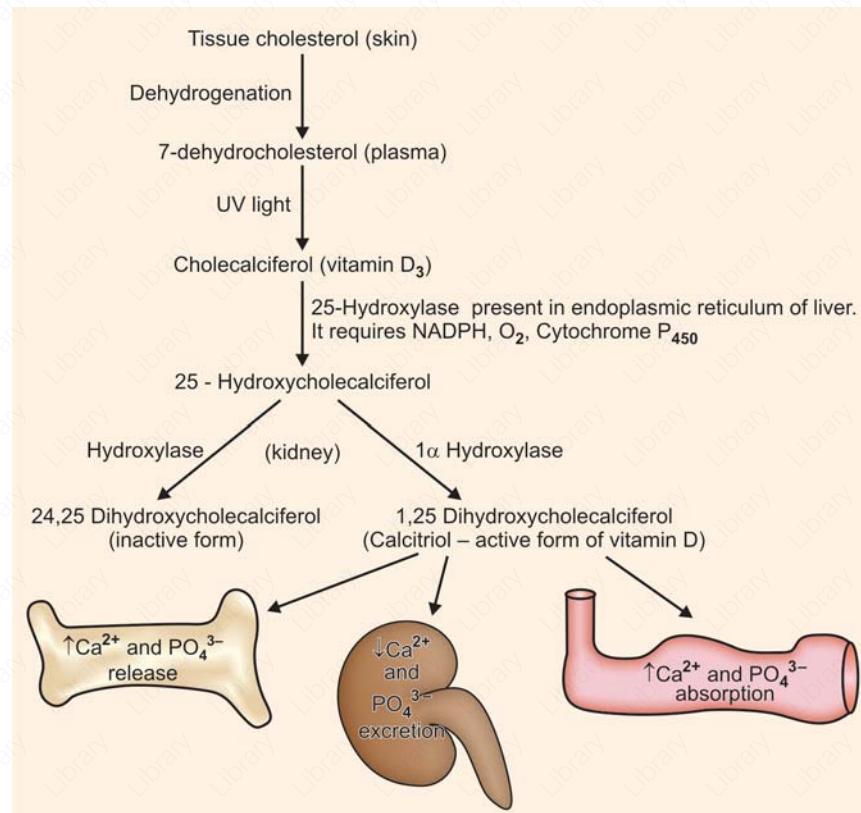
Marine fish liver oils like cod liver oil and shark liver oil are good sources. Sardines, egg yolk and liver and mushrooms contain small amounts.

RDA

10 µg or 400 International Units (IU)/day.

Absorption, Transport and Storage

Dietary vitamin D₂ and D₃ are absorbed in the small intestine in the presence of bile salts. In the enterocytes, absorbed vitamin D is incorporated into chylomicrons and enters circulation via the lymph. Vitamin D dissociates from chylomicrons to bind to a specific vitamin D binding protein (DBP) which has higher affinity for vitamin D₃. Different tissues take up vitamin D from DBP and vitamin D is stored in the liver and adipose tissue. Vitamin DBP can combine with different forms of vitamin D.

Synthesis of Biologically Active form of Vitamin D or Calcitriol

Regulation of Activation of Calcitriol

The activity of 1α - hydroxylase in the kidney is strictly controlled. 25-hydroxycholecalciferol entering the kidney is normally metabolized to 24, 25-dihydroxycholecalciferol unless 1α - hydroxylase is active. The activity of 1α - hydroxylase depends on parathyroid hormone, plasma calcium and plasma phosphate levels. Low level of plasma calcium stimulates the enzyme through the secretion of parathyroid hormone while high concentration of calcium in plasma inhibit the activity of 1α - hydroxylase. Reduced plasma phosphate concentration also activates 1α - hydroxylase but independent of parathyroid hormone.

Insulin, growth hormone, prolactin and estrogen increase the production of the active form of vitamin D. The most potent inhibitor of 1α hydroxylase is the production of 1,25 dihydroxycholecalciferol.

Functions

Calcitriol is a hormone rather than a vitamin because of its several hormone like properties. It can be synthesized in the body, is released in the circulation and has distinct target organs. Further, its mechanism of actions resembles the group II hormones (first messengers). Calcitriol (active form of Vitamin D) is required to maintain serum calcium and phosphate level in the body.

The predominant target organs for calcitriol are bone, kidney and intestine. It increases serum calcium and phosphate concentration by stimulating the following processes.

1. Mobilization of calcium and phosphorous from bone
2. Reabsorption of calcium and phosphorus from the kidney.
3. Absorption of calcium and phosphorus from intestine. Thus, calcitriol plays major role in calcium homeostasis. Pancreas, pituitary and thymus are also the additional target organs for calcitriol.

Calcitriol Acts on

- a. *Bone:* In the bones, calcitriol activates osteoblasts and thus help in bone formation. It deposits Ca^{2+} in the bone when there is sufficient amount of Ca^{2+} in the blood. Calcitriol also brings about bone demineralization or causes mobilization of Ca^{2+} from the bones to the blood. They do this by increasing the activity of osteoclastic cells. Demineralization is under control of parathyroid hormone, i.e. when serum Ca^{2+} and PO_4^{2-} level decreases, there is increase in synthesis of parathyroid hormone. Calcitriol with parathyroid hormone brings about bone demineralization.
- b. *Kidney:* Calcitriol increases the reabsorption of Ca^{2+} and PO_4^{2-} from the renal tubules. Hence, it decreases the excretion of Ca^{2+} and PO_4^{2-} .

- c. *Intestine:* It increases Ca^{2+} absorption by synthesizing Ca^{2+} binding proteins, which are intercellular receptors of Ca^{2+} . Calcitriol helps in transport of Ca^{2+} and formation of alkaline phosphatase which facilitates PO_4^{2-} absorption from the intestinal mucosal cell. All these effects of calcitriol help in maintaining serum Ca^{2+} and PO_4^{2-} level.

Deficiency

Blood calcium and phosphate levels are low (hypocalcemia and hypophosphatemia).

- 1. *Rickets:* In children vitamin D deficiency causes rickets. Deformation of bone (bowed legs, pigeon chest), softness in bones, bossing of frontal bones, enlarged cartilage tissues at the ends of long bones, swelling and pain at wrist, ankle, thinning and pitting of the dental enamel are observed. The ribs get beaded and look like a rosary and are called as rickety rosary.
- 2. *Osteomalacia:* Vitamin D deficiency causes osteomalacia in adults. It is seen in pregnant ladies and women in '*pardah*' in India. Skeletal pain is an early sign. Deformities of ribs, spine, pelvis and legs are seen. Bending of vertebral column and muscular weakness are observed in osteomalacia.
- 3. *Osteoporosis:* Vitamin D deficiency causes osteoporosis in old people. Photolysis of provitamins decreases with age. Decreases sex hormone production and decrease in photolysis of provitamins may lead to deficiency. Symptoms are bone pain and porous bones. Bone fractures are common.

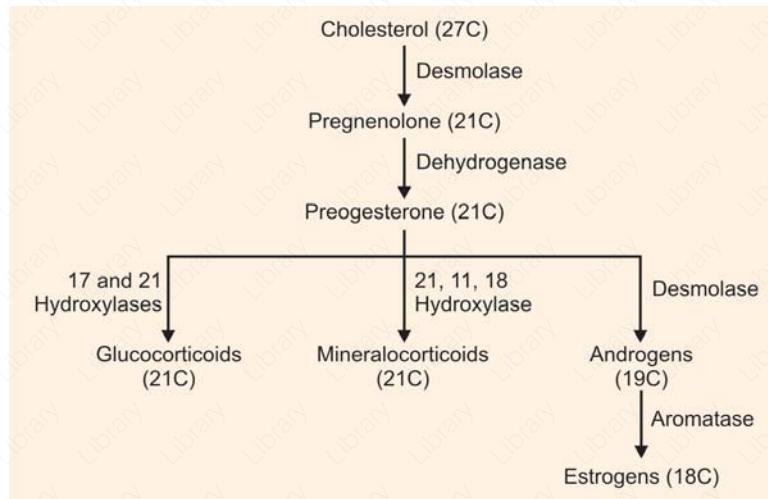
Other types of Rickets are:

- a. *Hypophosphatemic rickets:* Here there is a hereditary decrease in renal re-absorption of phosphate.
- b. *Vitamin D resistant rickets:* Is seen to be associated with Fanconi's syndrome where the renal tubular reabsorption of bicarbonate, phosphate, glucose and amino acids are also deficient. Metabolic acidosis is associated.
- c. *Renal rickets:* In kidney diseases, even if vitamin D is available, calcitriol is not synthesized.

Toxicity

Prolonged intake of high doses of vitamin D (above 155 units/day) may result in toxic symptom such as polyuria, intense thirst, confusion, difficulty in speaking and weight loss. Biochemical changes are hypercalcemia, hypokalemia and metabolic alkalosis.

Synthesis of Steroid Hormones



Estimation of Cholesterol

Serum cholesterol is determined by Zak's ferric chloride method as well as enzymatic method.

Clinical Significance

Normal serum cholesterol ranges from 150 to 240 mg/100 ml serum. This may be slightly higher in middle age and pregnancy. Increased levels of cholesterol in serum, is called hypercholesterolemia. This is seen in the following cases:

- Nephrosis
- Nephrotic syndrome
- Obstructive jaundice
- Myxedema
- Xanthochromatosis
- Glomerulonephritis (slight increase)
- Acute nephritis (marked increase in subacute stage)
- Coronary artery thrombosis and angina pectoris.

Decreased level is called hypcholesterolemia and seen in the following cases:

- Hyperthyroidism
- Pernicious and other anemia
- Malabsorption syndrome
- Severe wasting and acute infections
- Hemolytic jaundice

Estimation of HDL Cholesterol is carried out by four methods:

- a. Enzymatic method
- b. Phosphotungstate/Mg²⁺ method
- c. Heparin/Mn²⁺ method
- d. Using Leibermann Burchard reagent

Clinical Significance

1. The HDL or α -lipoproteins contain certain cholesterol and phospholipids as the main lipids.
2. The HDL particles carry about 20 percent of the total plasma cholesterol and this cholesterol has a clinical implication.
3. It has been observed that HDL-cholesterol is inversely associated with the development of ischemic heart diseases. Thus, persons with HDL-cholesterol elevated are less likely to develop ischemic heart disease.
4. The main function of HDL-cholesterol is transport of cholesterol.
5. The HDL serves in removing cholesterol from peripheral cells and transporting it back to the liver where most of the cholesterol excreted from the body is removed. So HDL is called as beneficial factor among the other lipoproteins concerned.

Normal Range of HDL

Males - 28 - 61 mg/dl
Females - 38 - 75 mg/dl

Reduced HDL levels are seen in:

1. Administration of androgens, propanol, neomycin
2. Maturity onset diabetes mellitus
3. Chronic renal dialysis
4. Nephrosis
5. Cystic fibrosis
6. Hepatocellular disease
7. Cigarette smokers

LDL Cholesterol

Estimation of LDL cholesterol is by enzymatic method: Serum is preferred for this estimation.

Clinical Significance

Increased LDL cholesterol increases the risk of coronary heart disease and atherosclerosis.

Normal range: 10 - 160 mg/dl.

Estimation of LDL Cholesterol and VLDL Cholesterol

Commonly total cholesterol, HDL cholesterol and triglycerides are estimated.

The remaining two (VLDL and LDL) are calculated from the above values as follows:

$$\text{VLDL cholesterol} = \frac{\text{Serum triglycerides}}{5}$$

LDL cholesterol = Total cholesterol - (HDL cholesterol + VLDL cholesterol).
Triglycerides are estimated by Glycerophosphate Oxidase method (GPO-PAP).

MULTIPLE CHOICE QUESTIONS

1. Precursor for the synthesis of cholesterol is:
A. Malonyl CoA B. HMG CoA
C. Succinyl CoA D. Acetyl CoA
2. Atherosclerosis results due to the deposition of _____ on the arterial walls:
A. Phospholipids B. Triglycerides
C. Cholesterol D. All the above
3. Calcitriol activates _____ to help in bone formation.
A. Osteoclasts B. Osteoblasts
C. Cholesterol D. Bile acid

ANSWERS

- 1.D 2.C 3.B

MOST LIKELY QUESTIONS**Long Essay**

1. What is the normal cholesterol level in plasma? Explain how the cholesterol is transported from liver to peripheral tissues and back? Give the causes for hypercholesterolemia.

Short Essay

1. Atherosclerosis

Short Answers

1. What is normal serum total cholesterol and HDL cholesterol level?
2. Cholesterol

4.6: LIPOPROTEINS AND THEIR FUNCTIONS

LIPOPROTEINS

Total plasma lipid is 750 – 1000 mg/dl.

Roughly 40 percent - Triglycerides
 20 percent - Cholesterol
 2 percent - Free fatty acids
 (38%) Rest - Phospholipids.

Since lipids are insoluble in water, they cannot be transported as such in plasma. Therefore, they are complexed with proteins to form lipoproteins (water-miscible). Protein part of lipoproteins is called apolipoprotein/ apoprotein.

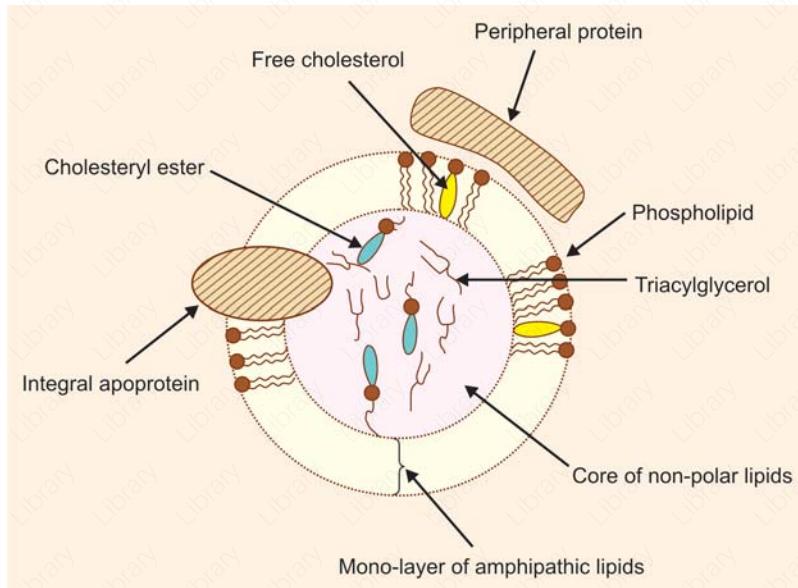


Fig. 4.6.1: Structure of a lipoprotein particle

Depending on the density (by Ultra Centrifuge) or on the Electrophoretic mobility, the lipoproteins in plasma are classified into six major types:

1. Chylomicrons
2. Very low density lipoproteins (VLDL) or Pre-Beta lipoproteins
3. Intermediate density lipoproteins (IDL) or Board-Beta lipoproteins.
4. Low density lipoproteins (LDL) or Beta-Lipoproteins.
5. High density lipoproteins (HDL) or Alpha – Lipoproteins.
6. Free fatty acid-albumin- cannot be separated by Electrophoresis.

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Composition and Functions of Lipoproteins

1. Chylomicrons : 99 percent lipid +1 percent proteins
Synthesis : Intestine
Function : Transport dietary TGs from intestine to various tissues such as, muscle , heart and adipose tissue.
Chylomicrons are the least in density and largest in size, among lipoproteins.
2. VLDL : 90 percent Lipid + 10 percent protein
Production : Liver and intestine
Function : Transport endogenously synthesized TGs to peripheral tissues.
3. LDL : (Bad cholesterol) 80 percent lipid + 20 percent protein.
Formation : From VLDL in blood circulation
Function : Transport cholesterol from liver to peripheral tissues.
4. HDL : (Good cholesterol) 45-70 percent lipid + 30-55 percent protein.
Synthesis : In liver
Function : Transport cholesterol from peripheral tissues to liver (Reverse cholesterol transport).

Apolipoproteins/Apoproteins

Apo A	Apo A - 1	Activator of LCAT (lecithin – cholesterol acyl transferase
Apo B	Apo B - 100 Apo B - 48	Acts as a ligand for LDL receptor Chylomicron secretion from intestine
Apo C	Apo C - 1 Apo C - 11	Possible activator of LCAT Activator of lipoprotein lipase
Apo D	Apo D Apo E	Ligand for chylomicron remnant receptor in liver and LDL receptor

MULTIPLE CHOICE QUESTIONS

1. Triacylglycerols in plasma lipoproteins may be hydrolyzed by:
A. Pancreatic lipase B. Gastric lipase
C. Lipoprotein lipase D. Hormone sensitive lipase
2. The plasma lipoprotein which transports maximum cholesterol is:
A. VLDL B. LDL
C. HDL D. Chylomicrons
3. _____ Lipoprotein helps in reverse cholesterol transport.
A. VLDL B. LDL
C. HDL D. Chylomicrons

ANSWERS

1. C 2. B 3. C

MOST LIKELY QUESTIONS**Long Essay**

- What are lipoproteins? Write the composition and functions of lipoproteins.

Short Essay

- Functions of lipoproteins.

Short Answer

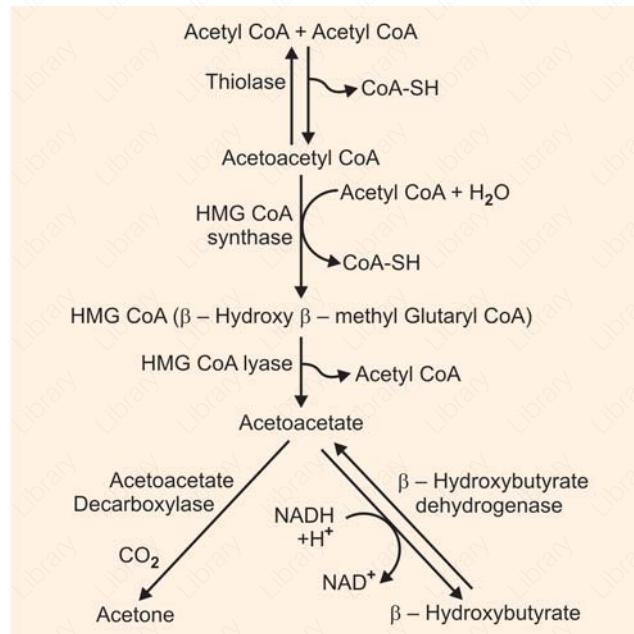
- Apolipoproteins.

4.7: KETONE BODY METABOLISM**1. Ketogenesis (Synthesis of ketone bodies)**

Acetyl CoA formed in the liver during oxidation of fatty acid may enter the TCA cycle or may be converted to: 'Ketone bodies' acetoacetate, β -hydroxy butyrate and acetone for export to other tissues.

Site: Liver

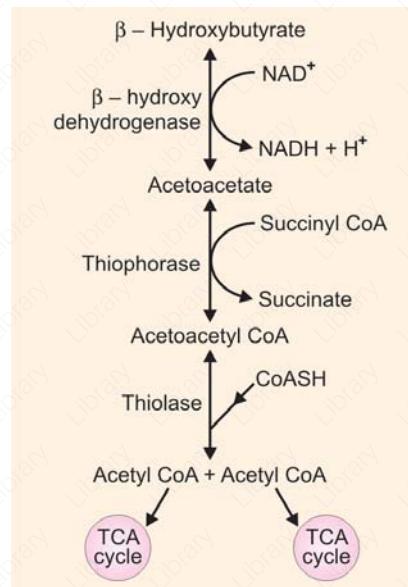
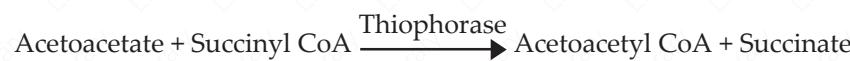
Sub-cellular site: Mitochondria

Formation of Ketone Bodies

Note: During starvation and untreated diabetes, thiolase catalyses the condensation of 2 acetyl CoA molecules to acetoacetyl CoA, the parent of three ketone bodies. These reactions occur in mitochondrial matrix.

2. Ketolysis (Breakdown of ketone bodies)

Ketone bodies formed in the liver are utilized by extrahepatic tissues as fuels. Heart muscle and renal cortex prefer ketone bodies as fuel. Skeletal muscle and brain can also use them as alternate sources of energy, if glucose is not available. Before utilization, acetoacetate is activated to acetoacetyl CoA by thiophorase enzyme.



β - hydroxybutyrate synthesized in the liver passes into the blood and thus to other tissues, where it is converted to acetyl CoA for energy production. It is first oxidized to acetoacetate, which is activated with coenzyme A donated from succinyl CoA, then split by thiolase.

3. Ketosis (Accumulation of ketone bodies in blood)

Extrahepatic tissues can metabolize ketone bodies formed in the liver. Normal ketone body level in blood is less than 1mg/dl. Normal ketone body in urine is traces only. Accumulation of ketone bodies in blood happens if the rate of synthesis exceeds the ability of extra hepatic tissues to utilize them. Ketosis leads to:

1. Excess ketone body in blood (Ketonemia).
 2. Excretion in urine (Ketonuria)
 3. Smell of acetone in breath.

All these three together constitute the condition known as 'Ketosis'.

Causes for Ketosis

1. Diabetes mellitus (DM)
 2. Starvation.

Diabetes mellitus: DM in its serious form is the most common cause for ketosis. Even though glucose is in plenty, insulin deficiency causes accelerated lipolysis and more fatty acids are released into circulation.

Fatty acids undergo β -oxidation to release acetyl CoA, which enter and get oxidized in TCA cycle only when carbohydrates are available. In diabetes, glucagon-insulin ratio is increased and key gluconeogenic enzymes are activated. Thus, oxaloacetate is diverted for gluconeogenesis; then citric acid cycle cannot function optimally. On one hand, acetyl CoA is generated in excess, on the other, its utilization is reduced. This excess acetyl CoA is channelized into ketogenic pathway. This leads to diabetic ketoacidosis.

Starvation: During starvation, the dietary supply of glucose is decreased. To provide alternate source of fuel, the rate of lipolysis increases. The high glucagon - insulin ratio prevailing under the conditions of starvation favors ketogenesis. The brain derives 75 percent of energy from ketone bodies under the conditions of fasting. Starvation ketosis occurs due to decreased availability of oxaloacetate for oxidation of acetyl CoA in citric acid cycle. Oxaloacetate is channelized to gluconeogenesis.

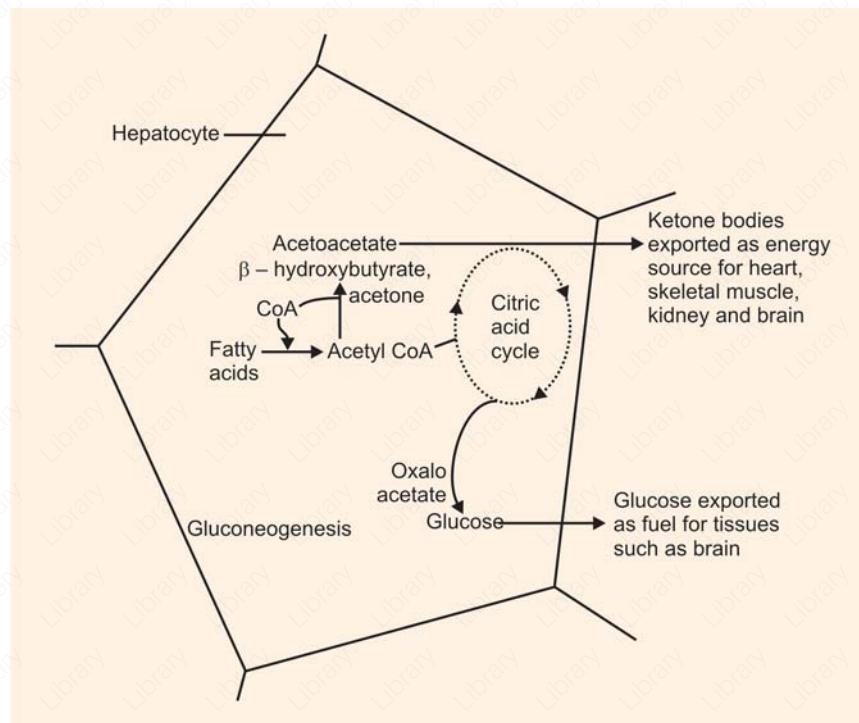
MULTIPLE CHOICE QUESTIONS

1. Ketosis can occur in:
 - Hypoglycemia
 - Diabetes mellitus
 - Semistarvation
 - Uncontrolled Diabetes mellitus
 2. Ketone bodies are synthesized in the liver but utilized by extrahepatic tissues. This is due to the absence of _____ enzyme in the liver.
 - Thiolase
 - Thiokinase
 - Thiophorase
 - Thioesterase
 3. In uncontrolled diabetes mellitus ketone bodies are formed due to:
 - Increase in glucagon-insulin ratio
 - Activation of key enzymes of gluconeogenesis
 - Diversion of oxaloacetate for gluconeogenesis
 - All the above

ANSWERS

- 1.D 2.C 3.D

Ketone Body Formation and Export from the Liver



MOST LIKELY QUESTIONS

Long Essay

1. Discuss ketone body metabolism. Write a note on ketosis.

Short Essays

1. Ketogenesis
2. Ketolysis

Short Answers

1. Ketosis
2. What are the causes of ketosis?

Chapter

5

Composition and Metabolism of Amino Acids and Proteins

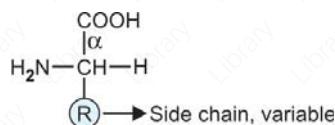
5.1: CHEMISTRY OF AMINO ACIDS AND PROTEINS

Amino acids are the basic structural units of proteins. Some exist in free form in human blood. More than 300 amino acids are found in nature. Only 20 amino acids are present in proteins and these 20 amino acids are called the standard amino acids. 21st amino acid is reported recently which is called selenocysteine.

Amino acids are group of organic compounds with 2 functional groups.

1. Amino - NH₂ (basic)
2. Carboxyl - COOH (acidic).

General Structure



L- α - amino acid

Proteins have only L - α amino acids in which NH₂ and COOH are attached to the same carbon atom.

CLASSIFICATION OF AMINO ACIDS

I. Based on Structure (of the side chain or R - group)

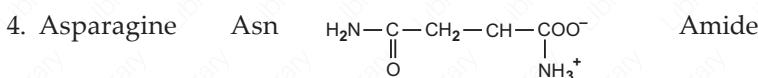
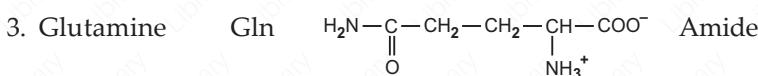
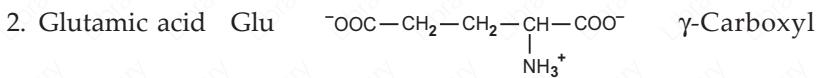
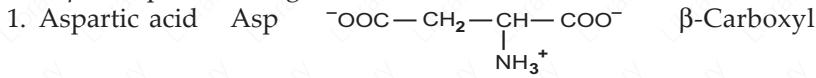
1. **Aliphatic amino acids** are made up of only C or H side chain.
Example: Glycine - simplest amino acid

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		<i>Symbol</i>	<i>Structure</i>	<i>Special group</i>
1.	Glycine	Gly	$\begin{array}{c} \text{H}-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	
2.	Alanine	Ala	$\begin{array}{c} \text{CH}_3-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	
3.	Valine	Val	$\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{CH}-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	Branched chain
4.	Leucine		$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{CH}-\text{CH}_2-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	Branched chain
5.	Isoleucine		$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{CH}_3-\text{CH}-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	Branched chain
2.	Hydroxy amino acids			
	Serine, Threonine			
	Serine	Ser	$\begin{array}{c} \text{CH}_2-\text{CH}-\text{COO}^- \\ \\ \text{OH} \quad \text{NH}_3^+ \end{array}$	Hydroxyl
	Threonine	Thr	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}-\text{CH}-\text{COO}^- \\ \quad \\ \text{OH} \quad \text{NH}_3^+ \end{array}$	Hydroxyl
3.	Sulphur containing amino acids			
	<i>Example: Cysteine, Methionine, Cystine</i>			
1.	Cysteine	Cys	$\begin{array}{c} \text{CH}_2-\text{CH}-\text{COO}^- \\ \\ \text{SH} \quad \text{NH}_3^+ \end{array}$	Sulfhydryl
2.	Cystine		$\begin{array}{c} \text{H}_2\text{C}-\text{CH}-\text{COO}^- \\ \\ \text{S} \quad \text{NH}_3^+ \\ \\ \text{S} \\ \\ \text{CH}_2-\text{CH}-\text{COO}^- \\ \\ \text{NH}_3^+ \end{array}$	Disulphide -S-S-
3.	Methionine	Met	$\begin{array}{c} \text{CH}_2-\text{CH}_2-\text{CH}-\text{COO}^- \\ \\ \text{S}-\text{CH}_3 \quad \text{NH}_3^+ \end{array}$	Thioether

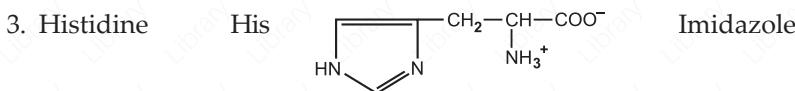
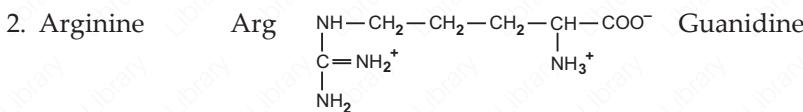
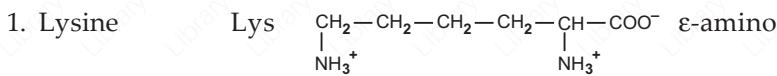
4. Acidic amino acids

Example: Aspartic acid, glutamic acid and their derivatives.



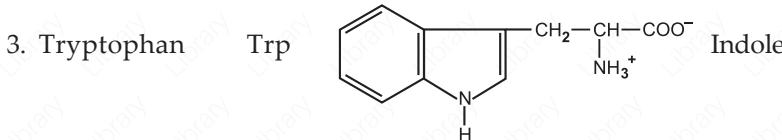
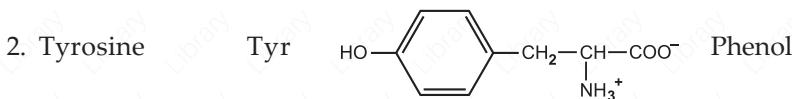
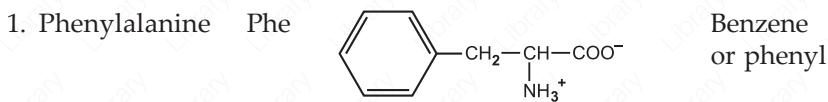
5. Basic amino acids

Example: Arginine, Histidine, Lysine ε δ γ β α



6. Aromatic amino acids

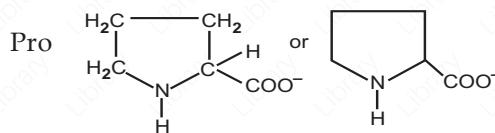
Example: Phenylalanine, Tyrosine, Tryptophan



7. Imino acid

Proline (it has imino group)

1. Proline



Pyrrolidine

II. Based on Number of -NH₂ and – COOH group present

1. Monoamino monocarboxylic acid.

Only one NH₂ and one COOH

Example: Glycine

2. Monoamino Dicarboxylic acids

Example: Glutamic acid

3. Diamino monocarboxylic acids

Example: Lysine

III. Based on Net Charge at Neutral pH (7.0)

1. Acidic amino acids are negatively charged.

Example: Aspartic acid, Glutamic acid

2. Basic amino acids are positively charged

Example: Arginine, Histidine, Lysine

3. Neutral amino acids have no net charge

Example: Glycine, Alanine, Valine, Leucine (all other 15)

IV. Based on polarity

1. Polar amino acids have hydrophilic (water loving) side chain.

i. Non ionic (uncharged)- They have OH group.

Example: Serine, Threonine, Cysteine, Tyrosine, Glutamine, Asparagine.

ii. Ionic (charged): Highly hydrophilic

a. Acidic

Example: Aspartic acid, Glutamic acid

b. Basic

Example: Arginine, Histidine, Lysine

2. Non-polar amino acids have hydrophobic side chains.

Example: Glycine, Alanine, Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Methionine, Proline.

V. Based on Nutritional Requirement

1. *Essential amino acids (indispensable)*: These are not synthesized in our body and must be provided in the diet. Lack of these amino acids in diet, leads to growth failure.

Example: Leucine, Isoleucine, Threonine, Lysine, Methionine, Phenylalanine, Tryptophan and Valine.

Semi-essential amino acids: These are synthesized in the body to some extent. They are essential for children. *Example:* Arginine, Histidine.

2. *Non-essential amino acids (dispensable):* These are synthesized in our body, so need not be present in the diet.

Example: Glycine, Serine, Tyrosine, Glutamic acid, Glutamine, Aspartic acid, Asparagine, Cysteine, Proline, Alanine.

VI. Based on Metabolic Fate

1. *Glucogenic amino acids:* Carbon skeleton of amino acid is converted to glucose or intermediate of TCA cycle. Non-essential amino acids are glucogenic. Example: Glycine, Alanine, Aspartic acid, Proline.
2. *Ketogenic amino acids:* Carbon skeleton of amino acid converted to ketone body or intermediates of fatty acid metabolism. Only one amino acid is purely ketogenic. Example: Leucine.
3. *Glucogenic and ketogenic amino acids:* Carbon skeleton of amino acids are partly glucogenic and partly ketogenic. Example: Phenylalanine, Tyrosine, Tryptophan, Isoleucine, Lysine. These amino acids are precursors for the synthesis of glucose as well as fat.

Non-Standard Amino Acids

1. *Derived amino acids:* α - Amino acids are modified after the protein is synthesized. Such amino acids are present in some proteins.
Example: 4 - Hydroxyproline and 5 - Hydroxylsine are found in collagen. γ - Carboxy glutamate is found in prothrombin. N - Methyl lysine is found in myosin.
2. *Non-protein amino acids:* Some amino acids are not present in proteins, but occur freely in body. They play important role in metabolism.
 - i. α - amino acids: Ornithine, citrulline, arginino succinic acid are the intermediates in biosynthesis of urea.
 - ii. Homocysteine and homoserine are the intermediates in methionine metabolism.
 - a. Non α - amino acids:
Example:
 1. β - Alanine is a part of co enzyme A
 2. γ - Aminobutyric acid (GABA) is an inhibitory neurotransmitter
 3. γ - Amino levulinic acid is an intermediate in heme synthesis.
 4. Taurine is a part of bile acids.

Properties of Amino Acids

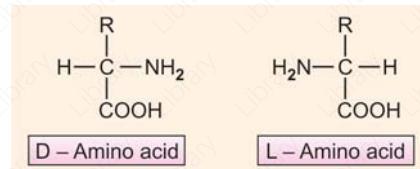
A. Physical Properties

- a. *Solubility:* All amino acids are soluble in H_2O , alcohol and insoluble in organic solvents (Example: benzene).

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- b. *Melting point*: Amino acids generally melt at higher temperatures often above 200°C.
- c. *Taste*: Amino acids may be sweet (Gly, Ala, Val), tasteless (Leu) or bitter (Arg, Ile). Sodium glutamate is a salt of glutamic acid and is used as a flavouring agent in food industry to increase taste and flavor (Mono sodium glutamate - Azinamoto).
- d. *Optical activity*: All standard amino acids except glycine are optically active, i.e. they can rotate plane of polarized light to the right or to the left. They possess asymmetric α carbon atom, i.e. four different groups are bonded to it.

One asymmetric carbon atom can exhibit two optical isomers. D-amino acid is the mirror image of L - amino acid. Certain D - amino acids are found in antibiotics and also in bacterial cell walls. Example: Gramicidin S contains D- amino acid. L - Amino acids are found in proteins.



Isoleucine and threonine have two asymmetric carbons and hence exhibit 4 isomers.

- e. *Charge (Acid-base) properties*: Amino acids are ionized in aqueous solutions. They are amphoteric in nature and can act as acids or bases. Therefore, they contain ionizable groups - COOH, NH₂. Charge of amino acid carried depends on pH of its surrounding medium.
 - i. *At neutral pH (7.0)*: Carboxy group of amino acid exists as COO⁻ and amino group as NH₃⁺, i.e. both groups are ionized to form zwitterion. It is an amino acid molecule with equal number of positive and negative charges.

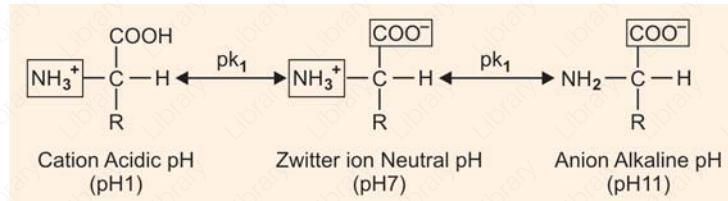
It is electrically neutral (net charge is zero) does not move in electrical field. It is the predominant form at pH 7.0.

- ii. *Isoelectric pH (P^I)*: pH at which net charge of an amino acid is zero, i.e. number of positive charges = number of negative charges. At P^I amino acids exist as zwitterions. So they do not move in electric field. They have minimum solubility (i.e. maximum tendency to precipitate).

	P^I
Glycine	6.0
Histidine	7.6
Lysine	9.7

- iii. *At acidic pH (<2)*: COOH remains undissociated - amino acids exist in cationic form (positively charged).
- iv. *At alkaline pH (>10)*: NH₃⁺ dissociates to - NH₂ groups. Amino acids exist in anionic form (negatively charged).

Different Forms of Amino Acids



pK_1 ($-COOH$) pH at which 50 percent amino acid molecules are in cationic form and 50 percent in zwitterion form.

pK_2 ($-NH_3^+$) pH at which 50 percent amino acid molecules are anionic form and 50 percent in zwitterion form.

$$P^I = \frac{pk_1 + pk_2}{2}$$

Buffering action is maximum at and around pk_1 or pk_2 .

Histidine has extra pK_3 (due to imidazole group) = 6.1 is an effective buffer at pH 7.4. It plays an unique role in enzymatic catalysis.

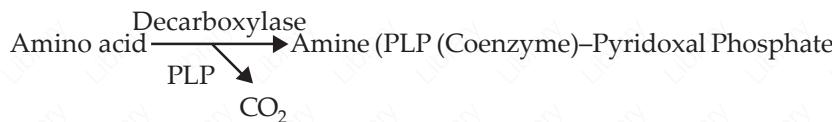
Amino acids have characteristic titration curve titration involves gradual addition or removal of protons (H^+).

f. **UV absorption:** Aromatic amino acids Phe, Tyr and Trp absorb UV light. Tryptophan absorbs UV light at 280 nm.

CHEMICAL PROPERTIES

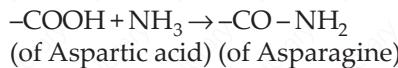
I. Reactions due to Carboxyl Groups

a. Decarboxylation



Decarboxylation produces biologically important amines such as histamine from histidine, GABA from glutamic acid, tyramine from tyrosine.

b. Amide formation



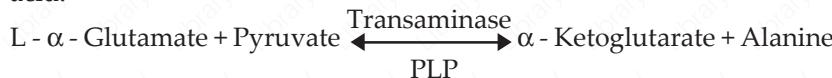
c. Salt and ester formation

Amino acids form salts ($-COONa$) with bases and esters ($-COOR^1$) with alcohols.

II. Reactions due to Amino Group

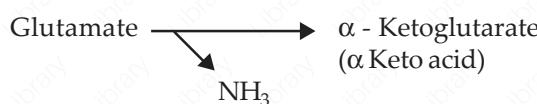
a. Transamination

It is the transfer of α - amino group of an α - amino acid to an α - keto acid.

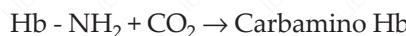


b. Deamination

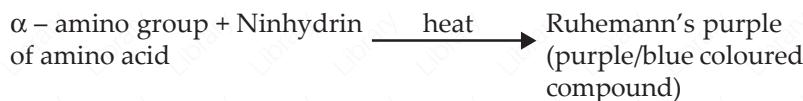
Here removal of α - amino group of amino acid takes place to form an α - ketoacid.



III. Formation of Carbamino Compounds



IV. Ninhydrin Reaction

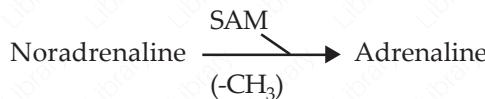


This test is done to detect α - amino acids. But proline does not answer this test, therefore it does not have amino group. It is an imino acid. It gives yellow color.

V. Reactions due to Side Chains

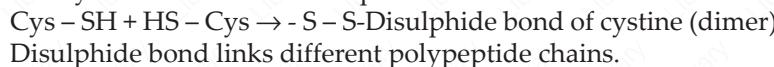
a. Transmethylation:

Transfer of methyl group from active form of methionine (S- Adenosyl Methionine SAM) to noradrenaline to form adrenaline.



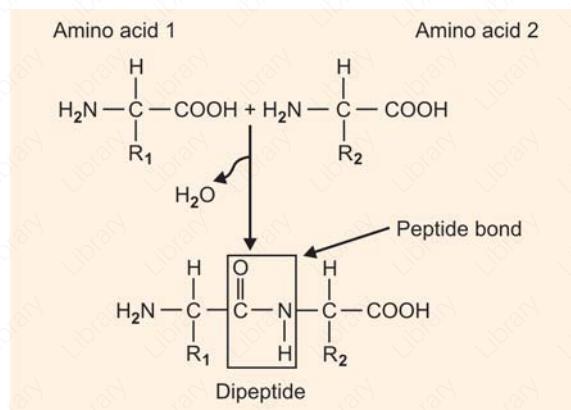
b. Formation of disulphide bond:

Here formation of disulphide bond between two cysteine molecules takes place.



VI. Peptide Bond Formation

α - Carboxyl group of one amino acid and α -amino group of another amino acid react together to form a peptide bond or CO - NH bond.



Dipeptide contains 2 amino acids and 1 peptide bond. Tripeptide contains 3 amino acids and 2 peptide bonds. Oligopeptide has 10 or less amino acids and polypeptide contains 10 - 50 (more than 10 up to 50) amino acids.

PROTEINS

The term protein is derived from the Greek words proteios meaning holding first place in living matter. Proteins make up 75 percent of total body weight. Proteins contain carbon, hydrogen, oxygen, nitrogen as major components and sulphur, phosphorus as minor components. Average nitrogen content of proteins is 16 percent by weight. Proteins are high molecular weight compounds. They are polymers of L- α -amino acids linked by peptide bonds. Proteins are structurally and functionally important.

Classification of Proteins

Protein has diverse functions.

A. Based on biological functions proteins are classified into:

Function	Examples
1. Structural	Collagen, elastin, ketatin
2. Catalytic (enzymes)	Hexokinase, pepsin, trypsin
3. Contractile	Actin, myosin
4. Transport	Hemoglobin, albumin, transferrin
5. Regulatory (Hormones)	Insulin, GH, ACTH
6. Protective (defence)	Immunoglobulins, interferons (antibodies)
7. Genetic	Histones
8. Storage	Ferritin (stores iron)
9. Buffers	Plasma proteins, hemoglobin
10. Transporters	$\text{Na}^+ - \text{K}^+$ ATPase

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- B. Based on shape proteins are classified into:
1. *Globular proteins*: Polypeptide chain folded into compact, spherical shape, easily soluble. Example: Albumin, hemoglobin, myoglobin.
 2. *Fibrous proteins*: Polypeptide chain extended along the axis is insoluble. Example: Collagen, elastin, keratin.
- C. Based on composition proteins are classified into:
- a. *Simple proteins*: Contain only amino acids. Simple proteins on hydrolysis yield amino acids. Example: Serum albumin, pepsin, trypsin.
Based on solubility: Simple proteins are subdivided
 - i. *Albumins*: Soluble in water and dilute salt solutions and coagulated by heat. Example: Serum albumin, ovalbumin (egg).
 - ii. *Globulins*: Insoluble in pure H₂O but soluble in dilute salt solutions. Example: Serum globulins.
 - iii. *Glutelins*: Soluble in dilute acids and alkalies. Example: glutenin of wheat.
 - iv. *Protamines*: Soluble in H₂O, dilute acids and alkalies. Example: Salmine of salmon fish.
 - v. *Histones*: Soluble in H₂O and dilute acids but insoluble in dilute ammonium hydroxide. Rich in basic amino acids. Example: Thymus histones.
 - vi. *Prolamines*: Soluble in 70 percent alcohol; insoluble in water, rich in proline. Example: Zein (of maize)
 - vii. *Scleroproteins*: Insoluble in water, dilute acids and alkalies. Example: Collagen, elastin, keratin.
 - b. *Conjugated (compound proteins)*: Contain non-protein part (prosthetic group) attached to protein part. Conjugated protein on hydrolysis yield protein and prosthetic group.

Types of conjugated proteins			
Subclass	Prosthetic group	Type of linkage	Example
1. Glycoproteins	Carbohydrates (<10%)	Covalent	<ul style="list-style-type: none">• Egg albumen• Immunoglobulins
2. Lipoproteins	Lipid	Hydrophobic interactions	<ul style="list-style-type: none">• Serum lipoproteins (HDL, LDL)• Membrane lipoprotein
3. Phosphoprotein	Phosphorus	Covalent	<ul style="list-style-type: none">• Casein of milk• Vitellin of egg yolk
4. Nucleoprotein	Nucleic acids (DNA or RNA)	Non-covalent	<ul style="list-style-type: none">• Nucleohistones of chromatin

Contd...

Contd...

Subclass	Prosthetic group	Type of linkage	Example
5. Chromoproteins: Colored substance.			
a. Hemoprotein	Heme	Noncovalent	<ul style="list-style-type: none"> • Hemoglobin • Myoglobin • Cytochromes • Succinate dehydrogenase
b. Flavoproteins	Flavin nucleotides FMN, FAD	Covalent	
c. Visual pigments	Retinal (Vitamin A)	Covalent	• Rhodopsin of retina
d. Metalloproteins	Metals Fe Cu Zn	Non-covalent	<ul style="list-style-type: none"> • Ferritin, cytochromes • Tyrosinase • Carbonic anhydrase

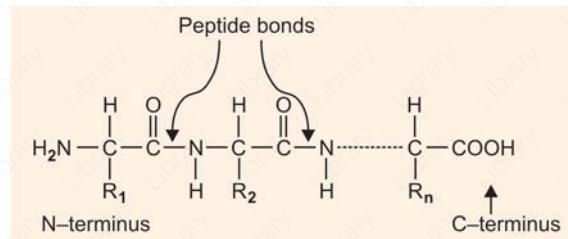
- c. *Derived proteins*: are derivatives of simple or conjugated proteins.
- Primary derived proteins* are denatured or coagulated proteins. Here peptide bonds are not hydrolyzed. Example: Coagulated albumin.
 - Secondary derived proteins*: are partially hydrolyzed (degraded) products of proteins. Example: proteases, peptones, gelatin, peptides.
- D. Based on nutritional value (based on their essential amino acid content) proteins are classified into:
- Complete proteins (Class I proteins) contain all 10 essential amino acids at proportions required by human body. They promote good growth. Example: Egg albumen, Casein of milk.
 - Partially incomplete proteins partially lack one or more essential amino acids. They promote moderate growth. Example: Pulse proteins (deficient in Met), Cereal proteins (deficient in Lys).
 - Incomplete proteins (poor proteins) completely lack one or more essential amino acids. They do not promote growth. Example: Zein from corn (lacks tryptophan, lysine).

Protein Structure (Conformation)

Proteins are polymers of L- α -amino acids. Linear polypeptide chains can be folded to specific 3D shape which is more stable. Function of a protein depends on its 3D structure, which is unique.

There are 4 levels of structural organization:

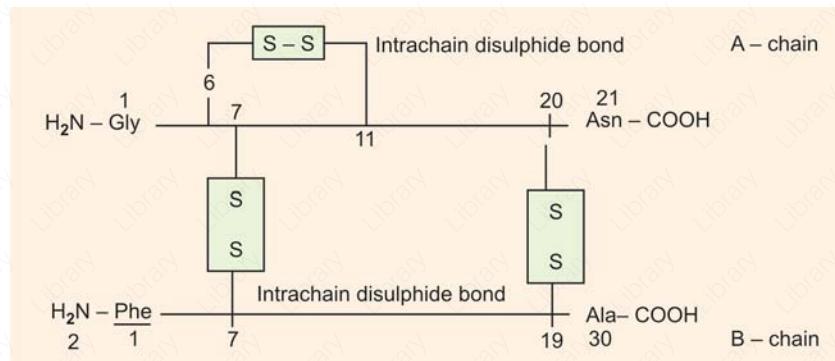
Primary structure: It refers to the linear sequence of amino acids linked by peptide bonds and locations of disulphide bonds (if any). Peptide bonds ($\text{CO} - \text{NH}$) and disulphide bonds ($\text{S} - \text{S}$) are covalent bonds or (covalent backbone). Primary structure is decided by genes. It influences higher levels of protein conformation.



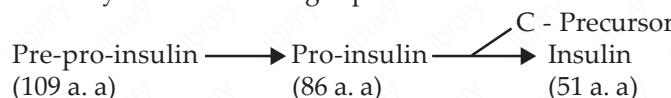
Peptide bonds: They are covalent bonds. They are rigid, planar and have partial double bond characteristics. C – N is in trans configuration.

Note: Change in a single amino acid of the primary structure can alter the properties of the protein. Example: Normal Hb becomes abnormal sickle cell Hb (HbS) when a single amino acid among 154 in β chain is changed. Sixth amino acid in β chain is glutamic acid in normal Hb and valine in abnormal HbS.

1. Primary Structure (covalent structure) of Insulin



Insulin is synthesized as larger precursor



C - Peptide is also called connecting peptide.

C - Peptide estimation is done to measure endogenous insulin.

2. Secondary Structure

- It is the folding of the polypeptide chain along its long axis into regular repeating structure.
- Secondary structure makes protein more compact.
- It adds some new properties to a protein increasing strength and flexibility.

4. It is maintained by numerous H - bonds between – NH and – C = O groups of amino acids within the protein.

Two common types of secondary structure are:

- α - Helix
- β - Pleated sheet

α - Helix: It is proposed by Pauling and Corney in 1951.

Features

Polypeptide backbone is coiled around a central axis, spontaneously. Numerous hydrogen bonds between -N - H and - C = O groups (that are four residues apart) stabilize the structure. Side chains of amino acids are extended outwards. Distance between two amino acid residues is 1.5 Å. Each turn has 3.6 amino acids. Pitch (rise/turn) = 5.4 Å. Every -N-H and -C = O participates in hydrogen bonding. Right handed α - helix is more common and more stable (Fig. 5.1.1).

Amino acids which do not allow formation of α - helix (terminators) are for example: proline, its derivative hydroxyproline. Amino acids which destabilize α - helix are acidic, basic amino acids. For example: aspartic acid, arginine.

Occurrence: Hemoglobin and myoglobin have abundant α - helical regions.

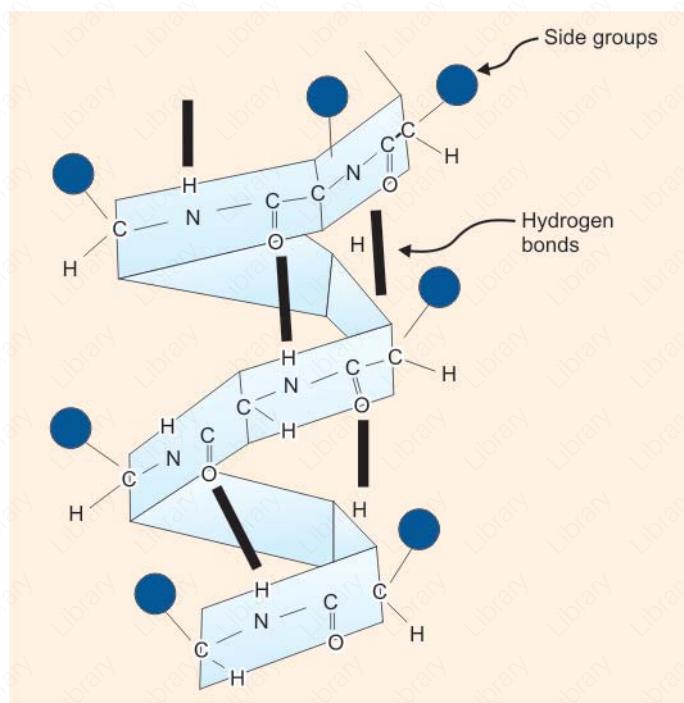


Fig. 5.1.1: The α -helix structure

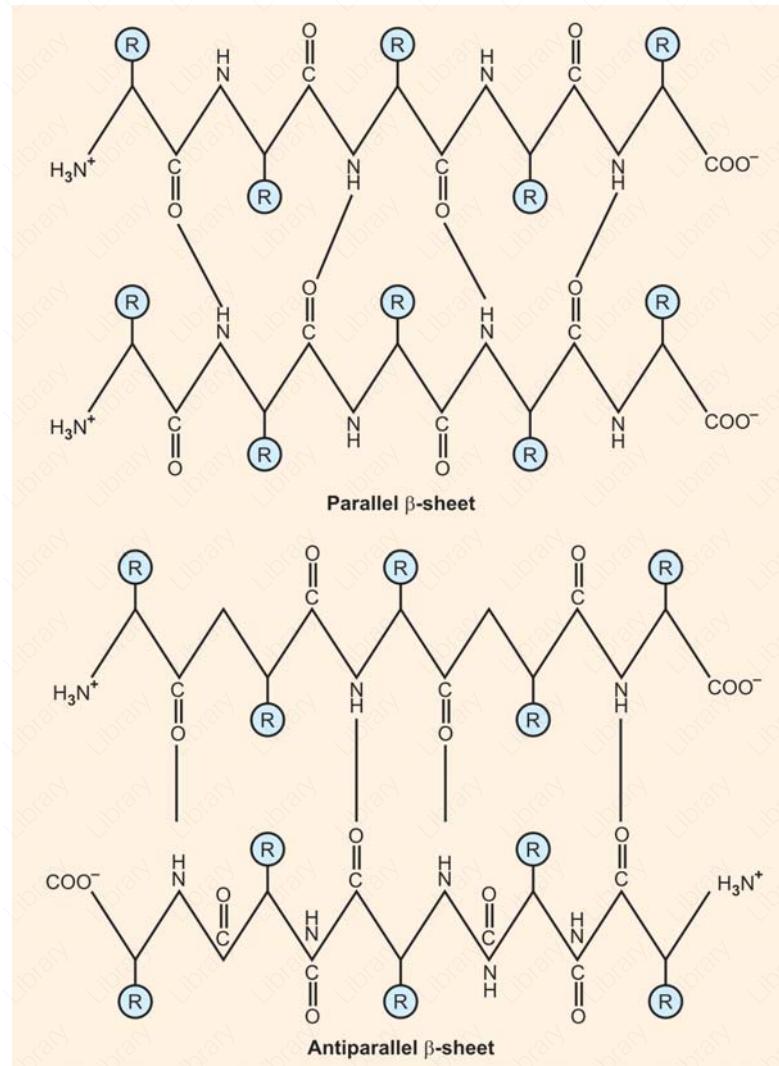
H bond: Bond formed by sharing a hydrogen between two electron donors.

<i>H-releasing groups (in proteins)</i>	<i>H-accepting groups (in proteins)</i>
-NH (imidazole)	-COO ⁻ (Asp, Glu)
-OH (Ser, Thr)	C=O (peptide)

β - Pleated Sheet

Features

Polypeptide chain is extended into zigzag structures. Two or more adjacent segment of chain line, up side by side to form sheet. The side chains are above or below the plane of the sheet.

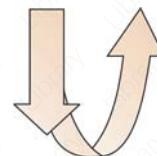


Numerous H- bonds between $-N-H$ and $-C=O$ groups of adjacent sheets, stabilize the structure. When adjacent strands run in same direction structure is parallel β - pleated sheets. When adjacent strands run in opposite direction, structure is antiparallel β - pleated sheet.

Occurrence: Seen in silk fibroin.

Random coils: These are the regions of proteins which are not organized as helices or parallel sheets.

Bends: β - Turn (β - bend or reverse turn) is a hairpin turn (U turn) of polypeptide. And it contains 4 residues Gly, Ser, Asp, Pro. It changes the direction of chain and connects anti parallel β - pleated sheets which make protein compact.



Loops: Loops contain 16 residues. They vary in size and shape. They connect adjacent units of chain and have biologically important functions. For example: Site for ligand interactions, antigen - binding site of antibodies.

Triple helix: Three left handed helices wrapped around each other make a triple helix. Collagen contains this structure. Collagen is the most abundant protein in mammals (bones, teeth, tendons, cartilages, skin and blood vessels.)

Tertiary structure: It denotes 3D arrangement of proteins and formed by interaction between amino acids far apart in chain. It consists of α - helices, β - pleated sheets, β turns, motifs and random coils. It is compact and most stable and stabilized by non-covalent bonds.

Bonds Responsible for Protein Structure

Non-covalent Bonds

1. *Hydrophobic interaction:* Association between non-polar side chains of amino acids.
2. *Electrostatic bonds (ionic bonds):* Formed between oppositely charged groups of amino acid side chain. For example: Between positively charged group of basic amino acids and negatively charged group of acidic amino acids.
3. *Hydrogen bonds:* They are formed by sharing of hydrogen atoms between nitrogen and carbonyl oxygen of different peptide bonds. Each hydrogen bond is weak but collectively they are strong.
4. *Van der Waals forces:* These are the non-covalent association between electrically neutral molecules. They are formed by the electrostatic interactions due to permanent or induced dipoles.

Covalent Bonds

Peptide and disulphide bonds are the strong bonds in protein structure.

Disulphide bonds: Disulphide bond is formed by the sulphydryl groups of two cysteine residues to produce cystine. Disulphide bonds are formed in a single polypeptide chain or between different polypeptides. These bonds are responsible for the stability of the proteins and their structural conformation.

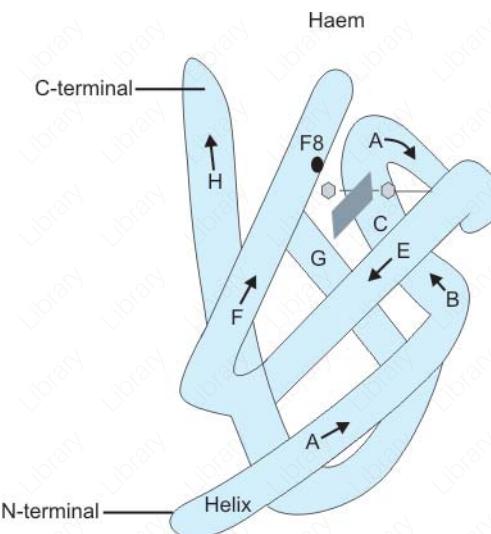


Fig. 5.1.2: Quarternary structure

Quaternary structure (Fig. 5.1.2): It is possessed by proteins containing two or more polypeptide chains (oligomeric proteins). Individual polypeptide chains are called monomers or subunits. For example: Dimer consists of 2 monomers and tetramer contains 4 monomers. Bonds that keep this structure are non-covalent such as, H bonds, electrostatic bonds, hydrophobic interaction and Van der Waals forces. Protein loses function when subunits are dissociated. Oligomeric proteins play significant role in regulation of metabolism.

- Techniques used to study protein structure are:
- X-ray diffraction
 - UV light spectroscopy
 - Nuclear magnetic resonance (NMR)

Physical Properties of Proteins

- Solubility:** Proteins exist as colloids in solution. They scatter light and exert osmotic pressure. Example: Plasma albumin exerts osmotic pressure.

2. *Molecular weight:* The proteins vary in their molecular weights depending upon the number of amino acid residues. For example: Insulin - 5,700; Albumin - 69,000; Hb -6500; Immunoglobulin (Ig) - 1,50,000.
3. *Shape:* There is a wide variation in the shape of proteins. For example: fibrinogen is elongated or fibrous.
4. *Isoelectric pH (P^I):* The nature of the amino acids determines the P^I of a protein. The acidic amino acids and basic amino acids strongly influenced the P^I . The proteins exist as zwitterions or dipolar ions at isoelectric pH. They are electrically neutral with minimum solubility, maximum precipitability and least buffering capacity.

Protein	- P^I
Casein	- 4.6
Human albumin	- 4.7
Urease	- 5.0
Human globulin	- 6.4
Human Hb	- 6.7
Lysozyme	- 11.0

5. *Precipitation of proteins:* Polar groups of proteins attract H_2O molecules around them to form shell of hydration. Proteins precipitate when their charges are neutralized or water of hydration around them is removed.

