NEW AGE

VIVA VOCE

Orals in Biochemistry

B. PRABHAKAR RAO

NEW AGE INTERNATIONAL PUBLISHERS

VIVA VOCE

Orals
in
Biochemistry

THIS PAGE IS BLANK

VIVA VOCE

Orals in Biochemistry

B. PRABHAKAR RAO
M.D., Professor of Biochemistry
Prathima Institute of Medical Sciences
Nagunur Road, Karimnagar

PUBLISHING FOR ONE WORLD

NEW AGE INTERNATIONAL (P) LIMITED, PUBLISHERS

New Delhi • Bangalore • Chennai • Cochin • Guwahati • Hyderabad
Jalandhar • Kolkata • Lucknow • Mumbai • Ranchi

Visit us at **www.newagepublishers.com**

Published by New Age International (P) Ltd., Publishers

ISBN (10) : 81-224-2296-9

ISBN (13) : 978-81-224-2296-2

PUBLISHING FOR ONE WORLD

NEW AGE INTERNATIONAL (P) LIMITED, PUBLISHERS
4835/24, Ansari Road, Daryaganj, New Delhi - 110002
Visit us at **www.newagepublishers.com**

to

The Great Saints
of the

world who strived hard for the existence of peace in the universe

THIS PAGE IS BLANK

Foreword

The author Professor Dr. B. Prabhakar Rao has vast experience as a teacher, administrator and clinical biochemist. Though there are several textbooks in Biochemistry written by Indian authors, this book differs from others in that it comprises of questions and answers in all aspects of Biochemistry. Almost all the commonly asked questions in viva voce examination in Biochemistry are covered by the author. Subjects like metabolism of xenobiotics, biochemistry of cancer and clinical biochemistry which are of biomedical importance are also covered in a lucid and simplified manner. This textbook which is a compendium of questions and answers in all topics of Biochemistry is not a substitute for a textbook of medical Biochemistry but certainly is an adjuvant to it. This book is useful not only to undergraduate and post-graduate students in Biochemistry in India but is also useful to those preparing for other examinations in other countries.

I earnestly hope that this book will receive appreciation from students and teachers.

C. SITA DEVI
MD, FAMS, FIMSA
Retired Principal & HOD of Biochemistry
Andhra Medical College,
Visakhapatnam.
and
Former Senior Consultant Biochemist
Medwin CDR, CARE Hospitals and Elbit Medical Diagnostics
Hyderabad.

THIS PAGE IS BLANK

Preface

The idea of writing this book of **VIVA VOCE/ORALS IN BIOCHEMISTRY** is that it should be much helpful to the MBBS and M.Sc. (Medical Science) students and post-graduate students for the preparation of examination. Though it is not a textbook of medical Biochemistry, it is framed in the form of questions and answers in a simplified way. This book will definitely help the students for the preparation of their examinations.

This book is also aimed at the level of general practitioners, clinicians and medical students and technicians for applying the knowledge of clinical biochemistry in the clinical side, as much emphasis is given in the clinical biochemistry. The list of clinical biochemistry topics include interpretation of laboratory data and biochemical features of some clinical diseases like metabolic syndrome, diabetes mellitus, anaemias, jaundice, porphyrias and the disturbances of electrolyte and acid base balance.

I am very much grateful to my philosopher and guide Professor C. Sita Devi, MD, FAMS, FIMSA, retired Principal and HOD of Biochemistry, Andhra Medical College, Visakhapatnam and former Senior Consultant, Medwin, CDR, CARE hospitals and Elbit Medical Diagnostics, Hyderabad for making valuable suggestions and writing foreword to my book. I would like to acknowledge my thanks to Dr. Md. Rafi, MD, Assistant Professor of Biochemistry, Dr. Julie Bhattacharya, Assistant Professor of Physiology and Mrs. S. Sangeetha, faculty member of Biochemistry, Prathima Institute of Medical Sciences, Nagunur, Karimnagar for making corrections in the typed manuscript and for their valuable suggestions. Lastly but not the least I thank Mrs. B. Chaitanya Lakshmi and Mr. V. Goverdhan for typing my manuscript and without their help this task would not have been possible.

B. PRABHAKAR RAO

THIS PAGE IS BLANK

Contents

Foreword ..... *vii*
Preface ..... *ix*

1. Chemistry of carbohydrates. 1
2. Chemistry of lipids. 7
3. Chemistry of proteins. 12
4. Enzymes. 22
5. Vitamins. 26
6. Bioenergetics and biological oxidation. 34
7. Metabolism of carbohydrates. 38
8. Lipid metabolism. 56
9. Protein metabolism. 69
10. Chemistry and metabolism of nucleic acids. 83
11. (i) Molecular biology – I – A Replication 93
    (ii) Molecular biology I – B Transcription 96
    (iii) Molecular biology I – C Translation 100
12. Molecular biology II – Control of gene expression. 110
13. Molecular biology III – Recombinant DNA technology. 112
14. Xenobiotics metabolism (Detoxification) and free radicals. 116
15. Biochemistry of cancer. 122
16. Nutrition. 128
17. Mineral metabolism. 136
18. Water, electrolytes and acid-base balance. 145
19. Plasma proteins and immunoglobulins. 159
20. Haemoglobin, porphyrins and bile pigments. 165
21. Hormones. 182

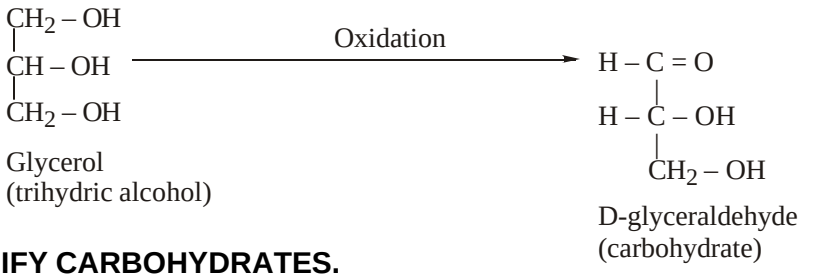
22. (i) Clinical biochemistry – I: Interpretations of Laboratory Investigations 200
(ii) Clinical biochemistry – II: Biochemical features of clinical disorders
(iii) Clinical biochemistry – III: Renal function tests.
23. Practicals in biochemistry. 216
Index 229

Chapter–1

Chemistry of Carbohydrates

1. DEFINE CARBOHYDRATES.

Ans. Carbohydrates are defined as aldehyde or keto derivatives of polyhydric alcohols. For example : Glycerol on oxidation is converted to D-glyceraldehyde, which is a carbohydrate derived from the trihydric alcohol (glycerol).



Glycerol
(trihydric alcohol)

D-glyceraldehyde
(carbohydrate)

2. CLASSIFY CARBOHYDRATES.

Ans. Carbohydrates are classified into

(*a*) Monosaccharides (simple sugars) example: glucose.

(*b*) Disaccharides (composed of two monosaccharides) example: Sucrose, Lactose.

(*c*) Oligosaccharides (consisting of 2–10 monosaccharides).

(*d*) Polysaccharides (consisting of more than 10 monosaccharides), example: Starch, glycogen.

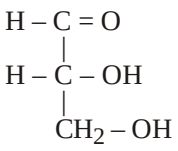
3. HOW MONOSACCHARIDES ARE CLASSIFIED? GIVE EXAMPLES.

Ans. Monosaccharides are sub classified depending on the number of 'C' atoms present in them and the nature of sugar group present in them

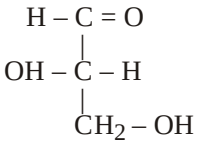
S.No.	*No. of 'C' atoms*	*Nature of sugar group (aldehyde) aldoses*	*Nature of sugar group (keto) ketoses*
1.	C_3 (Trioses)	D-Glyceraldehyde	Di-hydroxy acetone
2.	C_4 (Tetroses)	D-Erythrose	D-Erythrulose
3.	C_5 (Pentoses)	D-Ribose	D-Riboulose and D-xylulose
4.	C_6 (Hexoses)	D-glucose, D-galactose	D-Fructose
5.	C_7 (Heptoses)	—	D-Sedoheptulose

4. WHAT ARE 'D' AND 'L' STEREO ISOMERS?

Ans. Sugars which are related to 'D' glyceraldehyde in spatial configuration (structure) are called 'D' isomers and sugars which are related to L-glyceraldehyde in spatial configuration (structure) are called L isomers.



D-glyceraldehyde



L-glyceraldehyde

The orientation of H and OH groups of a sugar in carbon atom which is adjacent to last carbon atom are related to D-glyceraldehyde are called D-sugars. And the orientation of H and OH groups of a sugar in carbon atom which is adjacent to last carbon atom are related to L-glyceraldehyde are called L-sugars.

5. WHAT ARE OPTICAL ISOMERS?

Ans. The presence of asymmetric 'C' atom in the sugar confers optical activity on the compound. When a beam of polarised light is passed through optically active sugar the plane of polarised light is rotated to the right (Dextrorotatory) or to the left (Levorotatory). Dextrorotatory is designated as + sign and Levorotatory is designated as – sign.

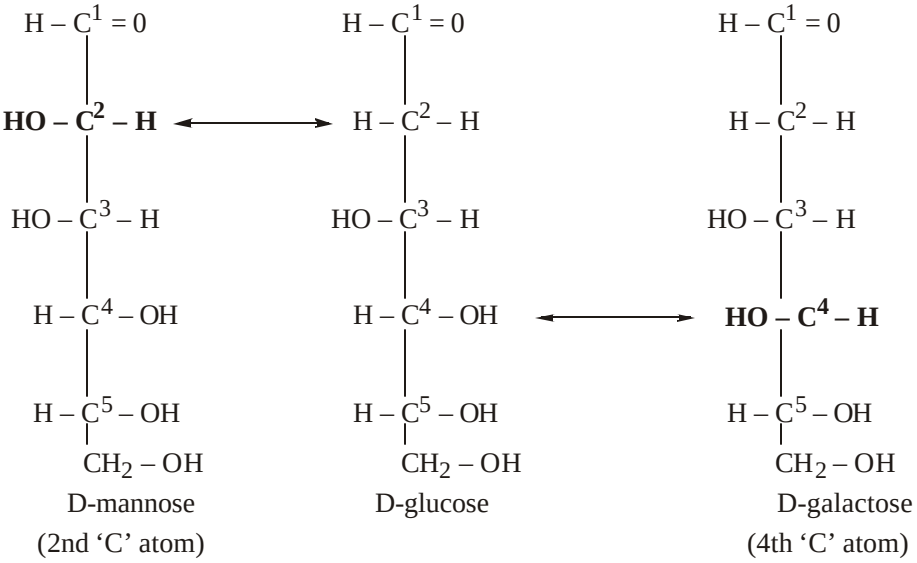
'D' glucose is a dextrorotatory therefore it is designated as D (+) glucose and L glucose is levorotatory and therefore it is designated as L(–) glucose.

6. WHAT ARE EPIMERS? GIVE EXAMPLES OF SUGARS AS EPIMERS?

Ans. When the stereo isomers (sugars) differing in the orientation of H^+ and OH^- groups in a single 'C' atom they are called epimers.

Example:

Glucose and galactose are epimers at 4th 'C' atom. Because they differ in the spatial configuration of OH and H at 4th 'C' atom. Similarly glucose and mannose are epimers of 2nd 'C' atom.



7. WHAT ARE THE DERIVATIVES OF MONOSACCHARIDES AND WHAT ARE THEIR FUNCTIONS?

Ans. The following are the derivatives of monosaccharides and their important biological functions.

S.No.	*Derivative*	*Formed by*	*Important functions*
1.	Glucuronic acid	Formed by oxidation of 6th 'C' atom of glucose	UDP glucuronic acid is involved in conjugation of bilirubin
2.	Glucosamime	2nd OH group of glucose is replaced by NH_2 group	These are present in the muco polysaccharides (Proteoglycans)
3.	Galactosamine	2nd OH group of galactose is replaced by NH_2 group	
4.	Deoxyribose	2nd oxygen of D–ribose is removed	Present in DNA
5.	Sorbitol	Reduction of aldehyde group of glucose by aldol reductase	Accumulate in diabetes mellitus and forms cataract
6.	Ribitol	Reduction of aldehyde group of ribose	Present in riboflavin (B_2)

8. WHAT IS MUTA ROTATION?

Ans. The gradual change in the specific rotation (optical activity) of a freshly prepared solution of monosaccharide until it remains constant on standing is called muta rotation.

Example:

α–D-glucose ⟶ Glucose at ⟶ β–D-glucose + 19°
+ 112° (specific rotation) equilibrium + 52.5° (specific rotation)

9. WHAT IS THE COMPOSITION OF THE FOLLOWING DISACCHARIDES AND HOW THE MONOSACCHARIDE UNITS ARE JOINED IN THEM AND WHAT IS THE BIOLOGICAL IMPORTANCE?

(I) SUCROSE (II) LACTOSE (III) MALTOSE

Ans.

S.No.	*Sugar*	*Composition and linkage*	*Biological importance*
1.	Sucrose (non reducing disaccharide)	α D–glucose and β D–fructose linked by α 1 β 2 glycosidic linkage.	Table sugar
2.	Lactose (reducing disaccharide)	β D–galactose and β D–glucose. β 1, 4 glycosidic linkage	Present in milk
3.	Maltose (reducing disaccharide)	2 α D–glucose units α 1, 4 glycosidic linkage.	Obtained by hydrolysis of starch

10. WHAT ARE THE SOLUBLE AND INSOLUBLE PORTIONS OF STARCH AND WHAT ARE THE DIFFERENCES BETWEEN THEM?

Ans. Starch is physically separated into two components. They are amylose and amylopectin. Differences between amylose and amylopectin.

S.No.	*Amylose*	*Amylopectin*
1.	Soluble portion of starch (10–20 %)	Insoluble portion of starch (80–90 %)
2.	Glucose units are joined by α 1, 4 glycosidic linkages and give straight chain structure	Glucose units are joined by α 1, 4 and α 1, 6 glycosidic linkage and give branching.
3.	Molecular weight is less	Molecular weight is more

11. WHAT ARE THE MAJOR DIFFERENCES BETWEEN AMYLOSE AND CELLULOSE?

Ans.

S. No.	*Amylose*	*Cellulose*
1.	A component of starch which is present in rice, wheat and pulses	Present in fibrous portion of plant (wood) and widely occurs in nature.
2.	It has nutritive value	It is not important from nutrition point of view. However it prevents the constipation by expulsion of feces.
3.	Glucose units are joined by α 1,4 glycosidic linkages and is hydrolysed by amylase	Glucose units are joined β 1,4 glycosidic linkages and not hydrolysed by amylase but hydrolysed by bacterial cellulose.

12. WHAT ARE THE MAJOR DIFFERENCES BETWEEN GLYCOGEN AND AMYLOPECTIN?

Ans.

S. No.	*Glycogen*	*Amylopectin*
1.	It is an animal polysaccharide stored in liver and muscle	It is a component of starch, which is a plant polysaccharide.
2.	Glucose units are joined by both α–1,4 and α–1,6 glycosidic linkages. The degree of branching is more i.e., about 0.09 (more) i.e., one end glucose unit for each 11 glucose units	Glucose units are joined by α 1,4 and α 1,6 glycosidic linkages. The degree of branching is 0.04 (less) i.e. one end group for each 25 glucose units.

13. WHAT ARE MUCOPOLYSACCHARIDES (GLYCOSAMINOGLYCANS) AND WHAT IS THEIR BIOLOGICAL IMPORTANCE?

Ans. Mucopolysaccharides are complex carbohydrates consisting of amino sugars and uronic acids. These are hyaluronic acid, heparin, and various chondriotin sulfates.

S.No.	*Name of the mucopolysaccharides*	*Repeating unit*	*Biological importance*
1.	Hyaluronic acid	N–acetyl glucosamine and D–glucuronic acid joined by β–1,4 and β–1,3 glycosidic linkages	Present in synovial fluid. It acts as a lubricant and shock absorbent in joints.

(*contd...*)

2.	Heparin	Repeating unit consists of sulfated glucosamine and sulfated iduronic acid joined by α1, 4 glycosidic linkages.	It is an anti coagulant present in liver, lung and blood.
3.	Chondroitin 4 sulfate	Repeating unit consists of β–glucuronic acid and N–acetyl galactosamine joined by β–1, 3 and β–1, 4 linkages. (Sulfated at 4th position).	Present in cartilage, bone and cornea.
4.	Chondroitin 6 sulfate	Structure is similar to chondroitin 4 sulfate except N–acetyl galactosamine is sulfated at 6th position.	Present in cartilage, bone and cornea.
5.	Dermatan sulfate	It is similar to chondriotin sulfate except the uronic acid is 'L' iduronic acid.	Present in skin.

Chapter–2

Chemistry of Lipids

1. DEFINE LIPIDS.

Ans. The lipids are heterogeneous group of compounds which are insoluble in water but soluble in non-polar solvents such as ether, chloroform and benzene. All lipids invariably contain fatty acids.

2. CLASSIFY LIPIDS.

Ans. Lipids are classified into three groups:

1. Simple lipids (Alcohol + Fatty acids i.e. glycerol + 3 FA's)
2. Complex lipids (Compound lipids) (Alcohol + FA's + groups)
   (*a*) Phospolipids (Alcohol + FA + Phosphoric acid + 'N' base/other group.
   (*b*) Glycolipids [Sphingosine (Alcohol) + FA + Carbohydrate].
3. Derived lipids: These are obtained by the hydrolysis of simple and complex lipids. Examples: Fatty acids, Glycerol, Steroids etc.

3. CLASSIFY THE FATTY ACIDS. GIVE EXAMPLES.

Ans. Fatty acids are classified into

(*a*) Saturated fatty acids.
(*b*) Unsaturated fatty acids.
(*c*) Cyclic fatty acids.

Saturated fatty acids:

All the naturally occuring fatty acids have even number of 'c' atoms. Most predominant fatty acids which occur in nature are:

(*i*) Palmitic acid (C16 fatty acid) $H_3C - (CH_2)_{14} - COOH$

(*ii*) Stearic acid (C18 fatty acid) $H_3C - (CH_2)_{16} - COOH$

Unsaturated fatty acids:

These contain one or more than one double bonds in them. They are:

(*i*) Oleic acid (most common unsaturated fatty acid) $\longrightarrow$ 18:1;9

(*ii*) Poly unsaturated fatty acids (PUFA) → Linoleic acid two (2) double bonds, Linolenic acid three (3) double bonds and Arachidonic acids four (4) double bonds.

4. WHAT ARE ESSENTIAL FATTY ACIDS?

Ans. The following polyunsaturated fatty acids are called essential fatty acids.

1. Linoleic ——→ 18:2;9,12
   (not synthesised in the body)
2. Linolenic ——→ 18:3;9,12,15
   (not synthesised in the body)
3. Arachidonic ——→ 20:4;5,8,11,14
   (semi essential. It can be synthesised from linoleic acid)

5. WHAT IS RANCIDITY?

Ans. The unpleasant odour and taste developed by natural fats upon ageing is called rancidity.

6. DEFINE SAPONIFICATION NUMBER, IODINE NUMBER AND ACID NUMBER.

Ans.

(*a*) **Saponification number:** It is defined as milligrams of KOH required to saponify one gram of fat.

(*b*) **Iodine number:** It is the grams of Iodine absorbed by 100 gms. of fat.

(*c*) **Acid number:** It is defined as milligrams of KOH required to neutralise the fatty acids present in one gram of fat. Acid number indicates degree of rancidity due to free acid.

7. WHAT ARE PHOSPHOLIPIDS? BRIEFLY OUTLINE THE STRUCTURE AND FUNCTIONS OF PHOSPHOLIPIDS.

Ans. Phospholipids are made up of alcohol, fatty acid, phosphoric acid and a nitrogenous base or other group. These are classified into two major types:

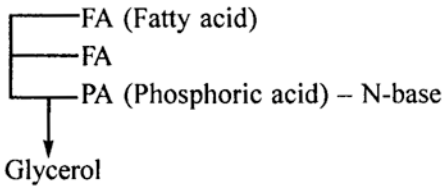
(*a*) Glycerophospholipids (Glycerol containing).

(*b*) Sphingophospholipids (Sphingosine containing).

Glycerophospholipids

These phospholipids contain a common substance phosphatidic acid + a 'N' base or other group.

Diagramatic Representation of Phospholipid



Phosphatidic acid is composed of glycerol + 2 FAs + Phosphoric acid. 'N' base is attached to Phosphoric acid residue in the phospolipids.

The following are the different types of glycerophospholipids which differ in the 'N' base.

S.No	*Name of the Phospholipid*	*'N' base/other group*	*Functions*
1.	Lecithin	Choline	(*a*) Present in cell membranes (*b*) Involved in the formation of cholesterol esters and lipo proteins (*c*) Involved in the formation of lung surfactant (dipalmitoyl lecithin) and the defect in its synthesis results in the development of RESPIRATORY DISTRESS SYNDROME (RDS) (*d*) Possesses amphipathic properties and forms micells in the digestion and absorption of lipids.
2.	Cephalin	Ethanolamine	Present in the biomembranes.
3.	Phosphatidyl serine	Serine	Present in the biomembranes.
4.	Phosphatidyl inositol	Inositol	By hormone agonist it is cleaved to 1. DAG and IP3 which act as second messengers in signal transduction
5.	Plasmalogens (Resembles cephalin)	Ist 'C' ether linkage (unsaturated alcohol)	10 % phospholipids of brain and muscle
6.	Cardiolipin (Di –phosphatidyl glycerol)	PA–glycerol –PA	Present in mitochondrial membranes

Sphingophospholipids (Sphingosine containing)

Spingomyelin contains

(*i*) Sphingosine (complex amino alcohol)

(*ii*) Fatty acid

(*iii*) Phosphoric acid

(*iv*) Choline

Sphingosine + FA is called ceramide.

Sphingomyelins are present in the nervous system.

8. WHAT ARE GLYCOLIPIDS AND WHAT ARE THEIR IMPORTANT FUNCTIONS?

Ans. Glycolipids contain ceramide (Sphingosine + FA) and sugar. These are:

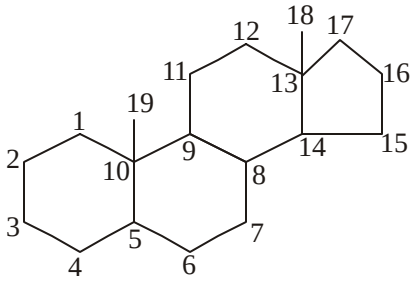
(*a*) Cerebrosides (Galacto or glucoceramides).

(*b*) Sulfatides (Sulpho galacto ceramide) present in myelin.

(*c*) Gangliosides (Glucosyl ceramide + sialic acid). Glycolipids are present in the nervous tissues and cell membranes.

9. WHAT IS THE BASIC STRUCTURE OF THE STEROID?

Ans. Steroid possesses cyclopentano, perhydro phenanthrene nucleus. Phenantherene has three A, B, C six membered rings to which a five membered cyclopentane ring is attached. The whole system is called CPP nucleus with 19 positions.



10. NAME THE BIOLOGICALLY IMPORTANT SUBSTANCES POSSESSING CPP NUCLEUS.

Ans. The following substances have CPP nucleus in their structures:

(*a*) Cholesterol.

(*b*) Provitamin $-D_3$ (7 dehydro cholesterol).

(*c*) Bile acids (cholic acid and chenodeoxy cholic acid).

(*d*) Steroid hormones (glucocorticoids, mineralocorticoids and sex hormones).

11. WHAT ARE THE GENERAL FEATURES OF STRUCTURE OF CHOLESTEROL.

Ans. Cholesterol is a 27 carbon containing compound. It has

(*a*) CPP nucleus.

(*b*) OH group at 3rd position.

(*c*) Δ^5 i.e. a double bond is present between 5th and 6th carbon atoms.

(*d*) A side chain at 17th position.

12. NAME THE IMPORTANT COLOR REACTION OF CHOLESTEROL AND WHAT IS ITS IMPORTANCE?

Ans. The important color reaction of cholesterol is LIEBERMANN BURCHARD REACTION. The chloroform solution of cholesterol is treated with acetic anhydride and sulphuric acid which gives a red color and this color quickly changes from blue to green. This reaction is the basis of quantitative estimation of cholesterol.

13. WHAT ARE EICOSANOIDS AND WHAT ARE THEIR FUNCTIONS?

Ans. The compounds derived from arachidonic acid are called Eicosanoids. These are

(*a*) Prostaglandins (PGs) → 6 primary PGs
→ 8 secondary PGs

Major functions **:** They cause contraction of pregnant uterus and therefore they can be used in the induction of labor or medical termination of pregnancy (MTP).

(*b*) *Prostacyclins* (PGI_2): These prevent platelet aggregation and act as vasodialators.

(*c*) *Thromboxanes* (TX_2): These cause platelet aggregation and forms thrombus.

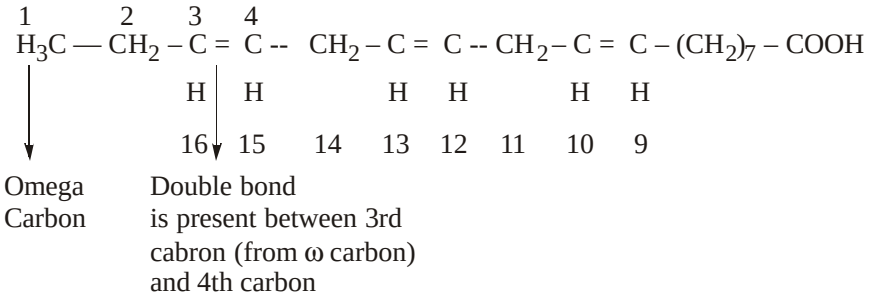
(*d*) *Leucotrienes* : These are formed as the products of mast cell degradation. SRS – A is formed in the anaphylaxis. This substance consists of leukotrienes C_4, D_4 and E_4.

14. WHAT ARE ω3 FATTY ACIDS? WHAT IS THE CLINICAL SIGNIFICANCE OF THESE FATTY ACIDS?

Ans. The ω3 fatty acids have double bond between 3rd carbon (3rd from ω carbon atom left side) and 4th carbon.

Examples:

α – Linolenic acid (present in plant oils).



18 : 3; 9, 12, 15

The other ω3 fatty acids are

(*a*) Eicosapentanoic acid 20:5; 5, 8, 11, 14, 17

(*b*) Docosa pentanoic acid 22:5; 7, 10, 13, 16, 19

(*c*) Docosahexanoic acid 22:6; 4, 7, 10, 13, 16, 19

a, b, c, are present in fish oils.

The ω3 fatty acids prevent thrombosis and hence these are used for the prevention of coronary artery heart disease (CAHD).

Chapter–3

Chemistry of Proteins

1. WHAT ARE THE DIFFERENT LEVELS OF ORGANIZATION OF STRUCTURE OF A PROTEIN?

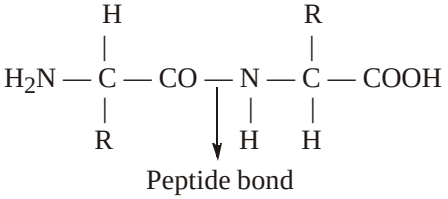
Ans.

(*a*) Primary structure.
(*b*) Secondary structure.
(*c*) Tertiary structure.
(*d*) Quaternary structure.

2. WHAT ARE THE MAIN FEATURES OF PRIMARY STRUCTURE OF A PROTEIN?

Ans. Primary structure comprises the sequence or order of amino acids in the polypeptide chains and location of disulphide bonds in them.

Peptide bond is the main force which maintains primary structure:

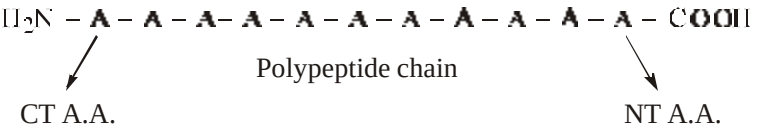


The polypeptide chain has:

(*i*) One 'N' terminal amino acid (Ist amino acid on left terminal of polypeptide chain having free amino group).

(*ii*) One 'C' terminal amino acid (last amino acid having free carboxyl group).

In between the amino acids are joined by peptide bonds.



3. WHAT IS SECONDARY STRUCTURE OF A PROTEIN AND GIVE EXAMPLES?

Ans. The regular folding and twisting of the polypeptide chain brought about by hydrogen bonds is called secondary structure of protein.

Examples:

(*i*) α– helix structure.
- (A) Fibrous proteins:
  - (*a*) Keratin of hair, nails and skin.
  - (*b*) Myosin and tropomyosin of muscles.
- (B) Globular proteins
  Hemoglobin

(*ii*) β–pleated sheet
Silk fibroin.

Salient features of α–helix

(*a*) The distance travelled per turn of helix is 0.54 nm and 3.6 amino acid residues take part.
(*b*) The distance between adjacent residues 0.15 nm.
(*c*) Proline and hydroxy proline disrupt helix.
(*d*) Hydrogen bonds and Van der Waal's forces stabilize α– helix structure.

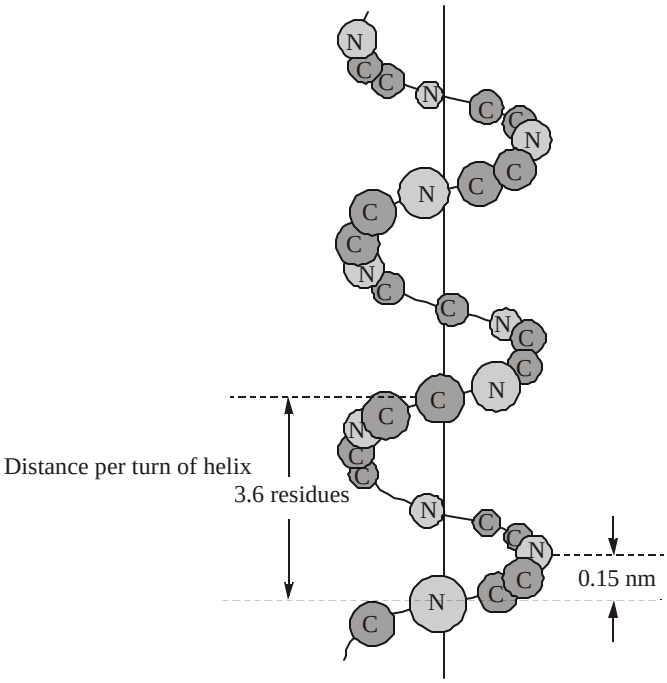


Figure 1 α–helix structure

Salient features of β– pleated sheet

(*a*) It is a sheet rather than rod.
(*b*) The distance between adjacent A.A is 3.5 Å.
(*c*) Sheets are composed of two (2) or more than two (2) polypeptide chains.
(*d*) And also anti-parallel sheets.
(*e*) Ans also in opposite direction.

(*f*) The arrangement of polypeptide chains in β–pleated sheet is parallel pleated sheet.
(*g*) Peptide chains are side by side and lie in the same direction.

4. WHAT ARE THE SALIENT FEATURES OF TRIPLE HELIX STRUCTURE OF COLLAGEN?

Ans. Collagen consists of approximately 1000 A.As. The repeating unit of collagen structure is represented by $(Gly–X–YN)_n$. About 100 of X positions are prolines and 100 of Y positions are hydroxy prolines.

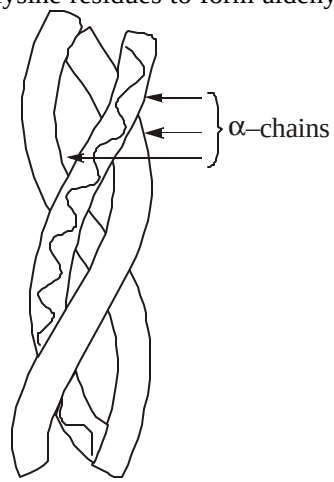
Due to presence of proline, hydroxy proline and glycine in the structure of collagen, these A.As prevent α– helix and β– pleated structure and forms triple helix structure. Collagen is composed of three (3) polypeptide chains. Each α chain is twisted into left–handed helix of three (3) residues per turn. Three (3) α chains then form a rod like structure.

Forces of triple helix

(*a*) *Hydrogen bonds*: Left handed helices are held together by interchain hydrogen bonds.
(*b*) Lysine or leucine bond.

Covalent cross links

Collagen fibers are further stabilized by the formation of covalent cross-links both within and between triple helical units. This occurs by lysyl oxidase which causes oxidative deamination of ε– NH_2 group of lysine and hydroxy lysine residues to form aldehydes.



Triple Helix

Figure 2. Triple Helix structure of collagen

5. WHAT ARE THE SALIENT FEATURES OF TERTIARY STRUCTURE OF A PROTEIN?

Ans. The looping and winding of the secondary structure of a protein by other associative forces between the amino acid residues which give three dimensional conformation is called tertiary structure.

In the tertiary structure, proteins fold into compact structure. The tertiary structure reflects overall shape of the molecule.

Example: Myoglobin.

The polypeptide chains are folded in such a way that hydrophobic side chains are burried inside and its polar side chains are on the surface (exterior).

Forces involved in the tertiary structure

(*a*) Hydrogen bonds.
(*b*) Hydrophobic interactions.
(*c*) Van der Waal's forces.
(*d*) Disulphide bonds.

6. What are the Salient Features of Quaternary Structure of a Protein?

Ans. The association of catalytically or functionally active small subunits of protein is called quaternary structure. The quaternary structure of protein is due to proteins having more than one polypeptide chain.

Examples:

(*i*) Hemoglobin (Hb–A): 4 polypeptide chains ($\alpha_2\beta_2$).
(*ii*) Lactate dehydrogenase (LDH) – 4 polypeptide chains (H or M).
(*iii*) Creatine kinase (CK) – 2 polypeptide chains (B or M).

Forces involved for the stabilization of quaternary structure

(*a*) Hydrophobic interactions.
(*b*) Hydrogen bonds.
(*c*) Ionic bonds.

7. WHAT ARE THE MAIN FEATURES OF STRUCTURE OF INSULIN?

Ans. Insulin is a protein hormone secreted by the β– cells of islets of Langerhans.

Insulin consists of two (2) polypeptide chains ('A' chain and 'B' chain) and composed of 51 A.As. The molecular weight is 5734.

Chain	*Total No. of A.As.*	*'N' terminal A.A.*	*'C' terminal A.A.*
A	21	Glycine	Asparazine
B	30	Phenylalanine	Threonine

Two chains are joined by (two) 2 inter disulphide bonds.

Ist bond is formed by 7th cystein of 'A'chain and 7th cystein of B chain.

2nd bond is formed by 20th cystein of 'A' chain and 19th cystein of 'B' chain.

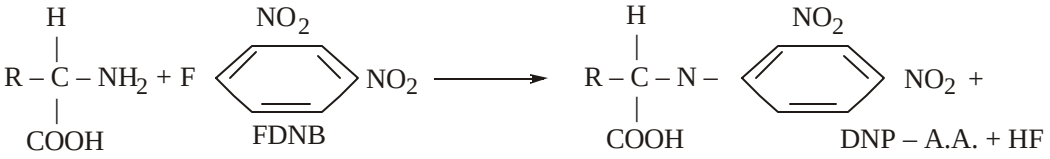
One intra disulphide bond is present in the 'A' chain formed by 6th cystein to 11th cystein.

8. What is end Group Analysis?

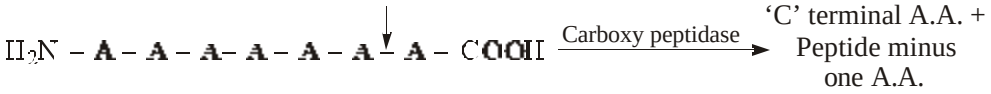
Ans. End group analysis is the method devised for the identification of 'N' terminal and 'C' terminal A.As. in the polypeptide chains of a protein.

9. GIVE ONE EXAMPLE OF IDENTIFICATION OF 'N' TERMINAL AMINO ACID AND 'C' TERMINAL AMINO ACID?

Ans. (A) Method for the identification of 'N' terminal amino acid.
The 'N' terminal amino acid reacts with Sanger's reagent (1 fluoro 2, 4 dinitrobenzene) and forms corresponding DNP – amino acid.



(B) Method for the identification of 'C' terminal amino acid.
'C' terminal A.A. is identified by treating with carboxy peptidase which splits terminal peptide bond and releases 'C' terminal A.A. which can be identified by chromatography.



10. WHAT ARE THE BASIC, ACIDIC, 'S' CONTAINING, HYDROXY, BRANCHED CHAIN AND AROMATIC AMINO ACIDS AND WHAT ARE THE IMINO ACIDS?

Ans.

S.No.	*Category of A.As.*	*Examples*
(*i*)	Basic	Lysine, arginine, ornithine, histidine (slightly basic)
(*ii*)	Acidic	Glutamic acid, aspartic acid
(*iii*)	'S' containing	Methionine, cysteine and cystine
(*iv*)	Branched chain	Valine, isoleucine and leucine
(*v*)	Hydroxy	Serine and threonine
(*vi*)	Aromatic	Phenylalanine, tyrosine and tryptophan
(*vii*)	Imino acids	Proline and hydroxy proline.

11. WHICH IS THE SIMPLEST AMINO ACID AND WHICH AMINO ACID LACKS THE ASYMMETRIC 'C' ATOM?

Ans. Glycine.

12. WHAT ARE THE BIOLOGICALLY IMPORTANT PEPTIDES AND WHAT ARE THEIR IMPORTANT FUNCTIONS?

Ans.

S.No.	*Name of the peptide*	*No. of A.As. present and is nature*	*Important functions*
(*i*)	Glutathione (GSH)	3 amino acids	Takes part in the reduction reactions and in the detoxification of H_2O_2 which maintains the integrity of RBC membrane.
(*ii*)	Thyrotrophin releasing hormone (TRH)	3 amino acids. It is produced in the hypothalamus	Releases TSH from the anterior pituitary gland
(*iii*)	Angiotensin – II	8 amino acids formed from the angiotensin I by the action of converting enzyme.	Stimulates aldosterone secretion from the zona gloumerulosa cells of adrenal cortex. Powerful vaso constrictor ($\uparrow\uparrow$ B.P)
(*iv*)	Vasopressin (ADH)	9 amino acids secreted by the posterior pituitary gland	Causes absorption of water from the renal tubules.
(*v*)	Angiotensin – I	Decapeptide (10 A.As.) formed by the action of renin on angiotensinogen (α_2 globulin)	It is converted to angiotensin – II by the CE which $\uparrow\uparrow$ BP (hypertension).

13. WHAT ARE SIMPLE PROTEINS?

Ans. Simple proteins consists of only amino acids. These are :

(*a*) Albumins: (Egg white) soluble in water. Precipitated by full saturation with $(NH_4)_2SO_4$.

(*b*) Globulins: Soluble in dilute neutral salt solution (egg yolk and myosin of muscle). It is precipitated by half saturation with $(NH_4)2\ SO_4$.

(*c*) Glutenins: Soluble in dilute acids and alkalis. Examples: Glutelin of wheat and oryzenin of rice.

(*d*) Prolamines are alcohol soluble proteins. Examples: Zein of corn and gliadin of wheat.

(*e*) Histones are basic proteins. Globin of hemoglobin and nucleohistone.

(*f*) Protamines are basic proteins and present in nucleoproteins of sperm (nucleoprotamines).

14. WHAT ARE CONJUGATED PROTEINS? GIVE EXAMPLES.

Ans. Conjugated proteins contain amino acids and a non–protein prosthetic group:

(*a*) **Chromoproteins –** Hemoglobin (Heme + globin).

(*b*) **Nucleoproteins –** Nucleic acid + proteins.

(*c*) **Glycoprotein –** Carbohydrate + protein.

(*d*) **Phosphoproteins –** Phosphoric acid + protein (casein of milk).

(*e*) **Lipoproteins –** Lipid + proteins (chylomicrons, VLDL, LDL etc.).

15. WHAT ARE DERIVED PROTEINS? GIVE EXAMPLES.

Ans. Derived proteins are formed by treatment with heat, acids and by the hydrolysis of native proteins.

Example :

Primary derived proteins – Denatured proteins and metaproteins.

Secondary derived proteins are formed by progressive hydrolysis of proteins.

Proteoses, peptones and peptides.

16. WHAT IS ELECTROPHORESIS AND WHAT ARE THE IMPORTANT APPLICATIONS OF ELECTROPHORESIS?

Ans. Movement of charged particle in the electric field either towards cathode or anode when subjected to an electric current is called electrophoresis.

The following factors influence the movement of particles during the electrophoresis.

(*a*) Electric current.

(*b*) Net charge of the particle.

(*c*) Size and shape of the particle.

(*d*) Type of supporting media.

(*e*) Buffer solution.

Important Applications of Electrophoresis

The technique of electrophoresis is used to separate and identify the

(*i*) Serum proteins

(*ii*) Serum lipoproteins

(*iii*) Blood hemoglobins

17. WHAT ARE THE DIFFERENT TYPES OF ELECTROPHORESIS?

Ans. (*a*) *Moving boundary electrophoresis*: This technique was first introduced by TISELIUS in 1937

(*b*) *Zone electrophoresis*: In this type of electrophoresis different types of supporting media are used. These are;

(*a*) Paper electrophoresis

(*i*) Whatman filter paper

(*ii*) Cellulose acetate

(*b*) Gel electrophoresis

(*i*) Agarose.

(*ii*) Polyacrylamide gel (used for the separation of isoenzymes).

(*iii*) SDS–PAGE.

(*iv*) Iso–electric focussing (proteins seperated in a medium possessing a stable pH gradient).

(*v*) Immuno electrophoresis (for the separation of immunoglobulins).

18. WHAT ARE THE MAIN STEPS INVOLVED IN THE SEPARATION OF SERUM PROTEINS BY AGAROSE GEL ELECTROPHORESIS?

Ans.

(*a*) Preparation of barbitone buffer (vernal buffer (pH 8.6). At pH 8.6 all the serum proteins carry the negative charge and they migrate from cathode to anode.

(*b*) Preparation of 1% agarose solution.

(*c*) Preparation of agarose gel slide (layer the molten agarose on the slide.

(*d*) Application of the serum sample with the help of cover slip.

(*e*) Filling of electrophoretic tank with the barbitone buffer and slide to be kept on the tank.

(*f*) Power supply to be switched on and 7 mA current to be passed per each slide for about 30 minutes.

(*g*) Slide to be kept in fixative solution for 30 minutes and then in the dehydrating solution for 4 hours.

(*h*) Slide to be kept in the incubator overnight at 37° C.

(*i*) Slide is stained with amido black stain for 30 minutes and then is destained with 7% acetic acid and should be washed with water.

(*j*) Quantitation is done by densitometer.

The following fractions of serum proteins are separated depending upon the rate of migration in order of rate of migration.

(*i*) Albumin ⟶ Fastest moving fraction

(*ii*) α1 globulin ⟶ Next fast moving fraction

(*iii*) α2 globulin ⟶ Just ahead of β globulins

(*iv*) β globulin ⟶ Just ahead of γ globulins

(*v*) Υ globulin ⟶ Least mobile fraction and it is almost near the point of application

19. WHAT IS CHROMATOGRAPHY AND WHAT ARE THE IMPORTANT APPLICATIONS OF CHROMATOGRAPHY?

Ans. Chromatography is a special technique by means of which a group of similar substances are separated by a continuous redistribution between two phases.

(*i*) Stationary phase
(*ii*) Moving phase

Many variety of attractive forces act between the stationary phase and the substances to be separated resulting in the separation of molecules.

Important Applications of Chromatography

This special technique is used for the separation and identification of

(*i*) Sugars (for the diagnosis of inborn errors of carbohydrate metabolism).
(*ii*) Amino acids (for the diagnosis inborn errors of amino acid metabolism, example: phenylketonuria (PKU)).
(*iii*) Lipid fractions mainly for the separation of phospholipids to find out
Lecithin: Spingomyelin ratio in the Hyaline membrane disease (RDS).

20. WHAT ARE THE DIFFERENT TYPES OF CHROMATOGRAPHY?

Ans. There are various types of chromatography.

(*a*) Paper chromatography (stationary phase is whatman filter paper and mobile phase is a solvent system. Example : Butanol, acetic acid and water) used for the separation of amino acids.
(*b*) Thin layer chromatography (glass plates coated with silica gel acts as stationary phase and solvent system as mobile phase). TLC is used for the separation of phospholipids and amino acids.
(*c*) *Gas liquid chromatography* (*GLC*): A separation technique in which the mobile phase is a gas. GLC is used for the separation of volatile substances like fatty acids.
(*d*) *Ion exchange chromatography*: In this technique separation is based in differences in the ion exchange affinities of the sample components.
(*e*) *High performance liquid chromatography* (*HPLC*): A type of liquid chromatography that uses an efficient column containing small particles of stationary phase. HPLC is carried out under liquid pressure in the chromatographic matrix. This improves speed and resolution for the efficient separation of substances.

21. WHAT ARE THE MAIN STEPS OF PAPER CHROMATOGRAPHY IN THE SEPARATION OF AMINOACIDS?

Ans.

(*i*) The chromatographic chamber is saturated with solvent system (butanol, acetic acid and water). The solvent system is kept on the upper portion of the chamber in the glass trough (descending paper chromatography).
(*ii*) A drop of the sample containing unknown amino acids is applied about 5 cms from the end of whatman filter paper. Known standard amino acids are also applied on the line of point of application.
(*iii*) The filter paper slip is dipped in the solvent system and is hung above downwards.
(*iv*) As the solvent moves from above downwards by the capillary action the amino acids also move depending upon their R_f values.

(*v*) Once the solvent reaches the other end of the paper the paper is removed from the chamber and is dried and sprayed with ninhydrin and acetone and heated. Purple spots of amino acids will be developed.

(*vi*) Amino acids will be identified by calculating R_f values.

(*vii*) Amino acids with large nonpolar groups move with a fastest rate. Phenylalanine, isoleucine and leucine. The amino acids with polar groups are least mobile amino acids.

22. WHAT IS R_f VALUE? WHAT IS THE IMPORTANCE OF R_F VALUE?

Ans. R_f value is the relative fraction value. This is calculated by the formula.

$$R_f \text{ value} = \frac{\text{Distance travelled by the substances (A.A)}}{\text{Distance travelled by the solvent front}}$$

By R_f value the unknown amino acids present in a sample can be identified. For instance the R_f value of unknown spot is *x* and the R_f value of leucine is also same *x*. The unknown spot is identified as leucine because its R_f value corresponds to the R_f value of leucine standard.

Chapter–4

Enzymes

1. WHAT ARE THE FACTORS INFLUENCING ENZYME CATALYZED REACTION?

Ans. Enzyme concentration, substrate concentration, temperature and pH.

2. WHAT IS THE EFFECT OF ENZYME CONCENTRATION?

Ans. When the other factors are kept constant the increase in enzyme concentration increases velocity. It maintains a linear relationship obeying Ist order kinetics.

3. WHAT IS THE EFFECT OF SUBSTRATE CONCENTRATION?

Ans. At low concentration of the substrate, the increase in substrate, increases the velocity until the enzyme is said to be saturated with the substrate molecules (Ist order kinetics). Beyond this point on further increase of substrate there is no increase of velocity (zero order kinetics).

4. WHAT IS K_M VALUE AND WHAT IS ITS IMPORTANCE?

Ans. It is the substrate concentration at which it gives half the maximum velocity.
Low K_m indicates high affinity towards substrate.
High K_m indicates low affinity towards substrate.
Example : Hexokinase – Low K_m
Glucokinase – High K_m

5. WHAT IS THE OPTIMUM TEMPERATURE OF ENZYMES PRESENT IN HUMAN BODY?

Ans. 37 – 45°C.

6. GIVE EXAMPLES OF OPTIMUM pH OF SOME OF ENZYMES?

Ans. Pepsin 1.5 – 2.0 Trypsin 8.0 ALP 9.5 – 10
ACP 4.9 – 5.0 Amylase 6.8–6.9

7. WHAT IS COMPETITIVE INHIBITION AND GIVE EXAMPLES?

Ans. In competitive inhibition, the inhibitor closely resemble the structure of substrate. It combines with enzyme and forms E.I. complex thus preventing products formation.

Examples: Succinate dehydrogenase (SDH) inhibited by Malonate Xanthine oxidase (X.O.) inhibited by allopurinol Folate reductase inhibited by methotrexate.

8. WHAT IS ALLOSTERIC INHIBITION? GIVE EXAMPLE OF ALLOSTERIC INHIBITOR AND ALLOSTERIC ACTIVATOR?

Ans. The inhibitor binds with enzyme at allosteric site and prevents the binding of substrate at substrate binding site.

	Enzyme	**Inhibitor**
Example :	Aspartate transcarbamoylase	CTP
	HMG–CoA reductase	Cholesterol

Activator	**Enzyme**
1. N– Acetyl glutamate	Carbamoyl phosphate synthase
2. Acetyl CoA	Pyruvate carboxylase

9. NAME THE CARDIAC ENZYMES. HOW DO YOU INTERPRET THEM IN AMI?

Ans. LDH, C.K. AST

Ist C.K. is raised (MBCK clinches the diagnosis).

Enzyme	**Start–rising**	**Peak**	**Persists upto**
C.K.	4 hrs	24 hrs	48–72 hours
AST	12 hrs	24 hrs	4th day
LDH	24 hrs.	72 hrs	8–10 days

Flipped LDH (Ratio of LDH_1:LDH_2 >1) clinches the diagnosis of AMI.

10. NAME THE ENZYMES WHICH ARE RAISED IN HEPATOCELLULAR JAUNDICE.

Ans. ALT and AST.

11. NAME THE ENZYMES WHICH ARE RAISED IN OBSTRUCTIVE JAUNDICE.

Ans. ALP, 5-NT, GGT.

12. NAME THE ENZYME WHICH IS RAISED IN ACUTE PANCREATITIS?

Ans. Serum amylase.

13. WHAT ARE ISO-ENZYMES?

Ans. Iso-enzymes are physically distinct forms of same catalytic activity. They differ in physical characters (like electrophoretic mobility) and chemical composition and are obtained from different sources.

14. GIVE EXAMPLES OF ISO–ENZYMES?

Ans. LDH (1 to 5) CK (1 to 3)

$LDH_1 – H_4$ $LDH_2 – H_3M$ $LDH_3 – H_2M_2$

$LDH_4 – HM_3$ $LDH_5 – M_4$

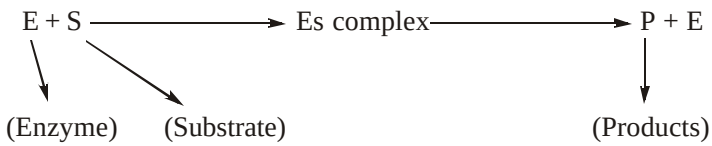
15. CLASSIFY THE ENZYMES ACCORDING TO I.U.B. SYSTEM OF CLASSIFICATION?

Ans. According to I.U.B. system enzymes are classified into 6 major classes. These are:

Sl.No.	*Major class*	*Action*	*Example*
1.	Oxido reductases	Catalyze the oxidation reduction reactions	Alcohol dehydrogenase
2.	Transferases	Transfer the groups from one substrate to other substrate	Aspartate amino transferase
3.	Hydrolases	Breaks the bonds by the introduction of water molecule	Amylase
4.	Lyases	Cleaves the substrate other than hydrolysis	Aldolase
5.	Isomerases	Interconverts various isomers.	Phosphotrio isomerase
6.	Ligases	Catalyse the synthetic reactions by break down of phosphate bonds	Glutamine synthetase

16. WHAT IS THE MECHANISM OF ACTION OF ENZYME?

Ans. According to MICHAELIS-MENTEN theory, enzyme combines with the substrate and forms enzyme – substrate complex, which immediately breaks down into products and liberates the enzyme.



17. WHAT IS CO-ENZYME? GIVE EXAMPLES OF CO-ENZYMES?

Ans. The enzymes become active only when they combine with the prosthetic groups. The prosthetic group is called co-enzyme

Apo enzyme + Co-enzyme – Holo enzyme

(Inactive) (Prosthetic group) – (Active)

The co-enzymes are non-protein moieties, dialisable, heat stable and low molecular weight substances. Most of the co-enzymes consist of one of the B-group vitamins.

Co-enzyme		B-group vitamins
TDP	–	B_1
FMN, FAD	–	B_2
NAD, NADP	–	Niacin

18. WHAT ARE THE METHODS OF REGULATION OF ENZYME ACTIVITY?

Ans.

(*a*) Allosteric regulation
- Activation
- Inhibition

(*b*) Covalent modification
- Phosphorylation
- Dephosphorylation

(*c*) Induction and repression.

Chapter–5

Vitamins

SOURCES OF VITAMINS

1. WHAT ARE THE FAT SOLUBLE VITAMINS?

Ans. A, D, E, K.

2. WHAT ARE THE WATER SOLUBLE VITAMINS?

Ans. B group vitamins (B_1, B_2, niacin, B_6, pantothenic acid, Biotin, lipoic acid, folic acid, B_{12}) and Vitamin C.

3. WHAT ARE THE SOURCES OF THE FOLLOWING VITAMINS?

Ans.

(*i*) Vit "A".
- (*a*) *Retinol*: Liver, egg yolk, whole milk, butter, chicken meat, fish (concentrates in fish liver oils).
- (*b*) *Carotenoids*: Dark green leafy vegetables like spinach etc., deep yellow vegetables like carrot, yellow fruits like mango and papaya.

(*ii*) Vit "D" fatty fish, fish liver oils, eggs, butter, liver, fortified milk with vit. D

(*iii*) Vit "E" – Sun flower oil, corn oil, cotton seed oil, wheat germ oil, grains, liver and nuts.

(*iv*) Vit "K".
K1 – Green leafy vegetables, cabbage and cauliflower etc.
K2 – Synthesised by intestinal micro organisms.

(*v*) B1 – Whole grains, dried legumes, liver and yeast.
B2 – Milk and milk products, liver, eggs, whole grains, dark green vegetables, poultry, etc.

(*vi*) Niacin – Meat, poultry, fish, whole grains, legumes and nuts.

(*vii*) B6 – Meat, poultry fish, nuts and bananas.

(*viii*) B12 – Liver muscle, meat, fish, eggs, milk and milk products.

(*ix*) Pantothenic acid – Liver, yeast, egg yolk and vegetables.

(*x*) Folic acid – Liver, dark green leafy vegetables like spinach, lettuce, dried beans, whole grains.

(*xi*) Vit. "C" – Citrus fruits like lemon and oranges, guava, gooseberry and fresh dark green leafy vegetables.

(*xii*) Biotin :

(*a*) Present in number of different foods.

(*b*) It can be synthesized by colonic bacteria.

RECOMMENDED DAILY ALLOWANCES (RDA)

4. WHAT ARE THE RDA OF THE FOLLOWING VITAMINS FOR HEALTHY ADULTS?

Ans.

Vitamin	*Male*	*Female*
Vit. A	1000 uG	800 uG
Vit. D	5–10 uG	5–10 uG
Vit. E	10 mg	8 mg
Vit. K	45–80 uG	45–65 uG
Vit. C	50–60 mg	50–60 mg
Vit. B1	1.2 – 1.5 mg	1–1.1 mg
Vit. B2	1.4 – 1.8 mg	1.2 – 1.3 mg
Niacin	15–20 mg	13–15 mg
Vit. B6	1.4 – 2 mg	1.4 – 1.6 mg
Pantothenic acid	5.0 mg	5.0 mg
Folic acid	150 – 200 uG	150–180 uG
Vit. B12	2.0 uG	2.0 uG
Biotin	30–100 uG/D	30–100 uG/D

DEFICIENCY MANIFESTATIONS OF VITAMINS

5. WHAT ARE THE DEFICIENCY MANIFESTATIONS OF THE FOLLOWING VITAMINS?

(*i*) Vitamin “A”.

Ans. Nyctalopia (night blindness), xerophthalmia, keratomalacia. Hyperkeratosis of the skin.

(*ii*) Vitamin “D”.

Ans. Rickets in children and osteomalacia in adults.

(*iii*) Vitamin "E".

Ans. Vitamin – E deficiency is rare, however, seen in severe and prolonged malabsorptive diseases. Anaemia in lactating women and new born infants, disorder of nervous system such as peripheral neuropathy, gait disturbance and ↓↓ vibration sensation and degeneration of posterior column of spinal.

(*iv*) Vitamin 'K'.

Ans. Haemorrhagic disease of new born. Deficiency can also occur when the gut is made sterile in preparative to gastro intestinal surgery by giving antibiotics.

(*v*) B_1.

Ans. (*a*) Wet beriberi (Heart failure).

(*b*) Dry beriberi (Peripheral neuropathy).

(*c*) Wernicke's encephalopathy (Ophthalmoplegia due to weakness of extraocular muscles, horizontal nystagmus, and ataxia etc.).

(*d*) Korsakoff syndrome (retrograde-amnesia and confabulation).

(*vi*) B_2.

Ans. Cheilosis, angular stomatitis, glossitis, seborrhoeic dermatitis, sore throat and odema of oral mucus membranes.

(*vii*) Niacin.

Ans. Pellagra (dermatitis, dementia and diarrhoea).

(*viii*) B_6.

Ans. Convulsions in infants and children due to ↓↓ GABA formation.

Adults

Peripheral neuritis in the treatment of TB with isoniazid and in alcoholics.

(*ix*) Pantothenic Acid.

Ans. Muscle cramps, paresthesia, ataxia, burning feet syndrome and G.I.T. disturbances.

(*x*) Folic Acid.

Ans. Macrocytic anemia (macro ovalocyte and hypersegmented neutrophils in the PBS examination

(*xi*) B_{12}

Ans. (*i*) Macrocytic anaemia (macro ovalocytes and hypersegmented neutrophils on PBS).

(*ii*) Sub–acute combined degeneration of spinal cord.

(*a*) Distal sensory neuropathy with glove and stocking sensory loss and paresthesia and areflexia.

(*b*) Difficulty in balance and walking.

(*xii*) Biotin : Human deficiency is rare.

Ans. (*a*) It has occurred in adults who have taken for long periods large amounts of raw egg-white which contains biotin antagonist avidin.

(*b*) The deficiency also occurs in persons taking poor diet or on patients on long term parenteral nutrition with biotin omitted from the fluids.

Deficiency manifestations :

(*a*) Scaly dermatitis.

(*b*) Alopecia.

(*c*) Paresthesia.
(*d*) Seborrhoeic dermatitis of infants.

(*xiii*) Vitamin 'C'.

Ans. Scurvy in infants and middle and old aged persons who live alone. Manifested by petechial haemorrhages, echymosis, swelling and bleeding of gums, loosening of teeth and delayed wound healing.

BIOCHEMICAL FUNCTIONS OF VITAMINS

6. WHAT ARE THE CO-ENZYMES CONTAINING 'B' GROUP VITAMINS?

Ans.

Co-enzyme	B group Vitamins
(*a*) TDP	B_1
(*b*) FMN and FAD	B_2
(*c*) NAD and NADP	Niacin
(*d*) Pyridoxal phosphate	B_6
(*e*) Co-enzyme – 'A'	Pantothenic acid
(*f*) Tetrahydro folic acid (FH_4)	Folic acid
(*g*) Methyl cobamide co-enzyme and 5 deoxyadenosyl cobamide coenzyme	B_{12}
(*h*) Biotin	Biotin
(*i*) Lipoic acid	Lipoic acid

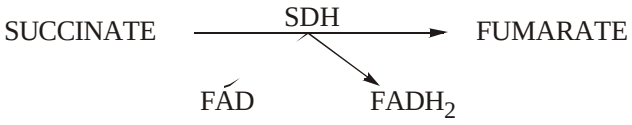
7. WHAT ARE THE FUNCTIONS OF CO–ENZYME CONTAINING 'B' GROUP VITAMINS?

Ans.

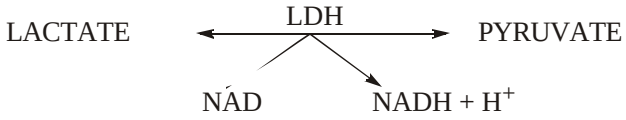
(*a*) TDP ⟶ TDP is one of the 5 co-enzymes required for PDH action.
(1) TDP is involved in the oxidative decarboxylation of α-keto acids.
Example: Pyruvate ⟶ CO_2 + Acetyl CoA.
(2) TDP is involved in the decarboxylation of pyruvate and forms active acetaldehyde which transfers acetyl group to LIPOIC acid.
(3) TDP is involved in the transketolation reaction. It is a co-enzyme for transketolase.

(*b*) FMN ⟶ B_2 containing co-enzyme. It is the second component of ETC. It accepts reducing equivalents from NADH and transfers to Co-Q in ETC.

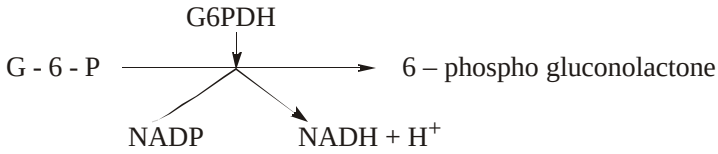
(*c*) FAD ⟶ B_2 containing co-enzyme.



(*d*) NAD ⟶ Niacin containing co–enzyme



(*e*) NADP ⟶ Niacin containing

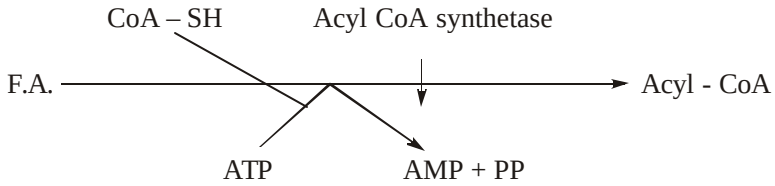


(*f*) Pyridoxal Phosphate: B_6 containing.