Precipitation method: By salting out, iso-electric precipitation, by organic solvents where proteins are dehydrated, by heavy metal ions, by anionic/alkaloidal reagents.

6. *Denaturation:* Denaturation is due to loss of secondary, tertiary and quaternary structures of native proteins by a variety of agents. Here peptide bonds are not broken while, hydrogen bond, ionic bond, hydrophobic interactions, Van der Waals force are broken. This involves a change in physical, chemical and biological properties of proteins molecules. Denaturation is usually irreversible. Denaturing agents: are of 2 types.

- a. *Physical agents:*
 - i. Heat
 - ii. High pressure
 - iii. Vigorous shaking
 - iv. X-rays
 - v. UV rays.
- b. *Chemical agents:*
 - i. Urea at high concentration
 - ii. Salicylates
 - iii. Strong acids and alkalis
 - iv. Organic solvents (ether, alcohol, acetone)



Biological Importance of Denaturation

- Loss of biological activity

For example:

- Enzyme loses enzymatic activity.
- Antibodies cannot bind antigens.

- Decrease in solubility

c. Increase in digestibility (HCl of gastric juice also denatures protein).

- Renaturation:* Denatured proteins are sometimes regain original structure when the physical agent is removed.

For example: Immunoglobulins (Ig) get denatured when exposed to 8 molar (8M) urea. When urea is removed by dialysis, Ig gets its original structure.

- Heat coagulation:* When heated at P^I, some proteins denature irreversibly to produce thick floating coagulum. For example: Albumin is easily coagulated.

Sanger's Reagent

It was earlier used to identify N-terminal amino acids of peptides. It is fluoro dinitro benzene (FDNB).

Principle

FDNB with the N-terminal amino acid gives rise to Dinitrophenyl derivative (yellow colored) which is separated by chromatography and identified.

Sanger's reagent has limited use. It can hydrolyze the peptide chain to amino acids.

MULTIPLE CHOICE QUESTIONS

- Example of a quality dietary protein is:
 - Gliadin of wheat
 - Zein of corn
 - Gelatin of collagen
 - Casein of milk
- The primary structure of proteins is due to:
 - Hydrogen bond
 - Peptide bond
 - Hydrophobic bond
 - S-S- bond

3. Peptide bond formation between two amino acids involve:
A. Dehydration B. Hydration
C. Condensation D. Electrostatic bonds
4. Most abundant protein in human body is:
A. Collagen B. Keratin
C. Myosin D. Albumin
5. Sulfur containing essential amino acid is:
A. Cysteine B. Cystine
C. Methionine D. Glycine

ANSWERS

1.D 2.B 3.A 4.A 5.C

MOST LIKELY QUESTIONS

Long Essays

1. Classify amino acids with suitable examples.
2. Classify proteins with suitable examples.
3. Explain the various levels of structural organization of proteins.

Short Essays

4. Primary structure of proteins.
5. Structure of collagen.
6. Secondary structure of proteins.
7. Derived proteins.
8. Protein precipitation.
9. Isoelectric pH of proteins and amino acids. What is its significance?
10. Conjugated proteins.

Short Answers

11. Aromatic amino acids.
12. Acidic amino acids.
13. Isoelectric pH.
14. Peptide bond.
15. Bonds responsible for higher orders of protein structure.
16. Quaternary structure of proteins.
17. Sanger's reagent.

5.2: DIGESTION AND ABSORPTION OF PROTEINS

DIGESTION

Dietary proteins are of plant and animal origin, and average intake is 50 - 100 g/day. About 30 - 100 g/day of endogenous protein is derived from the digestive enzymes and worn out cells of the digestive tract. Cooking denatures dietary proteins and so, it is easily digested. Proteins are degraded by 'hydrolases' which specifically cleave the peptide bonds, hence known as 'Peptidases' or 'Proteases'. These are divided into 2 groups:

1. Endopeptidases: They attack the internal peptide bonds and release peptide fragments,
Example: Pepsin, Trypsin.
2. Exopeptidases: They act on the peptide bonds of terminal amino acids. These are subdivided into (a) Carboxypeptidases (b) Aminopeptidases. Based on the amino acids of the active site, proteases can be classified as:
 - i. Serine proteases containing serine at the active site.
 - ii. Zinc proteases containing Zn^{+2} at the active site
 - iii. Carboxyproteases (acid proteases) have dicarboxylic amino acid at the active site.
 - iv. Thiolproteases contain cysteine at the active site. It is not found in human beings.

PROENZYMES

Some strong proteases are secreted as inactive proenzymes or zymogens. On arrival at the site of activity, one or more specific peptide bonds are hydrolyzed in the proenzyme either enzymatically or by pH changes. This yields the active enzymes and some inactive peptides called masking substances. For example: Inactive trypsinogen of the pancreatic juice is hydrolyzed to active trypsin in the intestinal lumen by enterokinase of intestinal juice when the intestinal contents have pH around 5.5.

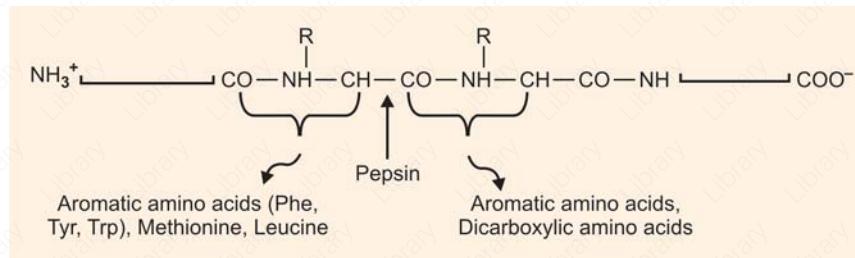
Stages of Protein Digestion

1. Digestion by gastric juice
 2. Digestion by pancreatic juice
 3. Digestion by intestinal juice
1. *Digestion by gastric juice:* Protein digestion does not take place in the oral cavity. In stomach, HCl, pepsin and rennin help in digestion of proteins. HCl along with intrinsic factor is secreted from the parietal cells of the gastric gland.

Rennin (Chymosin) - It is found in infants and absent in stomach of adults. It prevents rapid passage of milk from the stomach. It changes casein of milk irreversibly to a para-casein in presence of calcium. Para-casein is acted upon by pepsin.

Pepsin - Chief cells of gastric gland secrete pepsin. It is secreted in an inactive form known as pepsinogen. This gets activated initially by HCl by the removal of 44 amino acid residues from the N-terminal end. Later, pepsin itself activates remaining pepsinogen (autoactivation). Pepsin is active in the pH range of 1 - 3. Pepsin is an endopeptidase - carboxy protease (acid protease) with 2 aspartic acid residues in the active site. Proteoses and peptones are produced as a result of action of pepsin on proteins.

Site of Action of Pepsin



Functions of HCl

1. It activates pepsinogen to pepsin by removal of 44 amino acids from the N-terminal end.
2. It provides optimum pH (1 to 3) for pepsin action.
3. It destroys most organisms entering the GI tract due to low pH.
4. It causes hydrolysis of sucrose to glucose and fructose.
5. It causes denaturation of dietary proteins and acid metaproteins are formed.
6. It provides optimum acidic pH for the intrinsic factor - Vit B₁₂ complex absorption.
7. HCl releases Fe⁺³ from iron complexes of the diet.

Abnormal Conditions of Hydrochloric Acid Secretion

Achlorhydria: It is the absence of HCl in the gastric juice. It is seen in gastric carcinoma, atrophic gastritis, wasting diseases of stomach, chronic gastric ulcer leading to gastric atrophy. Achlorhydria prevents peptic digestion, reduces iron and vitamin absorption, delays emptying of stomach and leads to microbial fermentation in stomach resulting in flatulence and diarrhea.

Achylia gastrica: In this condition, gastric juice lacks in both acid and enzymes. It is observed in pernicious anemia, advanced gastric carcinoma, chronic gastric ulcer and gastritis. Failure of peptic digestion, retention and fermentation of food in the stomach, result in flatulence, diarrhea and megaloblastic anemia.

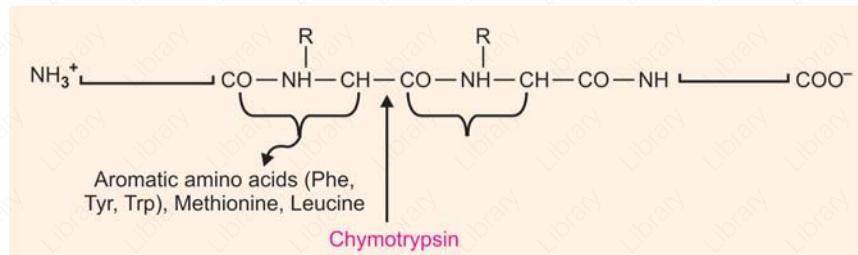
Hyperchlorhydria: This is an abnormally high secretion of gastric HCl, raising the free acidity of gastric contents and producing postprandial heart burns. It is found in gastric carcinoma, duodenal ulcer.

2. *Digestion by pancreatic juice:* Pancreatic juice contains 5 proteases. They are trypsin, chymotrypsin, elastase, carboxypeptidase A and B. Pepsin gets inactivated due to increased pH.

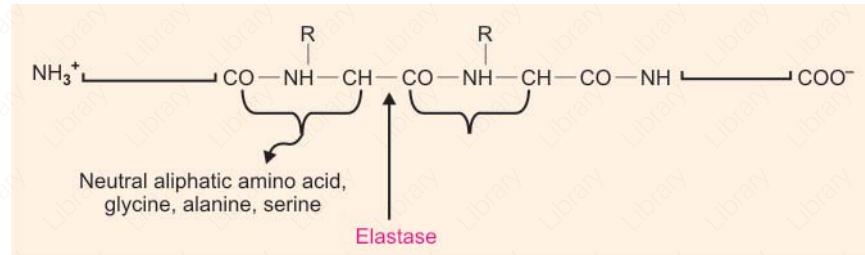
Trypsin - Secreted in an inactive trypsinogen form. Along with trypsin, there is trypsin inhibitor protein. At the site of action (i.e., in duodenum and jejunum), there is activation of trypsinogen to trypsin by enterokinase (enteropeptidase) enzyme of the intestinal juice. During activation, 6 amino acids from N - terminal end are removed from trypsinogen to form active trypsin. Trypsin is an endopeptidase. It belongs to serine proteases. It has autoactivation capacity. It is active in pH around 8.0. It helps in the activation of other pro-enzymes like chymotrypsinogen, proelastase, pro-carboxypeptidase A and B and prophospholipase A₂.

Chymotrypsin - It is secreted as inactive chymotrypsinogen and activated by trypsin. It is an endopeptidase, serine protease, active in the pH range of 7-8. Chymotrypsin action on protein leads to peptide formation.

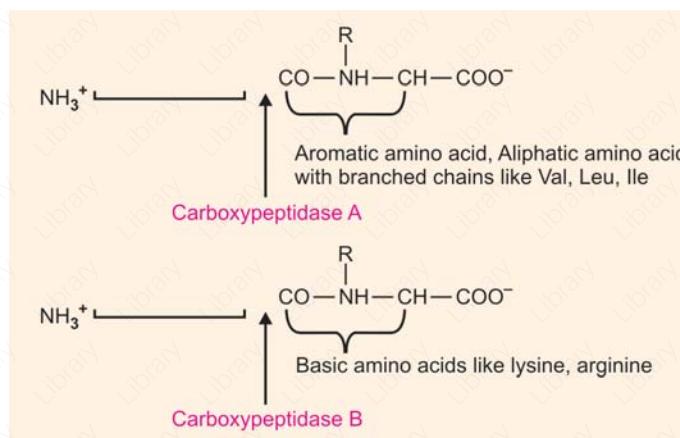
Site of Action



Elastase - It is secreted as inactive proelastase activated by trypsin. Elastase is an endopeptidase and serine protease. It acts mainly on elastin.

Site of Action

Carboxypeptidase A and B: They are secreted as inactive procarboxypeptidases and activated by trypsin. Both carboxypeptidases are Zinc proteases, exopeptidases, acting on C-terminal peptide bond. They are active in the pH range of 7.5 - 8.

Site of Action

3. **Digestion by intestinal juice:** Proteases in intestinal juice are, enteropeptidase (enterokinase), aminopeptidases, dipeptidases.

Enteropeptidase: It is an endopeptidase, a glycoprotein and initially anchored to the membrane of the luminal surface of the enterocytes. By the action of bile salts, it gets released into the duodenal lumen.



Aminopeptidases: They are glycoproteins anchored to the intestinal mucosal membrane. They act on amino terminus, release one amino acid at a time from the amino terminal end. Thus, they act as exopeptidases and cannot act on the dipeptides.

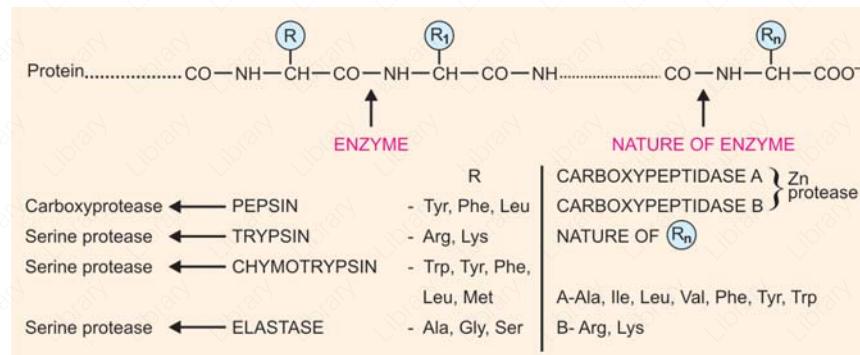
Example: Leucine aminopeptidase, proline aminopeptidase.

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Dipeptidases: Glycylglycine dipeptidase hydrolyzes dipeptide into two molecules of amino acids to complete the digestion of proteins. Dipeptidases are located mainly in the enterocyte cytoplasm and hence digestion is intracellular after the absorption of dipeptides.

Site of digestion	Enzymes	Type of enzyme	Proenzyme	Products formed
Stomach (gastric juice)	Pepsin Rennin	Carboxyprotease	Pepsinogen Prorennin	Proteoses, peptones and polypeptides
Pancreatic juice	Trypsin Chymotrypsin Proelastase Elastase Carboxy-peptidase A and B	Serine proteases Zinc protease	Trypsinogen Chymotrypsinogen Procarboxy peptidase A and B	Proteoses, peptones and polypeptides
Intestinal juice	Enterokinase Amino-peptidases Dipeptidases	Glycoproteins	Activates trypsinogen to trypsin	Aminopeptidases act from <i>amino terminal end</i> and remove one amino acid at a time from that end

Digestion of Proteins - Specificity of Enzymes— Cleavages of Peptide Bonds



Abnormalities Related to Digestion of Absorption of Proteins

- Hartnup's disease (Neutral amino aciduria):** It is characterized by the inability of intestinal and renal epithelial cells to absorb neutral amino acids. Tryptophan absorption is affected. Typical symptoms of pellagra are observed, such as dementia, diarrhea, dermatitis (3 D), due to impairment in conversion of tryptophan to NAD⁺ and NADP⁺ (Coenzymes of niacin).

2. *Peptic ulcer*: It is the collective name for gastric and duodenal ulcers. Ulceration occurs due to the autodigestion of mucosa by gastric secretions (Pepsin and HCl). An imbalance between the rate of gastric secretion and degree of protection by mucosal barrier and the neutralization of gastric HCl by duodenal HCO_3^- leads to peptic ulcer.
3. *Pancreatitis*: Inflammation of the pancreas is known as pancreatitis. Acute pancreatitis is caused by autodigestion of pancreas which is a life threatening disorder. Autodigestion is due to unusual conversion of proenzymes into active enzymes by trypsin. Normally this is prevented by trypsin inhibitor. Diagnosis is by measuring serum amylase (highly elevated). Chronic pancreatitis is due to excessive consumption of alcohol over a long period.

Absorption

Some dipeptides and L-oligopeptides are either first absorbed into enterocytes and hydrolyzed by intracellular peptidases into amino acid or first hydrolyzed by peptidases of microvillus membrane into amino acids which are then absorbed.

Mister cycle (Gamma glutamyl cycle): It is for the rapid transport of certain amino acids namely cysteine and glutamine.

Absorption of Amino Acids

It occurs mainly in the distal jejunum and ileum of small intestine. It is an energy requiring process. Rate of absorption of an L- amino acid is higher than that of its D - isomer. There are six different transport systems:

1. For short chain neutral amino acids (Ala, Ser, Thr)
2. For long chain, neutral and aromatic amino acids (Phe, Tyr, Ile, Leu, Val, Met)
3. For basic amino acids (Lys, Arg, Cystine)
4. For imino acids and glycine (Proline and Hydroxyproline)
5. For acidic amino acids (Asp, Glu)
6. For beta amino acids (β - Alanine and also taurine)

These transport systems are carrier mediated and ATP, sodium dependent symport systems.

MECHANISM

Absorption of L - amino acids is a Na^+ dependent active transport and requires ATP and specific transport proteins (Six of them are present). Both, L- amino acid and Na^+ ion attach to the carrier protein which is present on the mucosal surface of the microvillus membrane and form L-amino acid- carrier protein - Na^+ complex. The complex passes into the inner surface of the membrane and dissociates to liberate free amino acid and Na^+ . Carrier protein returns to the brush border while the amino acid is transferred by facilitated diffusion into the interstitial fluid and then to portal blood and enters the liver (Fig. 5.2.1).

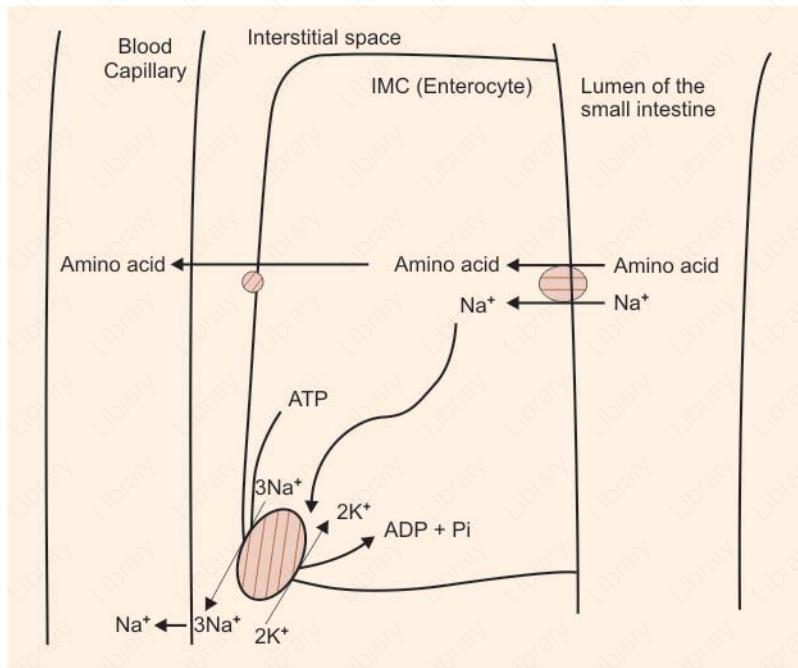


Fig. 5.2.1: Transport of amino acids across intestinal epithelium

On the other hand, Na^+ is actively pumped out of the cell membrane by the Na^+ pump. At the same time, ATP is hydrolyzed by ATPase to release energy required for this process.

MULTIPLE CHOICE QUESTIONS

1. Pancreatic proteolytic enzyme is:
 - A. Chymotrypsin
 - B. Amylase
 - C. Pepsin
 - D. Phospholipase
2. The disease characterized by impairment in the absorption of neutral amino acids is:
 - A. Steatorrhea
 - B. Pancreatitis
 - C. Hartnup's disease
 - D. Beri-beri
3. The enzyme that converts trypsinogen to trypsin is:
 - A. Pepsin
 - B. Elastase
 - C. Chymotrypsin
 - D. Enteropeptidase

ANSWERS

1. A 2. C 3. D

MOST LIKELY QUESTIONS

1. Digestion of proteins.
2. Name any four protein digesting enzymes.
3. How are dietary proteins digested in the human system?
4. Digestion and absorption of proteins.

5.3: AMINO ACID METABOLISM

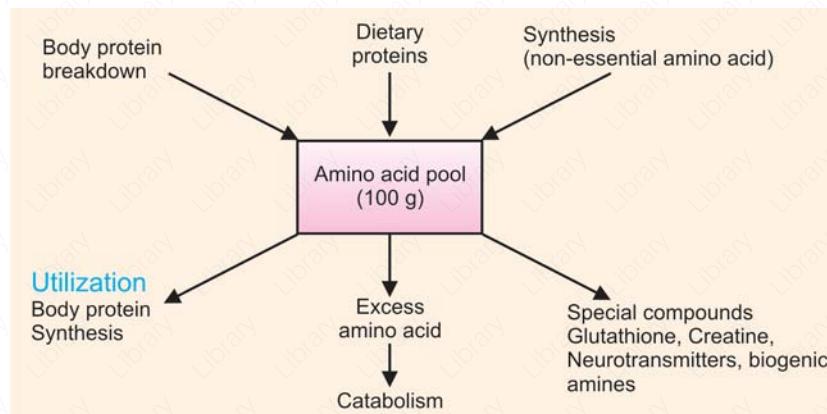
Most proteins in the body are constantly synthesized and degraded. The rate of synthesis is sufficient to replace the protein that is degraded. This facilitates the total amount of proteins in the body to remain nearly the same. This process is called protein turnover. It involves approximately 1-2 percent of body proteins per day. Predominantly protein turnover results from degradation of muscle proteins to amino acid. About 80 percent of the amino acid so liberated are recycled while the rest 20 percent are catabolized.

Nitrogen Balance

In an adult healthy person maintaining constant weight, the amount of intake of nitrogen in food is balanced by excretion of an equal amount of nitrogen in urine and feces. The person is said to be in nitrogen balance.

In the growing period and also during convalescence from illness when the person is putting on weight, the nitrogen intake will be more than the output, since some of the nitrogen is retained as tissue protein. This known as positive nitrogen balance. The reverse occurs in old age and during illness resulting in a negative nitrogen balance.

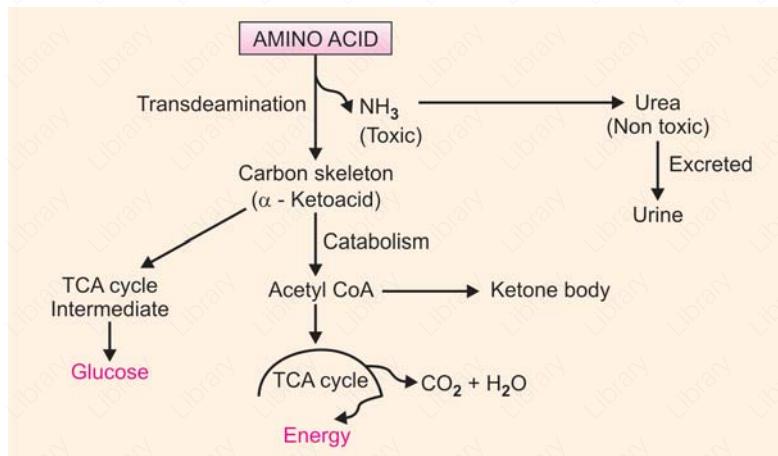
Sources



Note: Excess amino acids are not stored. (Philosophy is, use it or lose it!)

Catabolism of Amino Acids

Nitrogen catabolism consists of the removal of α -amino groups from amino acids as ammonia by transamination and deamination, and the conversion of the released ammonia to excretory end products such as urea and uric acid.



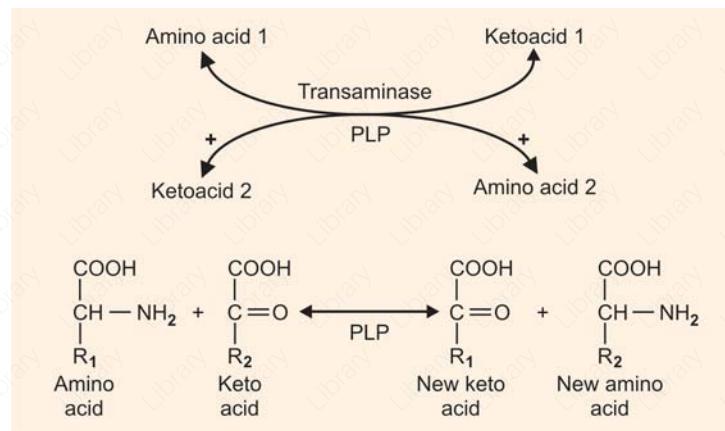
During the catabolism of amino acids, ammonia (NH_3) is formed in the body. In this way amino group of amino acids are removed.

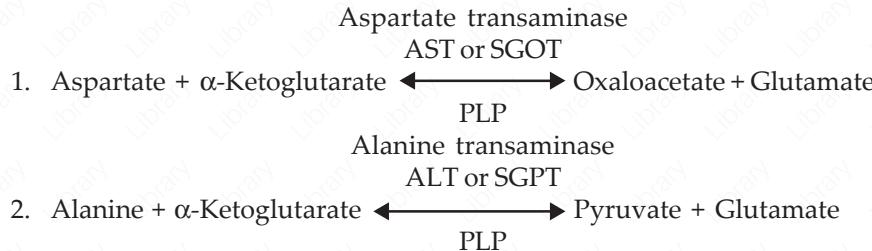
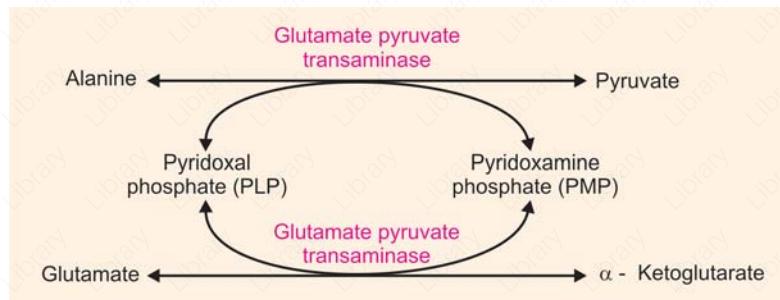
Transdeamination

It is the process of transamination of amino acids to form glutamate which is then deaminated in liver to release NH_3 .

Transamination

Here transfer of α - amino group from an amino acid to a ketoacid forming a new amino acid and a new ketoacid takes place. It is reversible.



Examples**Mechanism of Transamination**

PLP (coenzyme) is covalently linked to transaminase.

Significance of transamination role in both-synthesis and catabolism of amino acid:

1. Transamination is the first step in catabolism of amino acids.
2. Transamination causes synthesis of non-essential amino acids.

Example: Glutamate + Pyruvate $\xrightarrow{\text{ALT}}$ Alanine + α -KG

3. Transamination produces substrates for gluconeogenesis.

Example: Pyruvate, Oxaloacetate

4. Transamination maintains balance of different amino acids.

Clinical Importance of Transaminases

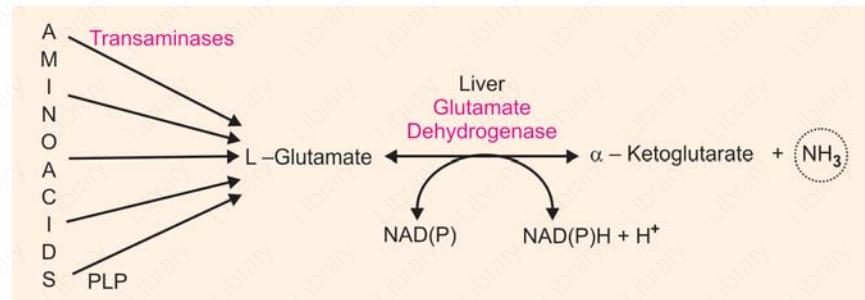
Enzyme	Disease in which serum level is increased
1. Aspartate transaminase AST or SGOT	Myocardial infarction
2. Alanine transaminase ALT or SGPT	Liver cell damage <i>Example:</i> Hepatitis

Deamination

It is a process of removal of amino group from an amino acid to form NH₃.

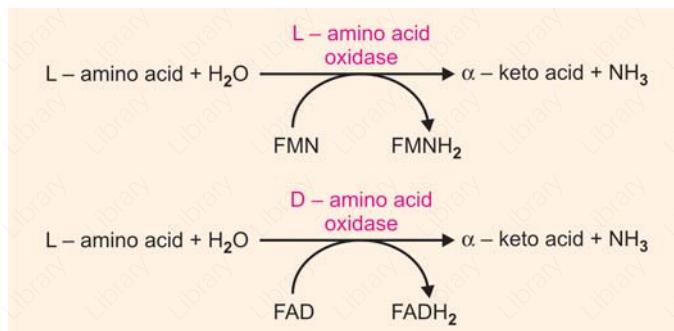
A. Oxidative Deamination

- a. *By Glutamate dehydrogenase:* Liberation of NH₃ from amino group of amino acid coupled with oxidation.



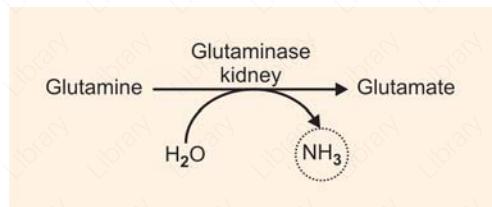
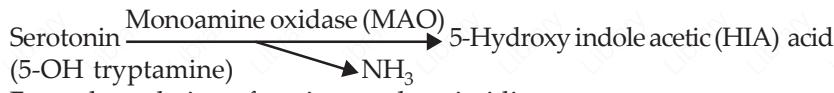
Amino acids transfer amino group to α - Ketoglutarate. Therefore, glutamate acts as a 'collection center' for amino groups from other amino acids. Glutamate undergoes oxidative deamination.

- b. By α - amino acid oxidases

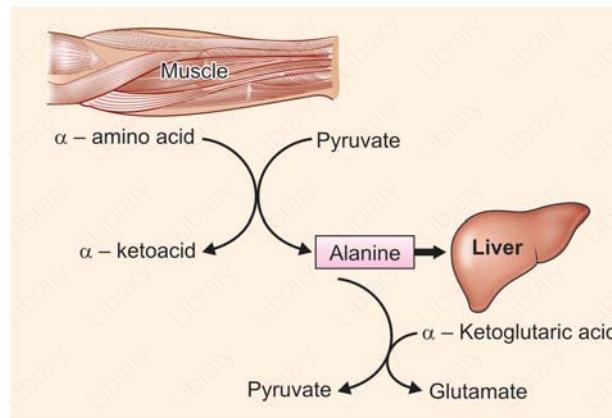
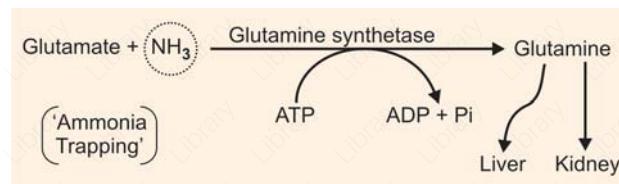


B. Non-Oxidative Deamination

1. Serine $\xrightarrow{\text{dehydratase}}$ Pyruvate + $\boxed{\text{NH}_3}$
 H_2O
 2. Threonine $\xrightarrow{\text{dehydratase}}$ α - keto butyric acid + $\boxed{\text{NH}_3}$
 H_2O
 3. Histidine $\xrightarrow{\text{Histidase}}$ Urocanic acid + $\boxed{\text{NH}_3}$

*Other Ways of NH₃ formation*1. *Hydrolysis of Glutamine*2. *From biogenic amines*

3. From degradation of purines and pyrimidines

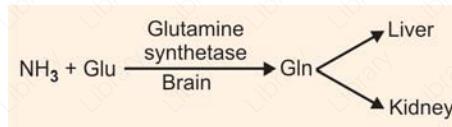
4. *Hydrolysis of Urea:***Transport Forms of NH₃**1. **As Alanine** (from muscle)2. **As Glutamine** (From brain, muscle)

Detoxification of NH₃ (Fate of NH₃)

Even slight increase in blood NH₃ is toxic to central nervous system (CNS).

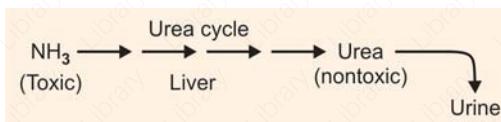
First line of defense against toxicity

'Ammonia trapping', i.e. glutamine formation



In liver and kidney, reaction is reversed by glutaminase to release NH₃.

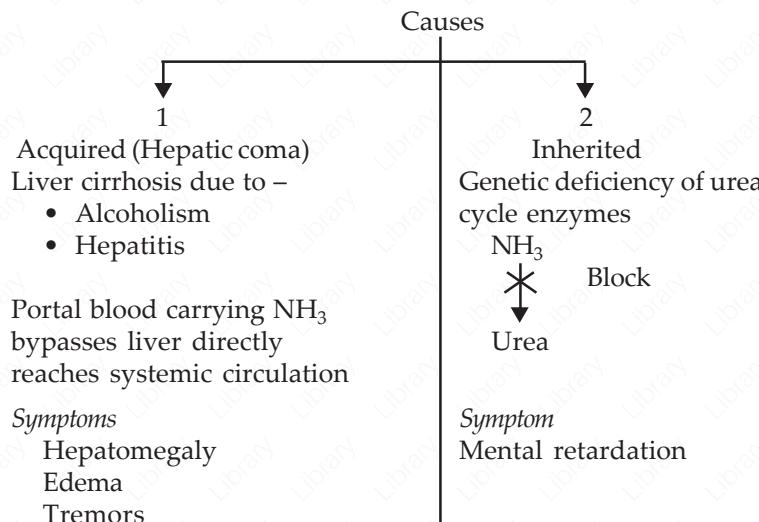
Second line of defense



Hyperammonemia (NH₃ toxicity)

Increased NH₃ level in blood is toxic to central nervous system.

Symptoms: Tremors, slurring of speech, blurring of vision, coma to death.



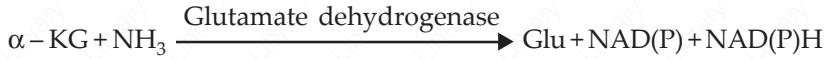
Mechanism of NH₃ toxicity: When blood NH₃ increases

- Glutamate gets depleted



Glutamate is a neurotransmitter itself and it is also the precursor of GABA.

2. α – ketoglutarate gets depleted



α - Ketoglutarate is a TCA cycle intermediate required for energy production and its depleted levels impair the TCA cycle. Therefore, decrease in energy level in brain. The toxic effects of ammonia on brain are therefore due to impairment in ATP formation.

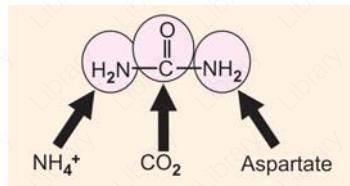
UREA CYCLE (ORNITHINE CYCLE)

The only organ where urea synthesis occurs is liver. Urea is the major excretory product in humans, accounting for an average of 86 percent of nitrogen eliminated. The rest of the nitrogen is eliminated as follows: 4.5 percent by creatinine, 2.8 percent as ammonium ions, 1.7 percent as uric acid and 5.0 percent as other compounds. About 30 g urea is excreted per day; the amount excreted is dependent on protein intake. Higher the protein intake more is the urea synthesis and excretion.

Site: Liver

Subcellular location: Partly mitochondrial (steps 1,2)
 Partly cytosolic (steps 3,4,5)
 (It is compartmentalized)

Sources of Atoms in Urea

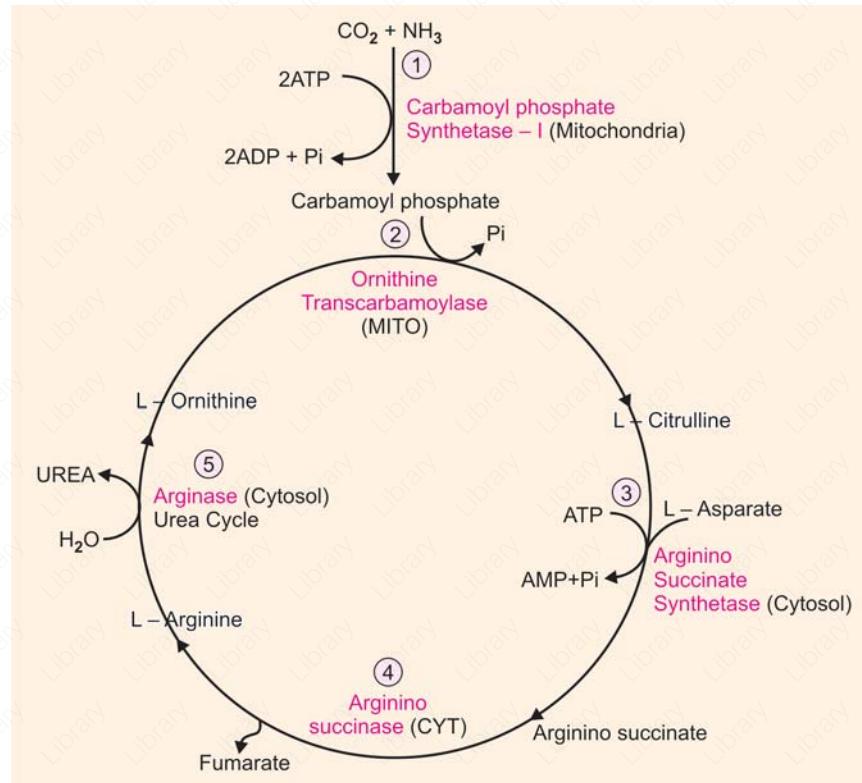


Significance: Detoxification of NH_3

Even slight increase in blood NH_3 is toxic to central nervous system. Ureotelic animals (humans, mammals) convert toxic NH_3 to nontoxic urea, water-soluble and excreted by kidneys.

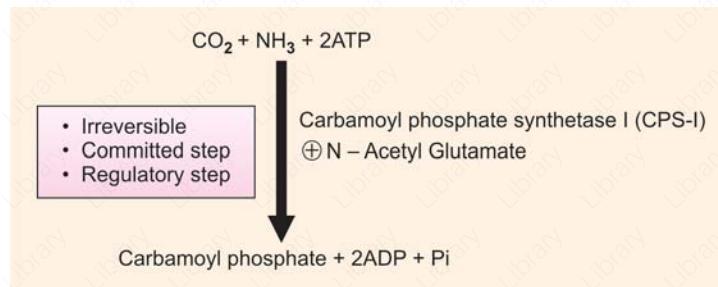
Biosynthesis of Urea

Urea cycle: Synonyms- Ornithine cycle, Kreb's-Hanseleit cycle, detoxification of NH_3 , disposal of NH_3 .

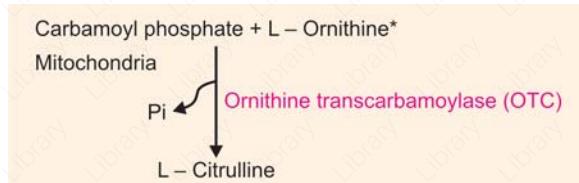


Reactions (steps)

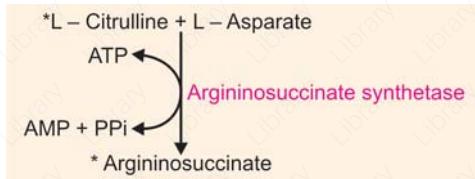
1. Synthesis of Carbamoyl Phosphate



2. Formation of L-Citrulline

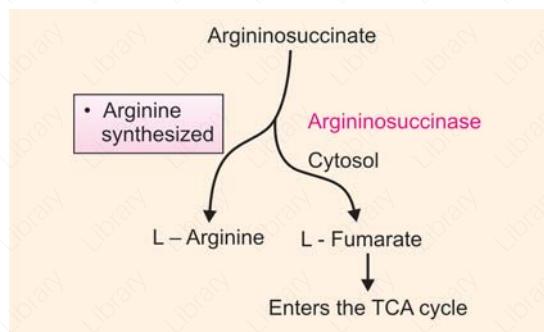


3. Synthesis of Argininosuccinate

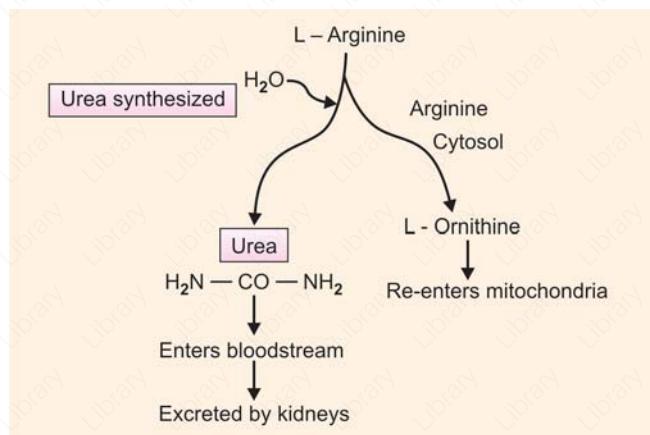


Note: Ornithine, citrulline, argininosuccinate are amino acids not found in proteins.

4. Cleavage of Argininosuccinate



5. Hydrolysis of L - Arginine



Normal blood urea = 20 – 40 mg/dL

Urinary urea = 15 – 30 g/day

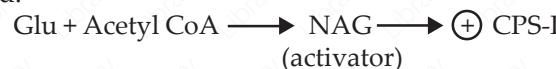
Blood urea increases in renal diseases

Blood urea nitrogen (BUN) = 7 – 8 mg/dL.

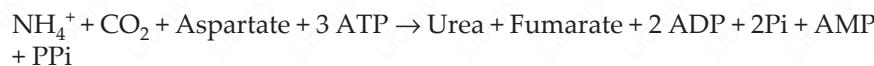
Regulation (Allosteric)

Rate-limiting step : Formation of carbamoyl phosphate
 Key regulatory enzyme : Carbamoyl phosphate synthetase I (CPS I)
 Allosteric activator : N - Acetyl Glutamate (NAG)

Increase in dietary protein and increased protein breakdown leads to increase in free amino acids. This decreases transamination and increases glutamic acid.



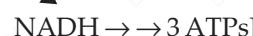
Overall Equation



Energetics

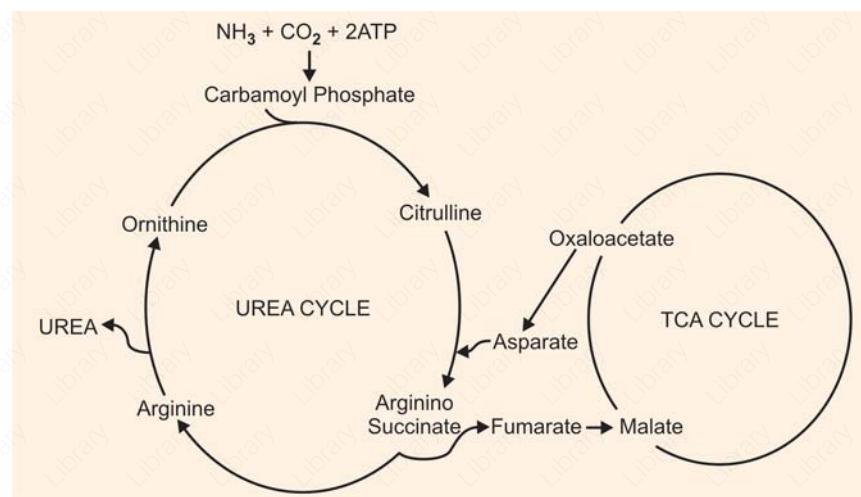
1. Synthesis of Carbamoyl phosphate \rightarrow 2 ATPs are consumed
2. Synthesis of Argininosuccinate \rightarrow 1 ATP is broken down to AMP which is equivalent to 2 high energy bonds \rightarrow 2 ATP consumed

Total expenditure of energy = 2 + 2 = 4 ATPs



Therefore, net expenditure = 4 ATP - 3 ATP = 1ATP

RELATION BETWEEN UREA AND TCA CYCLES "UREA BICYCLE"



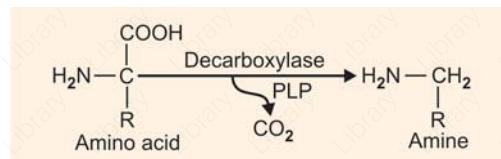
Disorders of Urea Cycle (Inborn errors)

Disorder	Defective enzyme	↑ Blood level of	Symptoms
Hyperammonemia Type I	Carbamoyl phosphate synthetase I	↑ NH ₃	• NH ₃ toxicity
Hyperammonemia Type II (X-linked)	Ornithine transcarbamoylase	↑ NH ₃	• Vomiting
Citrullinemia	Argininosuccinate synthetase	↑ Glutamine	• Irritability
Argininosuccinic aciduria	Argininosuccinase	↑ Citrulline	• Lethargy
Hyper argininemia	Arginase	↑ Arginino succinate	• Ataxia
		↑ Arginine	• Mental retardation

Management

- Intake of low protein diet
- Intake of frequent small meals (to avoid sudden increase in blood NH₃)
- Administration of Nabenzoate, L - Arg (to increase NH₃ excretion)

Decarboxylation of Amino Acids

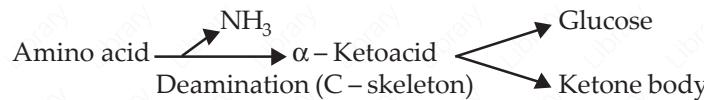


Decarboxylation of amino acids produces many 'Biogenic amines' with important functions.

Examples:

- Glutamate $\xrightarrow{\text{Decarboxylase}}$ γ - aminobutyric acid (GABA) Inhibitory neurotransmitter
- 5 - OH Tryptophan $\xrightarrow{\text{Decarboxylase}}$ Serotonin (Neurotransmitter, vasoconstrictor)
- Histidine $\xrightarrow{\text{Decarboxylase}}$ Histamine (Activates gastric secretion and mediates allergic response)
- Phenylalanine $\xrightarrow{\text{Decarboxylase}}$ Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ L-DOPA $\xrightarrow{\text{L-DOPA decarboxylase}}$ Dopamine (Neurotransmitter)

Catabolism of Carbon Skeleton of Amino Acids



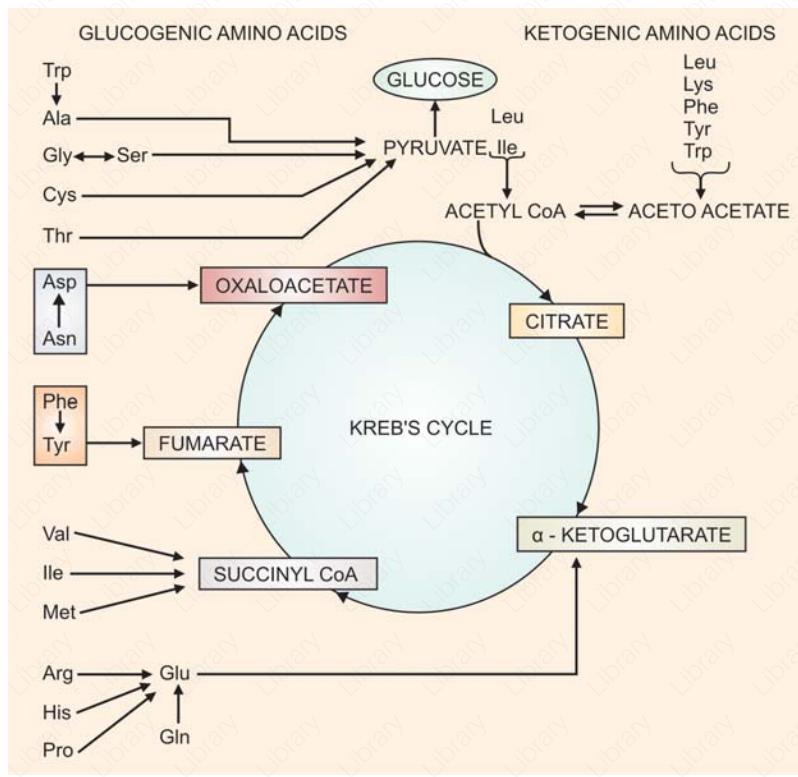
Glucogenic amino acids: They are catabolized to α -ketoglutarate, pyruvate, acetyl CoA, fumarate or succinyl CoA.

Example: Alanine $\xrightarrow{\text{Transamination}}$ Pyruvate $\xrightarrow{\text{Gluconeogenesis}}$ Glucose

Ketogenic amino acids: They are catabolized to acetyl CoA or acetoacetate (a ketone body).

Example: Leucine

Ketogenic	Both Glucogenic and Ketogenic	Glucogenic
Leucine	Phynylalanine Tyrosine Tryptophan Isoleucine Lysine	Glycine Serine Alanine Glutamic acid Aspartic acid Methionine and remaining 8 amino acids



Metabolic fates of carbon skeletons of amino acids

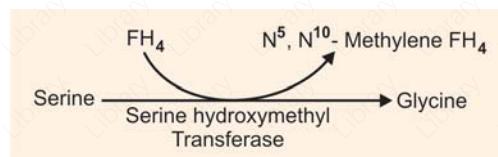
GLYCINE METABOLISM

Glycine is the simplest, optically inactive, neutral non-essential, glucogenic amino acid.

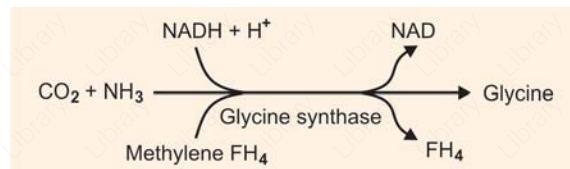
Metabolism of glycine includes its synthesis, catabolism, conversion to biologically important compounds and inborn errors.

A. Synthesis of Glycine

1. From Serine:



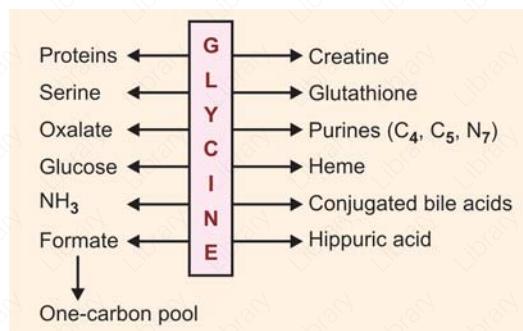
2. From CO_2 , NH_3 and Methylene FH_4



3. From Threonine:



B. Metabolic Fates of Glycine

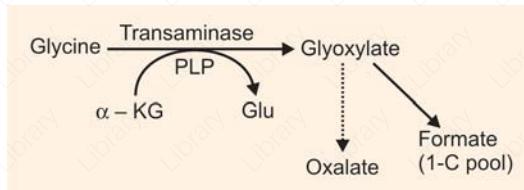


I. Protein Synthesis

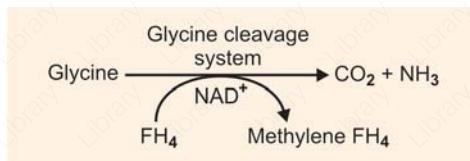
Example: Collagen is rich in Glycine (every 3rd residue is glycine)

II. Catabolism

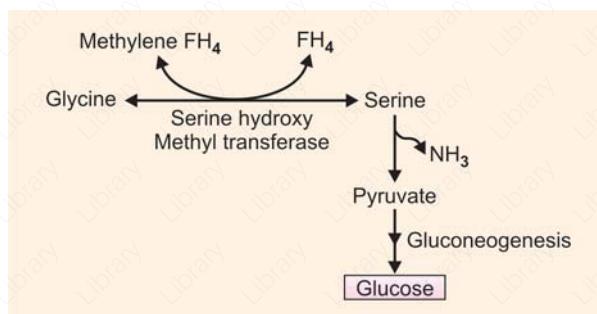
- Transamination to form glyoxylate



- Cleavage to CO_2 and NH_3



- Conversion to serine

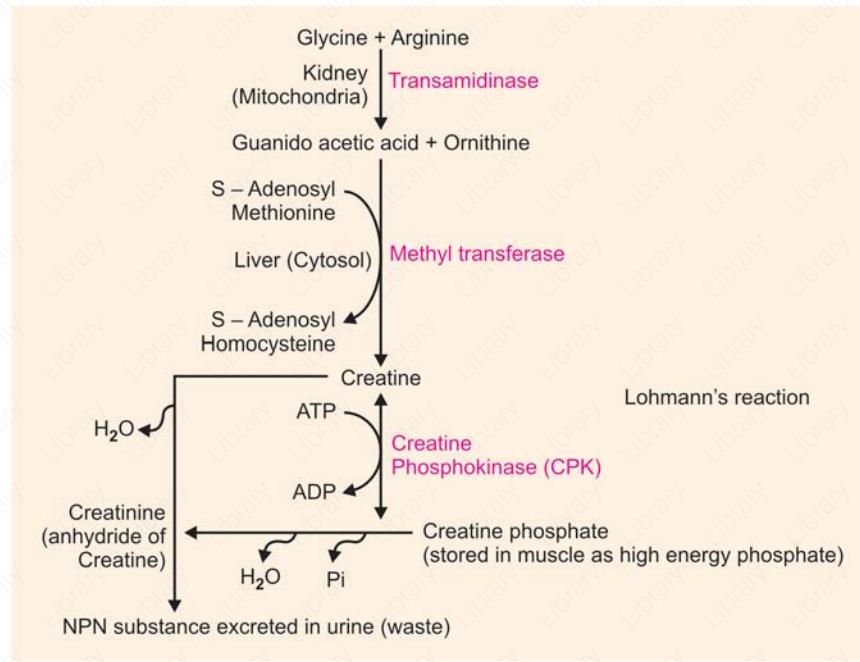


III. Conversion to Biologically Important Compounds

- Creatine
- Glutathione
- Purines
- Heme
- Hippuric acid
- Conjugated bile acids.

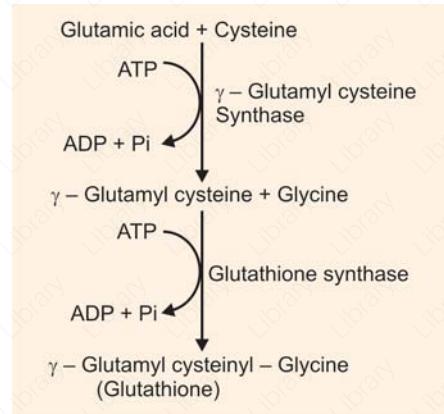
- Creatine:* It is found in muscle as creatine phosphate. It is a reserve store of energy for contracting muscle. Its synthesis requires 3 amino acids—Glycine, arginine and methionine (GAM).

Steps

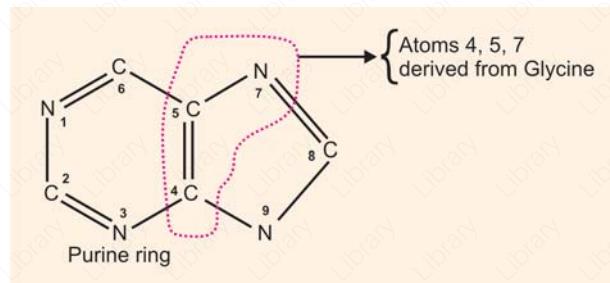


2. *Glutathione:* It is a tripeptide made of 3 amino acids - Glutamic acid, cysteine and glycine.

Synthesis

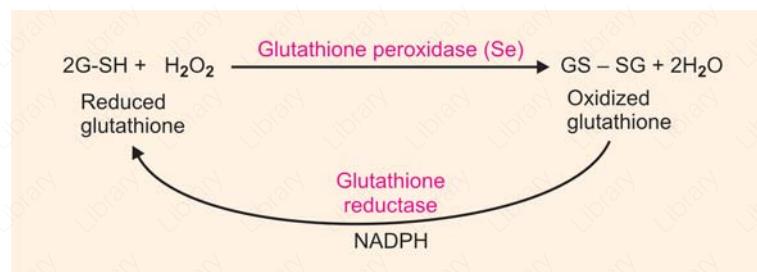


3. *Purines:* The entire glycine molecule is incorporated during formation of purine ring.

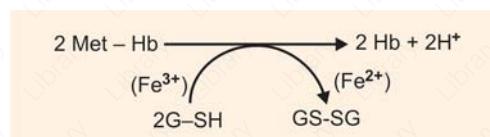


Functions of Glutathione G - SH

- i. *Acts as a reducing agent:* Role of glutathione in RBC
 - a. It destroys harmful peroxides and prevents damage



Otherwise, H_2O_2 which is free radial damages RBC.
b. It reduces Met Hb to Hb

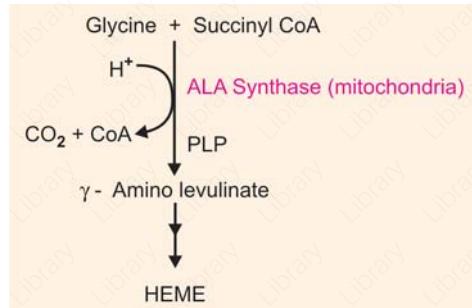


Therefore, GSH is essential to maintain integrity of RBC structure.

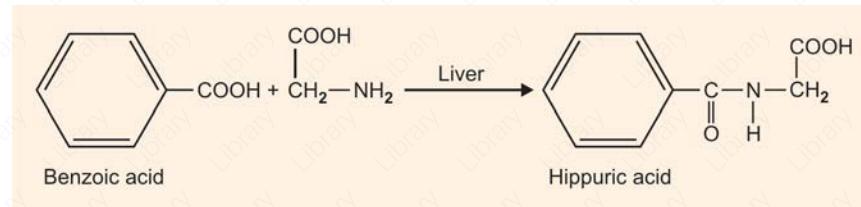
Decreased GSH increases hemolysis.

- Decreased GGT, increases hemolysis.

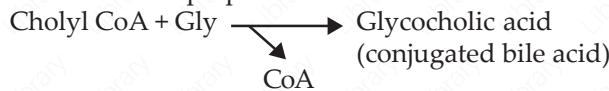
 - ii. *Coenzyme role of glutathione*
 - 1. It acts as a coenzyme for prostaglandin synthetase
 - 2. It inactivates Insulin by causing separation of A and B chains.
 - 3. It is required for transport and absorption of amino acid in GIT by Miester cycle.
 - 4. It detoxifies heavy metals, insecticides by transferring cysteinyl group.
 - 4. *Heme:* Heme is a prosthetic group of hemoglobin(Hb), myoglobin (Mb), cytochromes.

Synthesis:

5. Detoxification of Benzoic acid and other compounds, by conjugation

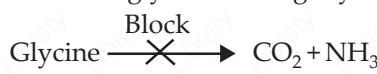


6. *Bile acids:* Glycine is required for conjugation with bile acids to make them more amphipathic.



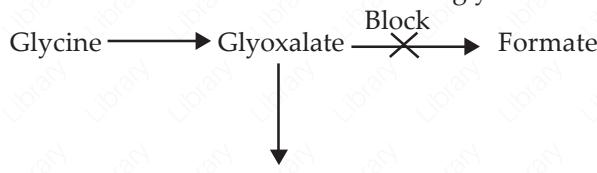
IV. Inborn Errors (Disorders)

1. Non-ketotic hyperglycinemia:
Defective glycine cleavage system



This increases blood glycine (inhibitory neurotransmitter) and that leads to severe mental retardation (even fatal). It is called primary hyperoxaluria.

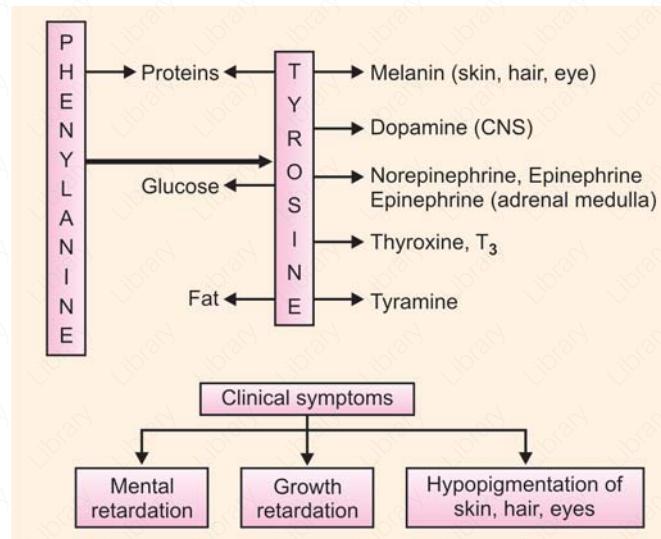
2. Increased formation of oxalates from glycine



Increase in oxalates leads to renal calculi and renal failure

3. *Glycinuria* results due to the defect in renal transporter for glycine. Decreased renal reabsorption of glycine leads to increased glycine in urine.

Metabolic Fates of Phenyl Alanine and Tyrosine



1. Seizures
2. Hyperactivity
3. Microcephaly
4. Delayed milestones

Reasons

Decreased neurotransmitters serotonin, catecholamines leads to decrease in melanin pigments.

Biochemical changes: Increase in blood phenylalanine ends up in the excretion of phenylketones in urine.

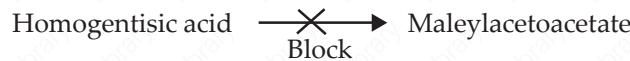
Diagnostic test in serum: Guthrie's screening test - *Bacillus subtilis*, needs phenylalanine to grow. In normal people phenylalanine is < 2 mg/dl. In patients suffering from phenylketonuria the level is > 20 mg/dl.

Diagnostic Tests in Urine

1. Phenylpyruvate in urine can be detected by ferric chloride test. It is not a specific test. A green color is obtained.
2. Paper chromatography

Management: Early detection in newborns is of supreme importance. Dietary phenylalanine should be decreased till age 5 and increase in dietary tyrosine should be encouraged.

Alkaptonuria results due to defective tyrosine catabolism. It is an autosomal recessive disorder, prevalence being 1 in 25,000. Deficiency or absence of homogentisate oxidase leads to alkapttonuria.



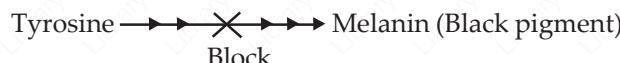
Homogentisate accumulates in tissues and blood and is excreted into urine. Homogentisate gets oxidized to benzoquinone acetate which undergoes polymerization to produce a black pigment called alkaptton, which accumulates in cartilage, bone and joints. Discoloration, pain in joints and various organs result in a condition known as ochronosis.

Symptoms: Blackening of urine on exposure to air (oxidation of homogentisic acid) and arthritis (in middle age).

Diagnosis: In urine, Benedict's test and ferric chloride tests are positive.

Treatment: Phenylalanine restricted diet is consumed.

Albinism: It is an autosomal recessive disorder leading to defective melanin synthesis. The most common cause of albinism is a defect in tyrosinase the enzyme most responsible for the synthesis of melanin.



Clinical signs: Hypopigmentation of skin, hair, eyes and photophobia (Intolerance to light). The person has increased sensitivity to sunlight and increased susceptibility to skin cancers.

Garrod's tetrad: Alkaptonuria, albinism, pentosuria and cystinuria.

Tyrosinemia: It is a condition where in blood tyrosine level is increased.

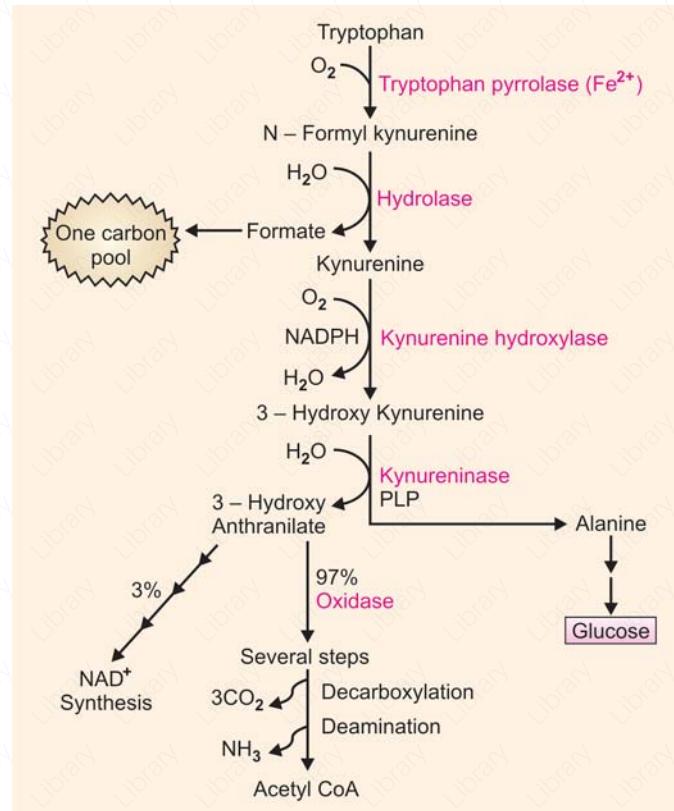
Defects in Tyrosine Catabolism

1. *Tyrosinemia:* Type I (tyrosinosis). Deficiency of fumaryl acetoacetate hydrolase causes tyrosinemia. In acute cases, clinical symptoms are vomiting, cabbage -like odour, liver failure. In chronic cases, cirrhosis, nephropathy and mild mental retardation are observed.
2. *Tyrosinemia:* Type II (Richner - Hanhart syndrome). Deficiency of tyrosine transaminase causes tyrosinemia type II and the clinical signs are skin and eye lesions and mental retardation.

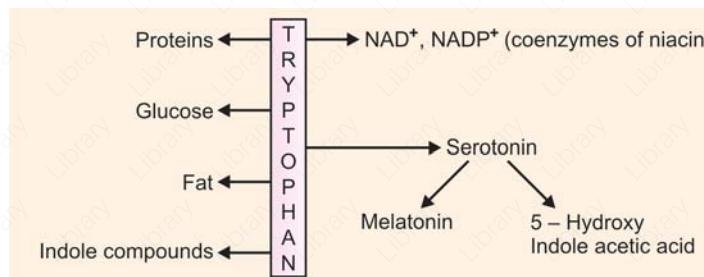
Tryptophan Metabolism

Tryptophan is an aromatic, essential amino acid with indole ring. It is partly glucogenic and partly ketogenic. Tryptophan gets converted to biologically important compounds.

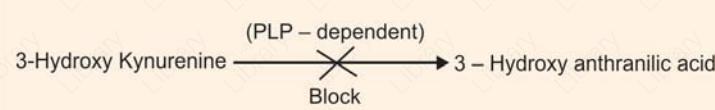
I. Catabolism: Kynurenine Pathway



Metabolic Fates of Tryptophan

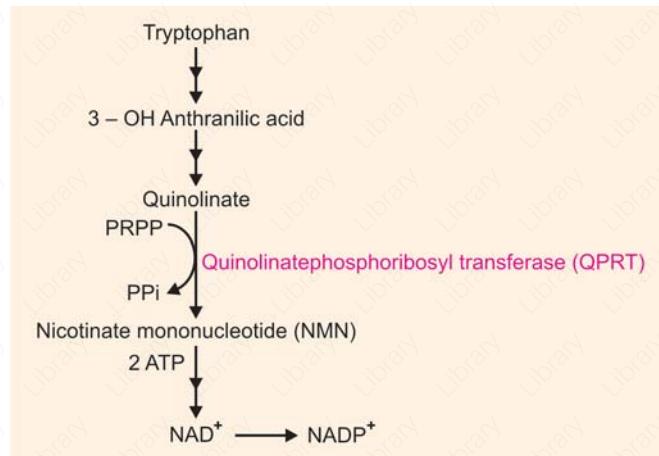


In Vitamin B₆ deficiency, 3-hydroxy kynurene gets accumulated and forms xanthurenic acid, which is excreted in urine and pellagra symptoms are observed.

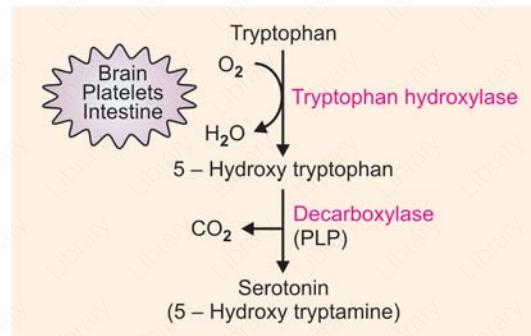


II. Conversion to Biologically Important Compounds

- Niacin (Vitamin B₃) quinolinate pathway: 60 mg of tryptophan produce 1 mg of niacin.



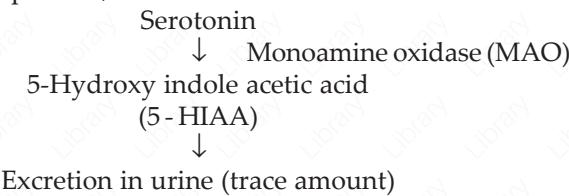
- Serotonin synthesis: It is synthesized in brain, platelets and intestine.



Functions

Serotonin is a neurotransmitter. It controls the behavioral patterns, sleep, blood pressure and body temperature. It is a powerful vasoconstrictor (increases BP) and results in smooth muscle contraction. It is a stimulant of the central nervous system.

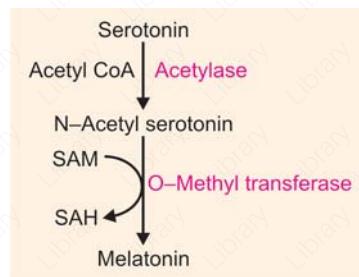
Note: Drugs affecting serotonin actions are used to treat depression, schizophrenia, etc.



Disorder: In carcinoid tumor which is a malignant GI tumor, serotonin production is increased. There is an increased excretion of 5 - HIAA in urine (diagnosis). Symptoms are hypertension, sweating, pellagra, etc.

3. Melatonin is a pineal gland hormone

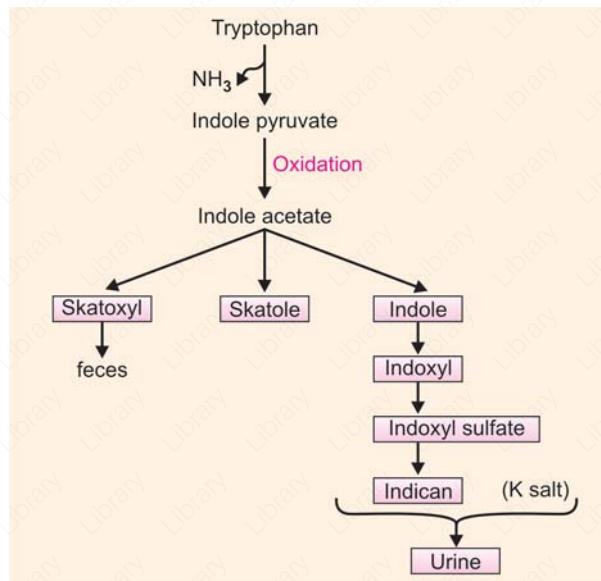
Synthesis:



Functions: It is involved in circadian rhythms or diurnal variations (24 hr. cyclic process) of the body. It plays a significant role in sleep and wake process.

Indole Compounds

Indole compounds are formed by intestinal bacterial putrefaction of tryptophan.



III. Inborn Errors of Tryptophan Metabolism

Hartnup's disease: Defective absorption of tryptophan (and other neutral amino acid) in intestine and kidney.

Symptoms: Dermatitis (increase in niacin synthesis), ataxia and neuro-psychiatric disease with sadistic and bizarre behavior (increase in serotonin level).

Diagnosis: In urine, tryptophan is excreted (neutral amino aciduria) and also indole compounds are excreted (Obermeyer test).

Estimation of Proteins

Quantitative estimations of proteins of foods and other biological materials are performed by the following methods:

1. Kjeldahl method
2. Colorimetric method
3. Electrophoretic method

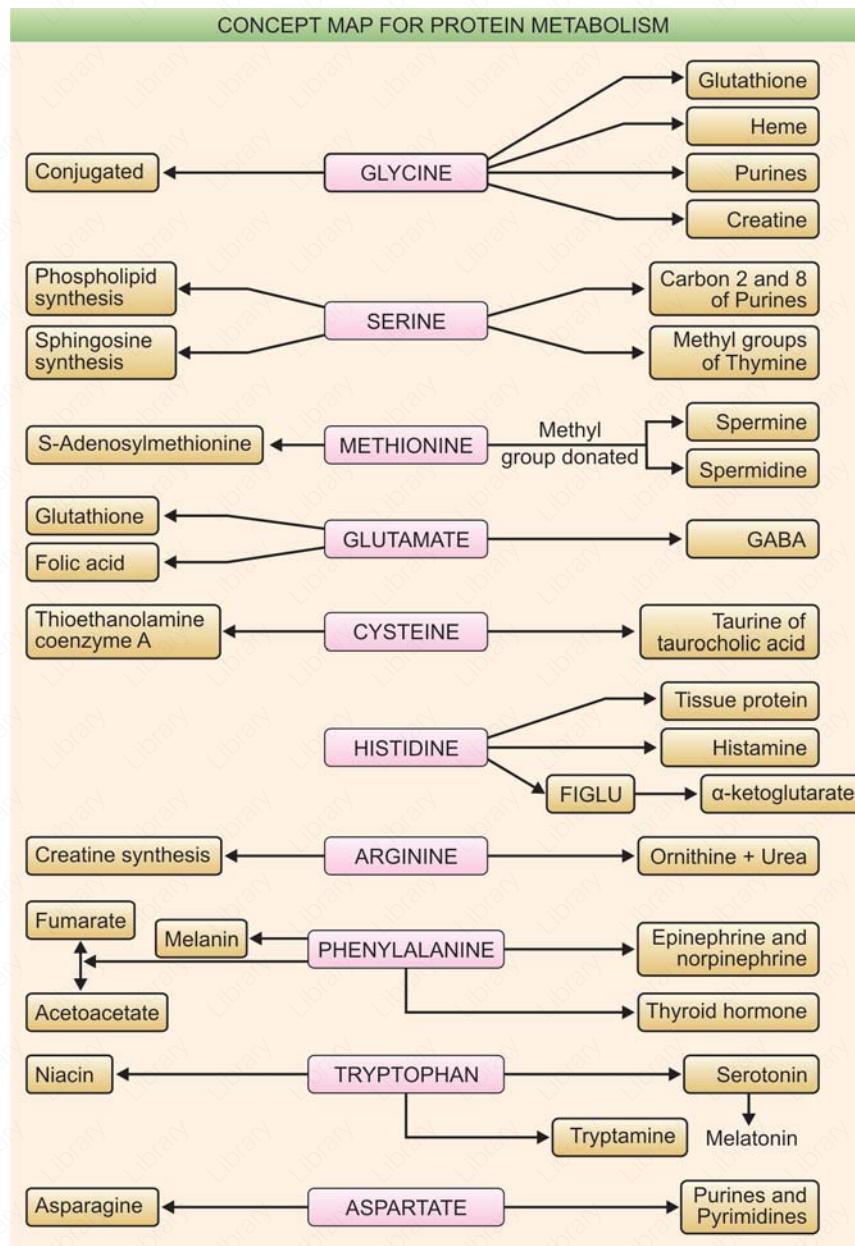
Albumin

They are frequently found in serum, muscle, milk and egg white.

Clinical Importance

1. The plasma albumin concentration falls somewhat in the later stages of normal pregnancy owing to hemodilution and decreased synthesis.
2. The major causes for abnormal decrease in albumin (hypoalbuminemia) are outlined:
 - i. Excessive loss in urine.
 - ii. Inadequate protein supply (dietary protein, restriction, vomiting, diarrhea).
 - iii. Impaired synthesis (liver dysfunction, chronic infection and severe anemia).
 - iv. Sudden plasma dilution (following sudden recovery from dehydration, infantile diarrhea).
3. In the prominent clinical manifestation of hypoalbuminemia edema occurs as a result of the decrease in plasma colloid osmotic pressure which favors retention of water in tissue spaces.
4. The investigation of albumin is of much importance not only in the case of edema but also of the state of liver function and of protein nutrition.
5. Pathological proteinuria may occur as a result of:
 - i. Increased glomerular permeability.
 - ii. Renal tubular damage with defective re-absorption of serum albumin (nephrosis).
 - iii. Disease of the lower urinary tract.
 - iv. Abnormal protein in the plasma.

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MULTIPLE CHOICE QUESTIONS

1. The site of synthesis of urea from ammonia and carbon dioxide is:
 - A. Kidney
 - B. Liver
 - C. Skin
 - D. Spleen

2. The number of ATP molecules required for the operation of the urea cycle is:
A. 1 B. 2
C. 3 D. 4
3. Glycine is not required for the synthesis of:
A. Hippuric acid B. Urea
C. Heme D. Creatine
4. When tyrosinase, is absent in melanocytes, the resulting inherited disorder is:
A. Tyrosinemia B. Phenylpyruvic oligophrenia
C. Albinism D. 3,4(OH)₂ phenylalaninemia
5. The amino acid required for the formation glutathione:
A. Lysine B. Histidine
C. Arginine D. Cysteine

ANSWERS

1. B 2. A 3. B 4. C 5. D

MOST LIKELY QUESTIONS

Long Essays

1. Explain urea cycle. What is its significance?
2. Explain the metabolism of glycine. Add a note on biologically important compounds formed from glycine.
3. Describe the metabolism of phenyl alanine and tyrosine. Add a note on inborn errors associated with it.
4. Describe metabolism of tryptophan. What are the biologically important compounds synthesized?

Short Essays

5. Urea cycle.
6. Formation of biologically important compounds from glycine.
7. Explain the synthesis and functions of glutathione.
8. Explain the synthesis of catecholamines. How are they catabolized?
9. Biologically important compounds synthesized from tyrosine.
10. What is creatine? How is it formed? What is its function?
11. Glucogenic and ketogenic amino acids.

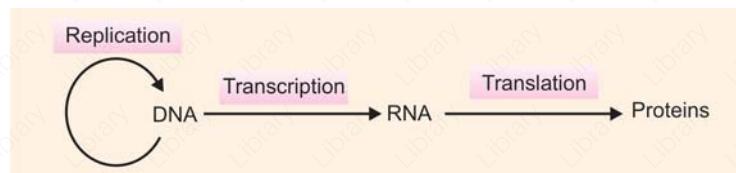
Short Answers

12. Glutathione.
13. Hartnup's disease.
14. Functions of tryptophan.

5.4: PROTEIN SYNTHESIS

Protein synthesis deals with the process of polypeptide chain formation in living organisms. It is the mechanism wherein information present in the mRNA sequence is converted into an amino acid sequence of a protein. Translation is another name for protein synthesis.

The Central Dogma



Replication: Synthesis of new DNA is known as replication. It is identical to parent DNA.

Transcription: Synthesis of RNA from DNA is known as transcription. Information is transferred from DNA to RNA.

Translation: Synthesis of proteins using information present in RNAs is known as translation.

Translation

It is a process of converting information stored in nucleic acid sequences into proteins. Sequences of mRNA (messenger RNA) are translated into unique sequence of amino acids in a polypeptide chain (linear order is preserved throughout). Genes store genetic information.

Why Translation?

All metabolic reactions are catalyzed by enzymes (proteins) and virtually all by different, specific enzymes. This includes energy-releasing reaction, energy-capturing actions, energy requiring reactions, anabolic reactions and catabolic reactions, immune response reactions, signal reception, transduction etc.

Further, proteins provide structure to cells and organism, such as the cytoskeleton of eukaryotic cells. It is essential to understand how proteins are synthesized in order to fully understand how they work in cells.

Some important terminologies repeatedly used in this chapter:

1. *Gene* is a section of DNA producing a functional product.
2. *Chromosome* is a physical linear sequence of DNA.
3. *Genome* is the entire collection of DNA for an organism.
4. *Humans* have 46 chromosome (3×10^9 bases are present)
5. *Prokaryotes* do not contain nucleus. So replication happens in cytoplasm.

Translation in Eukaryotes

Translation takes place in cytoplasm. There are exceptions wherein a few proteins are coded by mitochondrial (circular) DNA and chloroplastic DNA. It is performed on ribosomes.

Requirements

- a. Template - mRNA
- b. tRNAs (transfer RNAs)
- c. Linked to amino acids
- d. Ribosomes - with small and large subunits
- e. Many accessory proteins.
- f. Some energy (GTP hydrolysis)

mRNA: mRNA is a single stranded molecule of RNA that encodes sequence of the polypeptide. It is transcribed and processed in the nucleus and then exported into cytoplasm. Initiation of translation takes place on the binding site at the 3' end. Middle sequence is meant for coding. 3' End regulates stability of mRNA, which has polyadenine tail (poly A tail) with 200 - 300 nucleotides. It protects mRNA from degradation. 5' End consists of methyl guanosine cap. The function of this cap is to facilitate the initiation of translation, and protect the 5' end of mRNA from attack by 5' to 3' exonucleases.

tRNA: tRNA is the carrier of amino acids to the site of protein synthesis. There is at least one tRNA molecule to each of twenty amino acids required for protein synthesis. tRNA are stable molecules. Usually they consist of 50 - 100 nucleotides. 3' end has CCA base sequence in the acceptor arm. Hydroxyl group of adenine holds COOH group of amino acid. In TψC arm, uridine is modified to pseudouridine. tRNA binds to mRNA at this site. *Anticodon arm* is 5 nucleotides long. During translation mRNA is read from 5' to 3'direction. DHU arm consists mainly of dihydrouridine residues approximately 5 nucleotides. It helps in recognition of enzymes which add amino acid to tRNA.

Ribosomes are complex molecules and main machineries for protein synthesis. Ribosomes are found free in cytosol and some are attached to endoplasmic reticulum (rough ER). They are also located in mitochondria and chloroplasts of eukaryotic cells.

Ribosomes are made up of a larger and a smaller subunit. Larger subunit in eukaryotes consists of three types of rRNAs and proteins. Different molecules of ribosomes are held together entirely by ionic and hydrophobic bonds, so that they can easily be assembled. 80 S ribosomes bring about protein translation in eukaryotes. 80S ribosome is made up of 60S and 40S subunits.

60S Components are 5S rRNA, 28S rRNA, 5.8S rRNA and 45 different proteins.

40S Components are 18S rRNA and 33 proteins.

Genetic code: In the nucleotide sequence of mRNA, there are code words for amino acids. These code words are collectively known as genetic code. Hence, genetic information means code words for amino acids.

Genetic code is a triplet code. Each amino acid is coded by a sequence of three nucleotides. It is also known as codon. Example for codons: UUU codes for phenylalanine, AAA codes for lysine, CCC codes for proline, GCA codes for alanine.

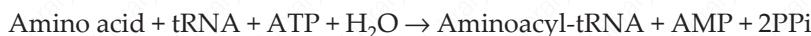
The codons are composed of four nucleotide bases namely, adenine, guanine, cytosine, uracil. These four bases produce 64 different combinations (4^3). The nucleotide sequence of the codon on mRNA is written from the 5' end to 3' end. Sixty one codons code for the 20 amino acids found in protein. The three codons UAA, UAG and UGA do not code for amino acids. They act as stop signals in protein synthesis and are called termination codons. The codons AUG and sometimes GUC are the chain initiating codons.

Process of Translation

Before initiation of translation, the amino acids should be added to tRNA and amino acids have to be activated. Activation is done by amino acyl tRNA synthetases. 20 amino acyl tRNA synthetases are present. These enzymes are highly specific for the amino acid and the corresponding tRNA. But there are about 30-100 tRNA. Hence one amino acid is recognized by more than one tRNA. One synthetase for each amino acid while a single synthetase may recognize multiple tRNA's.

Activation

Amino acid binds to ATP and adenylated amino acid is formed. Amino acid takes up AMP from ATP to become adenylated amino acid which is recognized by enzyme to form a complex and 2PPi are removed.



Transfer of Amino Acid to tRNA

Now complex goes in search of a specific tRNA and binds to it. Hydroxy group of CCA in 3' end of tRNA binds with COOH group of amino acid. Amino acyl synthetases are of 2 types. Class 1 synthetase which adds amino acid to 2' OH and class 2 synthetase adds amino acid to 3' OH. Class 2 synthetase is important in humans. Synthetases are very specific. In addition to binding they also edit. Amino acyl synthetase has proofreading side or its structure has an editing side, corrects only incorrect amino acid added to tRNA.

Translation

It has three steps:

1. Initiation
2. Elongation
3. Termination.

Initiation: Initiation of protein synthesis takes place when a ribosome has assembled on the mRNA and P site is occupied by the initiator codon. This complex is formed by the action of proteins known as initiation factors. There are above twelve different initiator factors which help in promoting the association of the small ribosomal subunit with the mRNA and a charged initiator tRNA.

Four steps are required for the specific initiation of translation.

1. Dissociation of ribosome into its 40S and 60S subunits.
2. Formation of a 43S pre-initiation complex with the initiator tRNA, GTP, eIF2 and the 40S subunit.
3. The mRNA is bound to the pre-initiation complex forming the 40S initiation complex.
4. Joining of the 60S ribosomal subunit to form 80S initiation complex.

Details of These Steps

Step 1: The initiator factor eIF1 and eIF2 bind to the 40S subunit which favors its dissociation from 60S subunit.

Step 2: The first step in the formation of 43S pre-initiation complex is binding of GTP to eIF2 which attaches to the activated initiator tRNA charged with methionine, thus forming a ternary complex. At the same time 40S subunit is complexed with eIF3 to form binary complexes. The binary and ternary complexes then join together to form 43S pre-initiation complex.

Step 3: Binding of mRNA to the pre-initiation complex is effected by the eIF4F. This factor is a complex of three proteins: eIF4E, 4A and 4G. This also ensures proper alignment of the mRNA on the 43S complex resulting in formation of the 40S initiation complex. The initiation complex moves along the mRNA till it encounters the first AUG triplet.

Step 4: The 40S initiation complex having migrated to AUG, now joins 60S subunit to form 80S complex. The joining is facilitated by eIF5. The energy needed to stimulate formation of 80S complex is provided by hydrolysis of GTP bound to eIF2.

With formation of the 80S complex, all initiation factors are released. At this stage the initiator tRNA is bound to the mRNA within the P site (peptide site) of ribosome. The other site within the ribosome to which incoming charged tRNAs bind is termed the A site (for amino acid site).

Hydrolysis of GTP drives the assembly of initiation complex, while hydrolysis of ATP drives movement of the complex down the mRNA.

Stage I: Initiation

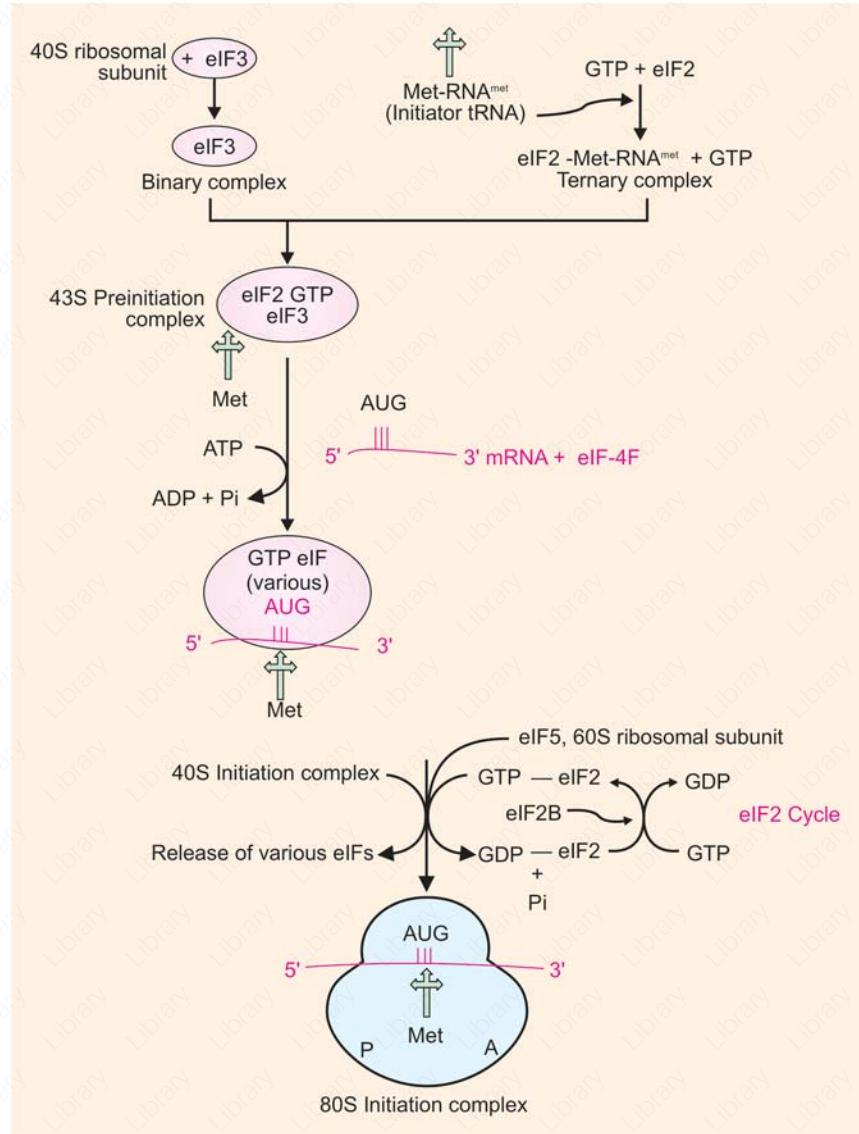


Fig. 5.4.1: The eukaryotic protein synthesis

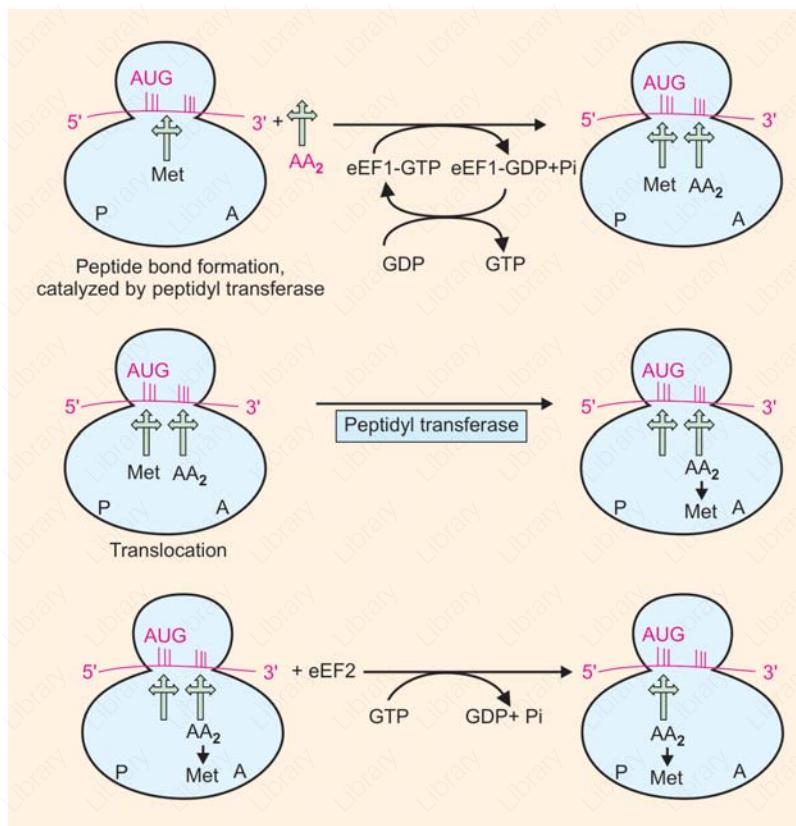
Elongation: Elongation process involves sequential addition of amino acids to the carboxyl end of the growing polypeptide chain by formation of peptide bonds. Elongation factors are termed eEF1 and eEF2, the former consists of two subunits bringing the charged tRNA molecule to ribosome. The eEF2 moves the ribosome one codon down the mRNA (translocation). eEF2 is the specific target for inactivation by diphtheria toxin.

Once the correct charged tRNA molecule is delivered to the A site of the ribosome, peptidyl transferase catalyzes the formation of a peptide bond between the amino acid in the A site. Translocation occurs next because the A site should be vacated in order to accept the next aminoacyl tRNA. During translocation the ribosome is moved along the mRNA such that the next codon mRNA resides under the A site. After translocation the eEF2 is released and the whole process is repeated for addition of next amino acid.

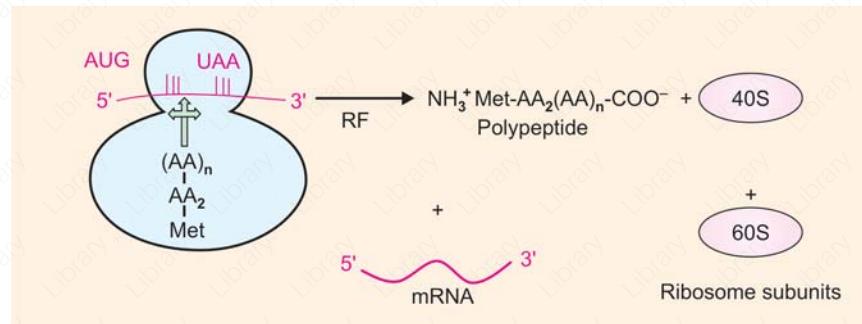
Termination: Termination of protein synthesis is accomplished when the A site of the ribosome reaches one of the stop codon of the mRNA. A eukaryotic releasing factor (RF) recognizes these codons and causes the protein that is attached to the last tRNA molecule in the P site to be released.

After termination the ribosomal subunits, tRNA and mRNA dissociate from each other. The eIF3 associates with the smaller subunit and this prevents its re-association with the 60S subunit in the absence of mRNA. Finally eIF2 binds to the smaller subunit setting the stage for translation of another mRNA.

Stage II: Elongation of Polypeptide: Binding of Incoming Aminoacyl-tRNA



Stage III: Termination



Inhibitors of Protein Synthesis

A number of antibiotics bind to various sites on ribosomes and interfere with individual steps of protein synthesis. Protein synthesis is mandatory for the growth and the survival of the cell. The use of antibiotics eliminates bacteria. Thus, antibiotics are potent tools with which infectious diseases are treated. Some of the antibiotics are discussed below:

1. **Streptomycin** binds to the 30S ribosomal subunit of prokaryotes, causes misreading of mRNA and thereby prevents formation of the initiation complex.
 2. **Tetracycline** binds to the 30S ribosomal subunit of prokaryotes and inhibits binding of aminoacyl-tRNA to the A site.
 3. **Chloramphenicol** competitively inhibits the peptidyl transferase activity in prokaryotes, thereby interfering with elongation of the peptide chain.
 4. **Erythromycin** binds to the 50S subunit of prokaryotes. It prevents translocation.
- These four compounds affect protein synthesis on 70S ribosomes of prokaryotes. Mitochondria also contain the 70S type ribosomes and so these compounds inhibit mitochondrial protein synthesis as well.
5. **Ricin and cyclohexamide** target eukaryotic 60S and 80S ribosomes, respectively.
 6. **Puromycin** has structural resemblance with the aminoacyl-tRNA. It forms a peptide bond with the growing peptide and prematurely terminates protein synthesis. It is active in both prokaryotes and eukaryotes.
 7. **Diphtheria toxin** prevents the translocation step in eukaryotes.
- This complex process of translation is identical in eukaryotes, but the factors are different. This explains the utility of antibiotics that preferentially inhibit protein synthesis in bacteria.

Post-translational Modifications

The nascent polypeptide chain undergoes many modifications which convert it to the biologically active form. During the modification and its release, the polypeptide chain is folded into its native conformation which depends on the amino acid sequence of the polypeptide. Maximum number of intra chain interaction takes place, such as hydrogen bonds, van der Waals forces, ionic and hydrophobic interactions. In this way, one-dimensional genetic message in the mRNA is changed into the three dimensional protein. In addition to folding several processing reactions also occur in the polypeptide chain such as, covalent modifications (phosphorylation, glycosylation, hydroxylation and addition of prosthetic groups), proteolytic processing, formation of disulphide cross links between sixteen residues.

MULTIPLE CHOICE QUESTIONS

1. The initiating codon in protein biosynthesis is:
A. UGA B. AUG
C. GAU D. AUA
2. Termination codons in protein synthesis is:
A. UAA B. UGA
C. UAG D. All the above
3. Diphtheria toxin prevents _____ step in eukaryotes.
A. Transcription B. Translation
C. Translocation D. Replication

ANSWERS

1. B 2. D 3. C

MOST LIKELY QUESTIONS

Long Essay

1. Describe protein synthesis.

Short Essays

2. What is a genetic code? Discuss the characteristic features of genetic code.
3. Give an account of inhibitors of translation.

Short Notes

4. Post-translational modifications.

5.5: TECHNIQUES

CHROMATOGRAPHY

Chromatography is a technique used to separate solutes on the basis of their differential distributions between two-phase solvent systems where one phase (the mobile phase) is caused to move over the other phase (the immobile or stationary phase). There are six types of chromatography, namely, partition, adsorption, ion-exchange, gel filtration, affinity, high performance liquid chromatography.

The chromatographic separations in practice may take one of the several forms; column chromatography in which the stationary phase is packed into glass columns; thin-layer chromatography in which the stationary phase is thinly coated onto glass plates and paper chromatography in which the stationary phase is supported by the cellulose fibers of a filter paper sheet.

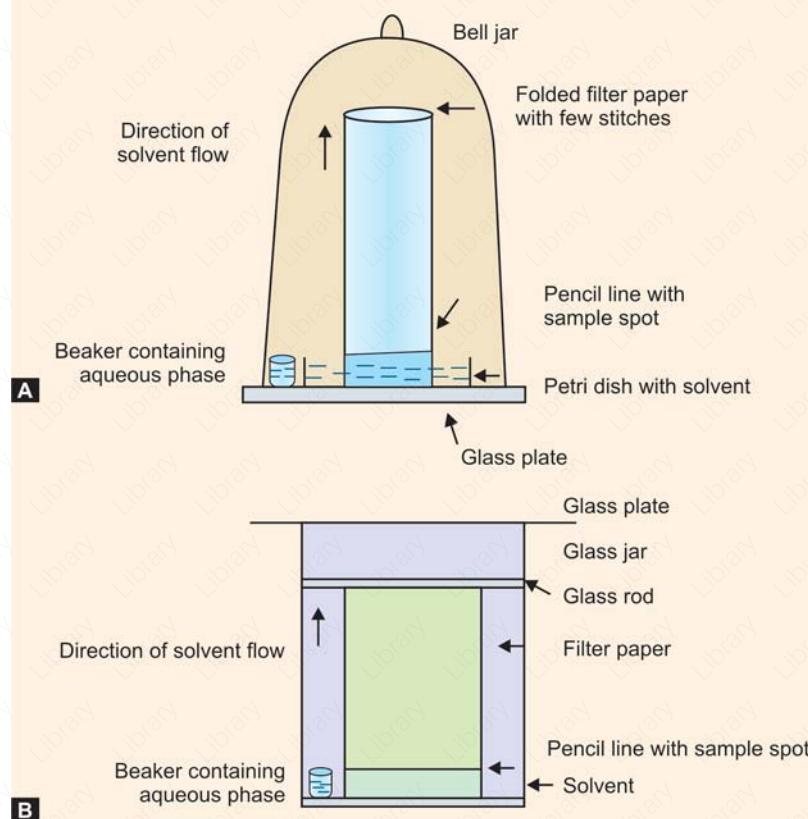
Paper chromatography is a partition chromatographic technique which has found widespread use. Over the cellulose supporting medium the solvents flow. Water is considered as a stationary phase, as it is bound to the polar cellulose and the organic solvent that runs over the hydrated cellulose fibers is the mobile phase.

Paper development: There are two techniques which may be employed for the development of paper chromatogram: (1) ascending and (2) descending method. The sample spots should be in a position just above the surface of the solvent so that, as the solvent moves vertically up the paper by capillary action, separation of the sample is achieved.

In the descending method the solvent moves downward under gravity. Although ascending chromatography is often preferred because of the simplicity of the set up, the flow of solvent is faster in the descending technique (Figs 5.5.1A and B and 5.5.2).

As the solvent passes through an area of the paper containing a solute, the solute will begin to partition itself between the aqueous and the organic phases in proportion to its relative solubilities in the two phases. More the soluble solute in the organic phase, the faster the solute will be carried along by the organic phase. Conversely, the greater the affinity for water, the slower the solute will move with respect to the solvent front. Thus, if several compounds possess different solubility characteristics, theoretically each will progress across the paper at a specific rate which is different from that of any of the other compounds. The distance the solute moves in relation to the distance the solvent moves, serves as a convenient means for identifying the solute. This relative rate of flow is the R_f value for the compound under the specified conditions of the experiment.

$$R_f = \frac{\text{Distance travelled by solute (measured from center of the spot)}}{\text{Distance travelled by solvent}}$$



Figs 5.5.1A and B: Apparatus for ascending chromatography section

Several compounds may have the same R_f value in a particular solvent system; these can be separated by running more than one chromatogram each with a different solvent system. It is always necessary to include known compounds in a chromatogram for comparative purposes.

ELECTROPHORESIS

Electrophoresis is the separation of the charged constituents of a solution by means of an electric current. Charged particle placed in an electrical field migrates towards the anode or the cathode depending upon the net charge carried by the particle. Molecules which have a similar charge will have different charge/mass ratio when they have differences in molecular weight. These differences form a sufficient basis for a differential migration.

Electrophoresis is a valuable diagnostic tool in clinical biochemistry laboratories and is most commonly used for the separation of proteins found in serum. It is also useful in the separation of different forms of hemoglobins, immunoglobulins and isoenzymes.

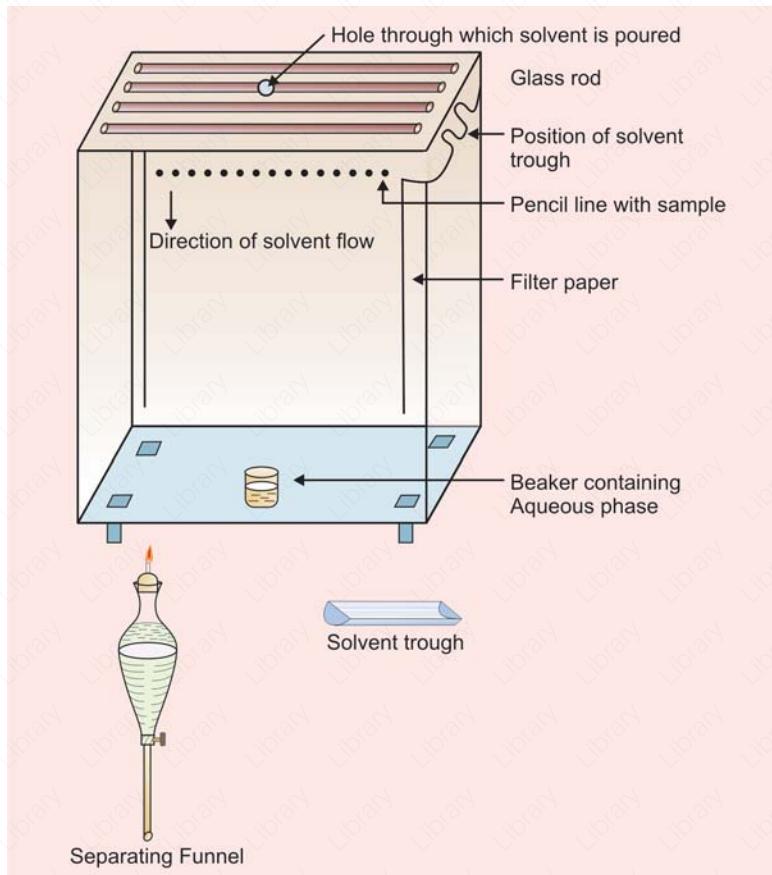


Fig. 5.5.2: Descending chromatography chamber

Agar, filter paper, cellulose acetate or agarose is used as support medium. They have large pores. At a pH of 8.6 all of the serum proteins carry a net negative charge and tend to migrate towards the anode (Gammaglobulins may stay at the point of application of serum or move a little towards the cathode). Albumin carries the largest charge and therefore, moves the fastest; the gammaglobulins have the smallest net charge. Serum proteins separate into five bands on cellulose acetate, etc. The five bands starting from anode are designated as albumin, α_1 - globulin, α_2 - globulin, β -globulin and γ -globulin. Six bands are formed on agarose (the β - globulin splitting to give β_1 and β_2).

The bands can be quantitated using densitometer.

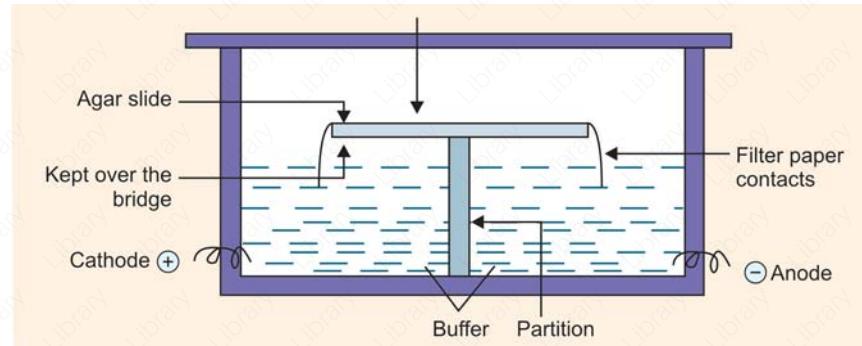


Fig. 5.5.3: Electrophoresis chamber

Electrophoretic Separation of Serum Proteins

Electrophoretic separation of serum proteins on agar gel equipment requires:

1. An electrophoresis chamber (Fig. 5.5.3)
2. Power pack (Power supply)

Agar gel reagents: Buffer, staining solution and wash solution.

Principle: Thin filter paper strip soaked in serum is applied onto the agar support medium and a voltage is applied. After a definite period of 'run' the agar slide is removed. The serum proteins would have separated into five distinct bands. Since they are colorless they have to be stained with the dye.

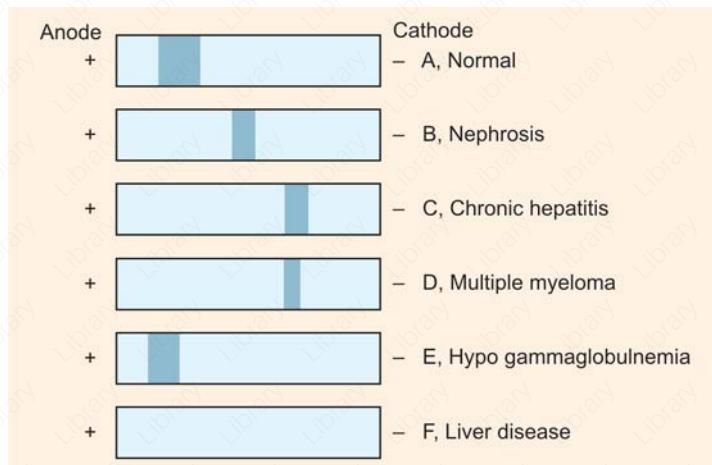


Fig. 5.5.4: Electrophoretograms (normal and some typical case) of serum proteins

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The intensities of the colored bands when compared with a normal pattern give an idea about the nature and relative concentrations of the resolved protein fractions.

Normal values: The normal adult has a serum protein concentration that varies from 6.0 to 8.2 g%. Upon separation by electrophoresis, the following concentration in g% and percentages are usually found:

Serum protein concentration		
Fraction	Concentration g%	Percent of total
Albumin	3.5 – 5.2	56
α_1 - globulin	0.1 – 0.4	3
α_2 - globulin	0.5 – 1.0	13.5
β - globulin	0.6 – 1.2	15.5
γ - globulin	0.6 – 1.6	12

The electrophoretogram of serum proteins is helpful in detecting changes in the individual protein fraction and in detecting abnormal bands in certain diseased conditions (Fig. 5.5.4).

The pattern in nephrosis reveals a low level of albumin and a marked rise of α_2 -globulin. A small increase in globulin can also be seen in this condition.

In chronic infection (hepatitis) a relative decrease in albumin with a notable elevation in γ -globulin is observed, presence of an abnormal band (M protein) usually between β and γ -globulin bands, closer to the γ -band.

MULTIPLE CHOICE QUESTIONS

1. Rf value in partition chromatography is:
A. Retention factor B. Retardation factor
C. Ratio of fronts D. All of the above
2. In electrophoresis at 8.6 pH all the serum proteins carry a net negative charge and tend to migrate towards:
A. Cathode B. Anode
C. Both anode and cathode D. Neither anode nor cathode

ANSWERS

1. D 2. B

MOST LIKELY QUESTIONS

1. What is chromatography? Discuss paper chromatography with suitable example
2. Define Electrophoresis. How do you separate serum proteins with the help of electrophoretic technique? Explain.

5.6: PROTEIN SEQUENCING

Proteins are found in every cell and are essential to every biological process. Protein structure is very complex. Determining a protein's structure involves first protein sequencing, i.e. determining the amino acid sequences of its constituent peptides; and also determining what conformation it adopts and whether it is complexed with any non-peptide molecules. The peptide is hydrolyzed into its constituent amino acids by heating it in 6 N HCl at 110°C for 24 hours. Cellular processes are understood well with the discovery of the structure and functions of proteins in living organisms. Discovering about the proteins, allows drugs which target specific metabolic pathways to be invented more easily.

The two major direct methods of protein sequencing are the Edman degradation reaction and mass spectrometry. It is also possible to generate an amino acid sequence from the DNA or mRNA sequence encoding the protein.

Determining Amino Acid Composition

It is often desirable to know the unordered amino acid composition of a protein prior to attempting to find the ordered sequence, as this knowledge can be used to facilitate the discovery of errors in the sequencing process or to distinguish between ambiguous results. Knowledge of the frequency of certain amino acids may also be used to choose which protease to use for digestion of the protein. A generalized method for doing this is as follows:

1. Hydrolyze a known quantity of protein into its constituent amino acids.
2. Separate the amino acids in some way.

Hydrolysis

Hydrolysis is done by heating a sample of the protein in 6 M HCl to 100–110°C for 24 hours or longer. Proteins with many bulky hydrophobic groups may require longer heating periods. However, these conditions are so vigorous that some amino acids (serine, threonine, tyrosine, tryptophan, glutamine and cystine) are degraded.

Separation

The amino acids can be separated by ion-exchange chromatography or hydrophobic interaction chromatography. An example of the ion-exchange chromatography is using sulfonated polystyrene as a matrix, adding the amino acids in acid solution and passing a buffer of steadily increasing pH through the column. Amino acids will be eluted when the pH reaches their respective isoelectric points. The hydrophobic interaction technique may be employed through the use of reversed phase chromatography. Many

commercially available C8 and C18 silica columns have demonstrated successful separation of amino acids in solution in less than 40 minutes through the use of an optimized elution gradient.

Quantitative Analysis

Once the amino acids have been separated, their respective quantities are determined by adding a reagent that will form a colored derivative. If the amounts of amino acids are in excess of 10 nanomol, ninhydrin can be used for this. It gives a yellow color when reacted with proline, and a vivid blue with other amino acids. The concentration of amino acid is proportional to the absorbance of the resulting solution. With very small quantities, down to 10 picomol, fluorescamine can be used as a marker: this forms a fluorescent derivative on reacting with an amino acid.

N-terminal Amino Acid Analysis

Determining which amino acid forms the N-terminus of a peptide chain is useful to aid the ordering of individual peptide sequences of fragments into a whole chain. The first round of Edman degradation is often contaminated by impurities and therefore does not give an accurate determination of the N-terminal amino acid. A generalized method for N-terminal amino acid analysis is as follows:

1. To react the peptide with a reagent. This will selectively label the terminal amino acid.
2. To hydrolyze the protein.
3. To determine the amino acid by chromatography and comparison with standards.

There are various reagents which can be used to label terminal amino acids. They all react with amino groups and will therefore also bind to amino groups in the side chains of amino acids such as lysine. For this reason it is necessary to be careful in interpreting chromatograms to ensure that the right spot is chosen.

Two of the more common reagents are **Sanger's reagent** (1-fluoro-2, 4-dinitrobenzene) and dansyl derivatives such as dansyl chloride. Phenyl isothiocyanate, the reagent for the Edman degradation, can also be used. The same questions apply here as in the determination of amino acid composition, with the exception that no stain is needed, since the reagents produce colored derivatives and only qualitative analysis is required. As a result, the amino acid need not have to be eluted from the chromatography column but just compared with a standard. Since any amino groups will react with the labeling reagent, ion exchange chromatography cannot be used. Instead, thin layer chromatography or high pressure liquid chromatography should be used.

C-terminal Amino Acid Analysis

The number of methods available for C-terminal amino acid analysis is much smaller than the number of available methods of N-terminal analysis. The most common method is to add carboxy-peptidases to a solution of the protein, take samples at regular intervals, and determine the terminal amino acid by analyzing a plot of amino acid concentrations against time.

Edman Degradation

The Edman degradation is a very important reaction for protein sequencing because it allows the ordered amino acid composition of a protein to be discovered. Automated Edman sequencers are now in widespread use, and are able to sequence peptides up to approximately 50 amino acids long. A reaction scheme for sequencing a protein by the Edman degradation follows some of the steps elaborated here:

1. Break any disulfide bridges in the protein by oxidizing with per-formic acid.
2. Separate and purify the individual chains of the protein complex, if there is more than one.
3. Determine the amino acid composition of each chain.
4. Determine the terminal amino acids of each chain.
5. Break each chain into fragments under 50 amino acids long.
6. Separate and purify the fragments.
7. Determine the sequence of each fragment.
8. Repeat with a different pattern of cleavage.
9. Construct the sequence of the overall protein.

Digestion into peptide fragments: Peptides longer than about 50-70 amino acids long cannot be sequenced reliably by the Edman degradation. Because of this, long protein chains need to be broken up into small fragments which can then be sequenced individually. Digestion is done either by endopeptidases such as trypsin or pepsin or by chemical reagents such as cyanogen bromide. Different enzymes give different cleavage patterns, and the overlap between fragments can be used to construct an overall sequence.

The Edman Degradation Reaction

The peptide to be sequenced is adsorbed onto a solid surface. One common substrate is glass fiber coated with polybrene, a cationic polymer. The Edman reagent, phenyl isothiocyanate (PTC), is added to the adsorbed peptide, together with a mildly basic buffer solution of 12 percent tri methylamine. This reacts with the amino group of the N-terminal amino acid.

The terminal amino acid derivative can then be selectively detached by the addition of anhydrous acid. The derivative then isomerizes to give a substituted phenyl thiohydantoin which can be washed off and identified by chromatography, and the cycle can be repeated. The efficiency of each step is about 98 percent, which allows about 50 amino acids to be reliably determined.

Limitations of the Edman Degradation

Because the Edman degradation proceeds from the N-terminus of the protein, it will not work if the N-terminal amino acid has been chemically modified or if it is concealed within the body of the protein. It also requires the use of either guesswork or a separate procedure to determine the positions of disulfide bridges.

Mass Spectrometry

The other major direct method by which the sequence of a protein can be determined is mass spectrometry. This method has been gaining popularity in recent years as a new technique and increasing computing power have facilitated it. In principle, mass spectrometry can sequence any size of protein, but the problem becomes computationally more difficult as the size increases. Peptides are also easier to prepare for mass spectrometry than whole proteins, because they are more soluble.

One method of delivering the peptides to the spectrometer is electrospray ionization. The protein is digested by an endo-protease, and the resulting solution is passed through a high pressure liquid chromatography column. At the end of this column, the solution is sprayed out of a narrow nozzle charged to a high positive potential into the mass spectrometer. The charge on the droplets causes them to fragment until only single ions remain. The peptides are then fragmented and the mass-charge ratios of the fragments measured. The mass spectrum is analyzed by computer and often compared against a database of previously sequenced proteins in order to determine the sequences of the fragments. This process is then repeated with a different digestion enzyme, and the overlaps in the sequences used to construct a sequence for the protein.

MOST LIKELY QUESTIONS

Short Essays

1. How do you determine amino acid composition?
2. Discuss Edman degradation.
3. How mass spectrometry is used in protein sequencing?

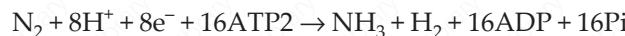
5.7: NITROGEN FIXATION

Nitrogen fixation is the process by which nitrogen is taken from its natural, relatively inert molecular form (N_2) in the atmosphere. It is converted into nitrogen compounds such as, ammonia, nitrate and nitrogen dioxide which are useful for other chemical processes.

Nitrogen fixation is performed naturally by a number of different prokaryotes, including bacteria, actinobacteria, and certain types of anaerobic bacteria. Microorganisms that fix nitrogen are called diazotrophs. Some higher plants, and some animals (termites), have formed associations with diazotrophs.

Nitrogen fixation also occurs as a result of non-biological processes. These include lightning, industrially through the Haber-Bosch Process, and combustion.

Nitrate in the natural environment is relatively rare. Microbes capable of using alternative nitrogen sources have an advantage and a subset of microbes is capable of obtaining the nitrogen they need from nitrogen gas. Nitrogen gas makes up about 79 percent of our atmosphere and is easily available. Molecular nitrogen is a stable un-reactive gas with a triple bond between the two atoms and the reduction of it to ammonia is an energy expensive process. A large amount of ATP, protons and electrons are required to reduce just one molecule of nitrogen gas.



Chemical equation for the reduction of nitrogen to ammonia by nitrogenase enzyme.

The enzyme that catalyzes the reaction is called nitrogenase and is made of two separate protein components, dinitrogenase reductase and dinitrogenase. Dinitrogenase reductase prepares and donates two high potential electrons at a time to dinitrogenase. It contains an Fe-S center that holds the electrons before donation. Dinitrogenase actually catalyzes the reduction of N_2 . The mechanism of reduction is unknown but is thought to involve three $2e^-$ transfers to nitrogen. Formation of hydrogen gas always accompanies the formation of ammonia by dinitrogenase and is a wasteful process. Some microorganisms have hydrogenases that recover this lost energy.

Associations

Nitrogen fixation is important to humans for two reasons. A better understanding of the biological process which takes place at pH 7.0 at around 37°C could lead to better chemical synthesis methods than those currently employed that require high temperatures and pressures. About 1 percent of the energy generated by humans is used to create nitrogen fertilizers; typically in the form of nitrate. Second, some nitrogen fixing microbes form cooperative (symbiotic) relationships with plants. The



Fig. 5.7.1: Legume root nodules

microbe fixes nitrogen for the plant and the plant provides carbon and energy for the microbes. Plants that are capable of forming these symbiotic relationships are called legumes. Many legumes are important crops including soybeans, peas and beans. A better understanding of the symbiosis and the biochemistry of nitrogen fixation should lead to improved crops and less need for nitrogen fertilizers that pollute our environment.

Regulation

Nitrogen fixation is costly to the microbe and because of this, its expression and activity are under tight control. In the presence of fixed forms of nitrogen, ammonia or nitrate, synthesis of the nitrogenase gene products, dinitrogenase and dinitrogenase reductase, is rapidly shut off. In some species, dinitrogenase reductase is covalently modified by the addition of an ADP-ribosyl unit.

Nitrogen assimilation is a fundamental biological process that occurs in plants and algae that are incapable of independent nitrogen fixation. The assimilation of nitrogen has marked effects on plant productivity, biomass, and crop yield, and nitrogen deficiency leads to a decrease in structural components. Initial conversion of nitrate to nitrite by nitrate reductase is followed by a reduction to ammonia by nitrite reductase. The ammonia is incorporated into glutamine as an amido nitrogen and is reductively transferred to 2-oxoglutarate to form 2 molecules of glutamate by glutamate synthase.

Photosynthetic nitrogen assimilation and associated carbon and respiratory metabolism focuses on the very latest developments in our understanding of how plants use light energy and fixed carbon to assimilate nitrate and ammonium into the organic compounds required for growth. From the partitioning of organic nitrogen within the photosynthetic apparatus, through the primary processes of reduction of nitrate and nitrite and the assimilation of ammonium and its cycling in photorespiration, the complex interactions inherent in the crosstalk between carbon and nitrogen assimilation are considered and exciting new developments such as nitric oxide production evaluated. Attention is paid throughout to the close coordination of photosynthetic and respiratory processes in nitrogen assimilation.

5.8: CHLOROPHYLL

Chlorophyll is a green pigment found in most plants, algae, and cyanobacteria. Its name is derived from Greek: *chloros* = green and *phyllon* = leaf. Chlorophyll absorbs light most strongly in the blue and red but poorly in the green portions of the electromagnetic spectrum, hence the green color of chlorophyll-containing tissues like plant leaves.

Chlorophyll and Photosynthesis

Plants obtain energy from light with the help of chlorophyll which is vital for photosynthesis.