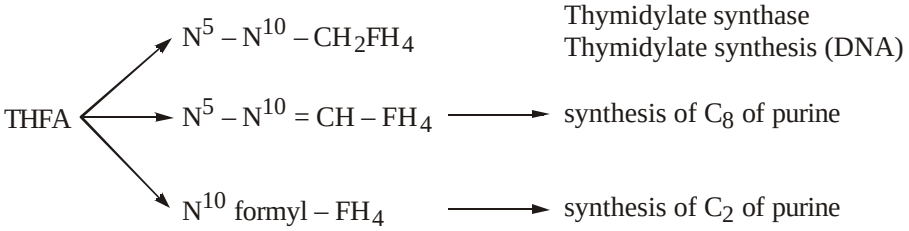
$$\text{Glutamate} \xrightarrow[\text{PLP } (B_6)]{\text{Glutamate – DC (decarboxylase)}} CO_2 + \text{GABA}$$

PLP is also required for transamination (TA) reaction and non-oxidative deamination reaction.

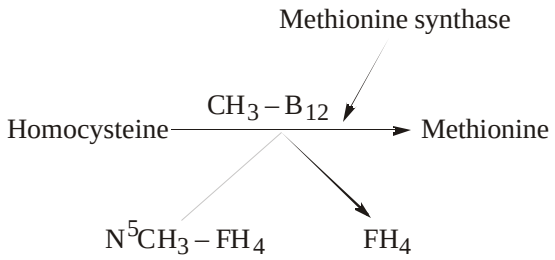
(*g*) CoA ⟶ Pantothenic acid containing



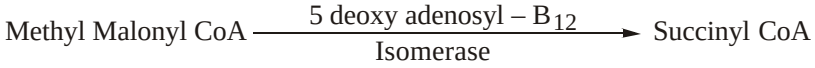
(*h*) Tetrahydrofolic acid ⟶ Folic acid.
One 'C' metabolism



(*i*) CH_3–B_{12} (methyl B_{12}) ⟶ B_{12} containing

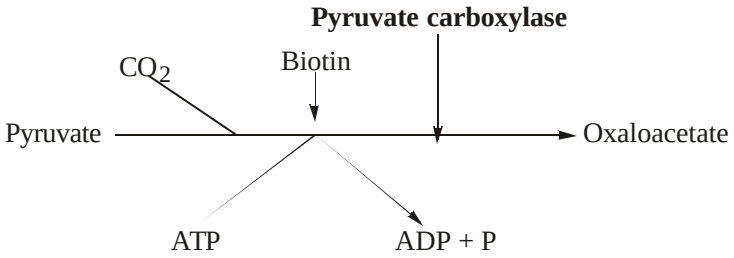


(*j*) 5 deoxy adenosyl – B_{12} ⟶ B_{12} containing

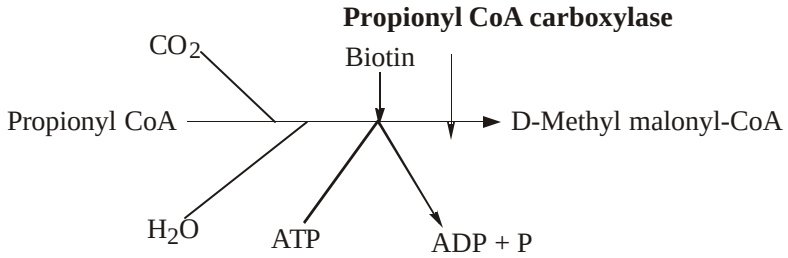


BIOTIN

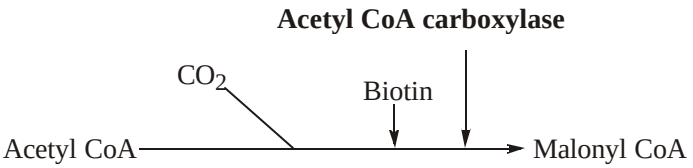
(*i*)



(*ii*)

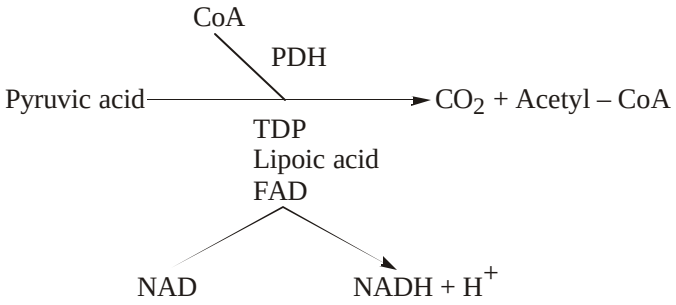


(*iii*)



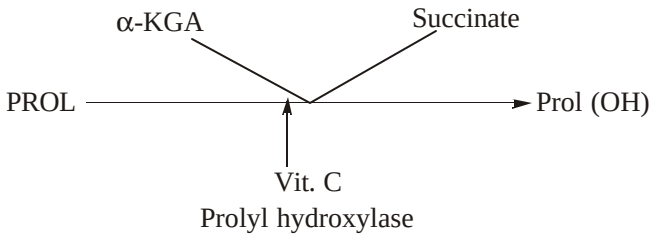
LIPOIC ACID

Lipoic acid is one of the co-enzymes required for the conversion of pyruvic acid to acetyl CoA. Its role is that it carries the acyl radical in the form of S–acetyl lipoate.



8. WHAT ARE THE BIOCHEMICAL FUNCTIONS OF VITAMIN C?

Ans. Vitamin C is required for the hydroxylation of proline to hydroxy proline and lysine to hydroxy lysine.

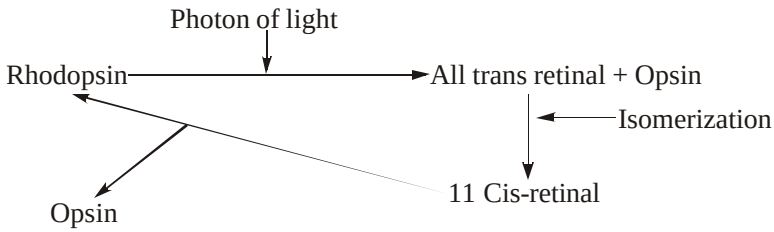


By this reaction pro-collagen is converted to collagen, which is required for the formation of intercellular cement substance of capillaries and other tissues.

Vitamin C acts as antioxidant in reducing oxidised Vitamin E and thus prevents free radical formation.

9. WHAT ARE THE FUNCTIONS OF VITAMIN A?

Ans. Vitamin A in the form of retinol is a component of the visual pigment (Rhodopsin) in the red cells, which is responsible for the vision in dim light.



(*a*) Retinol is required for the normal reproduction.
(*b*) Retinal is required for growth and differentiation.
(*c*) Generally Vitamin A is required for the maintenance of integrity of epithelium.
(*d*) Beta-carotene is an antioxidant and prevents the free radical formation.

10. WHAT IS THE ACTIVE METABOLITE OF VITAMIN D-3 ? HOW IS IT FORMED?

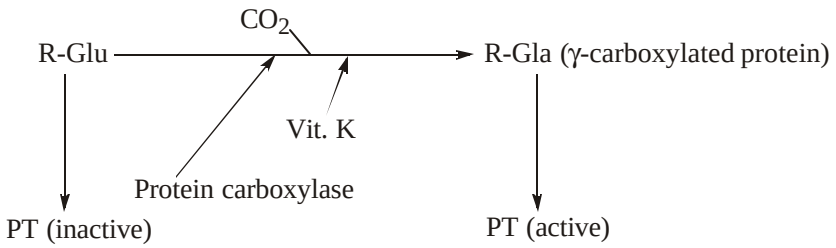
Ans. Calcitriol is the active metabolite. It is formed in two stages:
(*i*) Vitamin D_3 is taken up by the liver where it is hydroxylated on the 25th position by 25 hydoxylase (ER) to form 25 (OH) CC.
(*ii*) 25 (OH) CC is taken up by the kidney where it is further hydoxylated in position I by D_3-I α-hydroxylase (Mito) to form Calcitriol (CT).

11. WHAT ARE THE FUNCTIONS OF CALCITRIOL?

Ans. Calcitriol causes the formation of CBP by stimulating the transcription and translation processes in the intestinal cells. CBP in turn causes absorption of Ca^{++} and phosphate at brush border of intestine. The translocation of calcium takes place by CT against the concentration gradient.

12. WHAT ARE THE BIOCHEMICAL FUNCTIONS OF VITAMIN K?

Ans. Vitamin K acts a cofactor of the carboxylase that forms γ-carboxy glutamate in precursor proteins i.e., it helps in the formation of active prothrombin (PT) (Factor-II).



13. WHAT IS THE ACTION OF DICOUMAROL?

Ans. It is an anticoagulant. It is antagonist to Vitamin K action.

14. WHAT ARE THE FUNCTIONS OF VITAMIN E?

Ans. It is an antioxidant and prevents the free radical formation. Vitamin E and selenium act synergistically against lipid peroxides. Selenium is a cofactor required for GSH peroxidase.

Chapter–6

Bioenergetics and Biological Oxidation

1. WHAT ARE EXERGONIC AND ENDERGONIC REACTIONS? GIVE EXAMPLES?

Ans. When the reaction proceeds spontaneously accompanied by loss of free energy, it is called exergonic reaction. When the reaction proceeds accompanied by gain in free energy, it is called endergonic reaction.

2. GIVE EXAMPLES OF ENDERGONIC REACTION AND EXERGONIC REACTION?

Ans. Endergonic reaction :

(*i*) Muscular contraction.

(*ii*) Nervous excitation (transmission of nerve impulses).

(*iii*) Active transport.

(*iv*) Synthesis of substances.

In Endergonic the reaction proceeds only by the gain in free energy.

Exergonic reaction : $ATP \longrightarrow ADP + P$ (loss of free energy)

3. WHAT IS THE ΔG VALUE OF BREAK DOWN OF TERMINAL PHOSPHATE OF ATP?

Ans. – 7.3 Kcal /mol.

4. WHAT ARE THE HIGH ENERGY PHOSPHATE COMPOUNDS WHERE ΔG VALUE IS ABOVE – 7.3 K. CAL/MOL?

Ans. PEP, Carbamoyl-P, 1,3, Bisphosphoglycerate, Creatine-P.

5. WHAT ARE THE METHODS OF ATP FORMATION?

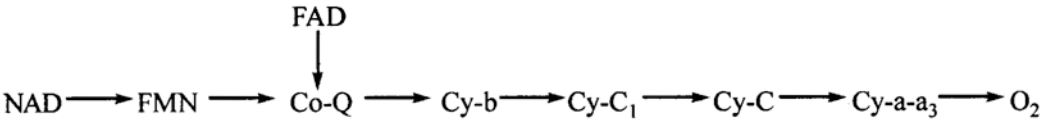
Ans. A. Substrate level phosphorylation.

B. Oxidative phosphorylation.

C. Break down of creatine-P coupled with ADP (Lohmann reaction).

6. WHAT ARE THE COMPONENTS OF ETC IN ORDER OF INCREASE IN REDOX POTENTIAL?

Ans.



7. WHAT IS OXIDATIVE PHOSPHORYLATION?

Ans. During the transport of reducing equivalents or electrons from one component to other components of ETC, the liberated free energy is trapped by phosphorylation to form ATP is called oxidative phosphorylation.

8. WHAT ARE THE SITES OF OXIDATIVE PHOSPHORYLATION IN ETC AND WHAT ARE THE INHIBITORS OF THESE SITES?

Ans. Site I ⟶ Complex I (NADH ⟶ Co–Q)
Site II ⟶ Complex III (Cy–b ⟶ C1)
Site III ⟶ Complex IV (Cy–a–a3)

Inhibitors of OP

Site I ⟶ Piericidin – A, Amobarbitol, Rotenone
Site II ⟶ BAL, Antimycin –A
Site III ⟶ H_2S, CO, CN

9. WHAT ARE UNCOUPLERS. GIVE EXAMPLES?

Ans. Uncouplers are the substances which dissociate oxidation in ETC from phosphorylation, so that oxidation proceeds without phosphorylation resulting in the dissipation of free energy in the form of heat.

Example : 2,4 Dinitrophenol.

10. WHAT IS THE CHEMIOSMOTIC THEORY OF OXIDATIVE PHOSPHORYLATION?

Ans. Mitchell's chemiosmotic theory postulates that the energy from oxidation of components of ETC is coupled to the translocation of hydrogen ions (Protons) from the inside to the outside of inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the H^+ ions is used to drive the mechanism responsible for the formation of ATP (membrane located ATP synthase).

11. WHAT IS CYTOCHROME OXIDASE AND WHAT IS ITS ACTION?

Ans. Cytochrome oxidase is a final component of ETC. It is a hemo protein consisting of two (2) heme groups. Each heme group has one Fe and Cu atoms. It is a complex of Cy–a–a3 and it accepts electrons from Cy–c and transfers to O_2 to form H_2O.

Inhibitors $\longrightarrow$ H_2S, CO, CN.

12. WHAT IS NADH – DEHYDROGENASE?

Ans. It is a Flavo protein consisting of FMN and FeS. It is the 2nd component of ETC. It accepts reducing equivalents from NADH and transfers to Co-Q.

13. NAME THE SUBSTRATES WHICH ARE OXIDIZED THROUGH NAD LINKED DH FOR ONWARD TRANSMISSION OF REDUCING EQUIVALENTS TO ETC.

Ans. TCA $\longrightarrow$ Isocitrate, α KGA, Malate.

Link reaction (PDH) $\longrightarrow$ Pyruvate

Lipid metabolism $\longrightarrow$ 3 Keto acyl-Co-A, 3-hydroxy butyrate

A.A. metabolism $\longrightarrow$ Glutamate, Proline.

14. NAME THE SUBSTRATES WHICH ARE OXIDIZED THROUGH FAD LINKED DH FOR ONWARD TRANSMISSION OF REDUCING EQUIVALENTS TO ETC.

Ans. Succinate, Acyl Co-A, Choline, Glycerol-3P.

15. WHAT IS CYTOCHROME P-450? WHERE IT IS UTILIZED?

Ans. Cytochrome P-450 is iron containing hemo protein. Microsomal Cy-p-450 is involved in the detoxification of drugs in the liver. Mitochondrial Cy-p-450 is involved in the hydroxylation reaction in the steroidogenic tissues and calcitriol formation in the kidney.

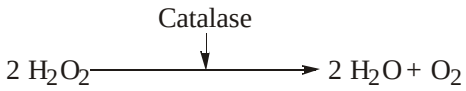
16. WHAT IS THE ACTION OF GSH – PEROXIDASE?

Ans. GSH – Peroxidase has selenium as a co-factor. It is involved in the detoxification of H_2O_2 and maintains the integrity of RBC membrane

$$2GSH + H_2O_2 \xrightarrow[\uparrow Se]{GSH - peroxidase} G - S - S - G + 2H_2O$$

17. WHAT IS THE ACTION OF CATALASE?

Ans. Catalase is involved in the detoxification of H_2O_2 formed by the action of A.A. oxidase.



18. WHAT ARE DIOXYGENASES? GIVE EXAMPLES?

Ans. Dioxygenases catalyse the reaction of incorporation of two (2) atoms of oxygen to the substrate.

Example: Tryptophan dioxygenase, Homogentisate dioxygenase, 3 hydroxy anthranilate dioxygenase.

19. WHAT ARE MONOOXYGENASES? GIVE EXAMPLES?

Ans. Monooxygenases catalyse the reaction of incorporation of one atom of oxygen to the substrate.

Example: Phenylalanine hydroxylase.

20. WHAT IS SUPEROXIDE DISMUTASE? WHAT IS ITS ACTION?

Ans. Superoxide dismutase is involved in the detoxification of superoxide anion ($O_2^{\cdot -}$). It is formed by the reoxidation of reduced flavins univalently by molecular oxygen.

Action of superoxide dismutase

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

Cytosolic enzyme has copper and zinc metals for its activity. Mitochondrial enzyme contains Mn^{2+}.

Chapter–7

Metabolism of Carbohydrates

DIGESTION AND ABSORPTION OF CARBOHYDRATES

1. WHAT IS THE ACTION OF THE FOLLOWING ENZYMES?

(*i*) Amylase

Ans. It splits α 1$\longrightarrow$4 glycosidic linkages (GL) of starch and glycogen and liberates the products of maltose, malto–triose and α 1$\longrightarrow$6 glucoside (α limit dextrin).

(*ii*) Maltase

Ans. It splits α 1 $\longrightarrow$4 glycosidic linkage of maltose, malto-triose and removes single glucose residues from oligosaccharides from non-reducing end.

(*iii*) Isomaltase

Ans. It splits 1$\longrightarrow$6 glycosidic linkage and removes the branches by liberating glucose.

(*iv*) Sucrase

Ans. It hydrolyses sucrose and liberates one molecule of glucose and one molecule of fructose.

(*v*) Lactase (β-galactosidase)

Ans. It hydrolyses lactose and liberates one molecule of glucose and one molecule of galactose.

2. WHAT IS LACTOSE INTOLERANCE?

Ans. The deficiency of enzyme lactase produces the disorder lactose intolerance. In this condition, lactose is not hydrolysed and is acted upon by the gut bacteria to produce organic acids which in turn give rise to diarrhoea. Benedicts Test is positive in stool samples.

There are two types of lactose intolerance :

(*i*) Congenital

(*ii*) Acquired

Acquired is due to sudden change in milk based diet, as lactase is an inducible enzyme.

3. WHAT ARE THE DIFFERENT TRANSPORTER SYSTEMS INVOLVED IN THE ABSORPTION OF GLUCOSE AND HOW THEY ACT?

Ans. There are two types of transporter systems available for the absorption of glucose.

(*i*) Sodium-dependent glucose transporter (SLGT-1) which binds both glucose and sodium at separate sides and transport them through the plasma membrane of the intestinal cell. Glucose

and sodium are released into cytosol. In this mechanism, sodium is transported down its concentration which drives the transport of glucose against the concentration gradient. The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a sodium pump that expels sodium from the cell in exchange for potassium.

(*ii*) The second transporter system is called sodium independent transporter GLUT 2, that facilitates transport of glucose out of the cell. This mechanism is called as facilitated transport.

4. NAME THE SUBSTANCE WHICH INHIBITS ACTIVE TRANSPORT OF GLUCOSE.

Ans. Ouabain (cardiac glycoside) which inhibits the sodium pump.

GLYCOLYSIS

5. DEFINE SUBSTRATE LEVEL PHOSPHORYLATION (SLP).

Ans. Formation of ATP at substrate level in glycolysis and TCA cycle called substrate level phosphorylation.

6. GIVE EXAMPLES OF SLP IN GLYCOLYSIS.

Ans. Conversion of :

(*a*) 1,3 bisphospho glycerate (BPG) to 3 phosphoglycerate.

(*b*) Phosphoenol pyruvate to pyruvate.

7. GIVE EXAMPLES OF SUBSTRATE LEVEL PHOSPHORYLATION (SLP) IN TCA CYCLE.

Ans. Conversion of succinyl Co-A to succinate.

8. WHAT ARE THE IRREVERSIBLE STEPS OF GLYCOLYSIS?

Ans. Conversion of :

(*a*) Glucose to glucose 6–P.

(*b*) Fructose 6–P to fructose 1, 6 BP.

(*c*) Phosphoenol pyruvate (PEP) to pyruvate.

9. WHAT ARE THE REACTIONS OF GLYCOLYSIS WHERE ATP IS CONSUMED?

Ans. Conversion of

(*a*) Glucose to glucose 6-P.

(*b*) Fructose 6-P to fructose 1, 6-BP.

10. WHAT ARE THE REACTIONS OF GLYCOLYSIS WHERE ATP IS FORMED?

Ans. Conversion of
(*a*) 1,3-bisphospho glycerate (BPG) to 3-phosphoglycerate.
(*b*) Phosphoenolpyruvate (PEP) to pyruvate.

11. NAME THE ENZYMES WHICH CATALYSE THE IRREVERSIBLE STEPS OF GLYCOLYSIS?

Ans. (*a*) Hexokinase/glucokinase.
(*b*) Phosphofructo kinase.
(*c*) Pyruvate kinase.

12. NAME THE ENZYMES WHICH CATALYSE THE REACTIONS OF SLP IN GLYCOLYSIS?

Ans. (*a*) Phosphoglycerate kinase.
(*b*) Pyruvate kinase.

13. WHAT ARE THE FATES OF PYRUVATE?

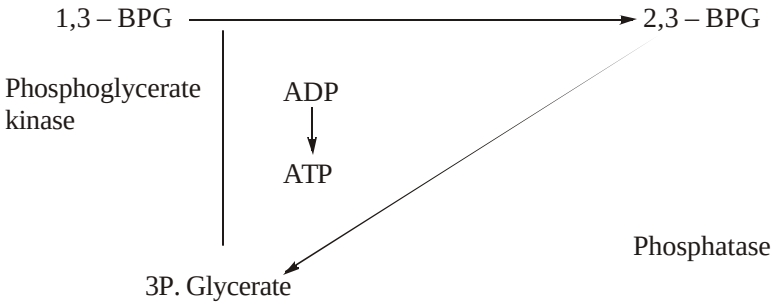
Ans. Conversion into:
(*a*) Lactate by LDH in cytosol.
(*b*) Acetyl Co-A by PDH in mitochondrion.
(*c*) Oxalo acetate by pyruvate carboxylase in mitochondrion.

14. NAME THE INHIBITORS OF GLYCOLYSIS AND AT WHAT LEVEL THEY ACT?

Ans. (1) Iodoacetate inhibits the enzyme Glyceraldehyde 3–P-DH.
(2) Fluoride inhibits the enzyme Enolase.

15. WHAT IS RAPAPORT – LEUBERING CYCLE? WHAT IS ITS SIGNIFICANCE?

Ans. It is by passed pathway of 1, 3, bisphospho glycerate. In this pathway 1, 3, bisphosphoglycerate (BPG) is converted to 2, 3 bisphosphoglycerate (BPG) by mutase and 2, 3, BPG is converted to 3 phosphoglycerate by phosphatase.



SIGNIFICANCE. In this pathway 2, 3, bisphosphoglycerate (BPG) is formed which is required for more delivery of oxygen during hypoxia as 2, 3, bisphosphoglycerate (BPG) decreases the affinity of oxygen to hemoglobin.

16. WHAT IS NET GAIN OF ATP IN ANAEROBIC GLYCOLYSIS?

Ans. No. of ATP produced = 4
No. of ATP utilized = –2
Net gain of ATP = 2

17. WHAT ARE THE KEY ENZYMES OF GLYCOLYSIS?

Ans. 1. Hexokinase/Glucokinase.
2. Phosphofructokinase.
3. Pyruvate kinase.

18. NAME THE MAIN ENZYME WHICH CONTROLS GLYCOLYSIS AND HOW IS GLYCOLYSIS REGULATED?

Ans. Phosphofructokinase. The activity of this enzyme controls the glycolysis. PFK –1 is inhibited by ATP and activated by AMP, **fructose 2,6 bisphosphate** and well fed state. Reverse is true in the opposite state.

19. WHAT IS THE OXIDATIVE REACTION IN THE GLYCOLYSIS?

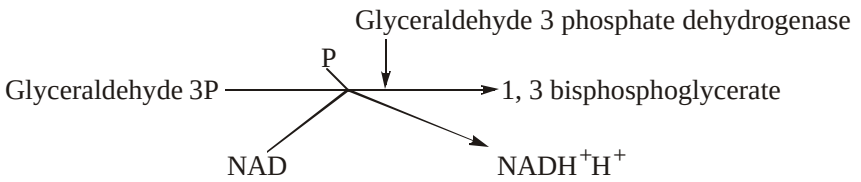
Ans. Conversion of 'glyceraldehyde 3P to 1, 3 bisphosphoglycerate (BPG) catalyzed by glyceraldehyde 3PDH and NAD acts as a co-enzyme and (P) is added.

20. WHAT IS THE ACTION OF

(*a*) Aldolase

Ans. It splits fructose 1, 6 bisphosphate to glyceraldehyde 3P+Dihydroxy acetone –P.

(*b*) Glyceraldehyde 3 phosphate dehydrogenase



(c) LDH

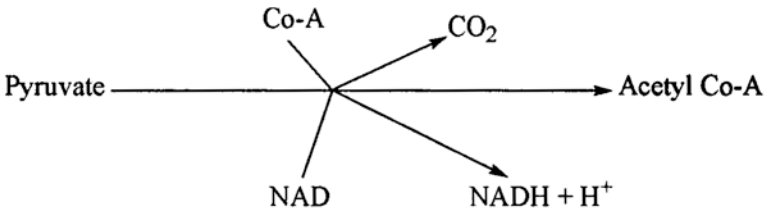
It interconverts Pyruvate $\xleftrightarrow{\text{LDH}}$ Lactate. NADH formed in earlier step of oxidation is utilised in this reaction.

21. WHAT IS THE LINK REACTION WHICH CONNECTS GLYCOLYSIS TO TCA CYCLE?

Ans. Conversion of Pyruvate to Acetyl Co-A catalysed by PDH. PDH is multienzyme complex composed of 3 enzymes and 5 co-enzymes.

3 enzymes	**5 co-enzymes**
(*i*) PDH	(*i*) TDP
(*ii*) Dihydrolipoyl transacetylase	(*ii*) Lipoic acid
(*iii*) Dihydrolipoyl –DH	(*iii*) Co-A
	(*iv*) FAD
	(*v*) NAD

Co-A and NAD directly participate in the reaction whereas other co-enzymes act as catalysts and carry molecules.



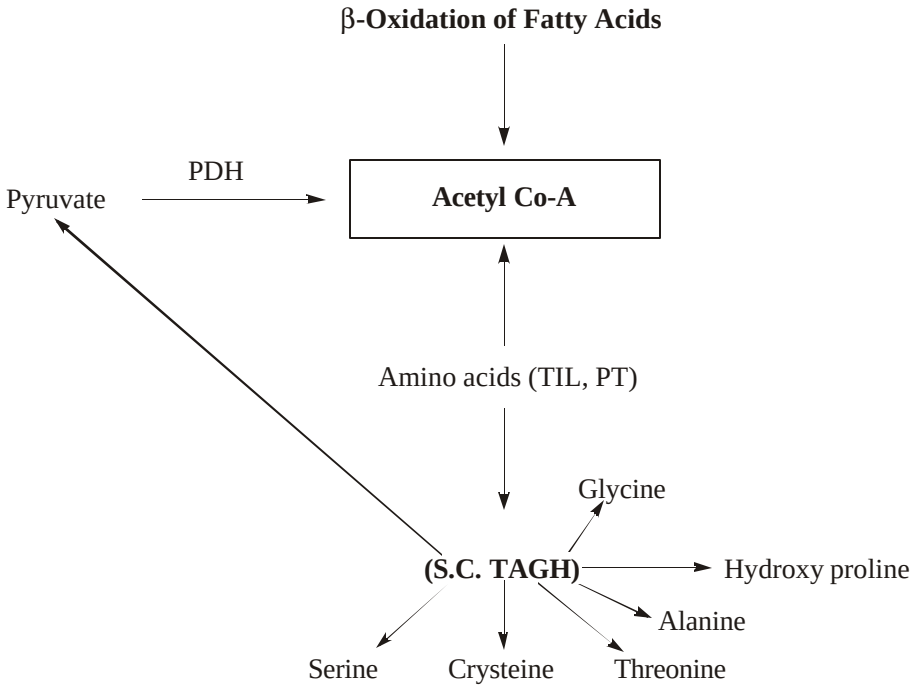
TCA CYCLE

22. WHAT ARE THE VARIOUS ROUTES OF ACETYL Co-A FORMATION?

Ans. (*i*)Conversion of pyruvate to Acetyl Co-A catalyzed by PDH.

(*ii*) End product of β–Oxidation of fatty acids.

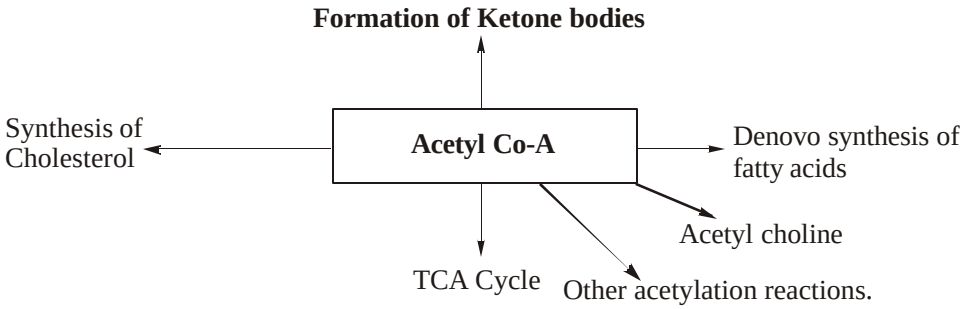
(*iii*) By catabolism of amino acids like leucine, isoleucine, phenylalanine, tyrosine, tryptophan, threonine and (TIL–PT) the amino acids which are converted to Pyruvate (S.C. TAGH).



23. WHAT ARE THE VARIOUS FATES OF ACETYL Co-A?

Ans. (*i*) Oxidation in TCA Cycle.
(*ii*) Denovo synthesis of fatty acids (Cytosol).
(*iii*) Synthesis of cholesterol.
(*iv*) Formation of ketone bodies.
(*v*) Formation of acetyl choline.
(*vi*) Other acetylation reactions.

FATES OF ACETYL-CoA



24. NAME THE INHIBITORS OF TCA CYCLE AND AT WHAT LEVEL THEY ACT?

Ans. (*i*) Fluoroacetate inhibits the enzyme aconitase, which converts citrate to Cis-aconitate.
(*ii*) Aresenate inhibits ∝ KGA–DH which converts ∝ KGA to succinyl Co-A.
(*iii*) Malonate inhibits SDH which converts succinate to furmarate.

25. WHAT ARE THE NAD DEPENDENT STEPS IN TCA CYCLE?

Ans. (*i*) Isocitrate–DH, which converts Isocitrate to oxalosuccinate.
(*ii*) ∝ KGA–DH, which converts ∝ KGA to succinyl Co-A.
(*iii*) Malate–DH which converts Malate to oxaloacetate.

26. WHAT IS THE FAD DEPENDENT STEP IN TCA CYCLE?

Ans. Succinate–DH which converts succinate to fumarate.

27. WHAT ARE THE IRREVERSIBLE STEPS OF TCA CYCLE AND NAME THE ENZYMES WHICH CATALYZE THESE STEPS?

Ans. (*i*) Condensation of OAA+ Acetyl Co-A to Citrate, catalyzed by citrate synthase.
(*ii*) Conversion of ∝ KGA to succinyl Co-A, catalyzed by ∝ KGA–DH.

28. WHAT IS THE NET GAIN OF ATP IN TCA CYCLE?

Ans. 12

Enzyme	*Co-enzyme*	*ATP generation*
1. Isocitrate DH	NADH	3 by (O.P.)
2. ∝ KGA DH	NADH	3 by (O.P.)
3. Succinate thiokinase		by SLP
4. SDH	$FADH_2$	2 by (O.P.)
5. MDH	NADH	3 by (O.P.)
	TOTAL	12 ATP

O.P. ⟶ Oxidative phosphorylation
SLP ⟶ Substrate level phosphorylation.

29. WHAT IS THE SIGNIFICANCE OF TCA CYCLE?

Ans. (*i*) It is the final common pathway for the oxidation of Carbohydrates, Fats and Amino Acids.
(*ii*) TCA plays as an amphibolic role.

(*a*) By oxidation it generates 12 ATP molecules.
(*b*) Intermediates of TCA cycle are involved in the synthetic reactions like :
(1) Gluconeogenesis (∝ KGA to OAA)
(2) Transamination (∝ KGA ⟶ glutamic acid)
OAA ⟶ Aspartic acid)
(3) Heme synthesis (succinyl Co-A)

30. HOW THE AMINO ACIDS ENTER INTO TCA CYCLE?

Ans. (*i*) SC TAGH ⟶ Pyruvate ⟶ Acetyl Co–A
(*ii*) PAGH ⟶ Glutamate ⟶ ∝ KGA
(*iii*) PT ⟶ Fumarate
(*iv*) VIM ⟶ Succinyl Co-A

P ⟶ Proline
A ⟶ Arginine
G ⟶ Glutamine
H ⟶ Histidine
V ⟶ Valine
I ⟶ Isoleucine
M ⟶ Methionine

31. NAME THE VITAMIN CONTAINING CO-ENZYMES TAKING PART IN TCA CYCLE & AT WHAT LEVEL THEY ACT?

Ans.
(*i*) B_1 containing co-enzyme TDP. It is the one of the co-enzymes required for the conversion of ∝ KGA to succinyl Co-A.
(*ii*) B_2 containing co-enzyme FAD. It takes part in the conversion of Succinate to Fumarate and ∝ KGA to succinyl Co-A.
(*iii*) Niacin containing co-enzyme NAD. It takes part in the conversion of
(*a*) Isocitrate to oxalosuccinate.
(*b*) ∝ KGA to succinyl Co-A.
(*c*) Malate to oxaloacetate.

GLUCONEOGENESIS

32. DEFINE GLUCONEOGENESIS?

Ans. Formation of glucose from non-carbohydrate sources like lactate, glycerol and glycogenic amino acids is called as gluconeogenesis.

33. TRACE THE MAIN PATHWAY OF GLUCONEOGENESIS FROM LACTATE TO GLUCOSE?

Ans.

Step 1 (Cytosol) Lactate to pyruvate by LDH.

Step 2 Pyruvate enters into mitochondrion and in the mitochondrion it is converted to oxalocetate by pyruvate carboxylase and biotin. ATP is broken to ADP + P.

Step 3 Oxaloacetate is converted to malate by malate dehydrogenase (MDH).

Step 4 Malate crosses the mitochondrion and enters the cytosol. It is converted back to oxaloacetate by MDH.

Step 5 Oxaloacetate is converted to PEP by PEP–CK and GTP is broken to GDP and CO_2 is liberated.

Step 6 PEP is converted to fructose 1, 6 bisphosphate by the reversal of glycolysis.

Step 7 Fructose 1, 6 bisphosphate is converted to fructose 6 P by fructose 1,6 bisphosphatase.

Step 8 Fructose 6 P is converted to glucose 6P by phosphohexose isomerase.

Step 9 Glucose 6 P is converted to glucose by glucose 6 phosphatase.

34. WHAT ARE THE KEY ENZYMES OF GLUCONEOGENESIS?

Ans. (*i*) Pyruvate carboxylase.

(*ii*) PEP–CK (Phosphoenol pyruvate-carboxy kinase).

(*iii*) Fructose 1,6 bisphosphatase.

(*iv*) Glucose-6 phosphatase.

35. TRACE THE PATHWAY OF GLUCONEOGENESIS FROM GLYCEROL TO GLUCOSE?

Ans. (*i*) Glycerol is converted to glycerol 3 P by glycerol kinase.

(*ii*) Glycerol 3P is next converted to dihydroxyacetone. P (DHAP) by glycerol 3P-DH.

(*iii*) DHAP is converted to glucose by the reversal of glycolysis and the key enzymes of gluconeogenesis.

36. TRACE THE PATHWAY OF GLUCONEOGENESIS FROM PROPIONATE TO GLUCOSE?

Ans.

(*i*) Propionate is activated to propionyl Co-A by acyl Co-A synthetase. ATP is broken to AMP +PP and Co-A is required for this reaction.

(*ii*) Propionyl Co-A is converted to D-methyl malonyl Co-A by CO_2 fixation reaction catalyzed by propionyl Co-A carboxylase and biotin.

(*iii*) D-Methyl malonyl Co-A is converted to L-methyl malonyl Co-A by recemase and L methyl malonyl Co-A is converted to succinyl Co-A by isomerase and B_{12} co-enzyme.

Succinyl Co-A ⟶ OAA ⟶ Main pathway of gluconeogenesis by key enzymes of gluconeogenesis and by the reversal of glycolysis to glucose.

37. HOW GLUCONEOGENESIS PATHWAY IS REGULATED?

Ans. (*i*) By induction and repression.

(*a*) Insulin causes repression of key enzymes of gluconeogenesis where as glucocorticoids and glucagon induce the synthesis of key enzymes of gluconeogenesis.

(*ii*) By covalent modification. Glucagon stimulates gluconeogenesis in liver by increasing the concentration of cAMP. Which in turn decreases the concentration of fructose 2, 6 BP.

(*iii*) Allosteric modification. Acetyl Co-A acts as a allostetric activator to pyruvate carboxylase and favours gluconeogenesis. Fatty acid oxidation increases acetyl Co-A, which favours gluconeogenesis.

38. NAME THE ORGANS WHERE GLUCONEOGENESIS TAKES PLACE.

Ans. Liver and kidney.

GLYCOGENESIS

39. DEFINE GLYCOGENESIS?

Ans. Formation of glycogen from glucose is called glycogenesis.

40. TRACE THE PATHWAY OF GLYCOGENESIS.

Ans.

Step 1: Glucose is converted to Glucose-6P by hexokinase (muscle) and glucokinase (liver).

Step 2: Glucose 6 P is converted to Glucose 1P by phosphoglucomutase.

Step 3: Glucose 1 P reacts with UTP and forms UDP glucose catalyzed by UDP glucose pyrophosphorylase.

Step 4: UDP glucose reacts with Glycogen primer and liberates UDP and Glycogen primer with one extra glucose unit catalyzed by glycogen synthase. The glucose is joined to glycogen primer by 1 $\longrightarrow$ 4 glycosidic linkage.

$$\underset{\text{Glycogen}}{UDPG + (C_6)_n} \longrightarrow \underset{\text{Glycogen plus one glucose unit}}{UDP + (C_6)_n + 1}$$

Step 5: By the action of branching enzyme a part of glycogen chain consisting of 6 glucose residues is removed and attached to neighbouring chain by 1 $\longrightarrow$ 6 glycosidic linkage. Thus it creates branch point.

41. WHAT IS THE KEY ENZYME OF GLYCOGENESIS?

Ans. Glycogen synthase.

42. HOW IS GLYCOGENESIS REGULATED?

Ans. Insulin stimulates glycogenesis and epinephrine in muscle and liver and glucagon in liver inhibits glycogenesis.

Glycogen synthase exists in two forms:

(*i*) Synthase – *a* (active) is in dephosphorylated form, and

(*ii*) Synthase – *b* (inactive) is in phosphorylated form.

The hormone triggered cAMP cascade mechanism in the phosphorylation and dephosphorylation of enzymes is similar to glycogenolysis process, so that at a given time either glycogenolysis is favoured by phosphorylation mechanism or glycogenesis is favoured by dephosphorylation.

GLYCOGENOLYSIS

43. WHAT IS GLYCOGENOLYSIS?

Ans. Breakdown of glycogen to glucose is called glycogenolysis. In the liver it is converted to glucose, but in the muscle it is broken to glucose 6 phosphate which later on enters into glycolysis during muscular contraction.

44. TRACE THE PATHWAY OF GLYCOGENOLYSIS.

Ans.

Step 1: Phosphorylase acts on glycogen and breaks the 1 ⟶ 4 glycosidic linkage in the presence of phosphate and liberates glucose 1 phosphate and glycogen minus one glucose unit.

$$(C_6)_n + Pi \longrightarrow (C_6)_{n-1} + \text{Glucose 1– Phosphate}$$

Glycogen — Glycogen minus one glucose

Step 2: α 1 ⟶ 4 glucan transferase transfers a trisaccharide unit from one branch to the other, exposing the α 1 ⟶ 6 linkage of branch.

Step 3: Debranching enzyme splits the 1 ⟶ 6 glycosidic linkage and remove the glucose residue of branch in the form of free glucose.

Step 4: Glucose 1 Phosphate is converted to Glucose 6 phosphate by mutase.

Step 5: In liver, glucose 6 phosphatase converts glucose 6-P to free glucose and liberates phosphate.

45. NAME THE ENZYME WHICH IS DEFICIENT IN THE FOLLOWING METABOLIC DISORDERS.

(*i*) Type I glycogen storage disease

Ans. Glucose 6 phosphatase. Von Gierke's disease.

(*ii*) Type II glycogen storage disease

Ans. Acid maltase. Pompe's disease.

(*iii*) Type III glycogen storage disease

Ans. Debranching enzyme. Forbe's or Cori's disease.

(*iv*) Type IV glycogen storage disease

Ans. Branching enzyme. Anderson's disease.

(*v*) Type V glycogen storage disease

Ans. Muscle phosphorylase. McArddle's syndrome.

(*vi*) Type VI glycogen storage disease

Ans. Liver phosphorylase. Her's disease.

46. WHAT ARE THE MAIN CLINICAL FEATURES OF THE FOLLOWING?

(*i*) Von Gierke's Disease

Ans. (*a*) Enlargement of liver and kidney due to accumulation of glycogen.
(*b*) Hypoglycemia.
(*c*) Ketosis.
(*d*) Hyperlipemia.

(*ii*) Pompe's disease

Ans. Heart failure due to accumulation of glycogen in lysozomes.

(*iii*) Anderson's disease

Ans. Cardiac and liver failure and death in first year of life.

(*iv*) McArdle's syndrome

Ans. Decreased tolerance to exercise. No lactate in blood after exercise.

47. WHAT IS THE KEY ENZYME OF GLYCOGENOLYSIS?

Ans. Phosphorylase.

48. HOW IS GLYCOGENOLYSIS REGULATED?

Ans. Hormones. Epinephrine in muscle and liver, glucogan in liver stimulates the glycogenolysis process and insulin inhibit the glycogenolysis process by the following mechanism.

Step 1: Epinephrine and glucogan activate adenylyl cylase to active adenylyl cylase.

Step 2: Active adenylyl cyclase converts ATP to cAMP.

Step 3: The cAMP activates protein kinase to active protein kinase (PK).

Step 4: Active PK activates phosphorylase kinase–b to phosphorylase kinase–a.

Step 5: Phosphorylase kinase–a activates phosphorylase–b to phosphorylase–a, where ATP is converted to ADP.

Step 6: Phosphorylase-a breaks glycogen to glucose 1 P in the presence of phosphate.

Action of hormone Insulin

Insulin has got reverse action. It activates Phosphodiesterase, which in turn destroys cAMP to AMP.

Dephosphorylation of enzymes are brought about by protein phosphatase

HMP – SHUNT PATHWAY

49. NAME THE ENZYMES WHICH CATALYSE THE OXIDATIVE REACTIONS IN THE–SHUNT PATHWAY AND GIVE THE REACTIONS CATALYSED BY THEM.

Ans. (*i*) Glucose 6 PDH converts glucose 6 P to 6 phosphogluconolactone. NADP is converted to $NADPH^+H^+$.

(*ii*) In the next step 6 phosphogluconate dehydrogenase converts 6 phosphogluconate to 3 keto 6 phosphogluconate. NADP is converted to $NADPH^+H^+$.

Significance

In these reactions NADPH are generated which are required for reductive synthesis of cholesterol and denovo synthesis of fatty acids.

50. WHAT IS THE SIGNIFICANCE OF HMP – SHUNT PATHWAY?

Ans.

(*i*) In the oxidative phase of HMP-shunt pathway, NADPH are generated. NADPH are utilised for the reductive synthesis of cholesterol and denovo synthesis of fatty acids.

(*ii*) In erythrocytes NADPH are utilised for the reduction of glutathione to reduced glutathione by glutathione reductase. Reduced glutathione is required for detoxification of H_2O_2.

(*iii*) In the non-oxidative phase of HMP–Shunt pathway, ribose 5 P is produced which is required for the synthesis of nucleic acids.

51. WHAT ARE THE CLINICAL FEATURES OF GLUCOSE 6 PDH DEFICIENCY?

Ans. Glucose 6 PDH deficiency produces hemolytic anemia and jaundice in the individuals taking the anti-malarial drugs like primaquin and quinine and other drugs like sulfonamides, dapsone and favabeans. The H_2O_2 damages the erythrocyte membrane and causes the hemolysis in this condition.

52. WHAT IS THE ACTION OF THE FOLLOWING ENZYMES?

(*a*) Transketolase

Ans. Converts Ribose 5 P + Xylulose 5 P to Sedoheptulose 7 P and Glyceraldehyde 3P, TDP is required as a co-enzyme.

(*b*) Transaldolase

Ans. Converts sedoheptulose 7 P + Glyceraldehyde 3P to Fructose 6 P + Erythrose 4P.

URONIC ACID PATHWAY

53. WHAT IS THE SIGNIFICANCE OF URONIC ACID PATHWAY?

Ans.

(*i*) In this pathway, UDP glucuronic acid is formed which is involved in the synthesis of proteoglycans and in the conjugation of bilirubin, steroid hormones and certain drugs.

(*ii*) In lower animals ascorbic acid is formed in this pathway by the action of enzyme L-gulono lactone oxidase.

54. WHAT IS ESSENTIAL PENTOSURIA?

Ans. Excretion of L-xylulose in urine is called essential pentosuria. In this condition L-xylulose is not converted to xylitol due to block in this reaction. It is a benign condition.

HOMEOSTASIS OF GLUCOSE AND METABOLISM OF OTHER HEXOSES

55. WHAT IS PASTEUR EFFECT?

Ans. The inhibition of glycolysis by respiration (aerobic condition) which is discovered by Louis Pasteur is called Pasteur effect. The inhibition of phospho fructo kinase by citrate and ATP accounts for much of the Pasteur effect.

56. WHAT IS CORI CYCLE?

Ans. Lactate formed by the breakdown of glucose in muscle is carried to liver through blood. In the liver lactate is converted to glucose by the enzymes of gluconeogenesis. Glucose from liver is brought to muscle through blood. These cyclical events are called Cori cycle.

57. WHAT IS GLUCOSE–ALANINE CYCLE?

Ans. During starvation alanine from muscle is carried to liver through blood. In the liver alanine is converted to pyruvate by ALT (transmination). Pyruvate is converted to glucose by the enzymes of gluconeogenesis. Glucose is brought to muscle and undergoes glycolysis to form pyruvate. Pyruvate by transmination is converted to alanine. These cyclical events are called glucose–alanine cycle.

Significance

During starvation the blood glucose level is maintained by this cycle.

58. WHAT IS NORMAL FASTING BLOOD GLUCOSE LEVEL?

Ans. Fasting — Serum/Plasma 70–110 mg/dl.

59. WHAT IS NORMAL POST PRANDIAL GLUCOSE LEVEL?

Ans. Post prandial — Serum/Plasma 110–140 mg/dl (in rare cases upto 160).

60. WHAT IS THE DIAGNOSTIC CRITERIA OF DIABETES MELLITUS?

Ans. (*i*) Fasting — Serum plasma > 125 mg/dl.
(*ii*) 2 hrs after oral glucose of 75 gms.
Serum/Plasma > 200 mg/dl.
(*iii*) Presence of glucose in urine.

61. WHAT IS THE CAUSE OF THE DIABETES MELLITUS SYNDROME?

Ans. Diabetes mellitus is caused by either by relative or absolute lack of hormone insulin which is secreted by beta cells of islets of langerhans.

62. WHAT ARE THE MAIN BIOCHEMICAL FEATURES OF DIABETES MELLITUS?

Ans. The metabolic derangements occur in diabetes mellitus by the decreased insulin : glucagon ratio.

(*i*) Carbohydrate metabolism	**Biochemical features**
(*a*) Hyperglycemia →	(*i*) Diminished uptake of glucose by tissues like muscle, adipose tissue and liver.
	(*ii*) Overproduction of glucose by
	(*a*) glycogenolysis due to ↑↑ activity of phosphorylase.
	(*b*) Gluconeogenesis due to ↑↑ activity of key enzymes of gluconeogenesis.
(*b*) Glycosuria →	Glycosuria is due to ↑↑ blood glucose level above renal threshold (180 mg/dl.).

(*ii*) Fat metabolism	**Biochemical Features**
(*a*) Plasma FFA →	Lipolysis due to ↑↑ activity of HS-lipase and ↓↓ lipogenesis.
(*b*) Ketosis and metabolic acidosis in untreated severe DM →	Oxidation of FAs and ↑↑↓↓ lipogenesis. ↓↓ bicarbonate level.
(*c*) Hypertriglyceridemia →	↑↑ Production of TAG leading to exudative xanthoma.

(*iii*) Protein metabolism Biochemical features

(*a*) Negative nitrogen balance ⟶ ⇓⇓ uptake of A.As. ⇓⇓ synthesis of proteins. Loss of muscle mass and muscle weakness.

(*b*) Hyperkelemia ⟶ ⇑⇑ Proteolysis

63. WHAT ARE THE DIAGNOSTIC TESTS OF DIABETES MELLITUS?

Ans. (*i*) GTT (*ii*) Fasting glucose
(*iii*) Post prandial glucose (*iv*) Urine sugar

64. WHAT IS GTT?

Ans. *Glucose tolerance test*: The tolerance to glucose load is tested by giving a known amount of oral glucose. This is called GTT. In GTT the patient is instructed to take.

(*i*) unrestricted amount of carbohydrate diet for atleast three days prior to the test, and

(*ii*) patient fasts overnight (12 hrs. fasting).

Procedure

A sample of blood is taken to measure the fasting blood glucose level. Fasting urine is collected and tested for the presence of sugar. 75 gms of glucose dissolved in 300 ml. of water is then given by mouth. Thereafter samples of blood and urine are collected at ½ hourly intervals for atleast 2 hrs. and then glucose content in blood is estimated and reducing sugar in urine is tested.

Interpretation of results

(*a*) (*i*) Normal – Fasting – serum/plasma < 100 mg/dl U.S. nil
(*ii*) 2 hr. after glucose – serum/plasma < 160 mg/dl U.S. nil

(*b*) (*i*) Diabetes mellitus – Fasting-serum/plasma > 125 mg/dl
(*ii*) 2 hrs. after glucose – serum/plasma > 200 mg/dl U.S. + +

65. NAME THE HORMONE WHICH LOWERS THE BLOOD SUGAR LEVEL (BSL) AND WHAT IS THE MODE OF ACTION?

Ans. Insulin. It lowers blood sugar level by

(*i*) ⇑⇑ uptake of glucose in the tissues like muscle, adipose tissue by recruiting GLUT-4 transporters from cytosol to plasma membrane.

(*ii*) ⇑⇑ Oxidation of glucose in glycolysis and TCA cycle by stimulating the key enzymes of glycolysis and PDH.

(*iii*) ⇑⇑ Glycogenesis by stimulating the enzyme glycogen synthase.

(*iv*) ⇓⇓ Glycogenolysis by inhibiting the enzyme phosphorylase.

(*v*) ⇓⇓ Gluconeogenesis by repression of the key enzymes of gluconeogenesis.

66. WHAT ARE THE HORMONES WHICH RAISE THE BLOOD SUGAR LEVEL AND WHAT IS THEIR MODE OF ACTION?

Ans. (*i*) Epinephrine by ↑↑ glycogenolysis in liver.
(*ii*) Glucagon by ↑↑ glycogenolysis and gluconeogenesis (GNG) in liver.
(*iii*) Glucocoriticoids by ↑↑ GNG in liver.
(*iv*) Growth hormone by ↓↓ uptake of the glucose in the tissues such as muscle and adipose tissue.

67. WHAT IS GALACTOSEMIA?

Ans. Inability to metabolise galactose by the deficiency of the enzmye galactose 1 Puridyl transferase is called galactosemia.

68. WHAT ARE THE CLINICAL FEATURES OF GALACTOSEMIA?

Ans.
(*i*) Galactose undergoes reduction to form galactitol which accumulates in lens causing cataract.
(*ii*) Galactose I P accumulates in liver and causes liver enlargement and jaundice due to unconjugated bilirubin.
(*iii*) There is galactosemia and galactosuria and severe mental retardation.

69. WHAT IS THE ACTION OF THE FOLLOWING ENZYMES?

(*i*) Galacto kinase
Ans. It brings about the phosphorylation of galactose to form galactose 1P, ATP is broken to ADP.
(*ii*) Galactose 1 P –uridyl transferase.
Ans. It converts galactose 1 P + UDP glucose to Glucose 1 P + UDP galactose.
(*iii*) UDP galactose epimerase.
Ans. It converts UDP glucose to UDP galactose.

Significance

By the action of this enzyme, galactose can be formed from glucose. Therefore pre-formed galactose is not required in the diet.

70. WHAT IS THE IMPORTANCE OF GALACTOSE?

Ans. Galactose is required for the synthesis of
(*a*) Lactose
(*b*) Cerebrosides
(*c*) Proteoglycans
(*d*) Glycoproteins

71. HOW IS LACTOSE SYNTHESIZED?

Ans. UDP galactose reacts with glucose and forms lactose in lactating mammary gland by the action of enzyme lactose synthase.

72. WHAT IS ESSENTIAL FRUCTOSURIA?

Ans. Excretion of fructose in urine by the deficiency of enzyme fructokinase is called essential fructosuria.

73. WHAT IS HEREDITARY FRUCTOSE INTOLERANCE?

Ans.

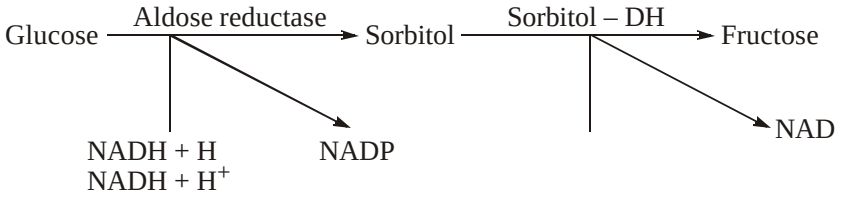
(*i*) Absence of enzyme aldolase B which gives rise to hereditary fructose intolerance. There is failure in conversion of fructose 1 P to dihydroxy acetone – P and glyceraldehyde. The clinical features are that the patient develops

(*a*) Hypoglycemia.

(*b*) Abdominal pain and vomiting.

74. WHAT IS POLYOL PATHWAY?

Ans. Glucose is converted to sorbitol by aldose reductase. Sorbitol is converted to fructose by sorbitol–DH.



75. WHAT IS THE IMPORTANCE OF POLYOL PATHWAY?

Ans. (*i*) Fructose is formed in semen by this pathway.

(*ii*) In the diabetes mellitus, this pathway is stimulated leading to the following complications of diabetes mellitus.

(*a*) Diabetic cataract (accumulation of sorbitol in lens).

(*b*) Diabetic neuropathy
(*c*) Diabetic retinopathy
} ⟶ Sorbitol ↓↓ the uptake of myoinositol.

Chapter–8

Lipid Metabolism – I

OXIDATION OF FATTY ACIDS AND METABOLISM OF KETONE BODIES

1. WHAT ARE THE DIFFERENT TYPES OF OXIDATION OF FATTY ACIDS AND WHICH ONE IS THE MAIN METHOD?

Ans. There are three different types of fatty acid oxidation:
1. α-Oxidation 2. β-Oxidation 3. ω-Oxidation
The β–oxidation is the main method.

2. WHAT ARE THE MAIN STEPS OF β-OXIDATION OF FATTY ACIDS?

Ans.
1. Activation of fatty acid to form acyl CoA. CoA is required and ATP is cleaved to AMP + PP.
2. Entry of acyl CoA into mitochondria with the help of carnitine.
3. Ist Oxidation in the mitochondria, involving FAD as a coenzyme.
4. Hydration (addition of water).
5. 2nd Oxidation involving NAD as a coenzyme.
6. Thiolytic cleavage with the help of CoA.

3. WHICH STEPS OF β-OXIDATION REQUIRE CoA?

Ans. Ist and last steps.

4. WHAT ARE THE OXIDATION STEPS IN β-OXIDATION?

Ans.
1. Conversion of acyl CoA to transenoyl CoA. The reaction is catalyzed by acyl-CoA DH.FAD is the coenzyme.
2. Conversion of β-hydroxy acyl CoA to 3 keto acyl CoA. NAD is the coenzyme.

5. WHAT IS THE NET GAIN OF ATP IN PALMITIC ACID OXIDATION?

Ans. Net gain = 129 Total production = 131

Palmitate enters 7 times into β-oxidation process.

7 × 5 = 35 No. of acetyl CoA = 8. (8 × 12=96)

96 + 35 = 131

Two (2) molecules are utilized in the activation,

Thus net gain = 131 – 2 = 129

6. WHAT ARE KETONE BODIES?

Ans. (*a*) Acetone (*b*) Aceto acetic acid (*c*) β-hydroxybutyric acid.

7. TRACE THE MAIN PATHWAY OF KETONE BODIES FORMATION.

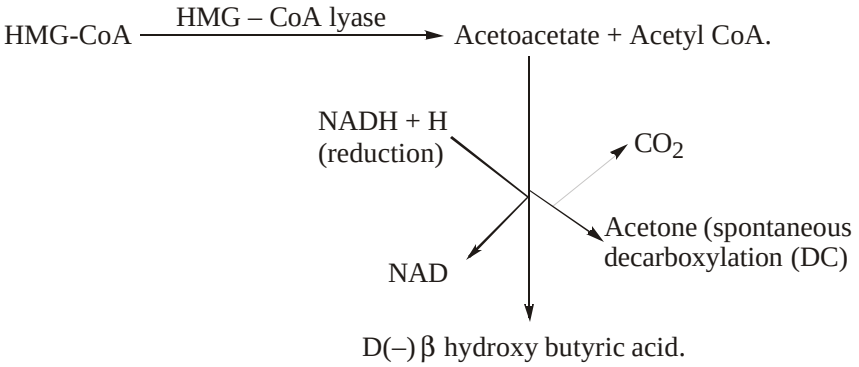
Ans. Two molecules of acetyl CoA combine by the reversal of thiolase action and form aceto acetyl CoA.

$$\text{Acetyl CoA + Acetyl CoA} \xrightarrow{\text{Thiolase}} \text{Aceto acetyl CoA + Co-A}$$

Aceto acetyl CoA combines with another molecule of acetyl CoA and forms HMG-CoA.

$$\text{Aceto acetyl CoA + acetyl CoA} \xrightarrow{\text{HMG–Co-A-synthase}} \text{HMG-CoA + CoA}$$

HMG-CoA by HMG-CoA lyase gives aceto acetate and acetyl CoA.



8. NAME THE TISSUES FOR THE KETONE BODIES PRODUCTION AND UTILIZATION.

Ans. Liver is the site for ketone bodies formation and muscle utilize ketone bodies.

9. NAME THE ENZYME REQUIRED FOR THE UTILIZATION OF KETONE BODIES.

Ans. Succinyl CoA-aceto acetate-CoA transferase.

10. WHAT IS KETOSIS AND WHAT ARE THE CAUSES OF KETOSIS?

Ans. Increased production of ketone bodies leading to ketonemia and ketonuria is called ketosis. Ketosis occurs by the ⇑⇑ mobilization of FFA from adipose tissue and ⇑⇑ oxidation in liver due to ⇓⇓ insulin : glucagon ratio.

Causes of Ketosis: (main causes)

1. Uncontrolled severe diabetes mellitus.
2. Prolonged fasting (starvation).

Other causes

1. Von Gierke's disease.

11. NAME THE TEST WHICH DETECTS KETONE BODIES?

Ans. Rothera's test.

12. WHAT IS THE NORMAL LEVELS OF K.B. IN BLOOD?

Ans. 1 mg/dl.

Lipid Metabolism – II

DENOVO SYNTHESIS OF FATTY ACIDS AND METABOLISM OF PROSTAGLANDINS AND LEUCOTRIENES

13. WHAT IS α-OXIDATION?

Ans. Oxidation of α-carbon (no. 2C) and the removal of one carbon at a time from the carboxyl end of the molecule is called α-oxidation. It occurs in brain.

14. WHAT IS ω-OXIDATION?

Ans. Oxidation of ω-methyl carbon (CH_3) to carboxyl (COOH) group forming dicarboxylic acid is called ω-Oxidation.

15. WHAT IS REFSUM'S DISEASE?

Ans. In Refsum's disease there is an inherited defect of ω-Oxidation. It is a neurological disorder caused by accumulation of phytanic acid formed from phytol.

16. NAME THE MULTI–ENZYME COMPLEX INVOLVED IN THE DE–NOVO SYNTHESIS OF FATTY ACIDS.

Ans. Fatty acid synthase complex.

17. WHAT ARE THE ENZYMES PRESENT IN FATTY ACID SYNTHASE COMPLEX?

Ans.
1. Keto acyl synthase
2. Acetyl transacylase
3. Malonyl transacylase
4. Hydratase
5. Enoyl reductase
6. Ketoacyl reductase
7. Thioesterase.

18. WHICH VITAMIN IS PRESENT IN THE FATTY ACID SYNTHASE COMPLEX?

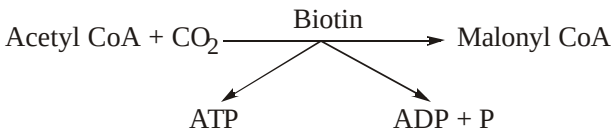
Ans. Pantothenic acid in the form of 4-phosphopantetheine which is present in ACP.

19. NAME THE IMPORTANT SUBSTANCES REQUIRED FOR THE DE-NOVO SYNTHESIS OF FATTY ACIDS.

Ans. NADPH, CO_2 (HCO_3), acetyl CoA.

20. WHAT IS THE KEY REGULATORY ENZYME WHICH CONTROLS DE-NOVO SYNTHESIS OF FATTY ACIDS AND WHAT IS ITS ACTION?

Ans. Acetyl CoA-carboxylase. It converts acetyl CoA to malonyl CoA.

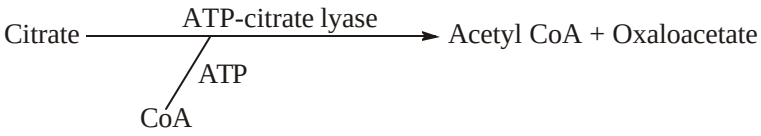


21. NAME THE TISSUES AND IN WHICH PORTION OF THE CELL THE DE-NOVO SYNTHESIS OF FATTY ACID OCCURS?

Ans. **Tissues:** Liver, kidney, lactating mammary gland, adipose tissue and lung; cytosol of the cells.

22. WHAT IS THE ACTION OF ATP CITRATE LYASE AND WHAT IS ITS IMPORTANCE?

Ans. In the cytosol it converts citrate to acetyl CoA and oxalo acetate.



It provides starting material acetyl CoA for denovo synthesis of fatty acids.

23. WHAT ARE EICOSANOIDS AND FROM WHICH SUBSTANCE THEY ARE FORMED?

Ans. The following are eicosanoids:

(*i*) Prosta glandins (PG).

(*ii*) Thromboxanes (TX).

(*iii*) Leukotrienes (LT).

These are formed from arachidonic acid.

24. NAME THE ENZYMES WHICH FORM PROSTAGLANDINS(PGs) AND LEUKOTRIENES (LTs)?

Ans. PG and thromboxanes are formed by the action of cyclooxygenase. LTs are formed by the action of lipoxygenase.

25. WHAT IS THE ACTION OF NSAIDS ON PROSTAGLANDIN METABOLISM?

Ans. NSAIDS like aspirin, indomethacin and ibuprofen inhibit cyclooxygenase.

26. WHAT ARE THE PRIMARY AND SECONDARY PGS?

Ans. Primary: PG–E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$, $F_{3\alpha}$
Secondary: PG–A_1, A_2, B_1, B_2, 19(OH)A_1, 19(OH)A_2, 19(OH)B_1 and 19(OH)B_2.

27. WHAT ARE THE 1st , 2nd AND 3rd DOUBLED BONDS IN PGs?

Ans. Ist $\longrightarrow C_{13}$, 2nd $\longrightarrow C_5$, 3rd $\longrightarrow C_{17}$

28. WHAT ARE THE IMPORTANT ACTIONS OF PGs?

Ans. (*a*) PG – E and Pg – A act as potent V.D. and $\downarrow\downarrow$ B.P.
(*b*) PG – E_1 and E_2 act as bronchodilators and has been used in the treatment of asthma.
(*c*) PGE, E_1 and $F_{2\alpha}$ cause contraction of uterus. PGE_2 at a rate of 0.54. μG/ml in the induction of labor and 5 mg/dl used in MTP.
(*d*) PG–E $\downarrow\downarrow$ lypolysis by lowering cAMP level.
(*e*) PGE and D play a role in inflammation and cause wheal formation.

29. WHAT ARE THE ACTIONS OF PGI_2 AND TX?

Ans. PGI_2 (*a*) $\downarrow\downarrow$ platelets aggregation.
(*b*) Causes vasodilatation (V.D.).
(*c*) $\downarrow\downarrow$ thrombus formation and it opposes TX action.
TX (*a*) $\uparrow\uparrow$ platelet aggregation.
(*b*) Causes vasoconstriction (V.C.).
(*c*) $\uparrow\uparrow$ thrombus formation.

30. WHAT IS THE ACTION OF ASPIRIN IN THROMBUS FORMATION?

Ans. Aspirin $\downarrow\downarrow$ thrombus formation by preventing platelet aggregation.

31. WHAT ARE THE DIFFERENT TYPES OF LTs AND WHAT ARE THEIR ACTIONS?

Ans. LT A_4, B_4, C_4, D_4 and E_4

B_4 stimulates chemotaxsis and chemo kinesis in neutrophils and eosinophils which are found in large numbers at the site of inflammation.

32. WHAT IS SRS-A AND WHAT IS ITS ACTION?

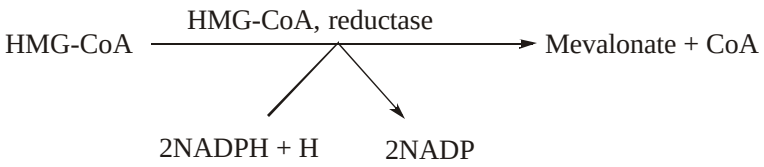
Ans. SRS-A is slow reacting substance of anaphylaxis, which is produced by mast cells during anaphylactic reaction. It consists of mixture of LTS. LTC_4, D_4 and E_4. It causes constriction of bronchial muscle and ↑↑ vascular permeability seen in anaphylactic and allergic reactions.

Lipid Metabolism – III

METABOLISM OF CHOLESTEROL AND LIPOPROTEINS AND LIPOTROPHIC FACTORS AND FATTY LIVER

33. NAME THE KEY REGULATORY ENZYME WHICH CONTROLS CHOLESTEROL SYNTHESIS AND WHAT IS ITS ACTION?

Ans. HMG-CoA reductase. It converts HMG-CoA to Mevalonate.
Two (2) molecules of NADPH are utilized



34. TRACE THE PATHWAY OF CHOLESTEROL SYNTHESIS FROM ACETYL CoA TO MEVALONATE.

Ans. Two (2) acetyl CoA combine to form acetoacetyl CoA by the action of thiolase. Acetoacetyl CoA combines with one more molecule of acetyl CoA by the action of HMG-CoA synthase and is converted to HMG-CoA. HMG-CoA is converted to mevalonate by the action of HMG-CoA reductase.

35. WHAT ARE THE MAIN STEPS OF SQUALENE FORMATION FROM MEVALONATE?

Ans. Mevalonate by series of phosphorylation converted to isoprenoid units. Isopentenyl D.P. and 3, 3-Dimetheyl allyl D.P. By the combination of these isoprenoid units these are converted to geranyl D.P., then farnesyl D.P. and lastly squalene. In the last step NADPH is required.

36. TRACE THE PATHWAY OF CHOLESTEROL FROM SQUALENE TO CHOLESTEROL?

Ans. Squalene is converted to Lanosterol, then to 14 Desmethyl Lanosterol ⟶ Zymosterol ⟶ Desmosterol and lastly to **cholesterol**.

37. WHAT IS THE DIFFERENCE BETWEEN HMG-CoA FORMATION IN KETONE BODIES SYNTHESIS AND CHOLESTEROL SYNTHESIS?

Ans. In ketone bodies (K.B.) formation HMG-CoA synthesis occurs in the mitochondria of liver cells. Whereas in cholesterol formation HMG-CoA synthesis occurs in extra mitochondrial portion of cells of many tissues.

38. WHAT ARE THE MAIN FATES OF CHOLESTEROL AND HOW IS IT UTILIZED?

Ans. The main fates of cholesterol are:

(*a*) It is converted to bile acids in the liver and secreted in the bile.

(*b*) It is converted to steroid hormones in the aderenal cortex, testes and ovary.

(*c*) It is converted to 7-Dehydrocholesterol and which in turn converted to Vitamin D_3 after exposure to sunlight.

39. WHAT ARE BILE ACIDS AND BILE SALTS?

Ans. Bile acids ⟶ 1. Cholic acids 2. Chenodeoxy cholic acid

Bile salts ⟶ 1. Sodium taurocholate 2. Sodium glycocholate.

40. WHAT ARE THE FUNCTIONS OF BILE SALTS?

Ans. They lower the surface tension and help in the emulsification of fats. They help in the absorption of fats and fat soluble vitamins by forming micelles. They accelerate the action of pancreatic lipase. They keep cholesterol in solution.

41. WHAT ARE THE CHOLESTEROL RICH FOODS AND CHOLESTEROL POOR FOODS?

Ans. Cholesterol rich foods ⟶ Liver, brain, heart and kidney, whole milk, cream, egg yolk (rich), animal fats,

Cholesterol poor foods ⟶ Cooked and raw vegetables, fruits, lemon juice, butter milk, skimmed milk, white of eggs, cereals, lean meats and lean fish.

42. NAME THE LIPOPROTEINS PRESENT IN PLASMA IN ORDER OF LOW DENSITY?

Ans. Chylomicrons (CM) – <0.95; VLDL – 0.96 – 1.006; LDL – 1.019 – 1.063; HDL – 1.063 – 1.21.

43. WHAT ARE THE METHODS OF SEPARATION OF LP?

Ans. Electrophoresis in order of fast movement ⟶ HDL(αLP) (fastest moving), VLDL (Pre-βLP), LDL (β-LP), CM (least mobile)
Ultracentrifugation in order of low density ⟶ CM, VLDL, LDL, HDL.

44. WHAT ARE THE MAIN LIPIDS PRESENT IN VARIOUS LPs?

Ans. CM ⟶ Exogenous–TAG (dietary fat), VLDL ⟶ Endogenous TAG formed in the liver.
LDL ⟶ 70% transport of cholesterol.
HDL ⟶ Phospholipid (PL) + Cholesterol (reverse transport of cholesterol from tissues to liver).

45. WHAT ARE THE DIFFERENT APOPROTEINS PRESENT IN DIFFERENT LPs?

Ans. A_1 ⟶ HDL and C.M.; APO–B_{100} ⟶ LDL, VLDL, IDL,
APO-B_{48} ⟶ CM and CM–remnant; APO C_1 and C_{II} ⟶ VLDL, HDL, C.M.
APO-E ⟶ VLDL, IDL, HDL, CM and CM-remnant.

46. WHAT ARE THE FUNCTIONS OF A_1, B_{100}, B_{48}, C_{II} AND E?

Ans. A_1 ⟶ (1) Reverse chol. Transport, (2) Activates LCAT, (3) Ligand for HDL receptor
B_{100} ⟶ (1) Ligand for LDL receptor. (2) VLDL secretion from liver.
B_{48} ⟶ CM secretion from intestine.
C_{II} ⟶ Activates LPL (Lipoprotein lipase).
E ⟶ Ligand for CMR receptor in liver and LDL receptor.

47. WHAT IS THE ACTION OF LPL?

Ans. LPL is present in the walls of blood capillaries of extra hepatic tissues, where it hydrolyses 90% of CM – TAG and VLDL – TAG to F.A.s and glycerol. By the action of this enzyme CM is converted to CMR and VLDL is converted to IDL.

48. WHAT IS THE ACTION OF LCAT (LECITHIN CHOLESTEROL ACYL TRANSFERASE)?

Ans. LCAT is the lecithin cholesterol acyl transferase. It converts plasma free cholesterol to cholesteryl ester and lecithin to lysolecithin. APO–A1 activates this enzyme. It converts discoidal HDL to spherical HDL.

$$\text{Cholesterol} + \text{Lecithin} \xrightarrow{\text{LCAT}} \text{Cholesteryl ester (C – E)} + \text{lysolecithin}$$

49. WHAT IS NORMAL SERUM TOTAL CHOLESTEROL LEVEL, LDL-CHOLESTEROL LEVEL AND HDL-CHOLESTEROL LEVEL?

Ans. Total cholesterol 150 – 180 mg/dl; HDL-C – 35 – 60 mg/dl; LDL-C – 90 – 130 mg/dl (but < 100 mg is ideal).

50. WHAT ARE THE FUNCTIONS OF HDL?

Ans. HDL_2 concentrations are inversely related to the incidence of coronary altherosclerosis. HDL is involved in the reverse transport of cholesterol from peripheral tissues to the liver, then it reduces the intra cellular cholesterol content (scavenging action of HDL).

51. WHAT ARE THE CAUSES OF HYPERCHOLESTEROLEMIA?

Ans. 1. Hypothyroidism (Myxoedema).
2. Nephrotic syndrome.
3. Obstructive jaundice/biliary cirrhosis.
4. Familial hypercholesterolemia (due to defect in the LDL receptors).

52. WHAT ARE THE HAZARDS OF HYPERCHOLESTEROLEMIA (HC)?

Ans. It causes atherosclerosis of coronary blood vessels ultimately resulting in AMI.

53. WHAT ARE THE MAIN TYPES OF HYPERLIPOPROTEINEMIAS (HLP) AND WHAT ARE THE CAUSES?

Ans. Type I–HLP: Due to deficiency of LPL and C_{II}. ↑↑ level of exogenous TAG.
Type IIa.–HLP: Familial hypercholesterolemia due to defect in LDL receptors.
Type IV HPL: Familial hypertriglyceridemia due to over production of VLDL. ↑↑ endogenous TAG.

54. NAME THE DRUGS WHICH LOWER CHOLESTEROL LEVEL?

Ans. HMG-CoA reductase inhibitors like Mevastatin and Lovastatin.

55. IN HEALTHY INDIVIDUAL BY A PROCESS OF SERUM L.P. ELECTROPHORESIS HOW MANY BANDS ARE SEPARATED OUT AND WHAT ARE THEY?

Ans. 2 bands. They are LDL (β-L.P.) and HDL (α-L.P.)

56. WHAT IS THE ACTION OF HORMONE SENSITIVE LIPASE AND WHICH HORMONES ACTIVATE IT AND WHICH HORMONES DECREASE ITS ACTIVITY?

Ans. Hormone sensitive lipase (HSL) is present in adipose tissue (A.T.) and it hydrolyses. AT – TAG to 3 F.A. and glycerol. It helps in mobilization of A.T. fat.

Hormones which activate HSL are ⟶ glucagon, GH, epinephrine, ACTH, T_4 (through the formation of cAMP). Hormones which ↓↓ HSL ⟶ insulin (by ↓↓ level of cAMP).

57. EXPLAIN THE ACTION OF FOLLOWING ENZYMES ? (a) Pancreatic lipase (b) Cholesteryl ester hydrolase (c) Phospholipase A_2

Ans. Pancreatic lipase ⟶ hydrolyses dietary TAG to 2 F.A. + 2–mono acyl glycerol + Glycerol.
Cholesterylester hydrolyase ⟶ hydrolyses C–E to C + F.A.
Phsopholipase A_2 ⟶ hydrolyses PL to lysophospholipid + F.A.

58. WHAT ARE THE MAIN STEPS OF ABSORPTION OF FAT?

Ans.

Ist step: Digestion of fat by pancreatic lipase and forms micelles with the help of bile salts which are absorbed into intestinal epithelium.

IInd step: In the intestinal epithelium TAG are reformed and incorporated in to APO B_{48} to form CM.

IIIrd step: CM are carried through the lacteals and ultimately enter into systemic circulation.

59. WHAT IS FATTY LIVER AND WHAT ARE THE CAUSES OF FATTY LIVER?

Ans. Accumulation of more than 5% of fat in the liver is called fatty liver.

Causes

(1) Starvation and feeding of high fat diet.
(2) Uncontrolled D.M.
(3) Deficiency of lipotropic factors (choline, methionine, betain and inositol).
(4) Alcoholism.
(5) Deficiency of Vit E, B_6 and pantothenic acid.
(6) CCl_4 poisoning.

60. WHAT ARE THE LIPOTROPIC FACTORS (L.F.) AND WHAT IS THE MODE OF ACTION OF THESE FACTORS?

Ans. Substances which prevent accumulation of fat are called as L.T. factors. These are choline, methionine, betain, inositol etc.

Mode of action: Deficiency of choline causes ↓↓ oxidation of F.A. by ↓↓ the level of carnitine. Deficiency of LT factors causes ↓↓ synthesis of LPs by ↓↓ level of PL and by ↓↓ synthesis of intra cellular membranes.

61. WHAT IS THE ACTION OF ALCOHOL IN CAUSING FATTY LIVER?

Ans. Alcohol is oxidised to acetaldehyde by alco .DH. This results in ↑↑ production of NADH, which in turn causes ↓↓ oxidation of F.A. and fatty liver.

62. WHAT ARE THE HAZARDS OF FATTY LIVER?

Ans. Fatty liver ultimately ends in cirrhosis of liver. Chronic accumulation of fat in liver causes fibrotic changes in liver cells and impair liver function, resulting in cirrhosis of liver.

Chapter–9

Protein Metabolism

DIGESTION AND ABSORPTION OF PROTEINS

1. WHAT ARE THE PROTEOLYTIC ENZYMES PRESENT IN G.I.T.?

Ans. (*a*) Endopeptidases ⟹ Pepsin, trypsin, chrymotrypsin and elastase.
(*b*) Exopeptidases ⟹ Carboxy-peptidase, amino-peptidase.

2. WHAT ARE THE PRECURSOR FORMS (ZYMOGEN) OF ABOVE ENZYMES AND HOW ARE THEY ACTIVATED AND WHAT ARE THEIR ACTIONS?

Ans.

1. Pepsinogen $\xrightarrow[\text{(Pepsin)}]{\text{HCL}}$ Pepsin
2. Trypsinogen $\xrightarrow{\text{Enterokinase}}$ Trypsin
3. Chymotrypsinogen $\xrightarrow{\text{Trypsin}}$ Chymotrypsin
4. Pro-Carboxypeptidase $\xrightarrow{\text{Trypsin}}$ Carboxypeptidase.

1.	Proteins	$\xrightarrow[\text{(optimum pH. 1-2)}]{\text{Pepsin}}$ Peptide	Specific action breaks peptide bonds formed by aromatic A.A.
2.	Proteins & Peptides	$\xrightarrow{\text{Trypsin}}$ Polypeptides and Di–Peptides	Breaks peptide bonds formed by basic A.As.
3.	Proteins & Peptides	$\xrightarrow{\text{Chymotrypsin}}$ Polypeptides and Di–Peptides	Breaks peptide bonds formed by carboxy groups of aromatic A.As.

4. Proteins —Elastase→ Polypeptides and Di-Peptides (Breaks peptide bonds formed by Neutral A.As.)

5. Polypeptides —Carboxypeptidase→ 'C' terminal amino acid + peptides minus one A.A.

3. BY WHAT MECHANISM AMINO ACIDS ARE ABSORBED?

Ans. Active transport. It requires transport proteins.

4. WHAT ARE THE DIFFERENT TRANSPORT SYSTEMS REQUIRED FOR THE ABSORPTION OF AMINO ACIDS?

Ans.
1. For neutral amino acids.
2. For basic amino acids.
3. For imino acid and glycine.
4. For acidic amino acids.
5. For β-amino acids.

METABOLISM OF AMINO ACIDS

5. WHAT ARE ESSENTIAL AMINO ACIDS?

Ans.
- M → Methionine
- P → Phenylalanine
- I → Iso-leucine
- L → Lysine
- L → Leucine
- Th → Threonine
- Try → Tryptophan
- V → Valine

For infants histidine is also required.
Arginine causes spermatogenesis.

6. WHICH AMINO ACID IS ONLY KETOGENIC?

Ans. Leucine.

7. WHICH AMINO ACIDS ARE GLUCOGENIC AND KETOGENIC?

Ans. Phenylalanine, Tyrosine, Tryptophan and Isoleucine.

8. WHAT ARE THE AMINO ACIDS WHICH ARE CONVERTED TO PYRUVATE?

Ans.

S → Serine
C → Cysteine
T → Threonine
A → Alanine
G → Glycine
H → Hydroxy proline

9. WHAT ARE THE AMINO ACIDS WHICH ARE CONVERTED TO SUCCINYL CoA?

Ans.

V → Valine
I → Iso-leucine
M → Methionine

10. WHAT ARE THE AMINO ACIDS. WHICH ARE CONVERTED TO α–KGA?

Ans.

P → Proline
A → Arginine
G → Glutamine
H → Histidine → Glutamate → α-KGA

11. WHAT IS TRANSAMINATION REACTION?

Ans. Transfer of NH_2 group from amino acid to keto acid, so that the amino acid becomes new keto acid and keto acid becomes new amino acid

Example:

$$\underset{\text{(A.A.)}}{\text{Aspartate}} + \underset{\text{(K.A.)}}{\alpha\text{-KGA}} \xrightleftharpoons[\text{PLP}(B_6)]{\text{AST}} \text{Oxalo acetate (K.A.)} + \text{Glutamate (A.A.)}$$

12. WHAT IS THE CLINICAL IMPORTANCE OF THE ENZYMES AST AND ALT.

Ans. AST ↑↑ in AMI and hepato cellular jaundice.
ALT ↑↑ in hepato cellular jaundice.

13. WHAT IS DEAMINATION REACTION?

Ans. Removal of NH_2 group from the amino acid in the form of NH_3 occurs so that amino acid becomes keto acid.

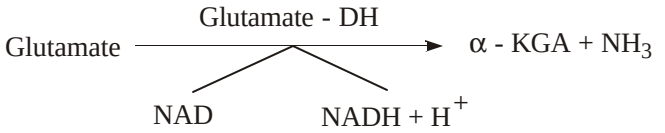
$$\text{Amino acid} \xrightarrow{\text{Deamination}} NH_3 + \text{keto acid}$$

14. WHAT ARE THE DIFFERENT TYPES OF DEAMINATION (D.A.) REACTIONS AND GIVE EXAMPLES?

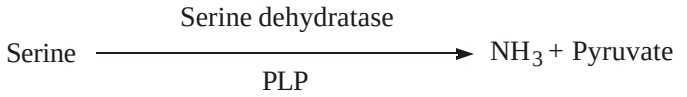
Ans. (*a*) Oxidative deamination (*b*) Non–oxidative deamination

Examples:

Oxidative Deamination



Non–oxidative Deamination



15. WHAT IS DECARBOXYLATION (DC) REACTION OF AMINO ACIDS AND GIVE EXAMPLES?

Ans. Removal of carboxyl group in the form of CO_2 from the A.A. is called D.C.

Example:

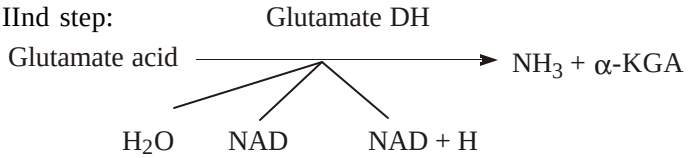
1. Glutamate —(Glutamate decarboxylase, PLP)→ CO_2 + GABA (γ-aminobutyric acid)
2. Histidine —(Histidine decarboxylase)→ CO_2 + Histamine

16. WHAT IS THE MAIN ROUTE OF REMOVAL OF NH_2 GROUP OF A.A. TO FORM NH_3? GIVE EXAMPLE.

Ans. Ist step:

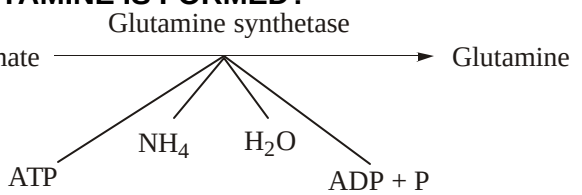
A.A. + α-KGA —(Transamination)→ Glutamic acid (G.A.) + K.A.

IInd step:



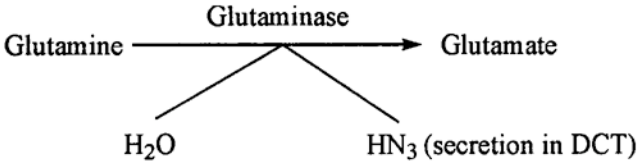
17. HOW GLUTAMINE IS FORMED?

Ans.



18. HOW GLUTAMINE IS CONVERTED TO GLUTAMATE AND WHAT IS THE IMPORTANCE OF THIS REACTION?

Ans.



NH_3 secretion occurs in DCT of kindney for the regulation of acid base balance. It conserves cations. ↑↑secretion of NH_3 occuring in metabolic acidosis.

UREA CYCLE

19. HOW MANY NITROGENS ARE PRESENT IN THE STRUCTURE OF UREA AND WHAT ARE THEIR SOURCES?

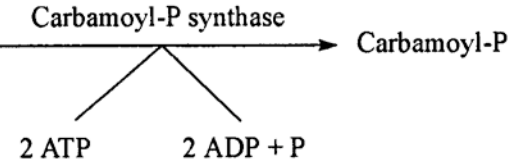
Ans. Two. One is derived from NH_3 and the other from aspartate.

20. NAME THE ENZYMES OF UREA CYCLE?

Ans.
1. Carbamoyl – P synthase – I.
2. Ornithine transcarbamoylase.
3. Argino succinate synthase.
4. Argino succinase.
5. Arginase.

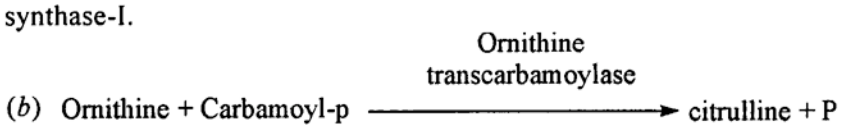
21. WHAT IS THE ACTION OF ABOVE ENZYMES?

Ans. (*a*)

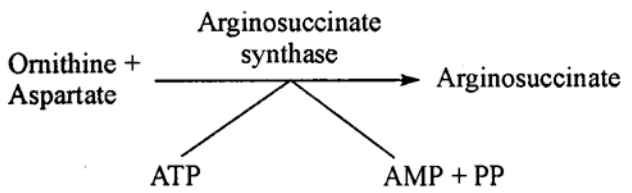


N-acetylglutamate (NAG) acts as a allosteric activator to carbamoyl–p synthase-I.

(*b*)



(*c*)



(*d*) Arginosuccinate $\xrightarrow{\text{Arginosuccinase}}$ Arginine + Fumarate

(*e*) Arginine $\xrightarrow[H_2O]{\text{Arginase}}$ Ornithine + Urea

22. WHAT IS THE MOL. WT. OF UREA?

Ans. 60.

23. WHAT IS THE FATE OF FUMARATE IN UREA CYCLE?

Ans. FUMARATE – Which is formed in urea cycle enters TCA and is converted to oxaloacetate

$$\text{oxaloacetate} \xrightarrow{\text{TA}} \text{aspartate (re-enters urea cycle).}$$

24. WHAT IS NORMAL BLOOD UREA LEVEL AND WHAT ARE THE CAUSES OF ↑↑ B.U. LEVEL?

Ans. Normal value 10–40 mg/dl.

↑↑ B.U.

1.	Pre-renal	Dehydration (vomiting and diarrhoea)
2.	Renal	Renal failure < ARF, CRF
3.	Post renal	Enlarged prostrate, renal calculus.

ACTIONS OF IMPORTANT ENZYMES OF PROTEIN METABOLISM

25. EXPLAIN THE REACTIONS CATALYZED BY THE FOLLOWING ENZYMES?

A. Serine Hydroxylmethyltransferase

Serine hydroxylmethyltransferase

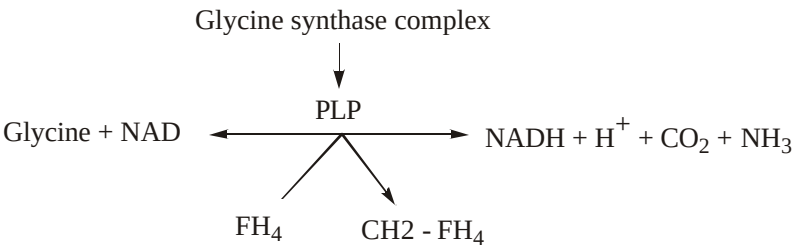
FH_4 FH_2–FH_4

Serine ⟷ Glycine

PLP

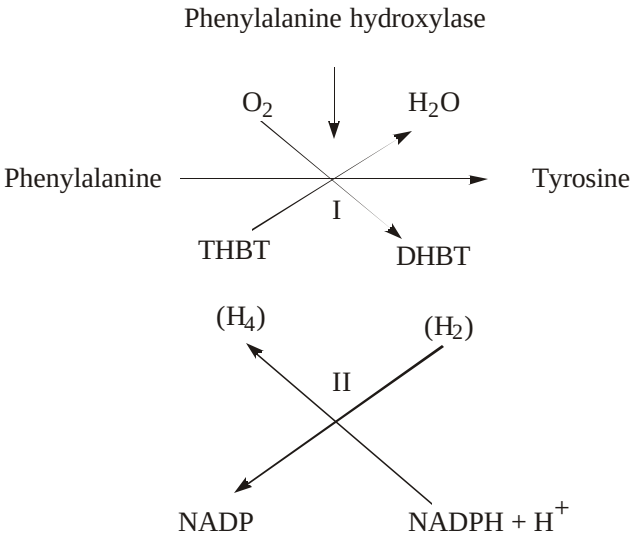
B. Glycine Synthase Complex

Ans.



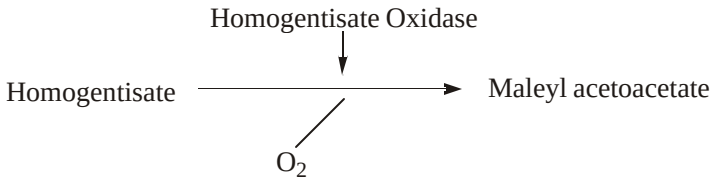
C. Phenylalanine Hydroxylase

Ans.



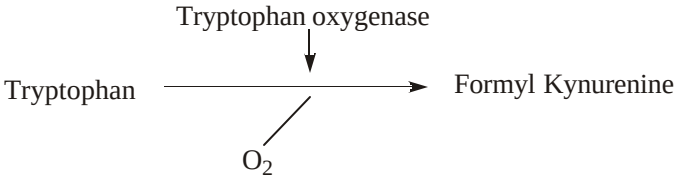
D. Homogentisate Oxidase

Ans.



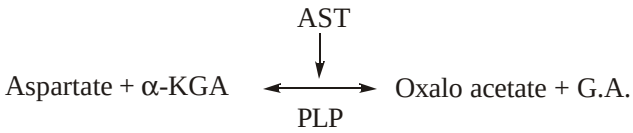
E. Tryptophan Oxygenase

Ans.



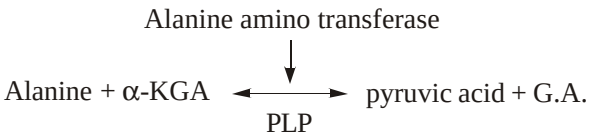
F. Aspartate Amino Transferase (AST)

Ans.



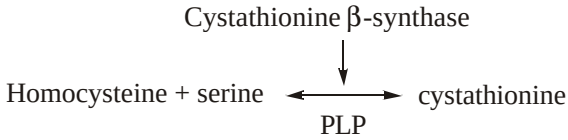
G. Alanine Amino Transferase (ALT)

Ans.



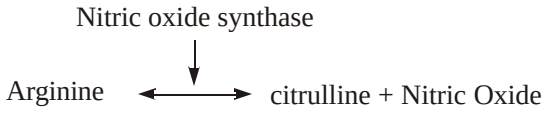
H. Cystathionine β-Synthase

Ans.



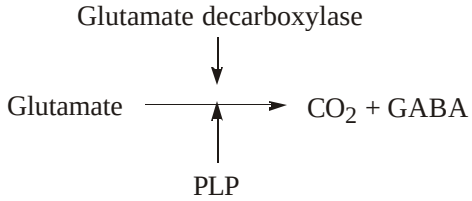
I. Nitric Oxide Synthase

Ans.



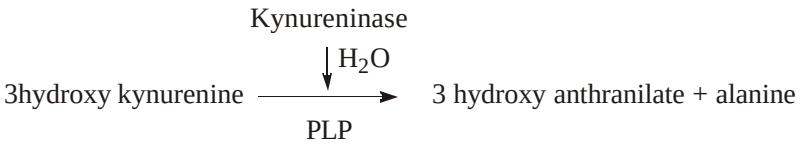
J. Glutamate Decarboxylase

Ans.



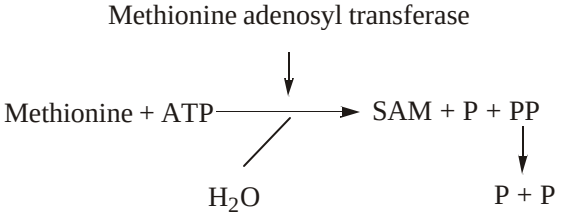
K. Kynureninase

Ans.



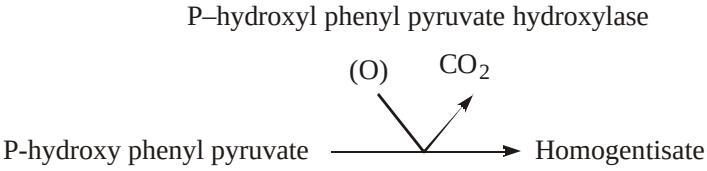
L. Methionine Adenosyl Transferase

Ans.



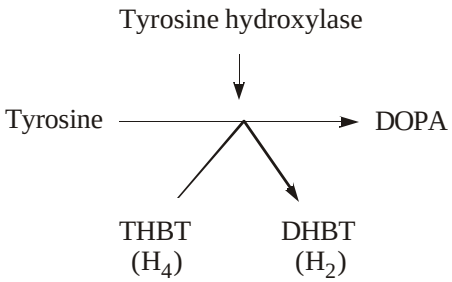
M. P–Hydroxyl Phenyl Pyruvate Hydroxylase

Ans.



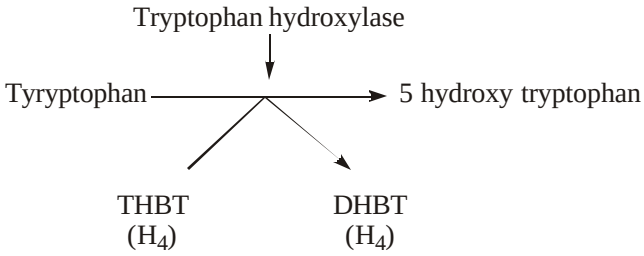
N. Tyrosine Hydroxylase

Ans.



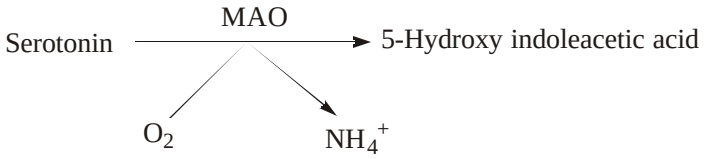
O. Tryptophan Hydroxylase

Ans.



P. Monoamine Oxidase

Ans.

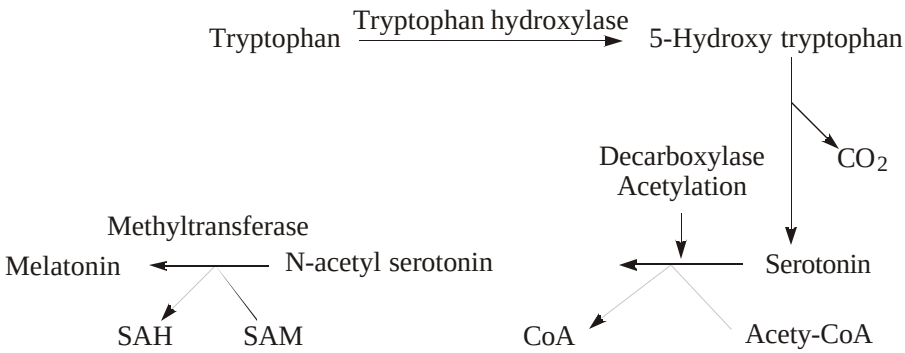


SYNTHESIS OF SPECIALISED PRODUCTS FORMED FROM THE AMINO ACIDS

26. TRACE THE PATHWAY OF

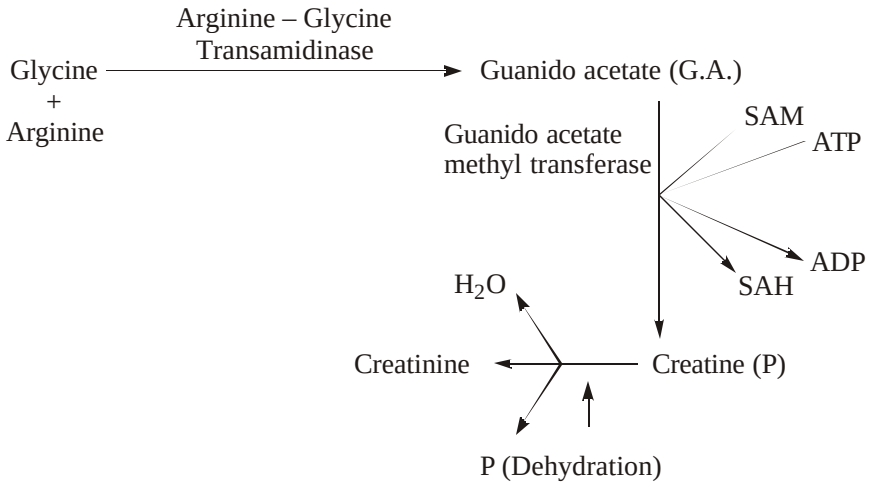
A. Formation of Serotonin and Melatonin

Ans.



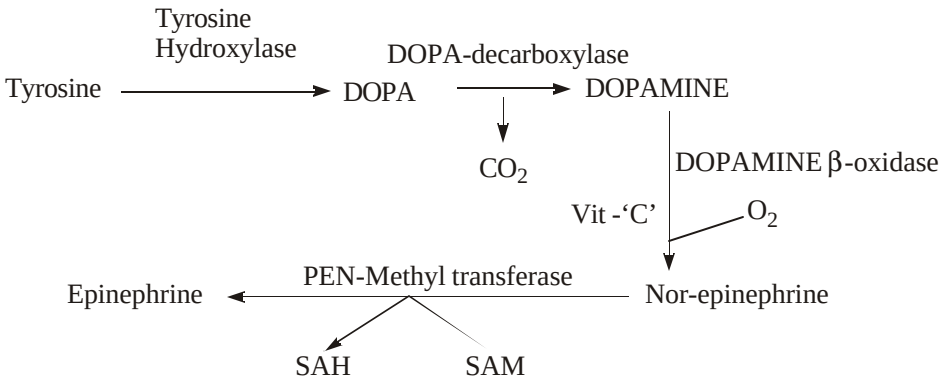
B. Formation of Creatine and Creatinine

Ans.



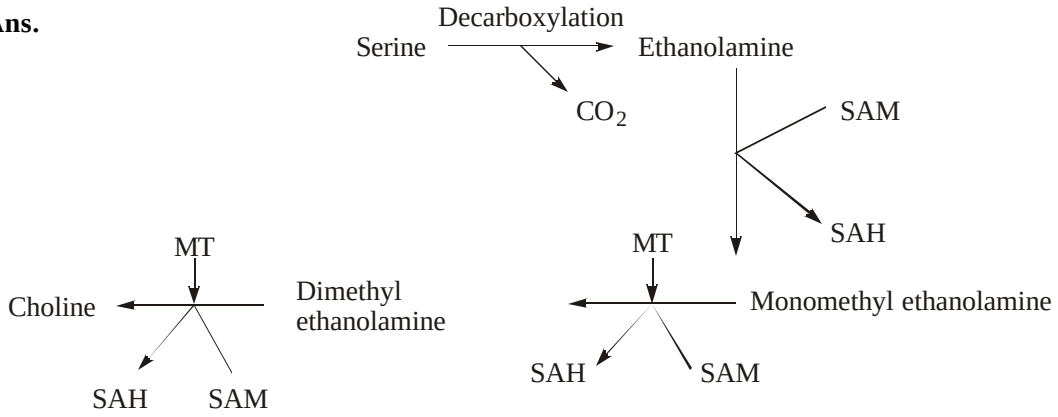
C. Formation of Catecholamines

Ans.



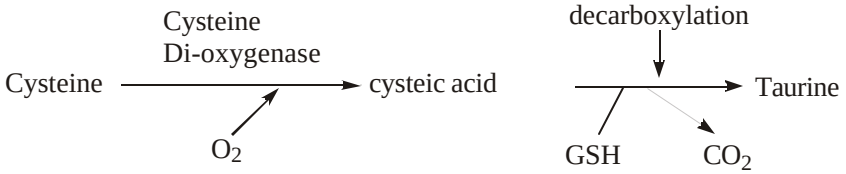
D. Formation of Choline

Ans.



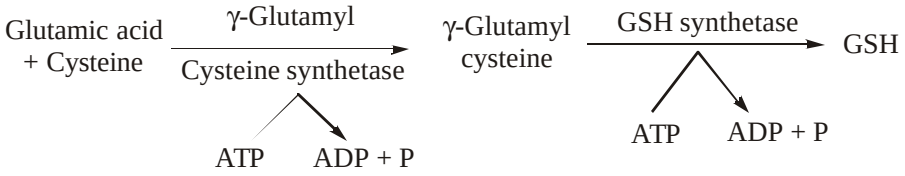
E. Formation of Taurine

Ans.



F. Formation of GSH

Ans.



G. What are the Specialized Products Formed From

Ans.

(*a*) Tyrosine → Dopamine, Norepinephrine and epinephrine, T_3, T_4, melanin.

(*b*) Glycine → Heme, Purine (C_4, C_5 and N_7), Glycocholate, Glutathione, Creatine.

(*c*) Tryptophan → Serotonin, Melatonin and Nicotinic acid.

(*d*) Glutamate → GABA, Glutamine, Glutathione.

(*e*) Cysteine → Glutathione, Taurine → Taurocholate, Thioethanolamine (a part of CoA-SH)

(*f*) Arginine → Nitric oxide. Creatine, ornithine → Polyamines.

(*g*) Aspartic acid → Pyrimidine (N_1, C_4, C_5, C_6) purine (N_1) one '*N*' of urea.

(*h*) Glutamine → Purine (N_3 and N_9), pyrimidine (N_3), urinary NH_3.

(*i*) Methionine → DC-SAM gives 1,3 diaminopropane portion of polyamines. By transmethylation, choline, epinephrine, creatine and melatonin are formed.

INBORN ERRORS OF THE METABOLISM OF AMINO ACIDS

27. NAME ENZYME DEFICIENCY AND IMPORTANT BIOCHEMICAL AND CLINICAL FEATURES OF THE FOLLOWING INBORN ERRORS OF METABOLISM.

Ans.

(*a*) P.K.U. → Enz → Phenylalanine hydroxylase.
- B.F. → Excretion of phenyl pyruvic acid, phenyl acetyl glutamine ↑↑ level of phenylalanine in blood > 20 mg/dl.
- C.F. → 1. Mental retardation.
  2. Mousy odour (phenyl acetate).
  3. Eczema (hypopigmentation).
  4. Convulsions.
- B.F. → Biochemical features.
- C.F. → Clinical features.

(*b*) Alkaptonuria → Enz → Homogentisate oxidase.
- B.F. → Excretion of black coloured alkaptone bodies in urine. Darkening of urine.
- C.F. → Occhronosis (deposition of alkaptone bodies in 3rd or 4th decade in intervertebral discs and pinna of ear).

(*c*) Cystinuria → Enz → Defect of transport protein for Cystine, Ly, Arg, Orn.
- B.F. → Excretion of cystine, Ly, Arg, Orn in urine.
- C.F. → Cystine calculi formation obstructive uropathy.

(*d*) Homocystinuria → Enz → Cystarthionine β-synthase
- B.F. → Excretion of homocysteine in urine. Defect in collagen formation in C.T.
- C.F. → Mental retardation, osteoporosis, flat foot, ectopialentis ↑↑ platelet adhesion → formation of thrombus.

(*e*) Primary hyperoxaluria

Enz → Defect in transamination of glyoxylate.
B.F. → ↑↑ excretion of oxalates in urine.
C.F. → Formation of oxalate stones, urolithiasis and nephrolithiasis renal colic and hematuria.

(*f*) Maple syrup urine disease

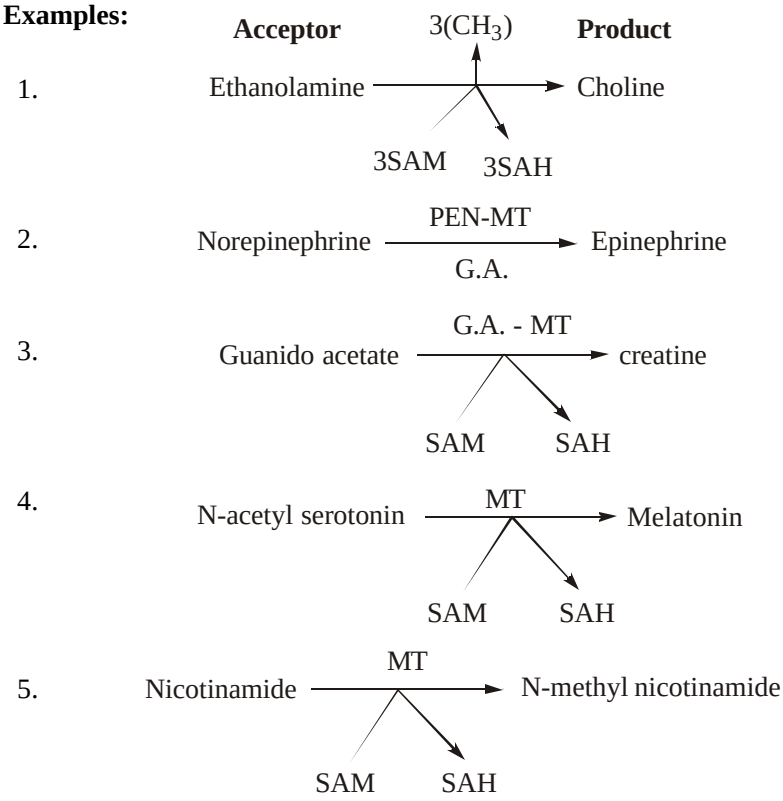
Enz → α-keto acid decarboxylase.
B.F. → ↑↑ level of branched chain amino acids and their K.A. in blood and ↑↑ excretion of them in urine. Odour of maple syrup.
C.F. → Disease starts in the Ist week of life. Seizures, mental retardation, failure to thrive, acidosis and coma.

TRANSMETHYLATION REACTIONS

28. WHAT ARE TRANSMETHYLATION REACTIONS? GIVE EXAMPLES.

Ans. The SAM acts as a labile methyl donor to acceptor substance.

Examples:



BIOLOGICAL FUNCTIONS OF SPECIALISED PRODUCTS OF AMINO ACIDS

29. WHAT ARE THE BIOLOGICAL FUNCTIONS OF

(*a*) Serotonin
- (*i*) Neuro transmitter (NT) in brain
- (*ii*) Induces sleep
- (*iii*) Psychic stimulator (mood elevator)
- (*iv*) Potent vasoconstrictor and stimulator of smooth muscle contraction.

(*b*) Nitric oxide (NO) ⟶
- (*i*) Potent vasodilator. A deficiency of NO is associated with hypertension.
- (*ii*) Inhibits platelet adhesion.

(*c*) Gama amino butyric acid (GABA) ⟶ Inhibitory N.T. in brain.

(*d*) Gultathione(GSH): It is present inside RBC and it maintains the integrity of RBC membrane by the action of GSH peroxidase.

$$H_2O_2 + \xrightarrow[\text{Se}]{\text{GSH-peroxidase}} 2H_2O + G - S - S - G \text{ (oxidized)}$$

2GSH (Red)

(*e*) Inactivation of insulin

$$\text{Insulin A} - \text{B} + 2\,\text{GSH} \xrightarrow{\text{Glutathione – insulin transhydrogenase}} \text{A} + \text{B} + G - S - S - G$$

(oxidised glutathione)

two chains of insulin

(*f*) Detoxification of drugs.

(g) Co-enzyme for the conversion of

$$\text{Maleyl acetoacetate} \xrightarrow{\text{GSH}} \text{Fumaryl acetoacetate}$$

(*h*)
$$Fe^{+++}\text{ (Ferric)} \xrightarrow{\text{GSH}} Fe^{++}\text{ (necessary for absorption).}$$

AMINO ACID LOAD TESTS

30. NAME THE A.A. LOAD TESTS TO ASSESS THE DEFICIENCY OF B-GROUP VITAMINS.

Ans.

	A.A. load Test		**Def. of Vitamin**
(*i*)	Histidine load test	⟶	Folic acid
(*ii*)	Tryptophan load test	⟶	B_6 Vitamin
(*iii*)	Valine load test	⟶	B_{12} Vitamin

Chapter–10

Chemistry and Metabolism of Nucleic Acids

CHEMISTRY OF NUCLEIC ACIDS

1. WHAT ARE THE PYRIMIDINE BASES PRESENT IN RNA AND DNA?

Ans.

Nucleic acid	Pyrimidine bases
RNA	Uracil, Cytosine (Uracil is exclusively present in RNA)
DNA	Thymine, Cytosine (Thymine is exclusively present in DNA).

2. WHAT ARE THE PURINE BASES PRESENT IN NUCLEIC ACIDS (RNA AND DNA)?

Ans.

Nucleic acid	Purine Bases
RNA	Adenine, Guanine
DNA	Adenine, Guanine

3. WHAT IS THE DIFFERENCE BETWEEN NUCLEOSIDE AND NUCLEOTIDE?

Ans.

Nucleoside	Nucleotide
Nitrogenous base + Pentose sugar (Phosphoric acid is absent)	Nitrogenous base + Pentose sugar + phosphoric acid (Phosphoric acid is present)

4. WHAT ARE BIOLOGICALLY IMPORTANT ADENINE NUCLEOTIDES?

Ans. ATP, ADP, AMP, cAMP.

5. WHAT ARE THE FUNCTIONS OF ATP?

Ans. The terminal two phosphates of ATP are high-energy phosphates. The break down of these phosphates results in larger quantity of free energy. Break down of ATP to ADP + P drives five important endergonic processes.

(*a*) Muscular contraction.
(*b*) Nervous excitation (transmission of nerve impulses).
(*c*) Active transport.
(*d*) Synthesis of important substances.
(*e*) Activation of metabolites.

6. WHAT IS CYCLIC AMP AND WHAT ARE ITS FUNCTIONS?

Ans. The cAMP is formed from ATP by adenylyl cyclase and destroyed by phosphodiesterase. It bears cyclic structure.

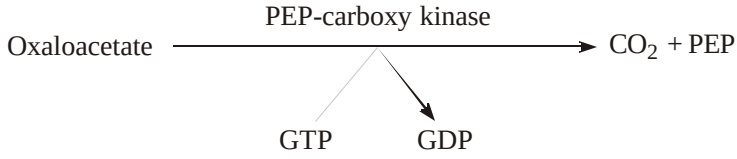
Functions: The cAMP acts as a second messenger. Many hormones act through the formation or destruction of cAMP. The action of hormone is attributed to the concentration of cAMP in the target cells.

7. WHAT ARE BIOLOGICALLY IMPORTANT GUANINE NUCLEOTIDES?

Ans. GTP, GDP, GMP, cGMP.

8. WHAT IS THE SPECIFIC ROLE OF GTP IN METABOLISM?

Ans. (*i*) GTP is required in the initiation, elongation and termination steps of protein synthesis.
(*ii*) GTP is required in the formation of phosphoenol Pyruvate (PEP) in the main gluconeogensis pathway.

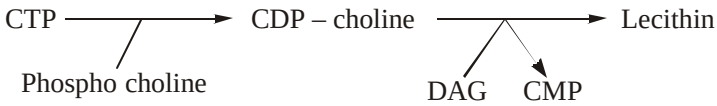


9. GIVE EXAMPLE WHERE cGMP IS UTILIZED?

Ans. Nitric oxide (NO) acts through the formation of cGMP. Nitric acid activates guanylyl cyclase, which results in the formation of cGMP. This inturn causes relaxation of smooth muscle of blood vessels (NO. → vasodilatation).

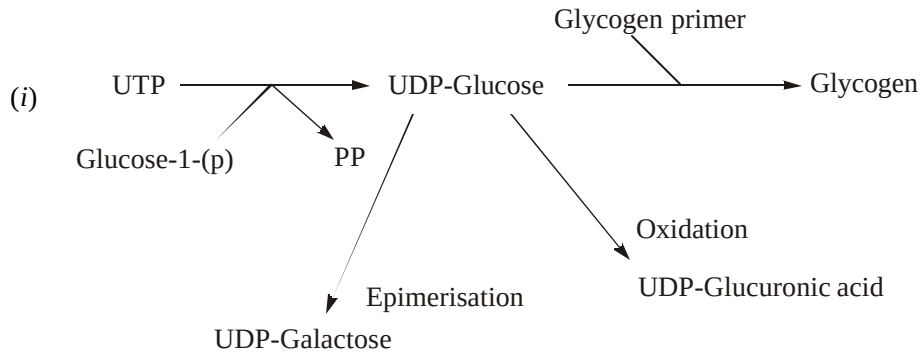
10. GIVE SPECIFIC ROLE OF CYTOSINE NUCLEOTIDES.

Ans. These are involved in the phospholipids formation



11. WHAT IS THE SPECIFIC ROLE OF URACIL NUCLEOTIDES?

Ans. Uracil nucleotides are required for the synthesis of glycogen, active glucuronic acid and in the interconversion of glucose to galactose.



12. WHAT ARE THE DIFFERENT TYPES OF RNA?

Ans. (*a*) mRNA ⟶ Messenger RNA
(*b*) tRNA ⟶ Transfer RNA
(*c*) rRNA ⟶ Ribosomal RNA
(*d*) sRNA ⟶ Small RNA

13. WHAT IS THE DIFFERENCE BETWEEN RNA AND DNA?

Ans.

RNA	DNA
(*a*) Single stranded molecule.	(*a*) Double stranded molecule and possesses double helix structure.
(*b*) Present in cytoplasm.	(*b*) Present mostly inside nucleus.
(*c*) Sugar: ⟶ ribose.	(*c*) Sugar: ⟶ Deoxy ribose.
(*d*) Purine bases : Adenine and Guanine.	(*d*) Purine bases: Adenine and Guanine.
(*e*) Pyrimidine bases cytosine and **uracil**.	(*e*) Pyrimidine bases cytosine and **thymine**.
(*f*) Adenine content is not equal to uracil and guanine content is not equal to cytosine.	(*f*) Adenine content is equal to thymine and guanine content is equal to cytosine.
(*g*) Easily destroyed by alkali.	(*g*) Alkali resistant.

14. WHAT ARE THE FUNCTIONS OF mRNA?

Ans. The mRNA acts as a messenger and carries the genetic information transcribed from DNA in the form of codons to the protein synthesising site (Ribosomes), where it acts as a template for the synthesis of protein.

15. WHAT ARE THE SALIENT FEATURES OF STRUCTURE OF mRNA?

Ans. (*i*) The mRNA is capped by 7– methyl GTP at 5 hydroxy terminus. The cap is involved in the recognition of mRNA by the translating machinery.

(*ii*) The 3 hydroxy terminus has poly – 'A' tail (20 – 250 adenylate residues). This maintains intra cellular stability and prevents the attack of 3-exonucleases.

(*iii*) It has 6-methyl adenylates and other methylated nucleotides.

16. WHAT ARE SALIENT FEATURES OF STRUCTURE OF t–RNA?

Ans. The t-RNA possesses clover leaf structure. It contains four arms.

(*a*) Acceptor arm CCA (5-3 direction) ⟶ Amino acid is attached to this arm.

(*b*) Anticodon arm. This arm has anticodon (complementary to codon bases). It recognizes codon of the template mRNA. The interaction of codon of mRNA and anticodon of t–RNA takes place on the ribosomes.

(*c*) D–arm is named due to the presence of dihydro uracil. It is required for the proper recognition of a given tRNA by its charging enzyme amino acyl tRNA synthetase.

17. WHAT ARE THE SALIENT FEATURES OF STRUCTURE OF DNA?

Ans. (*a*) DNA possesses double helix structure. Two polydeoxy nucleotide chains are twisted around one another in a right-handed double helix, similar to spiral stair case.

(*i*) Sugar and phosphates comprise hand rails.

(*ii*) Bases form the steps of stair case.

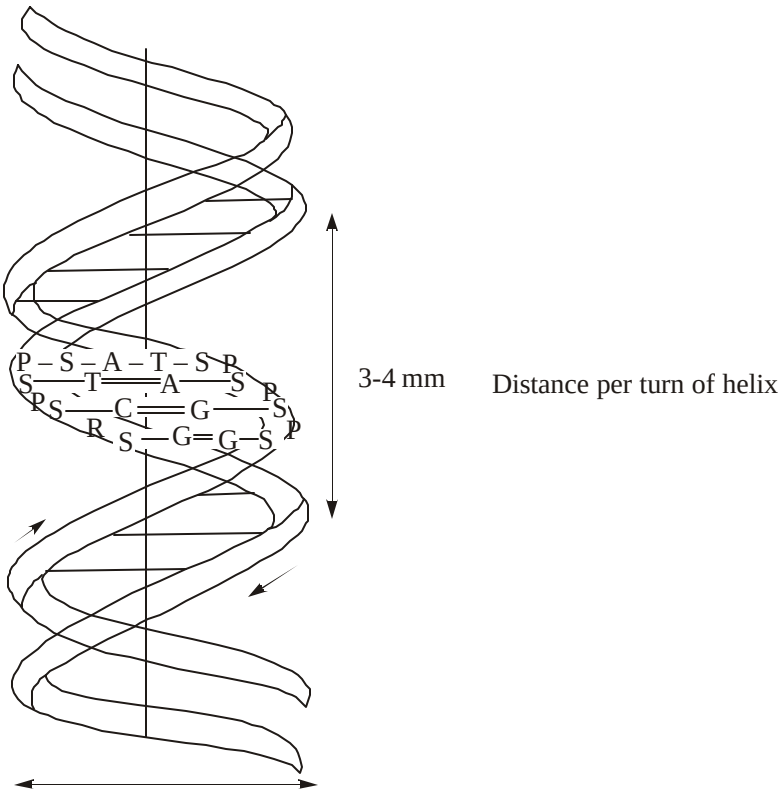


Figure 1 Double helical strucuture of DNA

(*b*) Base pairing is always maintained. A of one-chain pairs with T of opposite strand. G pairs with C.

(*c*) Two stands are held together by hydrogen bonds.
A is bonded to T by two hydrogen bonds A = T.
G is bonded to C by three hydrogen bonds G ≡ C.

(*d*) Two chains are anti-parallel to each other i.e., one strand runs in '3–5' direction and other '5–3' direction.

(*e*) The distance traveled by per turn of helix is 3.4 nm and 10 bases are present in it.

(*f*) Width of helix is 2.0 nm

METABOLISM OF PURINES AND PYRIMIDINES

18. WHAT ARE THE SOURCES OF PURINE RING?

Ans. Aspartate $\longrightarrow$ N_1
Formyl FH_4 (β-carbon of serine) $\longrightarrow$ C_2
Amide 'N' of glutamine $\longrightarrow$ N_3 and N_9
Glycine $\longrightarrow$ C_4, C_5, N_7
Respiratory CO_2 $\longrightarrow$ C_6
Methenyl FH_4 (β-carbon of serine) $\longrightarrow$ C_8

All positions are derived from amino acids, except C_6 which is formed from respiratory CO_2.

19. WHAT ARE THE SOURCES OF PYRIMIDINE RING?

Ans. Aspartate $\longrightarrow$ N_1, C_4, C_5, C_6
Glutamine as a source of NH_3 $\longrightarrow$ N_3
CO_2 $\longrightarrow$ C_2

20. NAME THE ENZYMES WHICH REGULATE SYNTHESIS OF PURINE AND WHAT ARE THE INHIBITORS OF THESE ENZYMES?

Ans. Regulation:

	Enzyme	Inhibitor
1.	PRPP-synthase	GMP and AMP
2.	PRPP-glutamylamido transferase	GMP and AMP

21. WHICH AMINO ACID AND NUCLEOTIDE IS REQUIRED FOR THE SYNTHESIS OF AMP FROM IMP AND GMP FROM IMP?

Ans.

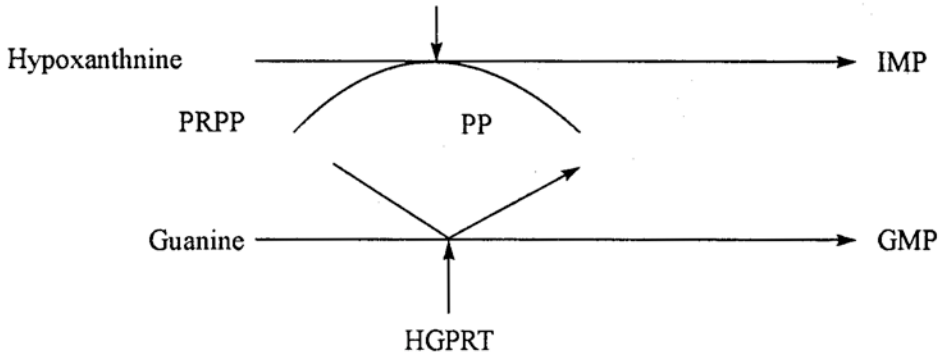
		Amino Acid	Nucleotide
(*a*)	IMP $\longrightarrow$ AMP	Aspartate	GTP
(*b*)	IMP $\longrightarrow$ GMP	Glutamine	ATP

22. WHAT IS PURINE SALVAGE PATHWAY AND WHAT IS THE SIGNIFICANCE OF THIS PATHWAY?

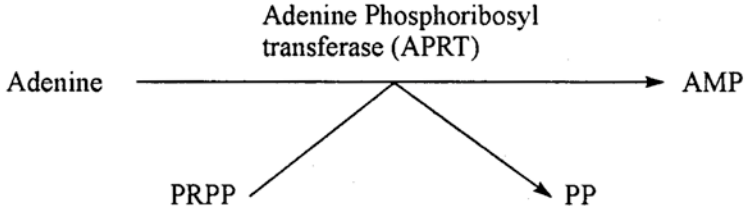
Ans. Conversion of purines to mononucleotides is called purine salvage pathway. This involves phosphoribosylation of free purine by PRPP.