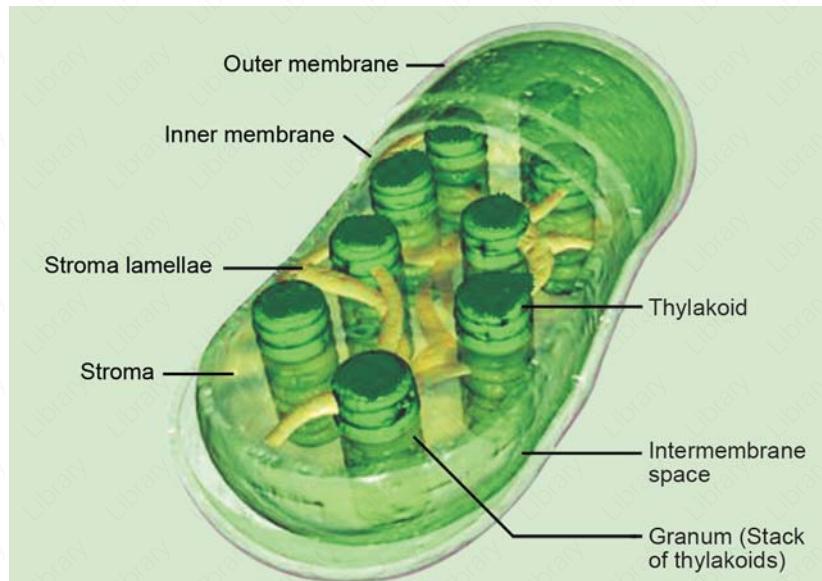


Fig. 5.8.1: Chloroplast

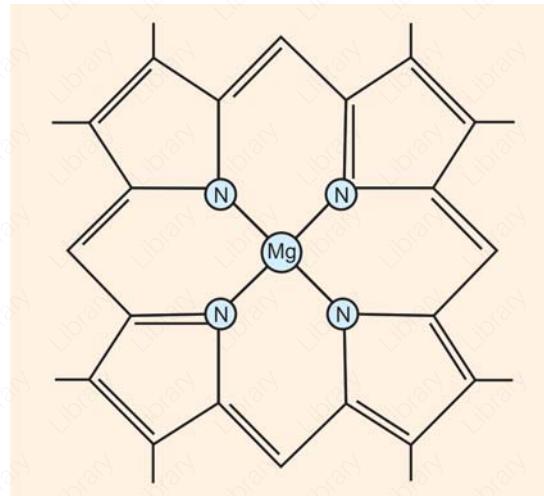
Chlorophyll molecules are specifically arranged in and around pigment protein complexes called photosystems. They are embedded in the thylakoid membranes of chloroplasts (Fig. 5.8.1). In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems. Because of chlorophyll's selectivity regarding the wavelength of light it absorbs, areas of a leaf containing the molecule will appear green. There are currently two accepted photosystem units, Photosystem II and Photosystem I. They have their own distinct reaction center chlorophylls, named P680 and P700 for photo system II and I respectively. These pigments are named after the wavelength (in nanometers) of their red-peak absorption maximum. The identity, function and spectral properties of the types of chlorophyll in each photo system are distinct and determined by each other and the protein structure surrounding them. Once extracted from the protein into a solvent such as acetone or methanol, these chlorophyll pigments can be separated in a simple paper chromatography experiment. Based on the number of polar groups between chlorophyll a and chlorophyll b, they will chemically separate out on the paper.

The function of the reaction center, chlorophyll is to use the energy absorbed by and transferred to it from the other chlorophyll pigments in the photosystems to undergo a charge separation. This is a specific redox reaction in which the chlorophyll donates an electron into a series of molecular intermediates called an electron transport chain. The charged reaction center chlorophyll ($P680^+$) is then reduced back to its ground state by accepting an electron.

In photosystem II, the electron which reduces $P680^+$ ultimately comes from the oxidation of water into O_2 and H^+ through several intermediates. This reaction shows how photosynthetic organisms like plants produce O_2 and is the source for practically all the O_2 in Earth's atmosphere.

Photosystem I typically works in series with photosystem II. Thus, the $P700^+$ of photosystem I is usually reduced, via many intermediates in the thylakoid membrane, by electrons ultimately from photosystem II. Electron transfer reactions in the thylakoid membranes are complex and the source of electrons used to reduce $P700^+$ can change. The electron flow produced by the reaction center chlorophyll pigments is used to shuttle H^+ ions across the thylakoid membrane. This sets up a chemiosmotic potential mainly used to produce ATP chemical energy. Those electrons ultimately reduce $NADP^+$ to NADPH a universal reductant used to reduce CO_2 into sugars as well as for other biosynthetic reductions.

Reaction center chlorophyll-protein complexes are capable of directly absorbing light and performing charge separation events without other chlorophyll pigments. Thus, the remaining chlorophylls in the photosystem and antenna pigment protein complexes associated with the photo systems cooperatively absorb and funnel light energy to the reaction center.

**Fig. 5.8.2: Chlorophyll**

Chlorosis

In plants, chlorophyll may be synthesized from succinyl-CoA and glycine, although the immediate precursor to chlorophyll *a* and *b* is protochlorophyll (Fig. 5.8.2).

Chlorosis is a condition in which leaves produce insufficient chlorophyll, turning them yellow. Chlorosis can be caused by a nutrient deficiency including iron - called iron chlorosis, or in a shortage of magnesium or nitrogen. Soil pH sometimes play a role in nutrient-caused chlorosis, many plants are adapted to grow in soils with specific pHs and their ability to absorb nutrients from the soil can be dependent on the soil pH. Chlorosis can also be caused by pathogens including viruses, bacteria and fungal infections or sap sucking insects.

5.9: ENZYMES

INTRODUCTION AND DEFINITION

Enzymes are colloidal, thermolabile (heat unstable), biological catalysts, protein in nature and synthesized by living cells. Substances which may be acted upon by an enzyme are called the substrates of that enzyme.

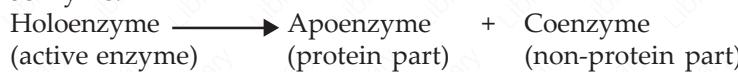
Biologic Catalysts

1. Enzymes share some of the properties of chemical catalysts.
 - a. They are neither consumed nor produced during the course of a reaction.
 - b. They do not cause reactions to take place. They speed up reactions that would ordinarily proceed, but at a much slower rate in their absence. In other words, they do not alter the equilibrium constants of reactions that they catalyze.
2. Enzymes differ from chemical catalysts in several ways.
 - a. Enzymes are mostly proteins.
 - b. They are highly specific for the reactions they catalyze and produce only the expected products from the given reactants or substrates.
 - c. They often show a high specificity toward one substrate, although some enzymes have a broader specificity, using more than one substrate.
 - d. They function within a moderate pH and temperature range.

In the body, enzymes are key determinants of rates of physiological events, thus play central role in health and disease. Life is not possible without enzymes. On every process in the body starting from digestion and absorption to DNA synthesis and cell division, is mediated by the enzymes. Most of the diseases, starting from indigestion and diarrhea, to cancer all have underlying defects in enzyme itself.

General Properties

Enzymes are either simple proteins or conjugated proteins. In conjugated proteins, the non-protein part of the molecule is called the prosthetic group (which cannot be separated) or co-enzyme (can be separated by dialysis from the enzyme). The protein part is called the apoenzyme and the complete molecule made up of apoenzyme and prosthetic group is called holoenzyme.



Some hydrolytic enzymes such as ribonuclease of bovine pancreas have a single polypeptide chain in the molecule and those are called monomeric enzymes. Many enzymes such as hexokinases and lactate dehydrogenase have more than one polypeptide chain or subunit in each molecule and are called oligomeric enzymes.

Sometimes several enzyme activities catalyzing different consecutive reactions are located at different sites of the same macromolecule. It cannot be fractionated into smaller molecules with individual enzyme activities. Such a macromolecule is considered as a multienzyme system. Example: Fatty acid synthase, pyruvate dehydrogenase.

Location

Intracellular: Nucleus, cytoplasmic, microsomal, lysosomal or mitochondrial.

Extracellular: Active outside the cell. *Example:* All digestive enzymes belong to this category.

Substrate and Products



The substance on which the enzyme acts is called as the substrate of the enzyme. The new substance that is produced by the reaction is called as product. In the above example, sucrose is the substrate, glucose and fructose are the products and sucrase is the enzyme.

Classification of Enzymes and Nomenclature

Nomenclature: Enzymes are usually named by adding the suffix: 'ase'

1. The action catalyzed by them, also indicating the substrate. *Example:* Glucose oxidase, alcohol dehydrogenase etc.
2. The substrate on which enzyme acts (usually these are hydrolytic enzymes). *Example:* Sucrase, urease, amylase etc.

Some enzymes discovered long ago were named after their source. *Example:* Ptyalin from saliva, papain from papaya. The names given to proteolytic enzymes are generally informative. *Example:* Pepsin, trypsin. All these names are called as trivial names of enzymes.

Classification of Enzymes

Even though enzymes were discovered much earlier, till 1956 there was no satisfactory classification. It was in 1956, IUB (International Union of Biochemists) appointed a commission called Enzyme Commission (EC) to make a satisfactory classification. This commission recommended a classification which was accepted by the union in 1964 and that was called IUB classification.

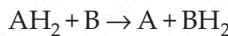
It is the most widely accepted classification. IUB classification is complex and cumbersome but it is unambiguous. According to this, the enzymes are divided into six major classes. Each class is subdivided into various sub-classes. Thus, each enzyme has been assigned an EC number. The classes, sub-classes etc are arranged in a fixed order by the commission.

The six major classes are as follows:

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

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1. *Oxidoreductases (EC 1):* They catalyze oxidation of one substrate with simultaneous reduction of another substrate.



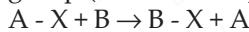
Oxidation → reduction

Example: Alcohol dehydrogenase (trivial name) and IUB name is alcohol-NAD-oxidoreductase. EC code is 1.1.1.1



Examples of oxidoreductases: LDH, cytochrome oxidase, L and D amino acid oxidases.

2. *Transferases (EC 2):* These are the enzymes catalyzing the transfer of a group (other than H₂) from one substrate to another.



Example:



Examples of transferases: Transaminases, transmethylases and phosphorylases.

3. *Hydrolases (EC 3):* These are the enzymes that cause cleavage of a molecule into two by the addition of H₂O. H₂O is also split into H and OH which gets added to broken ends of the substrate. Bonds that are cleaved are ester, ether, peptide or glycosidic bonds.

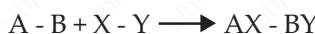


Examples of hydrolases: Lipase (triacylglycerol acyl hydrolase EC 3.1.1.3)

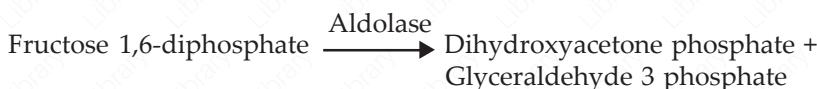
Choline esterase, acid phosphatase, alkaline phosphatase, urease, pepsin.

4. *Lyases (EC 4):* They catalyze the simple cleavage of molecule into two without the addition of H₂O or any other substance.

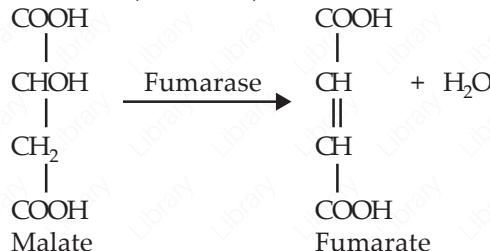
Addition \longrightarrow Elimination



a. *Example:* Aldolase (Ketose 1-phosphate aldehyde lyase EC 4.1.2.7)



b. Fumerase (EC 4.2.1.2)



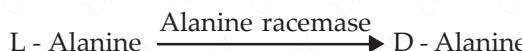
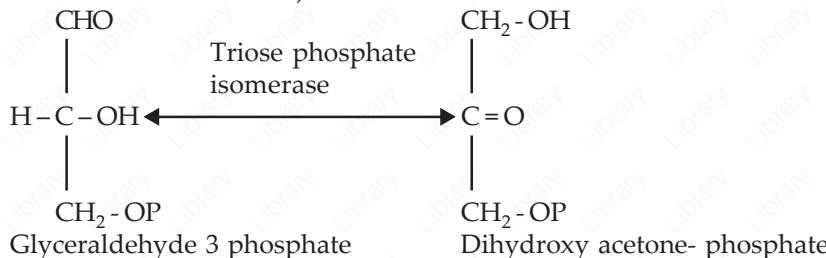
Fumarase name is misleading as it is not a hydrolase. In the body usually reverse reaction is favored.

Examples of lyases: Aldolase, fumarase, histidase.

5. *Isomerases (EC 5):* These enzymes catalyze intramolecular rearrangement producing isomers of the substrates. Inter conversion of isomers takes place.

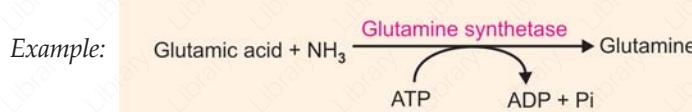


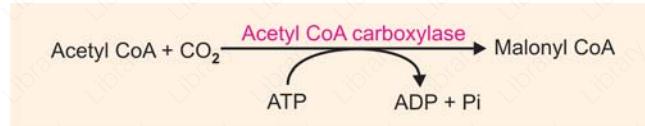
Example: Glyceraldehyde 3 phosphate \rightarrow Dihydroxy acetone- phosphate
Enzyme is triose phosphate isomerase (D glyceraldehyde 3-phosphate ketoisomerase EC 5.3.1.1).



Examples of isomerases: Retinene isomerase, glucose phosphate isomerase, triose phosphate isomerase, alanine racemase.

6. *Ligases (EC 6 Synthetases):* They catalyze the linking of two molecules usually with utilization of energy obtained by hydrolysis of ATP. Condensation is usually dependent on ATP.





Examples of ligases: Glutamine synthetase (EC 6.3.1.2), Acetyl CoA carboxylase (EC 6.4.1.2), succinate thiokinase.

ENZYME CATALYSIS

Introduction

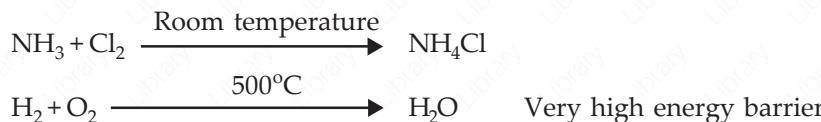
Biochemistry is mainly concerned with enzyme catalyzed reactions. Enzymes act by lowering the activation energy. Enzymes have immense catalytic power and they are highly specific both in the reactions catalyzed and in their choice of substrates. Catalytic activities of many enzymes are regulated. In a biosynthetic pathway, if the enzyme catalyses the first step, it is usually inhibited by the ultimate product. In many biochemical reactions, the energy of the reactants is converted with high efficiency into a different form.

Mechanism of Enzyme Action

Chemical reactions occur only when the reactants have sufficient energy to move about in such a way that they collide with each other and product is formed. Each reaction is said to have an energy barrier.

Activation Energy (Fig. 5.9.1)

The minimum energy that the reactants should possess to overcome the energy barrier is called activation energy. The best way to energize the reactant molecules is by heat.



Platinum: If used, reaction can proceed at much lower temperature.

Main function of a catalyst is to lower the activation energy for the reactions.

Enzymes as Biocatalysts: Enzymes lower the activation energy of reactions so that reactions can take place at body temperature.

Activation Energy Curve

Lowering of activation energy is achieved by providing an alternate reaction path which requires less activation energy (Fig. 5.9.2). This phenomenon is called 'tunneling through'.

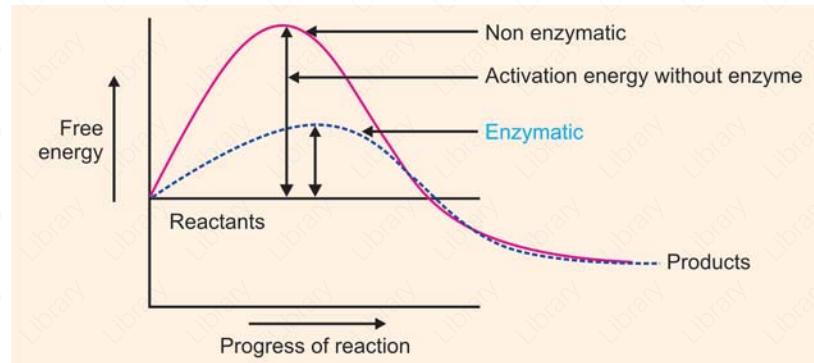


Fig. 5.9.1: Activation energy of a reaction with and without an enzyme

Schematic: Lowering Activation Energy

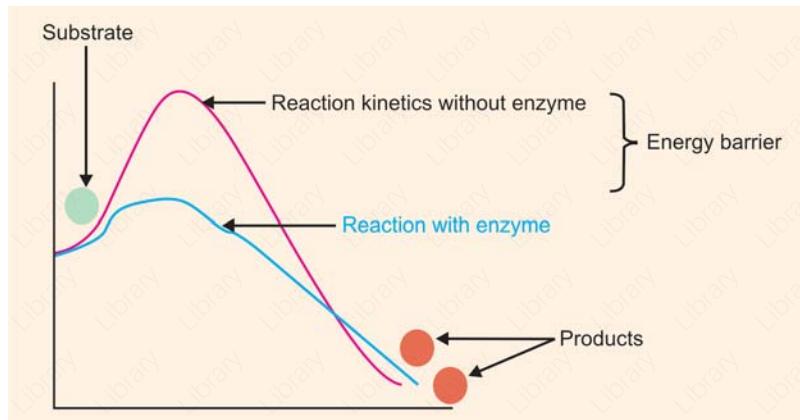


Fig. 5.9.2: Lowering of activation energy by enzymes

Reaction Equilibrium

Like any other catalyst, enzymes do not change the equilibrium of the reactions. Enzymes only increase the speed with which the reaction reaches equilibrium. Once the equilibrium is reached, the enzyme action virtually stops.

Enzymes in the Body

Enzymes never encounter the state of equilibrium since the products of the reactions are continuously being removed by other reactions. All the reactions in the body reach equilibrium immediately after death.

MICHAELIS - MENTEN THEORY

Mode of Action of Enzymes (Fig. 5.9.3)

Michaelis and Menten in 1913 suggested that enzyme binds with the substrate to form an unstable complex which breaks up into products liberating enzymes. Unstable product is called enzyme-substrate complex.

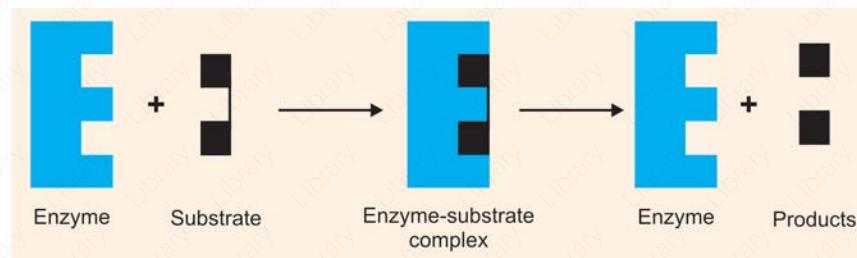


Fig. 5.9.3: ES Complex: Lock and Key Model

Template Hypothesis of Fischer (Fig. 5.9.4)

The active site of the enzyme where substrate binds is a rigid structure. Three dimensional structure of the active site is complementary to the substrate, thus only correct substrate can properly fit in just like Lock and Key.

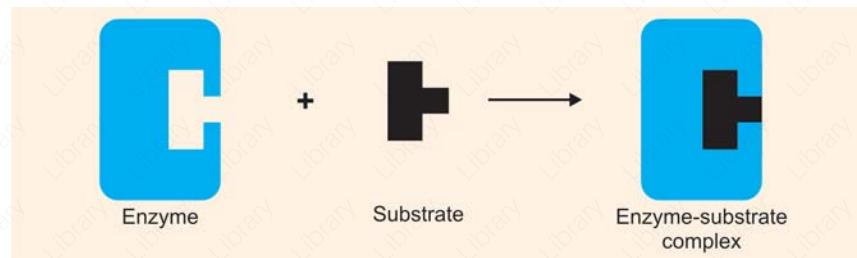


Fig. 5.9.4: Template hypothesis of Fischer

Features of the Model

1. Absolute substrate specificity.
2. Ordered binding of two or more substrates.

Drawback: Fails to explain the effect of allosteric modulators.

INDUCED FIT THEORY

Active site of the enzyme is not rigid and pre-shaped. It is a flexible structure which can undergo conformational changes when the correct substrate binds. The interaction of the substrate induces a fit or conformational change in the enzyme, so that substrate can correctly fit in and catalysis can be brought about. This is the most accepted theory (Fig. 5.9.5).

Features of the Model

1. Specificity.
2. Allosteric modulation.
3. Competitive inhibition on enzymes.

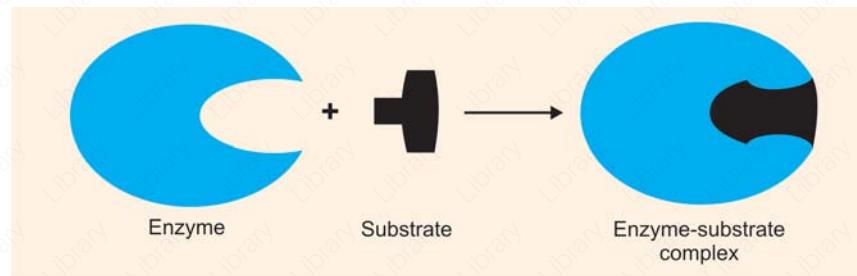


Fig. 5.9.5: ES Complex: Koshland's Model

Active Site (Fig. 5.9.6)

Small region on the enzyme where substrate binds and catalysis takes place.

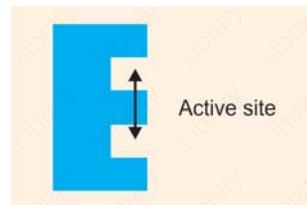


Fig. 5.9.6: Active site on the enzyme

Salient Features

Active site is seen as depression or cleft on enzyme molecule. It is made up of amino acids which are far apart in primary structure, but come closer to each other by conformational changes, brought about by substrate binding. It is a flexible structure.

Amino acids at active site are of three types:

1. Catalytic residues.
2. Contact residues.
3. Specificity residues.

Substrate - Binding Site

Depending on the catalytic residues at active site, enzymes can be serine proteases, zinc proteases, lysine enzymes, cysteine enzymes, histidine enzymes, aspartic enzymes. Specificity of the enzyme lies in the active site. Substrate (s) binds at the active site by weak non-covalent bonds. Enzyme activity is lost if active site is disrupted.

ACTIVE SITE OF LYSOZYME

Lysozyme

General purpose of hydrolytic enzyme is seen in body secretions such as saliva, tear and mucus. It hydrolyzes bacterial cell wall mucopolysaccharides and thereby kills the bacteria. It is a single polypeptide chain with 129 amino acids. It acts by hydrolyzing the β -1,4-glycosidic bond between N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG).



The active center can fit six carbohydrate units of which four (A,B,C and F) are adherent to the binding site. The catalytic site has one glutamic acid and one aspartic acid opposing the labile β -1, 4 linkage. The bond is attacked by the negative charge of the aspartic acid to form a carbonium ion, while the glutamic acid acts as a proton donor.

Enzyme Specificity

Enzymes are highly specific in their action when compared with the chemical catalysts. Three types of enzyme specificity are well recognized.

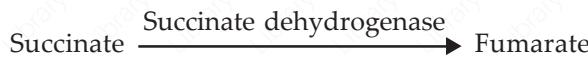
1. Stereospecificity
2. Reaction specificity
3. Substrate specificity.

Stereospecificity: Further divided into:

- a. *D-L specificity:* Enzymes of amino acid metabolism can act only on L-isomers. Enzymes of carbohydrate metabolism can act only on D-isomers.



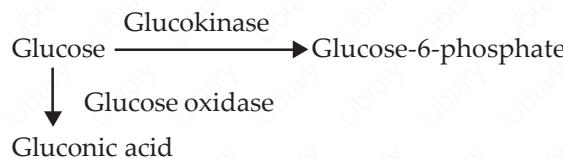
- b. *Cis-Trans specificity:* Enzymes can act only on one type of isomer.



Reaction and Substrate Specificity

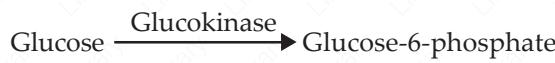
- a. *Reaction specificity:* Enzymes are specific to reaction they catalyze even though a substrate can undergo a variety of reactions.

Example:



- b. *Substrate specificity:* Absolute substrate specificity: Some enzymes are highly specific to only one substrate.

Example: Glucokinase.



Broad Substrate and Group Specificity

- a. *Broad substrate specificity:* Enzymes are specific to a group of structurally related substrates.

Example: Hexokinase



- b. *Group specificity:* Many enzymes can act only on substrates having particular groups or bonds. Example: Pepsin can hydrolyze peptide bonds formed by aromatic amino acids (Fig. 5.9.7).

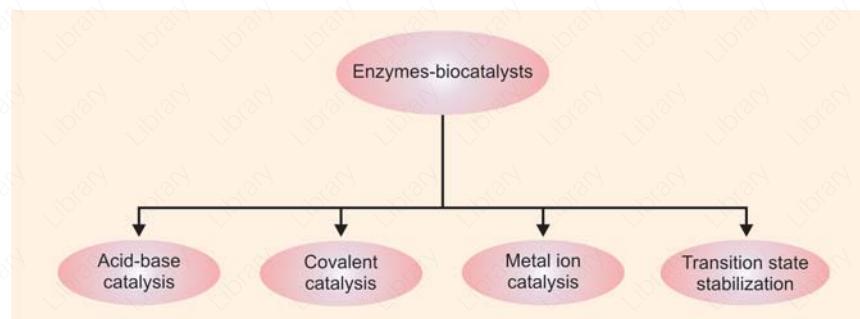


Fig. 5.9.7: Enzyme catalysis—concept map

Mechanisms of Catalysis

Acid-base catalysis: The catalytic activity of an enzyme may be sensitive to pH, since pH can influence the state of protonation of the side chain at the active site. At the physiological pH, the protonated form of histidine is the most important general acid while its conjugate base is an important general base.

Acids: Imidazole group of histidine, the -SH group of cysteine, the -OH group of tyrosine, and the •-amino group of lysine can also act as acids.

Bases: The general bases are carboxylic acid anions and the conjugate bases of the general acids.

Covalent Catalysis

This process accelerates the reaction rate through the transient formation of a catalyst-substrate covalent bond.

Formation: Usually this covalent bond is formed by the reaction of a nucleophilic (negatively charged) group on the catalyst with an electrophilic (positively charged) group on the substrate.

Good covalent catalysts: Functional groups in proteins, such as the •-amino group of lysine, the imidazole group of histidine, the -SH group of cysteine, the -COOH group of aspartate and the -OH group of serine, which have high polarity, are good covalent catalysts. Serine proteases, such as trypsin, chymotrypsin and thrombin are some of the examples of the enzymes acting by the covalent catalytic mechanism.

Metal Ion Catalysis

Metalloenzymes: The enzymes which require the presence of a metal ion for their catalytic activity are called metalloenzymes. They contain tightly bound metal ion cofactors, such as Fe^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} etc. Metal ions facilitate substrate binding and thus catalysis, by forming several kinds of bridge complexes of the enzyme, the metal and the substrate.

Example: Carbonic anhydrase contains Zn^{2+} which is implicated in the catalytic mechanism of the enzyme.

Transition State Stabilization

In an enzyme catalyzed reaction, the reactants and the products correspond to low free energy structures.

Transition state: The point of highest free energy is called as the transition state of the system, in which the reactants are partially converted to products. An enzyme binds in the transition state of the reaction it catalyzes, with great affinity than either of its substrate or the product.

Abzymes

Abzymes are the artificially synthesized catalytic antibodies against the enzyme substrate complex in the transition state of the reaction. Abzymes exhibit the same kind of substrate specificity as do enzymes and yield products with defined stereochemistry.

ENZYME KINETICS

Enzyme kinetics can be studied under three headings such as:

1. Factors affecting enzyme activity
2. Michaelis-Menten kinetics
3. Inhibition.

Factors Affecting Enzyme Activity

1. Concentration of the substrate and Michaelis-Menten kinetics
2. Temperature
3. pH
4. Product concentration.

Effect of Substrate Concentration (Fig. 5.9.8)

There are three phases:

1. Keeping all other factors constant, if substrate concentration is increased progressively, the velocity increases proportionately in initial phases.
2. But later with further increase in substrate concentration, increase in velocity is not proportionate.
3. When substrate concentration is increased further, the reaction attains maximal velocity (V_{max}) after which velocity of reaction is independent of substrate concentration.

A graph where substrate concentration [S] is plotted on the x-axis and velocity [V] on the y-axis.

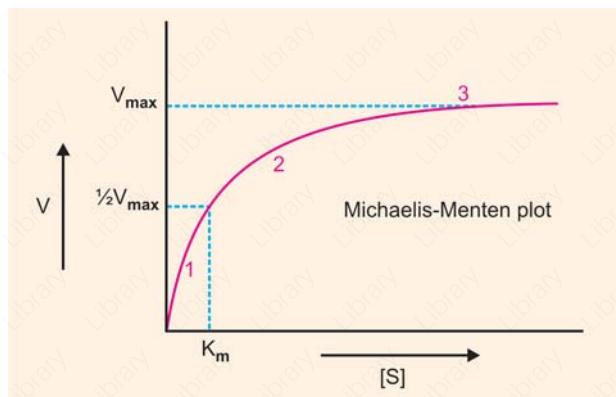


Fig. 5.9.8: Effects of substrate concentration

Hyperbolic substrate saturation kinetics: When such a graph is plotted, a rectangular hyperbola is obtained which has 3 parts.

1. Straight line where V is directly proportional to [S].
2. Curve where velocity increases with [S] but it is not proportionate.
3. Plateau where velocity is independent of [S].

Initially since concentration is low, most of the enzyme molecules remain idle, but as the concentration of [S] increases, more enzyme molecules come into the act increasing the velocity of the reaction. When concentration of substrate is still increased, all the enzymes are saturated and thus increase of [S] will not have any effect on the velocity of the reaction.

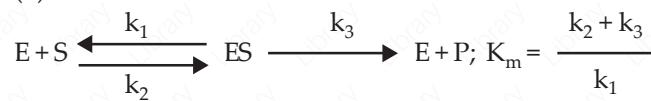
Michaelis-Menten Equation

The relationship between substrate concentration and reaction velocity can be derived by Michaelis-Menten equation.

Propositions of the Equation

An enzyme (E) forms enzyme-substrate complex (ES), with a single substrate (S).

The complex (ES) is broken down, into free enzyme (E) and the product (P).



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

V_0 - Initial reaction velocity

V_{\max} - Maximum velocity of a reaction

[S] - Concentration of the substrate

K_m - A constant, called Michaelis-Menten Constant

Michaelis-Menten Constant

K_m value

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

If velocity of the reaction is half the maximal velocity.

$$V = \frac{1}{2} V_{\max}$$

$$\frac{V_{\max}}{2} = \frac{V_{\max} [S]}{K_m + [S]}$$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

Thus, when velocity of the reaction is half of the maximum, K_m value equals substrate concentration.

Michaelis-Menten Plot

K_m value can be defined as the substrate concentration that would produce half the maximal velocity which is expressed in moles/liter. The substrate concentration corresponding to half the maximal velocity will give numerical value of K_m (Fig. 5.9.9).

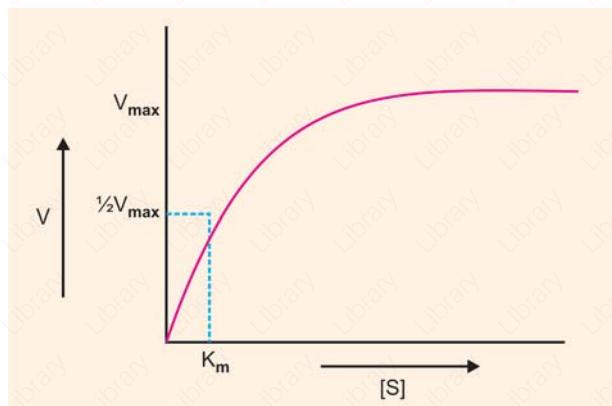


Fig. 5.9.9: Michaelis–Menten plot

Double-Reciprocal Plot

Drawback of Michaelis-Menten equation is that, it does not give a straight line. Hence, for determination of K_m value, velocities should be measured at 6-7 different substrate concentrations so as to get a hyperbola. Practically, it is difficult to obtain V_{max} at laboratory conditions. It makes determination of K_m value difficult.

To circumvent this problem, the Michaelis-Menten equation can be rearranged so as to give a straight line, where the number of velocity measurements can be reduced to minimum of two and then K_m can be calculated. A linear plot that is obtained by rearranging the equation is called as Lineweaver-Burk plot or double reciprocal plot.

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

Taking the reciprocal of both sides of the Michaelis-Menten equation.

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

Rearrangement of M-M Equation

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

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

$$\text{Therefore, } \frac{1}{V_0} = \frac{K_m}{V_{\max}} \times \frac{1}{[S]} + \frac{1}{V_{\max}}$$

This is an equation for the straight line as it conforms to the form, $y = mx + c$.

Lineweaver-Burk Plot (Fig. 5.9.10)

If $1/V_0$ is plotted against $1/[S]$, we get a straight line.

Slope of the line = K_m/V_{\max}

Intercept of the line = $1/V_{\max}$

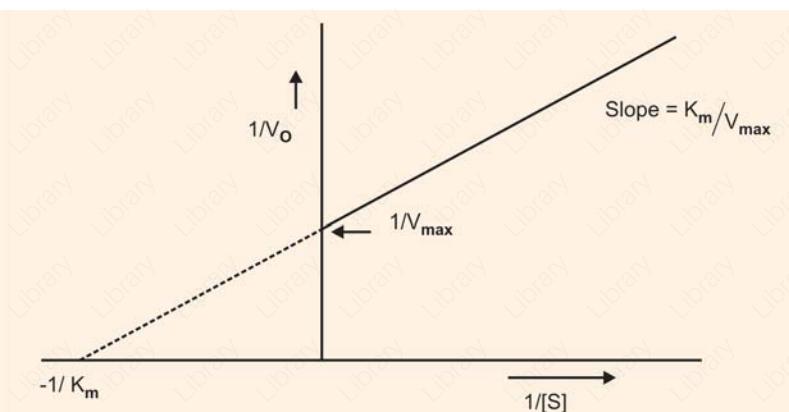
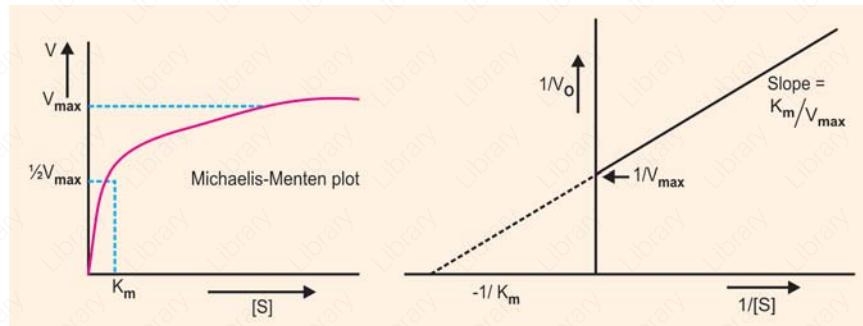
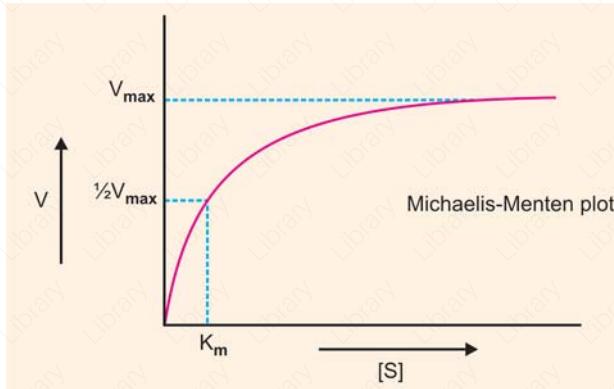


Fig. 5.9.10: Lineweaver-Burk plot

K_m Value: It is the substrate concentration at $\frac{1}{2}$ maximal velocity of an enzyme catalyzed reaction. It is calculated by plotting either Michaelis-Menten plot or Lineweaver-Burk plot (Fig. 5.9.11).

**Fig. 5.9.11:** Michaelis-Menten plot and Lineweaver-Burk plot

1. K_m is expressed in moles/liter.
2. K_m value is constant for a given enzyme with a particular substrate.
3. K_m signifies the affinity of an enzyme for a substrate.
4. Affinity is inversely related to K_m .
Affinity = $1 / K_m$, more the K_m value, lesser the affinity of enzyme towards the substrate.

Michaelis-Menten Plot and Order of Reaction (Fig. 5.9.12)**Fig. 5.9.12:** Order of reacting

The initial phase that is when $[S]$ is lesser than K_m , the velocity of the reaction is directly proportional to substrate concentration, then the reaction is said to be a 1st order reaction.

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

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]} \quad \text{Since } [S] < K_m$$

$$K_m + [S] = K_m$$

Zero Order Reaction

$$V_0 = [S]$$

$$V_0 \propto [S]$$

When the velocity of the reaction attains maximum, the rate of reaction is independent of $[S]$.

An enzyme catalyzed reaction, proceeding under V_{\max} conditions where the rate of reaction is independent of substrate concentration is said to be a **zero order reaction**.

When $[S] > K_m$

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

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

$$V_0 = V_{\max}$$

Sigmoid Kinetics

Some enzymes, which have many subunits, may not strictly follow the Michaelis-Menten kinetics. Each subunit behaves like a separate enzyme, though they are bound together. Binding of substrate on one subunit, enhances the affinity for binding on other subunits. It is called cooperative binding. Thus, affinity goes on increasing as the substrate goes on binding to the subunits and affinity of the last subunit is many more folds than the first subunit.

It was originally described for the hemoglobin but holds good for all enzymes having subunits. Such enzymes (allosteric) will not obey the Michaelis-Menten equation, when V is plotted against $[S]$, a sigmoid-shaped curve is obtained (Fig. 5.9.13).

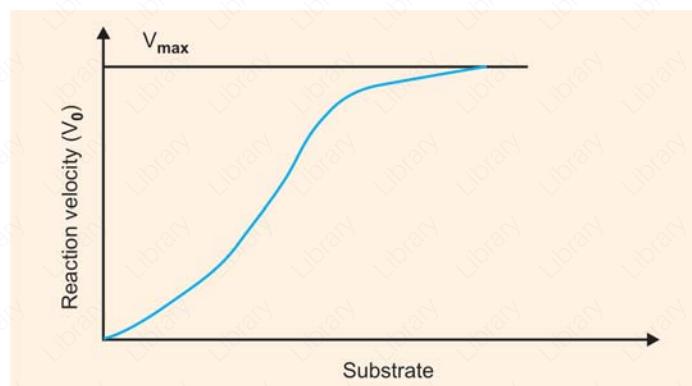


Fig. 5.9.13: Sigmoid kinetics

Effect of temperature: As the temperature increases, the velocity of enzyme catalyzing reaction also increases up to a particular temperature. Thereafter, increase in temperature decreases the velocity.

Optimum temperature: Temperature at which enzyme activity is maximum. As the temperature increases, kinetic energy of the reactant molecules increases and so, the reaction proceeds faster. For most of the enzymes, optimum temperature is near body temperature, 37°C (Fig. 5.9.14).

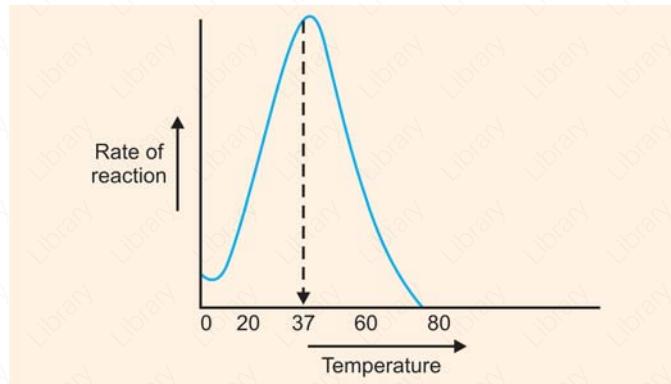


Fig. 5.9.14: Optimum temperature

With further rise in the temperature of the reaction, the enzyme which is a protein is denatured and results in loss of its activity. The relationship of the enzyme activity to a change in temperature is usually represented by a bell-shaped curve, the peak of which denotes optimum temperature.

Effect of pH: At optimum pH or pH range, enzyme activity is maximal and at higher or lower pH, enzyme activity decreases. Most of the intracellular enzymes have optimum pH in the neutral range, around 7. Pepsin and trypsin (proteolytic enzymes) have optimum pH of 2 and 8 respectively. A bell shaped curve is obtained when velocity is plotted against pH.

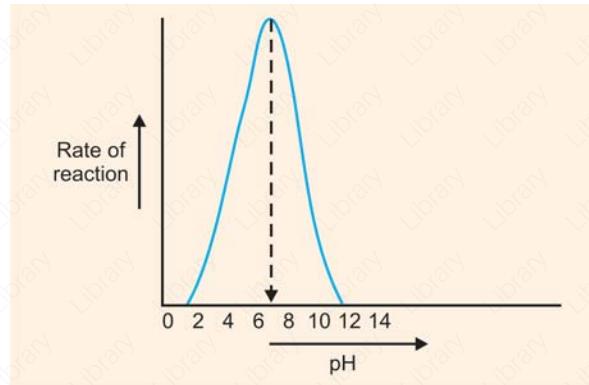


Fig. 5.9.15: Effect of pH

Change in pH may alter the ionization of both the substrate and active site of the enzyme. It may dissociate the apo-enzyme from the prosthetic group and even inactivate the enzyme by disturbing the ionic and hydrogen bonds stabilizing its 3-D structure.

Effect of product concentration: Reaction products may occupy the active site of the enzyme and make it unavailable for substrates. Rise in product concentration reduces the rate of an enzymatic reaction. Presence of activators, inhibitors and coenzymes also modify the enzyme activity.

Enzyme Inhibition

Inhibitors are the chemical substances which inhibit enzyme activity and reduce velocity of an enzyme catalyzed reaction. Enzyme inhibitors can be inorganic ions or organic substances. There are two broad classes of enzyme inhibitions:

1. Reversible
2. Irreversible

Reversible Inhibition

Reversible inhibition is further divided into three types:

- Competitive inhibition
- Non-competitive inhibition
- Uncompetitive inhibition

Competitive Inhibition

It is a common type of reversible inhibition. Inhibitor is a structural analogue of the substrate. A competitive inhibitor competes with the substrate for the active site of an enzyme. When inhibitor binds to the active site of enzyme, EI complex is formed. Affinity of the enzyme to the substrate decreases. But when substrate concentration is increased, it pushes the inhibitor from active site, then enzyme can form ES complex. And thus inhibition can be overcome. So it is reversible. K_m value is increased but V_{max} is not altered (Fig. 5.9.16).

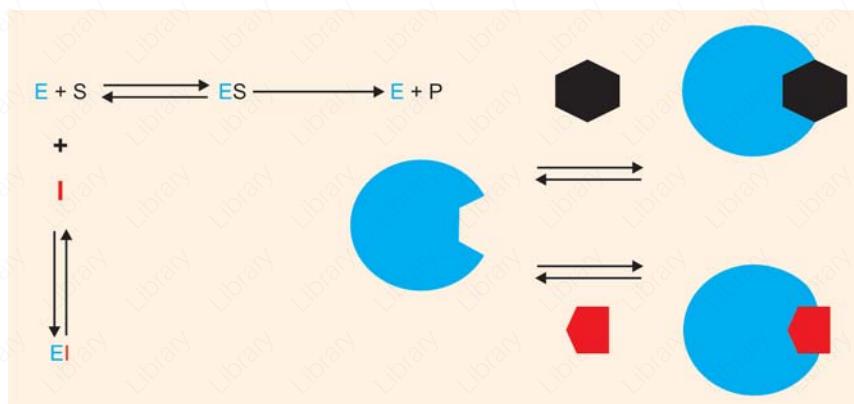
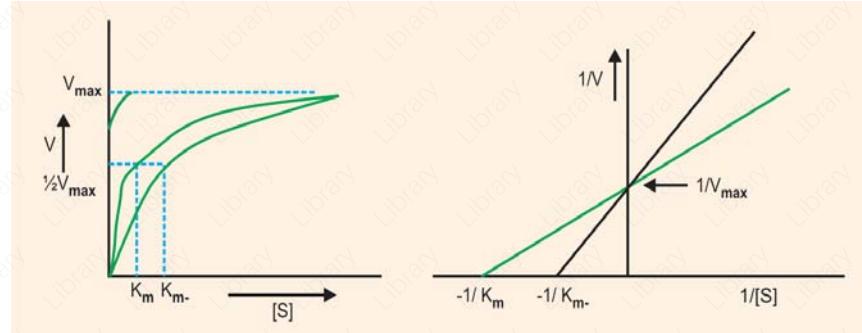
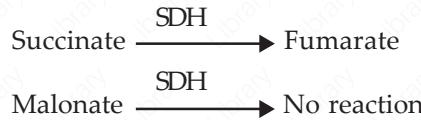


Fig. 5.9.16: Competitive inhibition—reversible

Plots - Competitive Inhibition (Fig. 5.9.17)**Fig. 5.9.17:** Competitive inhibition between Michaelis-Menten plot and Lineweaver-Burk plot

Example: Succinate dehydrogenase is inhibited by malonate since it structurally resembles succinate.



Medical importance: Competitive inhibition is of great medical importance. A large number of drugs act by competitive inhibition on several enzymes. Sulfa drugs have structural similarity to PABA (Para Amino Benzoic Acid). When these drugs are given, they compete for enzyme that converts PABA to folic acid. Thus, formation of folic acid is inhibited. Sulfa drugs are used as antibiotics. Folic acid is needed for cell division in bacteria.

Methotrexate bears structural similarity with Dihydrofolic acid (DFA) and competitively inhibits dihydrofolate reductase which converts DFA to TFA (Tetrahydrofolic acid). Methotrexate is used as an anti-cancer drug.

Ethanol is used in methanol poisoning. Ethanol is a structural analogue of methanol. Hence, alcohol dehydrogenase preferably binds with ethanol and thus formaldehyde formation is inhibited.

Non-competitive Inhibition

No competition occurs between substrate and inhibitor. Inhibitor does not bear structural resemblance to substrate. It binds at a site other than the active site on the enzyme surface. The non-competitive inhibitor can bind to either free enzyme or the ES complex. This brings about changes in the 3-D conformation of the enzyme, preventing the reaction from occurring (Fig. 5.9.17).

Schematic representation: The inhibitor effectively lowers the concentration of active enzyme and hence lowers the V_{max} . K_m value is not altered since it does not occupy the substrate binding site of the enzyme. *Example:* Lead

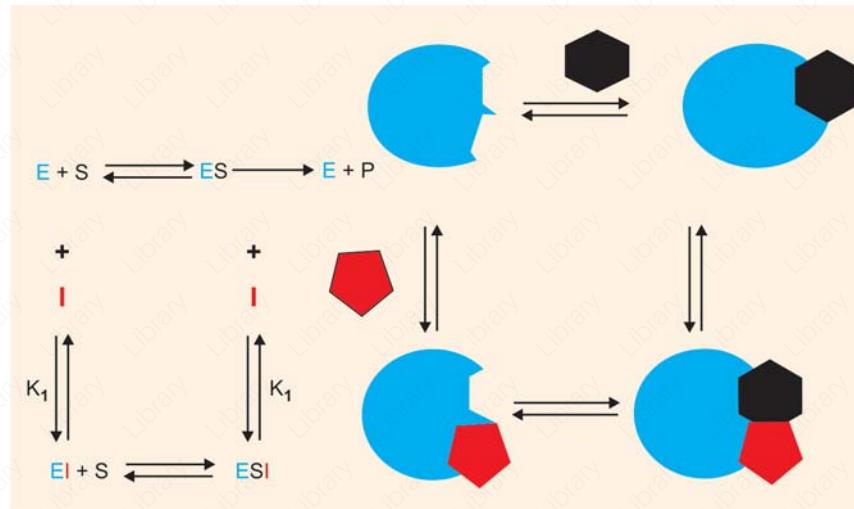


Fig. 5.9.17: Non-competitive inhibition

poisoning: Lead forms covalent bonds with the sulfhydryl side chains of cysteine in proteins. The binding of the heavy metals shows noncompetitive inhibition.

Uncompetitive Inhibition

It is a type of reversible inhibition, where the inhibitor binds to the enzyme-substrate complex and inhibits its dissociation into products. It does not bind to the free enzyme. Such an inhibitor may not resemble the substrate. In multisubstrate enzymes, the effect of an uncompetitive inhibitor is more complex. Both K_m and V_{max} are decreased.

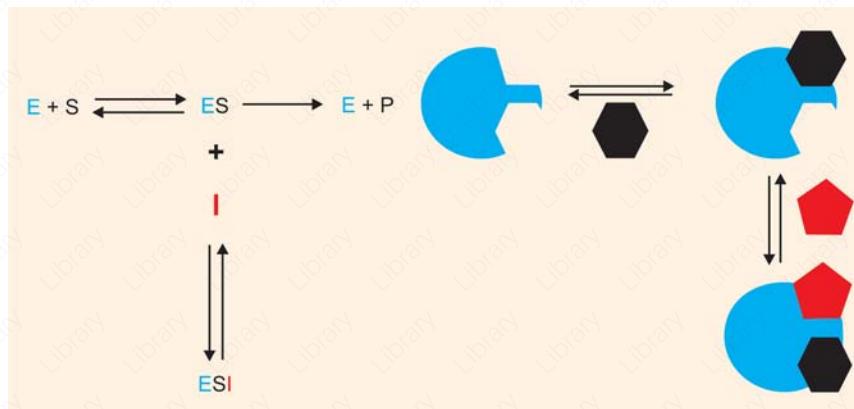


Fig. 5.9.18: Uncompetitive inhibition

Irreversible Inhibition

Irreversible inhibitors combine with or destroy a functional group on the enzyme that is essential for its activity. These inhibitors are covalently

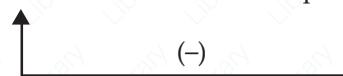
linked to the active site of the enzymes. Some of the inhibitors are iodoacetate, iodoacetamide and di-isopropylfluoro-phosphate (DFP).

Suicide Inhibition

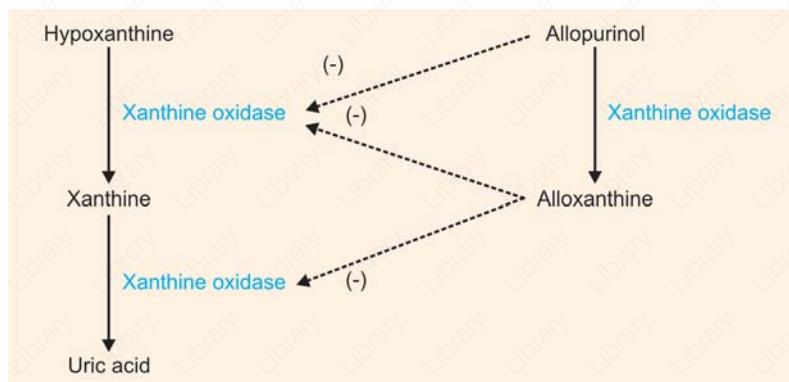
Suicide inhibitors are a very special class of irreversible inhibitors. These compounds become reactive only after they bind to the active site of a specific enzyme. Inhibitor is a structural analogue of the substrate. Inhibitor is acted upon by the enzyme itself to produce a product which is another substrate analogue having a more powerful inhibitory effect on the same enzyme.

Mechanism-based Inactivation

Inhibitor ————— Enzyme to be inhibited ————— More powerful inhibitor



Example: Suicide Inhibition: Inhibition of xanthine oxidase by allopurinol



Alloxanthine is a more powerful inhibitor than allopurinol.

Regulatory Properties of Enzymes

Regulatory enzymes are groups of enzymes working together in sequential pathways to carry out a given metabolic process. Example: Multi-reaction conversion of glucose into lactate in skeletal muscle. In each enzyme system, there is at least one enzyme that catalyzes the rate limiting reaction. It is known as a 'regulatory enzyme'. The activity of regulatory enzymes is modulated through various types of signal molecules, generally small metabolites or cofactors.

Classes of Regulatory Enzymes

1. Allosteric enzymes acting through reversible, non-covalent modulator.
2. Enzymes regulated by reversible covalent modification.

Features

1. The allosteric sites are different from its active site.
2. Regulatory substances are known as allosteric modulators.
3. Positive modulators are activators and negative modulators are inhibitors.

Irreversible Covalent Activation

Many digestive (proteolytic) enzymes are synthesized in the inactive form. Such inactive forms of enzymes are known as proenzymes or zymogens. Activation of zymogens involves irreversible covalent modification.

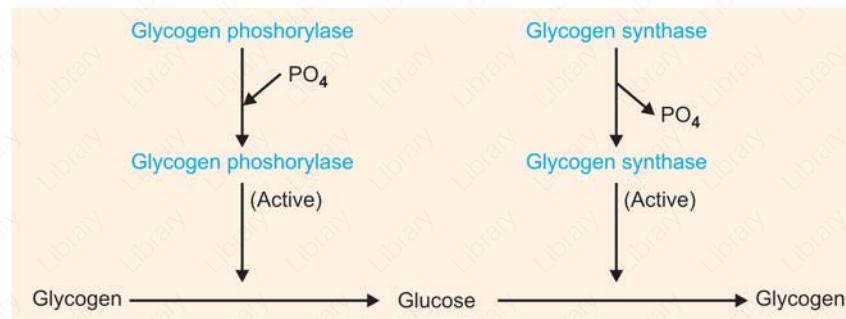
Example:

Zymogen	Active form
Trypsinogen	Trypsin
Pepsinogen	Pepsin
Procarboxypeptidase	Carboxypeptidase

Zymogen activation usually involves removal of a small peptide from the proenzyme, at a suitable pH. Once pepsin is formed, that itself will catalyze the conversion of further pepsinogen to pepsin. Secretion of enzymes in zymogen form prevents autolysis of organs.

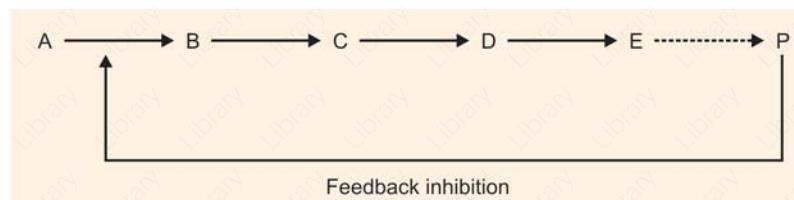
Reversible covalent modification: Alteration in enzyme activity brought about by either addition of a group or removal of a group involving a covalent bond. It can be reversible or irreversible. Reversible covalent modification usually involves either addition or removal of phosphate group. These are known as phosphorylation and dephosphorylation mechanisms. Some enzymes are active in phosphorylated state while some are active in dephosphorylated state. Example: Glycogen phosphorylase and glycogen synthase.

Glycogen phosphorylase is a key enzyme in glycogen breakdown, active in phosphorylated state but inactive in dephosphorylated state. Glycogen synthase is a key enzyme in glycogen synthesis, active in dephosphorylated state but inactive in phosphorylated state. Example: Covalent Modification- Glycogen synthesis and glycogen breakdown pathways are reciprocally regulated.



Allosteric modulation: Allosteric activator binds to the allosteric site and it causes favorable conformational changes in the active site and increases the affinity of the enzyme towards the substrate. This phenomenon is called **Allosteric modulation**. Allosteric modulator is structurally unrelated to substrate. Enzymes which exhibit allosteric modulation usually have multiple subunits and hence they obey Hill's kinetics. Allosteric enzymes can be of two types (as per their kinetics): **K-series**: Allosteric modulator changes the K_m value but V_{max} is not altered. Example: Aspartate transcarbamoylase and PFK (Phosphofructo kinase). **V-series**: V_{max} is altered but K_m is not changed. Example: Acetyl CoA carboxylase.

Feedback Inhibition: Examples of allosteric enzymes are acetyl CoA carboxylase, pyruvate kinase, phosphofructo kinase, aspartate transcarbamoylase. Allosteric inhibition is usually observed in the case of feedback inhibition or end product inhibition. When a substrate (A) is converted to an end product (P) through various intermediaries such as B, C, D etc, the end product inhibits the first enzyme of the pathway.



Classic examples: When the final product of the pathway inhibits the first reaction of the same pathway, such an inhibition is called as feedback inhibition. This provides an efficient mechanism by which metabolic pathways can be controlled in the body.

Example:

1. *Heme Biosynthesis:*



Heme acts as an allosteric inhibitor of ALA synthase.

2. *Cholesterol biosynthesis:*



Compartmentation: Localization of enzymes related to one pathway partly in mitochondria and partly in cytosol is called compartmentation. *Example:* Enzymes of urea cycle, enzymes of heme biosynthesis. When enzymes of a particular pathway are compartmentalized, for the pathway to proceed,

the intermediates have to be transported across mitochondrial membrane, which provides a point where controls can be exerted. Generally synthetic and breakdown pathways are operative in different organelles for the purpose of maximum economy and efficient regulation. *Example:* Fatty acid oxidation occurs in mitochondria, whereas fatty acid synthesis occurs in the cytosol.

Co-enzymes

Many reactions of substrates are catalyzed by enzymes only in the presence of a specific non-protein organic molecule called coenzyme. Co-enzymes combine with the apoenzyme (the protein part) to form holoenzyme. The co-enzymes are regarded as co-substrates.

They are heat stable, dialyzable non-protein organic molecules and the prosthetic groups of enzymes.

Classification

1. *Based on chemical characteristics:* Containing an aromatic hetero ring.
Example: ATP and its relatives, NAD, NADP, FMN, TPP, pyridoxal phosphate.
Containing a non-aromatic hetero ring, *Example:* Biotin, lipoic acid.
No hetero ring, *Example:* Sugar phosphate, co-enzyme Q.
2. *Based on functional characteristics:* Group transferring coenzymes. *Example:* ATP and its relatives, sugar phosphate, TPP, coenzyme A, pyridoxal phosphate, biotin.
Hydrogen transferring coenzymes, *Example:* NAD and NADP, FAD and FMN, coenzyme Q.
3. *Based on nutritional characteristics:* Containing B vitamins. *Example:* TPP, FAD, FMN, NAD, NADP, pyridoxal phosphate, biotin, coenzyme A, folic acid coenzyme, B₁₂ coenzyme.

Functions

1. They accept atoms or groups from substrate to transfer them to other molecules.
2. They are less specific than enzymes and thus same coenzyme can act in a number of different reactions.
3. NAD and NADP coenzymes function as hydrogen acceptors in dehydrogenase reactions.
4. Co-enzyme A carries acyl groups used in the oxidative decarboxylation of pyruvic acid and synthesis of fatty acids and acetylation.
5. TPP carries active aldehyde group.
6. Pyridoxal phosphate is involved in transamination reactions.
7. Tetrahydrofolic acid is a carrier of formate and is used in the synthesis of purines and pyrimidines.

Ribozymes

Enzymes made up of RNA are called ribozymes. They are the catalytic RNA molecules with sequence specific cleavage activity. They exhibit Michaelis-Menten kinetics and have high catalytic activity. They show high fidelity, specificity and low toxicity. They are the vestigial remnants. RNase-P is a ribozyme which generates the ends of tRNAs. Peptidyl transferase present in ribosomes is another example of ribozymes.

Significance

Ribozymes can recognize and cleave target RNA, hence they are tried in gene therapy to decrease translation of protein.

Isoenzymes

They are multiple forms of an enzyme. They catalyze the same reaction. Their tissue of origin is different. They differ in their physical and chemical properties such as structure, electrophoretic and immunological properties, K_m and V_{max} values, optimum pH, relative susceptibility to inhibitors and degree of denaturation.

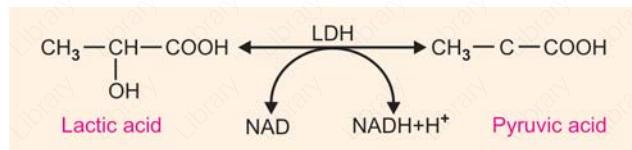
The differences between some isoenzymes are due to differences in the quaternary structure of the enzymes. For example, lactate dehydrogenase exists in five isozymic forms.

Isozymes having wide clinical applications are lactate dehydrogenase, creatine phosphokinase and alkaline phosphatase.

The most common mechanism for the formation of isoenzymes involves the arrangement of subunits, arising from different genetic loci, in-different combinations to form the active polymeric enzyme.

Example:

1. **Lactate dehydrogenase** catalyzes the conversion of lactate and pyruvate.



LDH is a tetramer. It has 4 polypeptide subunits. Each subunit may be one of the two types, known as the H type (heart type) and the M type (muscle type). LDH exists in serum in 5 distinct forms (isoenzymes), which have different proportions of H and M subunits. (LDH_1 to LDH_5).

<i>Isoenzyme</i>	<i>Subunits</i>	<i>(Tissues of origin)</i>
------------------	-----------------	----------------------------

- | | | |
|----------------|--------------------------|--|
| LDH_1 | - H_4 | \rightarrow Heart (increases in myocardial infarction) |
| LDH_2 | - H_3M | \rightarrow RBC |
| LDH_3 | - H_2M_2 | \rightarrow Brain |

LDH_4 - HM_3 → Liver

LDH_5 - M_4 → Skeletal muscle (increases in liver diseases)

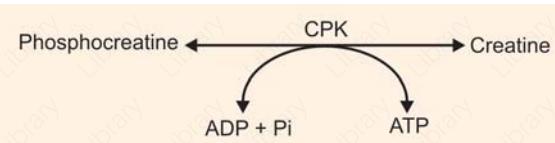
In healthy individuals, LDH_2 activity is higher. In M.I. patients, LDH_1 activity is seen in first 12 - 24 hours.

Lactate dehydrogenase in serum is determined by Spectrophotometric method of Wrobleksi and LaDue (1955), Mcqueen (1972) and Mactiners.

LDH is determined by Colorimetric methods of Amador et al (1963), King using dinitrophenyl hydrazine with lactate and INT and PMS with lactate as substrate.

Normal range of lactate dehydrogenase - 100 - 200 IU/L

2. **Creatine phosphokinase (CPK)** catalyzes the inter-conversion of phosphocreatine to creatine.



CPK exists as three isoenzymes. Each isoenzyme is a dimer composed of 2 subunits- M (muscle) or B (brain) or both.

CPK_1 BB Brain

CPK_2 MB Heart

CPK_3 MM Skeletal muscle

In healthy individuals, CPK_2 is undetectable. In MI patients, CPK_2 increases within 6 - 18 hours of infarction. Therefore CPK_2 (MB) is the earliest reliable indication of MI. Estimation of CPK is done by kinetic method.

Normal range of creatine phosphokinase :

Female - 10 - 80 U/L

Male - 15 - 100 U/L

3. **Alkaline phosphatase (ALP):** It is a monomer, a glycoprotein. The isoenzymes are due to difference in carbohydrate content (sialic acid residues). It is mainly produced by osteoblasts of bone. ALP hydrolyses phosphoric acid esters at optimum pH 9 - 10

Important isoenzymes of ALP are α_1 , ALP, α_2 heat labile ALP, α_2 heat stable ALP, pre- β -ALP, γ -ALP etc.

Increase in α_2 heat labile ALP suggests hepatitis. Increase in pre- β -ALP indicates bone diseases. Alkaline phosphatase is determined by King and King Method. Advantages of this method are simple and popular, it gives reproducible results and it does not require deproteinization of serum. It is increased in obstructive liver diseases (gallstones, malignancy, viral hepatitis), bone diseases (rickets, malignancy). Estimation of isoenzymes may be useful to differentiate liver disease from bone disease.

Normal value of ALP is 3 - 13 KAU/dl.

Acid phosphatase (ACP): Prostate gland is the richest source for ACP. Other sources are red blood cells, platelets, bone etc. ACP is estimated by 4-aminoantipyrine method and kit method.

Diagnostic significance of enzymes: Measurement of various enzymes in serum is helpful in the diagnosis of various diseases. Normally plasma contains 2 types of enzymes.

4. **Functional enzymes:** Present at all times in blood and have specific function to perform. *Example:* Enzymes of blood clotting, lipoprotein lipase.
5. **Non-functional enzymes:** Present in very low concentration (almost nil) in blood.

These do not show any specific function. But found in high concentration in tissues due to normal wear and tear of tissues. Leakage of enzymes from tissues into plasma occurs.

Non functional enzymes are of great significance clinically. When their concentration is abnormally high, it usually signifies the disease of the tissue or organ from which they arise, hence of diagnostic importance.

Diseases	Enzymes
Myocardial infarction	CPK, AST, LDH
Liver diseases	ALT, ALP, LDH, AST, 5'NT, GGT
Muscle diseases	CPK, LDH
Bone disease	ALP
Pancreatitis	Amylase and lipase
Prostate cancer	Acid phosphatase

Gamma Glutamyl transpeptidase (GGT): It is high in alcoholism and liver carcinoma. Moderate increase is observed in other liver diseases. Clinical usefulness is limited to diagnosis of alcoholism.

Transaminases

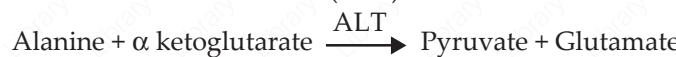
Aspartate aminotransferase (AST) or serum glutamate oxaloacetate transaminase (SGOT)



Main source of the enzyme is myocardial cells. Next source is liver.

Normal level of AST is 8 - 20 IU/L. AST is highly elevated in MI and moderately elevated in liver diseases.

Alanine Aminotransferase (ALT) or SGPT:



Main source is liver.

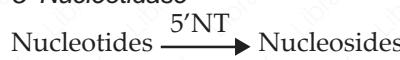
Normal level is 13 - 40 IU/L. Very high values are seen in acute hepatitis.

In liver diseases, rise of ALT is much more than AST. Moderate rise is found in liver cirrhosis.

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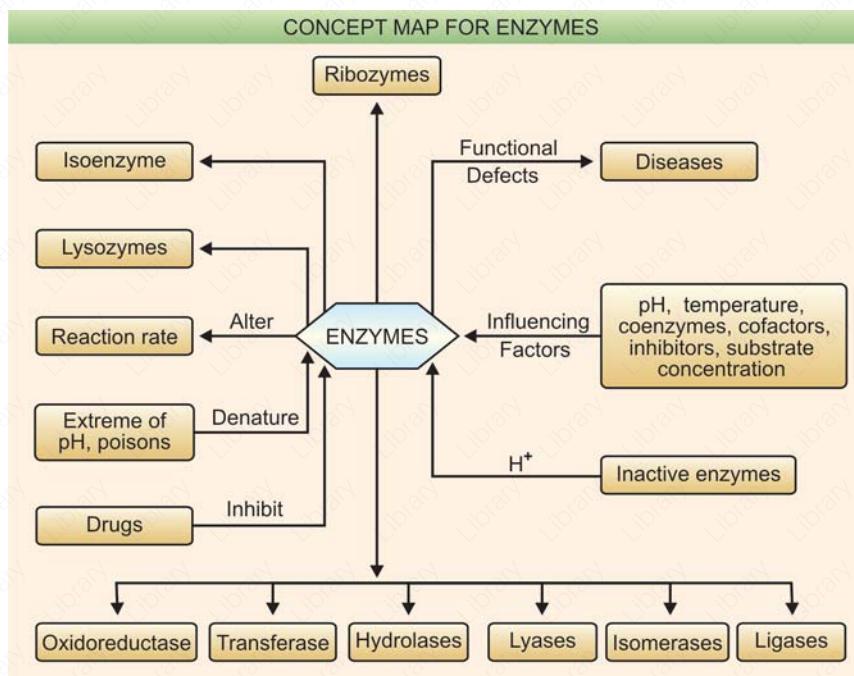
Serum transaminases are determined by kinetic method, DNP method, method of Reitman and Frankel, Sax and Moor method using disodium salt, continuous monitoring method.

5'-Nucleotidase



High values seen in obstructive liver diseases. More specific to liver (unlike ALP).

Normal level is 2 - 15 IU/L.



MULTIPLE CHOICE QUESTIONS

- Alcohol dehydrogenase belongs to the class of:
A. Oxidoreductases B. Hydrolases
C. Transferases D. Ligases
- The velocity of an enzyme reaction decreases after the optimum temperature is reached because:
A. ES complex is not formed B. The enzyme gets denatured
C. The substrate becomes D. The product begins to inhibit unstable
the reaction
- Competitive inhibition of an enzyme is due to:
A. Functional groups of substrate and inhibitor are same
B. Molecular weights of substrate and inhibitor are same
C. Structural similarity of both substrate and inhibitor
D. Structural similarity of both product and inhibitor

4. Relative increase of SGPT over SGOT is an index of:
A. Hepatitis B. Nephritis
C. Myocarditis D. Pancreatitis

5. The diagnostic serum enzyme in acute pancreatitis is:
A. Nucleotidase B. Aspartate amino transferase
C. Amylase D. Trypsin

ANSWERS

1. A 2. B 3. C 4. A 5. C

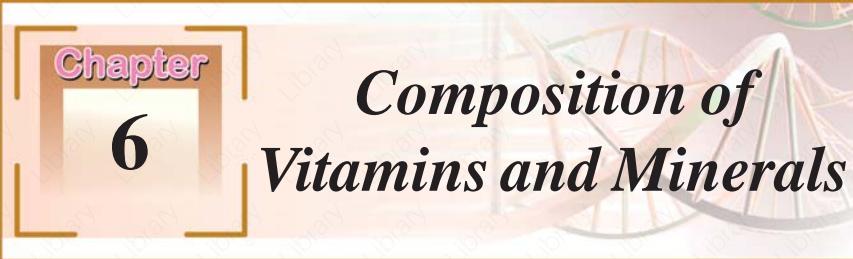
MOST LIKELY QUESTIONS

Long Essays

1. Explain factors affecting enzyme activities.
 2. Explain different types of enzyme inhibitions with suitable examples.
 3. Discuss clinically important enzymes in serum. State their normal levels.
 4. Describe how enzyme activity is regulated in living systems.

Short Essays

5. Classify enzymes with suitable examples.
 6. Factors affecting enzyme action.
 7. What is the effect of substrate concentration on enzyme action? Write Michaelis-Menten equation.
 8. Competitive inhibition.
 9. Discuss the theories to explain mechanism of enzyme action
 10. What is K_m value? Illustrate how it is altered in different types of enzyme inhibitions?
 11. What are isoenzymes? Give examples. What is their significance?
 12. List 5 clinically important enzymes in serum. State their normal levels and significance.
 13. Regulation of enzyme activity.
 14. Covalent modification of enzymes and its significance.
 15. Enzyme specificity.
 16. Effect of temperature on enzyme activity.
 17. Michaelis-Menton equation and K_m value.
 18. Suicide inhibition.
 19. Allosteric modulation.
 20. Creatine phosphokinase.
 21. Effect of pH on enzyme activity.
 22. Proenzymes.
 23. Name the enzymes that are raised in serum in following diseases:
 - i. Myocardial infarction
 - ii. Obstructive jaundice.



Chapter

6

Composition of Vitamins and Minerals

6.1: VITAMINS

Vitamins are organic nutrients that are required in small quantities to perform variety of specific biochemical functions of normal growth and health of human body. Vitamins generally cannot be synthesized by the body and must therefore be supplied by the diet.

Classification

Depending on the solubility, vitamins are classified into fat soluble and water soluble vitamins (Table 6.1.1).

Fat soluble vitamins which include vitamin A (retinol), vitamin D (cholecalciferol), vitamin E (tocopherol) and vitamin K.

Water soluble vitamins include vitamin C (ascorbic acid) and vitamin B complex. A number of water soluble vitamins are grouped in to B complex because they occur together in the same food source. Examples thiamine (B₁), riboflavin (B₂), niacin (B₃), pantothenic acid(B₅), pyridoxine (B₆), biotin (B₇), Folic acid and cyanocobalamin (B₁₂).

Table 6.1.1: Differences between water soluble and fat soluble vitamins

<i>Fat soluble</i>	<i>Water soluble</i>
1. Stored mainly in liver	They are normally not stored except vitamin B ₁₂
2. Deficiency manifestations are not immediate and they are manifested as soon as the stores are depleted.	Deficiency manifestations are immediate
3. They require specific carrier protein for their transport. Example: Vitamin A is transported bound to a protein called retinol binding protein.	They do not require specific carrier proteins
4. Some fat soluble vitamins like A and D act as steroid hormones and bring about translation of certain proteins.	

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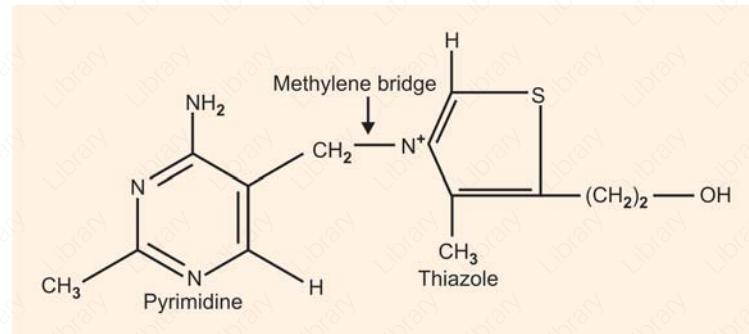
Fat soluble	Water soluble
5. They are toxic and even lethal when taken into excessive quantities and not usually excreted.	Water soluble vitamins are usually nontoxic since excess amounts are easily excreted in the urine.
6. Absorption of fat soluble vitamins require bile salts.	They function as precursors for coenzymes and antioxidants.
7. They function as coenzymes, hormones and antioxidants.	

WATER SOLUBLE VITAMINS

Thiamine (Vitamin B₁)

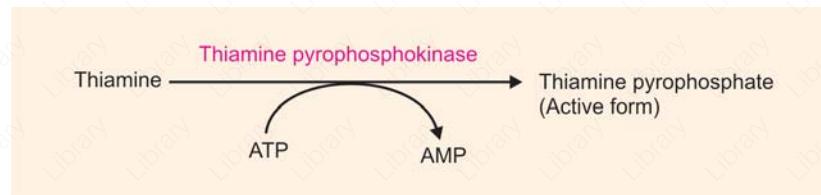
Thiamine is also called as the antiberiberi factor.

Structure



It is structurally made up of a substituted pyrimidine ring attached to a thiazole ring by methylene bridge. The active form of thiamine is thiamine pyrophosphate (TPP). Dietary thiamine is readily absorbed and phosphorylated to its active form TPP in the liver.

Conversion of Thiamine to TPP



Sources

Unpolished rice, outer coat of cereal grains, wheat, oil seeds, nuts, pulses, liver, kidney, egg yolk, yeast, milk. Thiamine is present in aleurone layer of cereals and pulses. Parboiling of rice fixes the aleurone layer and thus parboiled rice is a better source.

RDA

1 - 1.5 mg/day

Thiamine requirements are related to energy metabolism and therefore they are often expressed in terms of energy which is 0.4 mg/1000 kcal. Thiamine intake is increased with increased muscular activity, dietary carbohydrates, in pregnancy and lactation.

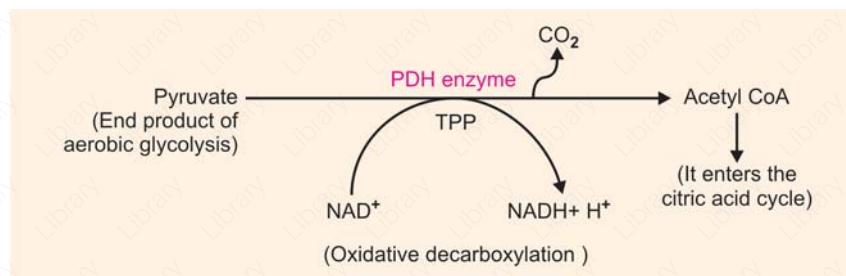
Absorption and Transport

It is absorbed in the small intestine by active transport mechanism and simple diffusion. Then it reaches the liver through circulation.

Functions

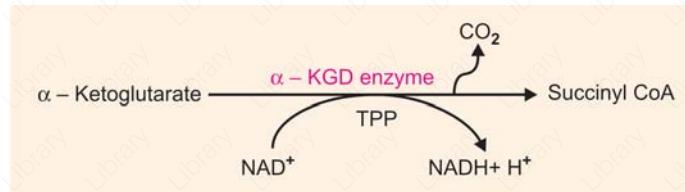
Thiamine pyrophosphate is a coenzyme involved in several enzymatic reactions mainly for oxidative decarboxylation and transketolase reactions as follows:

1. TPP acts as coenzyme of pyruvate dehydrogenase enzyme complex.

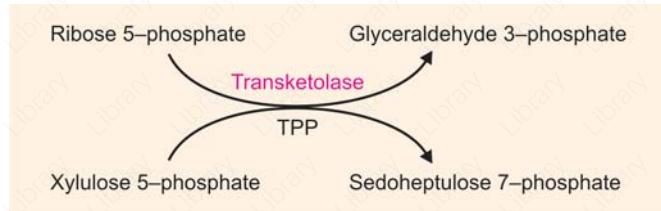


Pyruvate dehydrogenase multienzyme complex requires 5 coenzymes: NAD, FAD, TPP, co-enzyme A, lipoic Acid.

2. TPP is a coenzyme for α -ketoglutarate dehydrogenase enzyme complex which catalyses the conversion of α -ketoglutarate to succinyl CoA in TCA cycle.



3. TPP is a coenzyme for transketolase enzyme in the pentose phosphate pathway of glucose oxidation which produces ribose sugars and supplies NADPH necessary for a wide variety of redox and biosynthetic reactions.



4. TPP is also involved in decarboxylation reactions in the metabolism of branched chain amino acids.
 5. Thiamine triphosphate or TTP is known to be involved in nerve induction.

Deficiency

Thiamine deficiency includes neurological, cardiovascular and gastrointestinal disorders commonly referred to as "beriberi".

Types of Beriberi

Beriberi is of four types:

1. Dry beriberi (peripheral neuritis)
2. Wet beriberi (cardiac manifestation)
3. Cerebral beriberi (Wernicke-Korsakoff's syndrome)
4. Infantile beriberi
 1. Dry beriberi develops when the diet contains slightly less than the thiamine requirement. Symptoms include poor appetite, inflammation of peripheral nerves, wasting of limb muscle, unsteadiness and difficulty in walking, fatigue, deep muscle pain, pain on contact with the skin.
 2. Wet beriberi develops when the deficiency is more severe. In addition to neurologic manifestation cardiovascular symptoms and edema are more apparent.
 3. Cerebral beriberi occurs in chronic alcoholism and characterized by intelligence disturbance, ataxia, double vision and nystagmus (rapid involuntary movement of the eyes). If untreated it usually progresses to Korsakoff's psychosis which is irreversible and is characterized by loss of memory of recent events and inability to retain new information.
 4. Infantile beriberi is due to the low thiamine content of breast milk from deficient mother. It is characterized by tachycardia, anorexia, vomiting, convulsions, edema, aphonia (absence or loss of voice),

encephalopathy (various diseases that affect the functioning of the brain) and if not treated, death.

Antimetabolites

Thiamine can be destroyed if the diet contains thiaminase. It is present in raw fish and seafood.

Management

- Treatment must be started as soon as the diagnosis is made in the deficiency of thiamine. Complete rest is most essential and 50 mg thiamine should be given intramuscularly for 3 days. Thereafter 10 mg 3 times a day should be continued by mouth until convalescence is established.
- Wiernicke's encephalopathy should be treated with 50 mg thiamine hydrochloride by slow intravenous injection followed by 50 mg intramuscularly daily for a week. Confusion, disorientation and ophthalmoplegia should respond within 2 to 3 days. The memory disorder takes longer to improve.

RIBOFLAVIN (VITAMIN B₂)

Structure



It is 6,7 dimethyl - 9D - ribitol isoalloxazine consisting of an isoalloxazine ring with the ribitol side chain. Ribitol is an alcohol of ribose sugar. Riboflavin is relatively heat stable but decomposes in the presence of visible light (photosensitive). It is yellow, fluorescent and has capacity to absorb UV light.

Coenzyme Forms

- Flavin mononucleotide (FMN)
- Flavin adenine dinucleotide (FAD)

Riboflavin is converted to FMN in the intestinal mucosal cell, then to FAD in the liver.

Sources

Milk and milk products, fish, liver, meat, eggs, green leafy vegetables, whole cereals, legumes.

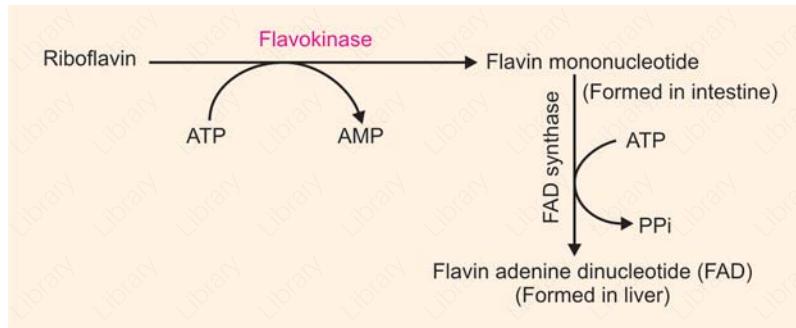
RDA

In adults, it is 1.3 to 1.7 mg/day. During pregnancy and lactation, it is more. Infants and children, it is 0.7 - 1.6 mg/day.

Absorption and Transport

It is absorbed in the small intestine and distributed to all tissues by circulation.

Conversion of Riboflavin to Flavin Nucleotides



Functions

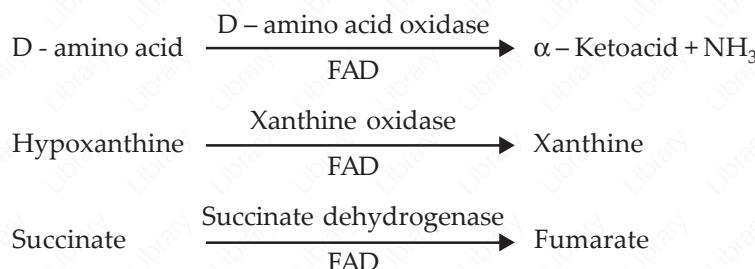
- Riboflavin is a precursor of coenzymes, FMN and FAD which are required by several oxidation - reduction reactions in metabolism. FMN and FAD are capable of reversibly accepting hydrogen atoms, forming FMNH_2 and FADH_2 . FMN and FAD serve as coenzymes for oxidoreductase enzymes. Some examples are given in Table 6.1.2.

Table 6.1.2: Examples of enzymes requiring FMN or FAD as a coenzyme and reaction where they are involved

<i>Flavoprotein enzyme</i>	<i>Pathway/reaction</i>
α -Amino acid oxidase	Deamination of amino acids
Xanthine oxidase	Purine degradation
Mitochondrial glycerol-3-phosphate dehydrogenase	Transfer of reducing equivalents from cytosol into mitochondria
Succinate dehydrogenase	Citric acid cycle
Acyl-CoA dehydrogenase	Fatty acid oxidation
NADH dehydrogenase	Respiratory chain in mitochondria
Pyruvate dehydrogenase and α -Ketoglutarate dehydrogenase	Oxidative decarboxylation of pyruvate and α -ketoglutarate

- ii. Riboflavin is needed for maintenance of mucosal, epithelial and ocular tissues.
- iii. Flavins are involved in the metabolism of iron, pyridoxine and folate.
- iv. Flavins are also involved in protection against peroxidation in metabolism of xenobiotics.

Some Reactions Requiring Coenzymes of Riboflavin



Deficiency

- i. Riboflavin deficiency can be seen in conditions such as malabsorption, malnutrition, anorexia and chronic alcoholism.
- ii. Drugs such as barbiturates may also cause riboflavin deficiency by inducing microsomal oxidation of the vitamin.
- iii. Riboflavin deficiency may occur in newborn infants with hyperbilirubinemia, who are treated by phototherapy, because of light sensitivity of riboflavin.

Ariboflavinosis

Deficiency of vitamin B results in ariboflavinosis, which is characterized by angular stomatitis (inflammation of the mouth), cheilosis (fissures at the angles of the mouth), glossitis (inflammation of the tongue), seborrheic dermatitis, vascularization of the cornea.

Laboratory diagnosis of riboflavin deficiency is difficult. Serum and urine riboflavin fall low only in severe deficiency. Erythrocyte enzyme activity measurements (glutathione reductase, a riboflavin-dependent enzyme) are used as index of riboflavin status.

Management

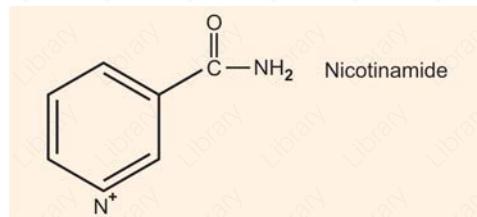
In the deficiency of riboflavin, the therapeutic dose of riboflavin is 5 mg 3 times a day by mouth. It gives the patient's urine a green fluorescence. Other B complex vitamins should also be given.

Niacin (Vitamin B₃)

Nicotinic acid and Nicotinamide have similar biochemical function as niacin.

Structure

Niacin is the simple derivative of pyridine and is thermostable and water soluble.



Active Forms

1. Nicotinamide Adenine Dinucleotide (NAD⁺)
2. Nicotinamide Adenine Dinucleotide Phosphate (NADP⁺)

Source

Yeast, liver, legumes and outer coats of cereals. It can be synthesized in the liver from tryptophan. We require 60 mg of tryptophan for the synthesis of 1 mg of niacin.

RDA

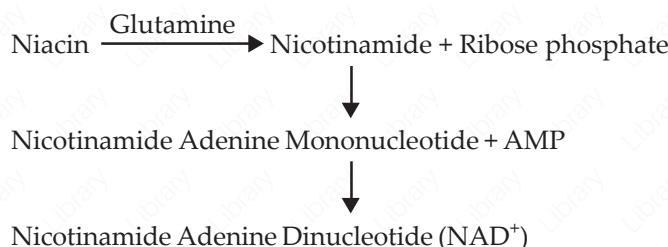
Adults : 15 - 20 mg/day

Children : 10 - 12 mg/day

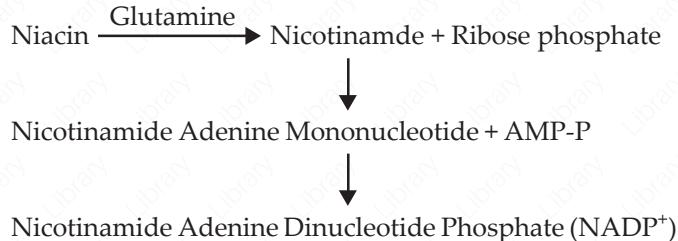
Absorption and Transport

Nicotinic acid and nicotinamide are absorbed in the small intestine and reach various tissues through circulation, where they are converted to NAD and NADP.

Conversion of Niacin to NAD



Conversion of Niacin to NADP



Biochemical Functions of Niacin

- i. Niacin in the form of nicotinamide is incorporated into the structure of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate (NADP^+).
- ii. NAD^+ and NADP^+ are involved in a great variety of oxidation and reduction reactions catalyzed by dehydrogenases in intermediary metabolism.
- iii. They are, therefore key components of many metabolic pathways of carbohydrates, lipids and proteins. Generally NAD^+ linked dehydrogenases catalyze oxidation-reduction reactions in oxidative pathways, Example: citric acid cycle and glycolysis.
- iv. NADP^+ linked dehydrogenases or reductases are often found in pathways concerned with reductive synthesis, Example: synthesis of cholesterol, fatty acid and pentose phosphate pathways.
- v. NAD Also seems to play a role in DNA repair and other cellular responses to DNA damage.

Deficiency

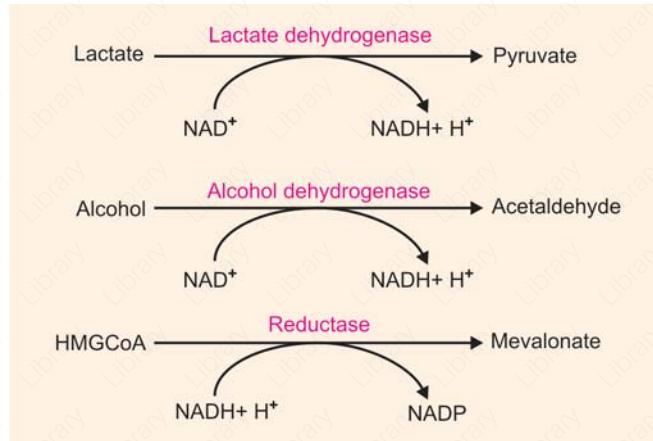
Pellagra

Deficiency of niacin in human causes pellagra, a disease involving the:

- i. Skin
- ii. Gastrointestinal tract and
- iii. Central nervous system.

The symptoms of pellagra are characterized by three "Ds":

- Dermatitis
- Diarrhea
- Dementia and if not treated death.

Reactions Requiring NAD⁺**Causes for Niacin Deficiency**

- High corn or maize diet:* Maize contains niacin in bound form and niacin is not easily absorbed.
- Kynureninase:* is a pyridoxal phosphate dependent enzyme. So conversion of tryptophan to niacin is not possible in pyridoxal deficiency.
- Increased jowar consumption:* Jowar is rich in leucine which inhibits conversion of tryptophan to niacin.
- Isoniazid therapy for tuberculosis:* Isoniazid inhibits conversion of tryptophan to niacin.
- Hartnup's disease:* Tryptophan absorption from intestine is defective in this disease. Tryptophan is excreted in urine in large quantities and thus lack of tryptophan leads to deficiency of niacin.
- Carcinoid syndrome:* The tumor utilizes major portion of available tryptophan for synthesis of serotonin. So tryptophan is unavailable.

Management

- In the deficiency of niacin, nicotinamide is given in a dose of 100 mg every 6 hours by mouth. The vitamin is well-absorbed but can be given parenterally. The response is very quick. The erythema of the skin diminishes and the diarrhea ceases within 24 hours. Improvement is also found in the patient's behavior and mental attitude.
- The deficient disease is developed due to a low intake of protein including tryptophan. Deficiencies of other B vitamins (riboflavin and vitamin B₆) are likely. Nicotinamide treatment should, therefore, be supplemented with a nutritious diet, high in protein.

- c. Vitamin B complex tablets should be given and iron, folic acid and vitamin B₁₂ may be necessary in addition, in some cases.
- d. Alcohol should be forbidden.

Prevention

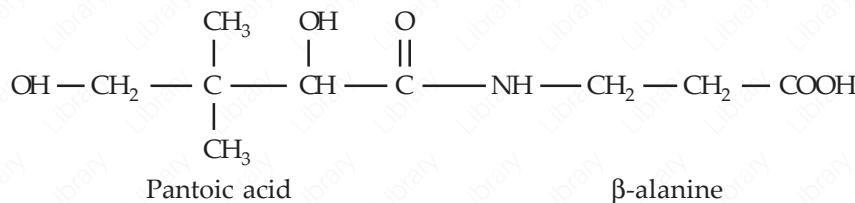
Pellagra can be prevented by enrichment of maize meal and bread with niacin.

Pantothenic Acid (Vitamin B₅)

It is widely distributed in foods. ('Pantothene' in Greek means everywhere).

Structure

It is made up of pantoic acid and β-alanine.



Active Forms

Coenzyme A (CoA - SH) and Acyl carrier protein (ACP).

Sources

Yeast, liver, eggs are the good sources of pantothenic acid. It is also synthesized by intestinal bacteria.

RDA

It is assumed to be about 10 mg/day.

Absorption and Transport

From dietary sources, pantothenic acid is released by intestinal phosphatases. Free pantothenate or its salts are freely absorbed in the intestine and circulate among various tissues.

Functions

Pantothenic acid is a component of Coenzyme A and acyl-carrier protein. The thiol (SH) group of CoA and ACP acts as a carrier of acyl groups.

1. Coenzyme A takes part in reactions concerned with:
 - i. Carrying acetyl group as acetyl CoA in TCA cycle.
 - ii. For β - oxidation and biosynthesis of fatty acids.

- iii. Acetylation reactions
 - iv. Synthesis of cholesterol
 - v. Utilization of ketone bodies
2. 4-Phosphopantetheine is a component of ACP (acyl carrier protein). ACP is a part of the enzyme required for fatty acid synthesis called fatty acid synthase, which is a multi enzyme complex.

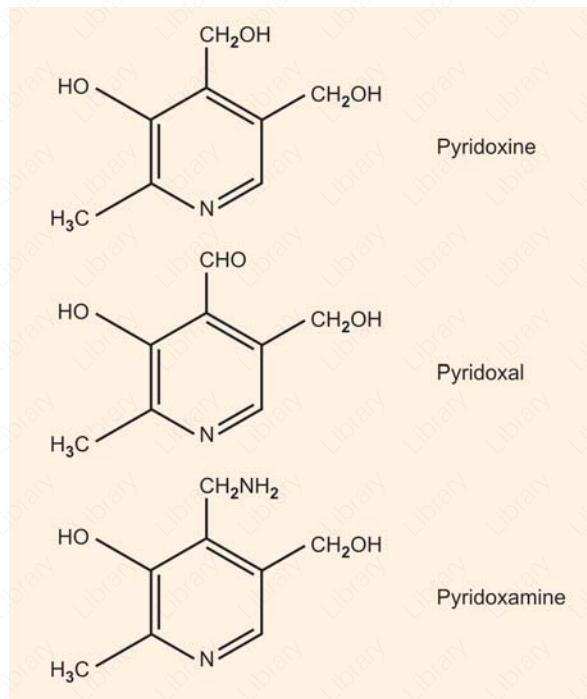
Deficiency

Deficiency is rare since pantothenic acid is widely distributed in foods except in malnourished prisoners of war where burning foot syndrome was reported. Clinical symptoms are burning sensation, headache, dizziness, gastrointestinal malfunction, fatigue and sleep disturbances.

PYRIDOXINE (VITAMIN B₆)

Structure

Vitamin B₆ consists of a mixture of three different closely related pyridine derivatives namely, pyridoxine, pyridoxal and pyridoxamine. All the three have equal vitamin activity since they can be interconverted in the body.



Active forms of vitamin B₆: Pyridoxal phosphate (PLP) is the active form of vitamin B₆. It is formed from phosphorylation of all three forms of vitamin B₆.

Sources

Yeast, liver, cereals, meat, poultry, fish, egg yolk, potatoes and vegetables.

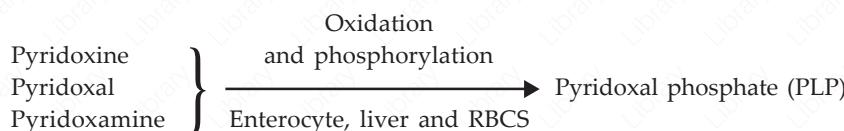
RDA

In adults: 1 to 2 mg/day. During pregnancy and lactation it is more.

Absorption and Transport

Pyridoxine is easily absorbed. It reaches various tissues through circulation. Pyridoxine is converted into pyridoxal and pyridoxamine in the tissues.

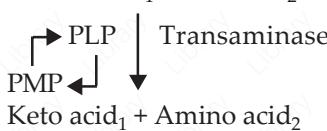
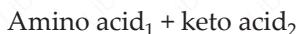
Conversion to its Active Form



Biochemical Functions

1. It is required for amino acid metabolism

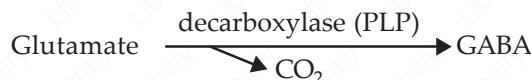
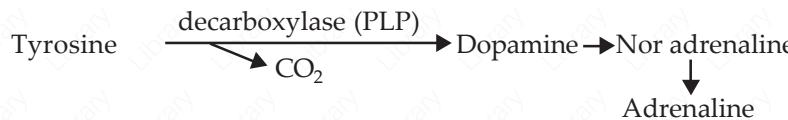
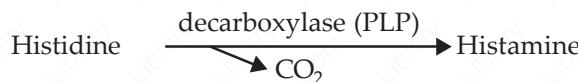
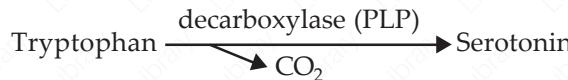
a. For transamination: Example: SGOT, SGPT



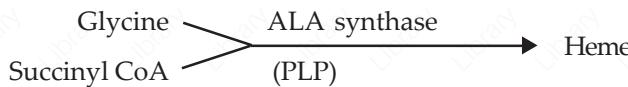
Example:



b. For decarboxylation:



- c. Required for heme synthesis:



- d. Required for Niacin synthesis:



- e. Required for degradation of cysteine:



- f. In glycogen metabolism, the glycogen phosphorylase requires PLP for the production of glucose from glycogen in both liver and muscle.

Deficiency

It causes neurological symptoms. As it is required for niacin synthesis, deficiency of pyridoxine may lead to pellagra.

Management

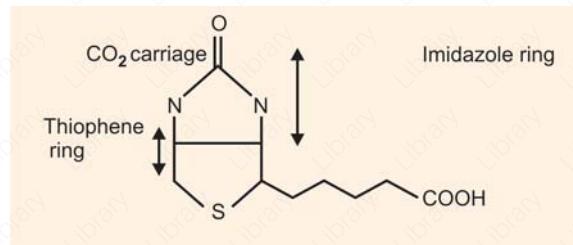
Vitamin B₆ deficiency can occur in women taking oral contraceptives and the mild depression as a result of this may be relieved by a small dose of pyridoxine.

BIOTIN

It is an imidazole derivative.

Structure

It consists of an imidazole ring fused with thiophene ring with a valeric acid side chain. Biotin as such is the active form.



It is widely distributed in foods. It is also synthesized by intestinal microflora. Yeast, egg yolk, liver, kidney, dried legumes, tomatoes are the important sources of biotin.

RDA

200- 300 µg/day for adults.

Absorption and Transport

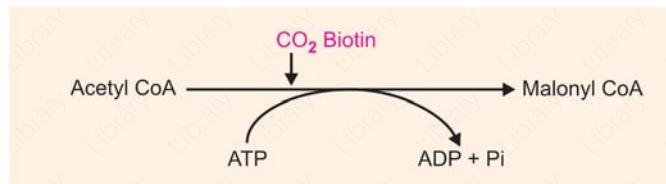
Biotin is absorbed in the small intestine. It reaches the liver and other tissues through circulation.

Functions

Biotin is a coenzyme of carboxylase reactions. It is a CO₂ transporter at N of imidazole ring.

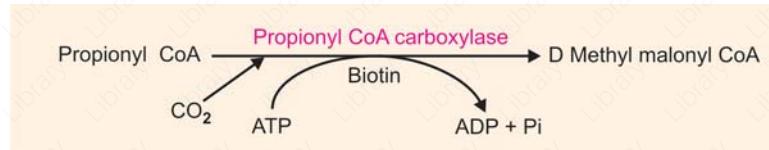
Carbon Dioxide Fixation Reactions

1.



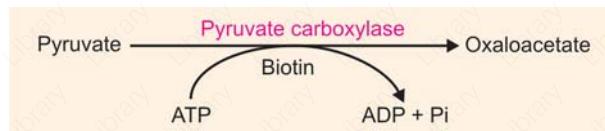
Acetyl CoA carboxylase enzyme (CO₂ from biotin and donated to Acetyl CoA) to form malonyl CoA.

2.



D-Methyl malonyl CoA can be converted to succinyl CoA which enters into TCA cycle.

3. Pyruvate to oxaloacetate by CO₂ fixation which then enters the TCA cycle.



4. β - Methyl crotonyl CoA $\xrightarrow[\text{Biotin}]{\text{Carboxylase}}$ D Methyl glutaconyl CoA
(In leucine metabolism)

Deficiency

Symptoms of biotin deficiency includes dermatitis, muscle pain, anorexia and hallucinations.

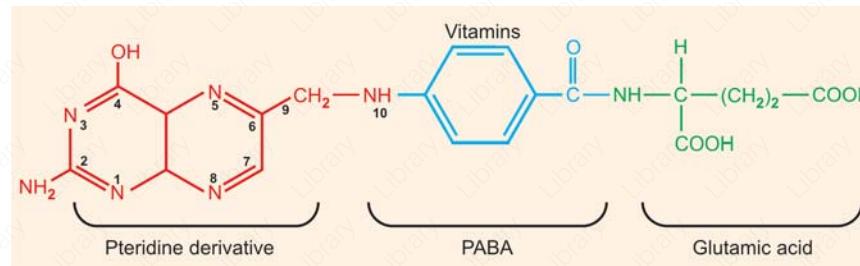
Biotin deficiency may be produced by large quantities of avidin, prolonged use of antibacterial drugs and in breast-fed young infants with persistent diarrhea.

Avidin is a glycoprotein present in raw egg white binds to biotin and prevents biotin absorption in the intestine. Cooking denatures avidin.

FOLIC ACID

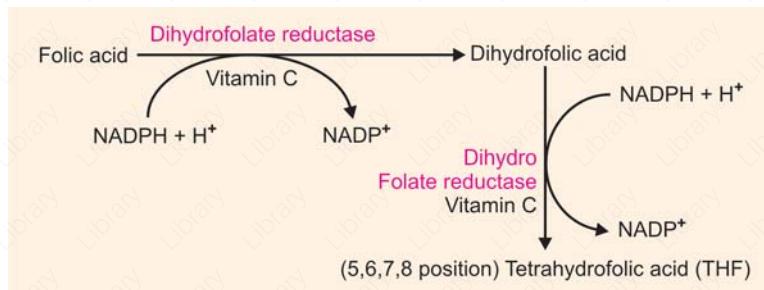
Structure

It is made up of 3 constituents. The pteridine group linked with para amino benzoic acid (PABA) is called pteroyl acid which is then attached to glutamic acid to form pteroyl glutamic acid or folic acid.



Active Form

Tetrahydrofolic acid is the active form of folic acid. Folate is enzymatically reduced in a two stage process in tissues to yield the dihydro and then tetrahydrofolate which requires vitamin C.



Sources

Yeast, liver, green leafy vegetables. Considerable amounts are also synthesized by intestinal bacteria.

RDA

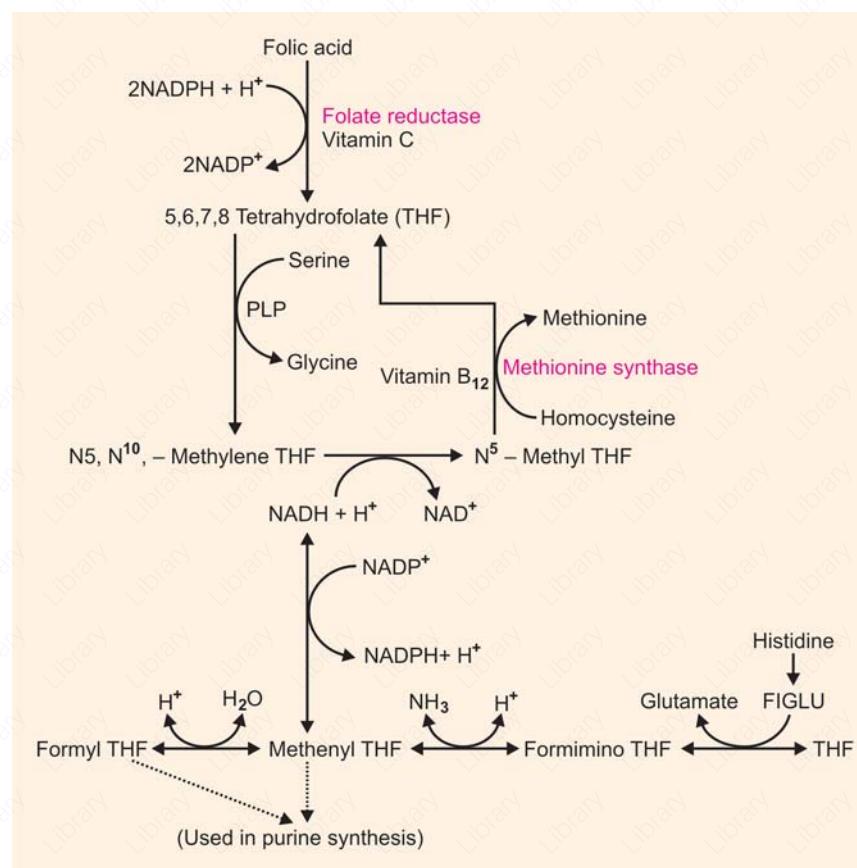
200 µg/day. During pregnancy and lactation requirement is more. Infants and children require less.

Absorption and Transport

Folic acid of natural foods has more glutamic acid residues. In the intestinal mucosal cells, lysosomal enzymes remove excess glutamate residues to form folic acid, which is reduced to tetrahydrofolate and methylated to N⁵-methyl tetrahydrofolate. Liver and other tissues take up the major circulating protein bound, methyl tetrahydrofolate and convert it to polyglutamate form after the transfer of methyl group.

Functions

Tetrahydrofolic acid helps in metabolism of one carbon compounds. It accepts one carbon from degradation reactions and donates in synthetic reactions. One carbon units are bound to one or both of the two nitrogens in the molecule, N⁵ and N¹⁰. The THF coenzyme serve as acceptors or donors of one carbon units in variety of reactions involved in nucleotide and amino acid metabolism. The one carbon units are formyl (CHO), formimino (CH=NH), methyl (CH₃), methylene (CH₂), methenyl (CH).



Note: Tetrahydrofolic acid as carrier of one carbon units. FIGLU = formiminoglutamate.

In the case of folate deficiency, FIGLU is accumulated in the body.

FIGLU test or Histidine load test: Histidine is normally metabolized to FIGLU from which formimino group is removed by THFA. Therefore in folate deficiency, FIGLU is excreted in urine. For the test 15 g of histidine is given orally, and urine is collected for 24 hours. In normal persons less than 35 mg of FIGLU is excreted under these conditions.

Inhibitors of Folate Reductase (Folate antagonists)

- i. *Sulphonamides*: These are structurally similar to P-amino benzoic acid (PABA) and inhibit folic acid synthesis in bacteria from PABA. Therefore, sulphonamides act as antibacterial agents.
- ii. *Pyrimethamine*: This inhibits folic acid synthesis in plasmodium and it is used as an anti-malarial drug.
- iii. *Aminopterin and Amethopterin*: These inhibit folate reductase, thus inhibiting the conversion of folic acid to THF and can be used as an anticancer drug for treatment of leukemias and choriocarcinomas.

Deficiency

Folate deficiency frequently occurs in pregnant women and in alcoholics. Clinical symptoms are:

- i. Megaloblastic anemia (macrocytic anemia): The deficiency of folate leads to impairment of conversion of homocysteine to methionine due to which synthesis of DNA is impaired. Hence, cell division and formation of nucleus of new erythrocytes are prevented. As a result megaloblasts are formed in place of normoblasts. Accumulation of these megaloblasts leads to megaloblastic anaemia.
- ii. Accumulation of FIGLU and excretion in urine.
- iii. In folate deficiency, if homocysteine level increases more than 6 micromoles/litre, it increases the risk of coronary artery diseases.
- iv. In pregnancy, folate deficiency increases birth defects like the neural tube defect.

Management

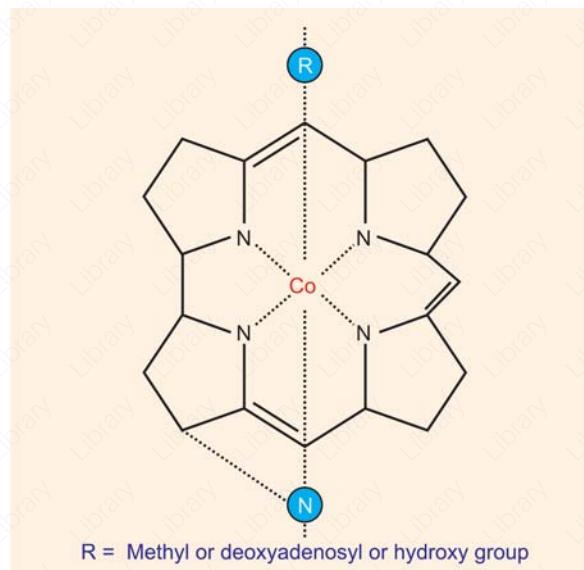
- a. In folic acid deficiency, treatment with a daily dose of 5 mg of folic acid by mouth is sufficient; 5 mg once a week is always adequate for maintenance therapy.
- b. Folic acid must never be given other than with vitamin B₁₂ in Addisonian pernicious anemia or other vitamin B₁₂ deficiency anemias because of the risk of aggravating or precipitating neurological features of vitamin B₁₂ depletion.

- c. Megaloblastic change due to vitamin B₁₂ deficiency is very rare in pregnancy. Therefore, it is reasonable to give folic acid supplements (350 µg daily) to all pregnant women.
- d. When a drug methotrexate inhibits dihydrofolate reductase, it is better to employ folinic acid to overcome the metabolic block. Folinic acid may be given as tablets, 15 mg daily orally or as an injection intravenously or intramuscularly at a dose of 3 mg per ml.
- e. Folinic acid mouthwashes are used to counteract the oral side-effects of folate antagonist drugs.
- f. Megaloblastic disorder caused by other cytotoxic drugs which inhibit DNA synthesis is not reversed by either vitamin B₁₂ or folic acid administration.
- g. Severe hemolytic crises require treatment by blood transfusion. Folic acid 5 mg daily orally is prescribed to support the increased erythropoiesis.

COBALAMIN (VITAMIN B₁₂)

It has a complex chemical structure. It is made up of four pyrrole ring system called corrin ring with a central cobalt atom held by four coordination bonds with the nitrogen of the pyrrole groups. The tetrapyrrole ring structure of corrin is similar to that of porphyrin to some extent.

Structure



Active Form

Active coenzyme forms are methylcobalamin and deoxyadenosylcobalamin.

Sources

It is mainly present in animal sources. Meat, eggs, liver, kidney, brain, fish are good sources. Milk and milk products are fair sources.

Vitamin B₁₂ is absent in plant foods. Humans obtain small amount of it from their intestinal flora.

RDA

Adults - 3 - 4 µg/day.

Absorption, Transport and Storage

The absorption of vitamin B₁₂ takes place in the ileum. A glycoprotein called intrinsic factor produced by gastric parietal cells is needed for absorption of vitamin B₁₂. Intrinsic factor combines with vitamin B₁₂ (extrinsic factor) and facilitates the absorption of the vitamin by cells of the ileum. Lack of intrinsic factor prevents the absorption of the vitamin B₁₂.

In the ileal cells, the intrinsic factor is released and the vitamin B₁₂ is transferred to a plasma transport protein which delivers vitamin B₁₂ to tissues.

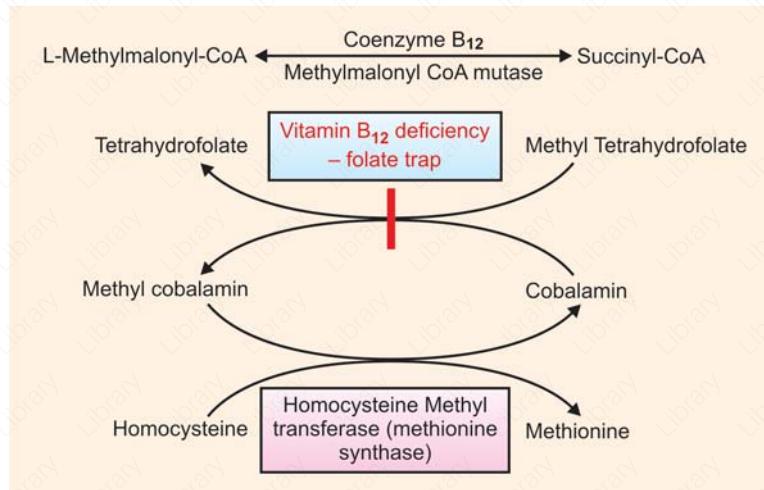
Vitamin B₁₂ is the only water soluble vitamin that is stored in significant amount in the liver, bone marrow and other tissues. Total body content of vitamin B₁₂ is 3 - 4 mg. In the liver it is stored as deoxy adenosyl cobalamin. Further liver cobalamins are secreted in the bile and undergo enterohepatic circulation.

Functions

Vitamin B₁₂ acts as coenzyme and it is called cobamide coenzyme. It is required in two reactions that lead to formation of succinyl CoA from methyl malonyl CoA and methionine from homocysteine.

Folate Trap

Methylation of homocysteine depends on methyl cobalamin and methyl-THF. When methyl-cobalamin is deficient the activity of methionine synthase is greatly reduced. Consequently, methyl-THF cannot be converted to THF and no other reaction used methyl-THF. Thus, most of folic acid of the body is irreversibly "trapped" as its methyl derivative (methyl-THF). This is called as folate trap.



This effectively creates deficiency state resembling true folate deficiency and an adequate supply of free THF is not available for carrying out reactions in which it normally participates, e.g. for the synthesis of purine and pyrimidine bases.

Deficiency

Cobalamin deficiency may arrive due to decreased absorption or decreased dietary intake.

1. *Pernicious anemia:* It is caused by a deficiency of intrinsic factor, which leads to decreased absorption of cobalamin. Pernicious anemia is an autoimmune disease with a strong familial background affecting people over 40 years of age.
2. *Atrophic gastritis:* It is a condition in which the gastric parietal cells that secrete intrinsic factor may themselves be destroyed, which leads to impaired absorption of cobalamin. It is also an autoimmune disease.
3. *Nutritional:* Cobalamin deficiency is very common among vegetarians of low socioeconomic groups who cannot afford milk and its products which are the only vegetarian sources of vitamin.
4. *Others:* These include iron deficiency anemia, infection with fish tapeworm or increased requirement in pregnancy.

Clinical Manifestations of Cobalamin

1. *Hematological abnormalities:* Megaloblastic anemia in which red cells are 25 - 60 percent larger than normal with fragile membranes and tendency to hemolyze. It is probably due to secondary deficiency of folate. It is a consequence of the accumulation of methyl tetrahydrofolate (folate trap) and so difficult to distinguish from anemia of folate deficiency.

2. *Neurological disorders:* Damage to nervous system, the sub acute combined degeneration is unique to cobalamin deficiency and is not associated with folate deficiency. There is demyelination and neuronal death affecting cerebral cortex and spinal cord. Symptoms include paresthesia (burning sensation) of extremities, unsteadiness in gait. Mild deficiency may cause depression, confusion and less alertness.

Laboratory diagnosis: Since cobalamin is required in only two reactions, its deficiency results in accumulation of methylmalonic acid (methylmalonic aciduria) and Homocysteine (Homocystinuria); both may be measured in laboratory. The plasma levels of cobalamin can be determined by bacteriological methods or radioimmunoassay.

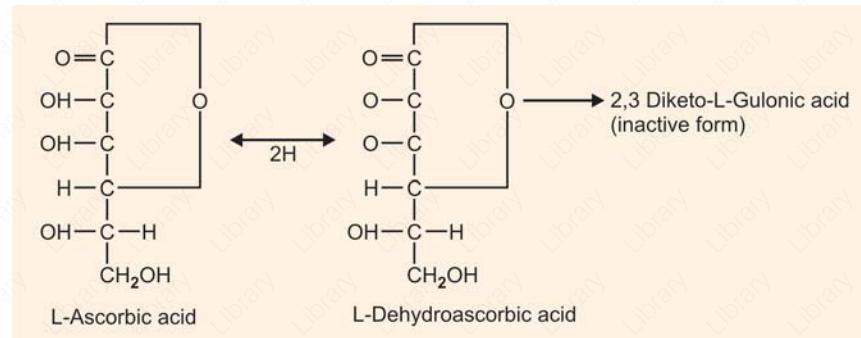
Management

- In the deficiency of vitamin B₁₂ hydroxy-cobalamin is given parenterally in a dosage of 1,000 µg twice during the first week, then 100 µg weekly for a further 6 doses. Within 48 hours of the first injection the bone marrow shows a striking change from a megaloblastic to a normoblastic state.
- In some patients the rapid regeneration of the blood depletes the iron reserves of the body and recovery is stopped. To prevent this, ferrous sulphate 200 mg daily should be given soon after the commencement of the treatment. A combined deficiency of vitamin B₁₂ and iron is found by the presence of macrocytosis and hypochromia.

VITAMIN C (ASCORBIC ACID)

It is a six-carbon sugar derivative. Humans cannot synthesize due to lack of the enzyme gluconolactone oxidase, although most animals can synthesize it from glucuronic acid. Ascorbic acid itself is its active form. It is sensitive to oxygen, metal ions, heat or light. It is excreted in urine as oxalic acid.

Structure



Sources

Citrus fruits, pineapple, mango, germinated legumes, guava, coriander leaves. Gooseberries are the rich sources of vitamin C.

RDA

Adult man and woman: 60 - 80 mg/day.

Absorption and Transport

Vitamin C is readily absorbed in the intestine via sodium dependent active transport mechanism. It reaches various body tissues through circulation. It freely enters various cells like, RBCs, WBCs, etc.

Functions

1. Ascorbic acid acts as an antioxidant and a free radical scavenger. It is a strong reducing agent and so protects carotenes, vitamin E and other B vitamins from dietary origin from oxidation.
2. *Collagen biosynthesis:* It is required for the hydroxylation of proline and lysine residues of collagen. It is also needed for proper bone and teeth formation.
3. *Steroid synthesis:* Vitamin C is involved in the hydroxylation reactions of steroids in adrenal cortex.
4. *Adrenaline synthesis:* It serves as a reducing agent in hydroxylation reactions in the synthesis of adrenaline and noradrenaline from tyrosine in adrenal medulla.
5. *Absorption of iron:* It facilitates the absorption of non-heme iron from intestine by reducing it to the Fe⁺⁺ (ferrous) state.
6. *Tryptophan metabolism:* Vitamin C has been implicated in the hydroxylation of tryptophan to serotonin.
7. *Folic acid metabolism:* Vitamin C reduces folic acid to THF. Thus, it helps in the maturation of RBC.
8. Vitamin C has a role to play in phagocytic action of leucocytes and helps in the formation of antibodies (i.e. it stimulates B cells to produce immunoglobulins).
9. In the liver, synthesis of bile acids from cholesterol requires vitamin C.
10. When given in large doses, it is found to reduce severity of cold.

Deficiency

1. In adults deficiency of vitamin C causes scurvy. Symptoms of scurvy are related to deficient collagen formation. These include: hemorrhages in various tissues due to capillary fragility, delay in wound healing, susceptible to infections, swollen joints, swollen gums and loosening of teeth.

2. In infants deficiency of vitamin C gives rise to infantile scurvy. It occurs in weaned infants who are fed on diets, low in vitamin C.

Management

- A dose of 250 mg of vitamin C by mouth 3 times daily may saturate the tissues quickly.
- No patient dies of scurvy with adequate treatment and recovery is usually rapid and complete.
- Old or solitary people who do not eat fruits and vegetables should be advised to take 50 mg ascorbic acid tablets daily.
- The requirement of vitamin C is increased in case of trauma, surgery and burns, infections, smoking and in intake of certain drugs- adrenocortical steroids, aspirin, indomethacin and tetracycline. So patients affected by these require more than the recommended intake.

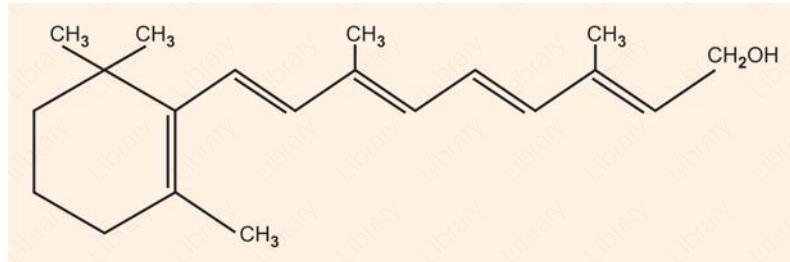
FAT SOLUBLE VITAMINS

Vitamin A

Structure

Vitamin A refers to three biologically active vitamers: retinol (an alcohol), retinal (an aldehyde) and retinoic acid (an acid). All these are found only in animals. They are polyisoprenoid compounds comprising of two distinct components.

- A cyclohexanyl ring
- A side chain made up of several isoprene units, attached to the cyclohexanyl ring.



Structure of vitamin A, retinol

The term retinoids is used to define these three compounds which are interconvertible. These are derived from the plant precursor molecule, β carotene, a provitamin form of vitamin A.

Sources

Animal sources: Fish liver oils such as cod, shark, mackerel, milk, butter, ghee, cheese, curd, liver, egg yolk.

Plant sources: Dark green leaves, such as spinach and vegetables, yellow and red fruits such as, carrots, tomatoes, pumpkin, drumstick and peaches, papaya, mango, jackfruit, banana and oranges.

RDA

Adults: 750 µg of retinol or 3 mg of carotene/day.

Absorption, Transport and Storage

Pancreatic esterase hydrolyses retinolesters of the diet to retinol and free fatty acids in presence of bile salts in the intestine. Mucosal cells absorb retinol and β-carotenes. Retinol generated from animal and plant sources is esterified with fatty acids and incorporated into chylomicrons in the enterocytes of intestine. The chylomicrons enter the blood via the lymph. All retinylesters are present in the chylomicron remnants formed from chylomicrons which are taken by the liver.