Significance

1. Requires less energy than denovo synthesis.
2. In brain, polymorphonuclear leucocytes and erythrocytes, this pathway is important, as denovo synthesis of purines is absent in them.
   (*a*) HGPRT (Hypoxanthine – guanine phosphoribosyl transferase)



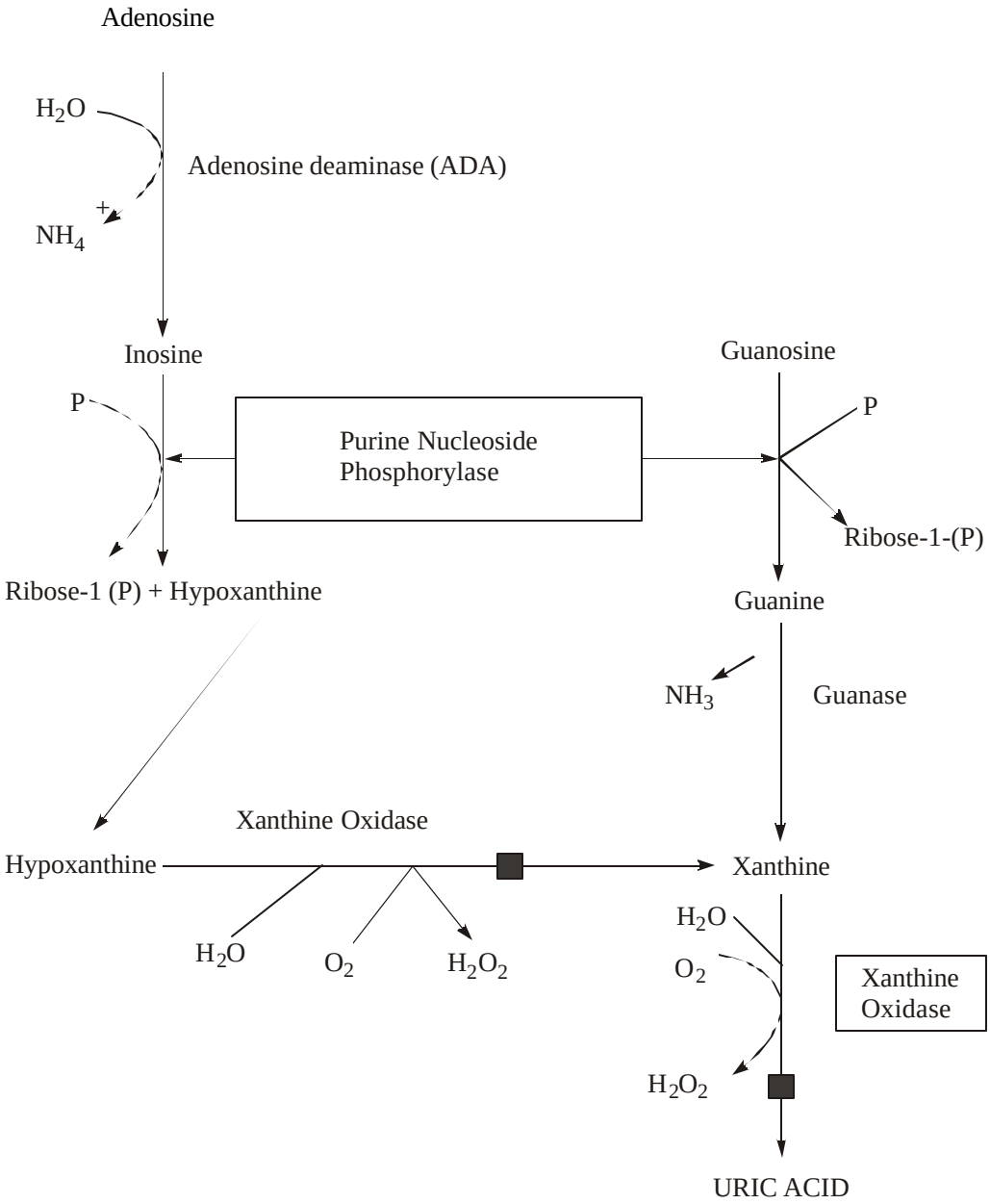
(*b*)



3. Deficiency of HGPRT results in the ↑↑ formation of uric acid due to ↑↑ level of PRPP. This in turn causes hyperuricemia and Gout. (Hyperuricemia ⟶ Gout).

23. TRACE THE PATHWAY OF URIC ACID SYNTHESIS FROM THE PURINE NUCLEOSIDES.

Ans.



- Xanthine oxidase is inhibited by allopurinol by competitive inhibition. Allopurinol is used in the treatment of Gout. This enzyme inhibits synthesis of uric acid.

24. WHAT IS NORMAL SERUM URIC ACID LEVEL?

Ans. 2-6 mg/dl.

25. WHAT IS GOUT?

Ans. Gout is caused by accumulation of monosodium urate monohydrate crystals in the joint resulting inflammation and ultimately leading to acute arthritis (First metatarsophalangeal joint of big toe is usually affected).

26. WHAT ARE THE CAUSES OF PRIMARY AND SECONDARY GOUT (HYPERURICEMIA)?

Ans. Causes of Primary Gout

(*a*) Deficiency of enzyme Hypoxanthine guanine Phosphoribosyl transferase.
(*b*) Overactivity of PRPP synthase due to resistant to feed back inhibition.
(*c*) Deficiency of enzyme Glucose-6-Phosphatase.
(*d*) Ch.Myeloproliferative or lympho proliferative disorders.
  (*i*) Polycythemia.
  (*ii*) Ch.Lymphatic.
(*e*) Risk factors (obesity and alcohol).

Causes of Secondary Gout

(*a*) Renal failure.
(*b*) Lactic acidosis (Alcohol).

27. WHAT ARE THE BIOCHEMICAL FEATURES OF GOUT?

Ans. (*a*) ↑↑ serum uric acid level >7.0 mg/dl.
(*b*) ↑↑ miscible uric acid pool (normal value 1200 mg). In Gout it is raised from 5,000 to 30,000 mg.
(*c*) Presence of mono sodium urate monohydrate crystals in the affected joint, which are observed as needle-like crystals under the polarized microscope.

28. WHAT IS LESCH NYHAN SYNDROME?

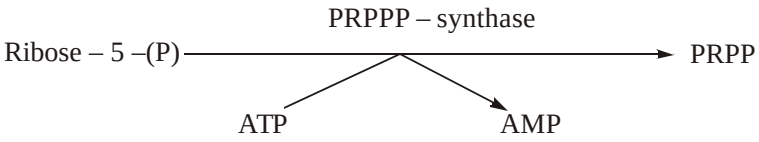
Ans. It is an X-linked disorder of purine metabolism. Lesch nyhan syndrome is caused by the complete absence of enzyme HGPRT.

The following clinical features are present:

(*a*) Hyper uricemia and Gout.
(*b*) Self-mutilation (biting of lips) and mental retardation.
(*c*) The neurological manifestation suggests the importance of salvage pathway in the brain.

29. WHAT IS PRPP? HOW IS IT SYNTHESIZED?

Ans. PRPP is Phosphoribosyl pyrophosphate. It is synthesized from ribose-5 (P)



30. WHAT IS THE ROLE OF PRPP?

Ans. PRPP is required for the synthesis of

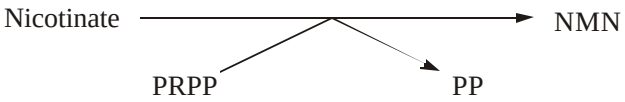
(*a*) Purines

(*b*) Pyrimidines

(*c*) Necleotide co-enzymes NAD and NADP.

31. AT WHAT STEP OF SYNTHESIS OF NAD, PRPP IS REQUIRED?

Ans. It is required for the conversion of Nicotinate to Nicotinate mono nucleotide (NMN).



32. WHAT ARE THE INHIBITORS OF PURINE SYNTHESIS AND PYRIMIDINE SYNTHESIS?

Ans. (*a*) **Inhibitors of purine synthesis**

(*i*) 6 – mercaptopurine : Inhibits conversion of adenylo succinate to AMP.

(*ii*) Azaserine: Inhibits incorporation of N_3 in the purine ring from glutamine.

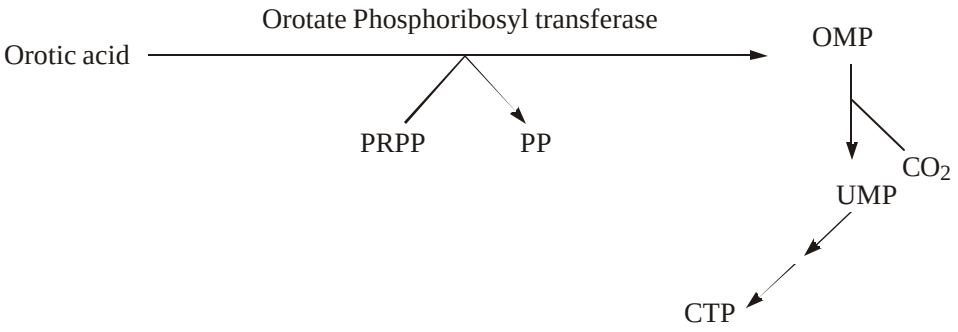
(*b*) **Inhibitors of pyrimidine synthesis**

(*i*) CTP inhibits aspartate transcarbamoylase and prevents formation of carbamoyl aspartate (feed back inhibition).

(*ii*) 5 – Fluoro uracil inhibits the synthesis of thymidylate.

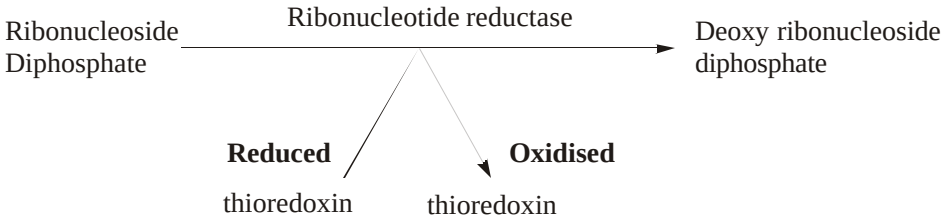
33. WHAT IS THE ROLE OF PRPP IN THE SYNTHESIS OF PYRIMIDINE?

Ans. PRPP is required in the conversion of orotic acid to orotidylic acid (OMP)... OMP later on converted to CTP by series of reactions.



34. HOW RIBOSE SUGAR OF A NUCLEOTIDE IS CONVERTED TO DEOXY RIBO-NUCLEOSIDE PHOSPHATE AND WHAT IS THE IMPORTANCE OF THIS REACTION?

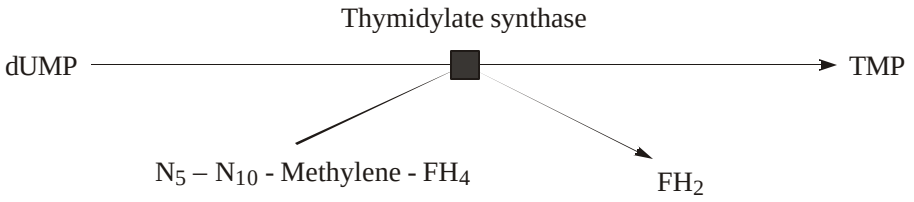
Ans.



This reaction is important for DNA synthesis.

35. HOW THYMIDYLATE IS SYNTHESIZED FROM DUMP AND WHAT IS THE IMPORTANCE OF THIS REACTION?

Ans.



This reaction is important for the synthesis of DNA.

- Thymidylate synthase is inhibited by 5-Fluoro uracil, which is used as anti-cancerous drug.

36. WHAT ARE THE END PRODUCTS FORMED FROM CYTOSINE, URACIL AND THYMINE?

Ans. (*i*) The end products formed from cytosine and uracil are :
β-alanine and $CO_2 + NH_3$.

(*ii*) The end products of thymine are :
β-Amino isobutyric acid and $CO_2 + NH_3$

Chapter–11

Molecular Biology – I

A–REPLICATION

1. WHAT IS REPLICATION?

Ans. Synthesis of daughter DNA by parent DNA during the cell division is called replication. In replication the genetic information is transmitted from the parent to offspring.

2. WHAT ARE THE CHARACTERISTIC FEATURES OF REPLICATION?

Ans. Replication is semi-conservative in nature. That means one strand of daughter DNA is derived from the parent and the other strand is newly synthesized.

3. WHAT ARE THE MAIN MATERIALS REQUIRED FOR REPLICATION AND WHAT IS THE OVER ALL REACTION?

Ans. The following materials are required for replication:

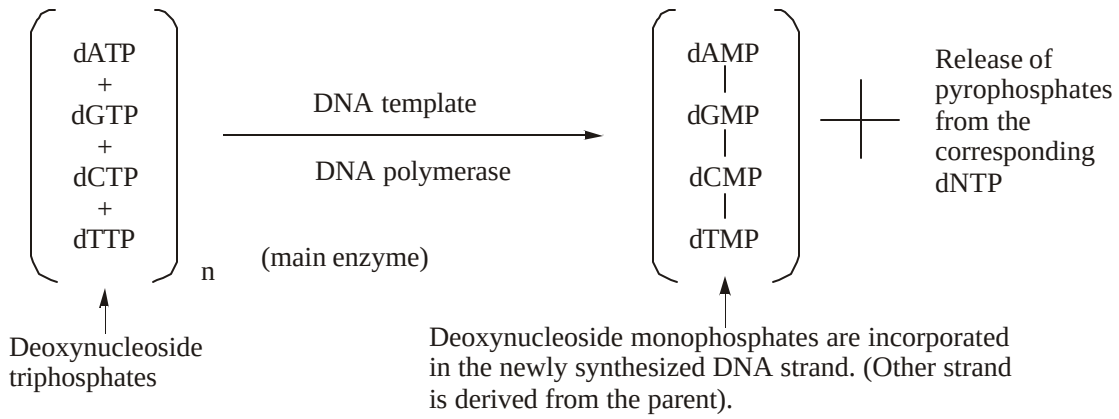
(*i*) Four types of deoxy nucleoside triphosphates (dATP, dGTP, dCTP and dTTP).

(*ii*) DNA template. Each strand of DNA acts as a template for the synthesis of complementary strand.

(*iii*) DNA polymerase.

(*iv*) Other enzymes and proteins.

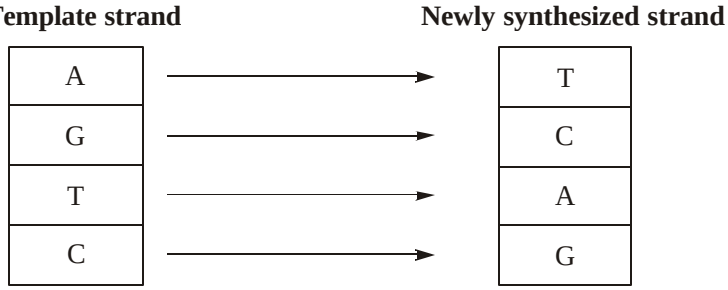
Over all reaction



In the above reaction pairing rule is strictly followed for the incorporation of dNMP.

For example :

Template strand directs ⟶ Incorporation of complementary bases in newly synthesized strand



4. WHAT ARE THE DIFFERENT TYPES OF ENZYMES AND PROTEINS REQUIRED FOR THE REPLICATION AND WHAT ARE THEIR FUNCTIONS?

Ans.

Enzymes and Proteins	Functions
(*i*) DNA polymerase	Polymerization of deoxynucleotides.
(*ii*) Helicase	Unwinding of double stranded DNA (dsDNA) to provide single stranded DNA (ssDNA) template.
(*iii*) Topoisomerase	To relieve torsional strain caused by unwinding.
(*iv*) DNA primase	Initiates synthesis of RNA primers.
(*v*) Single strand	Prevent premature reannealing of binding proteins ds DNA.
(*vi*) DNA ligase	Seals the single strand nick between the nascent chain and okazaki fragments on lagging strand.

5. WHAT ARE THE DIFFERENT TYPES OF DNA POLYMERASES IN PROKARYOTES AND EUKARYOTES AND WHAT ARE THEIR FUNCTIONS?

Ans.

Prokanryotes	*Eukaryotes*	*Functions of DNA polymerases*
I	a	Synthesis of lagging strand.
II	ε	DNA proof reading and repair.
	β	DNA repair.
	γ	Mitochondrial DNA synthesis.
III	δ	Leading strand synthesis.

6. WHAT ARE THE MAIN STEPS OF REPLICATION?

Ans. (*a*) Identification of origin of replication and binding of ori-binding protein at this site.

(*b*) Unwinding and separation of two strands of DNA, so that each strand serves as a template for the synthesis of new complementary stand.

(*c*) Formation of the replication fork by binding of required enzymes and proteins.

(*d*) Initiation of DNA synthesis and elongation. In the elongation step polymerization of new strand takes place from 5′ to 3′ direction and the template strand is read from 3′ to 5′ direction.

(*e*) The replication fork proceeds as DNA synthesis occurs continuously on leading strand by DNA polymerase and discontinuously on the lagging strand as okazaki fragments.

(*f*) When synthesis is complete in lagging strand the RNA pieces are removed and gaps are filled by deoxynucleotides (dNT) and the fragments are ligated by the DNA polymerase.

(*g*) Proof reading takes place by DNA polymerase II in prokaryotes and polymerase E in eukaryotes.

(*h*) Reconstitution of chromatin structure.

7. WHAT ARE OKAZAKI PIECES? HOW ARE THEY FORMED?

Ans. The small DNA molecules consisting of 150–250 nucleotides attached to RNA primer in lagging strand are called okazaki fragments.

An RNA primer consisting of 10–200 nucleotides length is synthesized by the DNA primase. By the action of DNA polymerase the RNA primer carries nucleophiric attack on the deoxynucleoside triphosphates and the corresponding deoxynucleoside monophosphates are joined to RNA primer by following the pairing rule. After the formation of many okazaki pieces the RNA primers are removed.

8. WHAT ARE THE MAIN CAUSES OF DAMAGE OF DNA AND HOW IS IT REPAIRED?

Ans. Main causes of damage of DNA

(*a*) Base alteration by UV light irradiation.

(*b*) Chain breaks by ionizing radiation and radio active disintegration.

Main steps of repair

(*a*) Removal of wrong base by DNA glycosylase.

(*b*) Endonuclease cuts the backbone near the defect (adjacent bases are removed).

(*c*) The gaps are filled by the correct bases by the action of DNA polymerase (repair enzyme) and DNA ligase.

9. WHAT IS XERODERMA PIGMENTOSUM?

Ans. It is an autosomal recessive genetic disorder. There is a defect of nucleotide excision repair in this condition.

The clinical features are :

Sensitivity to sunlight which causes blisters on the skin. There is a risk of developing skin cancer and death occurs in the second decade of life.

10. WHAT IS CELL CYCLE ? IN WHICH PHASE OF CELL CYCLE THE SYNTHESIS OF DNA OCCURS?

Ans. The events occurring during the period between two mitotic divisions is called cell cycle. Synthesis of DNA occurs in 'S' phase of cell cycle.

11. WHAT ARE THE DIFFERENT PHASES OF CELL CYCLE?

Ans. The following are the different phases of cell cycle.

(*i*) G_1phase (gap –1 period –12 hours).
(*ii*) 'S' phase (synthesis phase–6 to 8 hours).
(*iii*) G_2 phase (gap –2–4 to 5 hours).
(*iv*) M phase (mitotis–1 hours).

Total period in cell cycle is 20–22 hours duration.

MOLECULAR BIOLOGY – I

B–TRANSCRIPTION

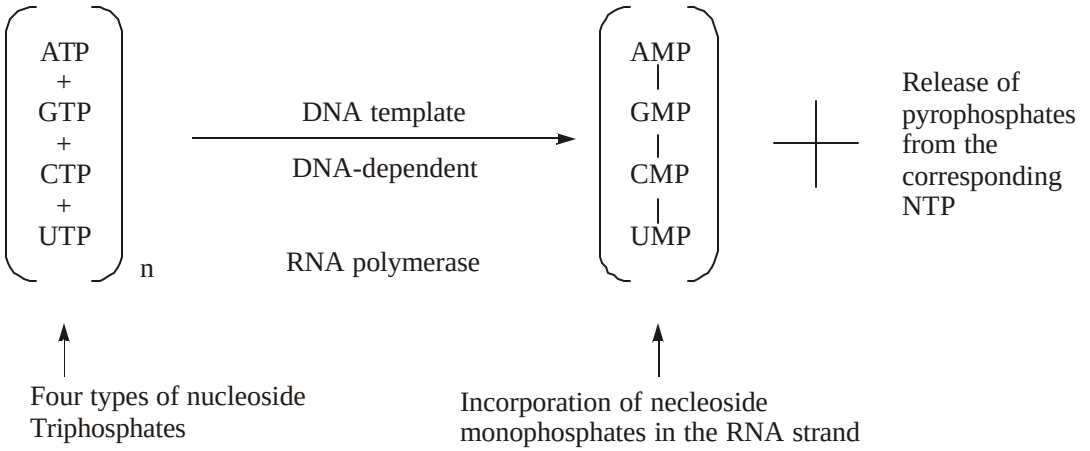
1. WHAT IS TRANSCRIPTION?

Ans. Synthesis of RNA by nuclear DNA is called as transcription.

2. WHAT ARE THE MAIN MATERIALS REQUIRED FOR TRANSCRIPTION AND WHAT IS THE OVER ALL REACTION?

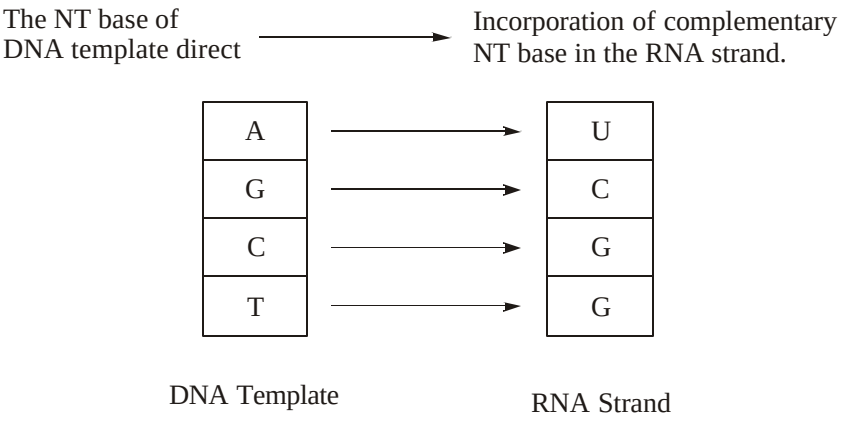
Ans. (*a*) DNA template.
(*b*) DNA dependent RNA polymerase.
(*c*) Four different types of nucleoside triphosphates (ATP, GTP, CTP, UTP).
(*d*) Sigma factor.
(*e*) Rho factor.

Over all reaction



In the above reaction pairing rule is strictly followed:

For Example



3. WHAT ARE THE DIFFERENT TYPES OF RNA POLYMERASES IN EUKARYOTES AND WHAT ARE THEIR FUNCTIONS?

Ans.

Polymerase of Eukaryotes	Functions
I or A	Synthesis of rRNA
II or B	Synthesis of mRNA
III or C	Synthesis of tRNA

4. WHAT IS THE INHIBITOR OF RNA POLYMERASE II?

Ans. Amanitin (Mushroom poison).

5. WHAT ARE THE SUBUNITS AND FACTORS PRESENT IN BACTERIAL RNA POLYMERASE?

Ans. Bacterial RNA polymerase contains two α, two β subunits, one sigma factor and two Zn^{++} molecules.

Functions: β-subunit fixes to the initiation site. The sigma factor recognizes the promoter site, of DNA template.

6. WHAT ARE THE NAMES OF TWO STRANDS OF DNA?

Ans. (*a*) *Template strand*: The strand that is transcribed in to a RNA molecule.

(*b*) *Coding strand*: The other DNA strand is called coding strand, because it has the exact sequence of nucleotides of primary transcript (except T in place of U).

7. DEFINE THE TERMS: PROMOTER SITE, TRANSCRIPTION UNIT AND PRIMARY TRANSCRIPT?

Ans. (*a*) **Promoter site:** It is the region of DNA where RNA polymerase binds to it.

(*b*) **Transcription unit:** The region of DNA present between promoter site and terminator site is called the transcription unit.

(*c*) **Primary transcript:** The RNA product which is synthesized from 5′ to 3′ direction is called primary transcript.

8. WHAT ARE THE MAIN STEPS OF RNA SYNTHESIS IN BACTERIA?

Ans. (*a*) **Template binding:** RNAP binds to promoter site of DNA. Sigma factor helps in the recognition of promoter site and binding of RNAP.

(*b*) **Initiation:** RNAP helps in the incorporation of Ist nucleotide (NT) in the RNA strand with the help of DNA template.

(*c*) **Chain elongation :** Successive residues of nucleotides are added by following pairing rule (i.e., complementary bases containing NT are added).

(*d*) **Chain termination:** When RNAP comes across Rho factor termination occurs. Rho factor recognizes termination signal. In the termination newly synthesized RNA and RNA polymerase are released from the template.

9. WHAT IS hnRNA?

Ans. The hnRNA is called heterogeneous nuclear RNA. It is a precursor form of mRNA, formed as native RNA in the nucleus as primary transcript. It's molecular weight is $>10^7$ where as mol.wt. of mRNA is $< 2 \times 10^6$. On further processing it is converted to mRNA.

10. HOW hnRNA IS CONVERTED TO mRNA?

Ans. The hnRNA undergoes post-transcriptional modification to form mRNA. The following changes take place during post-transcriptional modification.

(*i*) Addition of cap by a 7 methyl GTP at 5 hydroxyterminus.

(*ii*) Attachment of poly 'A' tail consisting of 20–250 nucleotides in length at 3 hydroxy terminus.

(*iii*) Internal nucleotides are methylated. Methylation takes place at N_6 of adenine residues and 2 hydroxy group of ribose.

(*iv*) Splicing: The hnRNA has two types of sequences of nucleotides.

(*a*) **Exons:** Sequence of NTs involved in the coding of amino acids.

(*b*) **Introns:** Intervening sequence of nucleotides which do not code any amino acid.

In the splicing process introns are removed and exons are ligated to form the mRNA molecule. Afterwards the mRNA molecule is transported to cytoplasm.

11. WHAT ARE THE MAIN EVENTS THAT OCCUR IN THE POSTTRANSCRIPTIONAL MODIFICATION?

Ans. In the post-transcriptional modification the processing of RNA primary transcript occurs primarily within nucleus.

The main events are:

(*a*) Nucleolytic and ligation reactions (splicing of exons).

(*b*) Terminal additions (addition of cap and poly 'A' tail in mRNA and attachment of CCA terminal to the tRNA).

(*c*) Nucleoside modification: Methylation. Deamination to produce unusual bases.

12. HOW THE PRECURSOR OF rRNA UNDERGOES PROCESSING?

Ans. The primary transcript of 45-s precursor of rRNA undergoes processing in the nucleolus to form 28s-rRNA, 18s-rRNA and 5.8s-RNA. The 5S-rRNA has a separate precursor.

In the processing the precursor RNA undergoes nucleolytic and methylation reactions. Nearly half the original primary transcript undergoes nucleolytic reaction.

13. WHAT IS TATA BOX OR PRIBNOW BOX AND WHAT IS ITS IMPORTANCE?

Ans. In bacterial DNA about 10 base pair up stream (–10) from the promoter site there is special sequence of nucleotides from 5 $\rightarrow$3 direction these are 5' TATAAT $^{3-}$. These have low melting temperature.

The TATAAT box acts as a signal for initiation of transcription.

14. WHAT ARE ENHANCERS AND SILENCERS?

Ans. Enhancers the rate of transcription and silencers $\downarrow\downarrow$ the rate of transcription. $\uparrow\uparrow$ They may be present either upstream or down stream of the promoter site point (usually about 1000 bp away).

15. WHAT IS REVERSE TRANSCRIPTASE?

Ans. Reverse transcriptase catalyzes the synthesis of DNA from a single stranded RNA template in retrovirus. This enzyme is also called as RNA dependent DNA polymerase.

Steps of DNA synthesis

Reverse transcriptase first synthesizes a DNA–RNA hybrid utilizing RNA template. In the next step ribonuclease – H degrades the RNA strand. The remaining DNA strand acts as a template and synthesizes complementary DNA strand and ultimately DNA molecule containing RNA genome is formed. HIV virus causing AIDS is a retrovirus.

16. WHAT ARE THE INHIBITORS OF RNA SYNTHESIS?

Ans.
1. Rifampicin (anti-tuberculous drug) binds with b-submit of prokaryotic RNAP and prevents transcription.
2. Amanitin inhibits eukaryotic RNA polymerase II and prevents transcription.
3. Actinomycin-D and mitomycin intercalate with DNA strands and prevent the transcription.

MOLECULAR BIOLOGY – I

C–TRANSLATION

1. WHAT IS THE STRUCTURE OF MAMMALIAN RIBOSOME AND WHAT ARE ITS FUNCTIONS?

Ans. Mammalian ribosome has Mol.wt 4.2×10^6 and sedimentation velocity of 80s (swedberg units). It is a cytoplasmic nucleoprotein and serves as the machinery for the synthesis of proteins. It has two subunits (60s and 40s).

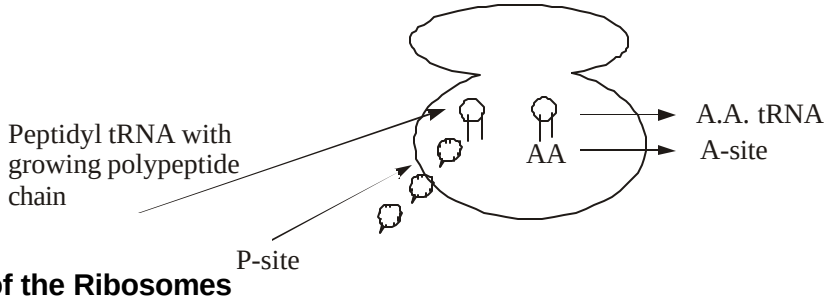
(*a*) 60s subunit (50 specific polypeptides)
Mol wt. 2.8×10.6
rRNA present: 5s, 5.8s and 28s

(*b*) 40s subunit (30 specific polypeptides)
Mol. wt 1.4×10^6
rRNA present: 18s

The two halves of ribosome consists of

A-site (Rt. half) $\rightarrow$ The aminoacyl tRNA enters the A site and attaches to this site.

P-site (left half) $\rightarrow$ The growing polyseptide chain with peptidyl tRNA attaches to this site.



Functions of the Ribosomes

The mRNA and tRNA interact on the ribosome and translate the message into a specific protein molecule.

2. WHAT IS rRNA AND WHAT ARE ITS FUNCTIONS?

Ans. The rRNA is called ribosomal RNA and these are present in ribosome. There are different types of rRNA present in the two subunits of ribosome. 18-s rRNA is present in 40-s ribosome and 5s, 5.8s and 28s rRNAs are present in 60-s ribosome.

Functions

1. rRNA are required for ribosomal assembly.
2. These help in binding of mRNA to ribosome.
3. The 28s rRNA performs peptidytransferase activity in the formation of peptide bond.

3. WHAT IS snRNA AND WHAT ARE ITS FUNCTIONS?

Ans. These are small nuclear RNAs present in the nucleolus as ribonucleic proteins.

Functions

1. The snRNAs are mostly involved in the processing of hnRNA to mRNA.
2. The snRNAs U1, U2, U4, U5 and U6 are involved in the removal of introns.
3. The U4 and U6 are required for poly – 'A' tail processing.

4. WHAT IS CODON AND WHAT IS ANTICODON?

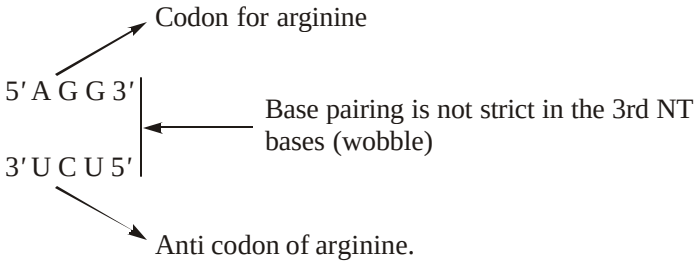
Ans. **CODON:** The sequence of three nucleotides from 5′ → 3′ direction on the mRNA strand is called codon. The codon of mRNA codes particular amino acid to be incorporated in the synthesis of proteins. The collection of codons is called genetic code.

ANTICODON: The sequence of three nucleotides from 3′ → 5′ direction in the anticodon arm is called anticodon. The nucleotides of anticodon are complementary to codon. Both the codon and anticodon interact on the ribosome by base pairing and this interaction is required for the polymerization of amino acids. The specificity of tRNA is due to the presence of anticodon in it. There are 20 different tRNAs having 20 different anticodons in them.

5. WHAT IS WOBBLE?

Ans. When the pairing of codon and anticodon in the 3rd nucleotide is not strict it is called wobble.

Example:



6. WHAT ARE THE SALIENT FEATURES OF GENETIC CODE?

Ans. (*i*) Genetic code is **degenerate**; multiple codons (more than one codon) coding the same amino acid is called degeneracy of codons. In general the 3rd NT in codon is not important.

Example:

Serine ⟶ 6 codons.

(*ii*) Genetic code is **unambiguous**: The given specific codon codes only one amino acid.

For Example:

UUU ⟶ codes only phenylalanine

AAA ⟶ codes only lysine

(*iii*) The genetic code is non-overlapping. The genetic code during the process of protein synthesis does not involve any overlap of codons.

(*iv*) Genetic code is not punctuated.

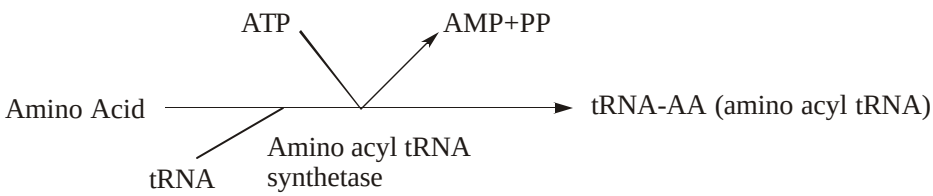
Once reading is commenced at a specific codon there is no punctuation between codons and the message is read continuously until it comes across the chain terminating codon.

(*v*) The genetic code is universal. This means; the triplet code is same in all the species.

7. HOW AMINOACYL tRNA IS FORMED?

Ans. For the polymerization of amino acids in the synthesis of proteins on the ribosome, it requires formation of aminoacyl tRNA.

The reaction for the formation of tRNA – AA is given below:



8. WHAT ARE THE MAIN STEPS OF PROTEIN SYNTHESIS?

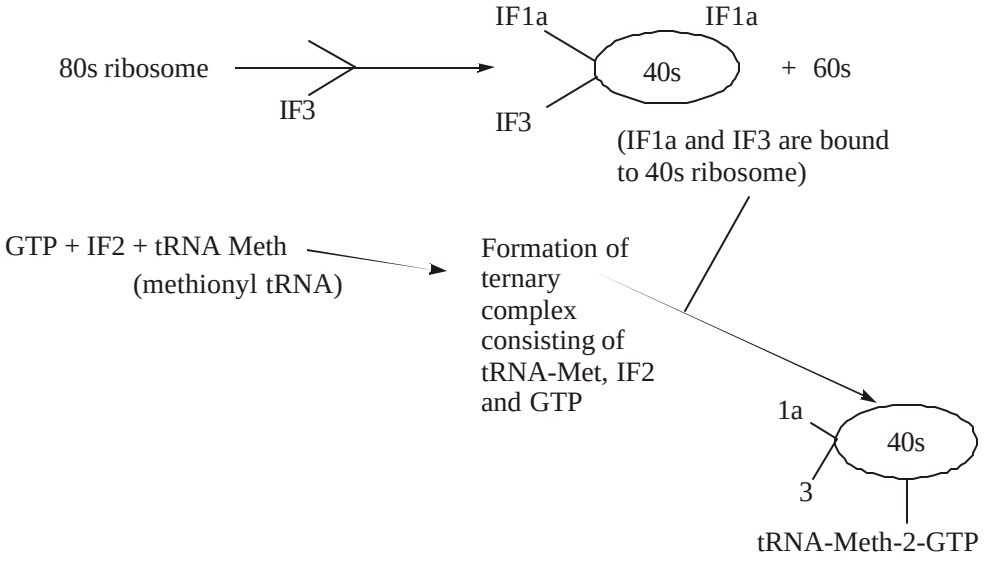
Ans. (*a*) Initiation.
(*b*) Elongation.
(*c*) Termination.
(*d*) Post translational modification.

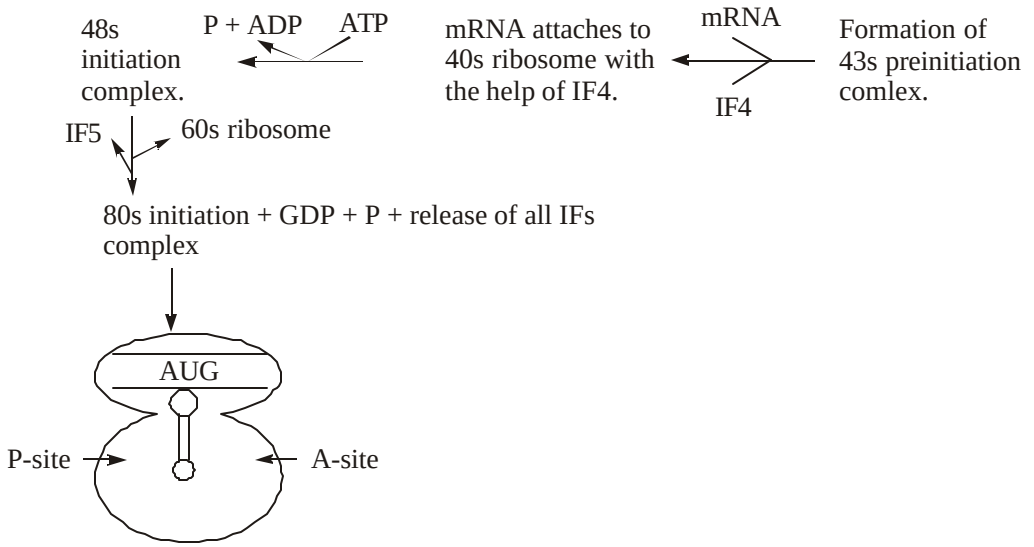
9. WHAT ARE THE MATERIALS REQUIRED FOR THE INITIATION STEP OF TRANSLATION IN EUKARYOTES?

Ans. (*a*) Initiation factors, 1a, 2, 3, 4, 5.
(*b*) 80s ribosome.
(*c*) ATP for the formation of 48s initiation complex.
(*d*) GTP for the formation of 80s initiation factor.

10. WHAT ARE THE MAIN STEPS OF INITIATION AND BRIEFLY MENTION THE REACTIONS THAT TAKE PLACE IN THEM?

Ans. MAIN STEPS
(*a*) Dissociation of 80s ribosome to 40s and 60s.
(*b*) Formation of 43s pre-initiation complex.
(*c*) Formation of 48s initiation complex.
(*d*) Formation of 80s initiation complex.





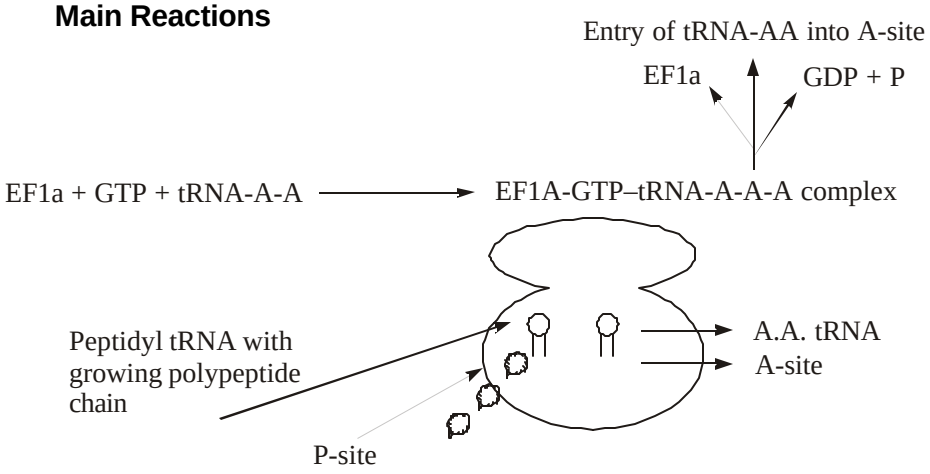
11. NAME THE FACTORS AND ENZYME REQUIRED FOR ELONGATION PROCESS OF TRANSLATION AND MENTION BRIEFLY THE REACTIONS INVOLVED IN IT.

Ans. (*a*) Elongation factors 1a and 2.
(*b*) GTP (2 molecules).
(*c*) Peptidyl transferase (the activity is present in 28s rRNA).

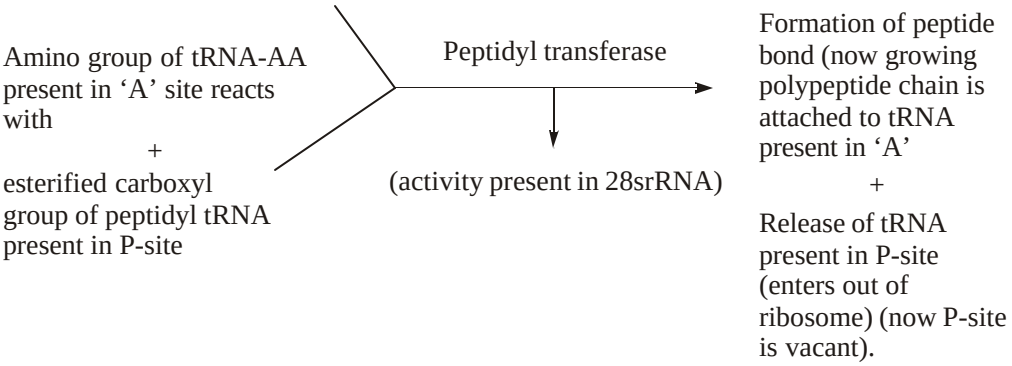
12. WHAT ARE THE STEPS OF ELONGATION PROCESS OF TRANSLATION?

Ans. (*a*) Binding of tRNA-AA to the A-site of ribosome.
(*b*) Peptide bond formation.
(*c*) Translocation.

Main Reactions

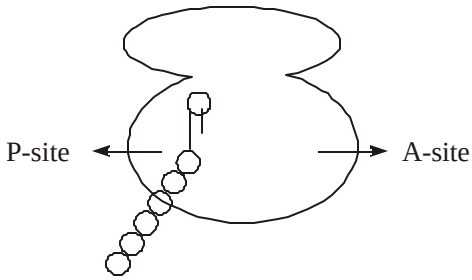


Formation of Peptide Bond



Translocation

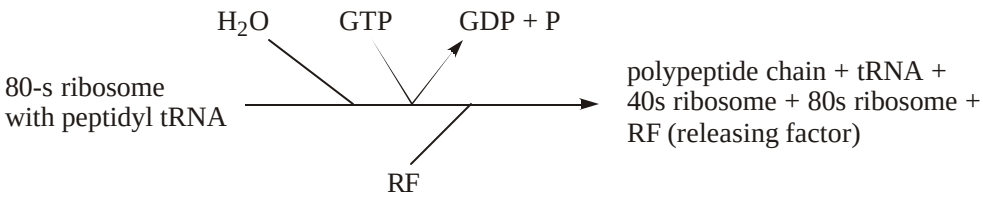
By translocation ribosome moves to next codon and peptidyl tRNA is shifted from 'A' site to 'P' site, so that 'A' site becomes vacant. For the translocation GTP is hydrocysed to GDP + P and EF_2 takes part in the reaction.



13. HOW TERMINATION OCCURS IN THE TRANSLATION PROCESS?

Ans. When ribosome comes across the chain terminating codon of mRNA (UUA, UAG, UGA) the releasing factor (RF) recognizes termination signal of protein synthesis in conjunction with GTP and water molecule and enter the 'A' site where it causes the hydrolysis of peptide bond releasing the polypeptide chain, tRNA and subunits of ribosome.

The Overall Reaction



14. WHAT IS POST-TRANSLATIONAL MODIFICATION?

Ans. The native protein further undergoes modification after termination. The native protein in the form of pre-pro protein is converted to actual protein by the removal of pre and pro peptides and it may further undergoes hydroxylation, glycosylation, methylation, carboxylation and acetylation reactions to form actual protein.

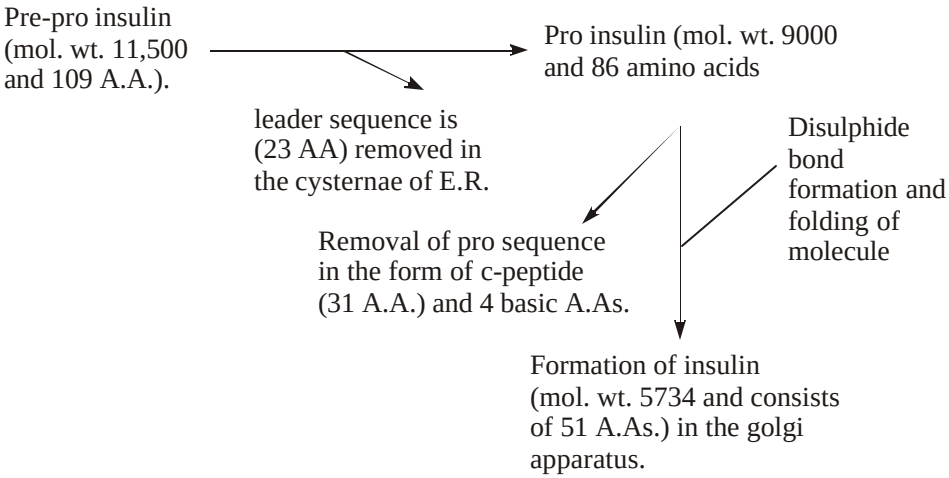
For Example

The native proteins in the form of pre-pro insulin and pre-pro collagen are converted to insulin and collagen by the following mechanism.

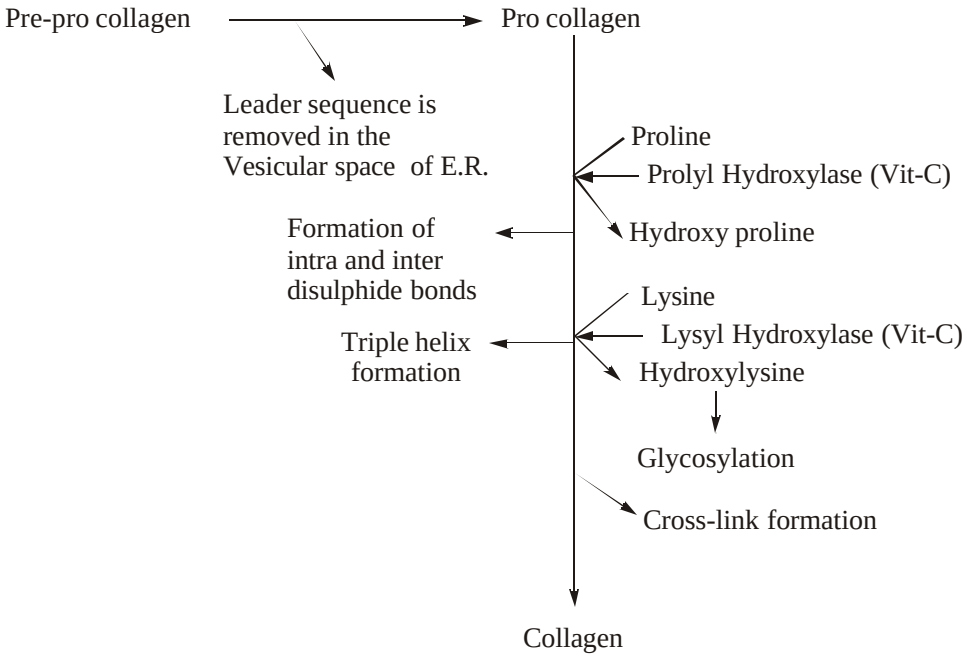
(*i*) In the pre-pro insulin the pre sequence of amino acids are called leader sequence consisting of 23 amino acids (A.A.) and pro sequence has 35 amino acids and actual insulin has 51 amino acids.

Removal of Leader Sequence

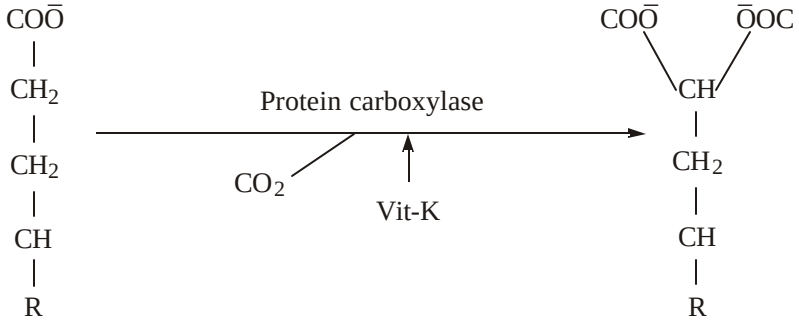
Leader sequence directs the molecule into cysternae of E.R. and then it is removed by hydrolytic cleavage.



2nd Example : Conversion of Pre-Pro Collagen to collagen



3rd Example: Conversion of Precursor of Prothrombin (PT) To Active Prothrombin



Precursor of PT (consisting of γ-glutamate residues (glu)

Active PT (γ-carboxy glutamate (glu residues are converted to gla residues)

15. WHAT ARE THE INHIBITORS OF PROTEIN SYNTHESIS IN PROKARYOTES AND EUKARYOTES AND WHAT IS THEIR MODE OF ACTION?

Ans. Inhibitors of prokaryotes

Inhibitor	Mode of action
(*a*) Tetracycline →	Binds to the 30s ribosome and prevents the attachment of tRNA-AA to 'A' site.

(*b*) Chloramphenicol → Inhibits peptidyl transferase activity.
(*c*) Erythromycin
(*d*) Clindamycin → Inhibits translocation.
(*e*) Streptomycin → Binds to 30s ribosome and inhibit the initiation step of translation.

Inhibitors of Eukaryotes

Inhibitor	Mode of Action
(*a*) Puromycin	Structural analog of tyrosinyl tRNA and causes premature termination of proteins.
(*b*) Diphtheria toxin	The fragment 'A' of diphtheria toxin causes transfer of ADP-ribose moiety from NAD to EF2 and inhibits protein synthesis.
(*c*) Ricin (from castor bean)	Inactivates 28s-rRNA and prevents protein synthesis.

16. WHAT ARE THE DIFFERENT TYPES OF MUTATION?

Ans. There are mainly two types of mutation

(*a*) Point mutation
- Transition (change of one purine to other purine or one pyrimidine to other pyrimidine bases).
- Transversion (change of purine to pyrimidine bases).

(*b*) Frame shift mutation
- Deletion of base
- Insertion of base.

17. WHAT ARE THE EFFECTS OF POINT MUTATION ON PROTEIN SYNTHESIS?

Ans. There are three effects of mutation on protein synthesis:

(*a*) No detectable effect	Same amino acid is coded due to degeneracy of genetic code.
(*b*) Missense effect	Three effects.
(*c*) Premature termination of proteins	Due to change of codon to chain terminating codon.

MISSENSE EFFECT: (Examples: abnormal haemoglobins)

(*i*) Physiological function is acceptable	Normal Hb β-chain 61 lysine Codon → AAA	Abnormal Hb-Hikari β-chain 61-asparazine AAU
(*ii*) Physiological function partially acceptable	Hb – A (normal) β-chain 6 → glutamic acid Condon → GAA	Hb-S (Sickle cell) β-chain 6 → valine GUA
(*iii*) Physiological function is unacceptable	Hb – A $(Fe)^{++}$ α-chain 58 → Histidine Codon → CAU →	Hb–M $(Fe)^{+++}$ α-chain 58 → Tyrosine UAU

Due to change of 58th distal histidine Ferrous iron (Fe^{++}) is converted to Ferric (Fe^{+++}) iron.

Chapter–12

Molecular Biology – II

CONTROL OF GENE EXPRESSION

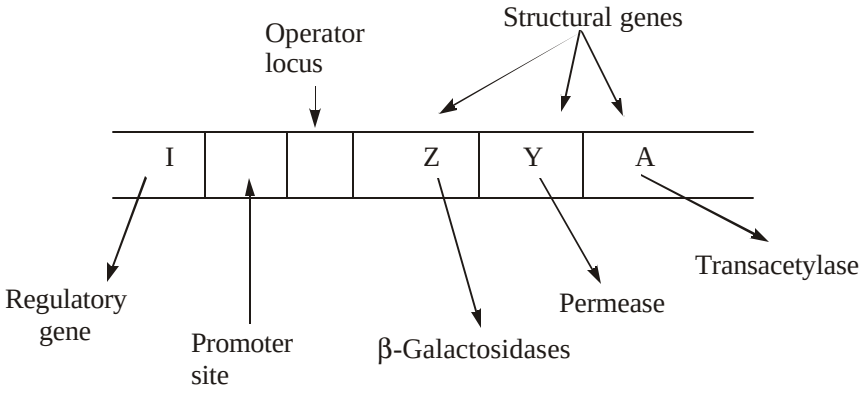
1. WHO PROPOSED OPERON HYPOTHESIS?

Ans. Jacob and Manod in 1961.

2. WHAT ARE THE COMPONENTS PRESENT IN THE LAC OPERON MODEL OF E.COLI?

Ans. The following components are present in sequential order in Lac operon model.

(*a*) Regulatory genes (I).
(*b*) Promoter site.
(*c*) Operator locus.
(*d*) Structural genes (Z, Y and A genes).



3. DEFINE THE TERMS 'REPRESSION' AND 'DEREPRESSION'.

Ans. In the presence of repressor molecule the expression of gene is decreased. This causes prevention of transcription and synthesis of proteins. This is called repression.

Reverse is true in case of derepression. In the derepression the repressor molecules become inactive in the presence of inducer and which in turn causes transcription and synthesis of proteins.

4. WHAT ARE THE FACTORS WHICH MODIFY THE GENE EXPRESSION?

Ans. Repressor molecules decrease the rate of gene expression whereas inducer or activator increase the rate of gene expression.

5. WHAT IS CATABOLITE GENE ACTIVATOR PROTEIN (CAP) AND WHAT IS ITS ACTION?

Ans. Catabolite gene activator protein (CAP) forms complex with Camp and this complex is required for the initiation of transcription.

6. WHAT IS CATABOLITE REPRESSION?

Ans. In the presence of glucose, Camp is not formed and therefore due to lack of CAP-Camp complex it causes repression by preventing transcription.

7. EXPLAIN THE OPERON CONCEPT IN THE ABSENCE OF INDUCER AND IN THE PRESENCE OF INDUCER?

Ans. **In the Absence of Inducer**

In the absence of inducer the regulatory gene produces repressor molecules which are attached to operator locus and prevents the binding of RNAP to promoter site. Thus it inhibits transcription and translation processes (no synthesis of enzymes).

In the Presence of Inducer

In the presence of inducer, (lactose), the inducer inactivates repressors and then by favouring transcription, it causes synthesis of β-galactosidase, transace and transacetylase enzymes.

Chapter–13

Molecular Biology – III

RECOMBINANT DNA TECHNOLOGY

1. WHAT ARE RESTRICTION ENDONUCLEASES?

Ans. Restriction endonucleases are also called restriction enzymes. These are endonucleases that cut both strands of DNA at specific sites consisting of specific base sequences. Presence of these enzymes in the given bacterium restricted the growth of bacteriophage (virus).

The names of restriction enzymes are derived from the bacteria from which they are isolated. For instance ECORI (From Escherichia Coli)

Ist letter corresponds to genus (E).

Next two letters indicate species (Co)

Next letter R indicates the strain.

Roman letter I indicates the order of discovery.

		Sequence cleaved
		⇓
1.	E.Co R-I	G A A T T C
	(Escherichia coli)	C T T A A G
		⇓ ⇑
2.	Bam HI	G G A T C C
	(Bacillus amylo	C C T A G G
	liquifaciens)	⇑

2. WHAT IS CHIMERIC DNA MOLECULE?

Ans. A DNA molecule containing sequences derived from two different species i.e., from plasmid DNA and human DNA.

3. WHAT IS A VECTOR?

Ans. A vector is a carrier of DNA molecule. It may be plasmid or bacteriophage or cosmid into which foreign DNA can be introduced for the purpose of cloning.

4. WHAT IS CLONING?

Ans. A clone is a large population of identical bacteria or cells that arise from a single parental cell. Cloning allows the production of a large number of DNA molecules.

5. WHAT IS SOUTHERN BLOT TRANSFER TECHNIQUE?

Ans. This is a technique used for transferring DNA from an agarose gel to nitrocellulose filter on which the DNA can be detected by a suitable probe of complementary DNA.

6. WHAT IS NORTHERN BLOT TRANSFER TECHNIQUE?

Ans. This is a technique used for transferring RNA from an agarose gel to a nitrocellulose filter, on which the RNA can be detected by suitable probe cDNA.

7. WHAT IS WESTERN BLOT TRANSFER TECHNIQUE?

Ans. This is a technique used for transferring protein from gel to nitro cellulose filter, on which the protein can be detected by a suitable probe antibody. Western blot transfer technique is most commonly used in the confirmation of detection of HIV infection in patients serum.

8. WHAT ARE THE MAIN STEPS OF RECOMBINANT DNA TECHNOLOGY (RDT)?

Ans. The following are the main steps of RDT.

(*a*) Preparation of specific human gene.

(*b*) Insertion of human gene into vector to form chimeric DNA molecule.

(*c*) Cloning of chimeric DNA.

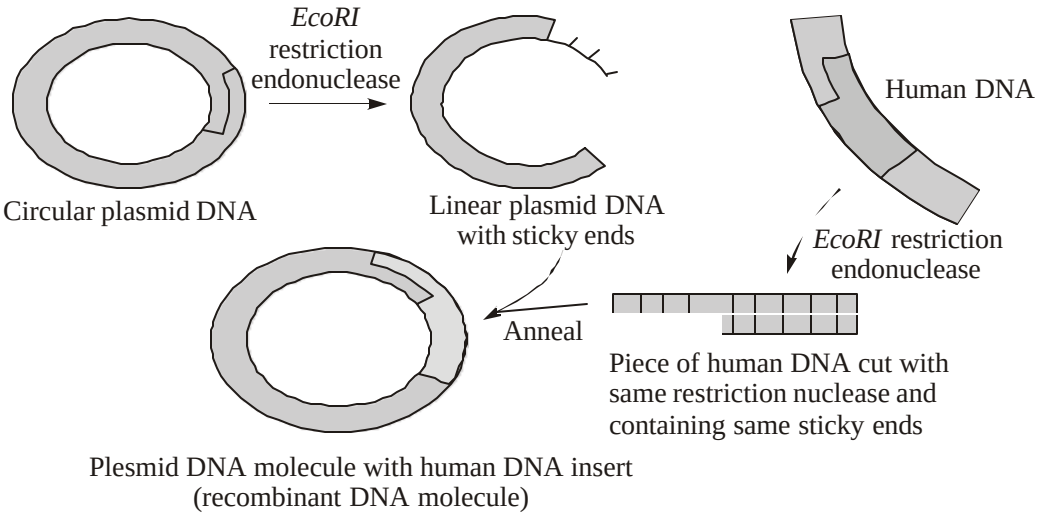
(*d*) Transfection of vector into the host.

9. HOW CHIMERIC DNA IS PREPARED?

Ans. (*a*) First the plasmid DNA is treated with restriction enzyme (ECO-RI) which cuts at specific sequence of NT bases.

(*b*) The human DNA is also treated with the same restriction enzyme which also cuts same sequence of NT bases.

(*c*) Cut piece of human DNA is inserted into cut piece of plasmid and annealed and joined by DNA ligase. This results in the formation of recombinant DNA molecule consisting of plasmid DNA plus cutpiece of human DNA. This is called chimeric DNA molecule.



10. WHAT IS POLYMERASE CHAIN REACTION (PCR)?

Ans. It is an enzymatic technique used for amplification of target DNA sequences by repeated copying. By PCR, the DNA can be amplified from a single strand of hair or single drop of blood or semen to several folds, so that it enables for the easy detection.

Materials Required for PCR

1. DNA sample (target sequence of DNA to be amplified).
2. Two oligonucleotide primers.
3. Heat stable DNA polymerase obtained from thermus aquaticus.

Steps of PCR

1. Heating of DNA sample for the separation of two strands.
2. Primers are allowed to bind to DNA.
3. Each strand of DNA acts as template and synthesizes complementary strand by the action of DNA polymerase (Taq polymerase).
4. Above steps are repeated to several cycles i.e., heat denaturation, annealing and polymerization etc.

Clinical Applications of PCR

1. The DNA of hair, single drop of blood or semen is amplified by PCR to solve medico legal problems in forensic medicine.
2. To detect latent virus (infectious agents like hepatitis C etc.).
3. To make prenatal genetic diagnosis.

Recombinant DNA Technology (RDT)

11. WHAT ARE THE IMPORTANT APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY?

Ans. The important applications of recombinant DNA technology are that by making use of this technology.

(*a*) Human proteins like insulin and growth hormones can be produced.

(*b*) Proteins for vaccines (hepatitis B) and diagnostic tests (AIDS) can be obtained.

(*c*) It offers a rational approach to understand the molecular basis of diseases like sickle cell disease, thalassemias and cystic fibrosis etc.

Chapter–14

Xenobiotics Metabolism (Detoxification) & Free Radicals

XENOBIOTIC METABOLISM

1. WHAT IS XENOBIOTIC?

Ans. Xenos means stranger. The xenobiotic is a molecule, foreign to the body such as foreign chemical, drug, food additive, pollutant or a compound produced in the body but has to be eliminated from the body compulsarily as it may cause toxic manifestations by its presence. All the xenobiotics are transformed to compounds of more readily excretable forms either in urine or bile.

2. NAME THE ORGAN IN WHICH THE XENOBIOTIC UNDERGO BIOTRANSFORMATION (DETOXIFICATION) ?

Ans. Liver.

3. WHAT ARE THE MAIN METHODS OF METABOLISM OF XENOBIOTICS?

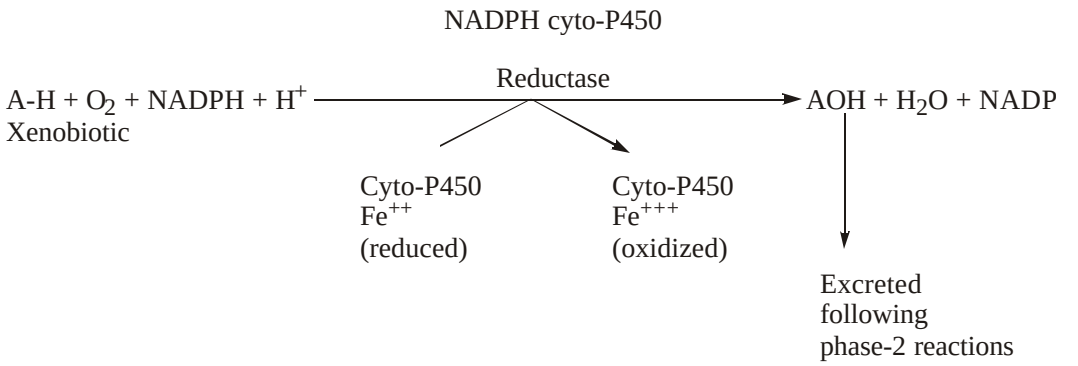
Ans. The metabolism of xenobiotics undergoes in two different phases. These are:

(*a*) *Phase I*: Involves hydroxylation reaction catalyzed by mono-oxygenases or cytochrome P450

(*b*) *Phase II*: The derivatives of Phase-I reaction are conjugated with the molecules like glucuronic acid, sulfate or glutathione etc.