Under normal condition, retinylesters are constantly broken down and resynthesized in the liver. Free retinol formed in the liver is transported to target cells as protein complexes, after it is reversibly bound to retinol-binding protein (RBP). The target cells take up RBP by a RBP specific receptor mediated process and the retinol is bound to cellular retinol binding protein (CRBP) which is subsequently oxidized to retinal and/or retinoic acid. Plasma transport of retinoic acid is accomplished by binding to albumin.

The hepatocytes dispatch retinol with RBP and can also store the surplus in the form of retinol esters. More than 90 percent of body's supply of vitamin A is stored in the liver cells.

Functions

- i. Retinal and retinol are involved in vision
- ii. Retinoic acid is involved in cellular differentiation, as a regulator of gene expression.

Role of vitamin A in vision: Retinal helps in dim light and bright light visions.

Rods and cones present in the retina are responsible for normal and colour vision. Rods are for vision in dim light and cones are for visual acuity and color vision. Rods contain visual pigment rhodopsin which is made up of 11-cis retinal and opsin, a glycoprotein. When light strikes, 11-cis retinal undergoes conversion to all trans retinal. At the same time apoprotein dissociates as opsin. The conversion of rhodopsin to opsin and

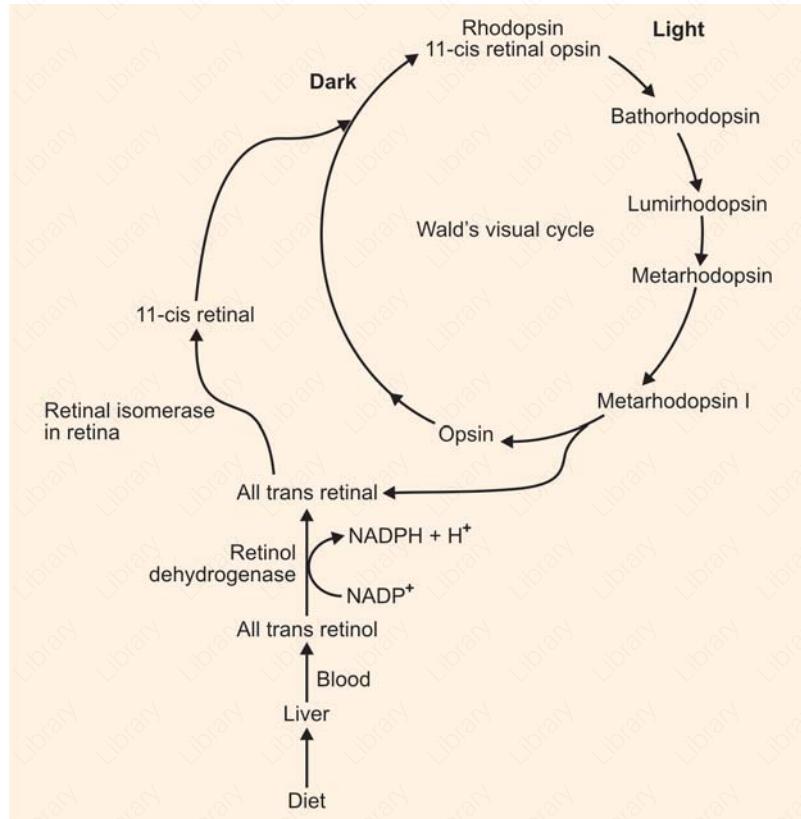


Fig. 6.1.1: Wald's visual cycle in rod cells

all trans retinal occurs through many intermediates such as, bathorhodopsin, lumirhodopsin and metarhodopsins. The isomerization is associated with the conformational change in the protein opsin.

In the first stage of visual process, a light signal is converted into atomic motion. In the next stage, this atomic motion is converted into nerve impulse that is transmitted by the optic nerve to the brain. This is followed by dissociation of all trans retinal from opsin which is immediately isomerized by retinal isomerase to 11-cis retinal. This combines with opsin to regenerate rhodopsin to complete the visual cycle.

The conversion of all trans retinal to 11-cis retinal is incomplete and hence most of the all trans retinal is converted to all trans retinol by retinol dehydrogenase and is stored in the liver. When required retinol reenters the circulation and is taken up by the retina. It is then converted back to 11-cis retinal which combines with opsin again to form rhodopsin.

Color vision: Color vision is mediated by 3 different retinal containing pigments in the cone cells. The three pigments are porphyropsin, iodopsin

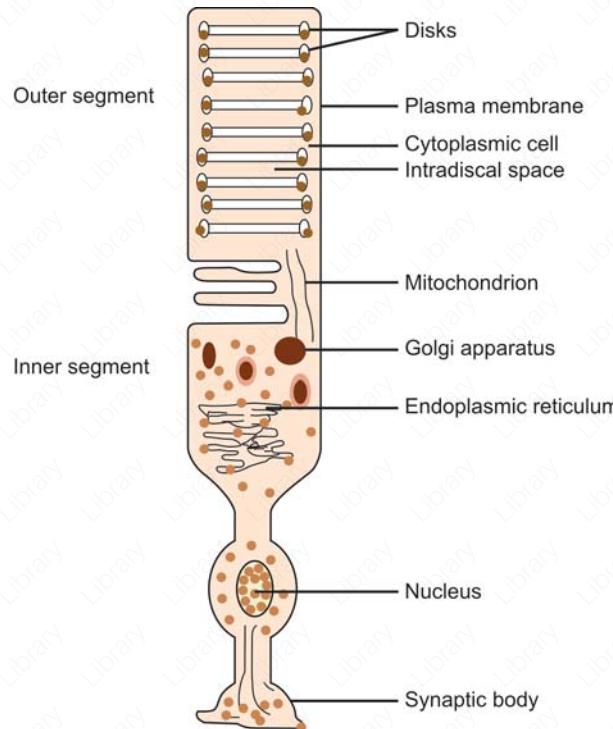


Fig. 6.1.2: A rod cell

and cyanopsin which are sensitive to three essential colours, red, blue and green respectively. When light strikes the retina, it bleaches one or more of these pigments depending on the color quality of the light. The pigments are converted to all trans retinal and opsin. This reaction gives rise to the nerve impulse which reads out in the brain as color, red if porphyropsin is split, green if iodopsin or blue if cyanopsin is split. If mixtures of the three are converted, the color read out in the brain depends on the proportions of the three split.

Cellular differentiation: Retinoic acid acts as a steroid hormone. It also promotes growth and differentiation to some extent, apart from regulating gene expression during embryonic development such as cell differentiation in spermatogenesis and differentiation of epithelial cells.

Antioxidant role of vitamin A: β - carotenes function as antioxidants at low oxygen partial pressure, which scavenge free radicals. They eliminate reactive oxygen species like superoxide anion radical, singlet oxygen etc. The antioxidant property of vitamin A accounts for its possible anticancer activity. Synthetic retinoids are found to prevent breast cancer and bladder

cancer. Retinoids prevent chemical carcinogenesis. High levels of dietary carotenoids are associated with decreased risk of cardiovascular disease.

Role in reproduction: Retinol is required in reproduction. This function is mediated by control of expression of certain genes by retinol bound to cellular retinol binding protein. Retinol and to some extent the retinal support spermatogenesis in males and prevents fetal resorption in females.

Determination of Vitamin A

Colorimetric determination utilizes the Carr-Price reaction, in which a blue colour is obtained when a solution of antimony trichloride in chloroform is added to the vitamin-containing mixture. This is used to determine the vitamin A content of blood plasma.

Effects

Vitamin A in excess intake causes toxicity.

Management

- a. On diagnosis in the deficiency of vitamin A a single large dose of 60 mg retinol as palmitate or acetate (200,000 I.U.) should be given orally. The oral dose should be repeated the next day and again prior to discharge.
- b. 55 mg retinol palmitate should be given by intramuscular injection if there is vomiting or severe diarrhea in addition to the deficiency of vitamin A.
- c. Antibiotics are of value in case of secondary bacterial infection.
- d. Local treatment of the eye will be required only if there is the presence of disorganization and in this case the services of an ophthalmic surgeon are essential.

Deficiency

Deficiency of vitamin A is of two types: Primary (dietary) or secondary. Causes of secondary deficiency include:

- a. Fat malabsorption
- b. Failure to synthesize chylomicrons
- c. Failure to cleave β carotene
- d. Impaired storage in diseased liver
- e. Failure to synthesize retinol binding protein (RBP).

The deficiency leads to the following clinical manifestations:

Effect on vision: Retinal is an essential component of the pigment rhodopsin (visual purple) on which the dim light vision depends. Therefore, lack of retinal may result in impairment of "dark adaptation". Inability to see in dim light (i.e. night blindness or nyctalopia) results because of elevation in the visual threshold. Prolonged deficiency leads to an irreversible loss of visual cells.

If the night blindness is not treated, it progresses to **xerophthalmia**. It is due to the pathological dryness of the conjunctiva and cornea, since there is a prolonged deficiency of retinoic acid required for growth and maintenance of epithelia.

Grayish white triangular plaques firmly adherent to the conjunctiva due to its increased thickness are called **Bitot's spots**.

Persistence of xerophthalmia progresses to **keratomalacia** (softening of the cornea). If there is bacterial infection ulceration and perforation of cornea occurs leading to **total blindness**.

Deficiency of vitamin A leads to:

- i. Failure of growth of bone and formation of teeth in children.
- ii. Effect on nerve growth is observed leading to degeneration of myelin sheath.
- iii. Keratinization of mucous secreting epithelial cells lining the respiratory and reproductive tracts occurs.
- iv. Deposition of keratin in skin gives rise to toad skin appearance.
- v. Degenerative testicles.
- vi. Degenerative changes in kidneys.

Toxicity

A single large dose of more than 300 mg leads to toxic effects. It is characterized by dry and pruritic skin, hepatomegaly, drowsiness, vomiting and raised intracranial pressure which sometimes mimics the symptoms of a brain tumor. Excessive intake can cause congenital malformation in the growing fetus and is teratogenic.

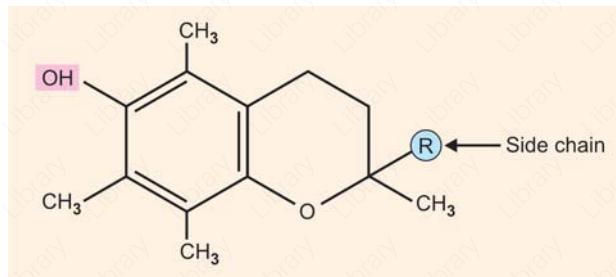
Vitamin D

(Discussed in Cholesterol Metabolism, Chapter 4.5).

Vitamin E (α -Tocopherol)

Vitamin E is an important antioxidant. The word tocopherol is derived from Greek word. *Tocos* means child birth.

Structure



The term vitamin E refers to a group of four compounds that exhibit vitamin E activity, α , β , γ and δ - tocopherol. All are viscous, light yellow oils that are heat stable but readily degraded by oxygen or ultraviolet light. They are derivatives of 6-hydroxyl chromane ring with phytol side chain of variable length. The most abundant and potent is α -tocopherol.

Sources

Vegetable oils (sunflower, soyabean, peanut, cotton seed, wheat germ, corn oil etc.).

RDA

An adult needs 10 mg/day

During pregnancy and lactation 12-13 mg/day.

Absorption, Transport and Storage

Dietary tocopherols are absorbed in small intestine in the presence of bile salts. Absorbed tocopherols are incorporated into chylomicron in the intestinal mucosal cells and enter the circulation via lymph.

In plasma, tocopherols are released from chylomicrons by lipoprotein lipase. Liver takes at half of tocopherol and it is stored. Skeletal muscle and adipose tissues also store vitamin E. From the liver tocopherols are transported to other tissues in β -lipoprotein (LDL).

Functions

- i. It acts as an antioxidant or free radical scavenger. It is present in the cell membrane along with the polyunsaturated fatty acids of phospholipids. It acts as a first line of defense against free radicals by acting as chain breaking antioxidants.

Mechanism of chain breaking action of Vitamin E:

It prevents propagation of free radicals.



$\text{ROO}^\bullet + \text{TOC-O}^\bullet \rightarrow \text{ROOH} + \text{Oxidized product of tocopherol}$
This oxidized product of tocopherol is conjugated with glucuronic acid and gets excreted in bile.

Tocopherol free radical which is formed can also be reduced with the help of vitamin C.

- ii. Vitamin E is required for fertility in experimental animals like rat.
- iii. Vitamin E increases synthesis of heme protein by increasing synthesis of ALA synthase and ALA dehydratase.
- iv. Vitamin E prevents dietary vitamin A and carotenes from oxidative damage.

- v. Vitamin E also helps to prevent oxidation of LDL. Oxidized LDL may be more atherogenic than native LDL. Thus, it protects against coronary heart disease.
- vi. Vitamin E boosts immune response, protects RBC from hemolysis, keeps structural and functional integrity of all cells, slows aging process and appears to offer protection against Alzheimer's disease. These effects appear to be by virtue of its antioxidant role.

Deficiency

Deficiency occurs rarely in adults because of its widespread distribution in foods. The major symptom of vitamin E deficiency in humans is hemolytic anemia due to increased susceptibility of erythrocytes to hemolysis.

A genetic defect in the formation of hepatic α -tocopherol transfer protein leading to the vitamin deficiency has been described, which is associated with defective lipid absorption or transport.

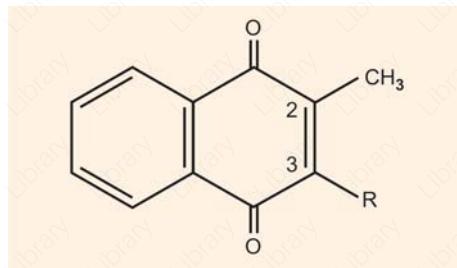
Toxicity

High doses of vitamin E depress coagulability. Patients with bleeding disorder and those receiving warfarin should be cautioned against its use.

Vitamin K

Vitamin K is called an anti-hemorrhagic factor, since its deficiency produces uncontrolled hemorrhages due to defect in blood coagulation (Danish: Koagulation).

Structure



R = H in Menadione

R = 20 C in Phylloquinone

R = 30 C in Menaquinone

It is the only fat soluble vitamin that acts as a coenzyme. There are two naturally occurring forms of vitamin K:

- i. Vitamin K₁ or phylloquinone, heat resistant and a reducing agent derived from plants.

- ii. Vitamin K₂ or menaquinone heat resistant and a reducing agent produced by microorganisms.
- iii. Vitamin K₃ or menadione is the synthetic product which is the alkylated form of vitamin K₂.

Sources

Cabbage, Cauliflower, Spinach, green vegetables are the excellent sources. Egg yolk, meat, liver, cheese and tomatoes are good sources. Intestinal bacteria also synthesize this vitamin in the intestinal tract.

RDA

In adults – 70 - 140 µg/day.

Absorption and Transport

Vitamin K of dietary origin is absorbed in the small intestine in presence of bile salts. Vitamin K is incorporated into chylomicrons after its absorption into the mucosal cells. It reaches the liver through the lymph. Liver distributes vitamin K to other tissues. It rarely accumulates in the liver and peripheral tissues.

Functions

- i. Vitamin K plays an important role in blood coagulation. Factors dependent on vitamin K are II (prothrombin), VII, IX and X all of which are synthesized in the liver as inactive zymogens.

Formation of matured clotting factors requires that, the glutamyl residues of the precursor proteins be converted to γ -carboxyglutamate residues by addition of carboxylate group. This reaction is dependent on vitamin K which serves as a coenzyme. γ -Carboxyglutamate has high affinity for calcium and is an effective calcium chelator. Binding of calcium to γ -carboxyl groups of prothrombin, promotes its conversion to thrombin, by blood clotting factors during the blood clotting process.

- ii. Vitamin K is also required for the γ -carboxylation of glutamate residues of another calcium binding protein, osteocalcin in bone. This carboxylation is also catalyzed by vitamin K dependent carboxylase.

Deficiency

Vitamin K deficiency is rare. It may be induced in the following ways:

- i. Due to treatment with antibiotics that eliminates normal intestinal flora.
- ii. In fat malabsorption syndromes.
- iii. In liver diseases.

- iv. By vitamin K antagonists such as dicoumarol or warfarin.
- v. In newborn infants, the placenta does not pass the vitamin K to the fetus efficiently and the gut is sterilized immediately after birth.
- vi. Vitamin K deficiency is associated with hemorrhagic disease.

Deficiency of vitamin K increases the time for blood coagulation, and so bleeding tendency is the prominent deficiency manifestation. Even a minor cut may cause prolonged bleeding. The only important deficiency sign is increase in prothrombin time (PT), and it is the most important laboratory test for the evaluation of vitamin K status.

Toxicity

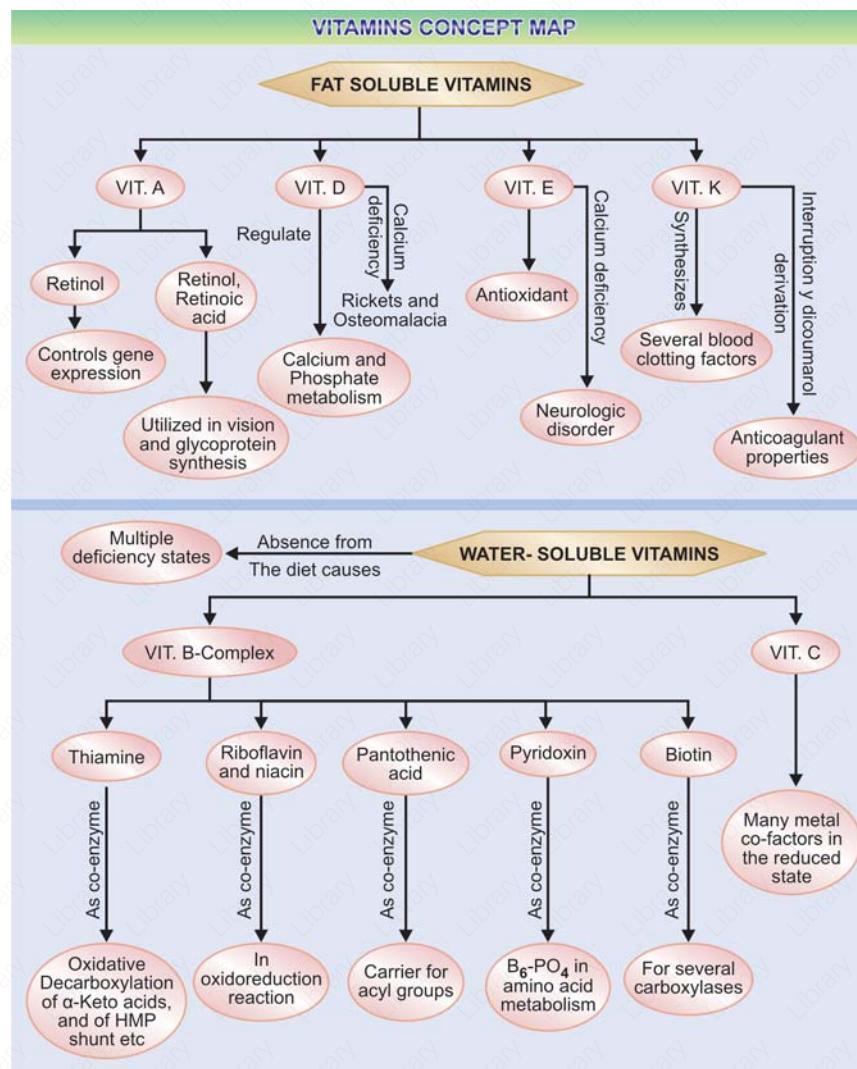
Administration of large quantities of menadione (vitamin K₃) may result in toxicity symptoms like, hemolytic anemia, obstructive jaundice in infants and breakdown of RBCS.

Management

- a. Vitamin K₁ is given routinely to newborn babies to prevent hemorrhagic disease of the newborn.
- b. Since dietary vitamin K is not absorbed in obstructive jaundice it is very important to administer the vitamin before biliary surgery.
- c. Warfarin and related anticoagulants act by antagonizing vitamin K.

MULTIPLE CHOICE QUESTIONS

1. The richest source of vitamin A is:
 - A. Milk
 - B. Yellow vegetables
 - C. Fish liver oil
 - D. Dark green leafy vegetables
2. The biochemical function of active vitamin D₃, i.e. calcitriol is effective in:
 - A. Small intestines
 - B. Bone
 - C. Kidney and bone
 - D. Kidney, bone and small intestine
3. The richest source of vitamin E is:
 - A. Milk
 - B. Wheat germ oil
 - C. Cheese
 - D. Lean meat
4. Spinach is a good source of:
 - A. Vitamin K
 - B. Vitamin E
 - C. Riboflavin
 - D. Thiamine
5. Early symptom of Beri-beri, a thiamine deficiency disease are:
 - A. Anorexia, Fatigue, tachycardia
 - B. Anorexia, polyneuritis, calf muscle weakness
 - C. Polyneuritis, muscular atrophy, insomnia
 - D. Polyneuritis, tachycardia, edema



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3. Give the dietary sources, RDA, functions and deficiency symptoms of vitamin C.
4. Give the dietary sources, RDA, functions and deficiency symptoms of vitamin B_{12}
5. Give the dietary sources, RDA, functions and deficiency symptoms of Folic acid.

Short Essays

6. Functions of vitamin C
7. Functions of vitamin B_1
8. Functions of vitamin B_2
9. Functions of vitamin B_3
10. Functions of vitamin B_5
11. Functions of vitamin B_6
12. Functions of vitamin B_{12}
13. Folic acid
14. Retinol
15. Scurvy
16. Vitamin K
17. Vitamin E
18. Coenzymes of niacin
19. Coenzymes of riboflavin
20. Night blindness
21. Beri-beri
22. Wald's visual cycle
23. Antioxidants

Short Answers

24. γ -Carboxylation
25. Sources of vitamin E
26. Deficiency symptoms of vitamin E
27. Deficiency of biotin
28. Avidin
29. Pellagra
30. Visual cycle
31. Folate trap
32. Rickets.

6.2: MINERAL METABOLISM

Major elements/macro-minerals are nutritionally important and their daily requirement is more than 100 mg. They are Calcium (Ca), Phosphorus (P), Magnesium (Mg), Sodium (Na), Potassium (K), Chloride (Cl^-) and Sulphur (S).

Minor elements/trace elements/micro-minerals and their daily requirement is less than 100 mg. They are Iron (Fe), Iodine (I), Copper (Cu), Manganese (Mn), Zinc (Zn), Cobalt (Co), Molybdenum (Mo), Selenium (Se) and Fluoride (F⁻).

Minerals which are necessary for the body, but their exact functions are not known. They are Chromium (Cr), Nickel (Ni), Bromine (Br), Lithium (Li) and Barium (Ba). Minerals are seen in tissues, non-essential and contaminate foodstuffs. Those are rubidium, silver, gold and bismuth. Minerals which are toxic and should be avoided are Aluminium (Al), Lead (Pb), Cadmium (Cd) and Mercury (Hg).

Calcium

Total calcium in the human body is about 1-1.5 kg, of which 99 percent is in bones. In ECF 1 percent calcium present is in the physiologically active form and responsible for biological functions.

Sources

Milk is the good source (100 mg/100 ml in cow's milk) and egg, fish, vegetables are the medium sources and small amount of calcium is found in cereals.

<i>Required daily allowance (RDA):</i>	Adults	- 500 mg
	Children	- 1200 mg
	Pregnancy and lactation	- 1500 mg

After the age of 50 years, calcium - 1500 mg and vitamin D - 20 µg/day.

Absorption

Calcium is absorbed in duodenum and proximal jejunum. It is an active and energy dependent process. It requires carrier protein (calbindin) and Ca⁺⁺ dependent ATPase. Absorption is influenced by several factors.

Factors Affecting Calcium Absorption

a. Increase in Calcium Absorption

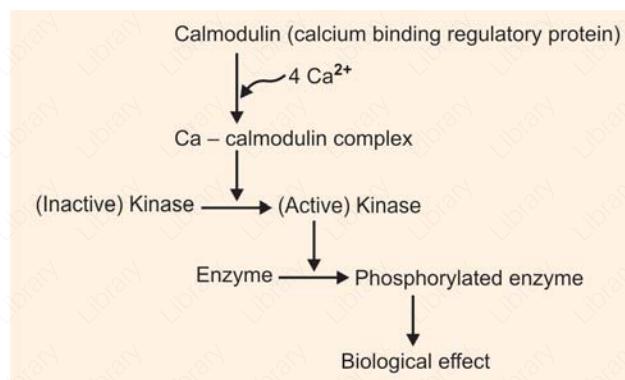
- i. Lactose of milk and other sugars get converted to organic acids and increase the absorption of Ca salts.
- ii. Amino acids such as lysine, arginine, histidine, tryptophan increase calcium absorption. Thus, calcium is absorbed more efficiently from high protein diets.
- iii. Vitamin D induces the synthesis of calcium binding protein in the intestinal epithelial cells and promotes calcium absorption.
- iv. Parathyroid hormone (PTH) stimulates calcium absorption indirectly via activating vitamin D.

b. *Decrease in Calcium Absorption*

- i. Phytic acid is a common constituent of many cereals, it binds dietary calcium forming insoluble complexes which are not absorbed by the intestine.
- ii. Obstruction of the bile duct or a defective bile salt supply leads to deficiency of vitamin D that impairs calcium absorption.
- iii. Coeliac disease will also interfere with vitamin D absorption and consequently the absorption of calcium.
- iv. Chronic renal failure is associated with impaired conversion of vitamin D to its active form and consequently absorption of calcium will also be compromised.

Functions of Calcium

1. *Constituent of bones and teeth:* Calcium is present in bones and teeth as hydroxyapatite and carbonate. Bones also act as reservoir for calcium in the body. Osteoblasts induce bone deposition and osteoclasts promote demineralization.
2. *Role in blood coagulation:* Ca^{2+} is factor IV and it helps in conversion of prothrombin to thrombin. Many other clotting factors are Ca dependant. Precipitation of calcium by oxalate or fluoride renders the blood non-coagulable.
3. *Nerves:* Calcium is essential for nerve impulse transmission from presynaptic to postsynaptic region.
4. *Role in enzyme action:* Activation of number of enzymes or binding proteins requires Ca^{2+} as a specific cofactor. Calcium ions stabilize the active conformation of the enzymes, such as glycogen synthase, adenylate cyclase, phospholipase A₂, some protein kinases, pyruvate dehydrogenase etc. The mechanism of action is summarized as follows:

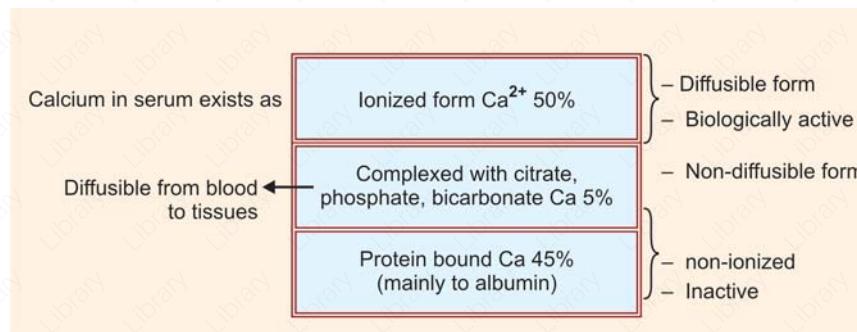


5. *Membrane permeability:* Ca^{2+} reduces membrane permeability to ions and water, by binding to calmodulin of cell membrane and changing the conformation and hydration of membrane proteins.

6. *Role in muscle contraction:* Ca^{2+} mediates excitation and contraction of muscle fibers. Ca^{2+} interacts with troponin C to trigger muscle contraction. Calcium also activates ATPase, increases the interaction between actin and myosin.
7. *Release of hormones:* Ca^{2+} facilitates the release of certain hormones like insulin, PTH, calcitonin from the endocrine glands.
8. *Contact inhibition:* Calcium is believed to be involved in cell to cell contact and adhesion of cells in a tissue. Increase in intracellular Ca^{2+} , closes the gap junctions which are the communicating channels between adjacent cells.
9. *Intracellular messenger:* Calcium mediates the action of certain hormones and hence regarded as a second messenger. *Example:* Epinephrine in liver glycogenolysis.
10. Calcium is required for cell motility, mitosis and other microfilament mediated processes.
11. Calcium is involved in membrane transport and for the maintenance of membrane integrity.
12. *Action on heart:* Calcium acts on myocardium and prolongs systole.

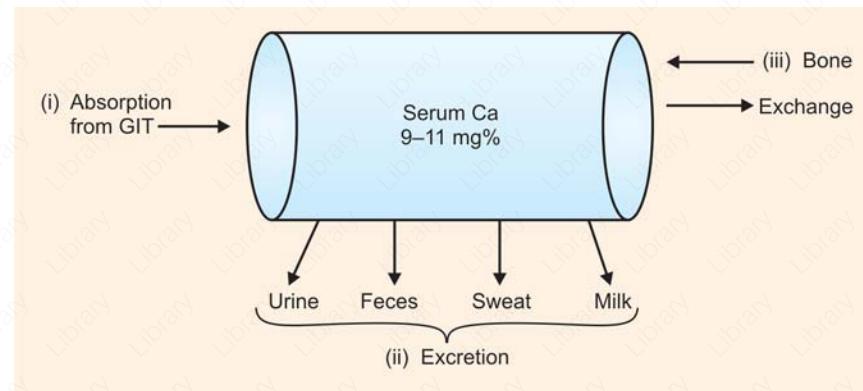
Serum Calcium

Normal range of serum calcium is 9 - 11 mg/dl. Calcium has got reciprocal relationship with serum phosphate level. The ionic product of $\text{Ca} \times \text{P} = 40$ in adults. The ionic product of $\text{Ca} \times \text{P} = 50$ in children.



Processes that alter serum calcium level are:

1. Absorption from GIT
2. Excretion via kidney, milk, faeces
3. Exchange of calcium between bone and serum



Factors that regulate serum calcium

1. PTH (Parathyroid hormone)
 2. Calcitriol (Active form of vitamin D - 1, 25 DHCC)
 3. Calcitonin
1. Parathyroid hormone (Fig. 6.2.1) is secreted by parathyroid glands when serum calcium level falls.

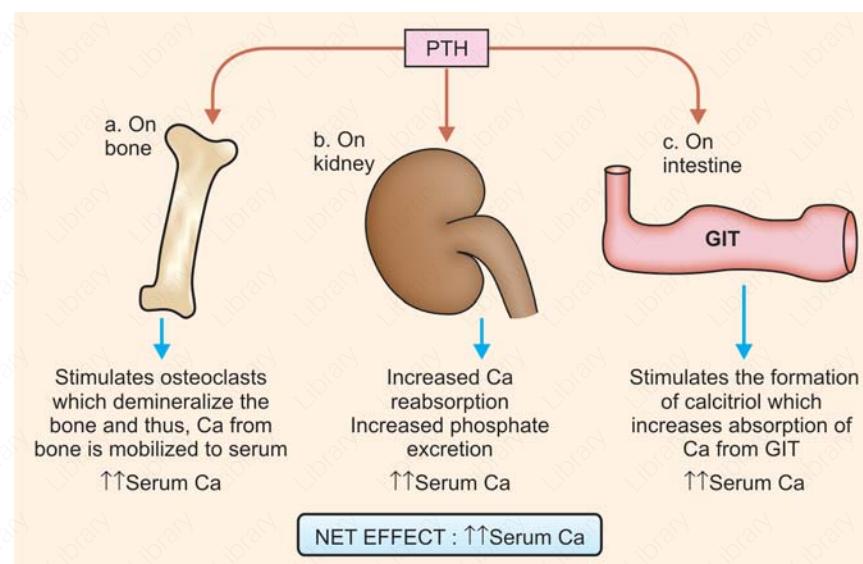


Fig. 6.2.1: Action of parathyroid hormone

2. Calcitriol (Active form of vitamin D) (Fig. 6.2.2)

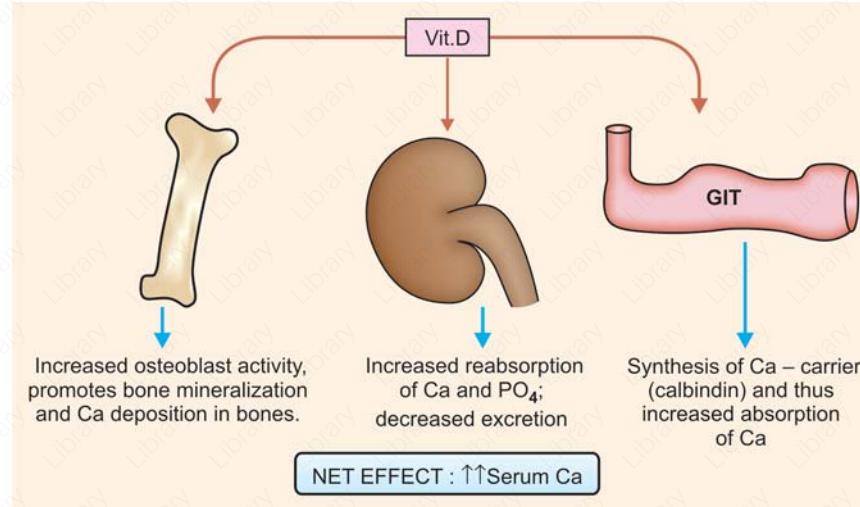


Fig. 6.2.2: Action of vitamin D

3. Calcitonin is secreted by parafollicular cells of thyroid, when serum Ca is increased. Here, actions are opposite to PTH and it inhibits resorption (demineralization) of bones (Fig. 6.2.3).

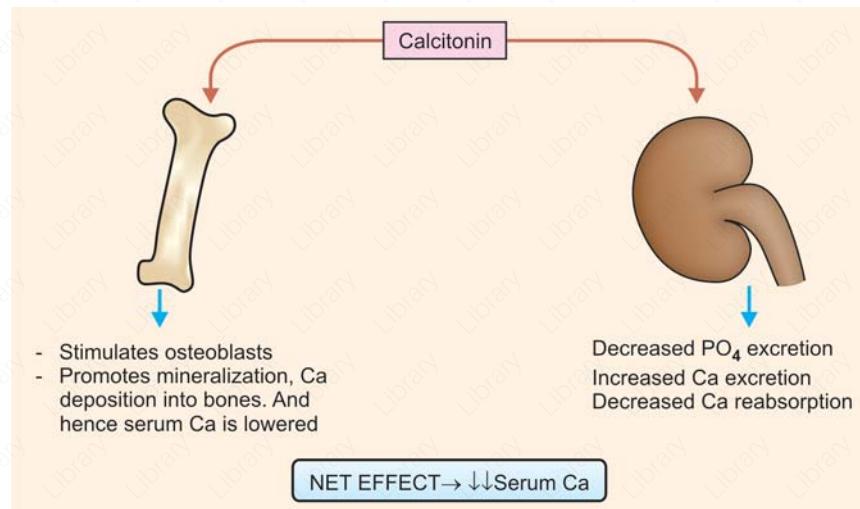


Fig. 6.2.3: Action of calcitonin

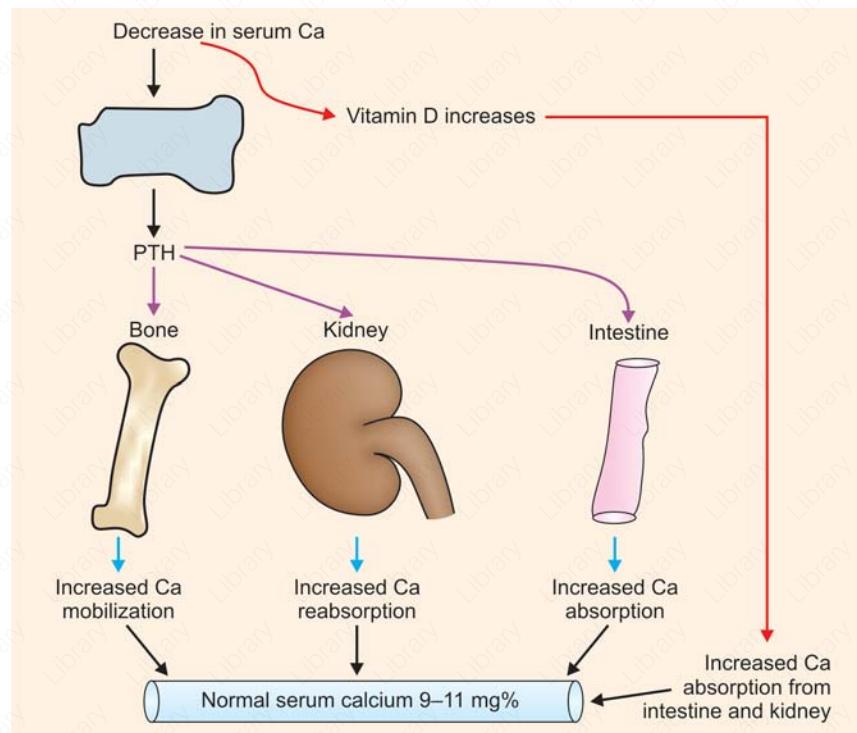


Fig. 6.2.4: Action of PTH and vitamin D when serum calcium decreases

ABNORMAL CALCIUM LEVELS

Hypocalcemia: Serum calcium level less than 8.8 mg/dl. This occurs in inadequate dietary intake, hypoparathyroidism, vitamin D deficiency (Rickets and Osteomalacia), renal failure. Hypocalcemic tetany results if serum calcium level is less than 7.5 mg/dl.

Hypercalcemia: Serum calcium level more than 11mg/dl. This occurs in hyperparathyroidism (because of tumor of parathyroid gland), increased PTH secretion leads to hypercalcemia. In hypervitaminosis D and osteoporosis, decrease in bone density in old age, leads to fractures.

Serum calcium is determined by Trinder Method and O-Cresolphthalein Complexone Method.

Interpretation

Normal value ranges from 9-11 mg/100 ml serum or 4.5 - 5.5 mEq/L.

A lower than the normal level of calcium may be seen in:

- Tetany
- Hypoparathyroidism
- Sprue

- Diarrhea
- Childhood rickets
- Calcium poor diet

Higher than the normal level of calcium is seen in:

- Primary hyperparathyroidism
- Vitamin D over dosage
- Bone tumors
- Renal diseases.

PHOSPHORUS

Total amount of phosphorus in the body is 1 kg, of which 80 percent is present in bones and teeth as hydroxyapatite and 10 percent in muscles. Rest is distributed in intracellular fluid (ICF) and extracellular fluid (ECF). Its concentration is greater in ICF than ECF. So, it is mainly "intracellular".

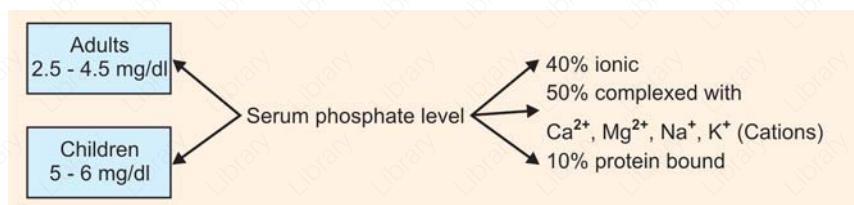
Sources: Milk, cereals, meat, egg, etc.

RDA: Adults : 500 - 800 mg

Absorption: Phosphates are mainly absorbed from jejunum. Calcitriol increases absorption and antacids decrease absorption.

Functions

1. Phosphates are required in the formation of bones and teeth along with Ca, gives strength and hardness to bones.
2. Phosphates are the constituents of phospholipids, lipoproteins, phosphoproteins, etc.
3. Role in ICF:
 - i. Formation of high energy phosphate compounds like ATP, GTP, CTP, creatine phosphate of muscles.
 - ii. Synthesis of coenzymes like NADP, TPP, PLP.
 - iii. Integral part of phosphate buffer system.
 - iv. Formation of phosphoesters like glucose 6-phosphate.
 - v. Regulation of enzyme activity by phosphorylation - dephosphorylation mechanism
 - vi. Phosphates help in the formation of phosphodiester bonds in DNA and RNA.



- Phosphate is excreted in urine - 500 mg/day.
- Regulation of serum phosphate is mainly by PTH which increases excretion of phosphate in urine.

Renal Rickets

Renal tissue damage is observed. Calcitriol synthesis is impaired. High vitamin D doses do not help. This can be treated by administration of calcitriol.

Rickets

It is a disorder of defective calcification of bones. Decreased vitamin D or dietary deficiency of calcium and phosphate or both. Increased alkaline phosphatase level is observed which is a characteristic feature of rickets.

Determination of serum inorganic phosphate is by Fiske-Subba Row Method.

Interpretation

- The phosphate concentration in serum is inversely proportional to the calcium concentration.
- The normal value of phosphate in serum for adults is 2.5 to 4.5 mg percent and for children 4-6 mg percent.
- Increased serum phosphate may be found in:
 - Hypoparathyroidism
 - Renal failure
- Low serum phosphate level may be found in:
 - Hyperparathyroidism
 - Calcium absorption disturbances
 - Vitamin D over dosage

MAGNESIUM

Total body Mg²⁺ is about 20 g, 75 percent of which is associated with calcium in bones. It is mainly intracellular.

Sources: Cereals, beans, leafy vegetables and fish.

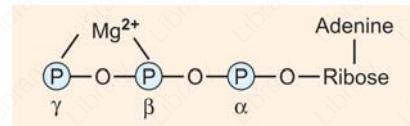
RDA: Men = 400 mg.

Women = 300 mg.

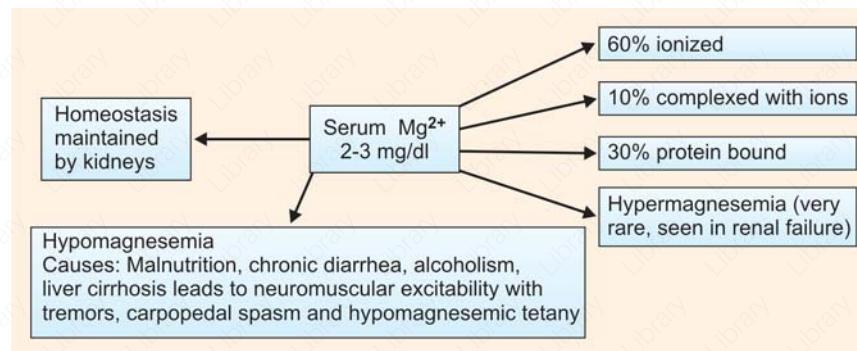
Increased amount acts as purgative.

Functions

- i. Magnesium is a constituent of bones and teeth. 70 percent of body Mg²⁺ is present as apatites in bones, enamel and dentin.
- ii. It decreases neuromuscular irritability.
- iii. Enzyme action: Co-factor of enzymes using ATP. Example: Glucokinase, phosphofructokinase.



- iv. Improves glucose tolerance.



ZINC

Total zinc (Zn) content of body is 2 grams. Out of which 60 percent in skeletal muscles and 30 percent in bones.

Rich dietary sources: Grains, beans, nuts, cheese, meat and shell fish.

RDA: 10 mg for adults and children.

Normal serum level of zinc is 100 $\mu\text{g}/\text{dl}$.

Functions

- i. More than 300 enzymes are Zn dependent. Some important examples are carbonic anhydrase, alkaline phosphatase, lactate dehydrogenase, ethanol dehydrogenase, carboxy peptidase, DNA and RNA polymerase, superoxide dismutase.
- ii. Zinc is present in "Gusten" protein in saliva, which is important for taste sensation.
- iii. Zinc is included in native structure of insulin.
- iv. It is an important element in wound healing as it is a necessary factor in the biosynthesis and integrity of connective tissue.
- v. In vitamin A metabolism, it stimulates vitamin A release from liver to blood.
- vi. Zinc is necessary for the growth and division of cells.
- vii. Zinc stabilizes structure of protein and nucleic acids.

Deficiency Manifestations

Zinc deficiency has many causes but malnutrition and malabsorption are the most common. Clinical symptoms include growth failure, hair loss, reduced taste acuity, hypogonadism and delayed wound healing.

Acrodermatitis (inflammation around mouth, nose, fingers, etc), enteropathica is a rare inherited disorder of zinc metabolism. It is due to an inherited defect in zinc absorption that causes low plasma zinc concentration and reduced total body content of zinc, it is manifested in infancy as skin rash.

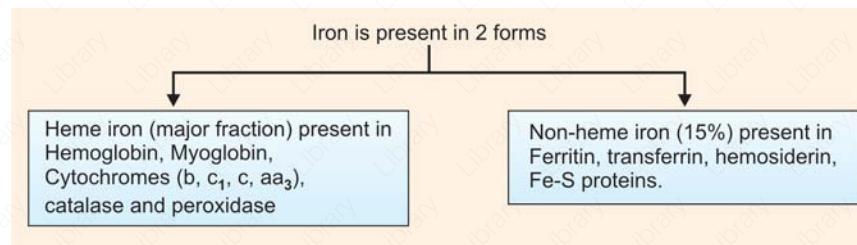
Zinc toxicity: Toxic manifestations if intake is more than 1000 mg/day.

Chronic toxicity: Gastric ulcer, pancreatitis, anemia, nausea and vomiting.

Acute toxicity: Fever, excessive salivation, headache, etc.

IRON (FE)

Iron is a trace element, total body Fe is about 3 - 5 grams.



Sources

Sources are meat, liver, eggs, green-leafy vegetables, cereals, legumes, nuts, jaggery, dates, chikku, etc.

RDA:	Adult man	- 10 mg
	Adult women	- 18 mg
	Pregnancy and lactation	- 40 mg

Functions: Iron helps mainly in the transport, storage and utilization of oxygen.

- As a constituent of hemoglobin and myoglobin (O₂ and CO₂ transport)
- Cellular respiration since Fe is a part of cytochromes and Fe-S proteins of respiratory chain.
- As a constituent of enzymes like catalase, peroxidase, tryptophan pyrrolase, succinate dehydrogenase.
- Iron is associated with effective immune competence of the body.

Iron absorption: only 10 percent (= 1 mg) of food iron is absorbed. Absorption takes place in duodenum and jejunum. Iron is present in Fe³⁺ (ferric) form, bound to proteins or organic acids of food. Iron absorption always occurs in ferrous (Fe²⁺) form. Gastric HCl, reducing substances like vitamin C, cysteine convert Fe³⁺ to Fe²⁺ and thus favor Fe absorption. Phytates and oxalates in the diet reduce iron absorption. Calcium, Copper, Zinc, lead and phosphates inhibit iron absorption.

Absorption, Storage and Utilization of Food Iron

See figure 6.2.5.

Storage of Iron

- Iron is stored in liver, spleen, bone marrow and intestinal mucosal cells in the form of "ferritin".
- Iron can also be stored as "hemosiderin", when all body ferritin is saturated. It occurs when iron is in excess (body overloaded with iron).

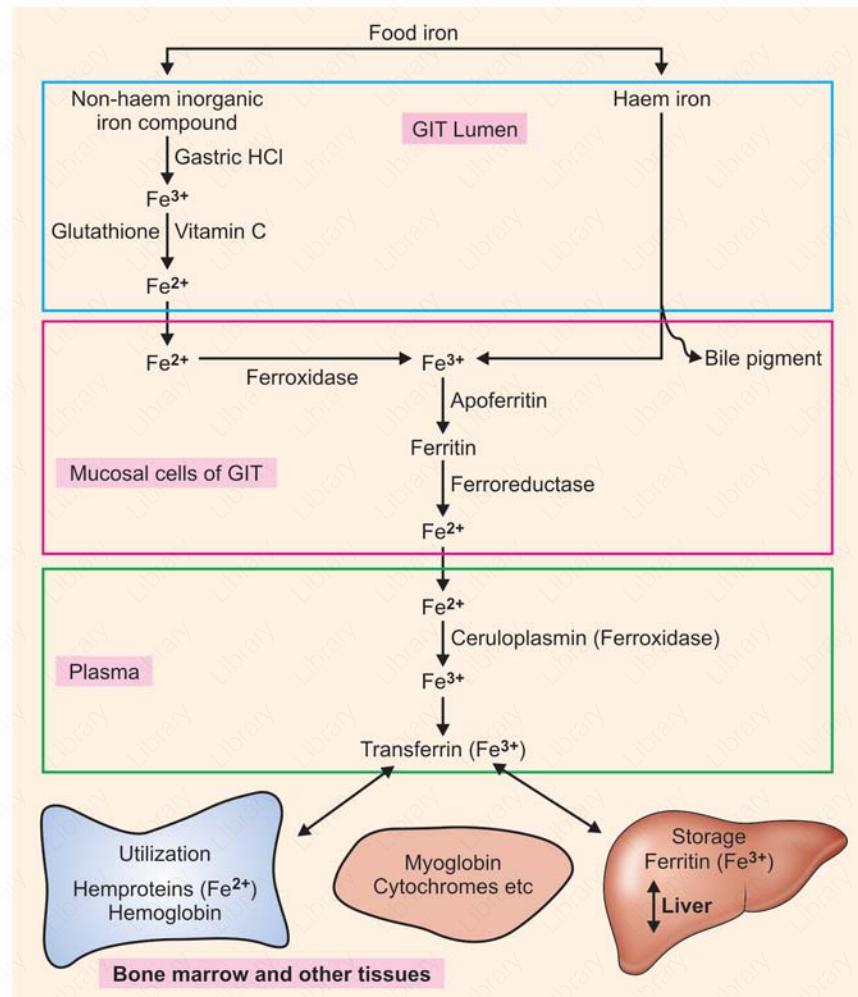


Fig. 6.2.5: Iron absorption and transport

Regulation of Body Iron

Fe is a highly conserved element in the body; efficiently utilized and reutilized. Excretion is negligible. Hence, Fe is called a one-way element (It can only get in, cannot get out). Iron level in the body is controlled at the absorption level. When there is excess iron, absorption is inhibited. When there is iron deficiency, absorption is highly increased ("mucosal block theory").

Iron Excretion: Negligible.

It can occur by:

- i. Blood loss due to accidental bleeding, menstruation, from GIT due to peptic ulcers and hookworm infestation.
- ii. By desquamation of intestinal mucosal cells.

Diseases associated with iron metabolism:

1. Iron deficiency causes anemia
2. Iron excess causes hemosiderosis

Iron deficiency anemia: Deficiency of iron in the blood gives rise to anemia and this condition is called iron deficiency anemia. It is common in India. Children, adolescent girls, pregnant and lactating women are susceptible to iron deficiency.

Microcytic hypochromic erythrocytes are present in the blood. The hemoglobin content is less than 9 g %.

Conditions are :

- i. Increased demand of iron, occurs in pregnancy
- ii. Increased loss of iron in hookworm infestation and hemorrhage
- iii. Reduced intake of iron, malnutrition, diet poor in iron.

Symptoms: Tiredness, apathy, repeated infections, growth retardation, breathlessness on exertion, giddiness and skin acquires a pale color. In severe cases finger nails become soft, spoon shaped and affected children tend to eat mud.

Treatment: Iron supplementation.

Iron excess: Hemosiderosis (Iron excess without cell damage).

Occurs due to:

- Repeated blood transfusions for patients of thalassemia, hemophilia, hemolytic anemia.
- Bantu tribe in South Africa suffers from hemosiderosis, due to increased intake of iron as they cook in iron pots.
- Genetic defect where iron absorption is increased.

Manifestations

Deposition of hemosiderin in various organs like spleen, liver, pancreas, kidney etc. affecting the functioning of organs.

Hemochromatosis: (Excess iron associated with injury to cells).

- It is a rare disease in which hemosiderin is deposited in tissues, resulting in yellowish brown discoloration of skin.
- Excessive intestinal absorption of iron due to genetic defect.

Bronze diabetes: The triad of liver cirrhosis, diabetes, yellow discoloration of skin leads to bronze diabetes.

Normal serum ferritin: 3-30 µg/dl in males
2-12 µg/dl in females

Normal transferrin level in plasma is 250 mg/dl, Total iron binding capacity (TIBC) is 400 mg/dl. This is provided by transferrin.

Normal serum iron: 100-150 µg/dl.

Normal blood iron: 5 mg/dl.

SODIUM

Sodium is the major cation of extracellular fluid (ECF). Total body sodium is about 4000 mEq. Out of which 50 percent in bones, 40 percent in ECF and 10 percent in soft tissues.

Sources: Table salt, vegetables, fruits, meat

RDA: 5 - 10 g.

Absorption: Sodium readily gets absorbed in the bloodstream via the epithelial cells by active transport.

Excretion: Major route of excretion is via kidneys. The rate of sodium excretion is related to the GFR. When the GFR falls, less sodium is excreted and vice versa.

Metabolic Functions

- i. It maintains the osmotic pressure and water balance.
- ii. It is a constituent of buffer and involved in the maintenance of acid base balance.
- iii. It maintains muscle and nerve irritability at the proper level.
- iv. Sodium is involved in cell membrane permeability.
- v. It is involved in the intestinal absorption of glucose.
- vi. It is necessary for initiating and maintaining heart beat.

Clinical Conditions

Decrease in sodium levels leads to dehydration, reduced blood volume and circulatory failure.

Increased sodium levels cause edema, increase in blood volume and blood pressure.

Hyponatremia: Decrease in serum Na⁺ level can occur in diarrhea, vomiting, diuretic therapy and Addison's disease (Adrenocortical insufficiency).

Hypernatremia: Increase in serum Na⁺ level can occur in increased secretion of aldosterone, increased secretion of glucocorticoids (Cushing's syndrome), excessive elimination of dilute urine in diabetes insipidus and pregnancy.

Patients with high blood pressure should restrict salt.

Determination of Sodium

Specimen: Serum, heparinized plasma, sweat, urine, feces, or gastrointestinal fluids are used for the assay.

1. Hemolyzed samples are not suitable for electrolyte analysis because RBC releases potassium which will cause a false increase in the K^+ values. Hemolysis causes a decrease in sodium values also.
2. Urine collection for electrolyte estimation should be made without any preservative.
3. Serum, plasma or urine must be stored at 2 - 8°C or frozen if the analysis is delayed.

Sodium may be determined by atomic absorption spectrophotometry (AAS), flame emission spectrophotometry (FES), electrochemically with a sodium ion selective electrode (ISE) or spectrophotometrically. FES and ISE assays are currently used because of their accuracy.

Reference intervals of sodium in serum: 136-145 mEq/L.

Deficiency

A nutritional deficiency is highly impossible since normal Indian diet contains about 5-10 g of sodium mainly in the form of table salt (sodium chloride). On a low sodium diet, the kidney decreases the excretion of sodium in urine. A low blood sodium triggers the kidney to release angiotensin which causes the adrenal cortex to secrete aldosterone, the latter induces the renal tubules to reabsorb sodium from the glomerular filtrate.

POTASSIUM (K)

- Potassium is the most important cation of intracellular fluid (ICF).
- Average concentration is 150 mEq/L of ICF.
- Extracellular potassium is important for its controlling influence upon neuromuscular irritability, cardiac muscle (a proper balance between potassium and calcium is essential for the contraction of heart muscle) and the operation of Na^+/K^+ -ATPase (Na^+ pump) against the concentration gradient.

Sources: Whole and skimmed milk, bananas, tomatoes, oranges, melons, potatoes, sweet potatoes, prunes, raisins, spinach, turnip greens, collard greens, kale, other green leafy vegetables, most peas and beans, and salt substitutes (potassium chloride).

RDA: About 4 gm.

Absorption: Normally, potassium is practically completely absorbed from the gastrointestinal tract.

Excretion: Potassium is normally eliminated almost entirely in the urine and the small amount in the feces. Aldosterone exerts an influence on potassium excretion.

Metabolic Functions

1. It is the principal cation of ICF.
2. Potassium is required for the functioning of nerves, skeletal muscles and cardiac muscles. Either decreased potassium or increased potassium levels finally cause cardiac arrest.
3. Potassium is required as a cofactor in several enzymatic reactions in the body.
4. Potassium is involved in acid-base balance.

Clinical Conditions

Hypokalemia

- It is the condition in which serum potassium is reduced
- This condition decreases the heartbeat and interferes with vital muscles such as those involved in respiration
- Hypokalemia can occur in any illness, wasting disease, intestinal fistulas and in diarrhea
- Aldosterone increases the excretion of potassium or administration of cortisone leads to hypokalemia
- Certain diuretics increase the excretion of potassium. It is, therefore, important to supplement enough potassium when these diuretics are used.

Hyperkalemia

- Elevated plasma potassium concentration is observed.
- It occurs in Addison's disease and in intravenous infusion of potassium at a rate excess of 25 mmol/hr.
- It also occurs in treatment using concentrated potassium solutions.

Causes

- Acute renal failure
- Chronic renal failure
- Glomerulonephritis
- Tissue trauma causing the cells to release potassium into the ECF includes burns, traumatic injury and intestinal bleeding.

Signs and Symptoms

- Fatigue
- Weakness
- Tingling
- Numbness
- Paralysis
- Palpitations and difficulty in breathing.

Assay of Potassium

1. Flame emission spectrophotometry (Flame photometry).
2. *Ion selective electrode method (ISE)*: Specific electrodes for sodium and potassium are used in this method. The above two methods are more popular and commonly used.
3. Atomic absorption spectrophotometry (AAS).
4. Spectrophotometric methods.

Normal Range

In serum – 3.5 - 5 mEq/L

In plasma – 3.5 - 4.5 mEq/L

- Serum potassium level above 7 mEq/L and below 2.5 mEq/L is serious, life threatening and requires immediate attention.
Specimen: Serum, heparinized plasma, sweat, urine, feces, or gastrointestinal fluids are used for the assay.
- Hemolyzed samples are not suitable for electrolyte analysis because of the release of potassium from the RBC which will cause a false increase in the potassium values.
- Urine collection for electrolyte estimation should be made without any preservative.
- Serum, plasma or urine must be stored at 2 - 4°C or frozen if the analysis is delayed.

CHLORIDE (Cl⁻)

Chloride is the major extracellular anion. As a component of sodium chloride, chloride ion is essential in acid-base equilibrium.

Sources: It is mainly available as sodium chloride.

RDA: The daily requirement in tropical countries:

Adults	– 10 - 20 g
Children	– 5 - 10 g
Women during pregnancy and lactation	– 10 - 15 g

Absorption: Normally, chloride is practically completely absorbed from the gastrointestinal tract.

Excretion: Chloride is chiefly eliminated in the urine. It is also excreted in the sweat. It is lost more during excessive sweating in hot climates under hard work. Its concentration in sweat is decreased by aldosterone.

Metabolic Functions

It is involved in maintaining osmotic pressure, proper body hydration and electric neutrality. Dietary chloride is almost completely absorbed by the intestine. It is filtered out by the glomerulus and passively reabsorbed

in conjunction with sodium by the proximal tubules. Excess chloride is excreted in the urine and through sweating. Excessive sweating stimulates aldosterone secretion, which acts on the sweat glands to conserve sodium and chloride.

Hypochloremia

A low serum chloride is associated with loss of gastric HCl due to prolonged vomiting, salt losing renal disease, in metabolic acidosis, etc.

Hyperchloremia

High serum chloride is seen in dehydration and decreased renal blood flow.

Determination of Chloride

During the assay of electrolytes high quality of distilled water is recommended for preparation of standards and diluting the samples. Chloride in the plasma or serum is determined by colorimetric method using mercuric thiocyanate and optical density of red color is measured at 480 nm wavelength.

Interpretation

The normal level is between 95-105 mEq/L. A higher than the normal may be seen in dehydration, respiratory acidosis, nephritis and Cushing's syndrome.

A lower than the normal level may be observed in diarrhea, vomiting, pyloric obstruction, Addison's disease, pulmonary emphysema, etc.

SELENIUM (Se)

Selenium is distributed in all tissues, higher amounts in renal cortex, pancreas, pituitary and liver.

Sources: Plant foods, cereals, fish and meat, food content of selenium depends upon selenium content of soil.

RDA: 50 - 100 µg

Absorption and excretion: The principal dietary forms of selenium selenocysteine and selenomethionine are absorbed from gastrointestinal tract. Selenium homeostasis is achieved by regulation of its excretion via urine.

Functions

- i. Selenium is a constituent of glutathione peroxidase which has a cellular antioxidant function protecting cell membrane against oxidative

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damage by H_2O and variety of hydroperoxides. Selenium is important in preventing lipid peroxidation and protecting cells against superoxide and some other free radicals.

- ii. Selenium is also a constituent of the enzyme that converts thyroxine to triiodothyronine.
- iii. Selenium is a synergistic antioxidant with vitamin E.
- iv. Selenium binds with heavy metals like Cd, Hg, Ag and minimizes their effects.