In phase 2 the molecules become more water-soluble and they are ultimately excreted in urine or bile.

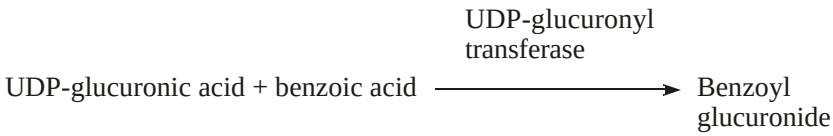
4. WHAT IS THE ROLE OF CYTOCHROME P450 IN THE METABOLISM OF XENOBIOTICS (BIOTRANSFORMATION)?



5. WHAT ARE THE DIFFERENT CONJUGATING AGENTS AND GIVE ONE EXAMPLE OF PHASE 2 REACTION IN EACH?

Ans. (*a*) **Glucuronic Acid**

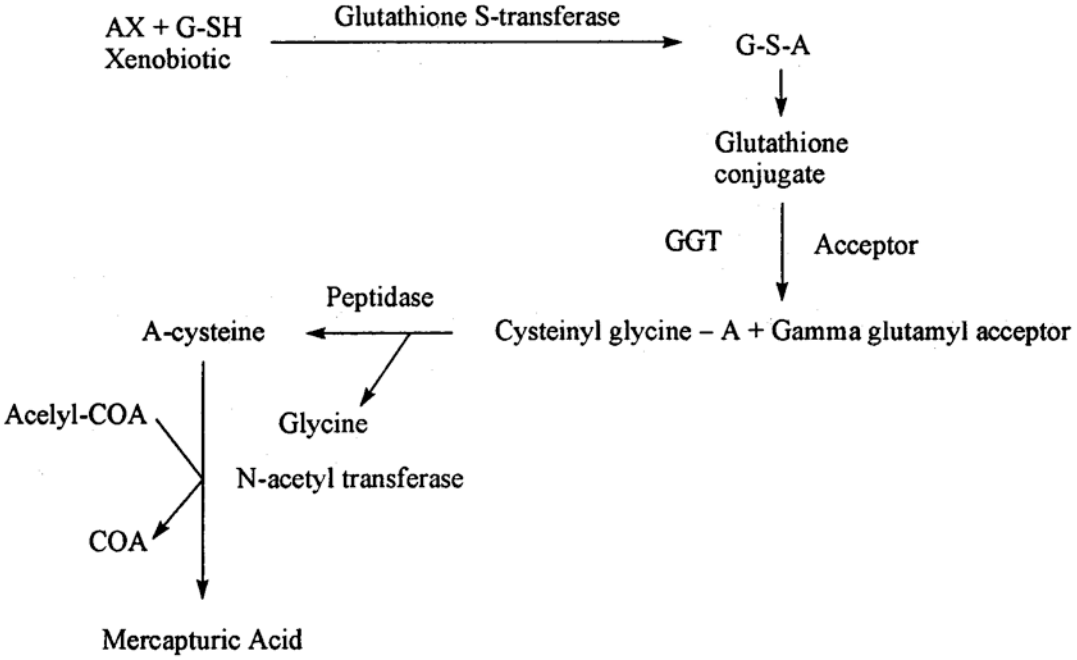
Glucuronic acid is required for converting the bilirubin to conjugated bilirubin (bilirubin diglucuronide). In xenobiotics metabolism it is used as a conjugating agent for benzoic acid and various drugs.



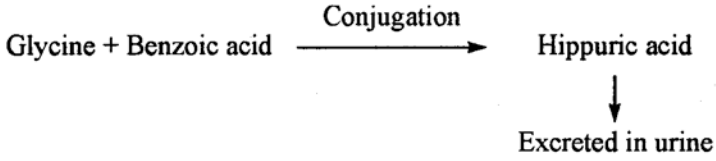
(*b*) **Sulphate (Sulfation)**

PAPS (Phosphoadenosyl phosphosulphate) acts as a sulphate donor.

(*c*) **Glutathione (GSH)**



(*d*) **Glycine**



(*e*) **Glutamine**

Phenyl acetate + glutamine ⟶ Phenyl acetyl glutamine

↓

(Abnormal metabolite formed in PKU)

(*f*) **Methylation**

Acceptor + SAM ⟶ S-adenosyl homocysteine + acceptor–CH_3
(methyl acceptor)

Example

Nicotinamide	⟶	N-methyl nicotinamide
Epinephrine	⟶	Metanephnine
Norepinephrine	⟶	Epinephnine
Pyridine	⟶	N-methyl pyridine

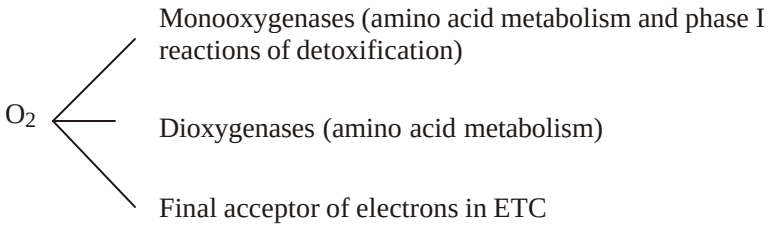
FREE RADICALS

6. WHAT ARE THE USES OF OXYGEN AND WHAT ARE THE HAZARDS OF OXYGEN?

Ans. Uses of oxygen (O_2)

(*a*) Oxygen is the final acceptor of electrons in the respiratory chain and cytochrome oxidase is the final component of ETC. It transfers electrons resulting from the oxidation of substrate molecule through the dehydrogenases to oxygen to form water. During the transport of electrons the free energy is trapped to form ATP molecules by oxidative phosphorylation.

(*b*) Oxygen is required for the reactions catalyzed by the monooxygenases and dioxygenases. In monooxygenases one oxygen atom and in dioxygenases two oxygen atoms are incorporated to substrates. These reactions play important role in the amino acid metabolism and monooxygenases are also required in phase I reactions of detoxification.



Hazards of Oxygen

Oxygen forms reactive oxygen species (ROS) which are harmful to health.

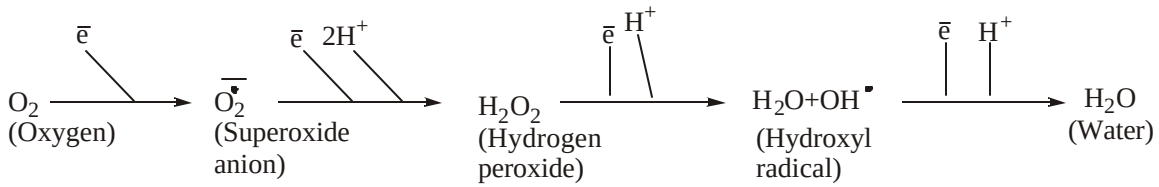
7. WHAT ARE THE REACTIVE OXYGEN SPECIES (ROS)?

Ans. The reactive oxygen species (ROS) are :

(*a*) Superoxide anion – $O^{\bullet}_2$.

(*b*) Hydroxyl radical – $OH^{\bullet}$.

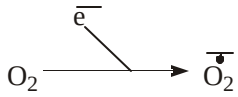
(*c*) Hydrogen peroxide – H_2O_2.



8. HOW SUPEROXIDE ANION IS FORMED AND HOW IS IT REMOVED?

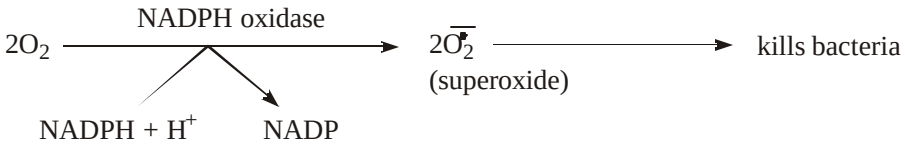
Ans. Formation of Superoxide Anion

Overall reaction



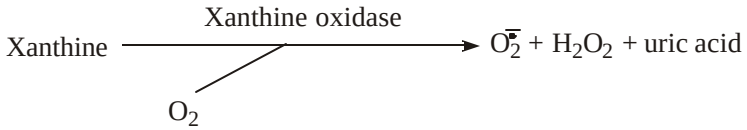
Example

(*a*) It is formed as a part of respiratory burst in leucocytes by NADPH oxidase.



Deficiency of NADPH-oxidase causes chronic granulomatous disease.

(*b*) Superoxide is also formed by the action of Xanthine oxidase

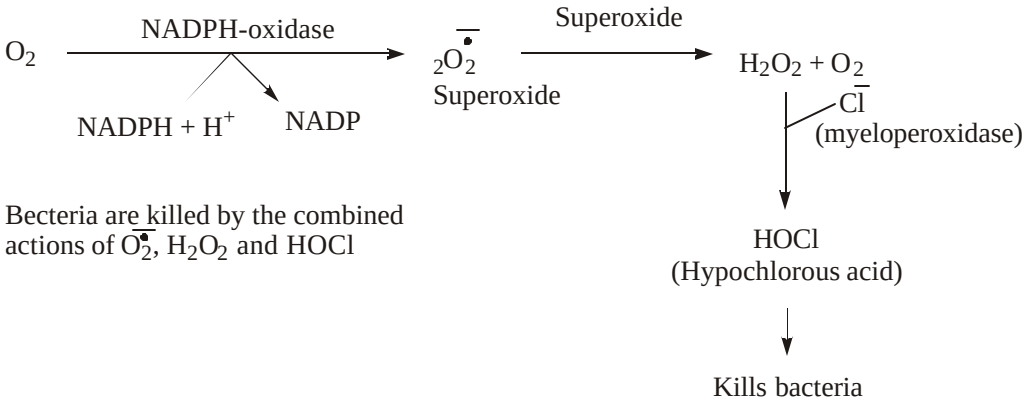


(*c*) Removal of Superoxide

9. WHAT IS RESPIRATORY BURST AND WHAT ARE THE REACTIONS THAT TAKE PLACE IN THE RESPIRATORY BURST?

Ans. During phagocytosis neutrophils and other phagocytic cells engulf bacteria and exhibit a rapid increase in oxygen consumption called as respiratory burst.

Reactions of Respiratory Burst



10. WHAT ARE FREE RADICALS AND WHAT ARE THE HAZARDS OF FREE RADICALS?

Ans. Free radicals have an unpaired electron in the outer orbital. The following are the free radicals:

(*i*) Superoxide anion ⟶ $\dot{O}_2^-$

(*ii*) Hydroxy radical ⟶ $OH^{\cdot}$

(*iii*) Hydroperoxy radical ⟶ $HO\dot{O}$

(*iv*) Lipid peroxide radical ⟶ $LO\dot{O}$

(*v*) Nitric oxide ⟶ $N\dot{O}$

NB: ROS which are not free radicals but cause damage ⟶ H_2O_2.

Hazards of Free Radicals

(*a*) Peroxidation of PUFA in plasma membrane leading to loss of membrane function.

(*b*) Proteins.

(*c*) DNA is damaged by strand breaks leading to inhibition of protein synthesis and enzyme synthesis.

11. WHAT ARE THE SCAVENGER SYSTEMS AVAILABLE FOR THE REMOVAL OF FREE RADICAL?

Ans. (*a*) **Enzymes**

(*i*) Superoxide dismutase ⟶ removes superoxide anion.

(*ii*) Glutathione peroxidase ⟶ involved in the detoxification of H_2O_2. This reaction helps in the maintenance of integrity of RBC membrane.

(*iii*) Catalase ⟶ removes H_2O_2

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$$

(*b*) **Antioxidants**

Free radicals/ROS		Antioxident involved in the removal of free radicals
(*i*) $O\dot{H}$ hydroxy free radical	⟶	Vit – E, β-carotene
(*ii*) $RO\dot{O}$ peroxy free radical	⟶	Vitamin 'E' and 'C'
(*iii*) $\dot{O}_2^-$ superoxide anion	⟶	Superoxide dismutase
(*iv*) H_2O_2 (ROS) not free radical	⟶	Catalase and glutathione peroxidase
(*v*) $LO\dot{O}$ lipid peroxides	⟶	Glutathione peroxidase

Chapter–15

BIOCHEMISTRY OF CANCER

ONCOGENES

1. DEFINE THE TERMS PROTOONCOGENES AND ONCOGENES.

Ans. **Protooncogenes**

Protooncogenes encode proteins that are involved in the control of cell growth.

Oncogenes

When protooncogenes are activated these become oncogenes. The activation of protooncogenes causes either alteration in the structure or alteration in the expression of protooncogenes to become oncogenes. Oncogenes are capable of producing cancers in susceptible cells.

2. CLASSIFY THE ONCOGENES AND GIVE ONE OR TWO EXAMPLES TO EACH CATEGORY.

Ans. Oncogenes are classified into five groups:

(*i*) Growth factors (GF).

(*ii*) Growth factor receptors (GF–R).

(*iii*) Signal transducers (ST).

(*iv*) Transcription factors (TF).

(*v*) Programmed cell death regulators (PCDR).

VARIOUS TYPES OF ONCOGENES

S.No.	*Oncogene*	*Function*	*Alteration in*	*Neoplasm*
1.	V-SIS	Growth factor	Constitutional production	Glioma fibrosarcoma
2.	ERBB2	Growth factor receptor	Point mutation	Breast, ovarian and stomach cancers
3.	MYC	Transcription factor	Amplification	Breast, colon and liver cancers
4.	HRAS	GTP-ase (Strand transducer)	Point mutation	Colon, lung and pancreas cancers
5.	ABL	Protein tyrosine kinase	DNA rearrangement and translocation of chromosome	Chronic myelogenous
6.	PCDR	Protein functions as antiapoptosic function	Chromosomal translocation	B-cell lymphomaa

3. WHAT ARE THE MECHANISMS BY MEANS OF WHICH THE PROTOONCOGENES ARE ACTIVATED TO FORM ONCOGENES, RESULTING IN THE DEVELOPMENT OF CANCER?

Ans. There are three mechanisms which cause activation of protooncogenes to oncogenes. These are:

(*i*) Gene amplification (*ii*) Point mutation (*iii*) Chromosomal translocation.

(*i*) **Gene Amplification**

Activation of protooncogene causes DNA sequence amplification leading to over expression of the gene product. Example : About 20% to 30% breast and ovarian cancers show with C-MYC amplification.

(*ii*) **Point Mutation**

Point mutation of c-γ ras protooncogene results in amino acid substitution at position 12 of p21 protein, thus causing the ↓↓ activity of GTP-ase leading to ↑↑ formation of cAMP and ↑↑ activity of the cAMP dependent protein kinase resulting in bladder cancer.

(*iii*) **Translocation of Chromosomes**

The patients of ch.myelogenous leukemia have philadelphia chromosome. This is formed by the reciprocal translocation of material with chromosome 9 to chromosome 22. The break on chromosome 22 occurs in the BCR (break point cluster region). This produces abnormal juxta position of BCR gene on chromosome 22 with part of C-ABL gene on chromosome 9 (fragment from chromosome 9). The juxta position results in chimeric BCR-ABL mRNA, which encodes ↑↑ activity of tyrosine kinase resulting in ch.myelogenous leukaemia.

4. WHAT ARE ONCO SUPPRESSOR GENES? GIVE EXAMPLES.

Ans. Onco suppressor genes in normal persons prevent the development of cancers. However, the mutation of them causes cancer. The tumour suppressor genes are given in the table:

TUMOUR SUPPRESSOR GENES

RB_1	Familial retino blastoma	Transcription regulator
TP53	50% of all cancers	Transcription factor. Regulates cell cycle and apoptosis.
APC	Familial adenomatous polyposes of colon, colorectal cancers	Regulates cell cycle and apoptosis
$BRCA_1$	Inherited breast and ovarian cancer	DNA repair
$BRCA_2$	Inherited breast cancer	DNA repair
WT_1	Wilms tumour.	Transcription factor

5. WHAT ARE TUMOUR MARKERS? GIVE EXAMPLES.

Ans. The tumour markers are present in serum or plasma. The estimation of these markers help in the management of patients with cancers. The various tumour markers are given in the table:

TUMOUR MARKERS

Hormones

Hormone	*Cancer*
Human chorionic gonadotrophin	1. Germ cell tumour (Teratoma, choriocarcinoma)
Calcitonin	Medullary cancer of thyroid
Catecholamines	Phaeochromocytoma

Oncofetal Antigens

Alphafetoproteins (AFP)	1. Hepatocellular carcinoma 2. Germ cell tumour (Teratoma, seminoma)
Carcino embryonic antigen (CEA) (GIT, lung and breast)	1. Adeno carcinoma of the colon 2. Cancer of pancreas 3. Lung, breast and ovarian cancer

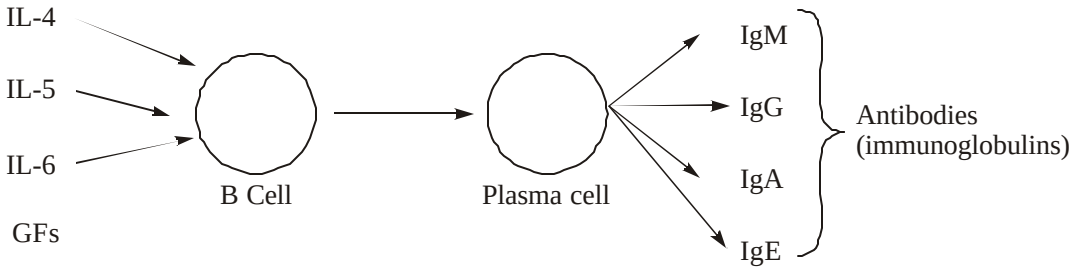
Enzymes

Prostatic acid phosphatase	*Prostatic cancer*
Neuron specific enolase	1. Small cell cancer of lung 2. Neuroblastoma
Prostate specific antigen	Prostatic cancer
Carbohydrate antigens	
CA-125	Ovarian cancer
CA-19–9	Colon, pancreatic and breast cancer
CA-15–3	Breast and ovarian cancers
CA-549 (High mol.wt glycoprotein)	Breast and ovarian cancers

GROWTH FACTORS

6. WHAT ARE GROWTH FACTORS AND WHAT ARE THEIR FUNCTIONS? GIVE EXAMPLES.

Ans. Growth factors exert mitogenic response and differentiation on their target cells. The target cells in the absence of growth factor become quiescent and are stimulated by the growth factors. Platelet derived GF(PDGF) released from the α-granules of platelets plays a role in normal wound healing. Various GFs play a role in the differentiation of stem cells to form various types of mature haematopoietic cells. For instance inter leukins (IL_4, IL_5, IL_6) are called B-cell growth factors which cause stimulation of B-cell growth and differentiation to form plasma cells and antibodies. These ILs are secreted by helper T cells (lymphocytes).



Growth inhibitory factors are also present in our body.

Example

Transforming growth factor β (TGF-β) exerts inhibitory effect on fibroblasts.

Growth factors act on the cell cycle and mitosis via transmembrane signal transduction.

The examples of GFs are given in the table:

SOME IMPORTANT GROWTH FACTORS

S.No.	*Growth factors*	*Functions*
1.	Epidermal growth factor (EGF)	Stimulates growth of epidermal and epithelial cells.
2.	Erythropoietin	Stimulates development of early erythropoietic cells.
3.	Interleukin-1 (IL-1)	Stimulates the production of IL-2 by helper T cells.
4.	Interleukin-2 (IL-2)	Stimulates growth of T cells.
5.	Interleukins (IL-4, IL-5 and IL-6)	Stimulates growth and differentiation of B-cells.
6.	Platelet derived growth factor (PDGF)	Stimulates growth of mesenchymal cells and accelerates wound healing.
7.	Nerve growth factor (NGF)	Stimulates growth of sympathetic and sensory neurons.
8.	Transforming growth factor – α (TGF-α)	Stimulates growth of epidermal and epithelial cells.
9.	Transforming growth factor – β (TGF-β)	Inhibition of fibroblasts.
10.	Granulocyte macrophage colony stimulating factor (GM-CSF)	Stimulates granulocytes and macrophages.
11.	Granulocyte colony stimulating factor (GSF)	Stimulates granulocyte.
12.	Thombopoietin	Stimulates proliferation of platelets.

ANTICANCEROUS DRUGS

7. NAME ANY TWO ANTICANCEROUS DRUGS AND WHAT IS THE MODE OF ACTION OF THESE DRUGS?

Ans. The following are the anticancerous drugs:

(*a*) Methotrexate.

(*b*) 5-fluorouracil.

Mode of Action

(*a*) **Methotrexate**

Methotrexate is folate antagonist. It has 10th position methyl group in its structure.

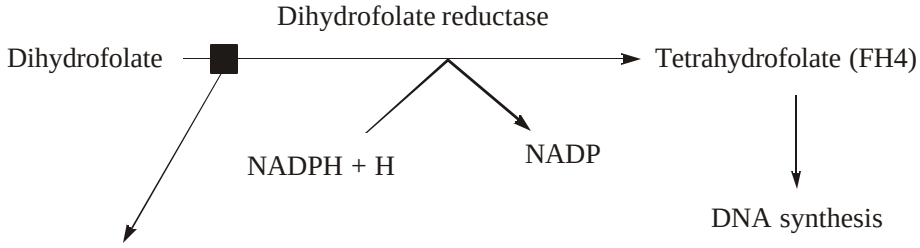
Normal

Tetrahydrofolate Methotrexate

$N^{10} - H$ $N^{10} - CH^3$

It inhibits dihydrofolate reductase by competitive inhibition.

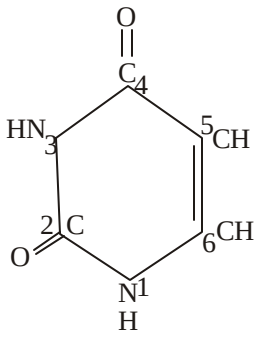
Mode of Action of Methotrexate



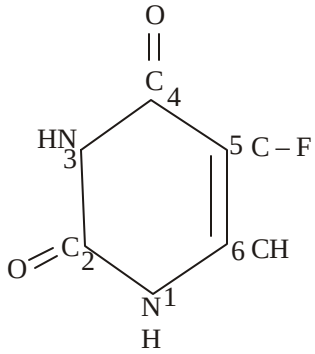
Methotrexate blocks
the reaction by competitive
inhibition and causes decrease synthesis of DNA resulting↓↓ cell
division and cell growth and cures cancer.

(*b*) **5-Fluorouracil**

5-Fluorouracil is analog of uracil.



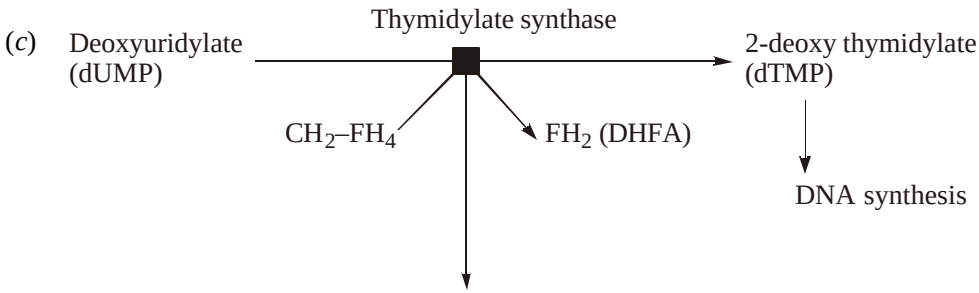
Uracil



5- Fluorouracil

It inhibits the enzyme thymidylate synthase by competitive inhibition.

Mode of Action of 5 – Fluorouracil



5-Fluorouracil blocks the reaction and prevents
the DNA synthesis and cures cancer.

Chapter–16

Nutrition

1. HOW DO YOU EXPRESS THE CALORIFIC VALUE OF FOOD?

Ans. The calorific value of food is expressed in terms of kilo calories per gram of food i.e., 1 Kcal is equivalent to 1000 calories (one calorie is the heat required to raise the temperature of 1 gram of water by 1ºC i.e., from 15ºC to 16ºC).

2. WHAT ARE THE PROXIMATE PRINCIPLES AND WHAT ARE THEIR CALORIFIC VALUES?

Ans. Proximate principles are the major foods, which undergo degradation for the purpose of providing the energy. These are:

	Proximate Principles		*Calorific value per gram*
(*i*)	Carbohydrates	–	4 Kcal
(*ii*)	Proteins	–	4 Kcal
(*iii*)	Fats	–	9 Kcal

3. WHAT IS RESPIRATORY QUOTIENT?

Ans. It is the ratio of volume of CO_2 produced to volume of oxygen consumed.

$$\text{R.Q.} = \frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}}$$

R.Q. Values are:

(*a*)	Carbohydrates	–	1
(*b*)	Proteins	–	0.8
(*c*)	Fats	–	0.7

(*d*) Mixed diet consisting of carbohydrates protein and fats. RQ value of mixed diet is 0.82 – 0.85.

4. WHAT IS B.M.R.?

Ans. B.M.R. is basal metabolic rate. It is the energy required for the basal metabolism when the individual is physically and mentally at rest in reclined position, and is fasting for 12 hours.

5. WHAT IS THE NORMAL VALUE OF BMR AND IN WHICH CONDITIONS IT IS RAISED AND DECREASED?

Ans. Normal value of BMR is 36 Kcal/Sq. metre of BSA (body surface area)/Hour.

BMR is Raised (⇑⇑) in

(*i*) Fever 1°c rise of temperature raises 7 Kcal of BMR.
(*ii*) Infants and growing children.
(*iii*) Men.
(*iv*) Cold climate.
(*v*) Physically active individuals.
(*vi*) Hyperthyroidism.

BMR is Decreased (⇓⇓) in

(*i*) Hypothyroidism.
(*ii*) Sleep.
(*iii*) Malnutrition.

6. WHAT IS SPECIFIC DYNAMIC ACTION?

Ans. The increase of heat production due to intake of food is called specific dynamic action (SDA). SDA of protein is more than that of carbohydrates and fats.

SDA of protein	20% to 30%
SDA of Fats	11%–13%
SDA of Carbohydrates	5% –6%

7. WHAT IS THE ROLE OF FIBER IN NUTRITION?

Ans. The indigestible carbohydrate in the diet is called FIBER.

Importance of Fiber in Nutrition

(*i*) Fiber increases bowel motility and helps in the propulsion of undigested food in the large intestine and ultimately helps in the excretion of feces. By virtue of these properties it prevents the constipation.
(*ii*) It decreases (⇓⇓) the incidence of colon cancers.
(*iii*) It decreases the absorption of cholesterol and eliminate it in the feces. In this way it ⇓⇓ plasma cholesterol level.
(*iv*) *Hypoglycemic effect*: It decreases absorption of glucose, thus it lowers post prandial blood glucose level.

8. WHAT IS THE IMPORTANCE OF POLYUNSATURATED FATTY ACIDS AND MONOUNSATURATED FATTY ACIDS IN NUTRITION? WHAT IS THE IDEAL RATIO OF SFA: MUFA: PUFA IN NUTRITION?

Ans. Importance of Polyunsaturated Fatty Acids in Nutrition

Polyunsaturated fatty acids are acids having two or more than two double bonds in them. They are:

1. Linoleic acid (ω6).
2. α-Linolenic acid (ω3).
3. Arachidonic acid.

PUFA are components of phospho lipids and form biomembranes. From the arachidonic acid, prostaglandins and leukotrienes are formed. They lower the plasma cholesterol level. The ω3 fatty acids (PUFA) decrease platelet aggregation and prevent thrombosis. By virtue of these properties PUFAs have anti-atherogenic effect. Moreover excess of PUFAs may lead to production of 'free radicals' and these may be injurious to cell. Therefore, PUFA content should not be more than 30% of total fats. The monounsaturated fatty acids (MUFA) have single double bond in them. These are :

Palmitoleic acid (16:1; 9)

Oleic acid (18:1; 9)

MUFAs lower plasma cholesterol level when substituted for saturated fat. MUFAs are present in olive oil, groundnut oil and sesame oil. MUFAs also ⇓⇓ the incidence of atherosclerosis. The ideal ratio of distribution of fatty acids in the nutrition are:

SFA: MUFA: PUFA $\longrightarrow$ 1: 1: 1

9. WHAT IS BIOLOGICAL VALUE OF PROTEIN? GIVE EXAMPLES OF PROTEINS OF HIGH BIOLOGICAL VALUE AND LOW BIOLOGICAL VALUE?

Ans. It is the ratio of amount of nitrogen retained to the nitrogen absorbed.

$$BV = \frac{\text{Amount of nitrogen retained}}{\text{Amount of nitrogen absorbed}} \times 100$$

The biological value depends on the ability of a protein to provide essential amino acids (EAA) to the body in sufficient amounts (optimum concentration) to meet the demands of the body. In general all the animal proteins are superior for they contain EAAs in optimum concentration.

Examples of Proteins of High Biological Value

Sl.No.	*Protein*	*B.V.*
1	Egg	96
2.	Milk	84
3.	Meat	80
4.	Fish	85

Examples of Proteins of Low B.V.

Sl.No.	*Protein*	*B.V.*
1.	Rice	64
2.	Wheat	58
3.	Bengal gram	58

Usually proteins of low B.V. lack one or two EAAs. For example wheat lacks lysine and corn lacks tryphotophan and lysine. The missing AAs are called limiting AAs.

10. WHAT IS THE METHOD OF SOLVING THE PROBLEM OF PERSON TAKING THE PROTEINS LACKING ANY EAA (LIMITING AA)?

Ans. The persons taking the proteins lacking the EAAs is solved by the method of mutual supplementation of proteins. For example pulses are deficient of methionine but rich in lysine and cereals are deficient of lysine but rich in methionine. Therefore, a combination of pulses plus cereal (chapatti + Dal/Rice + Dal) will complement each others deficiency and become equivalent protein of high biological value.

11. DEFINE THE TERMS: NET PROTEIN UTILIZATION (NPU) AND PROTEIN EFFICIENCY RATIO.

Ans. Net protein utilization

It is calculated by the formula

$$\text{NPU} = \frac{\text{Retained nitrogen}}{\text{Intake of nitrogen}} \times 100$$

The NPU is better index than BV and NPU indicates both availability and quality of a protein.

Protein Efficiency Ratio (PER)

It is the weight gain per gram of protein taken.

$$\text{PER} = \frac{\text{Gain in body weight (G)}}{\text{Protein fed (G)}}$$

Proteins of high PER $\longrightarrow$ Eggs, milk and meat have high PER > 2.5.

Proteins of low PER $\longrightarrow$ Rice, wheat, nuts have lower PER < 2.5.

12. WHAT IS NITROGEN BALANCE?

Ans. The relationship of intake of nitrogen ('N') and excretion of nitrogen is evaluated. There are three types in this evaluation.

(*i*) **Nitrogen equilibrium:** A normal healthy adult is in nitrogen equilibrium. When the intake of 'N' is equal to excretion of 'N' it is called nitrogen equilibrium .

'N' equilibrium $\longrightarrow$ intake of 'N' = Excretion of 'N'.

(*ii*) **Positive nitrogen balance:** When the intake of 'N' is greater than the excretion of 'N'it is called positive 'N' balance.

Positive 'N' balance ⟶ Intake of 'N' > than excretion of 'N'.

(*iii*) **Negative nitrogen balance:** When the excretion of 'N' is greater than intake of 'N' it is called negative 'N' balance.

Negative 'N' balance ⟶ Excretion of 'N' > intake of 'N'.

13. WHAT ARE THE CAUSES OF POSITIVE NITROGEN BALANCE AND NEGATIVE NITROGEN BALANCE?

Ans. Causes of positive nitrogen balance:

(*i*) Positive 'N' balance is observed in the conditions of tissue growth specially during the growing period.

Examples are :

(*a*) Growing children

(*b*) Convalescing persons due to regeneration of tissues.

(*c*) Pregnant women.

(*ii*) Negative 'N' balance is observed when dietary proteins intake is not adequate and when the person suffers from illness.

Examples are :

(*a*) Acute illness ⟶ Trauma, burns

(*b*) Chronic illness ⟶ Malignancy, uncontrolled diabetes mellitus.

(*c*) Protein deficiency.

BALANCED DIET

14. WHAT IS BALANCED DIET?

Ans. A balanced diet is defined as the diet consisting of required amounts of proximate principles (carbohydrates, fats and first class proteins with optimum concentration of EAAs) and required amounts of minerals and all vitamins for the maintenance of normal health, vitality, reproduction and well being.

15. WHAT ARE THE PRINCIPLES ADOPTED IN PLANNING THE BALANCED DIET?

Ans. The following general guidelines are adopted for planning the balanced diet:

1. Total energy requirement based on ideal body weight should be calculated.
   Ideal body weight × 30 or 35 Kcal/day.
   The main aim should be to maintain ideal body weight and normal BMI (Body Mass Index). Weight reduction to achieve a BMI of 25 or < 30 is essential in obese individuals.
2. The FAT intake should be < 20% of total calories.
3. The proportion of fatty acids in the fat should be SFA: MUFA: PUFA = 1: 1: 1 ratio. Ground nut oil, sesame or gingily oils contain sufficient amount of MUFA and PUFA.
   Sunflower oil contains large amount of PUFA.

4. Excess PUFA is not advisable as oxidation of PUFA may generate FREE RADICALS that may be injurious to cells. Therefore, PUFA content should not be more than 30% of total fats.
5. Fast foods should be avoided as these contain trans fatty acids which are injurious to health.
6. Cholesterol intake should be < 250 mg/day. Therefore, cholesterol rich foods like egg yolk, liver, brain should be avoided.
7. As far as possible animal fat including butter should be avoided.
8. Minimum amount of proteins for adults 1g/Kg body weight should be taken and for children 2G/Kg of body weight should be taken. Persons who take only vegetarian diet should take cereal (rice/wheat) + pulses + milk in sufficient amounts.
9. The remaining calories should be supplemented in the form of carbohydrates. Salt and sucrose should be avoided. (Avoid sweets and spices) and fiber content should be supplemented.
10. The diet should be supplemented with the plenty of green leafy vegetables, fruits and carrots etc.
11. RDA of vitamins and minerals should be fulfilled.

16. WHAT ARE THE PRINCIPLES ADOPTED FOR THE CALCULATION OF ENERGY REQUIREMENT OF INDIVIDUAL WITH MODERATE WORK WEIGHING 60 KG?

Ans. The following guidelines are adopted for the calculation of energy.

(*a*) Calculate the energy requirement for basal metabolism i.e., 24 Kcal/hour.

(*b*) Calculate approximately 40% BMR as extra work (moderate work).

(*c*) Calculate 10% of energy of (BMR + Mod.work) for SDA.

Actual calculation :

For BMR $24 \times 60 = 1440$ Kcal

For extra work 40% of BMR = 576

Subtotal	2016
For SDA 10% of subtotal	202

Total energy required 2218 Kcal/day.

ABNORMALITIES OF NUTRITION
PROTEIN ENERGY MALNUTRITION

17. WHAT IS PROTEIN ENERGY MALNUTRITION AND WHAT ARE THE CLINICAL AND BIOCHEMICAL FEATURES OF PEM?

Ans. Protein energy malnutrition is manifested primarily by inadequate dietary intake of protein and energy.

PEM occurs commonly among weaned infants and pre-school children in India, Africa and South America. These are classified into three groups:

(*i*) Kwashiorkor (Oedematous – PEM)

(*ii*) Nutritional marasmus (non-odematous – PEM)

(*iii*) Marasmus kwashiorkor (both).

Non-Oedematous PEM

Marasmus: It results primarily from inadequate energy intake. It is caused by severe deficiency of proteins and calories. The important features are:

(*i*) Growth retardation.

(*ii*) Severe wasting of muscles and hypotonia of muscles and loss of subcutaneous fat.

(*iii*) The skin loses turgor and becomes wrinkled.

Oedematous PEM

(*i*) Growth is markedly retarded.

(*ii*) Oedema (dependent parts are oedematous).

(*iii*) Muscle wasting.

(*iv*) Changes in the colour of skin and hair.

(*v*) Enlargement of liver.

(*vi*) Dermatitis is common.

(*vii*) Serum albumin level is decreased (↓↓).

(*viii*) Serum retinol binding protein (RBP) level is decreased (↓↓).

(*ix*) FFA ↑↑.

(*x*) Hypokalemia (K^+↓↓) and hypomagnesaemia (Mg^{++}↓↓).

OBESITY

18. WHAT IS OBESITY? WHAT ARE THE MAIN FEATURES OF OBESITY?

Ans. Obesity is a state of excess adipose tissue mass (↑↑ number and ↑↑ size of adipocytes occur. Obesity can result from ↑↑ energy intake or ↓↓ energy expenditure or a combination of two. Obesity is gauged by body mass index (BMI).

The normal value of (BMI) is between 20–25

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (meters)} \text{m}_2}$$

The BMI value of individuals in obesity usually is >30.

(*a*) Leptin is the product of ob-gene. It is secreted as a small peptide by adipocytes.

(*b*) Leptin receptors are present in specific regions of brain.

(*c*) The feeding behaviour is regulated by leptin. High leptin levels decrease food intake and increases energy expenditure.

(*d*) A defect in leptin or its receptor can lead to obesity.

19. WHAT ARE THE SPECIFIC SYNDROMES ASSOCIATED WITH OBESITY?

Ans. (*i*) Cushing's syndrome, (*ii*) Hypothyroidism, (*iii*) Insulinoma.

Chapter–17

Mineral Metabolism

SOURCES OF MINERALS

1. WHAT ARE THE SOURCES OF FOLLOWING MINERALS AND TRACE ELEMENTS?

Ans. (*a*) **Calcium:** Milk and milk products, seseme seeds and green leafy vegetables.

(*b*) **Phosphorus:** Milk, eggs, meat and fish.

(*c*) **Iron:** Organ meats (liver), meat, fish, egg yolk, dates, green leafy vegetables, legumes, nuts, enriched grains, jaggery.

(*d*) **Copper:** Meat, shell fish, nuts, legumes, whole grain.

(*e*) **Iodine:** Seafood and fish and water. Near the sea no iodine deficiency. Soil of mountain region contain less iodine.

Goitrogenous foods – cabbage, cauliflower, radish.

(*f*) **Zinc:** Seafood, oysters, meat, eggs, legumes and whole grains.

(*g*) **Magnesium:** Green leafy vegetables, legumes, nuts, whole grain, dried bean.

(*h*) **Manganese:** Best source –whole grains, tea, moderate – vegetables and fruits.

(*i*) **Fluoride:** Fluorinated water, fish and tea.

(*j*) **Chromium:** Meat, mushrooms, nuts, yeast, organ meats.

(*k*) **Molybdenum:** Milk, liver, legumes and cereals.

(*l*) **Selenium:** Seafood, kidney, liver, muscle meats.

2. WHAT IS THE RDA OF THE FOLLOWING MINERALS AND TRACE ELEMENTS?

Ans.

Mineral	*RDA of Children*	*RDA of Adults*	*Pregnancy Lactating women*
Calcium (mg/D)	500 – 800	19–50 Years 1000 Above 50 Y –1200	19–50Y – 1000
Phosphorus (mg/D)	400 – 500	700	700
Iron (mg/D)	7 – 10	Male : 10 Female: 5–18 18 – 50 year age groups	Preg – 27 Lac – 10
Copper mg/D	1 – 15	1.5 – 3.0	1.5 – 30
Iodine (mG/D)	90	150	Preg – 220 Lac – 290
Zinc (mg/D)	3 – 5	Male : 15 Female : 12	Preg – 15 Lac – 19
Magnesium (mg/D)	80 – 130	Male : 400 – 420 Female: 310 – 320	Preg – 350 – 400 Lac – 310 – 320
Manganese mg/D	1.2 – 1.5	Male : 2.3 Female: 1.8	Preg – 2 Lac – 2.6
Fluoride (mg/D)	1.0	Male : 3 – 4 Female: 3	Preg, Lac } 3 – 4
Chromium (μG/D)	11 – 15	Male : 35 Female: 25	Preg – 30 Lac – 45
Molybdenum (μg/D)	22	45	Preg – 50 Lac – 50
Selenium (μg/D)	20 – 30	55	Preg – 60 Lac – 50

FUNCTIONS AND DEFICIENCY MANIFESTATIONS OF MINERALS

3. WHAT ARE THE FUNCTIONS AND DEFICIENCY MANIFESTATIONS OF THE FOLLOWING MINERALS AND TRACE ELEMENTS?

Ans.

Mineral	*Functions*	*Deficiency manifestations/toxic manifestations*
(*a*) Copper (Cu)	(*i*) It is required for Hb synthesis. (*ii*) It is present in cytochrome oxidase which is final component of ETC	Excess copper storage occurs in lenticular nucleus, and liver resulting in hepatolenticular degeneration. It is caused by the deficiency of enzyme ceruloplasmin.

(*b*) Iodine	Synthesis of thyroid hormones T_3 and T_4 which are required for the maintenance of BMR	(*a*) Deficiency results in endemic goiter, cretinism. (*b*) Abnormalities of T_3, T_4 hypothyroidim ↓ T_4 hyperthyroidism ↑ T_4
(*c*) Zinc (Zn^{++})	It is present in the enzymes carbonic anhydrase, carboxy peptidase. Role of Zinc in Vitamin–'A' metabolism: ZINC deficiency interferes with 1. The mobilization of Vitamin–A from liver stores 2. Synthesis of Rhodopsin in the eye. Vitamin deficiency is exacerberated with concurrent zinc deficiency.	Deficiency causes: (*a*) Dwarfism. (*b*) Hypogonadism. (*c*) Sensory impairment – taste and smell. (*d*) Delayed wound healing and ↓↓ immune response.
(*d*) Magnesium (Mg^{++})	Constituent of bones and teeth. Co–factor for phosphorylation reactions. Required for the function of muscles and nerves.	Tremors and spasm.
(*e*) Manganese (Mn)	It is a constituent of several enzymes. Required for growth and reproduction.	Impaired growth and skeletal abnormalities, ↓↓ reproductive function
(*f*) Fluoride	Fluoride accumulates in bones and teeth increasing their hardness. Fluoride is a protective agent against the development of dental caries and its deficiency promotes the development of dental caries and tooth decay. Excessive amount of fluorides in drinkng water > 3–5 ppm causes dental fluorosis i.e., discolouration or mottling of children's teeth.	
(*g*) Chromium	Associated with glucose metabolism. Improves glucose uptake by tissues (glucose tolerance factor).	Deficiency causes disturbance of glucose metabolism.
(*h*) Molybdenum	It is a constituent of Xanthine oxidase.	Deficiency is unknown in humans.
(*i*) Selenium	It is present in glutathione peroxidase and helps in the detoxification of H_2O_2. It is a synergistic antioxidant with Vitamin – E.	Selenium is used in the treatment of Keshan's disease. The deficiency causes Keshan's disease which is an endemic cardiomyopathy found in children.

METABOLISM OF IRON

4. WHAT ARE THE FACTORS INFLUENCING IRON ABSORPTION?

Ans. The following factors influence iron absorption:

Factors Increasing (⇑⇑) Absorption of Iron

(*i*) Acidity in the stomach.
(*ii*) Heme iron is more absorbed than non-heme iron.
(*iii*) ⇑⇑ demand for iron (pregnancy, blood loss).

(*iv*) Reducing agents like vitamin C or glutathione.
(*v*) Low body stores of iron.

Factors Decreasing (⇓⇓) Absorption of Iron

(*i*) Phytic acid.
(*ii*) Oxalic acid.
(*iii*) Polyphenols in tea and coffee.
(*iv*) Full body stores of iron.
(*v*) Gastric HCl.
(*vi*) Infections.
(*vii*) Excess of other minerals Zn, Mn and Ca.

5. WHAT IS THE MECHANISM OF ABSORPTION OF IRON?

Ans. Iron absorption takes place in the proximal small intestine. The non-heme iron which is present in the ferric (Fe^{++}) form is dissociated by the acid. At the brush border of the absorptive cell. The ferric (Fe^{+++}) iron is converted to the ferrous form by a ferrireductase. In the first step it is transported across the membrane by a divalent metal transporter-1 DMLT-1 and enters into gut cell. Approximately 10% to 30% of ingested iron is absorbed. In the gut cell it has two fates:

(*i*) It may be stored as ferritin by combining with apoferritin.
(*ii*) Transported through the cell into plasma as transferrin. In the mucosal cells of the duodenum and proximal jejunum, iron is oxidised and bound to a transport protein to form plasma transferrin, for transport to body cells. Approximately about 20% to 35% of iron binding capacity of transferrin is filled.

6. WHAT ARE THE DIFFERENT FORMS OF IRON IN THE BODY AND WHAT ARE THEIR FUNCTIONS?

Ans. (*a*) **Transport iron in the form of transferrin:** It is involved in the transport of plasma iron to body cells (0.05 to 0.18 mg/dl).

(*b*) **Haemoglobin:** Most of the body iron about 70% is present in RBC as hemoglobin (Hb) and 5% in the muscle as myoglobin, Hb is involved in the transport of O_2 to tissues.

(*c*) **Ferritin (storage form of iron):** Within the cells iron is stored as ferritin. It is complexed to a protein called apo-ferritin and forms ferritin.

(*d*) Normal value of ferritin:

Males – 100 μG/L
Females – 30 μG/L

Iron deficiency anemia <15 μG/L.

ABNORMALITIES OF IRON METABOLISM

7. WHAT ARE THE ABNORMALITIES OF IRON METABOLISM?

Ans. Deficiency:

The deficiency of iron causes iron deficiency anemia. The main causes of iron deficiency anemia are:

(*a*) Pregnancy.

(*b*) Periods of rapid growth.

(*c*) Blood loss.

The major biochemical features are:

1. Peripheral smear shows hypochromic and microcytic cells.
2. ↓↓ hematocrit and ↓↓ Hb concentration.
3. Serum iron < 30 μG/dl (↓↓).
4. TIBC > 360 μG/dl (↑↑).
5. Ferritin < 15 μG/Lit (↓↓).

Iron Excess

The iron overload is called Haemochromatosis. This is manifested by increase in intestinal absorption resulting in deposition of excessive amounts of iron in parenchymal cells (liver, pancreas, heart) with eventual tissue damage.

(*a*) Congenital: It is caused by inheritance of mutant HFE gene.

(*b*) Acquired (Bantu siderosis)

The Bantu community cook their food in iron utensils.

Biochemcal Features

(*i*) Serum iron ↑↑.

(*ii*) Serum ferritin ↑↑.

METABOLISM OF CALCIUM

8. WHAT ARE THE MAIN FUNCTIONS OF CALCIUM?

Ans.

(*i*) **Formation of Bones and Teeth**: The total body content of calcium is approximately 1 Kg. Ninety nine percent (99%) is exclusively present in the bones where it forms with phosphate hydroxyapatite crystals which provide the structure and strength to the bones. Calcium is also required for the formation of teeth.

Mineralisation

Deposition of calcium and phosphate (mineralisation) occurs when $Ca^{++} \times PO_4^{-}$ iron product rises beyond a critical level (cut off level).

(*ii*) **Activation of Enzymes**

Four calcium ions bind calmodulin and cause activation of kinases which results in the phosphorylation of enzymes and bring the biological effect.

(*iii*) **Contraction of Muscles**

Calcium mediates excitation and contraction of muscle fibres. Contraction of muscles require binding of calcium to troponin.

(*iv*) **Coagulation of Blood**

Calcium plays important role in the coagulation of blood as a factor 4 of coagulation.

(*v*) **Nerve Excitability**

↑↑ plasma calcium level ↓↓ nerve excitability and ↓↓ plasma calcium level ↑↑ the excitability of nerves.

(*vi*) **Activation of Enzymes**

Calcium causes direct activation of some enzymes.

Examples : Pancreatic lipase.

(*vii*) **Action on Heart**

↑↑ calcium concentration ↑↑ contraction of heart and ↓↓ concentration causes ↓↓ contraction. Hypercalcemia can cause cardiac arrest in systole.

(*viii*) **Signal Transduction**

Calcium acts as a second messenger in signal transduction involving 'G' protein and inositol triphosphate (IP_3). Hormones like acetylcholine or ADH bound to receptor and cause release of IP_3 which in turn releases intracellular calcium into cytosol and bring the effects attributed to hormone.

9. WHAT IS THE MECHANISM OF ABSORPTION OF CALCIUM?

Ans. Most of the calcium is absorbed in the proximal small intestine. Less than half of dietary calcium is absorbed in adults.

Role of Calcitriol in the Absorption of Calcium and Phosphate

Vitamin D_3 is converted to 25 (OH) D in the liver. In the next step 25 (OH) D is bound to vitamin D binding protein and transported to kidney. In the kidney it is converted to 1, 25 $(OH)_2$ D (Calcitriol) the active metabolite of D. Calcitriol is bound to vitamin D binding protein and carried to intestine. In the intestine it is bound to nuclear receptor and causes the synthesis of calcium binding protein (CBP). CBP causes transport of Ca^{++} and PO_4^{3-} from the lumen of intestine to circulation. Thus CBP is directly responsible for the absorption of Ca^{++} and PO_4^{3-}.

10. WHAT ARE THE FACTORS INFLUENCING THE ABSORPTION OF CALCIUM?

Ans. Factors increasing absorption of calcium:

(*a*) Calcitriol.
(*b*) Parathyroid hormone ↑↑ calcium transport from the intestinal cells.
(*c*) During periods of rapid growth in children.
(*d*) Pregnancy.
(*e*) Lactation.
(*f*) Low pH.
(*g*) Amino acids especially lysine and arginine favour absorption.
(*h*) Lactose enhances calcium absorption through the action of Lactobacilli which produce lactic acid and lower pH.

Factors Decreasing the Absorption of Calcium

(*a*) Vitamin–D deficiency.
(*b*) Phytic acid present in cereals.
(*c*) Oxalates form insoluble calcium oxalates.
(*d*) Steatorrhoea, fatty acids form insoluble calcium salts of fatty acids.
(*e*) High phosphate.
(*f*) Alkalinity.
(*g*) In Chronic renal failure, formation of active metabolites of Vitamin D_3 (Calcitriol) is not formed leading to ↓↓ absorption.
(*h*) Advancing age.

11. WHAT IS NORMAL SERUM CALCIUM LEVEL AND WHAT ARE THE FACTORS WHICH REGULATE THE PLASMA CALCIUM LEVEL?

Ans. The normal serum calcium level lies between the range of 9 mg to 10.5 mg/dl. (< 11 mg/dl) The major factors which regulate plasma calcium are:

(*a*) Active metabolite of vitamin – D (Calcitriol).
(*b*) Parathornome (PTH).
(*c*) Calcitonin.

(*a*) **Calcitriol**

It is formed in the kidney by PTH which is secreted by low calcium level. Calcitriol ↑↑ absorption of calcium through the formation of CBP and raises the plasma calcium level.

(*b*) **PTH**

Low serum Ca^{++} level causes secretion of PTH by Parathyroid gland which causes resorption of bone and ↑↑ reabsorption of calcium from kidney tubules. By these mechanisms it raises plasma calcium level.

(*c*) **Calcitonin**

It is a peptide consisting of 32 amino acids. It is secreted by the parafollicular 'C' cells (originate from neural crest) of the thyroid gland. The secretion of calcitonin renders direct

control of blood calcium ($\uparrow\uparrow$ Ca^{++} stimulates secretion). It primarily inhibits osteoclast mediated bone resorption. It secondarily stimulates renal calcium clearance. By these mechanism it $\downarrow\downarrow$ plasma calcium level.

ABNORMALITIES OF CALCIUM METABOLISM

12. WHAT IS RENAL OSTEODYSTROPHY?

Ans. When substantial renal parenchyma is damaged, the formation of calcitriol is reduced and absorption of calcium is decreased resulting in hypocalcemia. Hypocalcemia inturn stimulates parathyroid gland to secrete PTH. PTH acts on bone and brings the changes in bone structure. This condition is called renal osteodystrophy.

13. WHAT ARE THE CAUSES OF HYPERCALCEMIA?

Ans. Increased serum calcium level is called hypercalcemia. The following are the causes of hypercalcemia.

1. Primary hyperparathyroidism (Solitary adenoma).
2. Familial hypocalciuric hypercalcemia (inappropriate secretion of PTH and excessive renal reabsorption of calcium).
3. Malignancy related hypercalcemia (cancer of lung and breast).
4. Vitamin D intoxication.
5. Sarcoidosis ($\uparrow\uparrow$ calcitriol synthesis).
6. Secondary hyperparathyroidism.

14. WHAT ARE THE DEFICIENCY MANIFESTATIONS OF CALCIUM?

Ans. The deficiency of calcium causes the following clinical conditions.

(*a*) Osteoporosis.
(*b*) Osteomalacia in adults (caused by $\downarrow\downarrow$ intake of vitamin-D).
(*c*) Rickets in children (caused by $\downarrow\downarrow$ intake of vitamin-D).
(*d*) Tetany (when ionised Ca^{++} is decreased).
(*e*) Retarded growth.
(*f*) Poor teeth and bone formation.

15. WHAT IS TETANY?

Ans. A decreased ionized calcium causes tetany. The important causes of tetany are :

(*a*) Hypoparathyroidism.
(*b*) Rickets.
(*c*) Alkalosis.

It is characterised by intermittent spastic contractions of distal muscles of hands and feet resulting in carpo-pedal spasm associated with tingling around mouth and distally in the

limbs. Tetany with carpo-pedal spasm is a common manifestation of

(*a*) Hypocalcemia.

(*b*) Respiratory alkalosis.

16. WHAT IS OSTEOPOROSIS?

Ans. The osteoporosis is characterised by mineral loss, occurs mainly in older persons, especially in post menopausal women. There is a negative calcium balance. Osteoporosis is corrected by exercise coupled with calcium supplements. Current therapy is administration of vitamin–D hormone, oestrogen and calcium supplements.

Chapter–18

Water, Electrolytes and Acid Base Balance

DISTRIBUTION OF WATER AND ELECTROLYTES

1. WHAT IS THE TOTAL BODY WATER OF AN ADULT AND HOW IS IT DISTRIBUTED?

Ans. In the average 70 kg adult human, the total body water is 60% of body weight or 42 ltrs. The body water is distributed mainly in the two compartments.

(*a*) Intracellular fluid (ICF) 40% of body weight (28 L).

(*b*) Extracellular fluid (ECF) 20% of body weight (14 L). This has two compartments.

(*i*) Interstitial tissue fluid 3/4th of ECF (11 L).

(*ii*) Plasma (intravascular fluid) 1/4th of ECF (3 L).

2. WHAT ARE THE ELECTROLYTES PRESENT IN THE PLASMA, INTERSTITIAL AND INTRACELLULAR FLUIDS AND WHAT ARE THEIR NORMAL VALUES?

Ans. There are two types of electrolytes present in the plasma, interstitial tissue fluids and intracellular fluids. These are cations which carry positive charge and anions which carry negative charge.

Electrolytes Present in Plasma

S.No.	*Cations*	*Concentration in meq./L*	*Anions*	*Concentration in meq./L*
1.	Sodium (Na^+)	143 (136–145)	Chloride (Cl^-)	103 (96–105)
2.	Potassium (K^+)	4.0 (3.7– 5.0)	Bicarbonate (HCO_3^-)	26 (24–28)
3.	Calcium (Ca^{++})	5.0	Phosphate (HPO_4^{-2})	2.0
4.	Magnesium (Mg^{++})	2.0	Sulphate (SO_4^{-2})	1.0
			Proteins$^-$	16
			Organic acids$^-$	5.0 (5–6)
	Total	154		154

Electrolytes Present in Interstitial Fluids

S.No.	*Cations*	*Concentration in mEq./L*	*Anions*	*Concentration in mEq./L*
1.	Sodium (Na^+)	145 (136 – 145)	Chloride (Cl^-)	116 (96–105)
2.	Potassium (K^+)	5.0 (3.7 – 5.0)	Bicarbonate (HCO_3^-)	27 (24–28)
3.	Calcium (Ca^{++})	3.0	Phosphate (HPO_4^{-2})	3.0
4.	Magnesium (Mg^{++})	2.0	Sulphate (SO_4^{-2})	2.0
			Proteins$^-$	1.0
			Organic acids$^-$	6.0 (5–6)
	Total	155		155

Electrolytes of ICF

S.No.	*Cations*	*Concentration in mEq./L*	*Anions*	*Concentration in mEq./L*
1.	Potassium (K^+)	150	HPO_4^{-2}	110
2.	Magnesium (Mg^{++})	40	Proteins$^-$	50
3.	Sodium (Na^+)	5.0	SO_4^{-2}	20
			HCO_3^-	10
			Cl^-	5.0
	Total	195		195

METABOLISM OF ELECTROLYTES

3. BRIEFLY OUTLINE THE NORMAL METABOLISM, PHYSIOLOGICAL FUNCTIONS AND RDA OF THE FOLLOWING ELECTROLYTES?

(*a*) Sodium (*b*) Potassium (*c*) Chlorides.

S.No.	*Electrolyte*	*Sources*	*Mineral metabolism*	*RDA and Functions*
1.	Sodium (Na^+)	Table salt (NaCl), milk, cheese, meat, egg, carrot, beans, spinach.	Readily absorbed by intestine. Excreted by kidney. Lost in feces and sweat. Reabsorption from DCT of kidney by the action of aldosterone. Deficiency leads to muscle cramps.	RDA adults 500 mg/D, infants 120–200 mg/D, children 225–500 mg/D 1. Major cation in extra cellular fluid (plasma). 2. Maintains water balance. 3. Maintains acid base balance. 4. Required for cell membrane permeability. 5. Plays important role in the absorption of glucose, amino acids and iodine through the transport proteins. 6. Maintains normal muscle and nerve irritability.
2.	Potassium	Oranges & bananas, dried fruits, vegetables, legumes, nuts, whole grains and meat.	Readily absorbed from the intestine and excreted by kidney. Aldosterone controls the excretion by kidney.	RDA Adults – 2000 mg/D Infants–500–700 mg/D Children– 1000–2000 mg/D 1. Major cation of intracellular fluid. 2. Maintains water balance and acid base balance. 3. Required for normal muscle and nerve irritability. 4. Low potassium diet causes prevalence of hypertension. That shows its role for the prevention of hypertension. 5. Maintains regular heart rhythm.
3.	Chloride	Table salt (NaCl), eggs, sea food, milk.	Deficiency leads to inbalance in gastric acidity and inbalance in blood pH	1. Major anion in extra cellular fluid. 2. Maintains water and acid base balance. 3. Chloride shift helps the transport of CO_2 from tissues to lungs. 4. Formation of gastric HCl. (Gastric acidity)

SODIUM POTASSIUM PUMP

4. WHAT IS SODIUM POTASSIUM PUMP? BRIEFLY EXPLAIN THE MECHANISM OF ACTION OF THIS PUMP.

Ans. Sodium Potassium pump keeps high concentration of Na^+ in ECF and high concentration of K^+ in ICF.

The $Na^+ – K^+$ pump is a transport process that pumps three (3) Na^+ ions from inside to outward through the cell membrane and two (2) K^+ ions from outside to inside. The membrane has a carrier protein which has three (3) receptor sites for binding Na^+ ions in the interior of the cell and has two (2) receptor sites for K^+ ions on the outside. The inside portion of the protein has ATP – ase activity which cleaves the ATP to ADP and phosphate and the liberated energy is responsible for the movement of Na^+ and K^+ ions. The $Na^+ – K^+$ pump is inhibited by ouabain, a cardiac glycoside. $Na^+ – K^+$ pump is responsible for the transmission of nerve and muscle signals.

5. WHAT ARE THE FACTORS WHICH REGULATE THE WATER AND ELECTROLYTE BALANCE?

Ans. Water balance is mainly regulated by proper intake and output (excretion) of water.

1. The intake of water is regulated by the mechanism of thirst. A thirst centre is located in the IIIrd ventricle.
2. Antidiuretic hormone (ADH) regulates the excretion of water by kidney. This hormone is a peptide containing 9 amino acids and is produced by the posterior pituitary gland. It facilitates reabsorption of water by tubules.
3. Atrial natriuretic peptide (ANP)
   The ↑↑ in intravascular volume causes the production of ANP from the cardiac atrium. This peptide stimulates excretion of Na^+ by the kidney (natriuresis). It ↓↓ vasoconstriction (↓↓ BP)
   It ↓↓ Renin and aldosterone secretion.
4. Aldosterone : It is produced by the zona glomerulosa of adrenal cortex which is secreted by Renin – Angiotensin system. Aldosterone ↑↑ reabsorption of Na^+ from the distal convoluted tubules of kidney.

ABNORMALITIES OF WATER METABOLISM

6. WHAT IS DEHYDRATION AND WHAT ARE THE CAUSES OF DEHYDRATION?

Ans. Dehydration is the condition of disturbance of water balance where the excretion (output) of water exceeds the intake of water and the following are the causes of dehydration of predominant water depletion.

A. Reduced intake.

Water unavailable to infants, aged persons.

B. ↑↑ loss from skin.
- (*a*) Fever
- (*b*) Hot environment

C. ↑↑ loss from respiratory tract
- (*a*) Hyperventilation
- (*b*) Fever

D. ↑↑ loss in urine due to marked impairment of urinary concentrating mechanism.
- (*a*) ADH deficiency (diabetes insipidus)
- (*b*) Nephrogenic diabetes insipidus (resistance to ADH action on renal tubules)
- (*c*) Hypercalcemia
- (*d*) K^+ depletion
- (*e*) Diabetes mellitus (solute diueresis)

HYPOKALEMIA

7. WHAT IS HYPOKALEMIA AND WHAT ARE THE CAUSES AND CLINICAL FEATURES OF HYPOKALEMIA?

Ans. Hypokalemia is defined as plasma concentration < 3.5 m.mol/Ltr. and may result from one or more of the following causes:

(*i*) Decreased intake
- (*a*) Starvation

(*ii*) Shift into cells (transport from ECF to cells)
- (*a*) metabolic alkalosis
- (*b*) Administration of glucose with insulin
- (*c*) β adrenergic agonist.