Deficiency manifestations: Selenium deficiency is associated in some parts of China with Keshan disease with cardiomyopathy that primarily affects children and women of child bearing age. Its most common symptoms include dizziness, loss of appetite, nausea, abnormal electrocardiograms and congestive heart failure.

Selenium toxicity (Selenosis): Excessive selenium intakes result in alkali reserve characterized by loss of hair and nails, skin lesions, liver and neuromuscular disorders that are usually fatal.

IODINE (I)

The adult human body contains about 50 mg of iodine. Eighty percent of it is present in thyroid gland as free iodine as well as in the form mono, di, tri and tetraiodothyronine. Skin and skeleton also contain small amounts of iodine. The blood plasma contains 4 - 8 μg of protein bound iodine (PBI) per 100 ml.

Sources: Sea food, drinking water, iodized table salt, onions, vegetables etc.

RDA: 100 - 150 μg

Absorption and excretion: Iodine in the diet is absorbed rapidly in the form of iodide from small intestine. Nearly 70 - 80 percent of iodine is excreted through bile, skin and saliva. Milk of lactating women also contains some iodine.

Ingredients in foodstuffs, which prevent utilization of iodine are called goiterogens. For example, cabbage and tapioca contain thiocyanate, which inhibits iodine uptake by thyroid.

Function

The most important role of iodine is in the synthesis of thyroid hormones, triiodothyronine (T_3) and tetraiodothyronine (T_4), which influence a large number of metabolic functions.

Deficiency Manifestation

Deficiency of iodine occurs in several regions of the world where the iodine content of soil and therefore of plants is low. A deficiency of iodine in children leads to cretinism and in adults endemic goiter.

Cretinism: Severe iodine deficiency in mother leads to intrauterine or neonatal hypothyroidism which results in cretinism in their children, condition characterized by mental retardation, slow body development, dwarfism and characteristic facial structure.

Goiter: It is an enlargement of the thyroid gland, with decreased thyroid hormone production. An iodine deficiency in adults stimulates the proliferation of thyroid epithelial cells, resulting in goiter. The thyroid gland collects iodine from the blood and uses it to make thyroid hormones. In iodine deficiency, thyroid gland undergoes compensatory enlargement in order to extract iodine from blood more efficiently.

COPPER (Cu)

A 70 kg human adult body contains approximately 80 mg of copper, 50 percent in muscles and 25 percent in bones.

Sources: Fish, meat (liver), lentils, dried legumes, nuts, leafy vegetables, potato and beet. Milk is a poor source of copper.

RDA: 2 - 5 mg.

Absorption and excretion: About 50 percent of the daily dietary copper is absorbed from the stomach and the small intestine. Absorbed copper is transported to the liver bound to albumin and exported to peripheral tissue mainly (about 90%) bound to ceruloplasmin and to a lesser extent 10 percent to albumin. The main route of excretion of copper is in the bile into the gut.

Transport: Copper enters the plasma and binds to serum albumin which is a direct reacting copper or transport form of copper.

Ceruloplasmin: It is a blue-colored glycoprotein, also called serum ferroxidase. It promotes oxidation of ferrous iron to ferric form, which is incorporated into transferrin.

Ceruloplasmin carries 4Cu^{2+} and 4Cu^+ ions per molecule. (Copper tightly bound with ceruloplasmin).

Normal serum level of ceruloplasmin = 25 - 50 mg/dl.

Normal serum copper concentration is usually between 10 - 22 μmole per litre of which 90 percent is bound to ceruloplasmin.

Functions

- i. Copper is an integral component of many metallo enzymes including ceruloplasmin (ferroxidase), cytochrome oxidase, superoxide dismutase, dopamine β hydroxylase Tyrosinase, tryptophan dioxygenase and lysyl oxidase. The major function of metallo protein involves oxidation reduction reactions.

ii. Copper plays an important role in iron absorption. Ceruloplasmin the major copper containing protein in plasma has ferroxidase activity that oxidizes ferrous ions to ferric state before its binding by plasma transferrin.

Deficiency manifestation: Both children and adults can develop symptomatic deficiency.

Signs of copper deficiency include neutropenia (Decreased number of neutrophils) and hypochromic anemia in the early stages.

Osteoporosis and various bone and joint abnormalities result due to impairment in copper dependent cross linking of bone collagen and connective tissue.

Depressed copper dependent tyrosinase activity which is required in the biosynthesis of skin pigment, melanin causes hypo pigmentation of skin.

Neurological abnormalities are observed by depressed cytochrome oxidase activity.

Inborn errors of copper metabolism: There are two inborn errors of copper metabolism:

1. Menke's syndrome
2. Wilson's disease.

Menke's Syndrome or Kinky Hair Disease

It is a genetic defect in transporting the absorbed copper across the serosal membrane of intestinal mucosal cells. It is characterized by depigmentation of skin and hair, seizures, mental retardation and vascular defects and kinky or twisted brittle hair due to loss of copper catalyzed disulfide bond formation.

Wilson's Disease (Hepatolenticular degeneration)

It is an inborn error of copper metabolism. It is an autosomal recessively inherited disorder in which excessive accumulation of copper occurs in tissues. Due to impaired copper excretion into bile, there occurs reabsorption of copper in the kidney and hepatic incorporation of copper into ceruloplasmin.

The gene encoding the copper binding ATPase in hepatocytes, which is required for excretion of copper from liver cells, may be defective. In its absence copper is not eliminated and is accumulated.

Copper accumulates particularly in liver, brain, kidney and eyes leading to copper toxicosis. Excessive deposition of copper in the brain, liver, kidney and eyes produces neurological symptoms, liver damage leading to cirrhosis, renal tubular damage and Kayser-Fleisher rings (brown pigment around the iris) at the edges of the cornea due to deposition of copper in the cornea. Urinary copper excretion is high with low serum concentrations.

FLUORINE (FLUORIDE)

In the form of fluoride, fluorine is found in bones and teeth.

Sources: Drinking water, tea, sea fish, vegetables, cheese, jowar.

RDA: 1 ppm of fluoride in drinking water (parts per million = 1 mg/1000 ml). Intake should not exceed 3 mg/day.

Functions

Role in dental health: Fluoride is present in traces in teeth. It helps in tooth development, maintenance and hardening of enamel. It prevents the development of dental caries. Fluoride forms protective Ca-F coating around the tooth. This coating gets converted to tough fluoroapatite layer around the tooth, thus making tooth resistant to decay by acids formed by bacteria.

Role in bone development: Trace amounts of fluoride acts as a catalyst in calcium hydroxyapatite formation.

Diseases

- a. Deficiency causes dental caries: Drinking water having less than 0.5 ppm of fluoride will cause dental caries in children.
- b. Fluoride excess: Causes "FLUOROSIS"
 - i. Fluoride level more than 2 ppm - Chronic intestinal upset, gastroenteritis, loss of appetite and loss of weight (systemic manifestation).
 - ii. Fluoride level more than 5 ppm - Mottling of enamel, discolouration of teeth with brown or yellow patches on the surface. This is dental fluorosis (Dental manifestation).
 - iii. Fluoride level more than 20 ppm - is toxic, causes pathological changes in bones, leading to alternate areas of osteoporosis and osteosclerosis with brittle bones. This is called skeletal fluorosis (Skeletal manifestation).
 - iv. A characteristic feature of skeletal fluorosis is "Genu Valgum" - advanced fluorosis with stiff joints.
 - v. In Karnataka, Andhra Pradesh, Punjab, Rajasthan, Uttar Pradesh, Delhi and Tamil Nadu, due to use of water from deep subsoil well which contains excess fluoride, fluorosis is widespread (Fluoride level increased to 50 µg/100 ml).
 - vi. Normal value is 4 µg/100 ml.
 - vii. High concentrations of fluoride inhibit Mg requiring enzymes like enolase of glycolysis, etc.

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MULTIPLE CHOICE QUESTIONS

1. The principal cation of extracellular fluid is:
A. Potassium B. Sodium
C. Calcium D. Phosphorus
2. Iron is transported in the plasma in a bound form to a protein called:
A. Ferritin B. Calbindin
C. Transferrin D. Calmodulin
3. The normal concentration of serum potassium:
A. 9 - 11 mg/dl B. 135 - 145 mEq/L
C. 25 - 50 mg/dl D. 3.5 - 5.0 mEq/L
4. Which element prevents the development of dental caries?
A. Chloride B. Calcium
C. Phosphorus D. Fluoride
5. Which element is involved in wound healing?
A. Zinc B. Copper
C. Magnesium D. Sodium

ANSWERS

1. B 2. C 3. D 4. D 5. A

MOST LIKELY QUESTIONS

Long Essays

1. Give the plasma levels of sodium. Explain the functions. Add a note on its imbalance.
2. Write an essay on the daily requirement, sources, functions and deficiency of calcium.
3. Write an essay on the functions, daily requirements, absorption and metabolism of iron.
4. Write an essay on the functions, daily requirements, sources and associated disorders of iron metabolism.
5. Give the normal values for serum calcium. Enumerate the factors regulating calcium equilibrium in the body.

Short Essays

6. Functions of calcium
7. Normal calcium levels and its hormonal regulation
8. Functions of phosphate
9. Selenium metabolism
10. Fluorine metabolism

11. Iodine metabolism
12. Copper metabolism
13. Absorption of iron
14. Tetany
15. Deficiency syndromes of iron
16. Absorption of calcium
17. Functions and deficiency of iron

Short Answers

18. Selenium
19. Functions of Magnesium
20. Ceruloplasmin
21. What are antioxidants? Give 4 examples
22. Fluoride functions
23. Give normal values in serum of the following: Ca^{++} , iron, Na^+ , K^+
24. Functions of copper
25. Functions of Zn
26. Functions of Iodine
27. Tetany
28. Hemochromatosis
29. Transferrin
30. Ferritin
31. Hyperphosphatemia



7.1: IMMUNITY

The term 'immunity' refers to the resistance exhibited by the host towards injury caused by microorganisms and their products. However, protection against infectious diseases is only one of the consequences of the immune response, which in its entirety is concerned with the reactions of the body against any foreign antigen.

Immunity against infectious diseases is of different types: Innate or native immunity is the resistance to infections which an individual possesses by virtue of his genetic and constitutional make-up. It is not affected by prior contact with microorganisms or immunization. It may be nonspecific, when it indicates a degree of resistance to infections in general, or specific where resistance to a particular pathogen is concerned.

Acquired Immunity

The resistance that an individual acquires during life is known as acquired immunity as distinct from inborn innate immunity. Acquired immunity is of two types, active and passive. Active immunity is resistance developed by an individual as a result of an antigenic stimulus. It is also known as adaptive immunity as it represents an adaptive response of the host to a specific pathogen or other antigen. This involves the active functioning of the host's immune apparatus leading to the synthesis of antibodies and the production of immunologically active cells. Active immunity sets in only after a latent period which is required for the immunological machinery to be set in motion.

Measurement of Immunity

The truly valid measurement of immunity is to test the resistance of an individual to a challenge by the pathogen. This is, however, not applicable since the challenge itself alters the state of immunity. It is, therefore, not possible to measure accurately the level of immunity in an individual. Estimates of immunity are generally made by statistical methods using large numbers of individuals.

A simple method of testing immunity is to relate its level to some convenient indicator, such as demonstration of the specific antibody. This is not always reliable as the immune response to a pathogen consists of the formation of antibodies to several antigens present in it, as also to the production of cellular immunity. The antibodies may be demonstrated by a variety of techniques such as agglutination, precipitation, complement fixation, hemagglutination inhibition, neutralization, ELISA and others.

Antigens

An antigen has been defined as any substance which, when introduced parenterally into the body, stimulates the production of an antibody with which it reacts specifically and in an observable manner. This traditional description of an antigen is no longer comprehensive enough in the light of current concepts about the immune response. Some antigens may not induce antibodies but may sensitize specific lymphocytes leading to cell mediated immunity or may cause immunological tolerance.

Biological Classes of Antigens

Depending on their ability to induce antibody formation, antigens are classified as T cell dependent (TD) and T cell independent (TI) antigens. Antibody production is the property of B lymphocytes. For the full expression of this function, however, the cooperation of T lymphocytes is necessary. Some antigens can directly stimulate antibody production by B cells, without the apparent participation of T cells. Such antigens are called TI antigens. Others that require T cell participation to generate an immune response are called TD antigens.

Antibodies - Immunoglobulins

Following the introduction of an antigen into an animal, certain substances called antibodies appear in the serum and tissue fluids, and react with the antigen specifically and in some observable manner. Depending on the observable reaction produced on mixing with antigens, the antibodies were designated variously as agglutinins, precipitins and so on.

Immune Response

The specific reactivity induced in a host by an antigenic stimulus is known as the immune response. In infectious disease it is generally equated with protection against invading microorganisms. But the immune response has a much wider scope and includes reactions against any antigen, living or nonliving. It may lead to consequences that are beneficial, indifferent or injurious to the host. It also includes the state of specific non-reactivity (tolerance) induced by certain types of antigenic stimuli. The immune response can be of two types - the humoral (antibody mediated) and the

cellular (cell mediated) types. The two are usually developed together, though at times one or the other may be predominant or exclusive. They usually act in conjunction but may sometimes act in opposition.

7.2: IMMUNE RESPONSE

Immune response is defined as the specific reactivity induced in a host following an antigenic stimulus.

Such specific reactivity is of two types:

1. Humoral (Antibody-mediated immunity — AMI)
2. Cellular (Cell-mediated immunity — CMI).

Humoral immunity depends on the production of specific antibodies against the antigens that stimulated their production. Antibodies thus formed will either neutralize the antigen or opsonize it and help in its phagocytosis. AMI provides primary defense against most extracellular bacterial infections. It is active against the viruses that infect through the respiratory or intestinal tracts. AMI also participates in the pathogenesis of immediate types of hypersensitivity reactions (Type I, II and III) and certain autoimmune diseases.

Cellular immunity depends on the lymphokines produced by the activated T lymphocytes (Helper T cells and cytotoxic T cells). CMI protects against intracellular bacteria like *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Brucella* spp. etc. It also participates in the pathogenesis of viral and fungal infections. CMI is involved in the rejection of grafts and immunological surveillance and immunity against cancer. It also participates in the pathogenesis of type IV hypersensitivity reaction and certain autoimmune diseases.

Both AMI and CMI responses are usually produced simultaneously, but often, one or the other may be the predominant type of response produced.

AMI Response

When the immune system is exposed to a particular antigen for the first time, the antibody response that occurs is called Primary Immune Response. Appreciable increase in antibody levels occurs only after a lag period of about 7 - 10 days. The titre of antibodies attained is not very high and the response is short lived. IgM is the initial antibody formed and later on there is switch over to IgG type of antibody response.

When the immune system is exposed to the same antigen again and subsequently, the antibody response that occurs is called Secondary Immune Response. During the secondary immune response, the lag period is very brief or negligible. The antibody levels are high and the response is prolonged. IgG is the predominant class of antibody produced during the secondary immune response (Fig. 7.2.1).

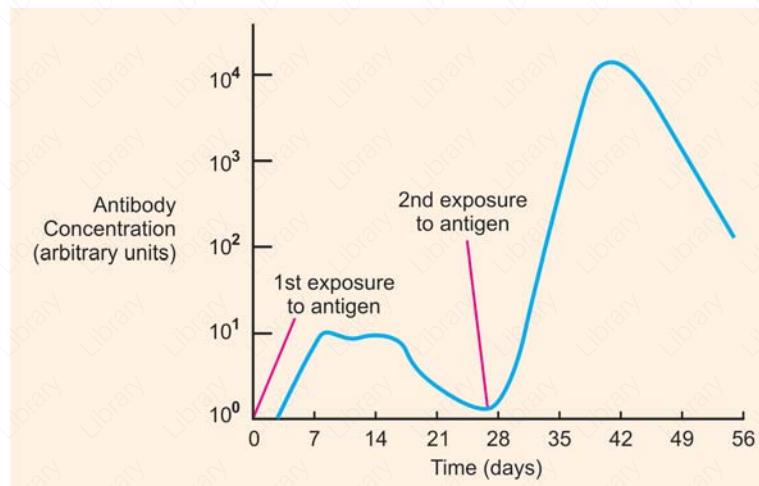


Fig. 7.2.1: Primary and secondary immune responses

Production of Antibodies

The antigen that enters the body is captured by antigen presenting cells (APCs) like macrophages, dendritic cells and Langerhan's cells. Antigen presenting cells ingest the antigen, process the antigen and the processed antigen is expressed on the surface of these cells and presented to T helper cells along with MHC - II molecules. T helper cells stimulate the appropriate clone of B lymphocytes with B cell receptor which is specific to the antigen. The stimulated B cells undergo clonal proliferation and differentiate into plasma cells that synthesise and secrete antibodies (Fig. 7.2.2).

Following antigenic stimulation (Primary Immune Response), a small proportion of B cells from the clone, instead of transforming into antibody producing plasma cells, develop into resting "memory cells" with a long life span. These are the cells that recognize the antigen during the subsequent exposure to the same antigen and bring about a prompt, powerful and more prolonged IgG antibody response (Secondary Immune Response).

Monoclonal Antibodies

During a microbial infection, the antibodies that are produced are heterogeneous as they are synthesized by several different clones of cells specific to different antigens of a particular microbe. Hence, they are called polyclonal antibodies.

A single clone of cells consists of all identical cells and produce antibodies directed against a single antigen or antigenic determinant. Such antibodies are called monoclonal antibodies.

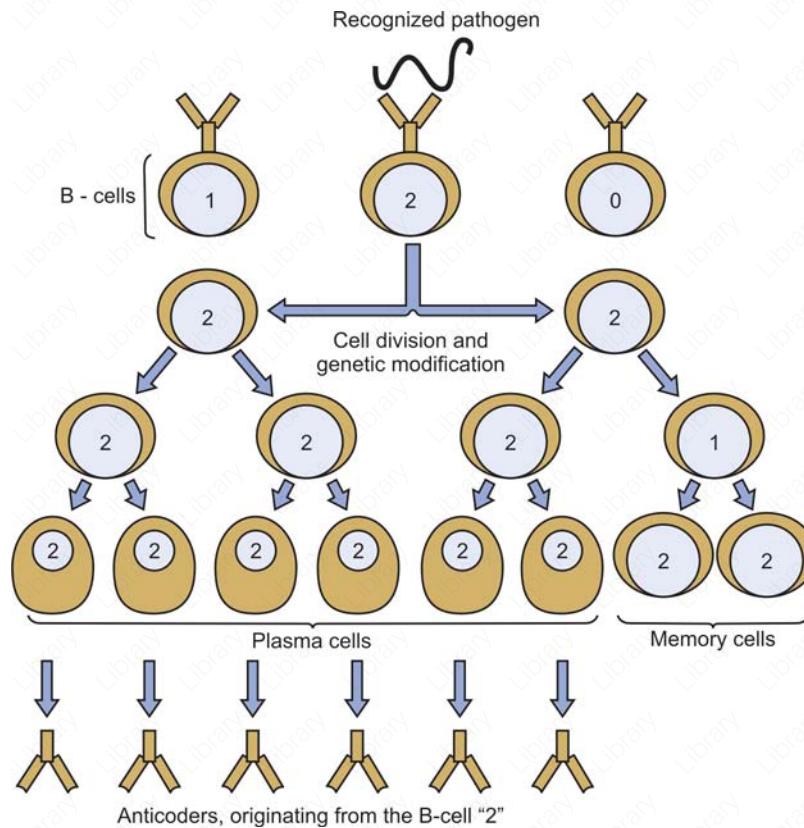


Fig. 7.2.2: Clonal proliferation

For production of such antibodies in the laboratory, antibody producing B cells are fused with immortal myeloma cells to produce hybrid cells. A collection of such cells is called hybridomas. The hybridomas retain the antibody producing capacity of the B lymphocytes and the ability of myeloma cells to undergo multiplication indefinitely, thereby providing an indefinite source of monoclonal antibodies in large quantities (Fig. 7.2.3).

Applications of Monoclonal Antibodies

1. In serological tests, for the detection of bacterial, viral and other antigens.
2. Production of specific antibodies.
3. Treatment of autoimmune diseases.
4. To prevent or treat allograft rejection or graft versus host reaction.

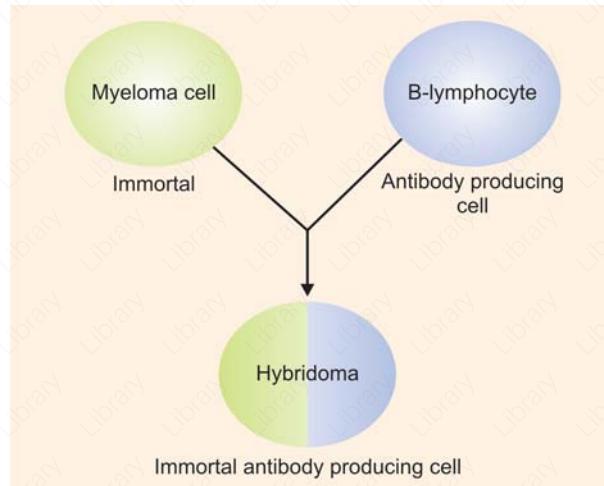


Fig. 7.2.3: Hybridoma technique

CMI Response

In a cell-mediated immune response, foreign antigen is presented by antigen presenting cells to T lymphocytes carrying T cell receptors specific to the encountered antigen. These sensitized T lymphocytes undergo blast transformation and differentiate into effector cells (Th, Tc) and memory cells. The activated lymphocytes release biologically active products called lymphokines which are responsible for the various manifestations of CMI response.

T helper 1 (Th1) cells help macrophages to kill intracellular microbes by releasing macrophage activating factors, particularly IFN γ . These factors activate the previously suppressed microbial mechanisms within the macrophages leading to the death of the intracellular microbes.

Cytotoxic T cells (Tc) recognize the virally infected cells when these cells express the virally derived antigens on their surface along with MHC-I molecules and release lymphokines to kill such cells.

Cytokines

Biologically active substances secreted by macrophages, lymphocytes and other cells are collectively called cytokines.

Lymphokines—produced by lymphocytes.

Monokines—produced by Monocytes/Macrophages.

Interleukins—chemical substances that function primarily as growth and differentiation factors. They exert a regulatory influence on other cells.

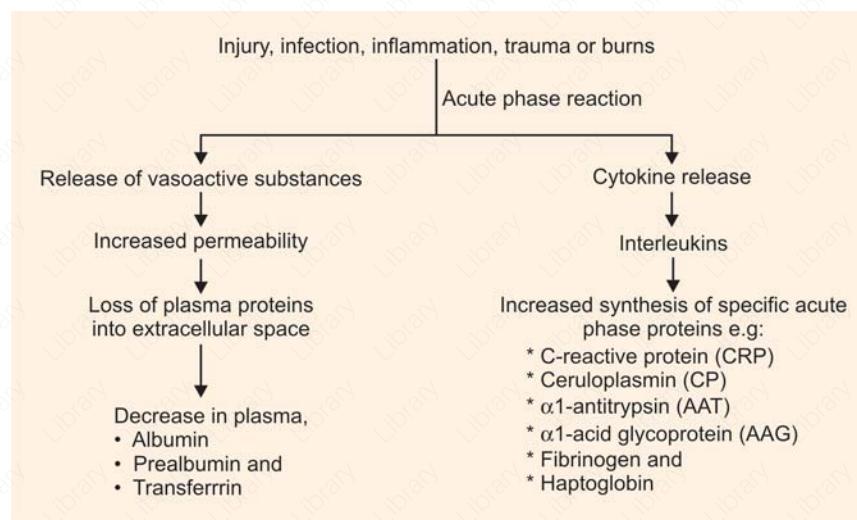


Fig. 7.2.4: Mechanism of acute phase response

Chemokines—are a group of cytokines which are chemotactic for neutrophils and macrophages, and hence attract these cells to the site of infection.

Cytokines include:

1. Interleukins (ILs): IL1, IL2.....IL23.
2. Interferons (IFNs): IFN α , IFN β and IFN γ .
3. Tumor Necrosis Factors (TNFs): TNF α and TNF β .
4. Colony Stimulating Factors (CSFs): GM-CSF, G-CSF, M-CSF.

Acute phase proteins: The concentration of these proteins increases during acute inflammation. They are α -antitrypsin, haptoglobin, ceruloplasmin, complement-3, fibrinogen and C-reactive protein. Their concentration increases in conditions like surgery, myocardial infarction, infections and tumors. Acute phase reaction is general to any infection. They all play a part in complex defensive process of inflammation. The synthesis of these proteins by the liver is triggered by interleukin at the site of injury (Fig. 7.2.4).

7.3: IMMUNOGLOBULINS (IG)

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

- The synthesis of specific immunoglobulin is stimulated by an antigen, a protein or complex carbohydrate that is foreign to the individual.

Structure of Immunoglobulin

Immunoglobulins are glycoproteins made up of 'light' (L) and 'heavy' (H) polypeptide chains. The term 'light' and 'heavy' refer to molecular weight. Light chains have a molecular weight of 25,000 daltons whereas heavy chains have a molecular weight of 50,000 to 70,000.

All immunoglobulins have the same basic structure. The basic immunoglobulin is a 'Y' shaped molecule and consist of four polypeptide chains: two H chains and two L chains (Fig. 7.3.1).

The four chains are linked by disulfide bonds. An individual antibody molecule always consists of identical H chains and identical L chains.

L chain may be either of two types, Kappa (κ) or Lambda (λ) but not both. The heavy chains may be of five types and are designated by Greek letter:

- Alpha (α)
- Gamma (γ)
- Delta (δ)
- Mu (μ) and
- Epsilon (ϵ)

Immunoglobulins are named as per their heavy chain type as IgA, IgG, IgD, IgM and IgE.

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

H chain consists of one variable (VL) and one constant (CL) domain or region. Domain is the loop formed by intrachain disulphide bonds between cysteine residues. Each domain has approximately 110 amino acid residues.

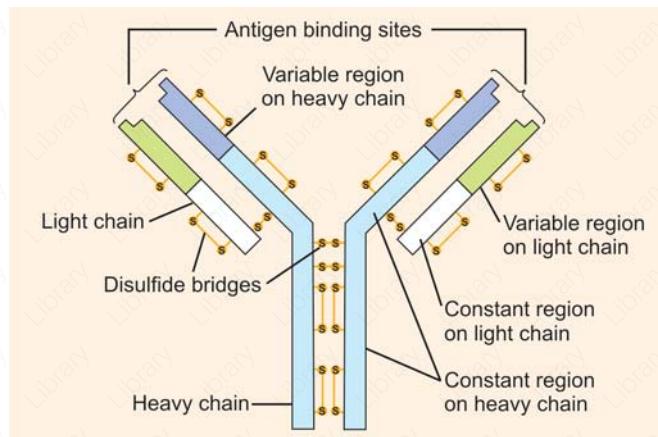


Fig. 7.3.1: Immunoglobulin

Different classes of Immunoglobulins corresponding to the type of heavy chains	
Ig classes	Types of H chains
IgG	γ (gamma)
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	ϵ (epsilon)

Most H-chains consist of one variable (VH) and three constant (CH-1, CH-2, and CH-3) domains. IgG and IgA have three CH domains whereas IgM and IgE have four. Each immunoglobulin molecule has hinge region between CH-1 and CH-2, which allows a better fit with the antigen surface. The variable regions of both the light and heavy chains form antigen binding site, whereas the constant region of heavy chain is responsible for various biologic functions, e.g. Complement (other components of the immune system) activation and binding to cell surface receptors. Complement is a collective term that consists of about 20 different proteins many of which are enzymes. Complement binding site is the CH-2 domain. The constant region of the light chain has no known biological function.

The variable regions of both L and H chains have three extremely variable amino acid sequences at the amino terminal end called hyper-variable region.

Enzyme (papain) digestion splits the immunoglobulin molecule into two fragments named as Fab (Fragment for antigen binding) and Fc (crystallizable fragment or fragment for complement binding). Fc is the constant part of the molecule and does not participate in antigen binding. However, the Fc fragment is associated with secondary effects after binding of complement.

Fab are the variable parts of the molecule and are the site of antigen binding. The variation in amino acid content allows a wide range of specific activity.

Functions of Immunoglobulins

Immunoglobulin G (IgG): It is the major immunoglobulin that constitutes about 75-80 percent of the total immunoglobulins circulation. It is the only type of Ig that can cross placenta and is localized in the vascular and extracellular compartments. IgG can enter the extravascular space and get involved in all the major functions such as:

1. It activates complement system
2. It plays a major role in neutralizing toxins
3. It enhances the phagocytosis of the bound antigen.

IgM: This is the first antibody produced when the immune system is challenged. IgM is the largest Ig, a pentamer that is confined to vascular spaces. It cannot traverse blood vessels due to its large size. IgM serves as receptor on the B lymphocytes and helps in establishing humoral immunity. It is the major antibody of primary immune responses. Natural antibodies are IgM in nature. The natural antibodies are produced without any antigenic stimulation. For example a person having blood group A will have anti B group antibodies in his circulation.

IgA: It is the major immunoglobulin in seromucous secretions, e.g. saliva, tears, sweat, walls of intestine and bronchial mucous. It is plenty in colostrum, the initial secretion from the mother's breast after child birth. IgA provides surface immunity by binding with antigen on outer epithelial surfaces. Thus preventing entry of foreign antigen into the body.

IgE: Its concentration in the plasma is the lowest among all the five Ig classes. Its concentration increases in responses to allergic reactions and is associated with immediate hyper sensitivity.

IgD: It is present on the surface of B lymphocytes and probably involved in the antigen recognition process.

MULTIPLE CHOICE QUESTIONS

1. The cells responsible for the production of immunoglobulins:
A. B-Lymphocytes B. T-Lymphocytes
C. Platelets D. Erythrocytes
2. The immunoglobulin that can cross the placenta and transfer the mother's immunity to the developing fetus:
A. IgE B. IgG
C. IgM D. IgA
3. The immunoglobulins that can bind with mast cells and release histamine:
A. IgG B. IgM
C. IgE D. IgA
4. The immunoglobulin present in most abundant quantity:
A. IgG B. IgA
C. IgM D. IgE
5. Name the immunoglobulin involved in body allergic reactions:
A. IgA B. IgE
C. IgD D. IgM

ANSWERS

1. A 2. B 3. C 4. A 5. B

MOST LIKELY QUESTIONS

Long Essays

1. Give an account of different types of immunoglobulins along with their functions.
2. Describe the structure of an immunoglobulin with a neatly labeled diagram.

Short Notes

3. Immunoglobulin G
4. Immunoglobulin M

7.4: ANTIGENS – HLA TYPING

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Expression of histocompatibility antigens is controlled by a group of genes called Major Histocompatibility Complex (MHC). MHC complex of genes is located on the short arm of the chromosome 6. MHC codes for MHC antigens or molecules (also called HLA or Human Leucocyte Antigens) which play vital role in immune recognition, including interactions between T lymphocytes and other cell types. It consists of three separate clusters of genes.

Class I - comprising of HLA - A, B and C loci.

Class II - (D region): consisting of DR, DQ and DP loci.

Class III - (Complement region): containing genes for complement components, C2 and C4 of the classical pathway as well as Properdin factor B of the alternate pathway and TNF α and β (Fig. 7.4.1).

Every individual inherits one set of HLA genes from each parent which is expressed as two haplotypes.

HLA Antigens

By virtue of genetic make up, every individual has unique antigens on the surface of their cells. These glycoprotein molecules encoded by the MHC

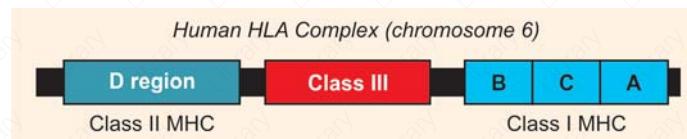


Fig. 7.4.1: MHC complex

genes are responsible for determining whether one tissue is compatible with another. These are called Human Leukocyte Antigens (HLA).

Class I genes code for HLA - A, B and C antigens.

Class II genes code for HLA - DP, DQ and DR antigens.

Class III genes code for C2, C4, factor B etc.

HLA Class I antigens are found on the surface of virtually all nucleated cells, abundantly on lymphoid cells and sparsely on the cells of liver, lung, kidney etc. HLA Class II antigens are more restricted in distribution—found only on cells of the immune system (mostly limited to macrophages and dendritic cells), activated T cells and on B cells.

Indications for HLA Typing

1. *Tissue transplantation:* HLA system is very useful in tissue typing and matching prior to transplantation. HLA typing is used primarily for testing compatibility between the recipients and potential donors before tissue transplantation.
2. *Disputed paternity:* Helpful in paternity determination.
3. *Anthropological survey:* As the prevalence of HLA types varies widely between different human races and ethnic groups.
4. *Disease association:* An association has been observed between HLA types and certain diseases. HLA B27 - Ankylosing Spondylitis; HLA DR4 - Rheumatoid Arthritis; HLA DR3 - many Auto-Immune conditions.

7.5: FREE RADICALS AND ANTIOXIDANTS

INTRODUCTION

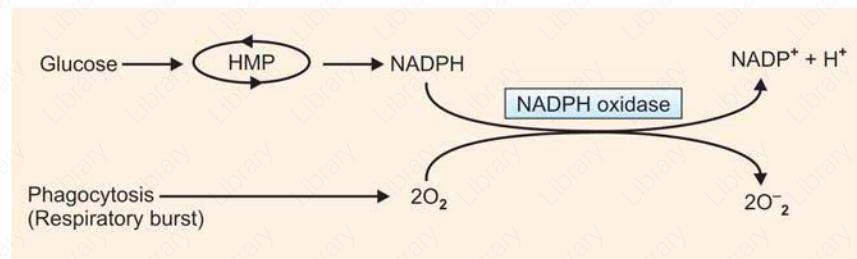
Free radicals are atoms or molecules containing one or more unpaired electrons in an outer most orbit. They are a highly reactive species and have a tendency either to lose an electron, thereby acting as reducing agents or gain an electron, acting as oxidizing agents. Thus, they can initiate chain reactions by extracting an electron from a neighboring molecule to complete its own orbit.

The most important radicals are derived from molecular oxygen and certain oxides of nitrogen especially nitric oxide. The free radicals may act as signaling molecules in physiological and biochemical activities or may provide defense against invading microorganisms. Failure of these protective mechanisms may lead to pathological conditions. Oxygen derived free radicals and related non-radical compounds (H_2O_2) are referred to as reactive oxygen species (ROS). Not all reactive oxygen species are free radicals, e.g. singlet oxygen and hydrogen peroxide.

Generation of Free Radicals

Free radicals are produced in the body in the following manner:

- They are constantly produced during the normal oxidation of foodstuffs, due to leaks in the electron transport chain in mitochondria. About 1- 4 percent of oxygen taken up in the body is converted as free radicals.



Generation of superoxide radicals in neutrophils by NADPH oxidase

- Some enzymes such as xanthine oxidase and aldehyde oxidase form superoxide anion radical or hydrogen peroxide.
- NADPH oxidase in the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) produce 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. This is a deliberate production of free radicals by the body. About 10 percent of the oxygen uptake by macrophage is used for free radicals generation. Along with the activation of macrophages, the consumption of oxygen by the cell is increased drastically. This is called respiratory burst.
- Macrophages also produce NO from arginine by the enzyme nitric oxide synthase. This is also an important anti-bacterial mechanism.
- Peroxidation is also catalyzed by lipo-oxygenase in platelets and leukocytes.
- Ionising radiation damages tissues by producing hydroxyl radicals, hydrogen peroxide and superoxide anion.
- $H_2O \xrightarrow{\text{(gamma, UV radiation)}} H + H$
- The capacity to produce tissue damage by H₂O₂ is minimal when compared to other free radicals (by definition, H₂O₂ is not a free radical). But in presence of free iron, H₂O₂ can generate OH (hydroxy radical) which is highly reactive. However, in the body iron is always bound to proteins (transferrin and ferritin), minimizing its catalytic role in hydroxyl radical production.

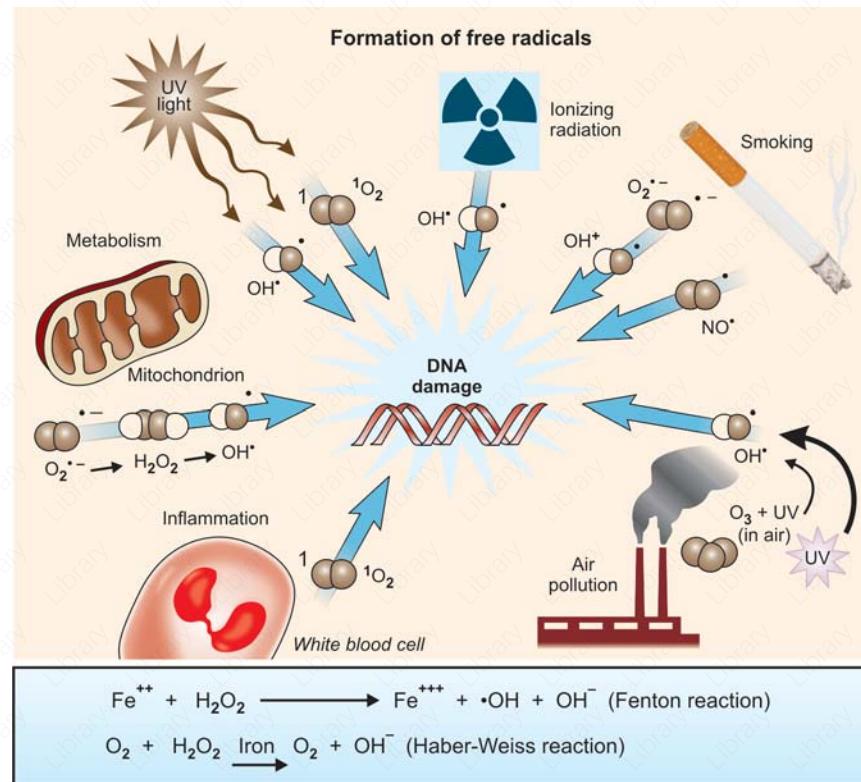


Fig. 7.5.1: Iron produces free radicals

9. Cigarette smoke contains high concentrations of various free radicals. Other toxic compounds such as carbon tetra chloride, drugs and inhalation of air pollutants will increase the production of free radicals.

Antioxidant Defense Mechanism

- Antioxidant defense mechanism is a protective mechanism of the cell that serves to minimize the toxic effect of free radicals.
- Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as pro-oxidants.
- On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation or opposing their actions are antioxidants.

Oxidative Stress

In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted towards the pro-oxidants when production of ROS is increased greatly, (e.g. following ingestion of certain chemical or drugs, exposure to ionizing radiation) or when levels of antioxidants are diminished. This state is called "oxidative stress" and can result in serious cell damage if the stress is massive or prolonged.

The involvement of free radicals in several diseased states is becoming well established. These include:

- Atherosclerosis
- Some forms of cancer
- Cataract formation, and other disorders of eye
- The diseases which involve the inflammatory response, such as rheumatoid arthritis
- Ulcerative colitis and
- Crohn's disease.

Action of Antioxidant

Different antioxidants act at different levels:

- They may prevent the initiation of chain reactions by removing free radicals.
- They may scavenge free radicals generated in chain reactions, thereby interrupting the chain sequence.
- They may remove peroxides, thereby preventing further generation of ROS

There are two main lines of defence against ROS:

- Enzymatic antioxidant systems also called scavenger enzymes.
- Non-enzymatic (nutrients) antioxidant systems.

Enzymatic Antioxidant System or Scavenger Enzymes

Such system include enzymes:

- Superoxide dismutase (SOD)
- Catalase
- Glutathione peroxidase

Super-oxide Dismutase (SOD)

It has two isomers:

- SOD-1 is a cytosolic copper and zinc containing enzymes, and
- SOD-2 is a mitochondrial manganese containing enzyme. These enzymes catalyze the following reactions:

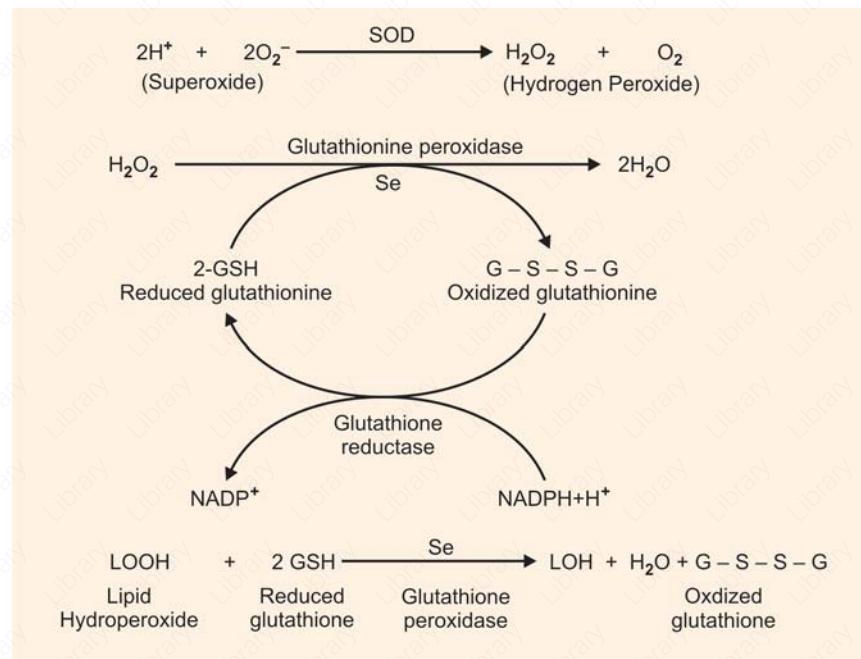
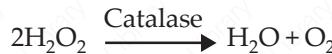


Fig. 7.5.2: Role of glutathione as an antioxidant

Catalase

Catalase is present in peroxisomes. The H_2O_2 which is generated by SOD can be scavenged by catalase.



Glutathione Peroxidase

Glutathione peroxidase is a selenium containing enzyme and can act on lipid hydroperoxides as well as H_2O_2 using glutathione (GSH) as the reducing agents.

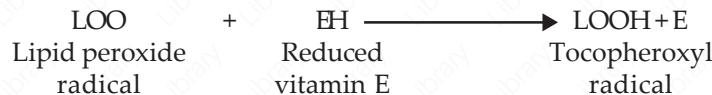
Non-enzymatic (Nutrients) Antioxidant System

Such systems include:

- **Vitamins:** e.g. vitamin E and C, carotenoids and flavonoids
- **Minerals:** e.g. manganese, copper, zinc or selenium.

Vitamins

Reduced form of vitamin E (EH) can disrupt the chain process by reacting with lipid peroxide radical and itself forming a free radical - tocopheroyl radical (E).



The resulting vitamin E radical is stable, as it is able to delocalize the unpaired electron within its structure and does not propagate the chain reaction.

Vitamin C, flavoprotein and carotenoids are able to generate vitamin E from reduced vitamin E, permitting the vitamin E once more to act as an antioxidant.

Vitamin C, β -carotene, flavoprotein are also able to reduce and detoxify oxygen intermediates in cells.

Minerals

The activity of the antioxidant enzymes depends on supply of minerals:

- Manganese
- Copper
- Zinc or
- Selenium

Therefore, it seems that both types of defence mechanisms depend ultimately on the supply of nutrients.

Figure 7.5.3 shows the interrelationship between antioxidant systems and Table 7.5.1 shows ROS and their antioxidants.

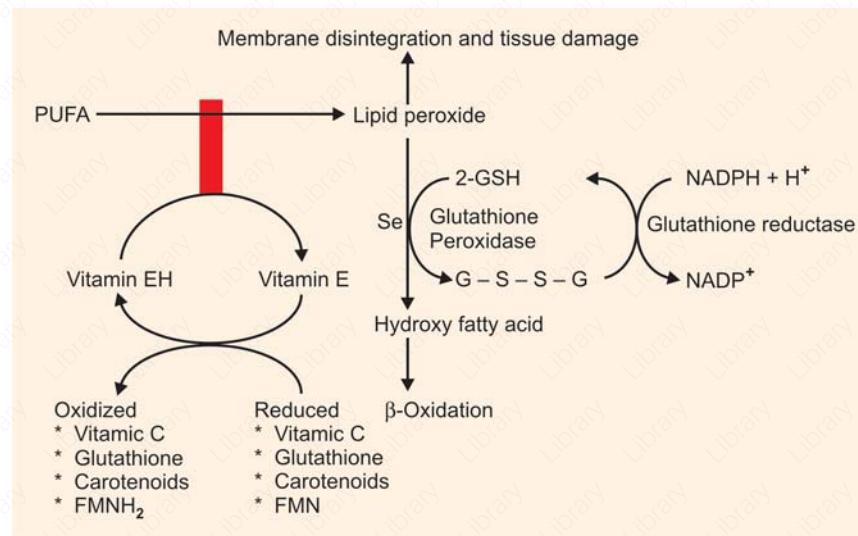


Fig. 7.5.3: Interrelationship between antioxidant system

Table 7.5.1: ROS and their antioxidants

<i>Reactive oxygen species</i>	<i>Antioxidant</i>
Superoxide free radical (O_2^-) and Hydroxyl free radical (OH)	Superoxide dismutase, Vitamin E β -Carotene
Hydrogen peroxide (H_2O_2)	Catalase, Glutathione peroxidase
Lipid peroxides (LOOH)	Glutathione peroxidase
Peroxy free radical (ROO)	Vitamin E and C

7.6: SPECIALIZED PROTEINS

COLLAGEN

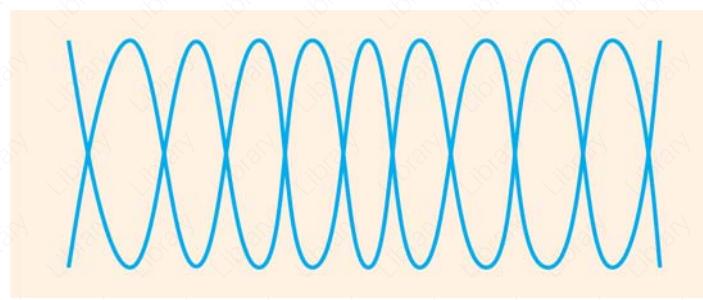
It is the most abundant protein in mammals (about 30% of total protein). It is found in connective tissues of tendons and cartilages and organic matrix of bones, teeth, skin, blood vessels and cornea.

Collagen is a structural, fibrous, insoluble protein. It has great tensile strength.

Structural Organization

- A. *Amino acid composition:* In collagen structure every third residue is glycine. It is also rich in proline and hydroxy proline (Hyp) and repeating sequence is Gly-Pro-Hyp.
- B. *Triple helical structure of tropocollagen fibril:* Collagen is not a single protein but a large protein family with closed to twenty members. Basic structural unit of all members is a trimer of polypeptides, called tropocollagen. It forms a characteristic triple helix. But the supermolecular structure of various collagen types differs, and they have different distributions and functions.

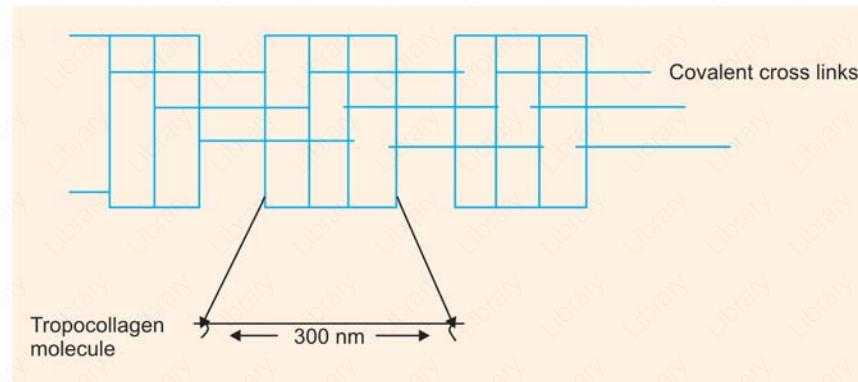
Tropocollagen is a rod shaped molecule, about 300 nm long and 1.5 nm thick. It consists of three helical polypeptide chains. These chains known as the α -chains are tightly wound around one another. Individual α -chains form a tight left handed triple helix with 3.6 amino acids per turn and a rise per amino acid of 0.15 nm.



Triple helix of tropocollagen

C. *Quarter staggered structure of collagen fiber:* Many tropocollagen fibrils are arranged in quarter-staggered way. Covalent cross link between lysine residues adds strength.

Quarter Staggered Arrangement in a Collagen Fiber



Note:- Vitamin C is required to convert proline to 4-hydroxyproline and lysine to 5 - hydroxy lysine. Therefore, in scurvy, disease due to vitamin C deficiency, there is defective collagen synthesis causing bleeding of gums, loose teeth, skin lesions and fragile blood vessels.

ELASTIN

Elastin is a fibrous element which unlike collagen, can be readily stretched. It is the major fibrous protein of yellow, elastic connective tissue of ligaments and large arteries.

The inextensibility of collagen suits it to forming extracellular matrices that have high tensile strength (Achilles tendon can withstand about 200 kg cm⁻²) or have ability to withstand compression (250 kg cm⁻² for the intervertebral discs). In some tissues (skin, ligaments and large arteries) there is requirement for elastic deformability. Such tissues have high content of elastic fibers (10% in skin to over 50% in large arteries and some ligaments). These fibers consist mainly of elastin.

The basic unit of elastin structure is tropoelastin, a polypeptide of 800-850 amino acid residues (MW 72000). Like tropocollagen, the tropoelastin has aberrant amino acid composition, with high proportions of glycine (31%), alanine (22%), and hydrophobic amino acids (40%). About 10-13 percent proline and 4-hydroxyproline are present, but there is no hydroxylysine. It has been proposed that tropoelastin units are present in the random coil conformation and are extensively cross-linked. This makes such a network kinetically free to stretch and recoil. Like in case of collagen, these cross-links are derived from lysine. Therefore, lysyl oxidase is required for the synthesis of elastin as well as that of collagen.

KERATINS

In an aqueous medium, the peptide chain first assumes a three-dimensional secondary structure consisting of a helically coiled, zigzag linear or mixed form. The secondary structure results from the steric relationship between amino acids located relatively near each other in the peptide chain. It is held and stabilized by hydrogen bonds between the carbonyl ($C=O$) and amide (NH) groups of peptide linkages in the peptide chain. Fibrous proteins have three main types of secondary structure.

- *Alpha-helix:* α -Keratins are abundant in hair, nail, wool and the stratum corneum of skin. The molecule is arranged into a regular coil called α -helix. The α -helical structure was first defined by Linus Pauling from his studies on fibrous proteins.

Features

The polypeptide backbone is coiled around a central axis. The α -helix is stabilized by hydrogen bonds that are formed between the peptide bonds. Each peptide bond $C=O$ is hydrogen bonded to the peptide bond $N-H$ four amino acid residues ahead of it. Each $C=O$ and each $N-H$ in the main chain is thus involved in hydrogen bonding. Distance between 2 amino acid residues is 1.5 \AA° . Each turn has 3.6 amino acids. Pitch (rise/turn) = $5.4\text{ \AA}^{\circ}\text{H}$.

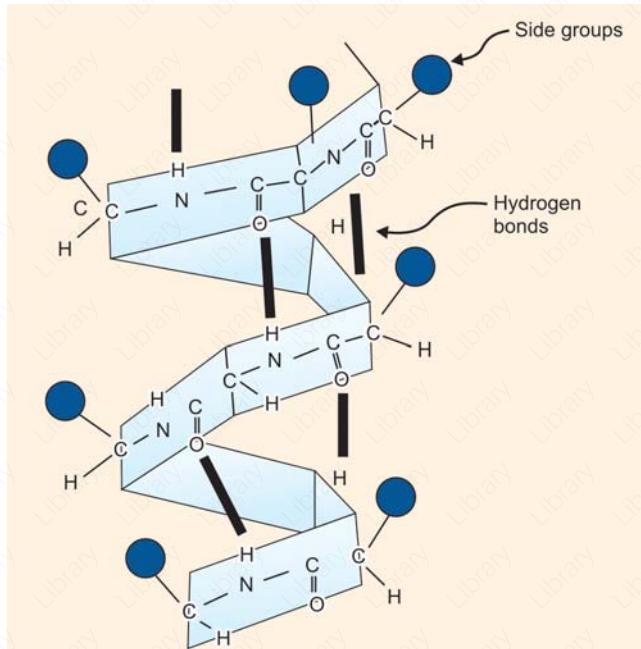


Fig. 7.6.1: The right-handed α -helix shows planar peptide bonds and hydrogen bonds between every fourth bond

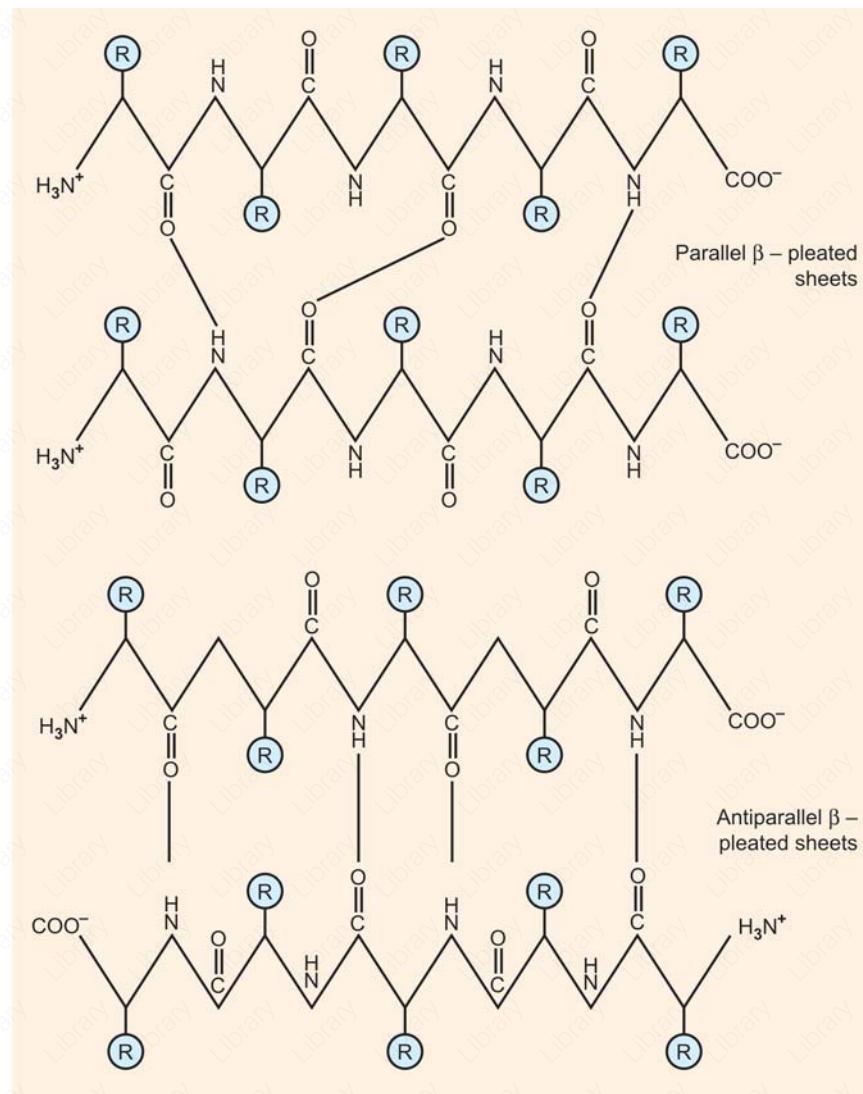
Every $-N-H$ and $-C=O$ participates in H - bonding.

H bond: Bond formed by sharing hydrogen between two electron donors.

H-releasing groups (in proteins)	H-accepting groups (in proteins)
-NH (imidazole)	-COO ⁻ (Asp, Glu)
-OH (Ser, Thr)	C=O (peptide)

Right handed α -helix is more common and more stable. Amino acids which do not allow formation of α -helix (terminators) are proline and its derivative hydroxy proline. Amino acids which destabilize α -helix are acidic, basic amino acids. Example: Aspartic acid, arginine.

Occurrence: Hb and Mb have abundant α -helical regions.





- b. *Beta-pleated sheet:* β -Keratins form the fibers of silk, spider's web and reptilian claw. The molecule is arranged into nonhelical, extended and zigzag linear strands, held closely side by side by hydrogen bonds between the amine-N and the carbonyl-O of peptide linkages of adjacent strands.

Features

Polypeptide chain is extended into zig-zag structures. Two or more adjacent segment of chain line up side by side to form sheet. Side chains are above or below the plane of the sheet. Numerous H- bonds between -N-H and -C=O groups of adjacent sheets, stabilize the structure. When adjacent strands run in same direction structure is parallel β -pleated sheets. When adjacent strands run in opposite direction, structure is antiparallel β -pleated sheet.

Occurrence: Seen in silk protein fibroin.

MYOSIN

Myosins are a large family of motor proteins found in eukaryotic tissues . They are responsible for actin -based motility. The term "myosin" was originally used to describe a group of similar, but non identical, ATPases found in striated and smooth muscle cells.

Domains

Most myosin molecules are composed of a head, neck and tail domain.

The head domain binds the filamentous actin and uses ATP hydrolysis to generate force and to "walk" along the filament towards the (+) end [with the exception of one family member, myosin VI, which moves towards the (-) end].

The neck domain acts as a linker and as a lever arm for transducing force generated by the catalytic motor domain. The neck domain can also serve as a binding site for myosin light chains which are distinct proteins that form part of a macromolecular complex and generally have regulatory functions.

The tail domain generally mediates interaction with cargo molecules and/or other myosin subunits . In some cases, the tail domain may play a role in regulating motor activity.

Skeletal Muscle Myosin

Skeletal muscle myosin, the most conspicuous of the myosin super family due to its abundance in muscle fibers, was the first to be discovered. This protein makes up part of the sarcomere and forms macromolecular filaments composed of multiple myosin subunits. Similar filament-forming myosin proteins were found in cardiac muscle, smooth muscle, and non-muscle cells.

Analysis of the amino acid sequences of different myosins shows great variability among the tail domains but strong conservation of head domain sequences. Presumably this is so; the myosins may interact, via their tails, with a large number of different cargoes, while the goal in each case to move along actin filaments - remains the same and therefore requires the same machinery in the motor. For example, the human genome contains over 40 different myosin genes.

These differences in shape also determine the speed at which myosins can move along actin filaments. The hydrolysis of ATP and the subsequent release of the phosphate group causes the "power stroke", in which the "lever arm" or "neck" region of the heavy chain is dragged forward. Since the power stroke always moves the lever arm by the same angle, the length of the lever arm determines how fast the cargo will move. A longer lever arm will cause the cargo to traverse a greater distance even though the lever arm undergoes the same angular displacement - just as a person with longer legs can move farther with each individual step. Myosin V, for example, has a much longer neck region than myosin II, and therefore moves 30-40 nanometers with each stroke as opposed to only 5-10.

Myosin Classes

Myosin I

Function of myosin I is unknown, but it is believed to be responsible for vesicle transport or the contraction vacuole of cells.

Myosin II

Myosin II is perhaps the best-studied example of these properties.

Myosin II contains two heavy chains, each about 2000 amino acids in length, which constitute the head and tail domains. Each of these heavy chains contains the N-terminal head domain, while the C-terminal tails take on a coiled-coil morphology, holding the two heavy chains together (imagine two snakes wrapped around each other, such as in a caduceus). Thus, myosin II has two heads.

It also contains 4 light chains (2 per head), which bind the heavy chains in the "neck" region between the head and tail.

In muscle cells, it is myosin II that is responsible for producing the contractile force. Here, the long coiled-coil tails of the individual myosin molecules join together, forming the thick filaments of the sarcomere. The force-producing head domains stick out from the side of the thick filament, ready to walk along the adjacent actin-based thin filaments in response to the proper chemical signals.

LENS PROTEINS

The lens, which is behind the iris, refracts light entering the eye through the pupil, thus focusing it on the retina. The perfect physicochemical balance of the lens proteins gives it transparency. Any alteration in the optical homogeneity of the lens or decrease in its transparency is known as a cataract. Senile cataract, a major cause of blindness worldwide, is an age associated condition. The term senile refers to the fact that no specific ophthalmic or metabolic diseases are known to precede or to be involved in this type of cataract.

On the other hand, diabetes is also considered a significant risk factor accelerating cataract formation. Increasing experimental evidences suggest that glycation of lens proteins is involved in cataract formation. Glycation of lens proteins, whereby glucose or other reducing sugars react with the α -amino group of lysine residues or amino terminal of proteins resulting in the formation of schiff base (SB). The SB undergoes an Amadori rearrangement via the Maillard reaction giving rise to a more stable ketoamine or Amadori product (early glycation products). At a later stage, the Amadori products undergo dehydration and rearrangement to form cross-links between adjacent proteins, resulting in protein aggregates or advanced glycation products (AGEs). These are susceptible to post-translational modification such as glycation, which is believed to enhance protein unfolding, changing not only the physicochemical properties of lens proteins, but also their functions.

This study was undertaken to compare the extent of glycation in nuclear lens crystallins in diabetic and various stages of senile cataracts.

7.7: ELECTROPHORESIS OF IMMUNOGLOBULINS

Electrophoresis is used to identify the presence of abnormal proteins, to identify the absence of normal proteins, and to determine when different groups of proteins are increased or decreased in serum. It is used to detect and identify monoclonal proteins — an excessive production of one specific immunoglobulin.

Serum protein electrophoresis may also be ordered when symptoms suggest an inflammatory condition, an autoimmune disease, an acute or chronic infection, a kidney or liver disorder, or a protein-losing condition. Urine protein electrophoresis may be ordered when there is protein detected in the urine or when the doctor suspects a monoclonal protein (Figs 7.7.1 and 7.7.2).

Immunofixation electrophoresis is usually carried out when the protein electrophoresis test shows the presence of an abnormal protein band that may be an immunoglobulin. The value of immunofixation electrophoresis is in the identification of the presence of a particular type of immunoglobulin.

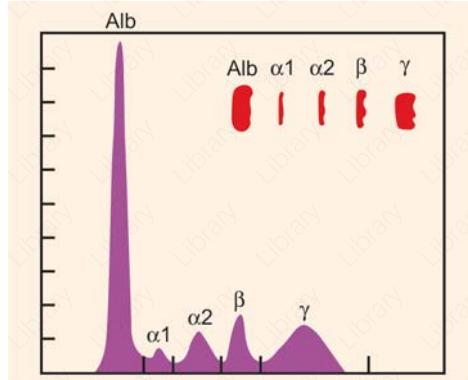


Fig. 7.7.1: Serum protein electrophoresis

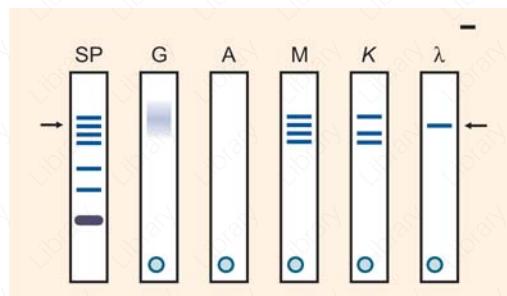


Fig. 7.7.2: Immunofixation electrophoresis

Once a disease or condition has been diagnosed, electrophoresis may be ordered at regular intervals to monitor the course of the disease and the effectiveness of treatment.



8.1: ENERGY REQUIREMENTS OF THE BODY

INTRODUCTION

Nutrition is the science that examines the qualitative and quantitative requirements of the diet necessary to maintain good health. Nutrient is the food constituent that body needs for well-being. Human nutrition can be divided into three categories such as under nutrition, over nutrition and ideal nutrition. In India the major concern is of under nutrition. Nutrients present in diet are carbohydrates, lipids, proteins, vitamins and minerals. Carbohydrates, lipids and proteins collectively referred to as macronutrients, since they are required in relatively larger quantities, i.e. several grams per day. Vitamins and minerals are required in much smaller quantities, i.e. few micrograms to milligrams per day and they are referred to as micronutrients.

Nutrition also deals with the aspects such as, what to eat, how much to eat and when to eat.

Calorie

A calorie is defined as the amount of heat required to raise the temperature of one gram of water by 1°C .

Kilocalorie (Cal/Kcal)

- It is 1,000 times the energy for calorie.
- The international unit of energy is the joule (107 ergs).
- Therefore, 1 kilo calorie = 4.2 kilo joules (kj).

Calorific Value

It is the amount of heat energy that is liberated by burning 1 gm of foodstuff completely in the presence of O_2 . It is usually expressed as kilocalories (Kcal). It is determined by "Bomb Calorimeter".

Calorific Values Expressed in Kcal/g

	<i>In the body</i>	<i>In bomb calorimeter</i>
Carbohydrate	-	4
Fat	-	9
Proteins	-	4
		5.6

Respiratory Quotient (RQ) for Foodstuff

It is the ratio of the volume of CO_2 produced to the volume of O_2 consumed, when a particular food is oxidized in the body.

$$\text{RQ} = \frac{\text{Volume of } \text{CO}_2 \text{ produced}}{\text{Volume of } \text{O}_2 \text{ consumed}}$$

RQ for carbohydrate	= 1
Lipids	= 0.7
Proteins	= 0.8
On a mixed diet RQ	= 0.85

Significance of RQ

1. It indicates the type of food that is being principally metabolized in the body.
2. In diabetes mellitus and starvation RQ decreases.

Example: If RQ is nearer to 1, then it means more of carbohydrates are metabolized.

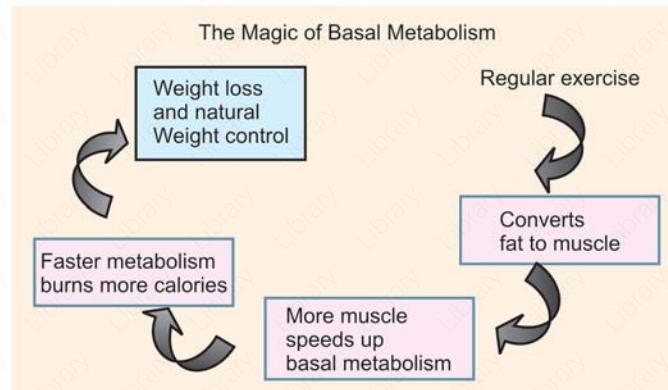
Energy Requirements of an Individual

Energy requirement of an individual is made up of several components.

They are:

1. Basal metabolic rate
2. Specific dynamic action of food
3. Various activities. However, for women, pregnancy and lactation are additional components of energy requirement.

Basal Metabolic Rate (BMR)



It is the minimum amount of energy required by the body at complete physical and mental rest in postabsorptive stage.

It is the minimum energy required to sustain life or vital functions like respiration, circulation, functions of viscera like liver, kidney, brain etc.

It is expressed as K cal/m²/hour

C = calorie; Kcal = Kilo calorie

Normal values (K cal/m²/hr) Males = 40 (35 – 38); Females = 35 (30 - 35).

Another way of expressing BMR: Normal = 24 K cal/kg body weight/day.

Conditions for Measuring BMR

1. Should be awake, but complete physical and mental rest.
2. Postabsorptive stage.
3. Environment should be thermoneutral (around 25°C).
4. Recumbent position (Lying down).

Determination of BMR by Indirect Calorimetry

Indirect calorimetry is based on the calorie equivalent of the volume of O₂ consumed by the person at the non-protein RQ, calculated from his respiratory gas exchange and urinary NPN during the interval.

- i. Benedict-Roth spirometer
- ii. Knipping spirometer.

Factors Influencing BMR

1. *Age*: BMR decreases with age. Children have a higher BMR and old people have lower BMR when compare to adults.
2. *Sex*: BMR is higher in males than females.
3. *Body surface area*: BMR is directly proportional to the body surface area. The larger the surface area the higher is the BMR.
4. *Environment*: In cold weather, BMR is high whereas in warm climate BMR is low.
5. Physiological conditions like pregnancy and lactation increases BMR whereas sleep decreases BMR.
6. *Racial differences*: Tropical races have lower BMR than European races.
7. *Body temperature*: With every 1°C rise in body temperature, BMR rises by 12 percent.
8. *Drugs*: Like caffeine will increase BMR.
9. *Hormone*: BMR changes due to thyroid hormones. Growth hormones increase BMR. Adrenaline secreted on cold exposure, quickly raises the BMR.

Pathological

Increase in BMR: Fever, hyperthyroidism, Cushing's syndrome, cancer, emphysema and hyperactivity of the pituitary.

Decrease in BMR: Starvation, hypothyroidism, Addison's disease and nephrotic syndrome.

Significance of BMR

1. Assessment of thyroid function.
2. Calculation of caloric requirement of a person.

Specific Dynamic Action (SDA)

After food intake, heat production rises more than what is expected to be produced by the catabolism of the ingested food. This additional heat output is called the specific dynamic action.

Extra heat production by the body over and above the actual calorific value of particular food called as "thermogenic action". The rise starts within 15 minutes of food intake and may continue upto 6 hours.

SDA Values

Proteins	- 30 percent
Fats	- 15 percent
Carbohydrate	- 5 percent
Mixed diet	- 10 percent

SDA may be useful in:

1. Processing the ingested food, so that it can be digested and assimilated.
2. Regulation of body temperature.

Significance

- i. Heat produced by SDA is not available for useful work. Therefore, while calculating calorie requirement of a person extra 10 percent calories should be added.
- ii. SDA is decreased in starvation and pregnancy.

Energy for Physical Activity

Energy required for different daily activities have been determined. It changes from individual to individual. There are 3 categories:

- a. *Sedentary (sitting, standing, dressing and reading):* Over and above BMR, add 30 percent of BMR
Example: Office work, teachers, doctors etc. 1.5 cal/kg body weight/hour energy is required.
- b. *Moderate (cycling, gardening, walking etc.):* Over and above BMR, add 40 percent of BMR
Example: Students, housewives, farm work etc. 2.5 cal/kg body weight/hour energy is required.

- c. *Heavy (swimming, running, wood cutting etc.):* Over and above BMR, add 50-80 percent of BMR

Example: Manual laborers, construction workers, athletes etc. 5 cal/kg body weight/hour energy is required.

Calculation of Calorie Requirement

$$\text{BMR} + \text{Calories of work} + \text{SDA}$$

Calculate energy requirement of a medical student weighing 60 kg.

$$\begin{aligned}\text{BMR} &= 24 \text{ K cal/kg body weight/day} \\ &= 24 \times 60 \\ &= 1440\end{aligned}$$

$$\text{SDA} = 10 \text{ percent (Mixed diet)}$$

$$\begin{aligned}\text{Calories of work} &= \frac{40}{100} \times 1440 \\ (\text{Physical activity}) &= 576 \\ &= 10 \text{ percent of 2016} \\ &= 200 \\ \text{Energy requirement} &= 1440 + 576 + 200 \\ &= 2216 \text{ calories/day}\end{aligned}$$

MULTIPLE CHOICE QUESTIONS

1. The endocrine organ most predominantly associated with BMR is:
A. Adrenal gland B. Pituitary gland
C. Thyroid gland D. Parathyroid gland
2. The specific dynamic action (SDA) is the greatest for the following foodstuff:
A. Protein B. Carbohydrate
C. Fat D. Vitamins
3. One calorie of energy is equivalent to _____ K Joules:
A. 4.128 B. 4.218
C. 4.812 D. 5.128
4. The major source of energy to the body is supplied by:
A. Fats B. Proteins
C. Nucleic acids D. Carbohydrates
5. The daily normal requirement of protein in an adult is:
A. 10 g/kg body weight/day B. 1 g/kg body weight/day
C. 100 g/kg body weight/day D. 1000 g/kg body weight/day

ANSWERS

1. C 2. A 3. A 4. D 5. B

MOSTLY LIKELY QUESTIONS

1. Biological value of protein.
2. Basal metabolic rate.
3. Which are the factors affecting basal metabolic rate?
4. Mention the calorific values for proteins, carbohydrates and fats.
5. What is respiratory quotient? Mention its significance.

8.2: NUTRITIONAL IMPORTANCE OF BIOMOLECULES

CLASSIFICATION OF FOOD

Food we eat contains several substances, both organic and inorganic. Some of them are essential and others, non-essential to the body. For growth and maintenance of the body, food must contain essential substances which are not produced in the body. They are known as nutrients. Some 40-50 chemical substances are essential for the human body and as such they must be present in the diet. They are divided into six major groups. Carbohydrates, lipids, proteins, vitamins, minerals and water.

Depending on the function:

1. *Energy yielding food*: Carbohydrates and lipids
2. *Body building food*: Proteins
3. *Protective food*: Vitamins and minerals
4. *Universal food*: Water

Major nutrients: Carbohydrates, lipids and proteins are required in large amounts.

Nutritional Importance of Carbohydrates

They are the main sources of energy and provide 50-80 percent of calories. Dietary carbohydrates are of two types: available carbohydrates and unavailable carbohydrates.

Available carbohydrates can be utilized and metabolized. For example, starch, glycogen, sugars etc. Unavailable carbohydrates cannot be assimilated by the body and they are called as dietary fibers. For example, Cellulose, hemicellulose, pectin, gums, lignin and mucilage.

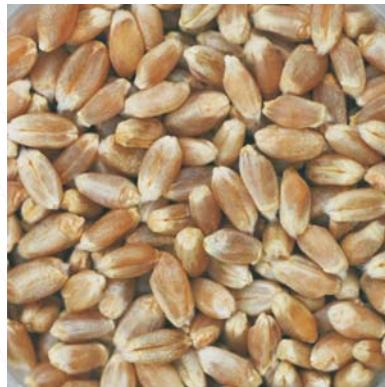
Functions of Carbohydrates

- i. Carbohydrates are the principal source of energy
- ii. Carbohydrates spare the proteins from being misused for caloric purpose

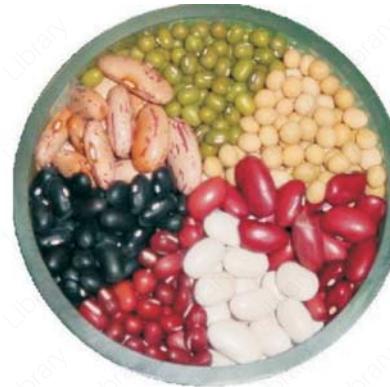
- iii. Absolute requirement by brain (glucose)
- iv. Required for metabolism of lipids in TCA cycle where in oxaloacetates are necessary
- v. Synthesis of nonessential amino acids (pyruvic acid serves as precursor)
- vi. Synthesis of pentoses
- vii. *Synthesis of fat:* excess consumption of carbohydrates leads to the formation of fat
- viii. Provide dietary fibers.

Sources of Carbohydrates

- i. Cereals—Rice and wheat—80 percent
- ii. Pulses—60 percent
- iii. Roots and tubers—40 percent
- iv. Refined sugars, *Example:* Sucrose—99 percent



Cereals – wheat



Pulses

High intake of refined sugars may lead to decrease in glucose tolerance, rise of plasma lipids (dangerous), dental caries and early satiety. Ideally carbohydrates should provide 60 - 65 percent of total calories. Excess carbohydrate consumption leads to obesity as carbohydrate is stored as fat.

Dietary Fiber

Sources: Always plant source. Fruits, leafy vegetables, whole wheat, legumes, rice bran, etc. are rich sources of fibers.

Definition: Complex carbohydrates that cannot be digested by humans.
Example: Cellulose, hemicelluloses, lignin, pectin.



Fruits

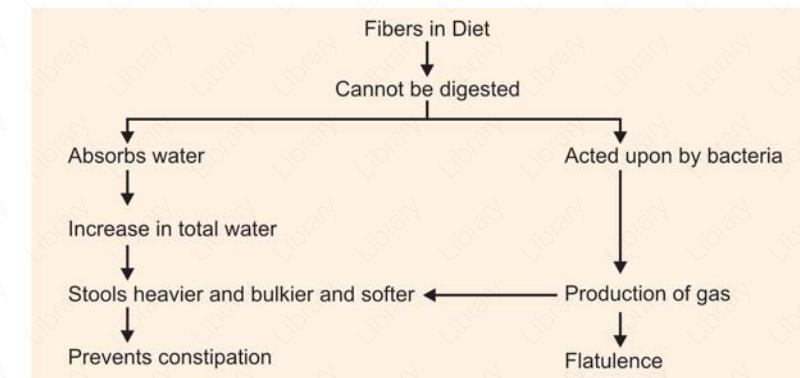


Vegetables

Beneficial Effects of Fiber

- i. Prevents constipation
- ii. Eliminates bacterial toxins
- iii. Decreased incidence of colon cancer
- iv. Improves glucose tolerance
- v. Reduces plasma cholesterol level
- vi. *Satiety value:* Fiber gives a sensation of stomach fullness and prevents consumption of excess calories to remain slim.

Mechanism



Adverse Effects of Fiber

- i. Digestion and absorption of protein is adversely affected.
 - ii. The intestinal absorption of certain minerals (calcium, phosphorus, magnesium etc) is decreased.
 - iii. Intestinal bacteria ferment some fibers causing flatulence and often discomfort.
- RDA = 30 g of fibers/day.

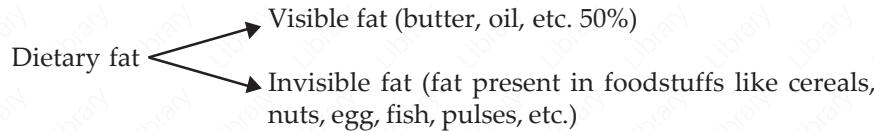


Nutritional Importance of Lipids

Triacylglycerols (Fat and oils) are the concentrated dietary source of fuel contributing upto 50 percent of the body energy requirements. Cholesterol and phospholipids from animal sources are also important in nutrition.

Functions of Lipids

- i. Lipids provide concentrated source of energy (about 10-40% of total calories).
- ii. Lipids provide essential fatty acids and fat soluble vitamins.
- iii. Lipids add palatability to food.



Points to Ponder

1. Diet should contain adequate amount of PUFA which is antiatherogenic. Hence, sunflower oil, cotton seed oil are preferred over saturated fats like coconut oil, butter, ghee, etc.
2. Excess PUFA is dangerous, since it generates free radicals in the body.
3. Lipids should provide 15-20 percent of total calories.
4. Intake of high cholesterol leads to atherosclerosis and heart attack.
5. Red meat consumption should be avoided.
6. Excess consumption of lipids results in obesity.

Nutritional Importance of Proteins

Proteins are the building blocks of body tissues. They provide 10-15 percent of total energy. Only protein is the source of essential amino acids.

Protein Requirement

For adult - 1 g/kg body weight
Children - 1.5 - 2 g/kg body weight
Pregnancy - 2 g/kg body weight

Nitrogen Balance

About 16 percent of weight of protein is nitrogen. Nitrogen balance refers to status of nitrogen content in the body. When intake of nitrogen is more than its excretion (through urine, feces, skin). Then nitrogen is retained in the body. It is termed as **positive nitrogen balance**.

Positive nitrogen balance is seen in the periods of active growth, hormonal influence of growth hormone, insulin, androgen, in pregnancy and when recovering after an illness.

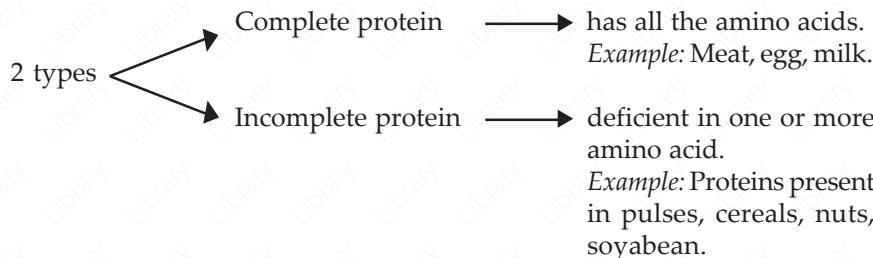
Excretion of nitrogen is more than the intake of nitrogen results in **negative nitrogen balance**.

It is seen in acute and chronic illness, protein deficient diet and influence of hormones like corticosteroids.

When intake of nitrogen = Excretion of nitrogen then the individual is said to be in **nitrogen balance** or equilibrium. Nitrogen balance is required for good health and adequate proteins should be supplied. Intestinal flora influences the nitrogen balance of an individual.

Criteria for Assessing the Quality of a Protein

Quality of a protein depends on its amino acid composition.



Limiting Amino Acid

If a protein is deficient in one or more essential amino acid, such amino acid is called as limiting amino acid. If such protein is exclusively fed to an individual, then he or she fails to grow.

Example: Cereals are deficient in lysine

Pulses are deficient in sulphur containing amino acid but rich in lysine.

Mutual Supplementation of Proteins

In the above example, when cereals and pulses are given together, they cancel deficiency of each other and that will provide excellent 1st class protein to the body. Hence, it is advisable to take variety of proteins in diet.

Practical Application of Mutual Supplementation

1. Rice + Sambar
(cereal) (pulse)
2. Roti or chapati + Dal
(cereal) (pulse)

Protein Indices

1. *Biological value (BV)*

$$BV = \frac{\text{Retained N}_2}{\text{Absorbed N}_2} \times 100$$

BV of egg protein = 90
Rice protein = 65

2. *Net protein utilization (NPU)*: Biological value of a protein does not cover nitrogen lost in digestion. In net protein utilization, it is included. It is defined as percentage of dietary nitrogen that is retained in the body.

$$NPU = \frac{\text{Retained N}_2}{\text{Intake N}_2} \times 100$$

NPU of egg = 91
Rice = 55

3. *Protein efficiency ratio (PER)*: It is a better index of protein quality than biological value. It is defined as weight gain per weight of protein eaten.

$$4. \text{Chemical score} = \frac{\text{mg of limiting amino acid/g protein}}{\text{mg of the same amino acid/g egg protein}} \times 100$$

Nutritive value of some common Indian foodstuffs.

Wheat: Taken in the form of rotis, chapati, puri, parotas, biscuits and bread. These are poor in lysine. These provide carbohydrates - 70 percent; protein - 10 percent; minerals, water and fiber.

Rice: Staple diet in South India. It is taken as rice as such dosa, apam. These provide protein - 7 percent; carbohydrates - 80 percent. Parboiled rice is rich in thiamine and minerals. Rice protein is poor in lysine. Rice should not be cooked in excess water to avoid loss of vitamins and proteins.

Maize: Staple diet of poor class. It gives carbohydrate - 70 percent; protein - 10 percent. Maize protein is deficient in tryptophan. Hence niacin synthesis is affected. Pellagra results if only maize is consumed.

Ragi: It is consumed in North Karnataka. it gives carbohydrate - 7 to 20 percent and protein 7 to 10 percent. Ragi is a good source of calcium.

Pulses: Include bengal gram, black gram, green gram and red gram. Preparations from these pulses are dhal, sambar, idlis, vada and dosa.

Horse gram: It gives 20 - 30 percent proteins and hence it is called 'poor mans meat'. It is deficient in 'S' containing amino acid and so taken with cereal. Horse gram gives first class protein, carbohydrate (60-65%) and minerals like calcium, iron, etc.

Soyabean: It is the richest source of plant protein. It has 40 percent protein, 20 percent fat and 20 percent carbohydrates. It contains all vitamins and minerals in adequate amounts. Now soya milk is available in the market.

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Vegetables: They mainly supply vitamins, minerals and fiber.

Content of water in vegetables : 80 - 90 percent

Content of carbohydrates : 2 - 6 percent

Content of fibers : 3 percent

Green leafy vegetables are rich in vitamin A, vitamin C, vitamin B complex, iron and calcium. Fat is present in traces.

Yellow vegetables like carrot, tomato are rich in vitamin A. Darker the colour of vegetables better is the composition of vitamin A. In order to retain nutrients in the vegetables, soaking of vegetables in water for a long time should be avoided. This facilitates retention of water soluble vitamins intact. Vegetables should be cooked with minimum water and cut into larger pieces to prevent loss of vitamins and minerals. Cooking of vegetables improves digestibility but vitamin C is lost.

Fruits: Fruits are delicious in nature and rich in vitamin A and minerals. They have adequate amount of carbohydrates and are rich in fiber. Citrus fruits are rich in vitamin C while yellow fruits are rich in vitamin A. Costly fruits need not be the best. *Example:* Nutritive value of Apple is much less when compared to banana.

Milk: Milk can be used as such, in the form of ice cream or curds. It is a complete and ideal food for all ages, as it contains all essential principles of a balanced diet. Milk is poor in vitamin C, iron and copper but rich in calcium and phosphorus. Milk protein is considered to be first class protein with high biological value.

	Human milk (g %)	Cow milk (g %)
Carbohydrates (as lactose)	6.8	4.6
Protein (casein, albumin, globulin)	2	4
Fat	3.6	3.5

Egg: It is a complete food with high cholesterol content. White of egg contains first class protein. Yellow of egg contains fat, cholesterol, vitamin, cobalt, phosphorus and iron. Raw egg contains avidin (anti vitamin) which inhibits biotin absorption.

Meat: It is a good source of protein with high biological value, consists of muscle fibers held by connective tissue. It gives 20 percent protein, 15 percent fat, some vitamin and iron. It is a poor source of calcium. Meat does not contain fiber. It should be adequately cooked before consumption.

Fish: Fish is free from carbohydrate just like meat. The fat content ranges from a trace to 5 percent. It is a fare source of B group vitamins. Fatty fish contains some vitamin A and D. Large fish is rich in phosphorus but deficient in calcium. Small fish eaten with bones are good sources of calcium. Fish contains 22 percent protein, 1.4 percent fat and 75 percent water.



Coconut: Coconut contains 20 percent carbohydrate and it is a good source of calcium.

Ground nut: 'Poor man's dry fruit'. It is a good source of protein (25%) and fat.

Beverages: Black tea has negligible caloric value. It has a stimulant and a diuretic effect. Strong tea disturbs gastric digestion due to tannic acid.

Coffee has no caloric value. It contains caffeine and tannic acid. It cannot be regarded as food unless taken with milk and sugar.

Cocoa as beverage is of little importance. It becomes nourishing when taken with milk and sugar. Chocolate consists of ground cocoa-nibs mixed with sugar. Starch and flavoring are frequently added.

MULTIPLE CHOICE QUESTIONS

1. Cereals are deficient in the essential amino acids:
A. Valine B. Lysine
C. Threonine D. Methionine
2. _____ is considered to be a complete food:
A. Fish B. Egg
C. Milk D. Meat
3. Citrus fruits are rich in _____.
A. Vitamin A B. Vitamin D
C. Vitamin C D. Vitamin K
4. Positive nitrogen balance is defined as:
A. Intake of nitrogen is more than its excretion
B. Intake of nitrogen is less than its excretion
C. Intake of nitrogen is equal to its excretion
D. None of the above
5. Fiber in diet is beneficial since:
A. It prevents constipation
B. It eliminates bacterial toxins
C. It improves glucose tolerance
D. All the above

ANSWERS

1. B 2. B 3. C 4. A 5. D

MOST LIKELY QUESTIONS

1. Name a cheap source of pro-vitamin A in diet.
2. Name the sulphur containing dietary essential amino acid.
3. Name four dietary essential amino acids.
4. How is parboiled rice nutritionally superior to raw rice?
5. Give an account of dietary fiber.

8.3: BALANCED DIET

The one which contains all required nutrients—carbohydrates, fats, proteins, vitamins and minerals in such quantities and proportions so as to meet the requirement for good health and vitality. It also makes provision for extra nutrients to withstand short duration of leanness.

The proportions of protein, fat and carbohydrate should approximately be 1:1:4 respectively.

Construction of Balanced Diet

A balanced diet (Fig. 8.3.1) should provide adequate

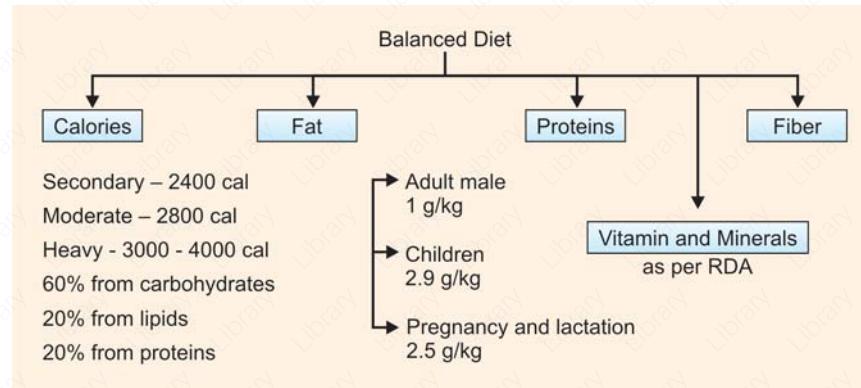
- i. Calories : Provided by cereals, fats, sugars, pulses.
 - ii. Protein : Obtained by monthly pulses, meat fish
 - iii. Vitamin and Minerals : Obtained by vegetables, fruits, egg, milk.
 - iv. Fiber : Vegetables and fruits
 - v. Water



Fig. 8.3.1: Balanced diet

Balanced diet for moderately active man:

1. Total calories : 2800 K cal/day (60% from carbohydrates, 20% from lipids)
 2. Proteins : 1 g/kg body weight
 3. Lipids : Adequate PUFA should be supplied
 4. Fiber : 30 g/day
 5. Vitamin and minerals : As per RDA



Diet Chart

<i>Dietary articles</i>	<i>Quantity in grams/day</i>
Rice/wheat	500
Pulses	65 (80 for vegetarian)
Green leafy vegetables	125
Other vegetables	75
Roots and tubers	100
Fruits	30
Milk	100 (200 for vegetarian)
Fat/oil	40
Meat/fish	30 (Ignore if vegetarian)
Egg	30 (Ignore if vegetarian)
Sugar/Jaggery	40

Balanced Diet Chart for Other Groups

<i>Food</i>	<i>Adult (Moderate)</i>	<i>Pregnancy</i>	<i>Lactating</i>
Rice/wheat	400	475	500
Pulses	55 (70 if veg.)	70	80
Green leafy	125	150	150
Other vegetables	75	75	75
Roots/tuber	75	75	75
Fruits	30	30	30
Milk	100 (200 for veg.)	225 (325 for veg.)	250 (350 for veg.)
Fat/oil	35	35	50
Meat/fish	30	30	30
Egg	30	30	30
Sugar	30	40	50
Total calories	2200 K cal	2500 K cal	2750 K cal

Values in calories of some common foods	
Apple	66/one
Banana	132/one full
Buffalo milk	109/100 ml
Butter	720/100 g
Cow milk	69/100 ml
Desi ghee	830 – 890/100 g
Egg	165/100 g
Fishes	50-150/100 g
Foods	Calories
Human milk	67/100 ml
Meat	100-450/100 g
Peas (cooked)	56/half cup
Potato	83/one medium size
Tomato (raw)	20/one medium size

In pregnancy: Calories increase by 300 cal/day and protein 15 g/day.

Lactation: Calories increase by 500 cal/day and protein 25 g/day

MULTIPLE CHOICE QUESTIONS

1. Recommended extra energy allowance for a pregnant woman in K cal/day is:
A. 100 B. 300
C. 200 D. 400
 2. Requirement of vitamin C per day for an adult:
A. 40 B. 60
C. 100 D. 125
 3. Sprouted green gram is a good source of the vitamin:
A. Thiamine B. Vitamin B₁₂
C. Vitamin C D. Niacin
 4. A continuous supply of energy to the body is necessary to meet the requirements of:
A. Basal metabolic rate B. Specific dynamic action
C. Physical activity D. All of them
 5. The nutrient required in greater amounts in menstruating women compared to men is:
A. Iron B. Copper
C. Calcium D. Zinc

ANSWERS

1. B 2. B 3. C 4. D 5. A

MOST LIKELY QUESTIONS

1. Explain the dietary care of a pregnant woman.
2. What is the nutritional value of ripe papaya fruit?
3. Write the importance of carbohydrates.
4. Add a note on protein and weight loss.
5. Write an essay on essential nutrients.

8.4: NUTRITIONAL IMPLICATIONS OF DISEASES

Since consumption of adequate amounts of nutrients is essential for growth and maintenance of body functions, intake of low quantities of nutrients affects the growth of the individual. Growing children pregnant and lactating women are more affected. The people of developing countries suffer from undernutrition while overnutrition is the major concern of the developed countries.

Nutritional Disorders

Diseases associated with malnutrition

1. Protein-energy malnutrition (PEM)
 - Marasmus
 - Kwashiorkor
2. Deficiency of vitamins and minerals
Example: Rickets, osteomalacia, iron deficiency anemia, beriberi, pellagra, scurvy, goiter etc.
3. Deficiency of essential fatty acids - Phrynoderma.

Disease of Overnutrition: Obesity

Obesity can be defined as a condition in which ingestion of food in excess of the body needs occurs resulting in abnormal increase of body weight.

Protein Energy Malnutrition (PEM)

It is a common nutritional disorder in developing countries, observed in infants and pre-school children. It is mainly seen in poor and illiterate community, mainly because of low intake of calories and proteins in the diet. Marasmus and Kwashiorkor are the two extreme forms of protein energy malnutrition.

Marasmus (Fig. 8.4.1)

It is a disease arising mainly because of 'Primary calorie deficiency' and 'secondary protein deficiency'. It affects children of less than one year.



Fig. 8.4.1: Marasmus

Causes

- i. Early weaning with very low calorie food such as rice water and dilute milk.
- ii. Repeated infections and diarrhea.

Features

Child looks highly emaciated with 'all skin and bone' appearance, sunken eyes, prominent ribs. Hence, it is called 'Wasting disease'. The child has marked muscle wasting, delayed milestones gets irritable and has 'monkey face'. Skin and hair are normal. Edema is not observed. Increased appetite for food is prominent in children.

Kwashiorkor (Disease of Deposed Child) (Fig. 8.4.2)

It is a disease arising mainly because of primary protein deficiency. It affects children of more than one year.

Causes

- i. Early weaning with low protein diet (starchy diet - No milk)
- ii. Repeated infections.



Fig. 8.4.2: Kwashiorkor

Features

Child looks lethargic and apathetic with puffy face because of edema (moon face). Child looks plump because of marked edema all over the body. Marked skin lesions are observed with desquamation of skin. Hair looks pale and thin. Appetite is lowered and serum albumin level is decreased.

<i>Feature</i>	<i>Marasmus</i>	<i>Kwashiorkor</i>
Age of onset	Less than 1 year	More than 1 year
Deficiency	Calorie	Protein
Edema	Absent	Present
Cause	Early weaning with low calorie food	Early weaning with low protein food
Appearance	Emaciated 'All skin and bone' Monkey face	Looks plump, moon face
Appetite	Normal or increased	Decreased
Skin and Hair	Normal	Lesions are seen Hair: Pale and thin
Muscle wasting	Marked	Present. But cannot make out (due to edema)
Serum albumin	Normal	Decreased
Serum cortisol	Increased	Decreased
Liver	Normal	Enlarged

Treatment of PEM

Treat the associated infection, institute proper diet, rich in proteins (2 g/kg/day) and calories, adequate in fat. Educating the parents is important.

Overnutrition: Obesity

It is the condition in which an excess of fat gets accumulated. In most cases it can be detected by visual inspection.

Obesity index: (OI) It can be calculated as W/H^2

Where W = Weight in kg and
 H = Height in meters

Grading of obesity: OI

Non-obese < 25; Grade I - 25-30; Grade II - 32-40 and Grade III >40.

Basis of Obesity

If the weight in men and women is more than 20 and 25 percent of body weight respectively, due to fat: Main factors responsible are over consumption of food and lack of exercise. For every 10 calories of excess 1 gram of fat is deposited and body weight is increased.

Obese people contain more adipocytes than normal individuals. Genetic predisposition has been suggested. If one parent is obese, 50 percent chances for children becoming obese (Fig. 8.4.3).

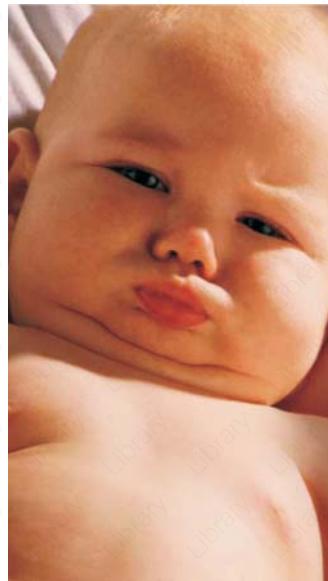


Fig. 8.4.3: Childhood obesity

Clinical Significance

Types of Obesity

1. Juvenile-onset obesity, adipose cells are more than normal.
 2. Mature onset obesity, adipose cells are normal but enlarged due to excessive fat accumulation. It is also called as hypertrophic obesity.

Endocrine Causes of Obesity

- Hypopituitarism
 - Hypothyroidism
 - Hypogonadism
 - Cushing's syndrome

Physiological Cause of Obesity

- Puberty
 - Pregnancy
 - Postmenopause in women

Side Effects of Obesity

- Hyperlipidemia
 - Increased tendency for heart attacks
 - Reduced life span

Treatment

- Reduction of intake of calories
 - Eating small meals with vegetables
 - Controlled exercise

Patients should appreciate that there are no slimming foods although advised in magazines, daily news papers, radio and television. All foods are fattening if taken in excess. Obese patients may be advised to walk, climb stairs, to swim and to do gardening.

MULTIPLE CHOICE QUESTIONS

1. The presenting features of Kwashiorkor are:
A. Edema B. Dermatitis
C. Anorexia D. Overweight
 2. All the following features are seen in marasmus, *except*:
A. Muscle wasting B. Growth retardation
C. Edema D. Anemia
 3. Obesity in a person has the health risk of:
A. Hypertension B. Coronary artery disease and stroke
C. Atherosclerosis D. All the above

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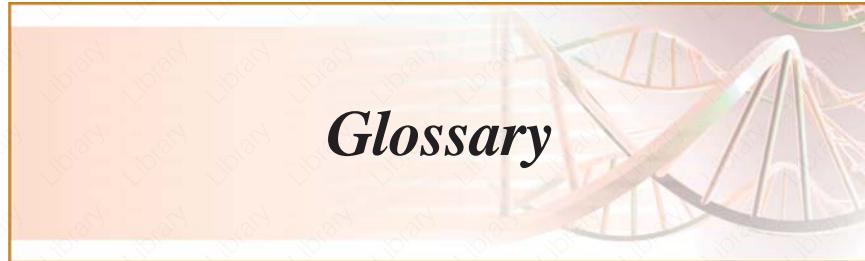
4. The biochemical parameter often used as an index for monitoring the recovery from Kwashiorkor is:
A. Serum potassium B. Plasma cholesterol
C. Plasma albumin D. Blood urea
5. Goiter is an iodine deficiency disease, associated with thyroid gland enlargement; this disease in the endemic areas may be controlled by:
A. Distribution of free iodized tablets to the people
B. Making available only iodized common salt to population
C. Iodizing drinking water
D. Supplying iodine rich vegetables in the market

ANSWERS

1. A 2. C 3. D 4. C 5. B

MOST LIKELY QUESTIONS

1. What nutrient deficiency in diet causes the Kwashiorkor disorder?
2. What is the difference between Marasmus and Kwashiorkor?
3. What is the cause of emaciated appearance?
4. Protein calorie malnutrition.
5. Obesity.



Glossary

A

Acidosis: Diminished capacity of the body to buffer protons in various respiratory/metabolic conditions.

Actin: A protein making up the thin filament of muscles.

Active site: It is the restricted part of a protein (enzyme) to which a substrate binds.

Active transport: Energy-driven, uphill transport of a solute across a membrane.

Adenosine diphosphate (ADP): A nucleotide serving as phosphate group acceptor in cellular respiration.

Adenosine triphosphate (ATP): A nucleotide serving as a phosphate group donor in metabolic reactions.

Aldose: A monosaccharide where terminal carbonyl carbon is an aldehyde.

Alkalosis: Diminished capacity of the body to buffer hydroxyl ions in various respiratory/metabolic conditions.

Allele: Allele is one of the several alternative forms of a gene, occupying a given locus on a chromosome.

Allosteric control: It refers to the ability of an interaction at one site of a protein to influence the activity of another site.

Allosteric enzyme: A regulatory enzyme whose catalytic activity is modulated by the non-covalent association with a specific metabolite at a site (other than the active site) known as the allosteric site.

Amino acid: α -Amino substituted carboxylic acid, the monomeric unit of protein.

Aminotransferases: Transaminases catalyzing the transfer of amino group from α -amino acid to α -keto acid.

Amphibolic pathway: A metabolic pathway used in catabolism as well as anabolism.

Amphipathic molecule: A molecule possessing polar as well as nonpolar domains. Amphipathic structures have two surfaces, one hydrophilic and the other hydrophobic, e.g. a lipid.

Amplification: Amplification refers to the production of additional copies of a chromosomal sequence, found as intrachromosomal or extrachromosomal DNA.

Anabolism: Phase of metabolism concerned with the energy requiring biosynthesis of compounds from smaller precursors.

Anaplerotic reaction: An enzymatic reaction capable of replenishing the supply of intermediates in the TCA cycle.

Annealing: The pairing of complementary single strands of DNA to form a double helix.

Anomers: Stereoisomers of a sugar differing in configuration about the carbonyl (anomeric) carbon atom.

Antibody: A defence protein (immunoglobulin) generated by B lymphocytes and capable of binding specifically to an antigen, to trigger the immune response.

Anticodon: A specific sequence (trinucleotide) in a t-RNA, complementary to a codon in an mRNA.

Antigen: A molecule capable of eliciting the synthesis of a specific antibody (immunoglobulin).

Antiport: Cotransport of two solutes across a membrane in opposite directions.

Apoenzyme: The protein portion of an enzyme.

Apolipoprotein (Apoprotein): The protein portion of lipoprotein.

Apoptosis: The capacity of a cell to undergo programmed cell death.

Asymmetric carbon atom: A carbon atom covalently bonded to four different groups and thus capable of existing in two different configurations.

Attenuation: It is the process of the regulation of termination of transcription that is involved in controlling the expression of some bacterial operons.

Attenuator: A specific RNA sequence functioning as a transcription terminator.

Autoradiography: The process which detects radioactively labeled molecules by their effect in creating an image on photographic film.

Avidin: The egg-white factor which can bind to biotin.

B

Back mutation: It reverses the effect of a mutation that had inactivated a gene.

Basal metabolic rate (BMR): The rate of oxygen consumption by an animal's body at complete physical and mental rest, 12-14 hrs, following the last meal.

Base-pair (bp): It is a partnership of A with T or G with C, in a DNA double helix.

Bile salts: Amphipathic steroid derivatives with detergent properties, helping in lipid digestion and absorption.

Biocytin: Active biotin, linked through an amide bond to a lysine residue.

Biopterin: A pterin-derived cofactor involved in some oxido-reduction processes.

B Lymphocytes (B Cells): These are the cells responsible for synthesizing antibodies.

Buffer: A system consisting of a conjugate acid-base pair and is capable of resisting change in pH.

C

Carotenoids: Lipid-soluble pigments made up of isoprene units.

Catabolism: Phase of metabolism concerned with the energy-yielding breakdown of larger molecules.

Catabolite activator protein (CAP): A positive regulatory protein activated by cAMP. It is needed for RNA polymerase to initiate transcription of certain catabolite-sensitive operons of *E. coli*.

Catecholamines: Amino derivatives of catechol acting as transmitters of hormonal or neuronal signals, viz. dopamine, Norepinephrine, epinephrine.

Central dogma: Flow of genetic information from DNA to RNA to protein.

Ceramide: Sphingosine derivative containing an acyl group attached to its amino group.

Cerebroside: Ceramide containing one sugar residue as a head group.

Chimeric molecule: A molecule, e.g. DNA, RNA or protein, containing sequences derived from two different species.

Chromatin: The filamentous complex of DNA and histones in the nucleus of interphase cells.

Chromatography: A process of separation of molecules based on partitioning between a mobile phase and a stationary phase.

Chromosome: A discrete unit of the genome carrying many genes, visible as a morphological entity only during cell division.

Chylomicron: A plasma lipoprotein carrying dietary TAGs from intestine to the other tissues.

Citric acid cycle (Krebs cycle, tricarboxylic acid cycle or TCA cycle): A cyclic system of enzymatic reactions for the oxidation of acetyl residues to carbon dioxide, formation of citrate being the first step.

Codon: A trinucleotide sequence (triplet) that codes for a specific amino acid or a termination signal.

Coenzyme: An organic cofactor, often containing a vitamin, required for the action of some enzyme.

Coenzyme A (CoA): A pantothenic acid containing coenzyme acting as an acyl group carrier.

Cofactor: An inorganic ion or a coenzyme required for enzyme activity.

Competitive inhibition: An enzyme inhibition reversed by increasing the substrate concentration.

Complementary DNA (cDNA): Single-stranded DNA complementary to an RNA, synthesized from it by reverse transcription *in vitro*.

β-Conformation: An extended, zig-zag arrangement of a polypeptide chain called β-pleated sheet.

Conjugated protein: A protein containing one or more prosthetic group.

Cotransport: The simultaneous transport of two solutes across a membrane, in the same (uniport) or opposite (antiport) direction.

Cyclic AMP (cAMP): A second messenger formed from ATP by the action of adenylate cyclase in which the phosphate group is joined to both 3' and 5' positions of ribose.

Cytoplasm: The material between the plasma membrane and the nucleus.

Cytoskeleton: The network of fibers in the cytoplasm of the eukaryotic cell.

Cytosol: The general volume of cytoplasm in which cell organelles are located.

D

Dalton: The weight of a single hydrogen atom (1.66×10^{-25} g).

Deamination: The enzymatic removal of amino groups from biomolecules.

Degeneracy: Degeneracy in the genetic code refers to the lack of an effect of many changes in the third base of the codon, on the represented amino acid.

Dehydrogenases: Enzymes catalyzing the removal of pair of hydrogen atoms from their substrates.

Denaturation: Denaturation of DNA describes its conversion from the double-stranded to the single stranded state, most often accomplished by heating.

Denaturation of protein describes its conversion from the physiological conformation to an inactive conformation.

De novo pathway: Synthesis of a molecule from simple precursors, distinct from a salvage pathway.

Deoxyribonucleic acid (DNA): The carrier of genetic information containing deoxyribonucleotides linked by 3', 5' – phosphodiester bonds.

Desaturases: Enzymes catalyzing the introduction of double bonds into fatty acids.

Dextrorotatory: A stereoisomer rotating plane-polarized light clockwise.

Diabetes mellitus: A metabolic disease with hyperglycemia due to absolute/relative insulin deficiency.

Diffusion: The net downhill movement of molecules.

Disaccharide: Two monosaccharides linked through a covalent glycosidic bond.

Disulfide bond: A covalent cross-linkage formed by two cysteinyl residues (-cys-cys-).

DNA library: A random collection of cloned DNA fragments including the entire genome of an organism.

DNA ligase: An enzyme catalyzing the formation of a phosphodiester bond between the 3' end of one DNA segment and the 5' end of another.

DNA polymerase: An enzyme catalyzing the template-dependent synthesis of DNA from deoxyribonucleotide precursors.

DNA replicase: Complex of all enzymes and proteins required, specifically, for replication.

DNA supercoiling: The coiling of DNA upon itself due to bending, underwinding or overwinding.

Domain: A distinct structural unit of a molecule.

Double helix: The coiled conformation of two antiparallel, complementary DNA strands.

Double reciprocal plot: Lineweaver-Burk plot of I/V versus $I/(S)$, allowing an accurate determination of V_{max} and K_m .

E

Electrophoresis: Movement of charged species in an electric field.

Elongation factors (EF in prokaryotes or eEF in eukaryotes): Specific proteins required in the elongation of polypeptides.

Enantiomers: Stereoisomers that are non-superimposable mirror images of each other.

Endocytosis: Invagination of plasma membrane leading to the uptake of extracellular material.

Endonuclease: Enzyme hydrolyzing internal phosphodiester bonds of a nucleic acid.

Enzyme: A protein/RNA catalyzing a specific reaction without affecting the equilibrium.

Equilibrium: The state of a system with no further net change occurring and has least free energy.

Epimers: Two stereoisomers differing in the configuration at an asymmetric center.

Epitope: The chemical group/antigenic determinant in an antigen to which an antibody binds.

Exergonic reaction: A reaction that releases free energy (exothermic for heat release).

Exocytosis: The fusion of an intracellular vesicle with the plasma membrane for the release of vesicular contents extracellularly.

Exon: A part of the gene that is represented in the mature RNA product.

Exonuclease: An enzyme hydrolyzing the terminal phosphodiester bond of a nucleic acid.

F

Facilitated diffusion: Diffusion of polar molecules through a membrane, mediated by a protein transporter.

Fatty acid: Long chain aliphatic carboxylic acid found in natural fats and oils.

Feedback inhibition: Inhibition of an enzyme early in a metabolic sequence by a product formed later in the sequence.

Fermentation: Exergoic breakdown of a nutrient without net oxidation.

Flavin adenine dinucleotide (FAD): Riboflavin containing coenzyme of some oxidoreductases.

Flavin mononucleotide (FMN): Riboflavin phosphate, a coenzyme for some oxidoreductases.

Flavoprotein: An enzyme with a flavin nucleotide as a tightly bound prosthetic group.

Fluidity: A property of membranes which indicates the ability of lipids to move laterally, within their monolayer.

Fluid-mosaic model: Biological membranes having fluid lipid bilayers with embedded proteins.

Frame shift mutation: An insertion/deletion of a base-pair altering the reading frame of codons.

Free energy: That portion of the total energy of a system that can do work at constant temperature and pressure.

Free radical: Highly reactive atom/group of atoms possessing an unpaired electron.

Furanose: A simple sugar having the five-membered furan ring.

G

Gangliosides: Neuronal sphingolipids having complex oligosaccharides as head groups.

Gene: Gene (cistron) is the segment of DNA encoding a functional RNA or protein.

Gene cluster: A group of adjacent genes those are identical or related.

Genetic code: The correspondence between triplets in DNA (or RNA) and amino acids in protein.

Genome: The entire genetic information contained within a cell/virus.

Genotype: It is the genetic constitution of an organism.

Gluconeogenesis (Neoglucogenesis): Synthesis of glucose from non-carbohydrate precursors.

Glycan: Synonym for polysaccharide.

Glycerophospholipd: An amphipathic lipid with a glycerol back done.

Glycolipids: A lipid containing a carbohydrate group.

Glycolysis: Catabolism of one glucose molecule into two molecules of pyruvate/lactate.

Glycoprotein: A protein containing a carbohydrate group.

Glycosaminoglycan: Synonymous with mucopolysaccharides, a heteropolysaccharide containing alternately repeating amino sugar and uronic acid.

Glycosidic bonds: Bonds between a sugar and another sugar/nonsugar residue through an intervening oxygen/nitrogen atom.

H

Hapten: A small molecule that acts as an antigen when conjugated to a protein.

Helicase: An enzyme catalyzing DNA strand separation.

α -Helix: A right-handed helical conformation of a polypeptide with maximum intrachain hydrogen bonding.

Heme (Haem): The iron-porphyrin prosthetic group of haemoproteins.

Henderson-Hasselbalch equation: An equation relating the pH, pKa and proton-acceptor/donor ratio in a solution.

Heterogenous nuclear RNA (hn RNA): It is the transcripts of nuclear genes made by RNA polymerase II.

Heteropolysaccharide: A polysaccharide having more than one type of monosaccharides.

Heterotropic enzyme: An allosteric enzyme modulated by a molecule other than the substrate.

Hexose: A simple sugar with six-carbon backbone.

Histones: Family of conserved DNA-binding proteins of eukaryotes that forms the nucleosomes. It forms the basic subunit of chromatin.

Holoenzyme: Complete enzyme with all necessary cofactors.

Homopolysaccharide: A polysaccharide with only one type of sugar (a polymer of same monosaccharides).

Homotropic enzyme: An allosteric enzyme modulated by its substrate.

Hormone: The first messenger secreted in minute amounts from an endocrine gland and carried by blood to a distant tissue.

Hybridization: The pairing of complementary RNA and DNA strands to give an RNA-DNA hybrid.

Hydrophilic: Polar molecule that easily dissolves in water.

Hydrophobic: Nonpolar molecule insoluble in water.

I

Immunoglobulin: A defense antibody protein generated against and capable of binding specifically to an antigen.

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Induced-fit model of enzyme binding site: A conformational change in an enzyme in response to substrate binding, making the enzyme catalytically active.

Initiation codon: The triplet of AUG (or GUG) in prokaryotes, coding for the first amino acid (methionine) in a polypeptide.

Interferon: Specific Glycoprotein with antiviral activity.

Intermediary metabolism: The combined activities of all the metabolic pathways that interconvert precursors, metabolites and products (excluding macromolecules).

Isoelectric pH: The pH at which a solute has no net electric charge.

Isoenzymes (Isozymes): Multiple forms of an enzyme catalyzing the same reaction but differing in amino acid sequence, K_m , V_{max} and regulatory properties.

J

Jaundice: Yellow discoloration of sclera, visible when serum bilirubin exceeds 2 mg/dl.

K

Keratin: Structural protein consisting of parallel polypeptide chains in α -helical conformation.

Ketogenic amino acid: Amino acid whose carbon skeleton can be a precursor of ketone bodies.

Ketone bodies: Fuel molecules (acetoacetate, β -hydroxybutyrate and acetone) produced by the liver and utilized by the peripheral tissues.

Ketone: A simple monosaccharide with a ketone as the carbonyl group.

Kinase: Enzyme catalyzing substrate phosphorylation by ATP.

L

Levorotatory: A stereoisomer rotating the plane of polarized light counter clockwise.

Lineweaver-Burk plot (Double reciprocal plot): A linear transformation of Michaelis-Menten equation allowing determination of V_{max} and K_m .

Lipid: Water-insoluble compound containing fatty acids, glycerol and phosphate.

Lipid bilayer: The form taken by concentration of lipids in which the hydrophobic fatty acids occupy the interior and the polar heads face the exterior.

Lipoprotein: A protein aggregate carrying water-insoluble lipids in the blood, the protein as an apolipoprotein (apoprotein).

Lyases: Enzymes catalyzing removal of a group from substrate forming a double bonds, or the addition of a group to a double bond.

M

Messenger RNA (mRNA): A class of RNA molecules, complementary to one strand of DNA, carrying genetic information from DNA to ribosomes.



Metabolism: The complete set of enzyme-catalyzed transformation of organic molecules in living cells. It includes both anabolism as well as catabolism.

Metalloprotein: A protein with some metal ion as a prosthetic group.

Micelle: An aggregate of amphipathic molecules with the nonpolar part towards the interior, and the polar part exposed to the surrounding aqueous environment.

Michaelis-Menten equation: The hyperbolic dependence of V_0 , the initial reaction velocity of an enzymatic reaction, on substrate concentration (S) which is given by:

$$V_0 = \frac{V_{\max} S}{(K_m + S)}$$

Microfilaments: Thin filaments, similar to actin, present in the cytoplasm with a contractile function.

Microtubules: Tubulin dimers found in cilia, flagella and centrosomes.

Mixed function oxidases: Enzymes using molecular oxygen to simultaneously oxidize a substrate and a cosubstrate.

Monoclonal antibodies: Antibodies produced by a cloned hybridoma cell, hence identical and directed against the same epitope.

Monosaccharide: A straight-chain carbohydrate with 3 or more carbon atoms.

Mucopolysaccharide: A heteropolysaccharide containing alternately repeating amino sugar and uronic acid.

Mutarotation: The change in specific rotation of a pyranose/furanose glycoside accompanying the equilibration of its α and β anomeric forms.

Myosin: The major contractile protein of thick filaments of muscle.

N

Negative feedback: A reaction product inhibiting an earlier step in a metabolic pathway.

Neurotransmitter: A low molecular weight compound secreted from a presynaptic neuron, transmitting nerve impulse by binding to postsynaptic neuron.

Nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NAD/NADP): Nicotinamide-containing coenzymes functioning as carriers of reducing equivalents during oxido-reduction.

Noncompetitive inhibition: Enzyme inhibition due to the binding of an inhibitor to the enzyme at a site distinct from substrate-binding site and not reversed by increasing the substrate concentration.

Nonessential amino acids: Amino acids synthesized in the human body from simple precursors and not essentially required in the diet.

Non-heme iron proteins: Proteins participating in oxido-reduction, containing iron but no porphyrin ring.

Nucleic acids: Polynucleotides (DNA, RNA) in which monomeric nucleotides are linked in a specific sequence by phosphodiester bond.

Nucleotide: A nucleoside phosphorylated at one of its pentose hydroxyl groups.

O

Oligomeric protein: A multi-subunit protein having two or more identical polypeptide chains.

Optical activity: The ability of a substance to rotate the plane of polarized light.

β -Oxidation: Successive oxidations at the β -carbon atom of fatty acyl CoA, producing acetyl CoA.

Oxidative phosphorylation: The enzymatic phosphorylation of ADP to ATP coupled to electron flow from a substrate to molecular oxygen.

Oxygenases: Enzymes catalyzing reactions where oxygen is incorporated into an acceptor molecule.

P

Palindrome: DNA segment in which the base sequences exhibit two fold rotational symmetry.

Pentose: A simple five-carbon sugar.

Pentose phosphate pathway (Hexose monophosphate shunt (HMP shunt): A pathway inter-converting hexoses and pentoses and a source of pentoses and NADPH.

Peptide bond: Covalent link between the α -amino group of an amino acid and the α -carboxyl group of another amino acid.

Peptidoglycan: Parallel heteropolysaccharides cross linked by short peptides in bacterial cell wall.

Peripheral proteins: Proteins loosely bound to a membrane by hydrogen bonds or electrostatic forces.

pH: The negative logarithm of the H^+ concentration in a solution.

Plasmalogen: A phospholipid with an ether substituent on the first carbon of glycerol.

Plasma membrane: The continuous membrane defining the boundary of every cell.

Polyclonal antibodies: A heterogenous pool of antibodies produced by different α -lymphocytes in response to an antigen, recognizing different epitopes.

Polymorphism: It refers to the simultaneous occurrence in the population of genomes showing allelic variations.

Polyribosome (Polysome): An mRNA associated with a series of ribosomes engaged in translation.

Polysaccharide: Polymer of monosaccharide units linked by glycosidic bonds.

Porphyrin: A nitrogenous compound with four substituted pyrroles linked covalently to form a ring, with a central metal atom.

Post-translational modification: The enzymatic processing of a polypeptide chain after translation.

Primary structure: The covalent backbone of a polymer including any inter chain/intra-chain covalent bonds.

Prostaglandins: A family of arachidonic acid derived metabolites, with local hormone-like properties.

Prosthetic group: A metal ion/organic molecule covalently bound to a protein and is essential for its activity.

Proteoglycan: A heteropolysaccharide joined to a polypeptide, the latter being a minor component.

Q

Quaternary structure: The three-dimensional structure of a multi subunit protein.

R

Racemic mixture: An equimolar mixture of D and L-stereoisomers of an optically active compound.

Receptor: A transmembrane protein, located in the plasma membrane, that binds a ligand in a domain on the extracellular side and as a result has a change in activity of the cytoplasmic domain.

Releasing factors: Hypothalamic hormones that stimulate the release of other hormones by the pituitary gland.

Ribosomal RNA (rRNA): A class of RNA present in ribosomes.

Ribozymes: Catalytic RNA molecules.

RNA polymerase: An enzyme catalyzing the synthesis of RNA from ribonucleoside 5-triphophates on a DNA template.

S

Saponification: Alkaline hydrolysis of neutral fat to release fatty acids as soaps.

Secondary structure: Regular, recurring spatial arrangements of adjacent monomers present in a polymer.

Second messenger: An intracellular effector molecule synthesized/activated in response to an extracellular signal, usually a hormone.

Sphingolipid: An amphipathic lipid with a sphingosine backbone to which a long-chain fatty acid and an alcohol are attached.

Stereoisomers: Compounds having the same chemical composition but different spatial arrangements.

Substrate level phosphorylation: ADP/GDP phosphorylation during a dehydrogenation reaction independent of oxidative phosphorylation.

Suicide inhibitor: A compound that is enzymatically activated and then inhibits the same enzyme.

Sympot: Cotransport of two solutes across a membrane in the same direction.

T

Termination factors (Release factors, RF in prokaryotes or eRF in eukaryotes): Cytosolic protein factors essential for releasing completed polypeptides from a ribosome.

Tertiary structure: The three-dimensional conformation of a polymer in its native folded state.

Thromboxanes: A class of arachidonic acid metabolites involved in platelet aggregation.

Transamination: Pyridoxal phosphate dependent enzymatic transfer of an amino group from an α -amino acid to an α -keto acid.

Transfer RNA (tRNA): A class of RNA molecules binding with a specific amino acid during initiation of translation.

U

Uniport: A transport system carrying only one solute.

V

Vitamin: An organic compound required in small amounts in the diet of some species, usually serving as a constituent of a coenzyme.

V_{max}: The maximum velocity of an enzymatic reaction at saturating substrate concentration.

W

Wax: Esters of long chain fatty acids with long chain alcohols, their melting point being higher than that of the triacylglycerols.

Wobble hypothesis: It accounts for the ability of a tRNA, to recognize more than one codon, by unusual pairing with the third base of a codon.

Z

Zwitterion: A dipolar ion with spatially separate positive and negative charges.

Zymogen: The inactive precursor for some enzymes, activation of which involves selective partial proteolysis.

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