(*iii*) Familial periodic paralysis (weakness or paralysis of muscles). Attack is precipitated by the injection of epinephrine or insulin. Attacks occur mostly at night.

(*iv*) Increased loss
- (*a*) Diarrhoea
- (*b*) ↑↑ Loss in sweat
- (*c*) Intake of diuretics

(*v*) ↑↑ excretion of K^+ by kidney
- (*a*) Primary hyperaldosteronism (Conn's syndrome, adrenal adenoma)
- (*b*) Secondary hyperaldosteronism
- (*c*) Cushings syndrome
- (*d*) Batters syndrome
- (*e*) Renal tubular acidosis

8. WHAT ARE THE CLINICAL FEATURES OF HYPOKALEMIA?

Ans. Symptoms occur when the serum K^+ value is < 3 m mol/l

(*i*) Fatigue, myalgia and muscular weakness of lower extremities due to more negative resting membrane potential.

(*ii*) More severe hypokalemia leads to progressive weakness, hypoventilation and paralysis of muscles.

(*iii*) ECG changes

(*a*) Early changes : Flattening or inversion of 'T' wave

(*b*) Prominent 'U' wave

(*c*) ST segement depression

Severe K^+ Depletion

(*a*) Prolonged PR interval (↑↑)

(*b*) Widening of QRS. Complex

(*c*) ↑↑ risk of ventricular arrythimia

(*i*) Hypokalemia predisposes to digitalis toxicity

(*ii*) Hypokalemia causes metabolic alkalosis (due to reabsorption of HCO_3)

HYPERKALEMIA

9. WHAT IS HYPERKALEMIA AND WHAT ARE THE CAUSES AND CLINICAL FEATURES OF HYPERKALEMIA?

Ans. When the plasma K^+ concentration is raised above 5 m mol/L it is called hyperkalemia. It may result either due to potassium release from cells or ↓↓ renal loss.

Causes of Hyperkalemia

1. Renal failure – Acute Renal failure
   chronic renal failure
2. Decreased K^+ – excretion

A. Imparied Na reabsorption

(*a*) Primary hypoaldosteronism ⟶ adrenal insufficiency

(*b*) Secondary hypoaldosteronism ⟶ hyporeninemia, ACE inhibitors

(*c*) Resistance to aldosterone ⟶ potassium sparing diuretics

B. Enhanced Cl^- reabsorption ⟶ cyclosporine

Clinical Features

1. Hyperkalemia partially depolarises the cell membrane which inturn impairs the membrane excitability resulting in

(*a*) Weakness and paresthesia of extremeties
(*b*) Flaccid paralysis and
(*c*) Hypoventilation

2. By enhancing NH_4 reabsorption it causes acidosis
3. Hyperkalemia causes cardiac toxicity giving rise to following ECG changes
   (*a*) Peaked 'T' wave ('T' wave becomes increasing taller with a narrow base)
   (*b*) QRS complex become broader
   (*c*) Prolonged PR interval
   (*d*) Loss of 'P' wave
   Terminal events usually ventricular fibrillation or asystole.

HYPONATREMIA

10. WHAT IS HYPONATREMIA AND WHAT ARE THE CAUSES OF HYPONATREMIA?

Ans. When the plasma Na^+ concentration is < 135 m mol/L it is called hyponatremia and the following are the causes of hyponatremia.

A. Loss of sodium in excess of water (hyponatremic dehydration).
   (*i*) Diabetic acidosis and coma
   (*ii*) Uremia from chronic renal insufficiency
   (*iii*) Protracted vomiting and diarrhoea
   (*iv*) Addison's disease (Adrenal failure)
   (*v*) Diuretics

B. Excessive retention of water (dilutional hyponatremia).
   (*i*) Heart failure
   (*ii*) Hepatic cirrhosis
   (*iii*) Nephrotic syndrome.

11. WHAT ARE THE CLINICAL FEATURES OF HYPONATREMIA?

Ans. Hyponatremia causes shifting of water to brain cells resulting in increased ICF volume of brain cells producing cerebral oedema. Therefore the symptoms are primarily neurological. The severity of symptoms depends upon the rapidity of onset and the degree of hyponatremia.

The symptoms are :

(*a*) Headache, lethargy and confusion
(*b*) Seizures and coma occurs when the plasma Na^+ falls below 120 m mol/L

HYPERNATREMIA

12. WHAT IS HYPERNATREMIA AND WHAT ARE THE CAUSES OF HYPERNATREMIA?

Ans. When the plasma Na^+ concentration is greater than 145 m mol/L it is called hypernatremia. Hypernatremia is a state of hyperosmolality as sodium is the principal cation of ECF. The following are the important causes of hypernatremia.

1. ⇑⇑ insensible loss of water (skin)
   (*a*) fever
   (*b*) Heat exposure
   (*c*) Severe burns
2. Diarrhoea (GIT loss)
3. Diabetes insipidus
4. Osmotic diuresis (Hyperglycemia and glycosuria)
5. Primary dessication syndrome (infants with moderate diarrhoea, elderly patients with fever and insensible loss of water unable to take fluids resulting in hypernatremia)
6. Brain injury with coma (subdural hematoma, subarachonid hemorrage)
7. Diabetic coma after therapy with insulin (shifting of intracellular Na^+ to ECF sodium value raising upto 160 m mol/L).

13. WHAT ARE THE CLINICAL FEATURES OF HYPERNATREMIA?

Ans. Hypernatremia causes shifting of water out of cells due to hypertonicity resulting in contracted ICF volume. This leads to cerebral hemorrage giving rise to following symptoms.

(*a*) Altered mental status
   (*a*) Weakness
   (*b*) Neuromuscular irritability
   (*c*) Focal neurologic deficits
   (d) Seizures and coma if the condition is severe.

ACID BASE BALANCE

14. WHAT IS THE NORMAL pH OF PLASMA AND WHAT ARE THE FACTORS WHICH MAINTAIN THE pH?

Ans. The normal pH of plasma lies between 7.35–7.45 and is approximately 7.4.

The following factors maintain pH of ECF (plasma).

1. Buffers
2. Lungs by controlling respiration
3. Renal regulation.

15. WHAT IS HENDERSON – HASSELBALCH EQUATION? WHAT ARE THE APPLICATIONS OF THIS EQUATION?

Ans. Henderson – Hasselbalch equation represents

$$pH = pK + \log \frac{salt}{acid}$$

Application of Henderson – Hasselbalch equation. This equation is applied to the buffer system of the body. It is most commonly applied to HCO_3/H_2CO_3 buffer system. The dissociation constant (pKa) of carbonic acid is 6.1.

Therefore At 7.4 pH

$$7.4 = 6.1 + \log \frac{salt}{acid}$$

$$7.4 - 6.1 = \log \frac{salt}{acid}$$

$$\log \frac{salt}{acid} = 1.3$$

Anti log of 1.3 = 20

Therefore salt/acid = 20/1 that means at 7.4 pH the ratio of HCO_3^- : H_2CO_3 is 20:1. The H_2CO_3 value can be calculated by the formula $\alpha \times PCO_2$ where α is solubility factor of CO_2 which is 0.03 and pCO_2 (partial pressure of CO_2) = 40 mm Hg. This means with pCO_2, the H_2CO_3 value can be calculated. By the blood gas analyser HCO_3^- value, pCO_2 and pH value can be measured and by means of these values acid base status of the patient can be found out.

BUFFERS

16. DEFINE BUFFER? WHAT ARE THE BUFFER SYSTEMS PRESENT IN PLASMA AND ERYTHROCYTES? HOW THE BUFFER SYSTEM PLAYS A ROLE IN THE MAINTENANCE OF ACID BASE BALANCE?

Ans. A buffer is a solution consisting of a mixture of a conjugated base and its weak acid.

Functions

1. Buffer resists the change of P^H inspite of addition of acid or base.
2. Buffer systems are mainly involved in the transport of H^+ ions from the site of their formation to the site of their elimination.

Examples

(*i*) Bicarbonate buffer system and hemoglobin are involved in the transport of H^+ ions from the tissues to the lungs.

Buffer Systems of Plasma

Salt (conjugated base)	$BHCO_3$	B. Protein	B_2HPO_4	B.organic acid
Weak acid	H_2CO_3	H. Protein	BH_2PO_4	H. organic acid

Buffer Systems of Erythrocytes

$\frac{BHCO_3}{H_2CO_3}$ $\frac{BHb}{HHb}$ $\frac{BHbO_2}{HHbO_2}$ $\frac{B_2HPO_4}{B.H_2PO_4}$ $\frac{B.\ Organic\ acid}{H.\ Organic\ acid}$

Mechanism of Action of Buffers

1. Role of bicarbonate buffer system in the regulation of acid base balance.
2. Role of Hb in the regulation of acid base balance.

(*i*) Role of bicarbonate buffer system in the regulation of acid base balance.

If acid (HCl, H_2SO_4, lactic acid) is added, the numerator of buffer system i.e., HCO_3 reacts with H^+ ion to form H_2CO_3

$HCO_3^- + H^+$ (from HCl) $\longrightarrow$ H_2CO_3

$\downarrow$

$H_2O + CO_2 \longrightarrow$ Expired in the air by the lungs.

If alkaline substance enters the ECF, the denominator of the buffer system i.e. H_2CO_3 reacts with alkali to form HCO_3^-

$H_2CO_3 + OH^-$ (from NaOH) $\longrightarrow$ $NaHCO_3 + H_2O$

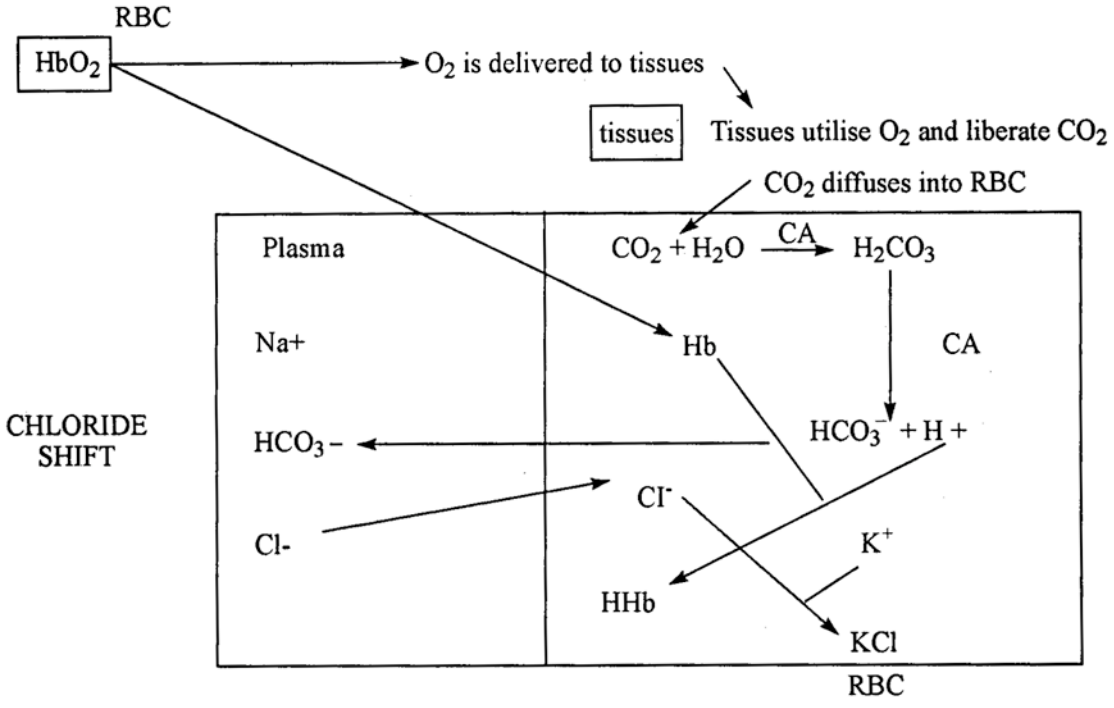
$\downarrow$

HCO_3^- is excreted by the kidney

(*ii*) Role of Hb in the regulation of acid base balance.

Changes in the Tissues

In the tissues PO_2 40 mm Hg and Hb is 75% saturated with O_2 therefore it delivers O_2 to tissues.

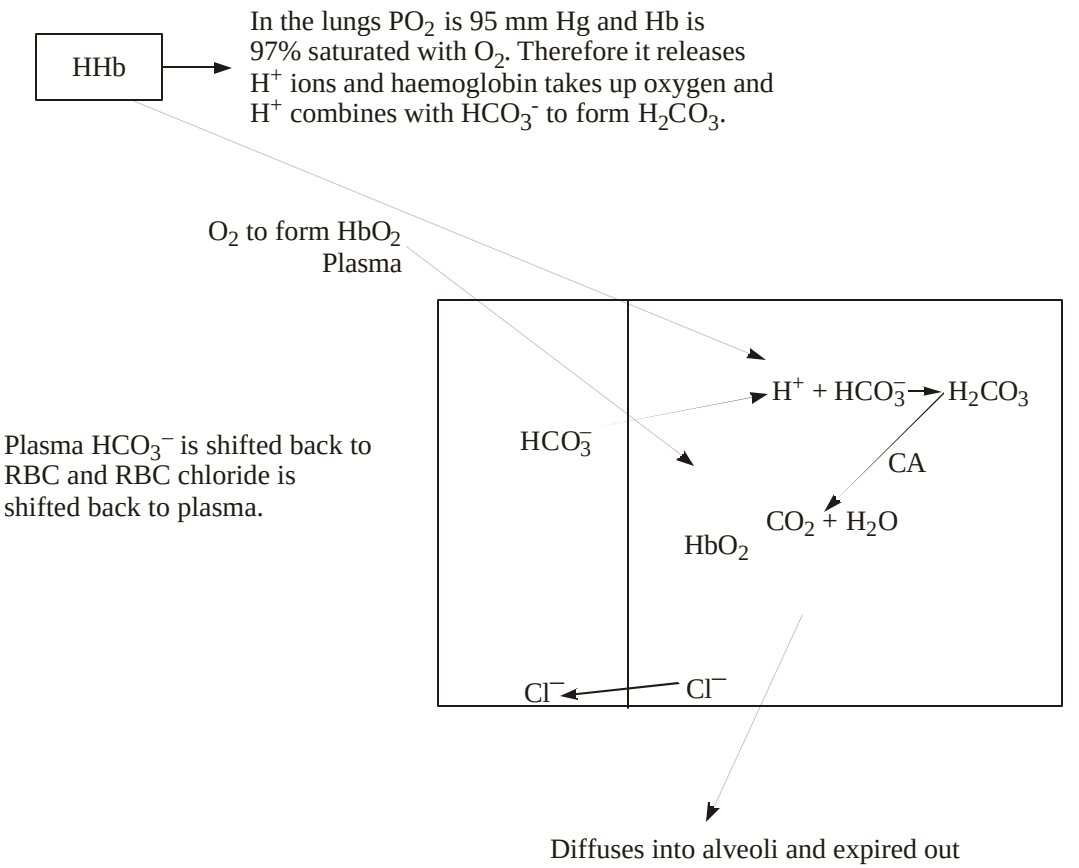


Isohydric transport of CO_2 from tissues to lungs involving chloride shift. Buffers play a role in the acid base balance.

Chloride Shift

Plasma chloride is shifted to RBC and RBC HCO_3^- is shifted to plasma. From the tissues H^+ ions are carried in the form of HHb (RBC) and HCO_3^- (Plasma) to the lungs.

Changes in the Lungs



RENAL REGULATION OF ACID BASE BALANCE

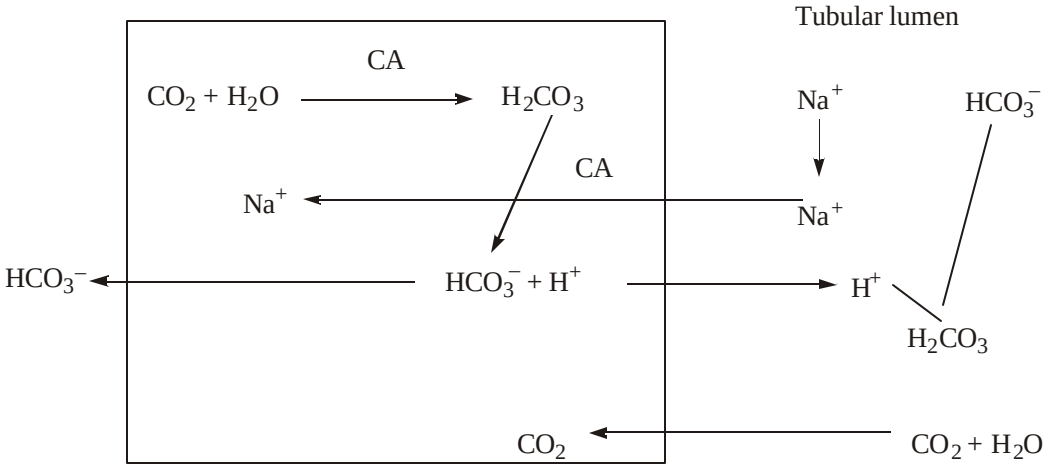
17. WHAT IS THE RENAL MECHANISM OF REGULATION OF ACID BASE BALANCE?

Ans. Kidney regulates acid base balance by three mechanisms.

1. Reabsorption of HCO_3^- at proxmial convoluted tubule (PCT)
2. Secretion of H^+ ions at distal convoluted tubule (DCT) coupled with phosphate buffer system (Titrable acidity)

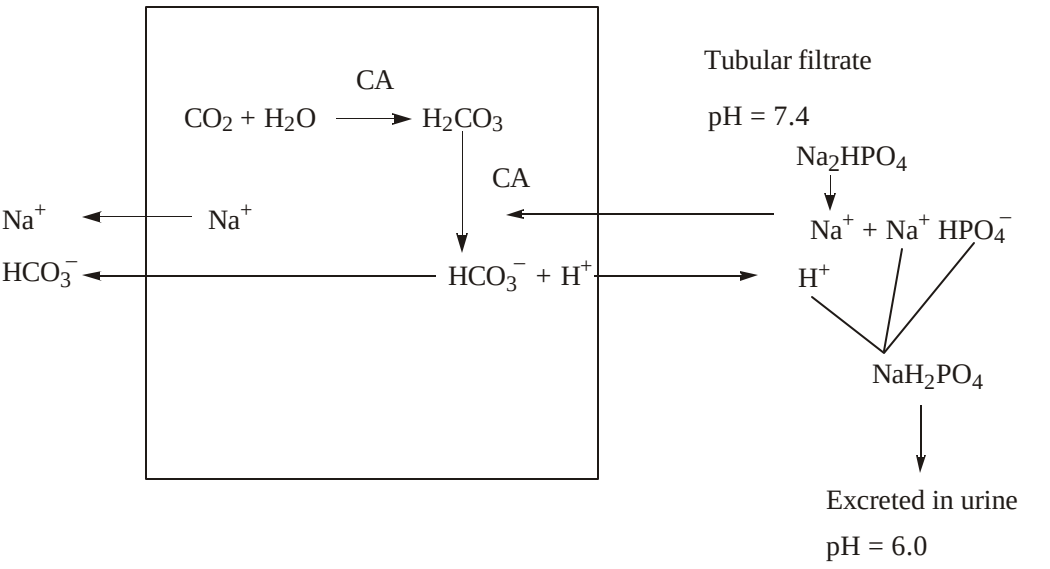
3. Secretion of ammonia at the distal convoluted tubule (DCT) (combined acidity). Reabsorption of HCO_3^- occurs in PCT.

A. The ↑↑ pCO_2 stimulates ↑↑ reabsorption of HCO_3 by PCT.



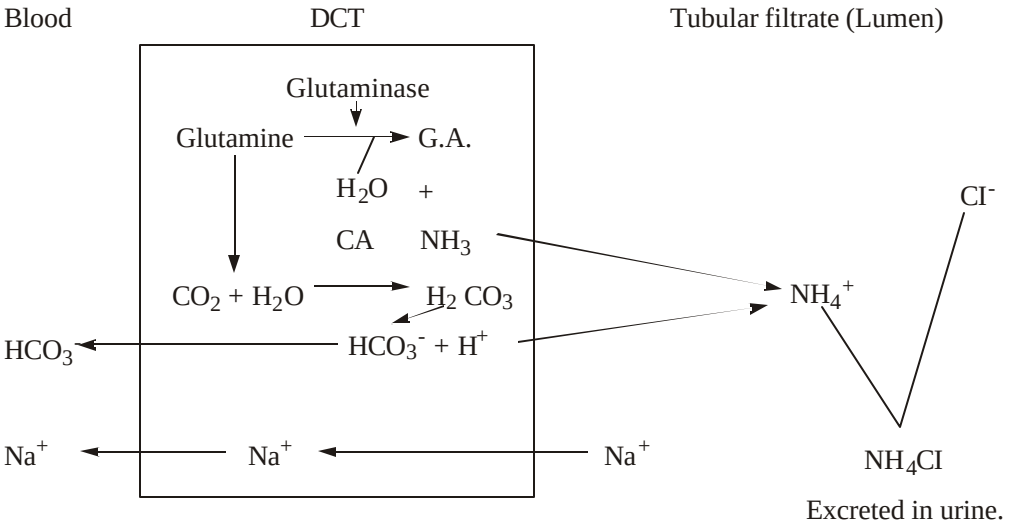
(*i*) H^+ ion is secreted by PCT cells by carbonic anhydrase and exchanges with lumen Na^+. Lumen HCO_3^- reacts with H^+ to form H_2CO_3 which is then dissociated into CO_2 and H_2O. CO_2 diffuses into the PCT cells.

B. Secretion of H^+ ions in the DCT coupled with phosphate buffer system. The secretion of H^+ ions and its excretion in the form of NaH_2PO_4 in urine is called titrable acidity.



H^+ ion secreted by DCT cells by CA is exchanged with Na^+ (cation) and ultimately the secreted H^+ ion is excreted in the form of NaH_2PO_4. The change of pH from 7.4–6.0 by the secretion of H^+ ions is called titrable acidity.

C. Secretion of ammonia



By the action of glutaminase, glutamine is converted to glutamic acid and NH_3. The H^+ ion secreted by DCT combines with NH_3 to form NH_4^+ ion which is later excreted in the form of NH_4Cl in urine. The acidity due to the secretion of ammonia is called combined acidity. In metabolic acidosis, the activity of glutaminase is increase to several folds.

ANION GAP

18. WHAT IS ANION GAP AND WHAT IS THE CLINICAL IMPORTANCE OF ANION GAP?

Ans. The sum of concentration of Na^+ and K^+ (cations) minus the sum of Cl^- and HCO_3^- is called as anion gap.

For example

138 + 4 = 142 m mol/L – (100 + 25 = 125)

$Na^+ + K^+$ sum of cations $Cl^- + HCO_3^-$ = sum of anions

= 17 m mol/L (unmeasured anions)

Anion gap

The anion gap is due to unmeasured anions such as PO_4^{-3}, SO_4^{-2} and proteins

Causes of ⇑⇑ anion gap

(*a*) Renal failure (CRF and ARF) due to retention of fixed acid.

(*b*) Diabetic ketoacidosis due to ⇑⇑ level of ketone bodies.

(*c*) Lactic acidosis (shock) due to ⇑⇑ level of lactic acid.

(*d*) Methanol intoxication.

ABNORMALITIES OF ACID BASE BALANCE

19. WHAT IS ACIDOSIS AND WHAT IS ALKALOSIS AND WHAT ARE THE DIFFERENT TYPES OF DISORDERS?

Ans. When the pH of blood is decreased below 7.30 either due to primary deficit of HCO_3^- or due to ↑↑ accumulation of H_2CO_3 (↑↑ PCO_2) is called acidosis.

When the P^H of blood is increased due to primary ↑↑ of HCO_3^- or due to primary deficit of H_2CO_3 (↓↓ pCO_2) is called alkalosis.

20. WHAT ARE THE CAUSES OF METABOLIC ACIDOSIS?

Ans. The following are the causes of metabolic acidosis.

(*a*) Diabetic ketoacidosis and starvation due to ↑↑ level of ketone bodies formation than normal (normal 1mg/dl). In this condition it is raised more than 70 mg/dl depending upon the condition.

(*b*) Renal failure (CRF and ARF) due to retention of fixed acid.

(*c*) Lactic aciodosis (shock) due to increased level of lactic acid.

(*d*) Methanol intoxication.

(*e*) Uretero sigmoidostomy.

(*f*) Diarrhoea.

(*g*) Renal tubular acidosis.

(*h*) Ammonium chloride administration.

21. WHAT ARE THE CAUSES OF RESPIRATORY ACIDOSIS?

Ans. The following are the causes of respiratory acidosis.

(*a*) Chronic obstructive pulmonary diseases (C.O.P.D.).

(*b*) Asthma.

(*c*) Emphysema.

(*d*) Adult respiratory distress syndrome.

(*e*) Hypoventilation.

(*f*) Drugs (Morphine).

Chapter–19

Plasma Proteins and Immunoglobulins

PLASMA PROTEINS

1. WHAT ARE PLASMA PROTEINS AND WHAT ARE THEIR FUNCTIONS?

Ans. The following are the plasma proteins

Sl.No.	*Plasma proteins*	*Mol.Wt.*	*Normal concentration in plasma*
1.	Albumin	69,000	3.5 to 5.5 G/dl
2.	Globulins	140,000	2.5 to 3 G/dl
3.	Fibrinogen	400,000	0.2 to 0.4 G/dl

Functions of Plasma Proteins

(*i*) **Osmotic Pressure due to Colloids**

About 80% of the total colloid osmotic pressure is exerted by albumin. The colloid osmotic pressure due to plasma protein is about 25 mm Hg. The ⇓⇓ plasma albumin (<2.0 G/dl) concentration causes oedema due to fall in osmotic pressure as the return of water into blood vessels is diminished.

The major function of albumin is to provide colloid osmotic pressure in the plasma, which in turn prevents plasma loss from the capillaries.

(*ii*) **Transport of Substances**

Albumin play important role in the transport of various water insoluble substances, such as bilirubin and free fatty acids.

(*iii*) **Buffer**

Albumin has maximum buffering capacity due to presence of histidine residues and this helps in the maintenance of acid base balance.

(*iv*) **Immunity**

Gamma globulins are involved in the natural and acquired immunity against invading organisms.

(*v*) **Coagulation**

Fibrinogen is involved in the formation of blood clot (coagulation).

(*vi*) **Nutritive Role**

Plasma proteins are used for the formation of tissue proteins (Nutritive role). When the tissues become depleted of proteins, the plasma proteins can act as a source for rapid replacement of the tissue proteins.

2. WHAT ARE THE DIFFERENT FRACTIONS OF SERUM PROTEINS SEPRATED BY ELECTROPHORESIS AND WHAT ARE THEIR PERCENTAGES?

Ans. Serum proteins are separated by agar gel electrophoresis in order of rate of movement.

Protein fraction	*Percentage*	*Rate of movement*
Albumin	55 – 65 %	Fastest mobile fraction
α_1 – globulin	2 – 4 %	Movement <than albumin
α_2 – globulin	6 – 12%	Movement <than α1 – globulin
β – globulin	8 – 12%	Movement <than α2 – globulin
γ – globulin	12 – 22%	Least mobile fraction

3. WHAT ARE THE PROTEINS PRESENT IN THE SERUM AND WHAT ARE THEIR FUNCTIONS?

Ans. The following proteins are present in the serum:

S.No.	*Protein separated by electrophoresis*	*Major functions*	*Altered in clinical disorders*
1.	Prealbumin (this minor band can be seen running ahead of albumin). Synthesized in liver.	(*a*) Binds Thyroxine. (*b*) It also binds retinol binding protein (RBP).	(*a*) Hepatitis. (*b*) Early Cirrhosis (*c*) Thyrotoxicosis.
2.	Albumin (fastest moving fraction) synthesized in the liver.	(*a*) Maintains osmotic pressure. (*b*) Transport of bilirubin and FFA. (*c*) Nutritive value and acts as a buffer.	Decreased in (*a*) Nephrotic syndrome. (*b*) Cirrhosis of liver. (*c*) Protein loosing enteropathy. (*d*) Malnutrition.
3.	Proteins present in α_1 band (*a*) α-fetoprotein formed in fetal liver.	Present in fetal and maternal blood and amniotic fluid.	$\uparrow\uparrow$ Level in maternal plasma and amniotic fluid in (*a*) Neural tube defect $\downarrow\downarrow$ serum level in hepatoma.
	(*b*) α_1 anti trypsin	Inhibitor of plasma proteases.	Decreased plasma level results in emphysema.
	(*c*) α_2 acid glyco protein.	Acute phase protein.	$\downarrow\downarrow$ plasma level in (*a*) Cirrhosis. (*b*) Nephrotic syndrome $\uparrow\uparrow$ plasma level in cancer patients.
4.	Proteins present in α_2 band (*a*) α_2 macroglobulin (major α_2 globulin).	It inhibits trypsin, chymotrypsin, plasmin and thrombin $\uparrow\uparrow$.	Plasma levels in (*a*) Nephrotic syndrome (in this condition α_2 band is prominent). (*b*) Cancers.

(*Contd...*)

	(*b*) Hepatoglobulin.	It binds with hemoglobin present in the plasma due to hemolysis and forms a complex which prevents its excretion in urine. The complex is removed by RE cells.	⇑⇑ levels seen in ⇑⇑ biliary obstruction, ⇓⇓ Level in hemolysis.
	(*c*) Ceruloplasmin.	Copper binding protein. Acute phase protein.	⇓⇓ level in Wilson's disease.
5.	Proteins present in β-band, (*a*) Transferrin synthesized in liver.	Transports iron in the plasma. Two molecules of iron per molecule of transferrin.	⇑⇑ levels are seen in iron deficiency anemia. ⇓⇓ levels in malnutrition.
	(*b*) 'C' reactive protein. It acts with 'C' polysaccharide of the cell wall of pneumo-coccus.	It is an acute phase protein.	⇑⇑ level seen in infection.
6.	Proteins present in the γ -band (*a*) Immunoglobulins.	Produced by B-lymphocytes in response to the presence of antigen. The main function of all immunoglobulins is to act as the first line of defense against foreign antigen (bacteria and virus).	(*a*) **Polyclonal gammopathy** (*i*) Many clones are stimulated. Generalised ⇑⇑ of all Ig observed in infecti- ons (a prominent γ- band present) (*ii*) ⇑⇑ immunoglobulins in (a) Chronic infections. (b) Collagen diseases. (c) Sarcoidosis. (b) **Monoclonal gammo pathy.** A single clone produces 1gG myeloma protein in multiple myeloma. The 'M' band is seen in γ -region or between γ and β regions, ⇑⇑ of 'M' protein in multiple myeloma.

4. WHAT ARE THE BENCE-JONES PROTEINS? IN WHICH CLINICAL DISORDER THESE PROTEINS ARE EXCRETED IN URINE?

Ans. These are proteins of low molecular weight having light chains of immunoglobulins excreted in the urine of patients with multiple myeloma. These proteins undergo precipitation when they are heated between 4ºC to 60ºC, redissolving at temperature higher than 80ºC and lower than 45ºC.

5. WHAT ARE THE IMMUNOGLOBULINS? OUTLINE THE MAIN FEATURES OF STRUCTURE OF IMMUNOGLOBULINS.

Ans. (*i*) The B-lymphocytes are responsible for the synthesis of humoral bodies called immunoglobulins.

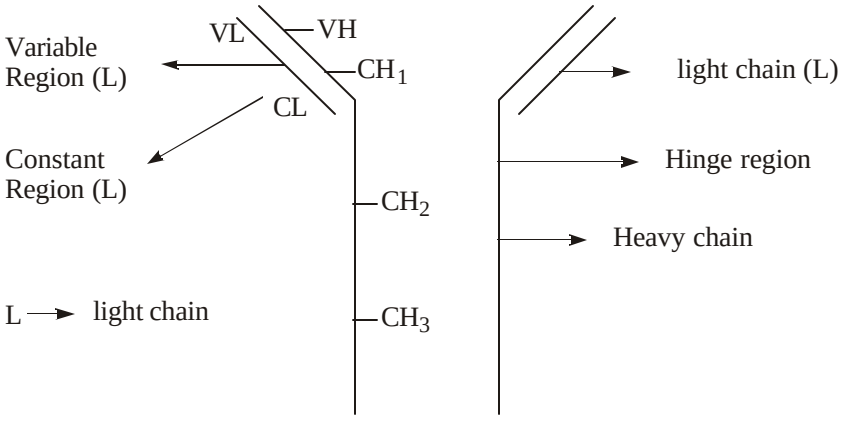
(*ii*) Immunoglobulins are classified into five types depending upon the nature of heavy chains present in them.

Classification of Immunoglobulins

Sl.No.	*Heavy chain*
1.	G ⟶ IgG [Immunoglobulin – G(gamma)]
2.	A ⟶ IgA [Immunoglobulin – A (Alpha)]
3.	M ⟶ IgM [Immunoglobulin – M (Mμ)]
4.	E ⟶ IgE [Immunoglobulin – E (Epsilon)]
5.	D ⟶ IgD [Immunoglobulin – D (Delta)]

STRUCTURE OF IMMUNOGLOBULINS

All immunoglobulins contain two light chains and two heavy chains. They possess Y shaped structure.



Structure of immunoglobulin:

CH ⟶ constant region (heavy chain)

VH ⟶ variable region (heavy chain)

Ig molecule binds the antigen at variable regions of H and L chains. Digestion of Ig by papain produces two antigen binding fragments (FAB) and one crystallizing fragment (Fc). Papain cleaves at hinge region (in between CH_1 and CH_2).

Types of Light Chains and Heavy Chains

There are two types of light chains:

(*i*) Kappa (K)

(*ii*) Lambda (λ)

There are five types of heavy chains based on which immunoglobulins are classified. They are called γ, α, μ, ε and δ chains.

6. WHAT ARE THE BIOLOGICAL FUNCTIONS OF IMMUNOGLOBULINS?

Ans. IgG comprises approximate by 75 to 85% of total immunoglobulins. There are four sub-classes. IgG_1, IgG_2, IgG_3 and IgG_4.

(*i*) Its serum level is 950 mg to 1250 mg/dl.

(*ii*) Crosses placenta to reach the fetus and provides the baby with passively acquired antibody during its early life.

(*iii*) Main antibody in the secondary immune response. Example: In the viral hepatitis caused by HAV the IgG antibodies appear in the plasma after the fall of Ig.M antibodies i.e., after 3 months of onset of clinical illness and persists for many years after infection.

(*iv*) Opsonizes bacteria: Stimulation of phagocytosis by an Ig molecule bound to surface of a foreign molecule (bacteria) is called opsonization (IgG – opsonin). IgG binds via Fc fragment macrophages, neutrophils, large granular lymphocytes. IgG molecules coat the bacteria so that their ingestion by phagocytes is facilitated.

(*v*) Complement activation. IgG binds complement at their Fc region and activate the complement cascade.

(*vi*) Neutralizes bacterial toxins such as those responsible for many of the clinical manifestations of diphtheria or tetanus.

IgM

(*a*) It is a macro molecular and predominantly intravascular antibody. It is made up of five immunoglobulin units linked with disulphide bonds together with a 'j' chain.

(*b*) Serum level: 70 to 170 mg/dl.

(*c*) Produced in primary response to an antigen. Example: In viral hepatitis caused by HAV, Ig M is produced during incubation period and at the onset of clinical illness but declines with in three months.

(*d*) Fixes complement. IgM is especially effective in activating complement to produce immunolysis of foreign cells.

(*e*) IgM antibodies are more efficient than IgG antibodies in linking particulate antigens together for agglutination and phagocytosis. The Fc portion of IgM molecule binds lymphocytes.

IgA

(*a*) IgA accounts for about 10–15% of the total serum immunoglobulins. Serum concentration: 150 to 260 mg.dl.

(*b*) Secretory IgA confers immunity to infection by enteric bacterial and viral organisms. It prevents attachment of bacteria and virus to mucus membrane.

(*c*) Does not fix complement.
(*d*) The Fc portion of IgA binds lymphocytes.

IgD

(*i*) IgD is found on the surface of immature B lymphocytes and may be involved in their maturation and regulation.
(*ii*) It is a marker for mature B cells.
(*iii*) Concentration in serum: 4 mg/dl.

IgE

(*i*) It binds firmly to mast cells and basophils and mediates immediate hypersensitivity reactions (in atopic individuals) causing release of mediators from mast cells and basophils upon exposure to antigen.
(*ii*) Defends against worm infections by causing release of eosinophils. IgE appears to be important in defense against helminth parasites.

COMPLEMENT SYSTEM

7. WHAT IS COMPLEMENT SYSTEMS?

Ans. There are 20 components present in serum, which make up complement system. This system is activated by antigen antibody complexes (IgG or IgM) or with 'C' reactive protein resulting complement cascade to destroy bacteria and virus. The activation of C_3 by the enzyme C_3 convertase is the central event of the complement sequence.

$$C_3 \longrightarrow C_3B \longrightarrow \boxed{C_5bC_6C_7C_8C_9C}\, C_{5b}\text{-}C_9$$ membrane attack complex-cell lysis.

Chapter–20

Haemoglobin, Porphyrins and Bile Pigments

CHEMISTRY OF PORPHYRINS

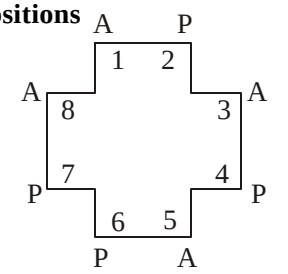
1. WHAT ARE THE NATURALLY OCCURRING AND BIOLOGICALLY IMPORTANT PORPHYRINS AND WHAT ARE THE FUNCTIONAL GROUPS PRESENT IN THEM?

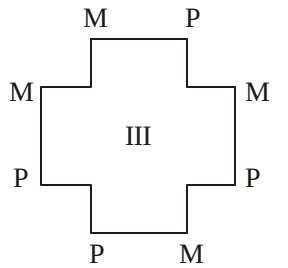
Ans. The naturally occurring and biologically important porphyrins are:

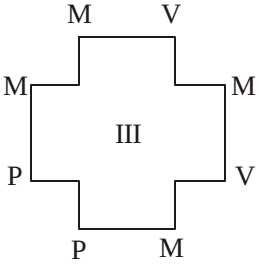
(*i*) Uroporphyrin – III
(*ii*) Coproporphyrin – III
(*iii*) Protoporphyrin – III

Functional Groups

S. No.	Porhyrin	Functional groups present in various positions
1.	Uroporphyrin – III	Acetate (CH_2COOH) in 1, 3, 5, 8 propionate in 2, 4, 6, 7 (CH_2–CH_2–COOH)
2.	Coproporphyrin – III	– Methyl (CH_3) – 1, 3, 5, 8 Propionate – 2, 4, 6, 7 (CH_2 – CH_2 – COOH)
3.	Protoporphyrin – III	– Methyl 1, 3, 5, 8 Vinyl 2, 4 (CH = CH_2) Propionate 6, 7







2. WHAT IS THE DIFFERENCE BETWEEN PORPHYRINOGENS AND PORPHYRINS? WHAT ARE THE CHARACTERISTIC FEATURES OF PORPHYRINS?

Ans.

Porphyrinogens	*Porphyrins*
(*a*) Reduced forms having 6 extra hydrogens (6H)	Oxidized forms formed by the removal of 6 hydrogens from porphyrinogens
(*b*) No double bonds	Have double bonds
(*c*) Colourless compounds	Porphyrins are coloured

CHARACTERISTIC FEATURES OF PORPHYRINS

(*i*) All porphyrins absorb light and produce sharp absorption band near 400 nm wave length. This is called soret band.

(*ii*) Solution of porphyrins emit strong red fluorescence due to double bonds present in them.

3. WHAT ARE THE MAIN COMPONENTS PRESENT IN NORMAL ADULT HAEMOGLOBIN (HB-A)?

Ans. The main components present in normal adult haemoglobin (Hb-A) are:

(*i*) Haeme (Iron protoporphyrin – III)

(*ii*) Globin – 4 polypeptide chains $\alpha_2\beta_2$

α-Chain has α-141 AAs.

β-Chain has β-146 AAs.

4. WHAT ARE THE PHYSIOLOGICALLY IMPORTANT HAEMOGLOBINS AND WHAT ARE THE STRUCTURAL DIFFERENCES AND CLINICAL SIGNIFICANCE OF THEM?

Ans. Physiologically important haemoglobins are:

Adult haemoglobin (Hb-A), fetal haemoglobin (Hb-F) and minor haemoglobin (Hb-A_2).

Structural Differences

Hb – A $\rightarrow \alpha_2\beta_2$, Hb – F $\rightarrow \alpha_2\gamma_2$, Hb A_2 – $\alpha_2\delta_2$

All these differ in the β, γ and δ chains. β, γ, δ have 146 A.As.

Clinical Significance

Sl. No.	*Name of Hb*	*Physiological significance and percentage*	*Clinical significance*
1.	Hb-A	Normal adult 97% to 98%	↓↓ (decreased percentage in (*a*) sickle cell anaemia (*b*) β-thalassemias (minor and major)
2.	Hb-F (fetal Hb)	(*a*) Normal adult has less than 1% (<1%) (*b*) Fetus has high percentage	↑↑ (increased percentage) in (*a*) Sickle cell anemia upto 15% (*b*) β-thalassemia major >90%.
3.	Hb-A2 (Minor Hb)	(*a*) Normal adult has 1 to 2.5 percent	↑↑ (increased percent) in (*a*) β-thalassemia minor >3 %

STRUCTURE OF HAEMOGLOBIN

5. WHAT ARE THE MAIN SALIENT FEATURES OF STRUCTURE OF Hb?

Ans. Hemoglobin is allosteric protein and is organized into primary, secondary, tertiary and quaternary structure.

Primary Structure: The adult hemoglobin Hb-A has four polypeptide chains. $\alpha_2\beta_2$ (two identical α-chains and two identical β-chains.

(*a*) The α-chains has 141 AAs.

(*b*) In α-chain, N-terminal A.A. is valine (Ist A.A.) and the C-terminal A.A. is arginine.

(*c*) In α-chain, proximal histidine is 87 and distal histidine is 58.

(*d*) The β-chain has 146 A.As.

(*e*) In β-chain the N-terminal A.A. is valine (Ist A.A) and the C-terminal A.A. is histidine.

(*f*) In β-chain proximal histidine is 92 and distal histidine is 63.

The AAs in polypeptide chains are joined by peptide linkages (CO-NH linkage).

Secondary Structure

Certain portions of the polypeptide chains of Hb form helices and other positions are not helical. This is called secondary structure of hemoglobin.

Haemoglobin has approximately 80% α-helical structure. Within the helix the 'CO' group of each amino acid is hydrogen bonded to the NH group of the amino acids. Hydrogen bonds maintain the α-helix structure of Hb.

Each β-chain of haemoglobin has eight right handed α-helices 'A' to H (A, B, C, D, E, F, G, H) similar to myoglobin. Each α-chain of Hb also has same helices similar to β-chain except the helix 'D' is deleted since 5 residues in the β-chain have no counter part in the α-chain.

Tertiary Structure

The α and β chains of Hb with its secondary structure are further folded and twisted to form the three dimensional arrangement of these polypeptide chains in the tertiary structure. In the tertiary structure each polypeptide chain is folded in such a way that its hydrophobic side chains (non-polar residues) are buried and its polar side chains are on the surface. This type of arrangement in the tertiary structure helps to form a protective hydrophobic packet for binding of heme so that, it protects reduced form of iron (Fe^{++}) of heme from oxidizing to ferric (Fe^{+++}) form, from the aqueous environment.

Hydrogen bonds, hydrophobic interactions, Van-der-Waals' forces and ionic bonds maintain tertiary structure.

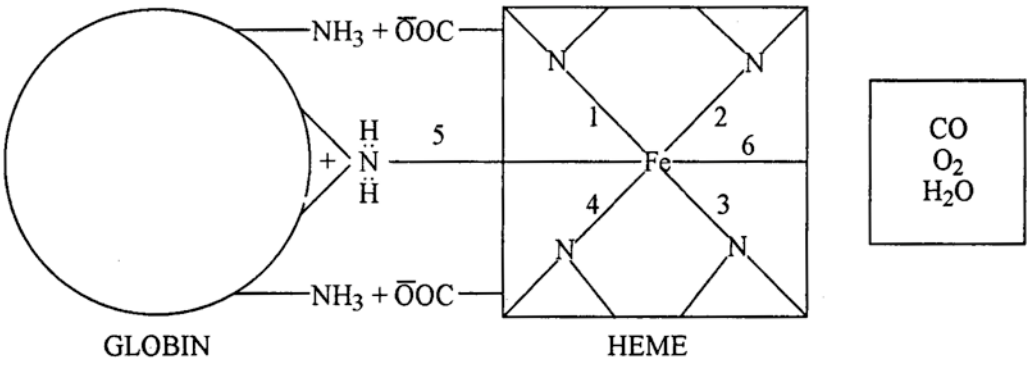


Fig. 1

Quaternary Structure

In the quaternary structure of Hb, the α and β chains (subunits) are arranged in three-dimensional complexes and this structure exhibits its allosteric properties.

Hydrogen bonds, salt bridges and Van-der-Waals' forces maintain the quaternary structure of Hb. The quaternary structure of Hb facilitates the cooperative binding of oxygen. That is, the binding of oxygen to one site facilitates further binding of O_2 easily at other sites.

6. WHAT IS THE MECHANISM OF OXYGEN BINDING BY HEMOGLOBIN AND WHAT IS THE ROLE OF HEME IN THIS MECHANISM?

Ans. The iron atom has six coordination bonds, in the hemoglobin. In the hemoglobin molecule the heme iron (Fe^{++}) is attached to four to (4) 'N' atoms of the heme ring by coordination bonds. The heme iron is attached to imidazole 'N' of proximal histidine (F8) by 5th coordination bond. In the Hb solution, valence 6-appears to be attached to H_2O and the oxygenation of Hb involves displacement of this water by O_2.

$$HHb.H_2O + O_2 \rightleftharpoons HbO_2 + H_2O$$

Diagram

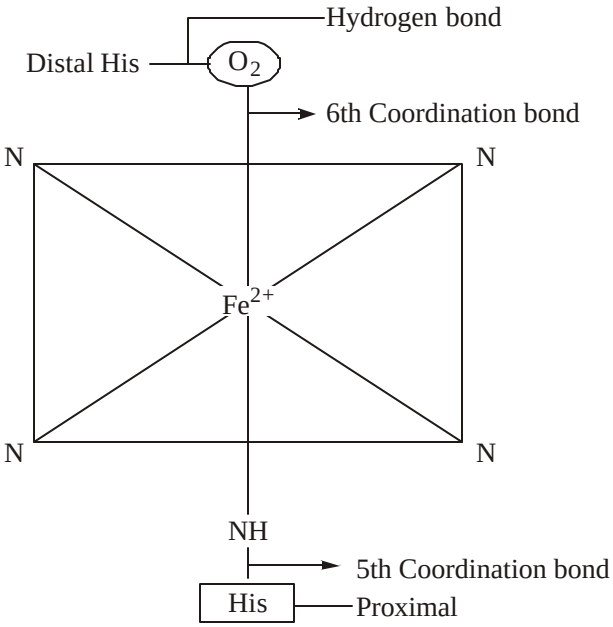


Fig. 2

The 5th coordination position is below the plane and the 6th co-ordination position is above the plane.

Hemoglobin exists in two states

(*i*) Taut state (T form) before binding of O_2.

(*ii*) Relaxed state (R-form) after binding of O_2.

Oxygenation of hemoglobin causes conformational changes in the tertiary and quaternary structure of Hb. A molecule of O_2 is bound first by α-chain. This facilitates further binding of oxygen by the remaining chains, (cooperative binding) as Hb is an allosteric protein. The following changes take place during the binding of O_2.

(*i*) On oxygenation, the iron atom of deoxy Hb (which lie 0.06 nm away the plane of the heme ring) move into the plane of heme ring.

(*ii*) Salt bridges are broken.

7. WHAT ARE THE FUNCTIONS OF HAEMOGLOBIN?

Ans. (*i*) Haemoglobin is involved in the transport of oxygen from the lungs to the peripheral tissues.

(*ii*) Transport of CO_2 and protons (H^+ ion) from the peripheral tissues to lungs.

8. WHAT IS OXYGEN DISSOCIATION CURVE AND WHAT ARE THE FACTORS INFLUENCING THE OXYGEN DISSOCIATION CURVE?

Ans. In oxygen dissociation curve pressure of O_2 in mmHg is plotted on the X-axis and percentage of saturation of O_2 is plotted on the Y-axis.

In the lungs, the pO_2 is 95 mm Hg and the oxygen saturation in the systemic arterial blood is 97 percent. Whereas in the tissues, pO_2 is 40 mmHg and the oxygen saturation is 75 percent. The shape of O_2 binding curve of Hb is sigmoidal (S-shaped) because O_2 binding is cooperative due to quaternary structure of Hb and the Hb is an allosteric protein.

The p50 value (partial pressure of O_2 that half saturates Hb) for Hb-A is 26 mm.Hg and P50 value for Hb-F is 20 Hg. This difference permits Hb-F to extract O_2 from the Hb-A of placental blood.

Factors Influencing Oxygen Dissociation Curve

The following factors decrease the affinity of Hb for oxygen and hence they shift the curve to the right.

(*i*) 2,3- bisphospho glycerate formed in the bypass pathway of glycolysis (Rapaport Leubering pathway). In RBCB ↑↑ 2,3 BPG is seen in the individuals climbing high altitude.

(*ii*) H^+ ions or protons

(*iii*) CO_2.

9. WHAT IS BOHR EFFECT?

Ans. In the lungs, as the blood passes through the lungs, CO_2 diffuses from the blood into the alveoli resulting ↓↓ blood CO_2 and ↓↓ H^+ ion concentration and shifts O_2 dissociation curve to the left (Hb binds more O_2).

In the tissue reverse is the true i.e., it shifts the curve to the right. Entry of CO_2 into blood ↑↑H^+ concentration → shifts the curve to right (Hb delivers more O_2 to the tissues).

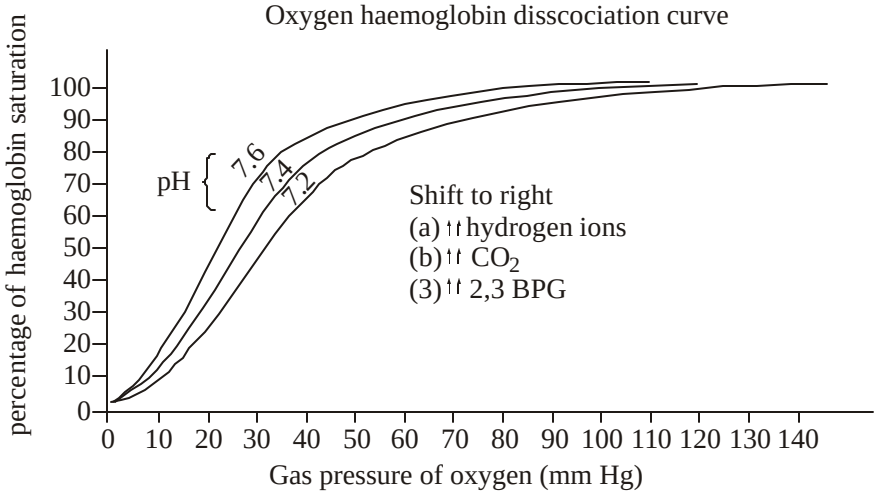


Fig. 3

10. WHAT ARE THE ABNORMAL HAEMOGLOBINS WHICH CAUSE THE CLINICAL DISORDERS AND WHAT ARE THE BIOCHEMICAL AND CLINICAL FEATURES OF THESE DISORDERS?

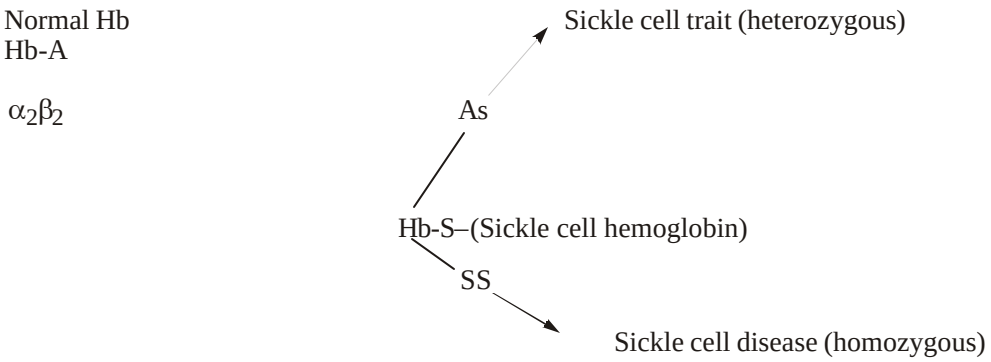
Ans. There are two types of abnormal haemoglobins, which cause main clinical disorders. These are:

(*i*) Sickle cell haemoglobin (Hb-S) causing sickle cell anaemia (hemolytic anaemia).

(*ii*) Thalassemias (β-thalassemia major) causing haemolytic anaemias.

SICKLE CELL HAEMOGLOBIN

Hemoglobin 'S' is formed due to point mutation in the gene resulting in the synthesis of an abnormal β-chain where the 6th glutamic acid is replaced by the valine



Sickle cell anemia is an autosomal recessive disorder in which an abnormal Hb (Hb-S) leads to Ch.hemolytic anemia. The abnormal hemoglobins is Hb-S $\rightarrow\alpha_2\beta_2^S$.

On the deoxygenated Hb-S a complement to stickypatch is present. The complementary surfaces allow deoxy Hb-S to polymerize and form tactoids.

The reduced from of Hb-A has half the solubility of oxyhemoglobin where as reduced form of Hb-S has 1/100th the solubility of oxyform. Based on this property sickling can be elicited in vitro by adding a reducing agent sodium metabisulphite to the blood.

The deoxy form of Hb-S forms polymers that damage the RBC membrane. Early stages → there will be reversible polymer formation. In the late stage, there will be irreversible sickling.

The rate of sickling is influenced by

(*i*) Concentration of Hb-S.

(*ii*) Red cell dehydration.

(*iii*) Presence of other hemoglobin with in the cells.

(*iv*) Acidosis.

(*v*) Hypoxia.

Clinical Features

(*a*) Jaundice (calcium bilirubinate).
(*b*) Gallstones.
(*c*) Poorly healing ulcer over the lower tibia.

Complications

(*i*) Hemolytic (co-existence G6PDH deficiencies, splenic sequestration of sickle cells).
(*ii*) Aplastic crisis (viral infection and folate deficiency).
(*iii*) Acute painful episodes (Acute vaso occlusion).

Laboratory Changes

(*i*) Hematocrit 20–30%
(*ii*) Peripheral blood smear irreversibly sickled cells (5–50%).
(*iii*) Reticulocytosis, nucleated RBC, howel Jolly bodies and target cells.
(*iv*) Indirect bilirubin level is high. By hemoglobin electrophoresis, Hb-S can be demonstrated.

	Hb-A	*Hb-S*	*Hb-A2*	*Hb-F*
As–trait	60%	40%	1 – 2 %	<1%
SS sick cell anemia	0	86.98%	1 – 3%	5 – 15%

Thalassemias

Thalassemia syndromes are inherited disorders of α or β globin biosynthesis. There are mainly two types:

(*i*) β-thalassemia major (homozygous).
(*ii*) β-thalassemia minor (heterozygous).

Mutations causing thalassemia can affect any pathway of globin gene expression such as transcription, processing of mRNA precursor, translation and post translational modifications. The most common cause is the mutation that results in defect in the splicing or premature termination of polypeptide chain.

In the β-thalassemia minor, anemia is minimal. In the β-thalassemia major, unbalanced α and β chains cause formation of inclusion bodies (unpaired α chains) which kill developing erythroblasts in the marrow. Shortening of red cell life span produce severe hemolytic anemia.

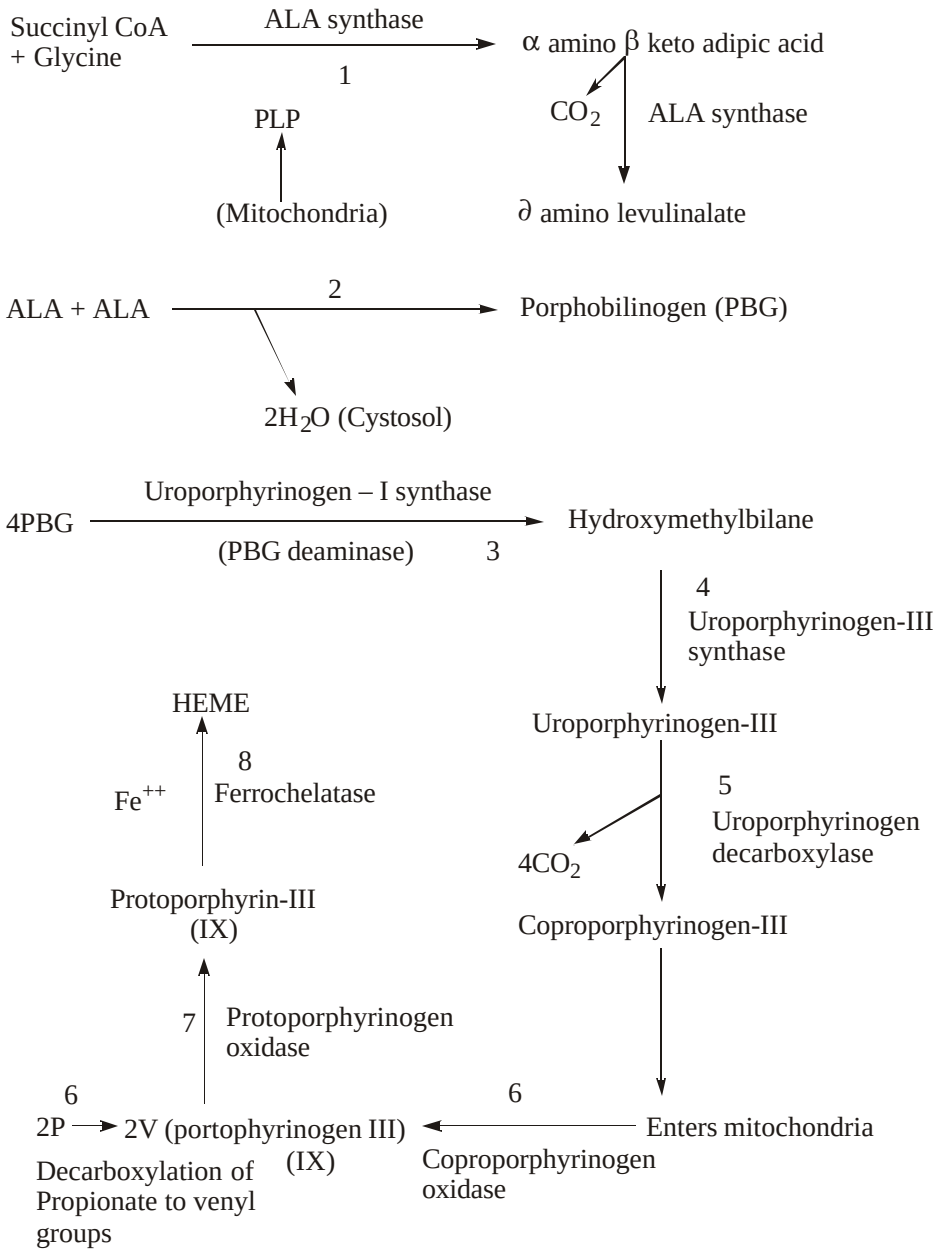
Laboratory Changes

(*i*) Microcyte out of proportion to the degree of anemia.
(*ii*) Abnormal red cell morphology with microcytes, acanthocytes and target cells.
In β-Thalassemia major – Hb-F level is raised to 90% to 96%
In β-Thalassemia minor – Hb-A2 level is raised to 4% to 8%

SYNTHESIS OF HEME

11. HOW IS HEME SYNTHESIZED?

Ans. The first step in heme synthesis occurs in mitochondrion. The starting materials succinyl CoA and glycine react to form δ amino levulenic acid which is catalyzed by the key regulatory enzyme ALA synthase.



12. HOW IS HEME SYNTHESIS REGULATED?

Ans. ALA synthase is the key regulatory enzyme of heme bio-synthesis. ALA synthase is subjected to both induction and repression. Both barobiturates and griseofulvin induce the ALA synthase. When the concentration of heme is raised this acts as a co-repressor and combines with the apo repressor which is formed by the regulatory gene to form the holorepressor and causes repression i.e. stops the synthesis of ALAsynthase. Low levels of heme has got opposite effect i.e. favours the syntheis of ALA synthase.

13. WHAT ARE THE DIFFERENT TYPES OF CIRCULATING BILIRUBINS AND WHAT ARE THEIR NORMAL LEVELS?

Ans. There are two types of circulating bilirubins. They are:

1. Unconjugated bilirubin which is insoluble and it gives indirect positive to Van den Bergh reaction.
2. Conjugated bilirubin (bilirubin diglucuronide) which is soluble in water and it gives immediate direct positive to Van den Bergh reaction.
3. Total bilirubin (unconjugated + conjugated bilirubin) value 0.3–1.0 mg/dl.
   Unconjugated bilirubin 90% of total bilirubin
   Conjugated bilirubin present in negligible amount.

PORPHYRIAS

14. WHAT ARE PORPHYRIAS? WHAT ARE THE BIOCHEMICAL AND CLINICAL FEATURES OF IMPORTANT PORPHYRIAS?

Ans. Sporadic excretion of porphyrins in moderate excess is called porphyrinuria. This occurs only in acquired diseases. Where as excretion of porphyrins and early metabolites of heme synthesis in well defined diseases due to defects of porphyrin metabolism is called porphyrias. Porphyrias are caused by the deficiency of any enzyme concerned with heme bio synthesis.

There are two types of porphyrias:

1. Erythropoietic porphyria
2. Hepatic porphyria

Biochemical and Clinical Features of Important Porphyrias

Deficiency of enzyme resulting in accumulation of metabolites of heme synthesis prior to the formation of porphyrinogens cause toxic effects in abdominal nerves and CNS resulting in ABDOMINAL PAIN and NEURO PSYCHIATRIC symptoms. Examples : Acute intermittent porphyria.

Where as enzyme blocks occurring in later pathway of heme bio-synthesis resulting in excretion of porphyrins cause photosensitivity (a reaction to visible light of 400 nm). This reaction occurs due to the presence of double bonds in their structure.

Example: congenital erythropoietic porphyria.

Acute Intermittent Porphyria

This hepatic porphyria is an autosomal dominant condition.

SITE – Liver

Enzyme deficiency	Uroporphyrinogen–I synthase (PBG–deaminase)
Products of heme metabolism excreted in urine	(*i*) porphobilinogen (PBG) (*ii*) ∂ Amino levulinic acid (ALA)
Precipitating factors	Alcohol, low calorie diets, porphyrinogenic drugs (barbiturates)

Clinical Features of Acute Attacks

(*i*) Severe abdominal cramps.
(*ii*) Neurological symptoms often with paralysis of muscles (Axonal degeneration).
(*iii*) Mental disturbance (Acute Psychosis).
(*iv*) Severe fever and leucocytosis.

The abdominal pain is due to the severe spasm of the intenstinal musculature. The nervous symptoms are both sensory and motor.

Biochemical Basis of Explanation of Symptoms

(*i*) ALA may inhibit (⇓⇓) ATP-ase in the nervous system leading to the development of neurological symptoms (ALA causes conduction paralysis).

Biochemical Investigations

(*i*) Urinary PBG excretion ⇑⇑ 50–200 mg/D (normal 0–4 mg /D)
(*ii*) Urinary ALA excretion ⇑⇑ 20–100 mg/D (normal 1–7 mg/D)

Congenital Erythropoietic Porphyria

It is an autosomal recessive disorder.

Enzyme deficiency ⟶ Uroporphyrinogen synthase
Products excreted in urine ⟶ Large amount of uroporphyrins and coproprophyrins

Clinical Features

(*i*) The outstanding clinical feature is severe skin lesions in the portions of the body exposed to sunlight.

Erythema ⟶ followed by large vesicles ⟶ become purrulent and necrotic ⟶ heal with deep mutilating and pigmented scars.

(*ii*) Pinkish–brown staining of teeth and bones due to the deposition of porphyrins. The teeth give fluorescence on exposure to long wave U.V. light.
(*iii*) Due to increase level of porphyrins in erythrocytes cause hemolysis and splenomegaly.
(*iv*) Accumulation of uroporphyrins and copro porphyrins (mostly type I) in bone marrow, erythrocytes, urine and feces.

Other Hepatic Porphyrias

S.No.	*Name of porphyria*	*Enzyme deficiency*	*Mode of inheritance*	*Photosensitivity*	*Neurovisceral symptoms*
1.	Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Autosomal dominant	Major clinical feature	Not observed
2.	Hereditary coproporphyria	Coproporphyrinogen oxidase	–do–	Minimal clinical feature	–do–
3.	Variegate porphyria	Protoporphyrinogen oxidase	–do–	–do–	–do–

METABOLISM OF BILE PIGMENTS

15. WHAT ARE BILE PIGMENTS? BRIEFLY OUTLINE THE METABOLISM OF BILE PIGMENTS?

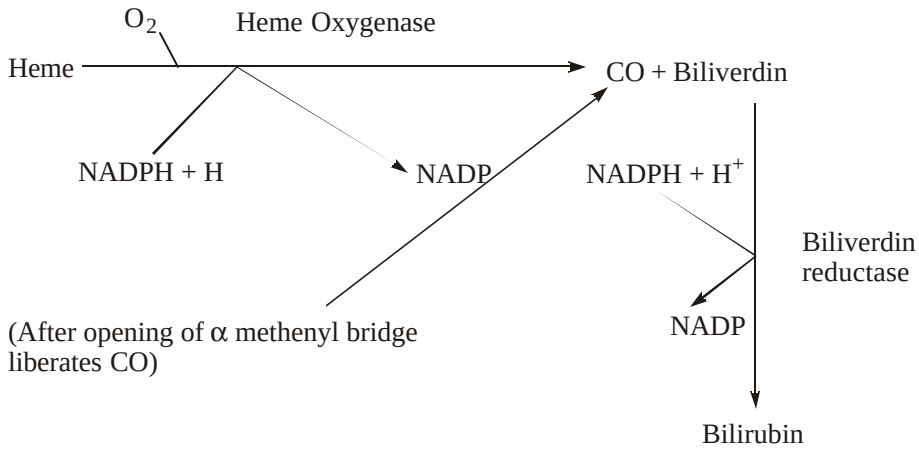
Ans. Bile pigments are:

(*i*) Biliverdin

(*ii*) Bilirubin (major circulating pigment)

Formation of Bile Pigments

After the normal life span (120 days) of erythrocytes (senescent red cells) they are destroyed by reticuloendothelial cells. Heme is dissociated from hemoglobin and is acted upon by the enzyme heme oxygenase.



The bilirubin is bound to albumin and transported to liver.

Metabolism of Bilirubin in the Liver

The process of bilirubin metabolism divided into three phases.

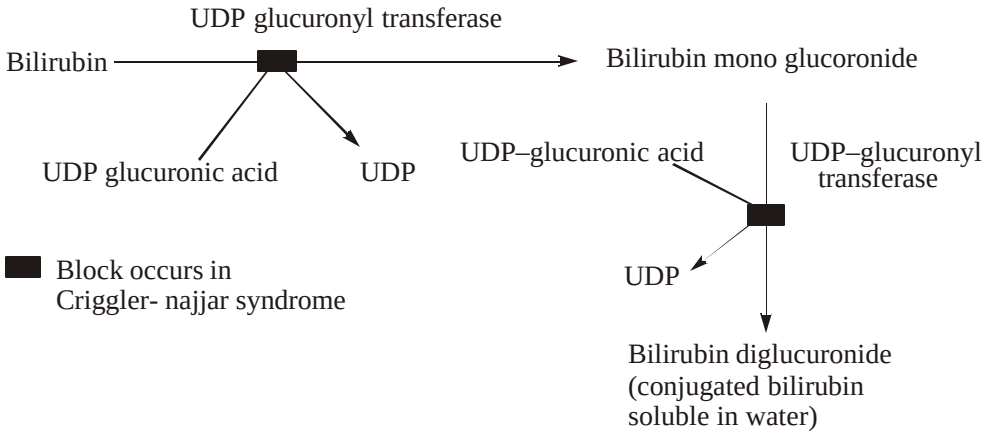
(*i*) Hepatic uptake.
(*ii*) Conjugation.
(*iii*) Excretion into bile.

Uptake

Bilirubin enters hepatocytes by facilitated transport mechanism. After uptake bilirubin is bound to glutathione-S–transferase.

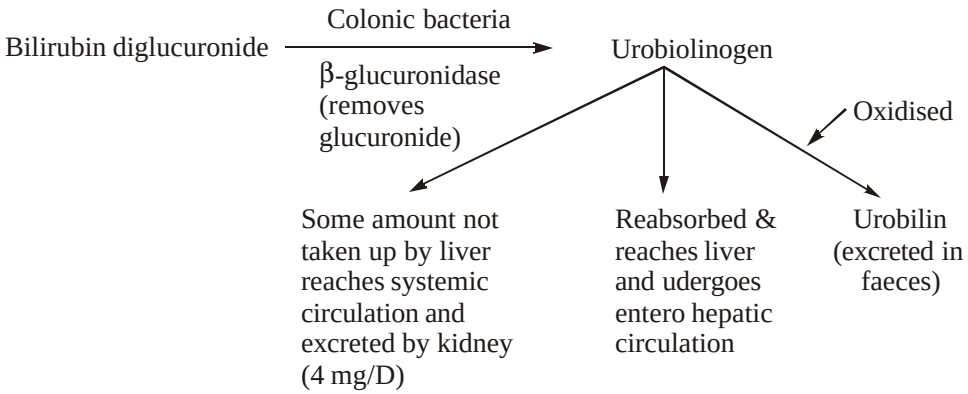
Conjugation

The unconjugated bilirubin is water insoluble and it is made into water soluble derivative by conjugation reaction.



Intestinal Phase of Bilirubin Metabolism

The conjugated bilirubin is secreted into bile and transported through the biliary ducts into the duodenum. It undergoes further cleavage by the colonic bacteria to form urobilinogen.



VAN DEN BERGH REACTION

16. WHAT IS VAN DEN BERGH REACTION AND WHAT IS ITS CLINICAL IMPORTANCE?

Ans. When diazotised sulphanilic acid is treated with equal volume of serum it gives a reddish purple colour (azo compound) immediately with in one minute, it is called Van den Bergh immediate direct positive.

If no colour is developed but on addition of alcohol if pink colour is developed it is called Van den Bergh indirect positive.

Interpretation

(*i*) Conjugated bilirubin gives immediate direct positive. It's level is raised either in
- (*a*) Intra hepatic obstruction i.e. obstruction within the canaliculi. It is called hepato cellular jaundice.
- (*b*) Extra hepatic obstruction i.e. obstruction in the common bile duct (Gall stones) it is called Cholestatic or obstructive jaundice.

(*ii*) Unconjugated bilirubin gives indirect positive.

It's level is raised in hemolytic jaundice.

JAUNDICE

17. WHAT IS JAUNDICE? WHAT ARE THE DIFFERENT TYPES OF JAUNDICE AND WHAT ARE THE MAJOR CAUSES?

Ans. Jaundice is yellowish discoloration of tissues (sclera) resulting from the deposition of bilirubin. The scleral icterus present when the serum bilirubin is 3 mg or > 3 mg/dl.

Classification of Jaundice

There are three different types of Jaundice:

I. Jaundice Caused by Raised Unconjugated Bilirubin
II. Hepatocellular Jaundice
III. Obstructive Jaundice

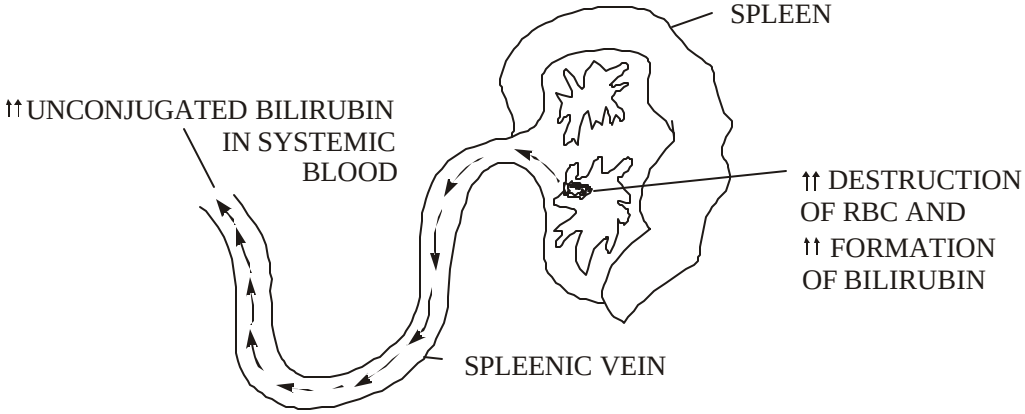
I. Jaundice Caused by Raised Unconjugated Bilirubin

A. **Hemolytic jaundice** (⇈ destruction of RBC). Hemolytic jaundice is caused by over production of bilirubin due to increased destruction of RBC. In this condition, unconjugated bilirubin level is raised.

Important causes are:

(*a*) Hemolytic anaemia.

(*b*) Neonatal physiological jaundice caused by ↑↑ hemolysis ↓↓ conjugation in the liver.



I. HEMOLYTIC JAUNDICE

Fig. 4

B. Jaundice with predominantly unconjugated bilirubinemia other than causes of hemolysis

(*i*) **Gilbert's disease :** Bilirubin 1– 4 mg/dl : (Impaired uptake of bilirubin and ↓↓ conjugation by liver due to ↓↓ activity of UDP glucuronyl transferase. It responds to phenobarbital.

(*ii*) **Criggler Najjar Syndrome–I**

(*a*) Complete absence of UDP–glucuronyl transferase. Therefore conjugation is absent.

(*b*) Response to phenobarbital is nil.

(*c*) No evidence of Hemolysis.

(*d*) Serum enzymes are normal.

Bilirubin : 20–45 mg/dl

(*iii*) **Criggler Najjar Syndrome–II**

(*a*) Markedly reduced activity of UDP glucuronyl transferase.

(*b*) Routine liver function tests are normal.

(*c*) Response to Phenobarbital is only 25%.

Bilirubin: 6–25 mg/dl

II. Hepato Cellular Jaundice

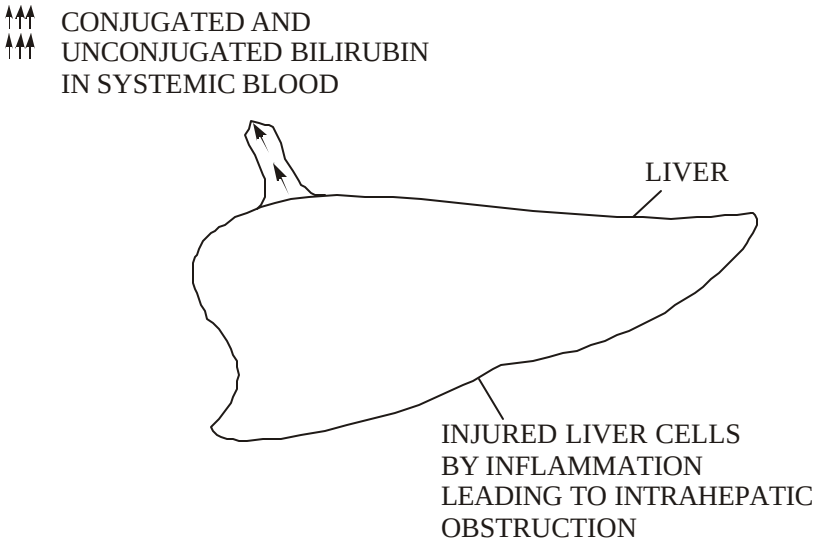
A. Important causes: *Viral hepatitis*: infections caused by various viruses (HAV, HBV, HCV, HDV and HEV). In this condition both types of serum bilirubins (conjugated and unconjugated bilirubins) are elevated.

Familial defect in hepatic excretory function.

B. Elevated conjugated bilirubin due to familial defect in hepatic excretory function.

(*a*) Dubin–Johnson syndrome

(*b*) Rotor syndrome



II. HEPATO CELLULAR JAUNDICE

Fig. 5

IV. Obstructive Jaundice

Due to predominantly hyper conjugated bilirubinemia (Extra hepatic obstruction)
Elevated conjugated bilirubin.

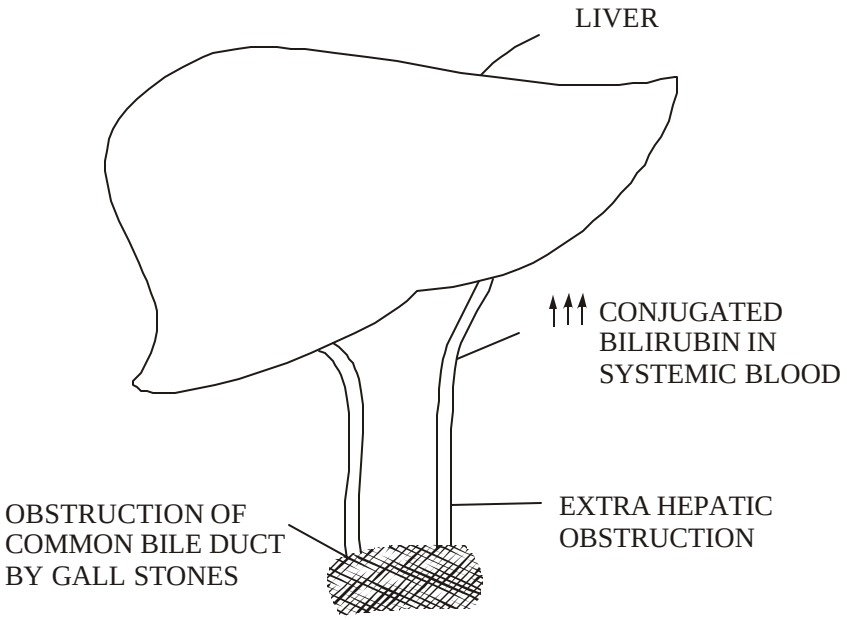
Causes

(*i*) Gall stones
(*ii*) Carcinoma of head of pancreas.

18. NAME THE IMPORTANT LIVER FUNCTION TESTS FOR THE INTERPRETATION OF DIFFERENT TYPES OF JAUNDICE?

Ans. The following are the important LFTs :

(*i*) Van den Bergh reaction
(*ii*) Serum total bilirubin
  (*a*) Direct bilirubin
  (*b*) Indirect bilirubin
(*iii*) Serum enzymes
  ALT, AST, ALP, GGT etc.
(*iv*) Total protein and A:G ratio.



III. OBSTRUCTIVE JAUNDICE

Fig. 6

Chapter–21

Hormones

MECHANISM OF ACTION OF HORMONES

1. WHAT ARE THE DIFFERENT METHODS OF MECHANISM OF ACTION OF VARIOUS HORMONES?

Ans. The following are some methods of mechanism of action of hormones.

(*a*) ***Group I hormones act through the binding of intracellular receptors***

(*i*) **Cytosolic receptors**

Glucocorticoids
Mineralcorticoids
Calcitriol.

(*ii*) **Nuclear receptors**

Thyroxine (T_4)
Triiodothyronine

(*b*) ***Group II hormones–that bind cell surface receptors***

(*i*) Second messenger is **cAMP**

Glucagon
Epinephrine
Tropic hormones (ACTH) MSH, hcGT, TSH etc.)

(*ii*) Second messenger is **cGMP**

Atrial natriuretic factor (ANF)
Nitric oxide (NO)

(*iii*) Second messenger is **Ca^{++} or phosphotidylinositides or both**

Releasing hormones (GHRH, TRH)
Vasopressin
Acetylcholine

(*iv*) Second messengers is **protein kinase**

Insulin
Growth hormone
Oxytocin
Prolactin

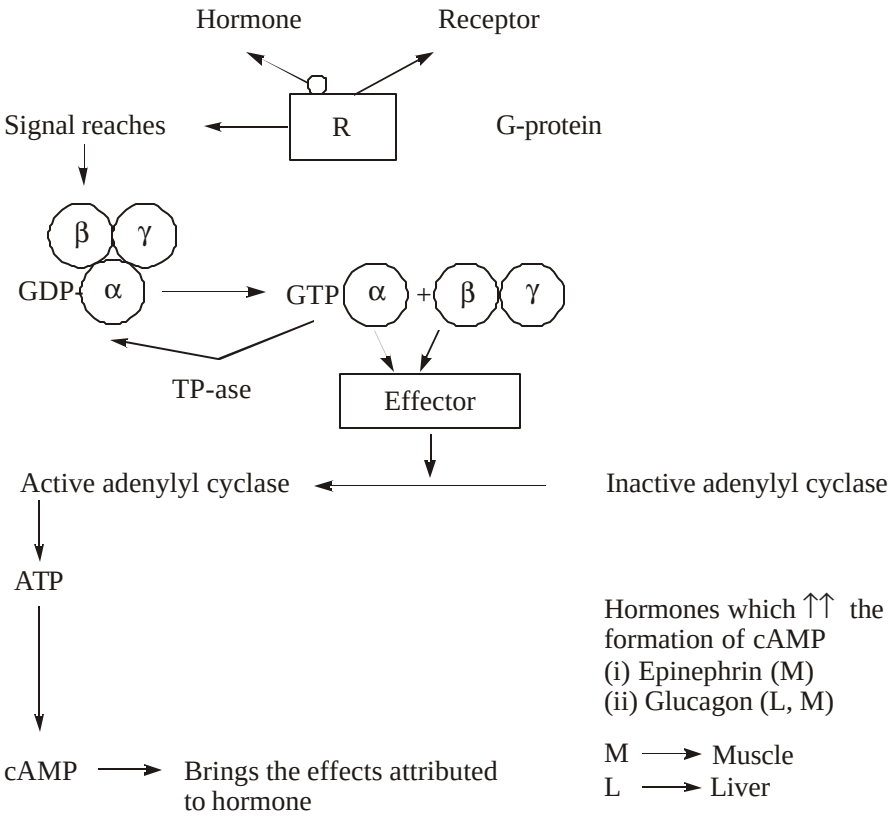
2. WHAT IS THE MECHANISM OF ACTION OF GROUP–I HORMONES WHICH ACT THOROUGH THE BINDING OF INTRACELLULAR RECEPTORS?

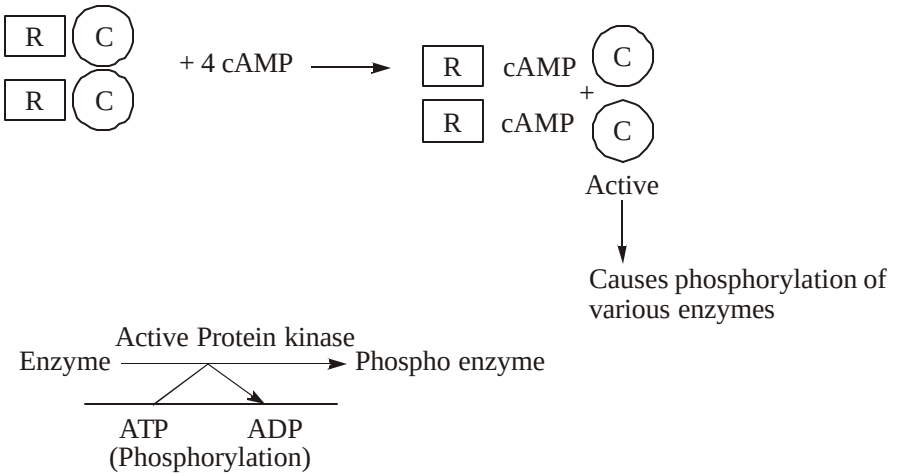
Ans. (*a*) Steroid hormones bind with a specific receptor protein in the cytosol. The hormone receptor complex at specific point of DNA (hormone response element) in the nucleus modifies the synthesis of protein (either ↑↑ synthesis or ↓↓ synthesis). Therefore, the level of protein brings the effects attributed to hormone.

(*b*) Thyroid hormone directly binds with receptor protein in the nucleus and then to HRE and causes modification of transcription and translation processes and synthesis of specific proteins.

3. WHAT IS THE MECHANISM OF ACTION OF GROUP–II HORMONES WHERE SECOND MESSENGER IS cAMP?

Ans. Hormone is bound to receptor and causes formation of cAMP as second messenger.





S.No.	*Enzyme*	*Effect of Phosphorylation*
1	Glycogen synthase (L, M) (Glycogenesis)	Inhibition
2.	Phosphorylase (L, M) (Glycogenolysis)	Stimulation
3.	Hormone sensitive lipase (Lipolysis on A.T (adipose tissue)	Stimulation

4. WHAT ARE THE DIFFERENT TYPES OF 'G' PROTEINS INVOLVED IN THE SIGNAL TRANSDUCTION AND THEIR MODE OF ACTION?

Ans. (*a*) Gs protein stimulate adenylyl cyclase and causes formation of cAMP $\longrightarrow \uparrow\uparrow$ level of cAMP.

(*b*) Gi protein inhibit adenylyl cyclase and cause $\downarrow\downarrow$ formation of cAMP $\longrightarrow \downarrow\downarrow$ level of cAMP.

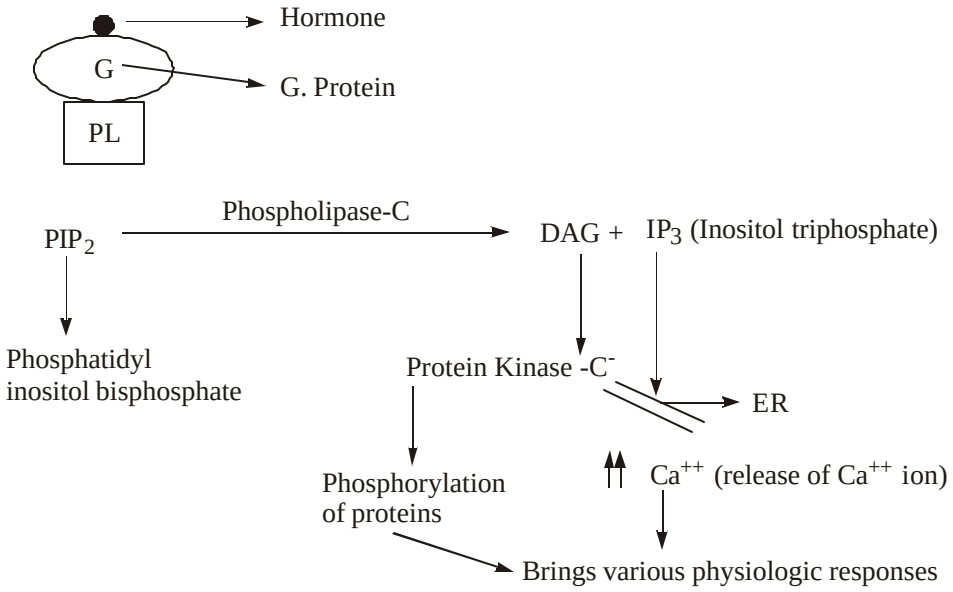
(*c*) Gq proteins stimulate phospholipase 'C' and cause formation of DAG + IP_3 (inositol triphosphate).

(*d*) Gt protein by the action of photons stimulates cGMP phospho diesterase and $\downarrow\downarrow$ the level of cGMP (photo-transduction signal in rods).

(*e*) Ga_{13} protein by the action of thrombin and other agonists stimulates Na^+ and H^+ exchange.

5. WHAT IS THE MECHANISM OF ACTION OF HORMONES INVOLVING PHOSPHOTIDYLINOSIDES?

Ans. Hormones like acetyl choline, ADH, α-1 adrenergic catecholamines bind with receptors coupled with a G protein.



PITUITARY AND HYPOTHALAMIC HORMONES

6. NAME THE HYPOTHALAMIC RELEASING AND INHIBITORY HORMONES AND WHAT ARE THEIR MAJOR FUNCTIONS?

Ans.

S.No.	*Hypothalamic releasing hormone*	*Major functions*
1.	Thyrotropin releasing hormone	Causes release of thyroid stimulating hormone from anterior pituitary gland (A.P.)
2.	Corticotropin releasing hormone	Causes release of ACTH by A.P.
3.	Growth hormone (GH) releasing hormone	Causes release of GH by A.P.
4.	Gonadotropin releasing hormone	Causes release of two gonadotropins LH and FSH by A.P.

S.No.	*Inhibitory hormones*	*Functions*
1.	GH inhibitory hormone (somatostatin)	Inhibits release of GH
2.	Prolactin inhibitory hormone	Inhibits prolactin secretion

7. WHAT ARE THE HORMONES SECRETED BY ANTERIOR PITUITARY AND WHAT ARE THEIR MAJOR FUNCTIONS?

S.No.	*Tropic hormones of anterior pituitary*	*Major Functions*
1.	Thyroid stimulating hormone (TSH)	Stimulates synthesis and secretion of thyroid hormones (T_3 and T_4).
2.	Adrenocorticotropic hormone (ACTH)	Stimulates synthesis and secretion of adrenal cortical hormones (cortisol androgen and aldosterone).
3.	Follicle stimulating hormone (FSH)	Causes growth of follicles in the ovaries and sperm maturation in the testes.
4.	Luteinizing hormone (LH)	Stimulates testosterone synthesis in the leydig cells of testes. Stimulates ovulation, formation of corpus luteum and oestrogen and progesterone synthesis in ovaries.
5.	Prolactin	Promotes development of female breasts and secretion of milk.
6.	GH	Stimulates overall growth of most of cells and tissues.

8. WHAT ARE THE HORMONES SECRETED BY POSTERIOR PITUITARY AND WHAT ARE THEIR MAJOR FUNCTIONS?

Ans.

S.No.	*Hormone of P.P.*	*Major Functions*
1.	Antidiuretic hormone (ADH) (Vasopressin)	↑↑ Water reabsorption by the kidney and causes vasoconstriction and ↑↑ B.P.
2.	Oxytocin	Stimulates uterine contraction and milk ejection from breast.

GROWTH HORMONE

9. WHAT IS GROWTH HORMONE AND WHAT ARE ITS BIOCHEMICAL FUNCTIONS?

Ans. Growth hormone is a single polypeptide chain having 91 amino acids and is secreted by anterior pituitary gland. Its action is mediated through the activation of tyrosine kinase and phosphorylation of the receptor on tyrosine residues resulting in the activation of a number of signaling pathways.

Biochemical Actions

1. Growth hormone is involved for postnatal growth and for normal carbohydrate, lipid, protein and mineral metabolism.
2. Its effects are mediated by interaction with IGF-I and IGF-II and cause growth promoting effects of GH. These GFs influence strong metabolic and mitogenic effects in cartilage, adipose tissue and muscles and thus promote growth.
3. Role of GH on Protein synthesis: GH causes protein synthesis in skeletal and other tissues by ↑↑ uptake of amino acids.
4. Role of GH on carbohydrate metabolism: Growth hormone ↓↓ glucose uptake in muscles and causes under utilization of glucose. It inhibits glycolysis.
5. Role of GH on lipid metabolism: It ↑↑ the activity of HS-lipase and causes ↑↑ mobilization of adipose tissue fat (↑↑ FFA). It also ↑↑ oxidation of fatty acids in the liver.

Role of GH on Mineral Metabolism and Bone Formation

GH through the IGF-I promotes a positive calcium(Ca^{++}), and phosphate (PO_4^{--}) and Mg^{++} metabolism and promotes growth of long bones at epiphyseal plates in growing children. It strongly stimulates osteoblasts which are responsible for the thickness of the bones. GH also causes the retention of Na^+, K^+ and Cl^-.

Role of GH on Mammary Gland

It causes stimulation of mammary gland and causes lactation.

Abnormalities of GH

Deficiency of GH causes in infants affect their growth and children become dwarfs.

Excess GH (↑↑)

(*a*) Before closure of epiphyseal plates ↑↑ GH causes GIGANTISM.

(*b*) After closure of epiphyseal plates it causes ACROMEGALY.

THYROID HORMONES

10. WHAT ARE THE THYROID HORMONES AND HOW ARE THEY SYNTHESIZED?

Ans. Thyroid hormones are:

(*a*) Thyroxine (T_4, tetra iodothyronine)

(*b*) Triiodothyronine (T_3)

Synthesis

Synthesis of thyroid hormones occur in various steps:

Step – I. Iodide Uptake

Iodide trapping by sodium iodide symporter (NIS). Transport of iodide into follicular cells of gland takes place against the concentration gradient.

(*a*) It requires the presence of sodium gradient across the basal membrane of the thyroid cell.

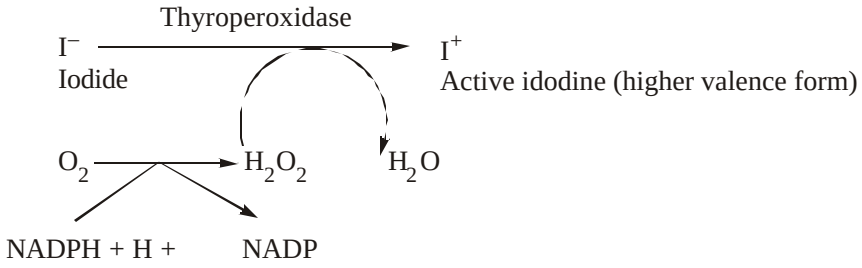
(*b*) This in turn facilitates down hill transport of $2Na^+$ ions and one iodide aion against the concentration gradient just like glucose transport. TSH causes the transcription of NIS gene.

Step – II. Oxidation and Organification of Iodide

(*a*) Iodide is trapped and transported to the apical membrane of thyroid follicular cells (TF cells).

(*b*) In the TF cells iodide is oxidized and then incorporated into tyrosine residues of thyroglobulin in the organification reaction.

Oxidation of iodide to higher valance form:



Structure of Thyroglobulin (TG)

(*a*) Glycoprotein : Its mol.wt is 660,000.

(*b*) It consists of 10% of carbohydrates and 115 tyrosine residues of which 35 tyrosine residues can be iodinated.

(*c*) By the iodination reaction monoiodo tyrosine (MIT) and diiodotyrosine (DIT) are formed.

(*d*) TSH stimulates and anti thyroid drugs inhibit the oxidation of iodide and organification reaction.

Step – III. Coupling Reaction

Coupling reaction is catalyzed by thyroperoxidase enzyme.

(*a*) DIT + DIT $\xrightarrow{\text{Thyroperoxidase}}$ Tetraiodothyronine (thyroxine or T4)

(*b*) MIT + DIT $\xrightarrow{\text{Thyroperoxidase}}$ Triiodothyronine (T3)

Step – IV. Storage and Release

The T3 and T4 attached to TG are stored in the follicular space as a colloid. In case of need TG with iodinated tyrosine residues enter the follicular cells and T3, T4 hormones are released by hydrolysis (proteolysis).

11. WHAT ARE THYROID BINDING PROTEINS?

Ans. (*a*) Thyroid binding globulin (TBG). It carries 80% of bound hormones.
(*b*) Thyroid binding pre-albumin (TBPA) or transthyretin (TTR). It binds 10% of T3 and T4.
(*c*) Albumin binds remaining percentage of hormones.

12. WHAT ARE THE CLINICAL CONDITIONS IN WHICH THE TBG LEVELS ARE ALTERED?

Ans. (*a*) TBG levels are increased in (↑↑).
(*i*) By the administration of oestrogen.
(*ii*) By the administration of birth control pills.
(*iii*) Pregnancy.
(*b*) TBG levels are decreased in (↓↓)
(*i*) By the administration of androgen.
(*ii*) Glucorticoid therapy.
(*iii*) Liver diseases.
(*iv*) Nephrotic syndrome.
(*v*) Malnutrition.

13. WHAT ARE THE CLINICAL ABNORMALITIES OF THYROID HORMONES AND HOW DO YOU INTERPRET THYROID FUNCTION TESTS IN THEM?

Ans. Clinical abnormalities of T3 and T4
(*a*) Hypothyroidism : ↓↓ levels of T3 and T4
(*b*) Hyperthyroidism : ↑↑ levels of T3 and T4

Interpretation of Thyroid Function Tests

The following tests are done in thyroid profile:
(*i*) TSH ⟶ Normal value 0.270 – 4.20 μIU/ml (electro chemi luminescence method).
(*ii*) T4 ⟶ Normal value 5.13 – 14.6 μG/dl (electrochemi luminescence method).
(*iii*) T3 ⟶ Normal value : 0.846 – 2.02 ng./ml. (electrochemi luminescence method).

Hypothyroidism

(*i*) TSH ↑↑↑
(*ii*) T3 ↓
(*iii*) T4 ↓↓

Hyperthyroidism

(*i*) TSH ↓↓
(*ii*) T3 ↑↑
(*iii*) T4 ↑↑↑

HORMONES REGULATING CALCIUM METABOLISM

(Please Refer To Chapter 17 'Calcium Metabolism' For Further Details)

14. NAME THE HORMONES WHICH REGULATE THE PLASMA CALCIUM LEVE.

Ans. The hormones which regulate the plasma calcium level are:

(*a*) Parathormone secreted by four parathyroid glands.

(*b*) Calcitriol formed in the kidney.

(*c*) Calcitonin formed by 'C' cells of thyroid gland.

15. HOW IS PARATHARMONE SYNTHESIZED?

Ans. Paratharmone (PTH) is synthesized in the four parathyroid glands as prepro PTH. It has 115 amino acids. Pre-sequence (leader sequence) has 25 A.As. and prosequence has 6 A.As. and actual PTH has 84 A.As. During transport of prepro-PTH to cisternal space the leader peptide is removed. Pro-PTH is then transported to golgi apparatus where an enzyme removes pro sequence to form mature PTH.

HORMONES OF ADRENAL CORTEX

16. NAME THE HORMONES SECRETED BY ADRENAL CORTEX .

Ans. Adrenal cortex has three zones. Each zone secrete separate hormones:

(*i*) Zona glomerulosa is the outer zone which secretes mineralocorticoids (aldosterone).

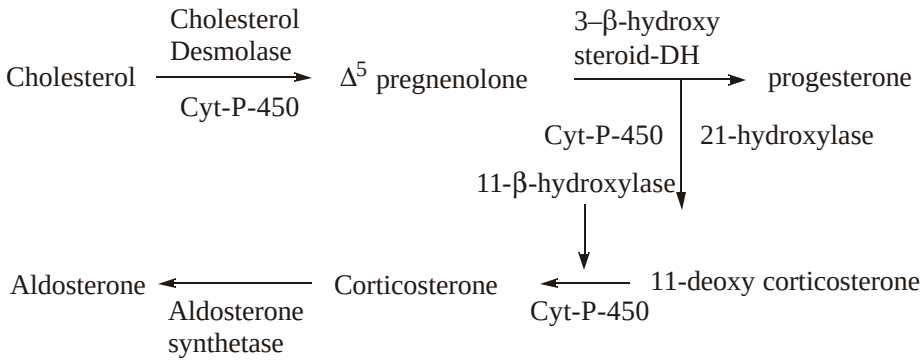
(*ii*) Zona fasciculata the middle zone produces glucocorticoids.

(*iii*) Zona reticularis the innermost zone produces androgen.

17. HOW ARE MINERALOCORTICOIDS SYNTHESIZED?

Ans. Cholesterol is the starting material for steroid hormone synthesis. The main enzyme aldosterone synthase is present in zona glomerulosa.

Upbtake: Uptake of cholesterol by the adrenal cortex is mediated by the LDL receptors.

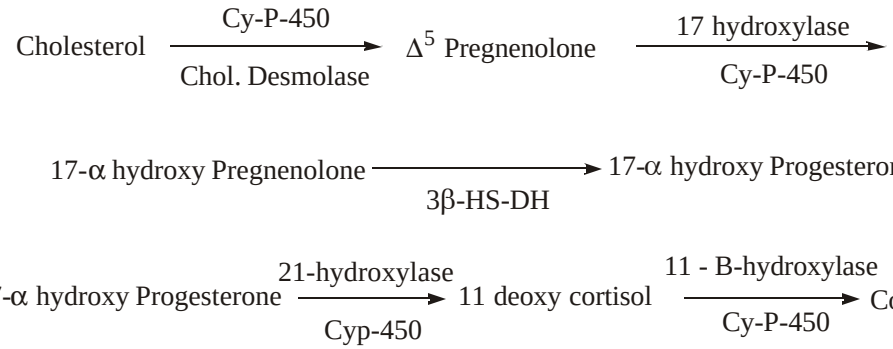


In the conversion of corticosterone to aldosterone 18 hydroxylation and dehydrogenation of corticosterone takes place for the formation of aldosterone.

NB: Hydroxylases require molecular oxygen and NADPH for the introduction of hydroxyl groups to steroids.

18. HOW ARE GLUCOCORTICOIDS SYNTHESIZED?

Ans. The main enzyme 17-hydroxylase is present in the zona fasciculata, which acts on Δ^5 pregnenolone



19. WHAT ARE MINERALOCORTICOIDS AND WHAT ARE THEIR MAJOR FUNCTIONS?

Ans. The following are mineralocorticoids:

(*a*) Aldosterone (90% activity of all mineralocorticoids. Highly potent in its action).

(*b*) Deoxycorticosterone (less secretion and 1/30th of activity of aldosterone).

(*c*) Corticosterone (slight mineralocorticoids activity).

Major Functions

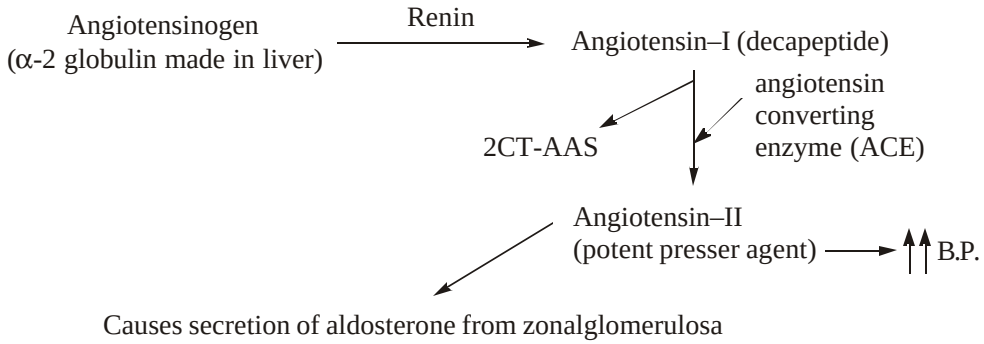
(*i*) It causes ↑↑ renal tubular resorption of sodium and excretion of potassium.

(*ii*) In the deficiency of aldosterone ↑↑ concentration of K^+ and ↓↓ concentration of Na^+ is present in ECF.

(*iii*) Excess aldosterone causes ↑↑ fluid volume and ↑↑ arterial pressure. Aldosterone secretion is regulated by the renin angiotensin system.

20. WHAT IS RENIN ANGIOTENSIN SYSTEM?

Ans. Renin is secreted by juxta glomerular cells of the renal afferent arteriole in the conditions of ↓↓ B.P. and salt depletion. It acts on angiotensinogen and ultimately causes the secretion of aldosterone.



21. WHAT ARE GLUCOCORTICOIDS AND WHAT ARE THEIR MAJOR FUNCTIONS.

Ans. The following are the glucocorticoids:

(*a*) Cortisol (95% activity of glucocorticoids).
(*b*) Corticosterone (4% of total glucocorticoids activity).
(*c*) Cortisone (synthetic, as potent as cortisol).
(*d*) Dexamethasone (synthetic 30 times potent than cortisol).

Major Functions

1. Effect on carbohydrate metabolism.
2. It stimulates gluconeogenesis by ↑↑ protein catabolism and ↑↑ uptake of A.A. by liver. It increases the activity of key enzymes of gluconeogenesis and amino transferases. It ↓↓ the utilization of glucose by cells.
3. Promote lipolysis in extremities but cause lipogenesis in face and trunk.
4. Suppresses immune response by the mechanism of lysis of lymphocytes.
5. Suppresses inflammatory response by ↓↓ circulatory leucocytes and the migration of tissue leucocytes.

Abnormalities

(*i*) **Primary aldosteronism (Conn's syndrome)**
Adrenal adenoma causes ↑↑ production of aldosterone.

Clinical Features

(*a*) Hypertension.
(*b*) Muscle weakness and fatigue due to depletion of K^+.

Biochemical Features

Hypokalemia due to ↑↑ excretion of K^+ by kidney.

(*ii*) **Secondary Aldosteronism**
Secondary aldosteronism is due to ↑↑ activity of renin angiotensin system.

(*iii*) **Addison's Disease**
(Primary adrenocortical deficiency) manifested by

(*a*) Hypotension.
(*b*) Hyperpigmentation.
(*c*) Fatigue and weakness.
(*d*) Weight loss.
(*e*) Electrolyte disturbance (hyperkalemia).

(*iv*) **Cushing's Syndrome**
Cushing's syndrome is due to ↑↑ production of cortisol caused by bilateral adrenal hyperplasia.

Clinical Features

(*a*) Hypertension.
(*b*) Truncal obesity.
(*c*) Fatigability and weakness.
(*d*) Osteoporosis.

Biochemical Features

(*a*) Glucosuria.
(*b*) Glucose intolerance (↑↑plasma glucose level).
(*c*) Hypokalemia.

HORMONES OF ADRENAL MEDULLA (CATECHOLAMINES)

22. WHAT ARE THE CATECHOLAMINES AND HOW ARE THEY SYNTHESIZED? WHAT ARE THE END PRODUCTS OF CATECHOLAMINES?

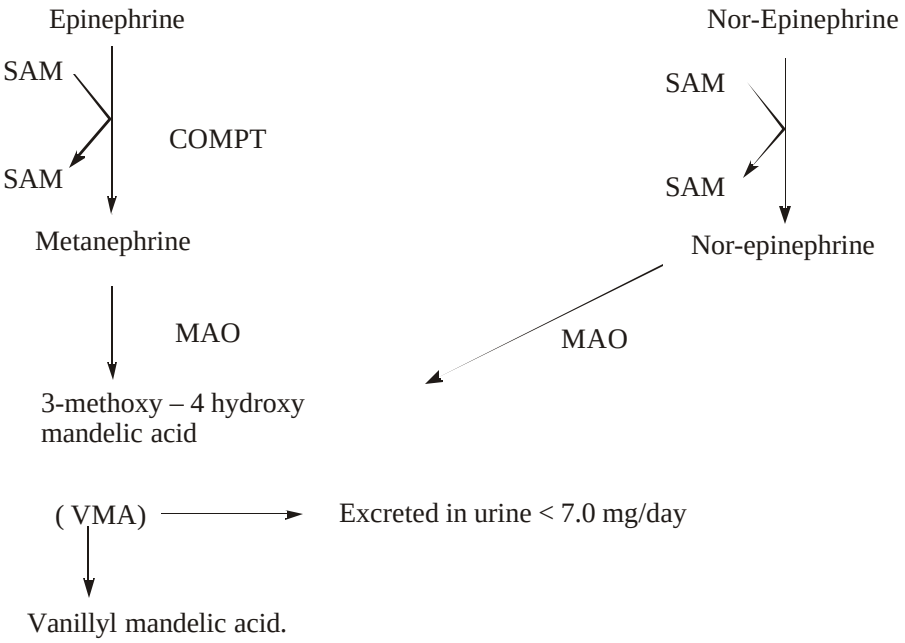
Ans. The catecholamines are:

(*a*) Epinephrine
(*b*) Norepinephrine
(*c*) Dopamine

Catecholamines are synthesized in the chromaffin cells of adrenal medulla. Synthesis of catecholamines are given in the A.A. metabolism.

In the adrenal medulla epinephrine forms 80% of catecholamines.

Catecholamines further undergo metabolism by the actions of enzymes COMPT and MAO.



23. WHAT ARE THE MAJOR FUNCTIONS OF CATECHOLAMINES?

Ans. There are two types of adrenergic receptors:

(*i*) α-receptors

(*ii*) β-receptors

Norepinephrine excites mainly α-receptors but it has less effect on β-receptors.

Epinephrine excites both α and β-adrenergic receptors but more predominantly excites β-receptors.

Major Functions of Dopamine

1. Specific dopaminergic receptors are present in the CNS and peripheral nervous system. Its major functions are that:
   (*a*) At low dose, causes renal and mesenteric vasodilatation, and
   (*b*) At high dose, causes vasoconstriction and cardiac stimulation.
   (*c*) Dopamine is used in the treatment of hypotension and shock.

Adrenergic Receptors and Functions

S.No.	*Functions of Alpha receptors*	*Functions of Beta receptors*
1	(*a*) Vasoconstriction	(*i*) Vasodilatation (β_2)
	(*b*) Iris dilatation (pupil dilatation)	(*ii*) ↑↑ heart rate and force of contraction (β_1)
	(*c*) Intestinal relaxation	(*iii*) Intestinal and uterus relaxation (β_2)
		(*iv*) Bronchodilatation (β_2)
		(*v*) Glycogenolysis and lipolysis (β_2)

Epinephrine

The epinephrine is released from adrenal medulla in response to fright, flight, exercise and hypoglycemia. The major functions of epinephrine are that it.

(*i*) ↑↑ heart rate and force of contraction of heart.

(*ii*) ↑↑ blood pressure.

(*iii*) Causes bronchodilatation.

(*iv*) ↑↑ glycogenolysis and lipolysis (mediated through the formation of cAMP).

(*v*) Epinephrine is specially used in the treatment of allergic reactions associated with anaphylaxis.

Norepinephrine

Norepinephrine is the neurotransmitter of post ganglionic sympathetic nerve endings and exerts its effects locally, in the immediate vicinity of its release.

The major action of nor-epinephrine is that it excites α-adrenergic receptors causing vasoconstriction (↑↑ BP) and it also excites β_1 receptors but to a minimum effect.

Abnormalities of Catecholamines

Pheochromocytoma (tumour of adrenal medulla). The tumour secretes excess amount of epinephrine and norepinephrine.

Clinical Features

(*a*) Paroxysmal hypertension (*b*) Tachycardia, and (*c*) Profuse sweating

Biochemical Features

↑↑ VMA excretion (>7mg/D)

INSULIN

24. HOW IS INSULIN SYNTHESIZED?

Ans. Answer is given in the Q. No. 2 in the post-translational modification of protein synthesis and also refer structure of insulin.

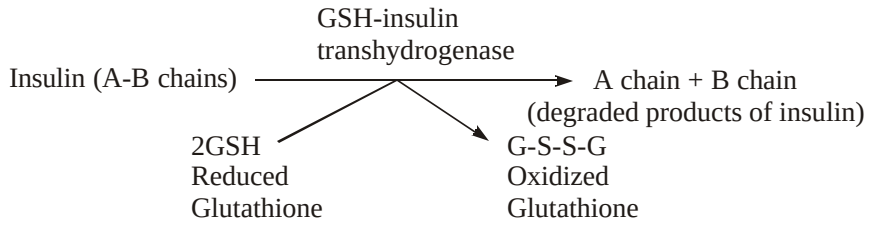
25. WHAT IS THE MECHANISM OF ACTION OF INSULIN?

Ans. Insulin is bound to its specific receptor on target tissues. It causes increased activity of tyrosine kinase leading to receptor autophospho-rylation and recruitment of intracellular signalling molecules such as insulin receptor substrates (IRS).

For instance, activation of phosphalidyl inositol 3- kinase (P1-3 kinase) pathway stimulates translocation of glucose transporters (GLUT-4) to the plasma membrane. GLUT- 4 is required for the uptake of glucose in the muscle and adipose tissue cells. Phosphorylation of other IRS molecules cause other metabolic effects.

26. HOW IS INSULIN METABOLIZED?

Ans. Insulin is degraded by the action of enzyme hepatic glutathione insulin transhydrogenase.



27. WHAT ARE THE METABOLIC ACTIONS OF INSULIN?

Ans. On carbohydrate metabolism it decreases blood sugar level by the following mechanisms.

(*a*) Increases uptake of glucose by the muscle and adipose tissue cells. It causes the recruitment of GLUT-4 transporter from cytosol to the plasma membrane.

(*b*) It stimulates glycogenesis and inhibits glycogenolysis and gluconeogenesis (ref. Q. No.18 of CHO metabolism).

(*c*) It increases oxidation of glucose in the glycolysis and TCA cycle (ref Q.No 18 of CHO metabolism).

On Lipid Metabolism

(*a*) It inhibits HS lipase and thus ↓↓ lipolysis acetylCoAcarboxylase.

(*b*) It stimulates lipogenesis by activating enzymes and ATP citrate lyase and by stimulating HMP shunt pathway it provides NADPH.

On Protein Metabolism

It ↑↑ uptake of amino acids by cells and favours protein synthesis.

Over All Effect

Insulin is an anabolic hormone because it causes glycogenesis, lipogenesis and protein synthesis.

Abnormalities of Insulin Metabolism

(*i*) **Deficiency:** Deficiency causes diabetes mellitus (↑↑ plasma glucose level >200 mg/dl)

(*ii*) **Excess Secretion:** Excess secretion occurs in the tumour insulinoma which causes hypoglycemia (↓↓ plasma glucose <50 mg/dl).

GLUCAGON

28. WHAT IS GLUCAGON AND WHAT ARE THE METABOLIC ACTIONS OF THIS HORMONE?

Ans. Glucagon is a single chain polypeptide consisting of 29 A.As. and secreted by α-cells of islets of langerhans. Glucose inhibits glucagon secretion. Glucagon is inactivated in the liver.

Metabolic Actions of Glucagon

Glucagon antagonises the actions of insulin.

On Carbohydrate Metabolism

Glucagon raises blood glucose level by the following mechanisms:

(*a*) Through the formation of cAMP it causes glycogenolysis.

(*b*) It stimulates gluconeogenesis by inducing the enzymes of gluconeogenesis.

On Lipid Metabolism (Lipolysis)

It activates enzyme HS-lipase through the formation of cAMP. Thus it causes $\uparrow\uparrow$ mobilization of adipose tissue fats and causes $\uparrow\uparrow$ FFA level.

Abnormalities of Glucagon Metabolism

The $\downarrow\downarrow$ insulin: glucagon ratio, results in the development of diabetes mellitus.

GASTRO INTESTINAL HORMONES

29. WHAT ARE THE GASTROINTESTINAL HORMONES AND WHAT ARE THEIR MAIN FUNCTIONS?

Ans. The following are the gastrointestinal hormones:

(*i*) **Gastrin**

Secreted by G cells of stomach antrum. It is a peptide consisting of 17 amino acids.

(*ii*) **SECRETIN**

Secreted by 'S' cells of the duodenum. It is a peptide consisting of 27 amino acids.

(*iii*) **Cholecystokinin**

Cholecystokinin–pancreozymin (CCK–PZ). Secreted by mucosa of small intestine. It is a peptide consisting of 33 amino acids.

(*iv*) **Gastric inhibitory peptide (GIP)**

Secreted by duodenal mucosa. It is a peptide consisting of 43 amino acids.

Secretogogues and Main Functions of GIT Hormones

S.No.	*G.I.T. Hormone*		*Main functions*
1.	Gastrin	Secretogogues ⇑⇑ secretion by bolus of food, acetyl choline and stimulation of vagus nerve. ⇓⇓ Secretion by GIP & VIP	Stimulates parietal and chief cells and causes secretion of HCl and pepsinogen.
2.	Secretin	⇑⇑ Secretion by acid chyme of food.	Stimulates exocrine portion of pancreas and causes secretion of HCO_3^- in the juice.
3.	CCK-PZ	⇑⇑ secretion by acid chyme of the food, peptides, AAs, MAG and fatty acids and glycerol.	Causes contraction of gall bladder to release bile into duodenum.
4.	GIP	⇑⇑ Secretion by fats entering duodenum.	Inhibits gastric motility and secretion.

SEX HORMONES

30. WHAT ARE THE MALE AND FEMALE SEX HORMONES AND WHAT ARE THEIR MAJOR FUNCTIONS?

Ans. The following are the male and female sex hormones:

Male Hormones

(I) Testosterone (C-19–steroid hormone secreted by Leydig cells of testes).

Functional Groups are

17th position 'OH' group.
3rd position keto group.

Female Sex Hormones

(*i*) Oestrogen (C-18 steroid hormone, secreted by ovarian follicular cells).

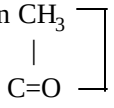
Functional Groups of Estradiol

Two OH groups on 3rd and 17th position.
'A' ring is aromatic.

(*ii*) Progesterone (C-21 steroid hormone. Secreted by ovarian follicular cells).

Functional Groups are

3rd position – C = O (keto group)
17th position side chain CH_3



Major Functions of Testosterone

(*a*) Causes growth and development of male internal genitalia.
(*b*) Causes spermatogenesis.
(*c*) Promote protein synthesis and cause +ve 'N' balance.
(*d*) Causes production of fructose which is richly present in semen.
(*e*) Promote formation of bone matrix proteins.

Major Function of Female Sex Hormones

Sl.No	*Hormone*	*Major Functions*
1.	Oestrogen (Oestradiol)	(*a*) Causes growth and development of female sex organs and development of secondary sex characters. (*b*) Maintains menstrual cycle. (*c*) Stimulates osteoblasts and causes skeletal growth and calcification. (*d*) ⇓⇓ total cholesterol and LDL-C and ⇑⇑ HDL-C. This results in the low incidence of CAHD. (*e*) ⇑⇑ fat deposition in adipose tissue.
2.	Progesterone	(*a*) Progesterone plays important role during luteal phase of menstrual cycle after ovulation.

Chapter–22

Clinical Biochemistry – I

INTERPRETATIONS OF LABORATORY INVESTIGATIONS

1. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Van den Bergh reaction	**–**	**Indirect positive**
Total Bilirubin	**–**	**12.6 mg %**
Conjugated Bilirubin	**–**	**0.8 mg %**
Unconjugated Bilirubin	**–**	**11.8 mg %**
ALT	**–**	**35 IU/L (N = <40 IU/L)**
AST	**–**	**105 KA units (N = 51 – 135 IU/L)**

Ans. It is a case of hemolytic jaundice. The hemolytic jaundice is caused by the over-production of bilirubin due to ↑↑ destruction of RBC.

The following points are in favour of hemolytic jaundice:

(*a*) Van den Bergh reaction is indirect positive and immediate direct negative.

(*b*) Unconjugated bilirubin ↑↑.

(*c*) ALT and AST are normal.

2. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Van den Bergh reaction	**–**	**Direct positive**
Total Bilirubin	**–**	**13.4 mg %**
Conjugated Bilirubin	**–**	**6.7 mg %**
Unconjugated Bilirubin	**–**	**6.7 mg %**
ALT	**–**	**304 IU/L (N=<40 IU/L)**
AST	**–**	**285 IU/L (N = 51 – 135 IU/L)**

Ans. It is a case of hepato cellular jaundice (intrahepatic obstruction). The hepato cellular jaundice is caused by the viral hepatitis and inflammation of hepatocytes results in the intrahepatic obstruction.

The following points are in favour of hepatocellular jaundice:

(*a*) Van den Bergh direct positive.

(*b*) Both conjugated and unconjugated bilirubin ↑↑.

(*c*) ALT ↑↑↑ and ALP ↑ (Moderately raised).

3. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Van den Bergh reaction	**–**	**Direct positive**
Total Bilirubin	**–**	**16.8 mg %**
Conjugated Bilirubin	**–**	**15.4 mg %**
Unconjugated Bilirubin	**–**	**1.4 mg %**
ALT	**–**	**40 Units (N = < 40 IU/L)**
AST	**–**	**400 IU/L (N = 51 – 135 IU/L)**

Ans. It is a case of obstructive or cholestatic jaundice (extra hepatic obstruction). The obstructive jaundice is caused by the obstruction in the common bile duct by gall stones etc.

(*a*) Van den Bergh direct positive.

(*b*) Mostly conjugated bilirubin ↑↑↑.

(*c*) ALT normal and ALP ↑↑↑ (very much raised).

The ↑↑↑ of ALP is observed in biliary obstruction. Usually ALP is raised more than 2.5 times the normal in obstructive jaundice.

4. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Van den Bergh reaction	**–**	**Indirect positive**
Total Bilirubin	**–**	**0.9 mg %**
Conjugated Bilirubin	**–**	**0.16 mg %**
Unconjugated Bilirubin	**–**	**0.74 mg %**
ALT	**–**	**35 IU/L (N=<40 IU/L)**
AST	**–**	**100 IU/L (N = 51 – 135 IU/L)**

Ans. It is a normal pattern because:

(*a*) All parameters are normal.

(*b*) Van den Bergh – Indirect positive and direct – Ve.

5. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Serum Urea	**:**	**203 mg %**	**(N=10 – 40 mg%)**
Serum Creatinine	**:**	**12.7 mg %**	**(N = < 1.5 mg%)**
Serum Uric Acid	**:**	**8.8 mg %**	**(N = < 7 mg%)**
Serum Inorganic Phosphorous	**:**	**6.2 mg %**	**(N = 3 – 4.5 mg%)**

Ans. It is a case of renal failure either ARF or CRF. In renal failure, NPN substances and phosphorus are not excreted by the kidney and retained in the body.

The following points are in favour of renal failure:

(*a*) All the NPN substances ↑↑↑ (urea, uric acid and creatinine).

(*b*) Serum phosphorus ↑↑↑.

6. THE FOLLOWING ARE SOME OF THE BIOCHEMICAL FINDINGS IN A PATIENT. WHAT IS YOUR PROBABLE DIAGNOSIS?

Serum Urea	**:**	**30 mg %**
Serum Creatinine	**:**	**1.0 mg %**
Serum Cholesterol	**:**	**560 mg % (N = 170 – 270 mg %)**
Total Plasma Protein	**:**	**4.5 g% (N = 6 – 8 g %)**
Albumin	**:**	**1.0 g %**
Globulin	**:**	**3.5 %**
Urine Protein	**:**	**10 g/Day**

Ans. It is a case of nephrotic syndrome. In nephrotic syndrome, the patient excretes large quantities of protein in urine more than 3G/day, which inevitably sets up a chain of events such as hypoproteinemia, lipemia etc.

The following points are in favour of nephrotic syndrome:

(*a*) Urine proteins > 3 G/Day (10 G.).

(*b*) ↓↓ Serum total protein and serum albumin.

(*c*) Serum cholesterol ↑↑↑.

(*d*) N.P.N. substances normal.

7. A 45 YEARS OLD MALE PATIENT WAS ADMITTED TO THE HOSPITAL WITH ACUTE ABDOMINAL PAIN. SERUM AMYLASE ESTIMATION GAVE A VALUE OF 400 IU/L (N = 35 – 140 IU/L). GIVE THE PROBABLE DIAGNOSIS?

Ans. It is a case of acute pancreatitis. Abdominal pain is the major symptom of acute pancreatitis and the serum amylase is widely used as a screening test for acute pancreatitis. The ↑↑↑ serum amylase in this patient clinches the diagnosis.

8. TWO PATIENTS WERE ADMITTED TO THE HOSPITAL WITH THE COMPLAINT OF CHEST PAIN. THE FOLLOWING INVESTIGATIONS WERE PERFORMED IN EACH OF THEM. WHAT IS YOUR DIAGNOSIS?

1st Patient

AST	**–**	**732 IU/L (N = 51 – 135 IU/L)**
CK	**–**	**3372 IU/L (N = < 2 00 IU/L)**
LDH	**–**	**1000 IU/L (N = 100 – 200 IU/L)**

2nd Patient

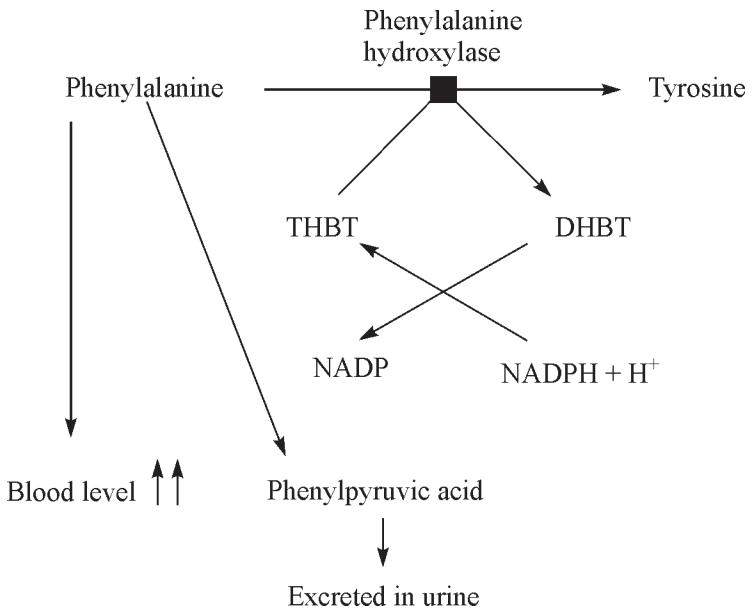
AST	**–**	**35 IU/L (N = 51 – 135 IU/L)**
CK	**–**	**80 IU/L (N = < 200 IU/L)**
LDH	**–**	**180 IU/L (N = 100 – 200 IU/L)**

Ans. **Ist Patient:** It is a case of acute myocardial infarction (AMI). Chest pain is the cardinal manifestation of AMI. Myacardial infarction causes a detectable rise in the plasma concentration of enzymes such as CK, AST and LDH. The ↑↑↑ cardiac enzymes in this patient clinches the diagnosis of AMI.

2nd Patient: It is not a case of AMI and chest pain is not due to AMI, as all the cardiac enzymes are (AST, CK and LDH) within normal limits.

9. A FAIR CHUBBY BOY WAS BROUGHT TO THE HOSPITAL WITH THE COMPLAINTS THAT HE SHOWED DELAYED DEVELOPMENT MILE STONES, MENTAL RETARDATION AND SEIZURES. BLOOD PHENYLALANINE VALUE WAS MORE THAN 20 MG/DL BY GUTHRIE BACTERIAL INHIBITION ASSAY AND FERRIC CHLORIDE TEST SHOWED BLUE GREEN COLOUR. WHAT IS YOUR PROBABLE DIAGNOSIS? WHAT IS THE CAUSE FOR THIS DISORDER?

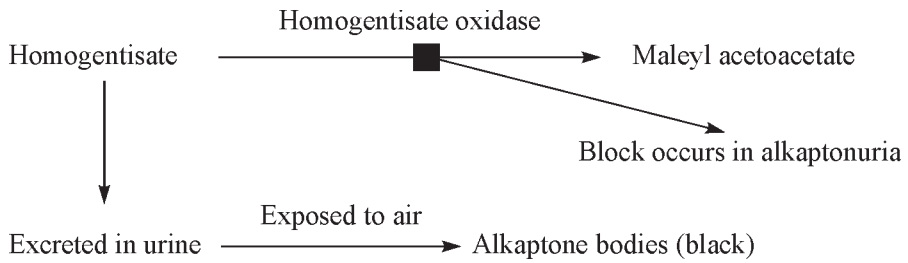
Ans. It is a case of phenylketonuria (PKU). It is caused by the deficiency of enzyme phenylalanine hydroxylase which converts phenylalanine to tyrosine. In the absence of this enzyme phenylalanine is not converted to tyrosine. Therefore, its level in blood is raised >20 mg. (Normal 1 mg/day) and phenylalanine is converted to phenylpruvic acid and excreted in urine which gives ferric chloride test +ve. The PKU patients develop mental retardation.



■ Block occurs in the deficiency of phenylalanine hydroxylase

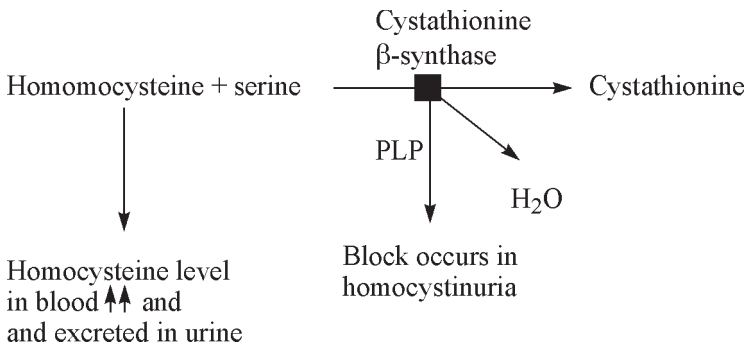
10. A MOTHER SOUGHT MEDICAL HELP FOR HER CHILD WITH THE COMPLAINT THAT DIAPERS USED FOR THE CHILD STAINED DARK URINE ON EXPOSURE BECOME DARKER. URINE REDUCES BENEDICT'S SOLUTION. GLUCOSE OXIDASE TEST WAS –VE. FERRIC CHLORIDE TEST SHOWED PURPLE BLACK COLOUR. WHAT IS YOUR PROBABLE DIAGNOSIS. WHAT IS THE CAUSE FOR THIS DISORDER?

Ans. It is a case of alkaptonuria. It is caused by the deficiency of enzyme homogentisate oxidase. This results in the excretion of homogentisic acid which is oxidized to black coloured alkaptone bodies on exposure to air. That is why the diapers used for child stained dark (black).



11. A 4 YEAR OLD GIRL WAS BROUGHT TO THE HOSPITAL WITH VISION PROBLEMS, AND THE MOTHER ALSO INDICATED THAT THE GIRL SHOWED DELAYED MILE STONES AND MENTAL RETARDATION. CLINICAL EXAMINATION REVEALED THAT SHE HAD LONG THIN BONES WITH SIGNS OF OSTEOPOROSIS. URINE CYANIDE NITROPRUSSIDE TEST WAS FOUND TO BE POSITIVE. WHAT IS YOUR INFERENCE ABOUT THIS CASE. WHAT IS THE CAUSE FOR THIS DISORDER.

Ans. It is a case of homocystinuria which is caused by the deficiency of enzyme cytathionine β- synthase. This enzyme catalyses the reaction of synthesis of cytathionine by making use of homocysteine and serine.



Homocysteine reacts with lysyl residues of collagen and interferes with cross-linking of collagen. Defect in collagen results in

(*a*) Ectopia lentis (vision problems).

(*b*) Osteoporosis (defect in bone formation).

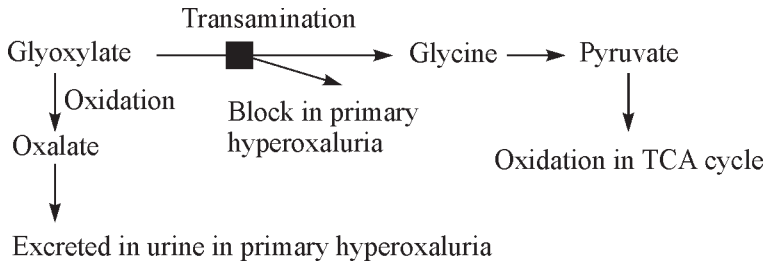
(*c*) The patient also develops mental retardation.

12. A 20 YEAR ADULT MALE WAS BROUGHT TO THE HOSPITAL WITH THE COMPLAINT OF HAEMATURIA AND FLANK PAIN (PAIN IN THE RENAL ANGLE). URINE EXAMINATION SHOWED POSITIVE CYANIDENITROPRUSSIDE TEST. URINE SHOWED HEXAGONAL CRYSTAL DEPOSIT. WHAT IS YOUR PROBABLE DIAGNOSIS. WHAT IS THE CAUSE FOR THIS DISORDER?

Ans. It is a case of cystinuria. It is caused by the defect of transport of basic amino acids (lysine, arginine and ornithine) and cystine. As a result of this, the patient excretes cystine, lysine, arginine and ornithine in urine. Cystine is sparingly soluble in water and therefore cystine calculi are formed which are hexagonal in shape. On account of cystine calculi, patient develops hematuria and pain in the renal angle.

13. A 2 YEAR OLD CHILD WAS BROUGHT TO THE HOSPITAL WITH THE COMPLAINT OF HAEMATURIA AND RENAL CALCULI. URINE EXAMINATION SHOWED PLENTY OF CALCIUM OXALATE CRYSTALS AND EXCRETION OF 150 MG OXALATE/DAY (NORMAL – <60 MG./DAY). WHAT IS YOUR PROBABLE DIAGNOSIS AND WHAT IS THE CAUSE FOR THIS DISORDER?

Ans. It is a case of primary hyperoxaluria. It is caused by the defect of transamination of glyoxylate (defect in glyoxylate metabolism) resulting in the oxidation of glyoxylate to oxalate.



14. FOLLOWING ARE THE FINDINGS IN A PATIENT BROUGHT TO THE HOSPITAL IN COMA STATE. WHAT IS YOUR PROBABLE DIAGNOSIS?

Plasma sugar	**–**	**400 mg/dl**
Benedict's test with urine	**–**	**brick red**
Rothera's test	**–**	**positive**
Serum HCO_3^-	**–**	**15 m Eq/l**
Plasma pH	**–**	**7.25**

Ans. It is a case of diabetic ketoacidosis developing coma. Diabetes mellitus is caused by either relative or absolute lack of insulin. Due to lack of insulin, glucose is excreted in urine (Benedict's test +ve) as sugar value is more than renal threshold. If the patient fails to take treatment, the value of ketone bodies is raised and excreted in urine (Rothera's test +ve). ↑↑ ketone bodies in blood leads to ketoacidosis, ↓↓ (serum HCO_3^-) and pH of blood is decreased. In ketoacidosis the patient goes into coma.

15. A CHILD WAS BROUGHT TO THE HOSPITAL WITH A COMPLAINT THAT THE CHILD WAS NOT GROWING WELL AND MILESTONES WERE DELAYED. THE CHILD RELUCTANT TO INGEST BREAST MILK AND MILK FORMULAS AND DEVELOPED COMPLAINTS OF VOMITING. ON EXAMINATION IT WAS FOUND TO HAVE CATARACT IN THE EYE. JAUNDICE AND HEPATOMEGALY. URINE EXAMINATION SHOWED REDUCTION WITH BENEDICTS REAGENT BUT NOT THE GLUCOSE OXIDASE. WHAT IS YOUR PROBABLE DIAGNOSIS AND WHAT IS THE CAUSE OF THIS DISORDER?

Ans. It is a case of galactosemia. It is caused by the deficiency of enzyme galactose 1–(P) uridyl transferase. Increased galactose level leads to its conversion into galactitol and accumulation of it in the lens resulting formation of cataract. Galactose–1–(P) accumulates in the liver leading to hepatomegaly and jaundice. Galactose is excreted in urine giving bendict's test +ve.

16. A BOY AGED 1 YEAR WAS BROUGHT TO THE HOSPITAL WITH A COMPLAINT OF PROTRUBERENT ABDOMEN. EXAMINATION SHOWED MARKED HEPATOMEGALY. THE FOLLOWING INVESTIGATIONS WERE PERFORMED.

TG	**–**	**1500 mg/dl**
URIC ACID	**–**	**9 mg/dl**
PLASMA SUGAR	**–**	**50 mg/dl**

THE PATIENT DEVELOPED LACTIC ACIDOSIS. WHAT IS YOUR PROBABLE DIAGNOSIS? WHAT IS THE CAUSE FOR THIS DISORDER?

Ans. It is a case of Von Gierke's disease. It is caused by the deficiency of enzyme glucose 6-phosphatase. As a result of this deficiency, glucose 6-(P) is converted to glycogen and stored in the liver causing hepatomegaly and protruded abdomen. Glucose 6-(P) is diverted to HMP–shunt pathway leading to formation of ribose – 5-(P) and ultimately excessive synthesis of uric acid (↑↑ level). Glucose 6-(P) is not converted to free glucose resulting in hypoglycemia (↓↓ plasma sugar). There is also an abnormality of lipid metabolism resulting ↑↑ level of TG.

17. AN ADULT MALE PATIENT WAS BROUGHT TO THE HOSPITAL WITH THE COMPLAINT OF PAIN AND CRAMPS FOLLOWING EXERCISE HE SHOWED A POSITIVE HISTORY OF MYOGLOBINURIA. SERUM CPK LEVEL WAS FOUND TO BE RAISED. WHAT IS YOUR PROBABLE DIAGNOSIS ? WHAT IS THE CAUSE OF THIS DISORDER?

Ans. It is a case of muscular dystrophy (duchenne) caused by the deficiency of dystrophin localizing to the sarcolemmal membrane. It is a x-linked recessive disorder.

In this disorder there is a progressive weakness of muscles particularly girdle muscles. The creatinine level is raised to 20–100 times normal. Patients may develop cardiomyopathy.

18. A 40 YEAR OLD MALE PATIENT WAS BROUGHT TO THE HOSPITAL WITH COMPLAINTS OF PAIN AND SWELLING OF THE BIG TOE. ON EXAMINATION IT WAS FOUND THAT THE MOVEMENT OF THE FIRST METATARSOPHALANGEAL JOINT WAS AFFECTED. INVESTIGATIONS SHOWED SERUM URIC ACID TO BE 9 MG/DL, RAISED ESR AND LEUKOCYTOSIS. BIOPSY OF THE JOINT SHOWED PRESENCE OF NEEDLE LIKE CRYSTALS. WHAT IS YOUR PROBABLE DIAGNOSIS OF THIS CASE AND WHAT IS THE CAUSE FOR THIS DISORDER?

Ans. It is a case of gouty arthritis. The following points are in favour of this clinical disorder.

(*i*) Serum uric acid level is raised 9.0 mg/dl. (normal 2–6 mg/dl).

(*ii*) Inflammation of Ist metatarsophalangeal joint of big toe. (Pain and swelling of big toe).

(*iii*) ESR is raised (↑↑) and leucocytosis (signs of inflammation).

(*iv*) Presence of needle like crystals of monosodium monourate.

The primary gout is caused by either the deficiency of HGPRT (hypoxanthine guanine phosphoribosyl transferase) or over activity of PRPP synthase.

19. A 25 YEAR OLD WOMEN WAS HOSPITALIZED WITH AN ACUTE MYOCARDIAL INFARCTION. SUBSEQUENTLY HER PLASMA CHOLESTEROL WAS FOUND TO BE 525 MG/DL, BUT HER PLASMA TRIGLYCERIDE CONCENTRATION WAS FOUND TO BE NORMAL. FURTHER ANALYSIS SHOWED THAT HER LDL LEVEL WAS HIGHLY ELEVATED. NODULAR SWELLING WERE SEEN ON ACHILLES TENDON (TENDON XANTHOMAS). CORONARY ANGIOGRAPHY INDICATED THE PRESENCE OF SEVERE ARTERIOSCLEROSIS IN ALL THREE CORONARY ARTERIES. WHAT IS YOUR PROBABLE DIAGNOSIS? WHAT IS THE CAUSE OF THE DISORDER?

Ans. It is a case of familial hypercholesterolemia (type IIa HLP). The following points are in favour of this clinical disorder.

(*i*) Serum cholesterol is raised 525 mg/dl (normal 150–200 mg/dl).

(*ii*) Due to ↑↑ cholesterol tendon xanthomas are formed.

(*iii*) LDL is raised and ↑↑ cholesterol is responsible for coronary atherosclerosis and AMI.

This clinical disorder is caused by the defect in LDL receptors, as a result of this the LDL is not taken up by the cells leading to ↑↑ level of cholesterol.

20. AN ADULT MALE PATIENT WAS BROUGHT TO THE HOSPITAL WITH COMPLAINT OF OBESITY. ON EXAMINATION IT WAS FOUND THAT HE HAD HYPERTENSION. BLOOD EXAMINATION SHOWED SUGAR LEVELS OF 310 MG%, SERUM URIC ACID OF 8.5 MG% AND FASTING TG OF 1100 MG%. WHAT IS YOUR PROBABLE DIAGNOSIS? WHAT IS THE CAUSE OF THIS DISORDER?

Ans. It is a case of familial hypertriglyceridemia (FHT). FHT is autosomal dominant disorder. The following points are in favour of this clinical disorder.

(*i*) Serum TG level is raised 1100 mg/dl (normal < 150 mg/dl).

(*ii*) ↑↑ TG level results in insulin resistance which causes:
- (*a*) obesity
- (*b*) hypertension
- (*c*) ↑↑ glucose tolerance (↑↑ blood sugar level). The ↑↑ TG is due to ↑↑ VLDL formation and ↓↓ clarance of VLDL.

21. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT.

pH	PCO_2	O_2–Satu	HCO_3^-	Base excess
7.419	41.4	95.6	26.2	–1.5

Ans. It is a case of normal person because all the values are within normal limits.

S. No.		*Normal*	*Individual value*
(*i*)	pH	7.35 – 7.45	7.419
(*ii*)	PCO_2	38–45	41.4
(*iii*)	O_2–Satu	94–100	95.6
(*iv*)	HCO_3^-	22–27	26.2
(*v*)	Base excess	–2 to +2	–1.5

22. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT. IF THERE IS ANY ABNORMALITY MENTION THE COMPENSATORY MECHANISM.

pH	PCO_2	O_2–Satu	HCO_3^-	Base excess
7.37	35	99.4	18.5	–5.6

Ans. It is a case of compensated metabolic acidosis. The following points are in favour of this clinical disorder.
- (*i*) PH become normal after compensation.
- (*ii*) ↓↓ HCO_3^-.
- (*iii*) ↓↓ base excess.
- (*iv*) It is compensated by hyperventilation causing ↓↓ PCO_2.

23. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT. IF THERE IS ANY ABNORMALITY MENTION THE COMPENSATORY MECHANISM.

pH	PCO_2	O_2–Satu	HCO_3^-	Base excess
7.056	82.4	86.1	22.6	–2.0

Ans. It is a case of uncompensated respiratory acidosis. The following points are in favour of this clinical disorder.
- (*i*) ↓↓ pH.
- (*ii*) ↑↑ PCO_2 (82.4).
- (*iii*) It is not compensated as the values of HCO_3^- and base excess are within normal limits.

24. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT. IF THERE IS ANY ABNORMALITY MENTION THE COMPENSATORY MECHANISM.

pH	PCO_2	O_2–Satu	HCO_3^-	Base excess
7.654	35	99.5	32.4	11.9

Ans. It is a case of uncompensated metabolic alkalosis. The following points are in favour of this clinical disorder.

(*i*) ↑↑ pH
(*ii*) ↑↑ HCO_3^-
(*iii*) ↑↑ base excess
(*iv*) It is not compensated as the PCO_2 value is not raised.

25. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT.

pH	PCO_2	HCO_2^-	Base excess
↓	N	↓	↓

Ans. It is a case of metabolic acidosis because the pH is decreased, primary ↓↓ of HCO_3 and base excess is decreased.

26. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT.

pH	PCO_2	HCO_3^-	Base excess
↓	↑	N	↓

Ans. It is a case of respiratory acidosis as the pH is decreased and primary elevation of PCO_2. Other parameters are normal.

27. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT.

pH	PCO_2	HCO_3^-	Base excess
↑	N	↑	↑

Ans. It is a case of metabolic alkalosis as pH is more than normal and HCO_3^- and base excess values are raised. The PCO_2 value is within normal limits.

28. INTERPRET THE FOLLOWING BLOOD GAS PARAMETERS AND COMMENT.

pH	PCO_2	PCO_3^-	Base excess
↑	↓	N	N

Ans. It is a case of respiratory alkalosis as pH is more than normal and PCO_2 value is decreased. The HCO_3 and base excess values are within normal limits.

CLINICAL BIOCHEMISTRY – II
BIOCHEMICAL FEATURES OF CLINICAL DISORDERS

29. EXPLAIN THE BIOCHEMICAL BASIS OF SYMPTOMS AND SIGNS OF DIABETIC COMA DUE TO KETOTICACIDOSIS AND NON KETOTIC HYPEROSMOLAR COMA IN DIABETES?

Ans. Diabetic Coma Due to Keto Acidosis (DKA)

(*i*) DKA is caused by the deficiency of insulin and excess of glucagon. Therefore ↓↓ insulin: glucagon ratio causes hyperglycemia by the hepatic over production (↑↑ gluconeogenesis and glycogenolysis) and peripheral under utilisation (↓↓ uptake of glucose in the muscle and adipose tissue cells) by the failure of recruitment of GLUT-4 transporter to plasma membrane.

(*ii*) The ↓↓ insulin: Glucagon ratio decreases delivery of fatty acids and amino acids to liver causing ketone body formation and over production of glucose.

(*iii*) The ↓↓ insulin: Glucagon ratio decreases fructose 2, 6 bisphosphate (↓↓ glycolysis) and insulin deficiency ↑↑ the activity of PEP-CK gluconeogenesis. Insulin deficiency decreases GLUT-4 transports. The activity of glucagon ↑↑ activity of carnitine palmitoyl transferase-I resulting in ↑↑ transport of fatty acids to mitochondria which inturn stimulates ↑↑ β-oxidation and ↑↑ formation of ketonebodies.

The ↑↑ ketone bodies decreases pH and HCO_3^- level leading to metabolic acidosis. The ↑↑ free fatty acids cause increase TAG formation in the liver and increase VLDL secretion. The ↓↓ insulin causes decreased activity of LPL resulting in hypertriglyceridemia and causes pancreatitis.

Symptoms and Signs

The patient of DKA develops the following symptoms:

(*a*) Nausea and vomiting .

(*b*) Abdominal pain (pancreatitis as stated above).

(*c*) Thirst, polyuria (osmotic diuresis) and develops dehydradation/hypotension/dry mucus membrane/reduced skin turgor. →↑↑ BUN

(*d*) The ↓↓ P^H (6.8 – 7.3) and HCO_3^- (< 10 mmol/l) causes metabolic acidosis resulting in kussmaul respiration and coma.

(*e*) There will be disturbed electrolyte balance (hyponatremia).

(*f*) Patient develops coma, due to ketoacidosis.

Diabetic Coma Due to Non Ketotic Hyper Osmolar State

1. Most commonly seen in elderly individuals with type–II DM. Insulin deficiency and inadequate fluid intake are the important causes.
2. Prominent feature is polyuria, orthostatic hypotension, altered mental status, lethargy and seizures ultimately resulting into coma.
3. Physical signs include
   (*a*) Hypotension.
   (*b*) Tachycardia.
   (*c*) Altered mental status.
   (*d*) Absence of symptoms and signs of DKA like nausea, vomiting, abdominal pain and Kussmaul respiration.
   (*e*) Serious infection and other precipitating factors.
   (*f*) Hyperglycemia is caused by hepatic over production (↑↑gluconeogenesis and glycogenolysis) and peripheral under utilisation (↓↓ uptake of glucose by muscle and adipose tissue cells) just like DKA. Hyperglycemia induces osmotic diuresis. The osmatic diuresis and ↓↓ intake of fluids decreases ⟶ intravascular volume depletion.
   (*g*) Absence of ketosis is due to lower level of counter regulatory hormone and ↓↓ levels of fatty acids and liver is less capable of ketone body synthesis.
   (*h*) Laboratory finding : Marked hyperglycemia.
   (*i*) Patient develops coma due to increase in osmolality and severe increase in dehydration (plasma glucose 1000 mg/dl).

Major Differences of DKA and NKHS

S. No.	*DKA (Diabetic Keto Acidosis)*	*NKHS (Nonketotic Hyperosmolar State)*
1.	Ketosis is present	Ketosis is absent
2.	Abdominal pain is present	Abdominal pain is absent
3.	Nausea and vomiting present	Nausea and vomiting absent
4.	Plasma glucose 350–650 mg/dl	Plasma glucose 700–1200 mg/dl
5.	Na^+ – 125 – 135 mmol/L.	Na^+ – 135 – 145 mmol/L.
6.	K^+ – Normal to ↑	K^+ – Normal
7.	Cl^- normal	Cl^- normal
8.	Creatinine slightly ↑	Creatinine moderate ↑
9.	$HCO3^-$ < 15 mmol/L.	$HCO3^-$ normal
10.	pH – 6.8 – 7.3	pH – > 7.3
11.	Anion gap ↑↑↑	Anion gap normal or slightly ↑ (due to Lactic acid)
12.	Osmolality 300 – 320	Osmolality 340 – 380

30. EXPLAIN THE BIOCHEMICAL BASIS OF SYMPTOMS AND SIGNS OF HEPATIC COMA DUE TO HEPATIC ENCEPHALOPATHY?

Ans. Hepatic coma due to encephalopathy is a complex neuropsychiatric syndrome characterized by disturbances of unconsciousness (coma), fluctuating neurologic signs and flapping tremors.

The hallmark of this syndrome is severe hepato cellular dysfunction or intra hepatic or extra hepatic shunting of portal venous blood into systemic circulation bypassing liver (portal systemic collateral shunts). As a result of these processes, various toxic metabolites are absorbed from the intestine and which are not detoxified by the liver and leads to metabolic abnormalities in the CNS.

Toxic Substances Which Accumulate in the Body

(*i*) **Ammonia:** Increased formation of NH_3 and its raised level in systemic blood is an important cause of encephalopathy. Many patients of hepatic encephalopathy have elevated NH_3 level.

Factors Influencing $\uparrow\uparrow$ NH_3 Formation:

(*a*) Bleeding from oesophageal varices due to the portal hypertension leads to $\uparrow\uparrow$ production of NH_3.

(*b*) Dietary proteins acted upon by colonic bacteria may cause $\uparrow\uparrow$ formation of NH_3.

(*c*) Systemic alkalosis causes an increase in the NH_3/NH_4^+ ratio thus enables crossing of NH_3 through blood brain barrier to accumulate in the CNS.

(*d*) Hypokalemia stimulates renal NH_3 production.

(*ii*) Mercaptans derived from intestinal metabolism of methionine.

(*iii*) Short chain fatty acids.

(*iv*) Phenol.

(*v*) Excessive concentration of GABA (inhibitory neurotransmitter) in the CNS reduces the consciousness.

(*vi*) False neurotransmitter (octopamine).

(*vii*) Excess manganese deposition may contribute the pathogenesis of hepatic encephalopathy.

(*viii*) Cerebral oedema is frequently present and contributes to clinical picture. (coma)

The Following Factors Precipitate Hepatic Encephalopathy

(*a*) Gastrointestinal bleeding

(*b*) Excess dietary protein

(*c*) Hypokalemia

(*d*) Constipation

(*e*) Alkalosis

(*f*) Hypoxia

(*g*) Hyponatremia

(*h*) Hypovolemia

(*i*) Infections

31. WHAT ARE THE CLINICAL AND BIOCHEMICAL FEATURES OF WILSON'S DISEASE?

Ans. Wilson's disease is characterised by the degeneration of the lenticular and caudate nuclei and liver.

Clinical and Biochemical Features

(*a*) The significant feature is the progressive cirrhosis.
(*b*) It is characterised by tremor, muscular rigidity, mental changes and evidence of hepatic dysfunction.
(*c*) Presence of greenish-brown rings in both eyes around the edge of the cornea (Kayer-Fleischer rings).
(*d*) Aminoaciduria (excretion of excess aminoacids in urine).
(*e*) Excessive deposition of copper in the tissues (5 to 20 times is the normal) particularly in brain, kidney and liver occurs due to the deficiency of ceruloplasmin which binds copper in plasma. The normal level of ceruloplasmin is 28 mg – 56 mg/dl.

32. WHAT ARE THE CLINICAL AND BIOCHEMICAL FEATURES OF IRON DEFICIENCY ANAEMIA?

Ans. Iron deficiency anaemia is present commonly in
(*a*) Pregnancy
(*b*) Adolescence
(*c*) Periods of rapid growth
(*d*) Blood loss (bleeding from GIT) and during menstruation

Clinical Features

The clinical features of IDA depends upon the severity of the deficiency and the chronicity of the condition.

Common Clinical Features

(*a*) Fatigue
(*b*) Pallor
(*c*) Reduced exercise capacity
(*d*) Koilonichia (spooning of finger nails)

Laboratory Diagnosis

(**A**) Peripheral smear shows hypochromic and microcytic cells. The Hb being visible as a pale ring at the periphery of thin cells. In more severe cases anisocytosis (variation in red blood cell size) and poikilocytosis (variation in shape of red cells) are seen.

	Normal	*IDA*
(*a*)	Hematocrit 40–45%	< 41% in males
		< 37% in females
	Hb – 14–16 gr/dl.	< 13.5 g/dl in males
		< 12 g/dl in females
(*b*)	SI–50–150 micrograms/dl.	< 30
(*c*)	TIBC 300 to 360 micrograms/dl.	> 360
(*d*)	Transferrin saturation 25–50%	< 10
(*e*)	Ferritin male 100 micrograms/ltr.	
	Female 30 micrograms/ltr.	< 15

CLINICAL BIOCHEMISTRY – III

RENAL FUNCTION TESTS

33. WHAT ARE THE RENAL FUNCTION TESTS?

Ans. There are two types of renal function tests:

(*i*) Tests to detect the intensity of the pathologic process that occurs in the kidney.
- (*a*) Presence of proteins in urine.
- (*b*) Presence of blood pigment in urine.
- (*c*) Presence of blood cells and casts in urine.

(*ii*) Tests to detect the functional capacity of kidney.
- (*a*) Concentration and dilution tests.
- (*b*) Clearance tests.
- (*c*) Concentration of NPN substances in plasma.
- (*d*) Concentration of electrolytes in plasma.

34. DEFINE THE TERM CLEARANCE OF THE SUBSTANCE. WHAT IS THE FORMULA APPLIED FOR THE CALCULATION OF CLEARANCE OF ANY SUBSTANCE?

Ans. Definition of clearance

The clearance of any substance is defined as the number of ml of plasma, which contains the amount of that substance excreted in a minute by the kidneys.

$$\text{Clearance} = \frac{\text{mg. of substance excreted per minute}}{\text{mg. of substance per ml. of plasma}}$$

$$C = \frac{UV}{P}$$

C ⟶ Clearance of a substance

U ⟶ Concentration of substance in urine

P ⟶ Concentration of substance in plasma
V ⟶ Volume of urine excreted per minute

35. WHAT ARE THE NORMAL VALUES OF

(*a*) Inulin clearance
(*b*) Urea clearance
(*c*) Endogenous creatinine clearance

Ans. (*a*) Inulin clearance – 125 ml to 130 ml/mt.
(*b*) Urea clearance
(*i*) Maximum clearance – 75 ml/mt.
(when the volume of urine excreted per minute is 2 ml or >2 ml/mt.)
(*ii*) Standard clearance – 54 ml/mt.
(when the volume of urine excreted per minute is <2 ml/mt.)
(*c*) Creatinine clearance:
(*i*) Males: Average clearance – 105 ml per 1.73 sq.m of BSA.
(*ii*) Females: Average clearance – 97 ml per 1.73 sq.m of BSA.

36. WHAT IS THE CLINICAL IMPORTANCE OF CREATININE CLEARANCE?

Ans. The value of creatinine clearance is decreased with the degree of renal insufficiency. The creatinine clearance is usually done to assess the early renal damage by nephrotoxic drugs such as aminoglycosides. In severe uremia the creatinine clearance is usually below 10 ml minute.

37. WHAT IS THE FILTRATION FRACTION AND WHAT IS ITS CLINICAL IMPORTANCE?

Ans. The ratio of GFR (glomerular filtration rate/ERPF (effective renal plasma flow) is called filtration fraction (FF).
The FF is determined by C_{in} (inulin clearance)/C_{PAH} (para amino hippurate clearance).

Clinical Significance of FF

The normal value of FF is 0.20
The FF is increased (↑↑) in
(*a*) Constriction of efferent arterioles
(*i*) Shock
(*ii*) Cardiac failure
(*iii*) Early hypertension
The FF is decreased (↓↓) in
(*a*) Constriction of afferent anterioles or dilatation of efferent arterioles.
(*b*) Acute glomerulonephritis (↓↓ permeability of glomerular filters).

Chapter–23

Practicals in Biochemistry

CARBOHYDRATES

1. WHAT IS A SUGAR?

Ans. Sugar is a white crystalline substance, soluble in water and sweet in taste. Sugars are classified into two types:

(*a*) Reducing sugars.

(*b*) Non-reducing sugars.

2. WHAT IS A REDUCING SUGAR? GIVE EXAMPLES.

Ans. Reducing sugar reduces cupric ions in the Benedict's and Fehling's solutions to cuprous ions and it answers these tests.

Examples

Reducing monosaccharide.

Aldohexose	:	Glucose
Keto hexose	:	Fructose
Reducing disaccharides	:	Lactose (milk sugar)
		Maltose (malt sugar, obtained by the hydrolysis of starch)

Reducing sugars contain sugar groups in their structure.

Example

(*i*) Aldelyde group $H - C(R^1) = O$ in aldoses

(*ii*) Keto group $C(R^1)(R^2) = O$ in ketoses

3. WHAT IS A NON-REDUCING SUGAR?

Ans. Non – reducing sugars do not have free sugar group in their structure and they fail to answer Benedicts' and Fehling's tests.

Example

Sucrose (table sugar)

4. WHAT ARE OSAZONES?

Ans. Reducing sugars react with pheynylhydrazine hydrochloride at 100°C for 30 minutes and form osazones. Reducing sugars can be identified by seeing the shape of osazones under microscope.

Example

Glucosazone	–	Needle shape crystals.
Lactosazone	–	Hedgehog shape crystals.
Maltosazone	–	Petals of flower (sun flower).

5. WHY GLUCOSE, FRUCTOSE AND MANNOSE FORM SAME SHAPE OF OSAZONES (NEEDLE SHAPE)?

Ans. The spatial configuration of atoms and groups in lower (four) carbons is same in them.

PROTEINS

6. WHAT ARE PROTEINS?

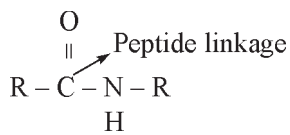
Ans. Proteins are polymers of amino acids. There are 20 different amino acids which are present in proteins. These amino acids are joined by peptide linkages.

7. WHAT ARE COLOUR REACTIONS OF A PROTEIN? GIVE EXAMPLES.

Ans. Certain specific reactions give particular colour due to presence of amino acids in their structures. These are called colour reactions.

Example

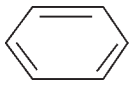
(*a*) Biuret Test ⟶ Gives violet colour. This is due to the presence of peptide linkages in the protein structure. It is a general test for the identification of a protein.



This test is called biuret because it was first demonstrated in the product called Biuret, which is formed by the decomposition of urea on heating at 180ºC.

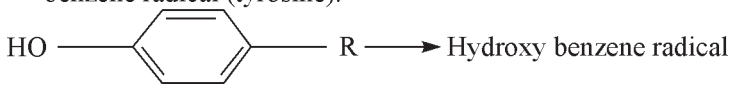
(*b*) Ninhydrin reaction ⟶ Blue colour due to presence of alpha-amino acid radical. It is also a general test for the identification of a protein.

(*c*) Xantho proteic reaction ⟶ Yellow colour due to presence of Benzoid radical containing amino acids (Phenylalanine, tyrosine, tryptophan)

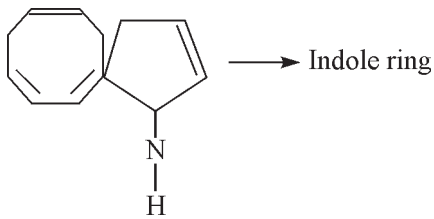


(*d*) Millon's reaction → Red colour due to presence of hydroxy benzene radical (tyrosine).

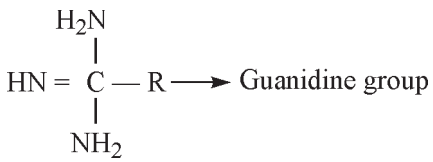
benzene radical (tyrosine).



(*e*) Aldelyde test: Purple colour due to presence of indole ring containing amino acid tryptophan.



(*f*) Sakaguchi test: Bright red colour due to presence of guanidine group containing amino acid (Arginine)



(*g*) Test for cysteine and cystine → black colour due to SH or S–S radicals.

8. CLASSIFY THE PROTEINS AND GIVE EXAMPLES.

Ans. Proteins are classified into :

1. Simple proteins → Example: Albumin (Egg white).
2. Conjugated proteins → Example: Casein (Phosphoprotein present in milk).
3. Derived proteins → Example: Peptones formed by the hydrolysis of a native protein.

9. WHAT ARE PROTEINS OF HIGH BIOLOGICAL VALUE?

Ans. Proteins of high biological value contain all the essential amino acids in optimum concentration.

Example

Albumin, Casein

10. WHAT ARE PROTEINS OF LOW BIOLOGICAL VALUE?

Ans. Proteins of low biological value lack one or two essential amino acids.

Example

Zein of corn lacks amino acids tryptophan and lysine.

11. WHAT ARE THE PROPERTIES OF PROTEINS AT ITS ISOELECTRIC pH (I.E.P.)?

Ans. (*a*) The proteins carry equal number of positive and negative charges, so that it is electrically neutral.
(*b*) The conductivity and osmotic pressure of a protein solution is at minimum.
(*c*) The solubility is at minimum.
(*d*) The viscosity is at minimum.

ABNORMAL URINE

12. WHAT ARE THE ABNORMAL SUBSTANCES WHICH ARE LIKELY TO BE EXCRETED IN VARIOUS DISEASES?

Ans. (*a*) Glucose
(*b*) Ketone bodies (acetone and acetoacetate)
(*c*) RBC (blood)
(*d*) Protein (albumin)
(*e*) Bile pigments (bilirubin)
(*f*) Bile salts

13. WHAT IS GLYCOSURIA (GLUCOSURIA)? WHAT ARE THE CAUSES OF THIS CONDITION?

Ans. Excretion of glucose in urine is called glycosuria. The causes are:
(*a*) Diabetes mellitus.
(*b*) Renal glycosuria (↓↓ renal threshold. Normal 180 mg/dl).

14. WHAT ARE THE CAUSES OF KETONURIA?

Ans. (*a*) Severe untreated diabetes mellitus.
(*b*) Starvation (prolonged fasting).

15. WHAT ARE THE CAUSES OF HAEMATURIA?

Ans. (*a*) Renal calculus (renal colic).
(*b*) Acute glomerulonephritis.

16. WHAT ARE THE CAUSES OF ALBUMINURIA?

Ans. (*a*) Nephrotic syndrome.
(*b*) Acute glomerulonephritis.

17. WHAT ARE THE CAUSES OF EXCRETION OF BILIRUBIN AND BILE SALTS?

Ans. (*a*) Obstructive jaundice < Intra hepatic / Extra hepatic

Intra hepatic – Viral hepatitis
Extra hepatic – Gallstones, carcinoma of head of pancreas.

N.P.N. SUBSTANCES

18. WHAT ARE THE N.P.N. SUBSTANCES?

Ans. The non-protein nitrogen (NPN) containing substances are :
(*a*) Urea (*b*) Uric Acid (*c*) Creatinine

19. WHAT IS NORMAL BLOOD UREA LEVEL AND NAME THE CLINICAL DISORDERS IN WHICH IT IS RAISED?

Ans. Normal value : 10 – 40 mg/dl
↑↑ Blood urea level.
Pre-renal – Dehydration (diarrhoea and vomiting).

Renal < ARF (Acute renal failure) / CRF (Chronic renal failure)

Post-Renal – Renal calculus and enlarged prostrate.

20. WHAT IS NORMAL URIC ACID LEVEL AND NAME THE CLINICAL DISORDERS IN WHICH IT IS RAISED?

Ans. Normal value : 2.0 – 6.0 mg/dl. Gout and renal failure.

21. WHAT IS NORMAL CREATININE LEVEL AND NAME THE CLINICAL DISORDERS IN WHICH IT IS RAISED?

Ans. Normal value : 0.6 – 1.2 mg/dl. Renal insufficiency and renal failure.

QUANTITATIVE ANALYSIS

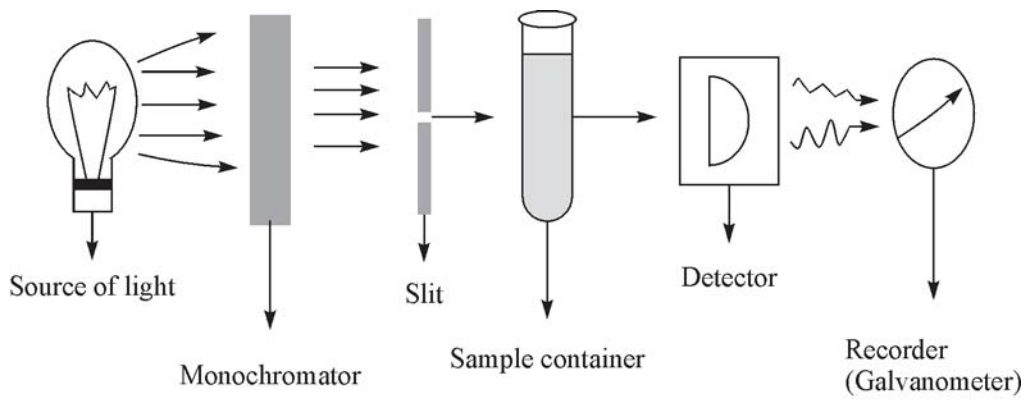
PHOTOELECTRIC COLORIMETER

22. WHAT ARE THE PARTS OF PHOTOELECTRIC COLORIMETER?

Ans. The following are the parts of photoelectric colorimeter:

The Parts of Photoelectric Colorimeter

1. **A Source of Light**
   Tungsten lamp is the source of visible light in the colorimeters (Spectrophotometers)
   (*a*) Tungsten lamp (400–700 nm).
   (*b*) Deuterium lamp – U.V. (200–400 nm).
2. **Monochromator**
   (Specific wave length of light) obtained by
   (*i*) Selective filters.
   (*ii*) Prisms.
   (*iii*) Diffraction by a grating.
3. **Slit**
   This is to allow a narrow beam of selected monochromatic light to pass through the sample solution.
4. **Cuvette**
   The glass container to keep the test solution.
5. **Photocells**
   Which convert quanta of radiation (light) to electrical energy, which may be amplified, detected and recorded.



Components of colorimeter/spectrophotometer

Fig. 1

23. WHAT IS BEERS LAW AND LAMBERT LAW?

Ans. Beer's law states that when monochromatic light passes through a coloured solution the amount of light transmitted decreases with the increase in concentration of the coloured substance i.e. the absorbance or optical density increases (↑↑) with the concentration of the substance.

Lambert's law states that when monochromatic light passes through a coloured solution the amount of light transmitted decreases with increase (↑↑) in optical path.

24. WHAT DO YOU RECORD WITH THE COLORIMETER?

Ans. The absorbance or optical density of the coloured solution.

25. WHAT ARE ANTICOAGULANTS?

Ans. Anticoagulants prevent the clotting of blood and these are used for the separation of plasma.

Example

Potassium Oxalate, Heparin, Sodium Citrate and EDTA.

26. WHAT IS THE DIFFERENCE BETWEEN SERUM AND PLASMA?

Ans. Fibrinogen is absent in serum and fibrinogen is present in plasma.

27. WHAT IS THE FORMULA FOR THE CALCULATION OF CONCENTRATION OF SUBSTANCE IN COLORIMETRY?

Ans. Formula for the calculation of concentration of substance in colorimetry.

(*i*) $$\frac{\text{O.D. of test} - \text{O.D. of Blank}}{\text{O.D. of standard} - \text{O.D. of blank}} \times \frac{\text{Concentration of standard}}{\text{Volume of fluid taken into test}} \times 100$$

(*ii*) When the volume of sample equals to the volume of standard, so that same conditions are fulfilled for both standard and test the following formula is applied.

$$\frac{T}{S} \times \text{Concentration of standard}$$

28. WHAT ARE THE FUNCTIONS OF THE FOLLOWING EQUIPMENTS?

Ans. **Centrifuge :** Used for the separation of serum or plasma.

Hot Air Oven : For drying the glassware after washing.

Incubator/37°C water bath : For the incubation of the sample with the reagents for allowing the reaction to occur.

29. HOW DO YOU WASH THE TEST TUBES, BEAKERS, VOLUMETRIC FLASKS, AND GLASS CYLINDERS ETC.?

Ans. Soak the glassware in the detergent solution for few hours and then wash them with the tap water. Afterwards rinse them with the distilled water and keep them in Hot Air Oven for one hour.

Pipettes

Keep them in the chromic acid over night and wash them with tap water, rinse them with the distilled water and keep them in Hot Air Oven for one hour.

30. WHAT IS END POINT ASSAY?

Ans. In End point assay the samples are treated with the reagent and incubated at 37ºC for a fixed time. The colour developed after incubation time at the end of reaction is compared in colorimeter and the concentration of the substance is calculated by applying the following formula.

$$\frac{T}{S} \times \text{Concentration of substance}$$

31. WHAT IS KINETIC ASSAY? AND HOW AN ENZYME ESTIMATION IS DONE BY KINETIC ASSAY?

Ans. In kinetic assay optimum pH is provided by the suitable buffer. In kinetic assay the sample is treated with buffer and other reagents which are required for reaction to occur and then it is incubated at 37°C water bath for one minute. The change in absorbance after one minute will be recorded in spectrophotometer and enzyme activity will be calculated by applying formula.

Formula : Change in absorbance × Factor per minute = Units per litre.

32. WHAT IS NORMAL FASTING PLASMA OR SERUM GLUCOSE LEVEL?

Ans. 70–110 mg/dl.

33. WHAT IS THE LATEST CRITERIA APPLIED FOR THE DIAGNOSIS OF DIABETES MELLITUS?

Ans. When fasting serum glucose is greater than 125 mg/dl and when 2 hours post prandial serum glucose is 200 mg/dl or more than 200 mg/dl, the patient is diagnosed as diabetes mellitus.

34. WHAT IS IMPAIRED GLUCOSE TOLERANCE?

Ans. When the fasting serum glucose is between 111 mg/dl to 125 mg/dl, it is called impaired glucose tolerance.

35. WHAT ARE THE DIFFERENT METHODS OF BLOOD SUGAR ESTIMATION?

Ans. 1. GOD–POD (glucose oxidase-peroxidase method).
2. Orthotoluidine method.
3. Folin and Wu method.

36. WHAT IS THE PRINCIPLE OF GOD-POD METHOD?

Ans. Test principle:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD (Glucose oxidase)}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + 4 - \text{aminophenazone} + \text{Phenol} \xrightarrow{\text{POD (peroxidase)}}$$

4-(p-benzoquinone-mono-imino) phenazone + $4H_2O$

The phenazone gives pink colour and the absorbance of this colour is read at 470–560 nm wave length in the colorimeter.

37. WHAT ARE THE DIFFERENT METHODS OF BLOOD UREA ESTIMATION?

Ans. 1. Berthelot reaction.
2. DAM (diacetylmonoxime) method.

38. WHAT IS THE PRINCIPLE OF BERTHELOT REACTION?

Ans. Urease splits urea into ammonia and carbondioxide. Ammonia released in this reaction reacts with hypochlorite and phenolic chromogen to produce green colour. The absorbance of this green colour is read at 585 nm (570–620) which is directly proportional to the concentration of urea in specimen.

$$NH_2 - CO - NH_2 \xrightarrow{H_2O,\ \text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenolic chromogen} \longrightarrow \text{Green colour.}$$

39. NAME THE METHOD OF ESTIMATION OF URINE CREATININE AND WHAT IS THE PRINCIPLE OF IT?

Ans. Creatinine in urine is determined by its reaction with picric acid in an alkaline medium to form the orange coloured tautomer of creatinine picrate (Jaffe's reaction). Since creatinine content of urine is high, it is suitably diluted. The intensity of orange colour is read using a green filter (520 nm).

40. WHAT IS THE NORMAL VALUE OF ENDOGENOUS CREATININE CLEARANCE TEST? GIVE ITS INTERPRETATION.

Ans. Normal value 91–130 ml/mt. ↓↓ Creatinine clearance indicates the renal insufficiency. In renal failure it is grossly decreased.

41. WHAT IS THE NORMAL EXCRETION OF CREATININE PER DAY?

Ans. 1–2 G/day.

42. WHAT IS THE PRINCIPLE OF SERUM TOTAL PROTEIN ESTIMATION?

Ans. The peptide bonds of protein react with cupric ions in alkaline solution to form blue-violet colour complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520–560 nm).

43. WHAT IS THE NORMAL VALUE OF SERUM TOTAL PROTEINS? GIVE ITS LINICAL INTERPRETATION.

Ans. Normal value of total protein – 6.0 – 8.0 g/dl
Normal value of total albumin – 3.5 – 5.0 g/dl
Normal value of total globulin – 2.0 – 3.5 g/dl

The concentration of total proteins and albumin are decreased in:

1. Nephrotic syndrome
2. Protein loosing enteropathy
3. Liver disease
4. Malnutrition.

44. WHAT ARE THE DIFFERENT FRACTIONS OBTAINED BY THE SEPARATION OF SERUM PROTEINS BY ELECTROPHORESIS IN ORDER OF RATE OF MIGRATION?

Ans. In order of rate of migration

(*a*) Albumin ⟶ Fastest moving fraction.
(*b*) α_1-globulin ⟶ Movement is less than albumin
(*c*) α_2-globulin ⟶ Movement is less than α_1-globulin
(*d*) β-globulin ⟶ Movement is less than α_2-globulin
(*e*) γ-globulin ⟶ Least mobile fraction.

45. WHAT IS THE ABNORMAL PATTERN IN THE SEPARATION OF SERUM PROTEINS BY ELECTROPHORESIS?

Ans. There are three possible abnormal patterns:

(*i*) **In polyclonal gammopathy**

The gamma band is most prominent. It is observed in:

(*a*) Chronic infections.
(*b*) Collagen diseases.
(*c*) Sarcoidosis.

(*ii*) **In monoclonal gammopathy (Multiple myeloma)**
The 'M' band is seen in γ regions or between γ and β regions.

(*iii*) **In nephrotic syndrome**
(*a*) α_2 band is prominent
(*b*) Albumin band is less prominent.

46. WHAT IS NORMAL A.G. RATIO? GIVE ITS INTERPRETATION.

Ans. 1.5:1
↓↓ A.G. ratio occurs in liver diseases.

CEREBRO SPINAL FLUID (C.S.F)

47. WHAT ARE THE NORMAL VALUES OF BIOCHEMICAL SUBSTANCES PRESENT IN C.S.F. AND GIVE THEIR CLINICAL INTERPRETATION?

Ans. (*a*) Reducing sugar – 40 – 70 mg/dl
↓↓ in Meningitis.
(*b*) Proteins – 10 – 40 mg/dl
↑↑ in Meningitis and obstruction to flow of C.S.F.
(*c*) Chlorides – 116 to 122 m.mol/dl
↓↓ in Meningitis.

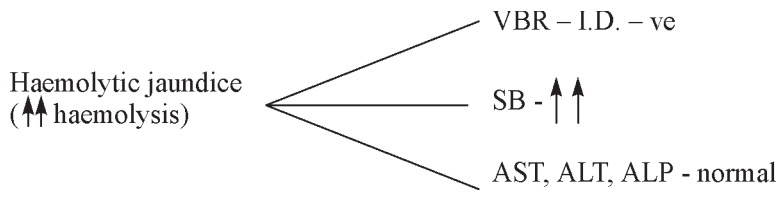
LIVER FUNCTION TESTS (L.F.T.)

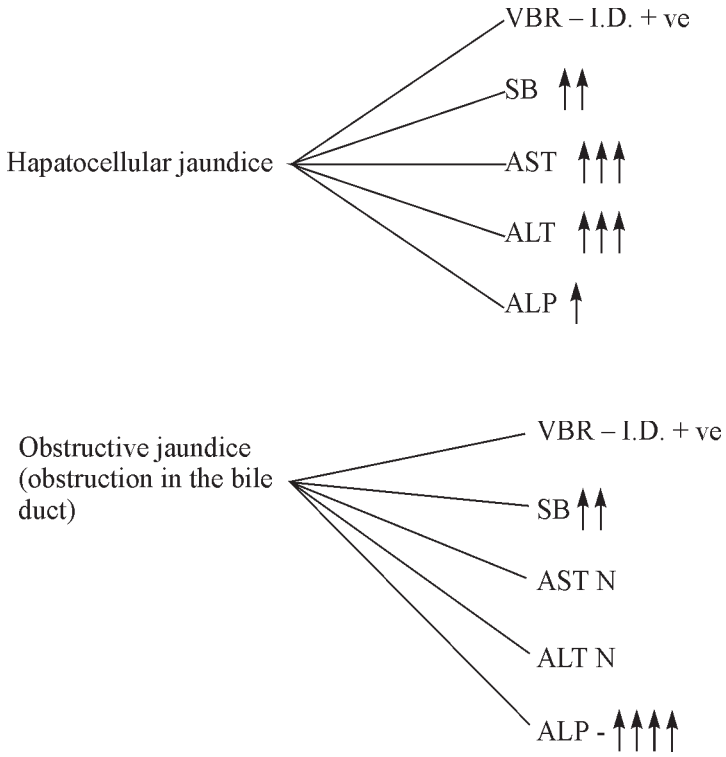
48. WHAT ARE THE IMPORTANT TESTS INCLUDED IN L.F.T.? GIVE THEIR NORMAL VALUES.

Ans. (*a*) Van den Bergh reaction (VBR): Immediate direct negative (I.D. –ve)
(*b*) Serum bilirubin (SB) – 0.2 – 0.8 mg/dl.
(*c*) (*i*) AST – 8–20 U/L
(*ii*) ALT – 10–40 U/L
(*iii*) ALP – 40–125 U/L

49. CLASSIFY THE JAUNDICE AND GIVE INTERPRETATION OF L.F.T. VALUES IN THEM.

Ans. Classification





LIPID PROFILE

50. WHAT ARE THE SUBSTANCES INCLUDED IN LIPID PROFILE AND GIVE THEIR NORMAL VALUES?

Ans.

1.	Serum cholesterol	150 – 180 mg/dl desirable	Borderline upto 200 mg/dl
2.	HDL-Cholesterol	40 – 60 mg/dl desirable	Lower side of borderline 35–39 mg/dl
3.	LDL-Cholesterol	Below 100 mg/dl desirable	Borderline upto 130 mg/dl
4.	Triglycerides (TAG)	60–150 mg/dl	

51. GIVE CLINICAL INTERPRETATION OF SERUM CHOLESTEROL LEVEL.

Ans. ↑↑ cholesterol level observed in:

1. Hypothyroidism (Myxoedema).
2. Nephrotic syndrome.
3. Primary biliary cirrhosis and biliary obstruction.
4. Familial hypercholesterolemia.

THIS PAGE IS BLANK

Index

α-Helix structure 13
α-oxidation 56, 59
β-Oxidation 56
β-pleated sheet 13

A
Acetyl-CoA 43
Acid-base balance 152, 153
Albumin 159, 160
Allosteric inhibition 23
Amino-acids 16
Amylopectn 4, 5
Amylose 4, 5
Anaemia-iron def. 213, 214
Anion Gap 157
Anti-cancerous drugs 126, 127
Anticodon 101
ATP 83

B
Beer's law 222
Bence-jones protein 161
Bile pigments 176, 177
Bile salts, Bile acids 64
Biochemical function 29-33
Bioenergetics & biological oxidation 34-37
Biotin 31
Biuret test 217
Blood sugar 53, 54
BMR 128,129
Bohr Effect 170
Buffers 153
C
Calcitriol 33
Calcium 142-144
Cancer 122
Carbohydrates-chemistry 1-6
Cardiac enzymes 23
Catecholamines 193-195
Chemi-osmotic theory 35
Chimertic DNA 112, 113
Chloride shift 155
Cholesterol 63,64
Chromatography 19-27
Citric acid cycle
Clinical Biochemistry 200-215
Codon 101
Coenzyme 35
Coenzymes 24, 25
Competitive inhibition 22
Complement system 164
Conjugated protein 18
Conjugation 117, 118
Cori's cycle 51
Corticosteroids 191-193
CPP nucleus 10
Creatinine 78
Creatinine clearance test 215
CSF 226
Cyclic AMP 84
Cytochrome oxidase 36
Cytochrome P450 36

D
De novo synthesis of fatty acids 57
Deamination 71, 72
Decarboxylation 72
Deficiency manifestations 27-29
Derived proteins 18
Detoxification 116
Diabetes mellitus 52-53
Diabetic ketoacidosis 210
DNA 85-86
DNA Polymerase 94

E
Eicosanoids 11, 60
Electrolytes 146-148
Electrophoresis 18-19
Enzymes Actions 74-77
Enzymes classification 24
Epimers 2
Essential amino acids 70
Essential fatty acids 8
Essential fructosuria 55
Essential pentosuria 51
ETC 35

F
Familial hypercholesterolemia 66
Fatty acid synthase complex 59
Fatty acids 7
Fatty liver 67,68
Free energy 34
Free radicals 119,120
Fructose 1, 55

G
GABA 82
Galactose 54
Gene amplification 123
Genetic code 102
GI Hormones 197
Glucagon 197
Glucocorticoids 192
Gluconeogenesis 45-47
Glucose 51
Glucose 6 PD Deficiency 50
Glucose-Alanine cycle 51
Glutathione (GSH-Peroxidase) 17, 36, 118
Glycogenesis 47-48
Glycogenolysis 48-49
Glycolipids 9
Glycolsis 39
Glycosuria 219
Gout 90
Growth factors 125, 126
Growth Hormone 186,187
GTT 53
Gylcogen 5

H
HDL 66
Heme synthesis 173-174
Hemoglobin 165-169
Henderson-Haselbach equation 153
Hepatic coma 212
HMP shunt 50
Hormone sensitive Lipase 66
Hormones 182-199
Hypercalcemia 143
Hyperkalemia 151
Hypernatremia 151-152
Hypocalcemia 143-144
Hypocholesterolemia 66
Hypokalemia 149, 150

I
Immunoglobulin 162-164
Inborn errors of metabolism 80
Insulin 15, 53, 195, 196
Iron 138-140
Iso-electric pH 219
Iso-enzymes 23
Isomers 2

J
Jaundice 178-180

K
K_M value 22
Kerbs cycle 172
Ketone bodies 57
Ketosis 58
Kwashiorkor 134

L
Lactose 4
Lactose intolerance 38
Lambert's law 222
LCAT 65
Lesch-nyhan 90
Leukotrienes 61
Libermann-Burchard reaction 10
Link reaction 42
Lipid profile 227
Lipids classification 7
Lipoic acid 32
Lipoproteins 64,65
Liptropic factors 67-69
Liver function tests 180-226

M
Maltose 4
Mc Ardle's disease 49
Mineral metabolism 136-144
Mineralocorticoids 190
Mucopolysaccharides 5
Muscle dystrophy 206
Mutarotation 3
Mutation 108

N
Net Protein utilization 132
Ninhydrin reaction 218
Nitrogen balance 131-132
Northern blot 113
Nucleic acids 83-84
Nutrition 128-135

O
Obesity 134-135
Of amino acids
Okazaki fragments 95
Omega fatty acids 11
Omega oxidation 59
Oncogenes 122
Oncosupperessor genes 123
Operon concept 110-111
Optical isomers 2
Osazone formation 217
Osteoporosis 144
Oxidative phosphorylation 33

P
Pantothemic acid 27
PAPS 117
Pasteur effect 51
PCR 104
PEM 133-134
Pentose-phosphate pathway 50
Phospholipids 8-9
Photoelectric colorimeter 221
Plasma lipid profile 227
Plasma proteins 159-167
Polyol pathway 55
Porphyias 174-176
Post transcriptionalmodifcation 99
Post translational modification 106
Pribnow box 99
Prostaglandin 61
Protein efficiency ration (PER) 131
Protein synthesis 103-105
Protein-structures 12-15
Proteolytic enzymes 69-70
PRPP 91

PUFA 130
Purines 83
Pyrimidines 83

Q
Quaternary structure 15

R
Rapaport-leubring cycle 40
RDA 27
Reactive Oxygen Species (ROS) 119
Recombinant DNA technology 113,115
Refsum's disease 59
Renal function tests 214
Replication 93-95
Respiratory Burst 120
Respiratory quotient 128
Restriction endonuclease 102
Reverse transcriptase 100
RNA 85
Rothera's test 58

S
Salvage pathway 88
Sanger's reaction 16
SDA 129
Sex Hormones 198-199
Sickle cell haemoglobin 171
Sodium-potassium pump 148
Sources of purine ring 87
Southern blot 113
SRS-A 62
Stereo isomers 2
Substrate level phosphorylation 39
Sucrose 4
Superoxide dismutase 34, 37

T
TATA BOX 99
TCA cycle 42-44
Tetany 143
Thalassemias 172
Thyroid hormones 187-189
Trancription 96-97
Transamination 71
Translation 100-101
Transmethylation 81
Tumour markers 124-125

U
Urea cycle 73-74
Uric acid 89-90
Uronic acid pathway 51

V
Van-Den Bergh Reaction 178
Vector 112
Vitamins-A, B, C, D, E, K, sources 26-27
Von-Gierke's disease 49

W
Water 145-146
Watson and Crick model 86
Western Blot 113
Wobbling phenomenon 102

X
Xenobiotics 116
Xenobiotics 116
Xeroderma pigmentosum 95-96
Xeroderma pigmentosum 95-96

Z
Zinc 136-138